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# Biodiesel

## Feedstocks, Production and Applications

*Edited by Zhen Fang*

# BIODIESEL - FEEDSTOCKS, PRODUCTION AND APPLICATIONS

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# Meet the editor

Prof. Dr. Zhen FANG is leader and founder of biomass group, Chinese Academy of Sciences, Xishuangbanna Tropical Botanical Garden. He is also an adjunct full Professor of Life Sciences, University of Science and Technology of China. He is the inventor of "fast hydrolysis" process (US patent#: 8268126). His speciality is in thermal/biochemical conversion of biomass, nanocatalyst synthesis and their applications, and pretreatment methods of biomass for biorefineries. He obtained his PhD degrees from China Agricultural University (Ph.D. Biological & Agricultural Engineering, 1991, Beijing) and McGill University (Ph.D. Materials Engineering, 2003, Montreal).

# Contents

# Preface

Biodiesel is renewable, biodegradable, nontoxic and carbon-neutral. Biodiesel production has been commercialized in Europe and United States, and its use is expanding dramatically worldwide. Although there are many books that focus on biodiesel, there is the need for a comprehensive text that considers development of biodiesel systems from the production of feedstocks and their processing technologies to the comprehensive applications of both by-products and biodiesel.

This book includes 17 chapters contributed by experts around world on biodiesel. The chapters are categorized into 4 parts: Feedstocks, Biodiesel production, By-product applications, Biodiesel applications in engines.

Part 1 (Chapters 1-5) focuses on feedstocks. Chapters 1 and 2 cover the growth of microalgae and algae for the production of biodiesel and other biofuels. Chapter 3 introduces the major diseases of biodiesel plant – Jatropha curcas L. during its plantation. Chapter 4 briefly reviews biodiesel feedstocks and their processing technologies. Chapter 5 studies some of non traditional seed oils (e.g., safflower and milk thistle) for the production of biodiesel.

Part 2 (Chapters 6-9) covers biodiesel production methods. Chapter 6 gives an overview of biodiesel production and its properties, and includes discussion on metallic corrosion from biodiesel and novel analytical methods for contaminants. Ultrasonic process, lipase applications and supercritical ethanol approaches in biodiesel production are introduced and discussed in detail in Chapters 7-9.

Part 3 (Chapters 10-13) shows applications of byproducts. Approaches for the detection of toxic compounds in Jatropha and castor seed cakes are reviewed in Chapter 10. Bio-detoxification of Jatropha cake as animal feed is introduced in Chapter 11. Chapters 12 and 13 describe the processes and reactors to convert glycerol to methanol and biogas.

Part 4 (Chapters 14-17) presents applications of biodiesel in engines. Chapters 14-16 review the practical use, combustion modeling of biodiesel as well as application of blending liquid biofuels (e.g., butanol, rapeseed oil) in engines. Finally, Chapter 17 gives examples of particulate emissions from diesel engines fuelled with waste cooking oil derived biodiesel.

This book offers reviews of state-of-the-art research and applications on biodiesel. It should be of interest for students, researchers, scientists and technologists in biodiesel.

I would like to thank all the contributing authors for their time and efforts in the careful construction of the chapters and for making this project realizable. It is certain that the careers of many young scientists and engineers will benefit from careful study of these works and that this will lead to further advances in science and technology of biodiesel.

I am also grateful to Ms. Iva Simcic (Publishing Process Manager) for her encouragement and guidelines during my preparation of the book.

Finally, I would like to express my deepest gratitude towards my family for their kind cooperation and encouragement, which help me in completion of this project.

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# Feedstocks

Chapter 1

# Potential Production of Biofuel from Microalgae Biomass Produced in Wastewater

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Valeriano A. Corbellini and Eduardo A. Lobo

Additional information is available at the end of the chapter

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## 1. Introduction

Microalgae are the principal primary producers of oxygen in the world and exhibit enormous potential for biotechnological industries. Microalgae cultivation is an efficient option for wastewater bioremediation, and these microorganisms are particularly efficient at recovering high levels of nitrogen, inorganic phosphorus, and heavy metals from effluent. Furthermore, microalgae are responsible for the reduction of $CO_2$ from gaseous effluent and from the atmosphere. In general, the microalgae biomass can be used for the production of pigments, lipids, foods, and renewable energy [1].

Much of the biotechnological potential of microalgae is derived from the production of important compounds from their biomass. The biodiversity of the compounds derived from these microorganisms permits the development of new research and future technological advances that will produce as yet unknown benefits [2].

Microalgae grow in open systems (turf scrubber system, raceways, and tanks) and in closed systems (vertical (bubble column) or horizontal tubular photobioreactors, flat panels, biocoils, and bags). The closed systems favor the efficient control of the growth of these microorganisms because they allow for improved monitoring of the growth parameters [3-4].

Because microalgae contain a large amount of lipids, another important application of microalgae is biodiesel production [5]. In addition, after hydrolysis, the residual biomass can potentially be used for bioethanol production [6]. These options for microalgae uses are promising for reducing the environmental impact of a number of industries; however, there

is a need for optimizing a number of parameters, such as increasing the lipid fraction and the availability of nutrients [7].

Notably, the microalgae biomass can produce biodiesel [5], bioethanol [6], biogas, biohydrogen [8-9] and bio-oils [10], as shown in Figure 1.

The productivity per unit area of microalgae is high compared to conventional processes for the production of raw materials for biofuels, and microalgae represent an important reserve of oil, carbohydrates, proteins, and other cellular substances that can be technologically exploited [2,11]. According to Brown *et al*. [12], 90-95% of the microalgae dry biomass is composed of proteins, carbohydrates, lipids, and minerals.

An advantage of culturing algae is that the application of pesticides is not required. Furthermore, after the extraction of the oil, by-products, such as proteins and the residual biomass, can be used as fertilizer [13]. Alternatively, the residual biomass can be fermented to produce bioethanol and biomethane [14]. Other applications include burning the biomass to produce energy [15].

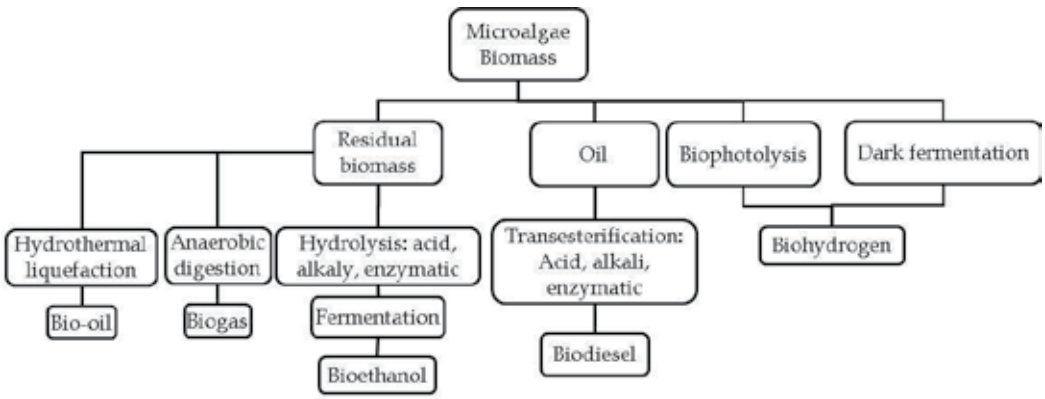


**Figure 1.** Diagram of the principal microalgae biomass transformation processes for biofuel production.

The cultivation of microalgae does not compete with other cropsfor space in agricultural areas, which immediately excludes them from the "biofuels versus food" controversy. Similar to other oil crops, microalgae exhibit a high oil productivity potential, which can reach up to 100,000 L $he^{-1}$. This productivity is excellent compared to more productive crops, such as palm, which yield 5,959 L $he^{-1}$ and thus contribute to the alleviation of the environmental and economic problems associated with this industry[16].

Although the productivity of microalgae for biofuel production is lower than traditional methods, there is increasing interest and initiatives regarding the potential production of microalgae in conjunction with wastewater treatment, and a number of experts favor this option for microalgae production as the most plausible for commercial application in the short term [17].

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## 2. Wastewater microalgae production

Photosynthetic microorganisms use pollutants as nutritional resources and grow in accordance with environmental conditions, such as light, temperature, pH, salinity, and the presence of inhibitors [18]. The eutrophication process (increases in nitrogen and inorganic phosphorus) of water can be used as a biological treatment when the microalgae grow in a controlled system. Furthermore, these microorganisms facilitate the removal of heavy metals and other organic contaminants from water [19-22].

In general, the use of microalgae can be combined with other treatment processes or as an additional step in the process to increase efficiency. Therefore, microalgae are an option for wastewater treatments that use processes such as oxidation [23], coagulation and flocculation [24], filtration [25], ozonation [26], chlorination [27], and reverse osmosis [28], among others. Treatments using these methods separately often prove efficient for the removal of pollutants; however, methods that are more practical, environmentally friendly, and produce less waste are desirable. In this case, the combination of traditional methods with microalgae bioremediation is promising [29]. The bioremediation process promoted by open systems, such as high rate algal ponds, combines microalgae production with wastewater treatment. In addition, the control of microalgae species, parasites, and natural bioflocculation is important for cost reduction during the production of the microorganism [20, 30].

Many microalgae species grow under inhospitable conditions and present several possibilities for wastewater treatments. All microalgae production generates biomass, which must be used in a suitable manner [31-32].

Microalgae are typically cultivated in photobioreactors, such as open systems (turf scrubbers, open ponds, raceway ponds, and tanks) or closed system (tubular photobioreactors, flat panels, and coil systems). The closed systems allow for increased control of the environmental variables and are more effective at controlling the growth conditions. Therefore, the specific cultivation and input of $CO_2$ are more successful. However, open systems can be more efficient when using wastewater, and low energy costs are achieved for many microalgae species grown in effluents in open systems [33-35]. Because of the necessity for renewable energy and the constant search for efficient wastewater treatment systems at a low cost, the use of microalgae offers a system that combines wastewater bioremediation, $CO_2$ recovery, and biofuel production.

In turf scrubber systems, high rates of nutrient (phosphorus and nitrogen) removal are observed. This phenomenon was observed in the biomass retained in the prototype turf scrubber system used in three rivers in Chesapeake Bay, USA. The time of year was crucial for the bioremediation of excess nutrients in the river water, and the best results demonstrated the removal of 65% of the total nitrogen and up to 55% of the total phosphorus, both of which were fixed in the biomass [32].

Compared to other systems, such as tanks and photobioreactors (Fig. 2), the algae turf scrubber system is an alternative for the final treatment of wastewater. The turf scrubber system offers numerous advantageous characteristics, such as temperature control in regions with

high solar incidence and the development of a microorganism community using microalgae, other bacteria, and fungi that promote nutrient removal. Under these conditions, it is possible to obtain biomass with the potential for producing biofuels. However, sufficient levels of oil in the biomass are an important consideration for the production of other biofuels, such as bioethanol, bio-oil, and biogas, among others, which would achieve the complete exploitation of the biomass.

Considering the possibility of using all the biomass, photobioreactors can be used to produce feedstock for biofuel, such as biodiesel and bioethanol, because the oil level of the biomass produced in closed systems is greater than in open systems. Table 1 shows the results obtained using a mixed system and a similar tubular photobioreactor with microalgae *Desmodesmus subspicatus* in the same effluent [36-37].

**Figure 2.** A) Mixed system prototype for microalgae production using a (1) scrubber, (2) tank, and (3) photobioreactor. B) Microalgae biomass in a mixed system separated by electroflotation [36].

| Parameters | Mixed system | | Photobioreactor | |
|---|---|---|---|---|
| | without $CO_2$ | with $CO_2$ | without $CO_2$ | with $CO_2$ |
| Cultivation Days | 20 | 15 | 7 | 7 |
| Maximum Cell Division (x$10^6$ cell $mL^{-1}$) | 25.48 ± 0.02 | 26.97 ± 0.21 | 8.49 ± 1.02 | 25.98± 1.57 |
| Average Cell Division (K) | 0.29 ± 0.48 | 0.16 ± 0.33 | -0.12 ± 0.60 | 0.34 ± 0.40 |
| Biomass (g $L^{-1}$) | 0.62 ± 0.11 | 0.72 ± 0.15 | 0.18 ± 5.65 | 1.41 ± 1.40 |
| Lipids (%) | 1.36 ± 0.29 | 6.07 ± 0.12 | 18.73 ± 0.25 | 12.00 ± 0.28 |

**Table 1.** Microalgae biomass growth and total lipids in a mixed system and a tubular photobioreactor [36-37].

The removal of nutrients from the effluent produced excellent results using the genus *Scenedesmus*, as shown in Table 2. Other studies have also produced promising results. According to Ai *et al*. [38], the cultivation of *Spirulina platensis* in photobioreactors was satisfactory because of the photosynthetic performance. The pH, temperature, and dissolved oxygen levels

were controlled effectively; however, continuous operation was required to ensure the reliability of photosynthetic performance in the photobioreactor.

The cultivation of the diatom *Chaetoceros calcitrans* in photobioreactors exhibited high growth rates; the maximum specific growth rate (μ) achievable was $9.65 \times 10^{-2}$ $h^{-1}$ and $8.88 \times 10^{6}$ cells $mL^{-1}$ in semicontinuous and batch systems, respectively. Even with a lower incidence of light, the results for the production of biomass were good [39].

The cultivation of microalgae *Chlorella* sp. in a semicontinuous photobioreactor produced a satisfactory level of biomass production ($1.445 \pm 0.015$ g $L^{-1}$ of dry cells). The growth, productivity and the amount of $CO_2$ removed obtained under conditions of increased control of the culture and a high concentration of inoculum using cells already adapted to the system increased the $CO_2$ assimilation[33]. The growth rate is also influenced by the concentration of microalgae until reaching an optimum concentration under the operational conditions used [40].

Therefore, microalgae can produce 3-10 times more energy per hectare than other land cultures and are associated with $CO_2$ mitigation and wastewater depollution [41]. Microalgae production is a promising alternative to land plants for reducing environmental impacts; however, the optimization of a number of the production parameters that are important for the viability of the process must be considered, such as the increase in lipid production [7].

| Microalgae | System | Removal (%) | |
|---|---|---|---|
| | | Nitrogen | Phosphorus |
| *Melosira* sp.; *Lygnbya* sp.; *Spirogyra* sp.; *Ulothrix* sp.; *Microspora* sp.; *Claophora* sp.; (seasonal succession) [32] | Turf scrubber | 65 | 45-55 |
| *Chlorella* sp.; *Euglena* sp.; *Spirogyra* sp.; *Scenedesmus* sp.; *Desmodesmus* sp.; *Pseudokirchneriella* sp.; *Phormidium* sp.; *Nitzschia* sp.[36] | Mix | 99 | 65 |
| *Scenedesmus* sp. [42] | Photobioreactor | 98 | 98 |
| *Scenedesmus* sp. [43] | Immobilized cell | 70 | 94 |
| *Chlamydomonas sp.* [44] | Photobioreactor | 100 | 33 |
| *Scenedesmus obliquus* [45] | Immobilized cell | 100 | - |
| *Scenedesmus obliquus* [46] | Photobioreactor | 100 | 98 |

**Table 2.** Use of microalgae grown in different systems for the removal of nitrogen and phosphorus from wastewater.

The bioremediation of wastewater using microalgae is a promising option because it reduces the application of the chemical compounds required in conventional mechanical methods, such as centrifugation, gravity settling, flotation, and tangential filtration [21].

The feasibility of using microalgae for bioremediation is directly related to the production of biofuels because of the high oil content. Without the high oil levels, using other bacteria for

this purpose would be more advantageous because there are limitations to the removal of organic matter by microalgae. In the literature, emphasis is placed on the ability of microalgae to remove heavy metals from industrial effluents [47].

## 3. Biofuels

The term biofuel refers to solid, liquid, or gaseous fuels derived from renewable raw materials. The use of microalgal biomass for the production of energy involves the same procedures used for terrestrial biomass. Among the factors that influence the choice of the conversion process are the type and amount of raw material biomass, the type of energy desired, and the desired economic return from the product [30].

Microalgae have been investigated for the production of numerous biofuels including biodiesel, which is obtained by the extraction and transformation of the lipid material, bioethanol, which is produced from the sugars, starch, and carbohydrate residues in general, biogas, and bio-hydrogen, among others (Fig. 3) [8].

Between 1978 and 1996, the Office of Fuels Development at the U.S. Department of Energy developed extensive research programs to produce renewable fuels from algae. The main objective of the program, known as The Aquatic Species Program (ASP), was to produce biodiesel from algae with a high lipid content grown in tanks that utilize $CO_2$ waste from coal-based power plants. After nearly two decades, many advances have been made in manipulating the metabolism of algae and the engineering of microalgae production systems. The study included consideration of the production of fuels, such as methane gas, ethanol and biodiesel, and the direct burning of the algal biomass to produce steam or electricity [48].

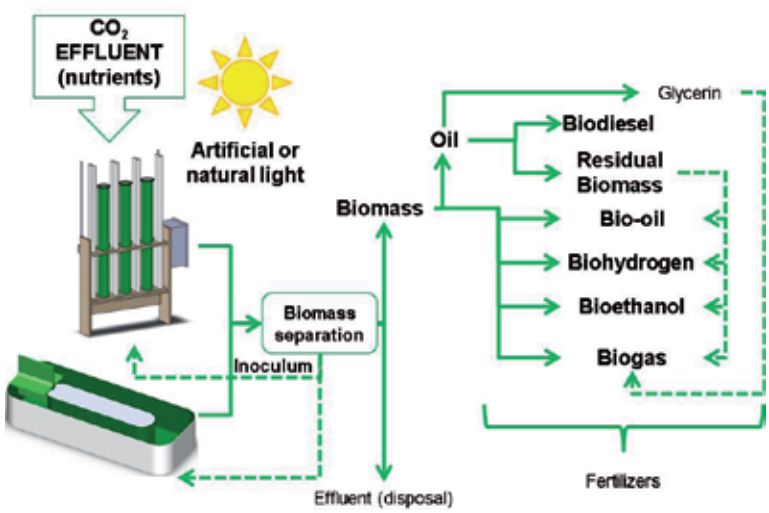


**Figure 3.** Utilization scheme for the microalgae biomass produced in wastewater.

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### 3.1. Biodiesel

The choice of raw material is a critical factor contributing to the final cost of biodiesel andaccounts for 50-85% of the total cost of the fuel. Therefore, to minimize the cost of this biofuel, it is important to assess the raw material in terms of yield, quality, and the utilization of the by-products [49-50].

A positive aspect of the production of biodiesel from microalgae is the area of land needed for production. For example, to supply 50% of the fuel used by the transportation sector in the U.S. using palm oil, which is derived from a plant with a high oil yield per hectare, would require 24% of the total agricultural area available in the country. In contrast, if the oil from microalgae grown in photobioreactors was used, it would require only 1-3% of the total cultivation area [49].

The biochemical composition of the algal biomass can be manipulated through variations in the growth conditions, which can significantly alter the oil content and composition of the microorganism [51]. Biodiesel produced from microalgae has a fatty acid composition (14 to 22 carbon atoms) that is similar to the vegetable oils used for biodiesel production [51-52].

The biodiesel produced from microalgae contains unsaturated fatty acids [53], and when the biomass is obtained from wastewater and is composed of a mixture of microalgae genera, it can exhibit various fatty acids profiles. Bjerk [36] produced biodiesel using a mixed system containing the microalgae genera *Chlorella* sp., *Euglena* sp., *Spirogyra* sp., *Scenedesmus* sp., *Desmodesmus* sp., *Pseudokirchneriella* sp., *Phormidium* sp. (cyanobacteria), and *Nitzschia* sp., identified by microscopy in accordance with Bicudo and Menezes [54]. The $CO_2$ input, the stress exerted by the nutrient composition, and the existence of a screen to fix the filamentous algae contributed to differential growth and differences in the fatty acid profiles (Table 3). Consequently, the biodiesel produced was relatively stable in the presence of oxygen.

In this mixed system, a difference between the fatty acid profiles of the biomass obtained in the photobioreactor compared to the biomass obtained on the screen was observed. The biomass from the screen contained the filamentous algae genera, and the oil did not contain linoleic acid.

This observation is important for biodiesel production because the oil produced was less unsaturated. The iodine index reflects this trend; oils from species such as *Spirulina maxima and Nanochloropsis* sp. have iodine indices between 50 and 70 mg $I_2$ $g^{-1}$ of oil, whereas in species such as *Dunaliella tertiolecta* and *Neochloris oleobundans*, the iodine index is greater than 100 mg $I_2$ $g^{-1}$ of oil [56].

The composition and proportion of fatty acids in the microalgae oil depends on the species used, the nutritional composition of the medium, and other cultivation conditions [57].

Table 4 shows the microalgae commonly used for oil production. The literature lacks information regarding the iodine index or the composition of saturated and unsaturated fatty acids, which could help identify the appropriate microalgae species for biodiesel production. Information on numerous parameters is important, such as the oil unsaturation levels, the productivity of the microalgae in the respective effluents, the growth rate, and the total

biomass composition. Using this information, a decision can be made regarding the economic and environmental feasibility of producing biodiesel and adequately allocating the waste.

| Fatty acids* | without CO2 (%) | with CO2 (%) | with $CO_2$ (screen) (%) |
|---|---|---|---|
| Caprylic (C8:0) | 0.05 | 0.08 | - |
| Myristic (C14:0) | 1.93 | 1.60 | 1.85 |
| Pentadecanoic (C15:0) | 0.50 | 0.44 | 0.52 |
| Palmitoleic (C16:1) | 1.28 | 2.02 | 4.20 |
| Palmitic (C16:0) | 29.58 | 24.68 | 32.50 |
| Margaric (C17:0) | 0.89 | 0.62 | 1.02 |
| Linoleic (C 18:2) | 15.12 | 9.51 | - |
| Oleic (C 18:1n-9) | 26.60 | 39.94 | 20.19 |
| Estearic (C 18:0) | 9.75 | 9.69 | 12.16 |
| Araquidic (C 20:0) | 0.70 | 1.43 | 1.72 |
| Saturated and unsaturated not identified** | 13.6 | 9.97 | 25.84 |

*The oil extraction method was adapted from the Bligh and Dyer (1959) method described by Gressler [37] using *Desmodesmussubspicatus* and the transesterification method described by Porte *et al.* [55] on a laboratorial scale.

**Table 3.** Relative proportion (%) of fatty acid methyl esters found in microalgae biomass cultivated in wastewater with and without $CO_2$ in a mixed system.

Among the microalgae shown in Table 4 that have an oil content that makes them competitive with land crops, twelve species (*Achnanthes sp., Chlorella sorokiniana, Chlorella* sp., *Chlorella vulgaris, Ellipsoidion sp., Neochloris oleoabundans, Nitzschia sp., Scenedemus quadricauda, Scenedemus sp., Schizochytrium sp., Skeletonoma costatum, and Skeletonoma sp.*) are from fresh water and can be investigated for the bioremediation of common urban and industrial effluents that do not have high salinity and contain pollutants that can be used as nutrients for the microorganisms. Because of their potential for oil production, a number of these microalgae species have been used for the production of biodiesel on a laboratory scale, although their potential industrial use associated with the bioremediation of industrial effluents is unknown. Studies using *Chlamydomonas* sp. [47] cultured in wastewater produced a rate of 18.4% oil and a fatty acid profile suitable for biodiesel production in addition to an excellent rate of nutrient removal (nitrogen and phosphorus).

| Microalgae | Oil (%) | Microalgae | Oil (%) |
|---|---|---|---|
| *Achnanthes* sp. | 44.5 | *Nannochloris* sp. | 20.0–35.0 |
| *Ankistrodesmus* sp. | 24.0–31.0 | *Nannochloropsis oculata* | 22.7–29.7 |
| *Botryococcus braunii* | 25–75 | *Nannochloropsis* sp. | 12.0-68.0 |
| *Chaetoceros calcitrans* | 39.8 | *Neochloris oleoabundans* | 35.0–54.0 |
| *Chaetoceros muelleri* | 33.6 | *Nitzschia* sp. | 45.0–47.0 |
| *Chlorella sorokiniana* | 19.3 | *Phaeodactylum tricornutum* | 18.7 |
| *Chlorella* sp. | 18.7–32 | *Pavlova lutheri* | 35.5 40.2 |
| *Chlorella vulgaris* | 19.2 | *Pavlova salina* | 30.9- 49.4 |
| *Chlorococcum* sp. | 19.3 | *Phaeodactylum tricornutum* | 18.0–57.0 |
| *Chlamydomonas* sp. | 18.4 | *Synechocystis aquatilis* | 18.5 |
| *Crypthecodinium cohnii* | 20.0 | *Scenedemus quadricauda* | 18.4 |
| *Cylindrotheca* sp. | 16–37 | *Scenedemus* sp. | 21.1 |
| *Dunaliella primolecta* | 23.0 | *Schizochytrium* sp. | 50.0–77.0 |
| *Ellipsoidion* sp. | 27.4 | *Skeletonoma costatum* | 21.0 |
| *Heterosigma* sp. | 39.9 | *Skeletonoma* sp. | 31.8 |
| *Isochrysis*sp. | 22.4-33 | *Tetraselmis sueica* | 15.0–23.0 |
| *Isochrysis galbana* | 7.0-40.0 | *Thalassioria pseudonana* | 20.6 |
| *Monallanthus salina* | >20.0 | *Thalassiosira* sp. | 17.8 |

Adapted from [5,16,44,52,58-60], considering the values found under the respective production condition.

**Table 4.** Oil-producing microalgae with potential for biodiesel production.

### 3.2. Bioethanol

Bioethanol production from microalgae has received remarkable attention because of the high photosynthetic rates, the large biodiversity and variability of their biochemical composition, and the rapid biomass production exhibited by these microorganisms [1].

Furthermore, bioethanol derived from microalgae biomass is an option that demonstrates the greatest potential. John *et al*. [61] assessed microalgae biomass as a raw material for bioethanol production and argued that it is a sustainable alternative for the production of renewable biofuels. Examples of the genera of microalgae that fit the parameters for bioethanol production include the following: *Chlorella, Dunaliella, Chlamydomonas, Scenedesmus, Arthrospira,* and *Spirulina.* These microorganisms are suitable because they contain large amounts of starch and glycogen, which are essential factors for the production of bioethanol. The carbohydrate composition of these genera can be 70% of the biomass [62].

Traditionally, bioethanol is produced through the fermentation of sugar and starch, which are produced from different sources, such as sugarcane, maize, or a number of other grains [62].

After the oil extraction, the residual biomass contains carbohydrates that can be used for bioethanol production. This process represents a second-generation bioethanol and may be an alternative to the sugar cane ethanol produced in Brazil and corn or beet ethanol produced in other countries. The process requires pretreatment with a hydrolysis step before fermentation [63-65].

In bioethanol production, the processes vary depending on the type of biomass and involve the pretreatment of the biomass, saccharification, fermentation, and recovery of the product. The pretreatment of the biomass is a critical process because it is essential for the formation of the sugars used in the fermentation process (Table 5). Before the traditional fermentation process, acid hydrolysis is widely used for the conversion of carbohydrates from the cell wall into simple sugars. The acid pretreatment is efficient and involves low energy consumption [63].

Other techniques, such as enzymatic digestion [74] or gamma radiation [75], are interesting alternatives for increasing the chemical hydrolysis to render it more sustainable. Through analysis of the process in terms of energy, mass, and residue generation, it is possible to determine the best route. With enzymatic hydrolysis, the process can be renewable. Another technique for pretreatment of the biomass is hydrolysis mediated by fungi. Bjerk [36] investigated the *Aspergillus* genera for this purpose, and the bioethanol produced was monitored by gas chromatography using a headspace autosampler. The study demonstrated that seven strains (four isolates from *A. niger*, one from *A. terreus*, one from *A. fumigatus,* and one from *Aspergillus* sp.) were more efficient at hydrolyzing the residual biomass.

However, it is worth noting the importance of developing a well-designed and efficient system for the cultivation of these microorganisms, which can remove compounds that cause impurities in the final product. In addition, more studies should be undertaken to select strains that are resistant to adverse conditions, especially studies related to genetic engineering.

According to Yoon *et al*. [75], the use of gamma radiation is of potential interest for the hydrolysis of the microalgae biomass because compared to chemical or enzymatic digestion, gamma radiation raised the concentration of sugar reducers, and the saccharification yield was 0.235 g $L^{-1}$ when gamma radiation was combined with acid hydrolysis. Acid hydrolysis alone produced a saccharification yield of only 0.017 g $L^{-1}$.

| Microalgae | Pre treatment | Reaction condition | | Fermenter | Bioethanol yield (%) | Ref. |
|---|---|---|---|---|---|---|
| | | Temp. (°C) | Time (min) | | | |
| *Chlamydomonas reinhardtii** | acid | 110 | 30 | *Saccharomyces cerevisiae* | 29.2 | [66] |
| *Chlorococcum* sp. | alkaline | 120 | 30 | *Saccharomyces cerevisiae* | 26.1 | [67] |
| | acid | 140 | 30 | *Saccharomyces cerevisiae* | 10-35 | [68] |
| *Chlorococcum humicola* | acid | 160 | 15 | *Saccharomyces cerevisiae* | 52 | [63] |
| *Nizimuddinia zanardini*** | acid | 120 | 45 | - | - | [69] |
| *Kappaphycus alvarezii* | acid | 100 | 60 | *Saccharomyces cerevisiae* | 2.46 | [70] |
| *Scenedesmus obliquus**** | acid | 120 | 30 | - | - | [71] |
| *Spirogyra* | alkaline | - | 120 | *Saccharomyces cerevisiae* | 20 | [72] |
| | enzymatic | - | - | *Saccharomyces cerevisiae* | 4.42 | [73] |
| | enzymatic | - | - | *Zymomonas mobilis* | 9.7 | |

Glucose yield: * 58%; **70.2%; *** 14.7%

**Table 5.** Conditions of bioethanol production from microalgae.

### 3.3. Other biofuels

Several articles describe the thermochemical processing of algal biomass using gasification [63,76] liquefaction [77], pyrolysis [78], hydrogenation [79], and biochemical processing, such as fermentation [80-81]. However, engineering processes have not been investigated as a potential biotechnological method for the production of other biofuels from microalgae.

Currently, the energy derived from biomass is considered one of the best energy sources and can be converted into various forms depending on the need and the technology used, and biogas is chief among the forms of energy produced by biomass. [82].

Anaerobic digestion for biogas production is a promising energy route because it provides numerous environmental benefits. Biogas is produced through the anaerobic digestion of organic waste, drastically reducing the emission of greenhouse gases. As an added benefit, the

by-products of fermentation, which are rich in nutrients, can be recycled for agricultural purposes. Adding anaerobic digestion to the use of biomass waste from which the oil has been removed produces an environmental gain and results in the complete exhaustion of the possible uses for the biomass. This strategy enables biomass waste to be an end-of-pipe technology for industrial processes that generate high amounts of organic matter containing phosphorus and nitrogen. A proposed system for this purpose is shown in Figure 4, which represents a simplification of the work performed by Chen *et al*. [83] and Ehimen *et al.* [84].

Therefore, using the residual microalgae biomass as a source of biogas is similar to other agricultural residue uses [85] in which the organic substrate is converted into biogas through anaerobic digestion, producing a gas mixture containing a higher percentage of carbon dioxide and methane [86].

The use of microalgae for biomethane production is significant because fermentation exhibits high stability and high conversion rates, which makes the process of bioenergy production more economically viable. For example, Feinberg (1984) (cited in Harun*et al*. [87]) considered exploiting *Tetraselmissuecica* for biomethane production in conjunction with the possibilities of producing other biofuels. The production of the following biofuels were proposed: biomethane alone (using total protein, carbohydrate, and lipids); biomethane and bioethanol (using carbohydrate for bioethanol production and protein and lipids for biomethane production); biomethane and biodiesel (using carbohydrate and protein for biomethane production and lipids for biodiesel production); and biomethane, biodiesel, and biomethanol (using carbohydrate for bioethanol production; lipids for biodiesel production, and proteins for biomethane production).

Harun *et al*. [47] also reported that the main factors influencing the process are the amount of the organic load, the temperature of the medium, the pH, and the retention time in the bioreactors, with long retention periods combined with high organic loads exhibiting greater effectiveness for biomethane production.

Converti *et al*. [82] demonstrated this effect, reporting the increased production of total biogas at 0.39 ± 0.02 $m^3$ $kg^{-1}$ of dissolved organic carbon after 50 days of maturation and 0.30 ± 0.02 $m^3$ of biomethane.

When considering total biomass use, in addition to biogas, it is possible to produce biohydrogen and bio-oils using enzymatic and chemical processes.

The chemical processes that can be used for hydrogen production include gasification, partial oxidation of oil, and water electrolysis. In the literature, cyanobacteria are primarily used for the production of biohydrogen through a biological method, and the reaction is catalyzed by nitrogenases and hydrogenases [88]. Studies with *Anabaena* sp. also demonstrate that this biomass is promising for the production of biohydrogen and that adequate levels of air, water, minerals, and light are necessary because the process can be photosynthetic [9,89].

Bio-oil can be produced from any biomass, and for microalgae, a number of investigations have been performed using *Chlamydomonas, Chlorella, Scenedesmus* [90], *Chlorella vulgaris* [91-92], *Scenedesmus dimorphus, Spirulina platensis, Chlorogloeopsis fritschiiwer* [91], *Nannochloropsis oculata* [93], *Chlorella minutissima* [94], and *Dunaliella tertiolecta* [10].

http://dx.doi.org/10.5772/52439

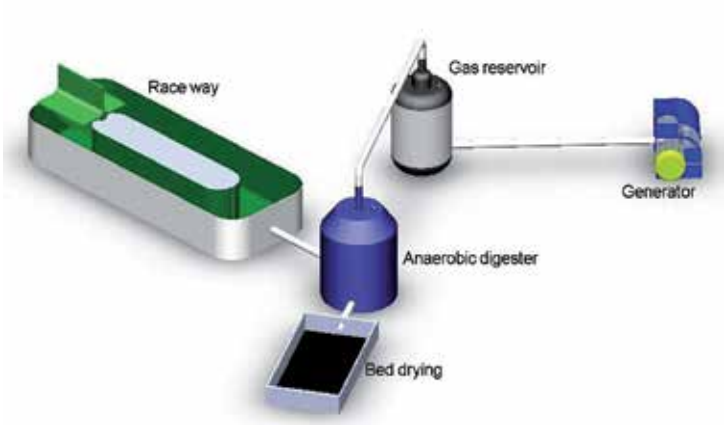


**Figure 4.** Anaerobic digestion of biomass waste in a unit of bioenergy production associated with an effluent treatment plant.

These initiatives highlight the potential use of hydrothermal liquefaction, which is a process that converts the biomass into bio-oil at a temperature range of 200-350°C and pressures of 15-20 MPa. According to Biller et al. [91], yields of 27-47% are possible, taking into account that microalgae can be produced using recycled nutrients, providing greater sustainability to the system.

A different bio-oil can be produced using pyrolysis in which the oil composition features compounds exhibiting boiling points lower than the hydrothermal liquefaction product [93]. In pyrolysis, the nitrogen content of the microalgae is converted into NOx during combustion. NOx is an undesirable emission that increases depending on the microalgae and their protein content; however, NOx emissions can be reduced by 42% using a hydrothermal pretreatment process.

In terms of waste recovery, the use of *Dunaliella tertiolecta* cake under various catalyst dosage conditions, temperatures, and times were used in hydrothermal liquefaction, and the yield was 25.8% using 5% sodium carbonate as catalyst at 360°C [10].

Therefore, in addition to producing microalgae in urban or industrial effluents, it is possible that after the extraction of the oil for biodiesel production and the production of bioethanol from carbohydrates, biogas or bio-oil can be produced from the waste material.

## 4. Conclusions

This chapter reviews the initiatives for biofuel production from microalgae cultivated in wastewaters. The exploitation of the total microalgae biomass was considered, and the potential for biodiesel and bioethanol production was explored.

The various systems for microalgae production using wastewater and the consequences for biodiesel and bioethanol production were discussed in detail.

Microalgae have been used to produce biodiesel and bioethanol with excellent results; however, the use of microalgae must be expanded to include bioremediation combined with biofuel production. The commercial initiatives for this purpose will depend on the composition and volume of the effluent, on the selected microalgae species, and on the temperature and light conditions of the region. The initiatives will also depend on the particular biofuel of interest to the region or that required for local consumption. Therefore, each situation must be analyzed on an individual basis, and there is no single model; however, because of the wide biodiversity of microalgae and the extensive ongoing research capacity of many countries, it is likely that a conditions for viable microalgae production can be achieved anywhere.

Finally, it should be noted that microalgae that are adapted to the environment could produce biomass that, depending on the composition of cells, can be used as the raw material for the production of one or more biofuels.

The research and development of microalgae production in urban or industrial effluents involve principles of sustainable development, clean technology, and the ecology of the productive sectors, prioritizing preventive and remediation steps with the decreased use of energy and inputs. Therefore, there is an emphasis on the methods of treatment, the transformation processes, and the biotechnological products (biofuels), prioritizing the use of wastewater for biomass and bioenergy production. These developments will decrease the impact on activities of anthropogenic origin from the industrial, commercial and service sectors, among others.

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Chapter 2

# Algal Biorefinery for Biodiesel Production

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Additional information is available at the end of the chapter

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## 1. Introduction

In recent years, the rapid depletion of fossil fuels, increase in energy demand, global warming, increase in price of fossil fuels depends on economic and political behaviors increased orientation to alternative energy sources. In this context, biodiesel that is one of the renewable alternative energy sources draws attention because of its useful features such as easily biodegradable and environmentally friendly. However, biodiesel production from oil crops does not meet the required demand of vehicle fuel, and recently it is not economic and feasible. It needs to be improved to produce more economically to be able to compete with diesel in the market. Vegetable oils and crops which biodiesel produced from are a kind of human food sources and the shortage on food source cause to go up prices and make the biodiesel high-priced. To meet the requirements, the interest on algae is increased day by day since this technology has potential to meet global demand [1]. Microalgae have higher productivity per area and no need for farm field to grow as opposed to oil crops and animal fat. Microalgae use sunlight to reduce $CO_2$ to biofuels, foods, fertilizers, and valuable products. Furthermore, microalgae can be used to get different types of biofuels. Using microalgae as fuel source is not a novel idea but recently the prices of diesel and global warming hit this solution to the top [2].

Microalgae have lots of advantages for biodiesel production over other raw materials such as crops, waste cooking oils, and so on. Microalgae have short doubling time which is around 12-24 h since they have a simple structure and capable to high photosynthetic efficiency and they contain much more amount of oil than other oil crops that can be used as oil source for biodiesel production. Compared with the oil yields from various oil crops such as corn (172 L/ha), soybean (446 L/ha), canola (1190 L/ha), jatropha (1892 L/ha), coconut (2689 L/ha) and oil palm (5959 L/ha), oil yield from microalgae is very high as 136900 L/ha and 58700 L/ha for 70% oil in biomass and 30% oil in biomass, respectively [2-4].

The other significant feature is that algae can grow everywhere and every season in a year since there are thousands of algae species that have different adaptations and different properties. They can grow in saltwater, freshwater, lakes, deserts, marginal lands, etc. In addition to biodiesel production, algae can be also used as feedstock to produce different valuable products such as fertilizer, energy, neutraceuticals, protein, animal feed etc. The other significant property is that microalgae can remove some heavy metals, phosphorous, and nitrogen from water during its growth. Algae also clean up the water. Moreover, microalgae sequester lots of carbon by photosynthesis. Utilization of carbon dioxide by algae is significantly lowering the risk for greenhouse gas effects. Lastly, usage of microalgae for biodiesel almost cancels out the carbon dioxide and sulfur release to atmosphere [5]. These reasons mentioned above are enough to believe that microalgae can take the place of fossil fuels completely.

There are many of microalgae studies for biodiesel production. Because the most of the scientists believe that microalgae will take the place of the petroleum diesel, however, algal biodiesel production is not feasible yet since there is no much commercial or large scale production of microalgae for biodiesel. That is why most of the works are focused on decreasing the cost of biodiesel production or make it competitive versus petroleum diesel. Surely, until these improvements are achieved, algal biodiesel can not be an accurate alternative. The current problems making biodiesel expensive can be improved with some innovations. The first of all is about the algae strain which is also first step of algal biodiesel production. The algae strain should be better than recent ones. There are natural many kinds of algae strains and isolation of new natural algae strain may help procedure to be cost effective. The algae strain has to have high lipid productivity and adaptability to new environments. These features let it produce more and obtain more oil content [6, 7]. As an example, if the flue gas is used as carbon dioxide source, microalgae have to be adapted for this situation so that it can tolerate the high concentration of $SO_x$, $NO_x$, and other gases [8]. That will reduce the cost and increase the biomass growth rate. The other important innovation should focus on cultivation of algae. The large-scale production is one of the most cost-intense parts. The innovative thinking should show a tendency to lower the cost of operation and capital for cultivation systems. As it is explained below, open ponds are the cheapest way but the efficiency of them has to be worked on. Moreover, the closed photobioreactors (PBR) are also being improved for a cheaper way to control and lighten the system. Furthermore, microalgae can be fixed in a cultivation system with an immobilization technique to get higher biomass. The last way to lower the cost is to produce sub-products from microalgae beyond biodiesel. There are lots of high value products and sub-products produced from microalgae such as biogas [9, 10], biobutanol, acetone [11], Omega 3 oil [12], eicosapentaenoic acid [13], livestock feed [14], pharmaceuticals and cosmetics [15, 16]. Especially sub-products can be preferred for economic support of main process.

For example, recovery of methane from microalgae pulp after biodiesel production develops renewability of conversion of microalgae biomass to biodiesel process as much as it makes the cost of process and environmental effects less. The microalgae pulps after oil removed contain significant amounts of protein and carbohydrate that can convert to biogas by anaerobic fermentation. Conversion of algal waste to biogas by anaerobic fermentation will play a dual role for renewable energy production and also sustainable development of microalgal biodiesel industry [17, 18].

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Algae can be also used in bioethanol production. Algae are more uniform and continuous than terrestrial plant, due to lack of functional parts such as root and leaf composition. Their cell walls made of polysaccharides that can hydrolyze to the sugar. For this reason, microalgae can be used as carbon source in fermentation process. Ethanol produced by fermentation can be purified for using as a fuel, $CO_2$ as a nutrient may also be recycled to algae culture to grow microalgae [19, 20].

In this chapter, algae production methods that cover the algae strain and location selection, algae cultivation, harvesting, oil extraction, and algal biodiesel production processes are presented in detail with alternatives. New progresses in this area are also explained.

## 2. Algae strains and properties

Algae are simple organisms including chlorophyll. They can be found in seas, soils and lakes wherever they can use the light for their photosynthesis. There are two types of main algae groups. The first group is macro algae, which includes green, brown and red algae. The second group is microalgae as phytoplankton in the coasts, lakes and oceans, which includes diatoms, dynoflagellates, green and brownish flagellate, and blue-green algae [21].

The classification of algae can be done in many ways since there is a millions of kind. Also there is no standard on classification so you can see different types of classification. The taxonomic group of algae can be given as follow: *Archaeplastida, Chlorophyta*(green algae), *Rhodophyta*(red algae), *Glaucophyta, Chlorarachniophytes, Euglenids, Heterokonts, Bacillariophyceae*(diatoms), *Axodine, Bolidomonas, Eustigmatophyceae, Phaeophyceae*(brown algae), *Chrysophyceae*(golden algae), *Raphidophyceae, Synurophyceae, Xanthophyceae*(yellow-green algae), *Cryptophyta, Dinoflagellates, Haptophyta*[22].

Algae are the most common wide photosynthetic bacteria ecologically. To grow algae some parameters such as amount and quality of ingredients, light, pH, turbulence, salinity, and temperature become prominent. Macro (nitrate, phosphate, silicate) and micro (some metals, B1, B12 and biotin vitamins) elements are required in the growth of algae. Light intensity has also an important role, the light demand changes up to microalgae density and type of microalgae. The other parameter pH is mostly between 7 and 9 for most of algae strains and mostly the optimum range is 8. 2-8. 7. The last parameter salinity should be between 20-24 ppt. Moreover, nitrogen also affects the growth of some algae strains as such as green algae [22-25].

### 2.1. Macroalgae

Macroalgae are adapted to life in ocean and it is a plant mostly seen on the costal strips. There are plenty of macro algae types. Algae can be classified as brown, red, and green based on type of pigments. Recently, several brown algae types have been used in the industry and energy production as an alternative source to fossil fuels, and green algae is also studied to produce biodiesel [26].

Brown algae have xanthophyll pigments and fucoxanthin, which results the colour of brown algae. These substances mask the other pigments [27]. Polysaccharides and higher alcohols are nutrition reserves of brown algae but the main carbohydrate reserve is laminarin. The cell walls of brown algae are made of cellulose and alginic acid. Brown algae have a lot of features such as: Cytotoxic and antitumor activity, Antifungal activity, Anti-inflammatory activity, Antiviral activity, Protection against herbivorous animals (fish, sea urchins), Antioxidant activity [21, 28, 29]. Composition of brown algae can vary according to species, their location, salinity and season. According to analysis, brown algae contain about 85% high moisture and 25 % high sodium carbonate [26].

Green algae contain chlorophyll a and b. Presence of these pigments makes green color of the green algae. There are a few reports about second metabolites of green algae. [21]. Moisture content of green algae is higher than brown algae but they have similar sodium carbonate content. Green algae species can access higher sugar levels and this makes them useful energy sources. They also have high cellulose content [26]. Green algae have a lot of features such as: Anti-inflammatory substances, Cytotoxic and immunosuppressive activities, Antibacterial activity, Antiviral activity, Antifungal activity [30].

Red Algae have phycoerythrin and phycothcyanin pigments that make red color of these algae. These pigments mask the other pigments. The cell walls of red algae made of cellulose, agar and carrageenan [27]. There are approximately 8000 red algae species. In comparison of the other algae species, red algae are considered as the most important active metabolite resource. They have a lot of features such as: Cytotoxic activities, Antiviral activity, Anti-inflammatory activity, Antimicrobial activity, Free radical scavenger activity [21, 31].

### 2.2. Microalgae

There are at least 30000 microalgae species in the world. Microalgae are mostly defined as unicellular photosynthetic cells but some complex associations create larger colonies. This is a heterogenic group, which contains prokaryotic organisms similar to bacteria and eukaryotic cells [26, 32]. Microalgae production is concentrated on particular species, which have special tolerance for extreme conditions in their growth. This situation enables the production in open ponds and canals. In future, microalgae production will focus on more advanced species for the demand of energy and pure monocultures which have specific capabilities like production of carbohydrate, lipid or hydrogen will be cultivated [33]. According to use of algae, biomass of microalgae has variable chemical composition. They can be rich or balanced composition of protein, lipid and sugar. Microalgae selection should be made according to desired biofuels. Microalgae have important lipid content even in the extreme conditions they reach higher lipid content [26].

Green algae or diatoms are the most used microalgae species for production of alternative energy derives. Just a handful of these species has commercial importance. This group contains *Chlorella, Spirulina, Dunaliella* and *Haematococcus*. Only *Dunaliella* is a dominant sea species. These are usually cultivated for extraction of high value component like pigments or proteins [26].

Blue-green algae (cyanobacteria) have a lot of common structural features with bacteria. They are classified as algae because they contain chlorophyll and other components. They have also nitrogenic components because all of the prokaryote species convert atmospheric nitrogen to ammonium [21, 34]. Morphologically blue green algae can have filamentous, conical or unicellular shape. They have a lot of features such as: anticancer and cytotoxic activities, antibacterial activity, antifungal activity, immunosuppressive activity [21, 35, 36].

*Pyrrhophyta (Dinoflagellates)* are unicellular organisms, which are classified as primitive algae. Large amount concentrations of these organisms exist in ocean surface and they cause fish deaths. Also because of their pigments, dinoflagellates give the water brown to red coloration in the sea [34, 37]. Particular dinoflagellate species produce toxin in case of consumed by species such as shellfish. Consumption of contaminated shellfish by humans can cause a lot of health problems including death [21].

*Bacillariophyceae* (Diatoms) are the most versatile and frequent family. They are more feasible for large-scale productions due to short doubling time and easy to grow. Unlike *Dinoflagellates* they create less second metabolites [38].

Microalgae are investigated as biodiesel feedstock because of their high photosynthetic efficiency, their ability to produce lipids. Macroalgae usually don't contain lipids too much and they are taken into consideration for the natural sugars and other carbohydrates that they contain. These contents can be fermented to produce alcohol-based fuels or biogas.

### 2.3. Lipid content of microalgae species

As the structure of many microalgae species can accumulate significant amounts of lipid and provide high oil yield. Their average lipid contents can be reached to 77% of dry biomass under some certain conditions [39]. Table 1 shows lipid content of some microalgae species.

| Microalgae | Oil content (dry weight %) |
|---|---|
| *Botryococcus braunii* | 25-75 |
| *Chlorella protothecoides* | 14-57 |
| *Crypthecodinium cohnii* | 20-51 |
| *Dunaliella tertiolecta* | 16-71 |
| *Nannochloris sp.* | 20-56 |
| *Neochloris oleoabundans* | 29-65 |
| *Phaeodactylum tricornutum* | 18-57 |
| *Schizochytrium sp.* | 50-77 |
| *Skeletonema coastatum* | 13-51 |

**Table 1.** Lipid content of some microalgae species [15, 39, 40-45].

Also high productivity is very important beside high oil content. As shown in table 1, microalgal lipid content can reach 77% by weight of dry biomass but it is observed that there can be low productivity of *Botryococcusbraunii*, however, *Chlorella* appears to be a good choice in biodiesel production, since it has high productivity though lower oil content [39].

Lipid content can be affected by several parameters such as nutrition, environment, cultivation phases and conditions growth can affect fatty acid composition [32], Fatty acid composition is important in microalgae selection because it has a significant effect on biodiesel properties. For example, if unsaturated fatty acid content is high in algal oils and their presence reduces the efficiency of esterification to produce biodiesel [39].

Value chain stages of biodiesel production from microalgae can be given as algae and site selection, algae cultivation, harvesting, filtration, dewatering, oil extraction and biodiesel production [39].

## 3. Biodiesel production from microalgae

The selection of species depends on some factors like ability to usage of nutrition or grow under specific environment conditions. All these parameters should be evaluated for biodiesel production.

### 3.1. Selection of algae strain and location

To make algal biodiesel cost effective lots of researchers keep going on algae culturing. The criteria to select location and sources are mentioned below [46]:

- Water sources and demand, salinity, content
- The region information such as topography, geology
- Weather conditions, isolation, evaporation
- Availability of carbon and food resources

The next decision should be on the algae culturing process type. It can be either batch or continuous process. Depending on microalgae strain, environmental conditions, availability of nutrition and moreover industrial pollutions the process type has to be selected. The devices and apparatuses also have to be adjusted for these conditions and nutrients [39].

Algae strains have different contents, different doubling time (the total biomass per time and volume) and resistance to change in environmental conditions. Biodiesel production directly depends on the oil content of microalgae and its efficiency. So that, even the process and culturing systems are selected perfectly, time and other related factors plays an important role [39].

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### 3.2. Methods used for algae growth

Not only the microalgae strain is important for efficiency of oil but also growing conditions are important. There are different ways to grow algae. Each type of microalgae has a different mechanism which let them to respond different weather and environmental conditions [39, 47]. Different growing conditions affect the microalgae doubling time. There are 4 growing type basically: phototrophic, heterotrophic, mixotrophic, and photo heterotrophic. All of them will be explained in detail.

#### *3.2.1. Phototrophic growth*

Microalgae are mostly thought to be phototrophic since it requires light [48]. Phototrophic growing method is based on using light and carbon dioxide to produce chemical energy during photosynthesis. This is the most common way used to grow microalgae. The best advantage of the process is using carbon dioxide as a carbon source to grow or produce fatty acid. Since carbon dioxide is only the carbon source, locations close to fabrics and companies could be selected to procure carbon dioxide. If it is compared to other growing types, phototrophic method has the lowest contamination risk [49].

#### *3.2.2. Heterototrophic growth*

Some microalgae are not able to grow phototrophic conditions but they can grow in dark using organic carbon as a carbon source like bacteria. If microalgae is using organic carbon these microalgae are heterotrophic growing algae. Heterotrophic growth has advantages over phototrophic growth because light is not required. The biggest problem with the phototrophic is the light penetration when the density of the culture gets higher. In that way one of the biggest problems is solved with heterotrophic growth. Heterotrophic growth will be more cost effective compared to phototrophic growth [48]. And this method is said the most practical and promising way to increase the productivity [50-52]. Also higher oil rates and efficiency can be obtained when the algae grow heterotrophic, but the contamination risk is much higher compared to phototrophic [49].

Microalgae uses different organic carbon sources such as glucose, acetate, glycerol, fructose, sucrose, lactose, galactose, and mannose, especially growth with sugar is more efficient [49].

Mostly the organism growing heterotrophic should have adaptation property to new habitat as soon as possible since when culturing to new media the lag phase should be too short, and durability during processing in fermenters and other machines [48].

#### *3.2.3. Mixotrophic growth*

Mixotrophic growth is a combination of phototrophic and heterotrophic growth. Mixotrophic growth is using organic and inorganic carbon and the process requires light because of photosynthesis. Thus the microalgae have ability to live in both conditions. Microalgae uses organic compounds and carbon dioxide as a carbon source and the released carbon dioxide are also captured with the photosynthesis. Although mixotrophic-growing meth-

od mostly is not preferred compared to heterotrophic and phototrophic growth [49], because of other advantages even so mixotrophic method is applied in some studies. For example; Park *et al.* found that biomass and lipid productivities were boosted by mixotrophic cultivation [53]. Bhatnagar et al. found the mixotrophic growth of some microalgae strains resulted in 3–10 times more biomass production compared to that obtained under phototrophic growth conditions [54].

*3.2.4. Photoheterototrophic growth*

When microalgae use organic compounds as carbon sources, sometimes it requires light. The main difference between mixothrophic and photoheterotrophic is that mixotrophic growth using organic compounds as energy sources, as photoheterotrophic growth requires light as energy source. This method is mostly used for production of some beneficial metabolites; however, it is rarely used for biodiesel production [49]. Metabolisms can split into groups due to pH changes. *Chlorella vulgaris, Haematococcus pluvialis, Arthrospira (Spirulina)* platensis strains are the examples for the growth by mixothrophic, phototrophic and heterotrophic methods. *Selenastrum capricornutum* and *Scenedesmus acutus* are able to grow in phototrophic, heterotrophic, photoheterotrophic conditions [47].

Algae require more than organic carbon, sugar, protein, oil or any carbon sources. Algae cannot grow without vitamins, salts, or some other nutrients (nitrogen and phosphor). Moreover, there are lots of parameters has to be controlled during algae growth to maximize and stabilize the production. Some of these parameters are oxygen rate, carbon dioxide rate, pH, heat, light intensity and so on. When appropriate weather conditions and enough nutrients are provided microalgae grow faster. Mostly doubling time is between 3. 5 h and 24 h [39].

As a result, if we compare different methods mentioned above for microalgae growth;Heterotrophic growth is much better than the others for the application of biodiesel. These methods can produce more oil than other growing types. However, heterotrophic cultures may contaminate especially in open pond systems and result in big problems in large-scale production. Moreover, organic carbon as a carbon source is an expensive raw material and makes the process cost higher. Phototrophic growth is an easily scalable and mostly uses the carbon dioxide from exhaust gas for the production of oil. However, the efficiency of the oil is lower than heterotrophic growth because the biomass doubling time is higher and total biomass rate is lower at the end. Phototrophic method mostly preferred to set a cost effective system [49].

*3.2.5. Conditions for growth of algae*

*3.2.5.1. Light*

The microalgae growing photosynthetically needs light and the light intensity is the most significant limiting factor. Algae culture systems mostly use both sun and lamp light. Mostly lamp-lightened algae culture systems uses wider screens to be able to absorb more light

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from the system. For photosynthetic production, at least 50 % of the volume of PBR has to get enough light [55]. Open raceway ponds, plate, plate PBR, Vertical-column PBRs, Internally-illuminated PBRs, inclined tubular type, horizontal/continuous type, bubble column and air-lift PBRs are the systems used for photosynthetic algae growth. Plate photo bioreactor is more efficient than tubular photo bioreactor because the light can penetrate to bottom more in plate design. Recent works are on closed system photobioreactors to improve the capacity. Some works are done to increase the capacity; however the light penetration becomes a major problem. Light source for open ponds is only Sun. That is why the alteration is not possible for raceway ponds. The depth of the pond that the only thing can be changed. Thus mostly researches are going on closed systems to optimize light emission. Mostly photobioreactors in lab scale are lightened by fluorescence lights from inside and outside [56]. The light wavelength should be between 600-700 nm to maximize the photosynthesis. Light intensity depends on microalgae density. Higher algae density requires higher light intensity. Light also affects the lipid content. Yeesang and Cheirsilp reported that the lipid contents in all strains increased with increasing light intensity in their study [57].

Changes in light intensity and quality can alter biofuel quality [58]. Each type of microalgae has its own optimal light absorbing point. If this point exceeds the optimum point, microalgae light absorption ratio decreases. After a specific point, light decreases the biomass production and this is called photoinhibition. Photoinhibition processes depend on time and after stress of light for a few minute biomass loss starts. 10-20 min later more than 50 % damage can be seen. Cheirsilp and Torpee investigated the effect of light intensity on growth and lipid content of marine *Chlorella sp.* and *Nannochloropsis sp.* The growth of marine *Chlorella sp.* increased when the light intensity was increased from 2000 to 8000 lux. But up to 10000 lux its growth decreased. They reported that this could be some extent of effect from photoinhibition. The growth of *Nannochloropsis sp.* continuously increased up to the maximum level when increasing light intensity up to a maximum light intensity of 10000 lux. [59]. High light intensity limited algal growth, but gave the benefit of higher lipid content and yield. It can be seen in Ruangsomboon's study whose cultures exposed to low light intensity showed a higher biomass compared to others [60].

To increase the microalgae production, photoinhibition should be cut off or exceed to high light intense. In addition, photorespiration decreases the photosynthetic efficiency. Therefore the process has to avoid photorespiration. Photorespiration occurs when the oxygen concentration increases depending on carbon dioxide [56].

Sara et al. investigated the light effects on microalgae. The research was done by using red and blue lasers as light source for photosynthetic growth of green algae. The results showed that the both blue and red lasers increased the algae cell count [61].

Allen and Arnon tested the effect of light on green algae growth. The light intensity was around 16000 lux. There were two samples. One of the samples was analyzed under 11 h darkness and 13 h light. The other sample was analyzed under light for 24 h and the results showed that the growth rate was same. However after 5 days the growth rate for the sample with 24 h light was declined [62].

The effects of light on *Parietochloris incisa* was analyzed by Solovchenko et al. The results showed that best growth was seen on high light (400 μmol photons $m^{-2}$ $s^{-1}$). With high light condition, total fatty acid and arachidonic amount was increased due to increase in biomass [63].

Another study (Yeh et al. ) was focused on effects of different light sources on microalgae (*C. vulgaris)* growth. In the sutdy, three different light sources was used which are tungsten lamp, fluorescent lamp (TL5), fluorescent lamp (helix lamp). The results showed that fluorescence lamps were much better for algae growth. In an other study by Floreto et al., it was mentioned that high light intensity increased the palmitic acid and most fatty acids ratio [64].

*3.2.5.2. Carbon dioxide*

Carbon dioxide is the natural carbon source of the microalgae culture. Oxygen is releasing depending on decreasing carbon amount and it is delivered to the medium. Carbon dioxide is an general carbon source for photosynthetic microalgae. When the carbon amounts get low, oxygen is produced by photolysis of water and released to media. Since algae lives in high carbon dioxide concentration, greenhouse gases, nitrogen dioxide and atmospheric pollutants came from different sources became a food for algae. The exhausted gases can feed algae production facilities from fossil fuels and also its efficiency would be increased. Works on usage of stack gases as carbon source were done but the toxicity of the stack gas components couldn't be documented well. The amount of carbon dioxide required for the growth relates to type of microalgae and photo bioreactor. Some types of algae strains are able to keep growing in high carbon dioxide conditions, in contrast for faster growth lower carbon dioxide concentration is required [56]. Widjaja studied the effect of $CO_2$ on growth and it was seen that this effect correlates directly to the lipid productivity since growth was enhanced tremendously by increasing the $CO_2$ concentration [65]. $CO_2$ requirement can change up to strains. VirthieBhola et al. reported in their studies that at 15% $CO_2$ concentration there is a 3-fold decline in biomass yield when compared to the yield produced at a 4% $CO_2$ concentration. This suggests that the strain under study could not endure $CO_2$ concentrations greater than 4% [66]. Also Ebrahimzadeh et al. reported that increasing $CO_2$ injection had a significant effect on microalgae growth [67]. $CO_2$ input is also important. Sonnekus reported that the $CO_2$ should make up 0. 2 -5% of the total gas flow and being careful about the $CO_2$ input does not lower the pH of the culture [68].

*3.2.5.3. Heat*

Algal growth is also dependent on temperature. For maximum growth there is a need to know the optimal temperature. The temperature changes also lipid production and composition [69]. The degree of unsaturation of algal membrane lipids increases if cultures are maintained at temperatures below their optimum [70]. Other than this temperature is significant for dissolubility of carbon particles, which helps carbon to be used for photosynthesis. Heat effects respiration and photorespiration more than photosynthesis. However, if carbon dioxide and light are the limiting factor, the effect of heat is not significant anymore. Optimal temperature for microalgae cultures is between 20-24 °C. This can be different according to media composition,

type of culture and strain. The most general cultured microalgae can tolerate the temperature between 16- 27 °C. The temperatures lower than 16 °C will increase the duplication time and higher than 35 °C will have a fatal effect on algae [56]. However, these ranges can be changed by environmental factors such as salinity, pH, carbon dioxide etc.

In the study of Floreta etal., the factors affecting algae growth were determined. Temperature effect was determined with salinity simultaneously. The results showed that low temperature (15 °C) with high salinity is the best choice. Low temperature increases the level of oleic and linoleic fatty acids. Moreover, high salinity increases the amount of $C_{16}$ and $C_{18}$ poly-unsaturated fatty acids [71].

*3.2.5.4. pH*

Microalgae require different pH values according to the media. During high pH concentration, the carbon dioxide might be limiting factor for growth and photosynthesis. The most used pH range for algal growth is around 7-9. The optimal pH for algae is between 8. 2- 8. 7. But it can change with different strains. For example, Weissel and Stadler studied with *Cryptomonas sp.* which showed positive population growth rates over a wide pH range, from 4. 4 to 9. 65 [72]. Appropriate pH can be adjusted by ventilation or gassing. There is a complex relationship between $CO_2$ concentration and pH in microalgal bioreactor systems, owing to the underlying chemical equilibrium among such chemical species as $CO_2$, $H_2CO_3$, $HCO_3$ and $CO_3$. Increasing $CO_2$ concentrations can increase biomass productivity, but will also decrease pH and this causes important effect upon microalgal physiology [73]. Water contaminated with a high pH has negative effects on algal abundance [74]. If there is not enough $CO_2$ gas supply, algae will utilize carbonate to maintain its growth [75].

Although high concentration of carbon dioxide provides high biomass efficiency, on the other side higher contamination risk and effect of low pH on microalgae physiology occurs [56].

Except the parameters mentioned above; there are also some parameters which affect on algal growth or lipid accumulation. Nitrogen, phosphorus and salinity can be examples for these parameters [76]. Widjaja et al. studied about nitrogen starvation effect on lipid accumulation. They reported that longer time of nitrogen starvation obviously resulted in higher accumulation of lipid inside the cells. Under all $CO_2$ concentrations, the lipid content tend to increase when the algae was exposed to nitrogen starvation condition that total lipid content was higher than lipid obtained during normal nutrition [75]. Ruangsomboon found the highest biomass concentration was found under the highest phosphorus concentration [60]. Li Xinet all. have reported in their study that lipid productivity was not at its highest when the lipid content was highest under nitrogen or phosphorus limitation [77]. Yeesang and Cheirsilp also studied about nitrogen and salinity effect. They found an increase in algal biomass under nitrogen-rich condition for all strains and in the absence of a nitrogen source, no growth was observed. They reported that although some loss in algal biomass was found, the lipid contents of four strains increased. They also noticed that growth and lipid accumulation by these microalgae could be affected by salinity. Under nitrogen-rich condition, all strains survived at high salinity but growth of some strains decreased [57, 78].

### 3.3. Microalgae cultivation systems

Cultivating microalgae can be achieved in open systems like lakes and ponds and in high controlled closed systems called photobioreactor. A bioreactor is defined as a system, which carries out biological conversion. Photobioreactors are reactors, which used for prototroph to grow inside or photo biological reactions to occur [79].

#### *3.3.1. Open ponds*

Generally open ponds are used in microalgae cultivation. Open ponds have various shapes and forms and certain advantages and disadvantages. In the scientific investigations and industrial applications, raceway ponds, shallow big ponds, circular ponds tanks and closed ponds are used [80]. Area where pool exist is critical factor for selection of pond type. Ponds become local climate function due to lack of control in open ponds [80, 81]. Therefore, area contributes to the success. Open ponds are limited by key growth parameters, which include light intensity, temperature, pH and dissolved $O_2$ concentration. Another problem seen in open ponds is contamination. It limits cultivation system of algae, which can grow under certain conditions [79].

Cost of cultivation systems is an important factor for comparison of open and closed systems. Construction, operation and maintaining costs are less than photobioreactors in ponds and these systems are simpler than the others [79, 82].

#### *3.3.2. Photobioreactors*

Nowadays researches are made for designing photobioreactors due to cultivating microalgae. Photobioreactors offer better control than open systems [2]. Their controlled environment allows high yield for cultivating.

Productivity is the most important indicator for bioreactor technology. It is very difficult to compare productivity of bioreactors due to various strains and scale of microalgae [80].

Photobioreactors basically can be tubular and flat type. When it is compared with the other bioreactors, tubular reactors considered as more suitable for open cultivating. Large illumination surface of reactor, which made of transparent tubes, is the main factor to being suitable for cultivation. Tubes can be adjusted in various types, adjustments convenience is depend to the specification of system.

A general configuration includes straight line and coiling tubes [83]. Reactor geometry is also important, tubular reactors can be vertical, horizontal or inclined shape. There are important differences between configurations of vertical and horizontal. Vertical designs provide more mass transfer and reduce energy consumption; horizontal designs can be scaled but needs more space. There are more studies about tubular photobioreactors but usually flat type photobioreactors is preferred because it can offer high cell density [84]. In addition, this type of reactors is advantageous due to low energy consumption and high mass transfer capacity, reduction of oxygen increases, high photosynthetic efficiency, no dark volumes when compared with the other photobioreactors. Suitable reactor design should

be provided with maximum cell mass. Various flat-plate photobioreactor designs are made of glass, thick transparent PVC materials and V-shape and inclined. Although the other designs are cheap and easy to construct, glass and PVC is more transparent for maximum light penetration [80, 84-86].

*3.3.2.1. Flat-plate photobioreactors*

These systems have large illuminated surfaces. Generally these photobioreactors are made of transparent materials to utilize the solar light with maximum degree. Dissolved oxygen concentration is low compared to the horizontal tubular photobioreactors. In this system high photosynthetic activity can achieve. Although it is very suitable for culturing algae but it has some limitations [83].

*3.3.2.2. Tubular photobioreactors*

Most of tubular photobioreactors are made of glass or plastic tubes. They can be horizontal, serpentine, vertical, near horizontal, conical and inclined photobioreactors. Ventilation and mixing is generally performed by pump or ventilation systems. Tubular photobioreactor is suitable with their illuminated surfaces. But one of the important limitations of this system is poor mass transfer. It is a problem when photobioreactor is scaled. Also photoinhibition is seen in photobioreactors [83, 87].

If there is not sufficient mixing system cells don't have enough light for their growth. Developing mixing systems can provide effective light distribution.

Also controlling culture temperature is very difficult in these systems. Thermostat can be used but it is expensive and hard to control. Also cells can attach the walls of tubes. Long tubular photobioreactors are characterized with transfer of oxygen and $CO_2$ [83, 88].

Vertical column photobioreactors are low cost, easily constructed and compact systems. They are promising for large scale of algae production. Bubble column and airlift photobioreactors can reach specific growth rate [56].

*3.3.2.3. Internally illuminated photobioreactors*

Florescent lamps can illuminate some photobioreactors internally. Photobioreactor is equipped with wheels for mixing algal cultures. Sprayer provides air and CO2 to culture. This type of photobioreactors can utilize solar light and artificial light [90]. When solar light intensity is low (night or cloudy day) artificial light is used. Also in some researches, it is told that solar light can be collected and distributed with optic fibers in cylindrical photobioreactors [91]. Another advantages of this system are can be sterilized with heat under pressure and minimizing the contamination [56, 83].

*3.3.2.4. Pyramid photobioreactor*

The Pyramid photobioreactor is using fully controlled and automatic system that increases the production rate. With this system, it is easy to grow any microalgae at any climate

conditions. The design is in pyramid shape to absorb light more effectively. As mentioned above, light is one of the significant parameters affecting algae growth rate and with this recent system algae can be supplied with optimal light intensity. That is why the shape of the system is the last innovation for production step. So, having optimal light intensity during high microalgae production decreases the energy consumption. The body design is angled to reduce to pump costs by using air-lifting method and decrease the deformation on cell walls. Thermo-isolated and high technologic materials are used to avoid energy lost and over heating [92].

### 3.4. Biocoil microalgae production system

Biocoil is a holozoic tubular photobioreactor which made of plastic tubes with small diameter (between 2.4-5 cm), centrifuges, diaphragm pumps or peristaltic pumping are utilized in this system. Biocoil design provides equal mixing and reduces the attachment of algae to the walls. It automates the production process. It is not suitable for all algae species. Some of algae species damages by circulation system and some of them attach to the internal surface of tubes and affects algae production negative. In this system, when the level of algae increases maximum degree, because of the light limitation photosynthesis can slow. Biocoil systems with utilizing solar light in or outsides can executable. Light is given with an angle so algal cell can utilize better and photosynthesis occurs easily [89, 93, 94].

#### *3.4.1. Design of culture growth systems*

Depends of local conditions and suitable materials various culture systems can be designed by various sizes, shapes of construction material, slope and mixing type. These factors affect performance, cost and resistance. To construct suitable photobioreactor material has main importance. Materials like plastic or glass relax and rigid shouldn't be toxic, they should have mechanical power, resistance, chemical stability and low cost. Tubular photobioreactors are the most suitable ones for open culture systems. They have big illumination surface, good biomass productivity and they aren't expensive because they are made of glass or plastic tubes. Flat-type photobioreactors are made of transparent materials to utilize solar light energy in maximum degree. This type of photobioreactors allows good immobilization of algae and they are cleaned easily [56]. Pond walls and deep side can made of simple sand, clay, brick or cement even PVC, glass fiber or polyurethane. For coating mostly long lasting plastic membrane is used. (e. g., 1-2 mm thick, UV-resistant, PVC or polyethylene sheets) sometimes to lower the cost uncoating ponds are used but that time some problems occur like contamination, a layer of mud and sand [39].

#### *3.4.2. Mixing*

Mixing is a process for increasing the productivity of biomass in photobioreactors. Mixing provides distribution of light intensity, sufficient $CO_2$ transfer and maintains uniform pH. Mixing is necessary for preventing algae sedimentation and avoiding cell attachment to the reactor wall. Mixing is also provides equal light and nutrients to all cells and increases the gas transfer between culture medium and air [95]. The second of priority measures is carbon

supply for using in photosynthesis. In very dense cultures, $CO_2$ from air (includes 0.035 % of $CO_2$) and bubbles during the culture can be limited for algal growth. $CO_2$ addition creates a buffer for the result of changing pH in the water [56].

Poor mixing allows cells to clumping like different size of aggregates; therefore it leads 3 phase (solid-liquid-gas) system in reactor. This situation tends to reduce the mass transfer. But all algae cannot tolerate agitation. Because they are sensitive to hydrodynamic stress. High mixing rate can cause the damaging of cells. Mixing in bubble column and air lift reactors can characterize with axial dispersion coefficient, mixing time, circulation time and Bodenstein number [96]. Analysis of mixing in bubble column shows it has shorter time than airlift reactors. Bubbles beyond the suction pipe provide less blurry area and causes better exposure to the light. In addition, existence of suction pipe in airlift reactors causes more effective mixing because internal loop provides a circulation. Airlift reactor gives information about fluid flow and high gas-liquid mass transfer rate. Bubble column causes unbalance cell density and these causes to death of algae [56, 97].

### *3.4.3. Light penetration*

Another key of successfully scale up is light penetration. IIumination in the photobioreactor affects biomass composition, growth rate and products. Microalgae need light for their photosynthesis [98]. Photosynthetic active radiation wave changes about 400-700 nm and this is equal to the visible light [99]. In intense cultures, light gradient changes over the photobioreactor radius due to the weakening of the light. Reduction of light intensity related to wave length, cell concentration, photobioreactor geometry and distance of the light transmittance. Light intensity in photobioreactor related to light way, cell concentration and light which emits by microalgae [56].

### *3.4.4. Gas injection*

Supplement of $CO_2$ by bubbles is an important factor to be considered in designs. Injection of $CO_2$ bases on giving $CO_2$ to photobioreactor artificially. Researches show that rich ventilation of $CO_2$ provides $CO_2$ to algae, supports deooxygenation of suspension, to improve cycling provides mixing and limits the light inhibition [100]. But high ventilation rate leads to higher cost that is why in large scale of microalgae production it is not recommended. These researches results for microalgae production necessary optimum aeration rate of $CO_2$ gas. Includes about 5% or 10% of $CO_2$ (v/v), rate of 0.025-1 vvm [100]. Volume of air/medium/time is found cost effective for air mass culture [56].

### *3.4.5. Comparison of open and closed culture systems*

Open and closed culture systems have advantages and disadvantages. Construction and operation of open culture systems are cheaper and they are more resistant than closed reactors and have large production capacity [101]. Ponds use more energy to homogenize to nutrients and to utilize the solar energy for growth their water level cannot be less than 15 cm [41]. Ponds are exposing to air conditions because water temperature evaporation and illu-

mination cannot be controlled. They produce large amounts of microalgae but they need larger areas than closed systems and they are open to other contaminations from the other microalgae and bacteria. Also when atmosphere has only 0. 03-0.06 % of $CO_2$, mass transfer limitation slows the growth of microalgae cell.

Photobioreactors are flexible systems, which can operate for biological and physiological characteristics of cultured microalgae. It can be possible to produce microalgae, which cannot produce in ponds. Exchange of gas and contaminants between atmosphere and cultured cells in photobioreactor is limited or blocked by reactor walls [39]. Depends on the shape and design, photobioreactors have more advantages than open ponds. Culture conditions and growth parameters can be controlled better, it prevents evaporation, reduces loss of $CO_2$, provides high microalgae density or cell concentration, high yield, creates more safe and preserved environment, prevents contamination. Despite the advantages, photobioreactors have problems to be solved. Over heating, biological pollution, accumulation of oxygen, difficulty of scale-up, high cost of construction and operation and cell damage because of shear stress and degradation of material in photo phase are main problems in photobioreactors [39].

Comparing photobioreactors and open ponds is not easy because growth of algae related to al lot of different factors. Three parameters are considered in algae production units for yield [41]:

- Volumetric productivity (VP): productivity per unit reactor volume (expressed as g/L. d).
- Areal productivity (AP): productivity per unit of ground area occupied by the reactor (expressed as $g/m^2d$).
- Illuminated surface productivity (ISP): productivity per unit of reactor illuminated surface area (expressed as $g/m^2d$).

According to researches closed systems don't provide advantage for areal productivity but provide volumetric productivity (8 times) and cell concentration (16 times) more than open ponds [39, 41].

### *3.4.6. Comparison of batch and continuous process*

Photobioreactors can be operated in batch or continuous process. There are a lot of advantages for using continuous bioreactors than batch bioreactors. Continuous bioreactors provide more control than batch bioreactors. Growth rates can be regulating in long time periods, can be saved and with variable dilution rates biomass concentration can be controlled. With steady state continuous bioreactors results is more dependable, products can be easily produced and can be reached desired product quality. Continuous reactions offer many opportunities for system research and analysis [102].

But some type of bioreactors is not suitable for continuous process. For some productions, cell aggregation and wall growth can inhibit the steady state growth. Another problem is loss of original product strain in time. Mixtures viscosity and heterogenic nature make diffi-

cult for maintaining filamentous organisms. Long growth periods increase the contamination risks [83].

### 3.5. Harvesting alternatives

There are several ways to harvest microalgae and dry them. Some main harvesting methods are sedimentation, flocculation and filtration.

*Sedimentation*: When a particle moves continuously in a phase, the velocity is affected by two factors. First of them is increasing the velocity because the density gradient between particle and fluid create buoyant force. At the end, buoyant force gets equal to dragging force and particle starts moving with a constant velocity. The same idea is applied to collect microalgae from the ponds. Gravity force is used for settling of suspended particles in fluid. This method is cheap and easy. However, the particles suspended in the fluid have to be incompressible. The problem with the *Scenedesmus sp.* and *Chlorella sp.* is that they are compressible. That is why sedimentation cannot be used for these types [103]. For low value products, sedimentation might be used if it is improved with flocculation [104].

*Flocculation:* is also used for harvesting microalgae. The general idea is microalgae carries negative charge on it and if the flocculants disappear the negative charge, algae starts coagulation. Some used flocculants are $Al_2(SO_4)_3$, $FeCl_3$, $Fe_2(SO_4)_3$ [105].

*Filtration:* This is one of the most competitive methods for the collection of algae. There are different types of filtrations, for example, dead end, microfiltration, ultrafiltration, pressure filter and vacuum filter. Mostly filtrations require the liquid media with algae to come through filtration. Filter can be fed until a thick layer of microalgae is collected on the screen. This method is very expensive for especially microalgae. The pore sizes of the filters are the most important part. If the pore size is bigger than algae you cannot collect it. In contrast, if the pore size is too small it might result in decrease of the flow rate and block the pores [106].

### 3.6. Extraction of lipid from microalgae

There are a lot of methods for extraction of lipid from microalgae but the most common techniques are oil presses, liquid-liquid extraction (solvent extraction), supercritical fluid extraction (SFE) and ultrasonic techniques. Oil presses are usually used for extracting of lipids from nuts and seeds. The same process and devices can be used for lipid extraction from microalgae. For the purpose of this process to be effective, firstly microalgae must be dried. Presses use pressure for breaking cells and removing oil [107]. This method can extract 75% of oil but in longer extraction times it is less effective [80].

Solvent extraction is more successful for extracting lipids from microalgae. In this method organic solvents such as hexane, acetone, and chloroform are added in the algae paste. Solubility of oil is higher in organic solvents than water. Therefore solvent breaks the cell wall and extracts oil easily. Solvent extraction continues with distillation process for separating oil from the solvent [108]. Hexane is cheap and has high extraction capacity. For this reason it is reported to be the most effective solvent in extractions.

In addition to this studies, 2 stage process using ethanol improves lipid extraction. The yield of recovery of oil reaches about 80%. Butanol is also effective in extraction of lysophospholipids. But evaporation of butanol is difficult and there are some impurities because of its high polarity [80].

Supercritical extraction uses high pressure and temperature for breaking cells. This method is widely used and efficient for extraction time. Studies reported that temperature and pressure don't affect the yield of components but it affects extraction rate. Similar effects are seen in SFE system and solvent extraction [109].

Another method is using ultrasonic techniques. In this method microalgae is exposed to high intensity ultrasonic waves and these waves creates bubbles around the cell. Shock waves are emitted by collapsing bubbles. It breaks cell wall and desired components release to the solution. This method is also improves the extraction rate with the same way. This technique is widely used in laboratory scale but in commercially scale there is not enough information about cost and applicability [110, 80].

### 3.7. Biodiesel Production from Oil

After extraction there are 4 main methods for producing biodiesel: direct used and mixing with raw oils; microemulsion; pyrolysis and transesterification.

#### *3.7.1. Dilution*

This is a dilution method that certain proportion of vegetable and waste oils blended with diesel fuel and another solvent. The most used oils for producing biodiesel with this way are waste oils and vegetable oils like sunflower and rapeseed.

Direct use or blending generally considered being unsatisfactory and impractical for both direct and indirect diesel engines. There are specific problems such as high viscosity, acid composition, free fatty-acid content, gum formation because of oxidation, polymerization during storage and combustion, carbon deposits and also lubricating-oil thickening [111].

Dilution of vegetable oils with solvents lowers the viscosity. The viscosity of oil can be lowered by blending with pure ethanol [112]. The low viscosity is good for better performance of engine, which decreases with increasing the percentage of diesel [33]. In this method there is no chemical process and viscosity can be lower but there are also carbon deposits and lube pollution problems to be solved. To solve problems caused by high viscosity, micro-emulsion, pyrolysis and transesterification methods are used [113].

#### *3.7.2. Micro-emulsion*

It is defined that the size of 1-150 nm, the two immiscible liquid organic mixtures with ionic or non-ionic, self-formed stable colloidal distribution. With this method it is possible to form alternative diesel fuels except petroleum [28]. In this method vegetable oils with an ester and dispersant (co-solvent), or of vegetable oils, an alcohol and a surfactant, with or without diesel fuels can be used to make a microemulsion. Due to their alcohol contents, microemul-

sions have lower volumetric heating values than diesel fuels. But these alcohols have high latent heats of vaporization and also tend to cool the combustion chamber, which cause a reduction of nozzle coking. A microemulsion made of methanol and vegetable oils can perform like diesel fuels [111]. To solve the problem of the high viscosity of vegetable oils, microemulsions with solvents and immiscible liquids, such as methanol, ethanol, 1-butanol and ionic or non-ionic amphiphiles have been studied [114].

*3.7.3. Pyrolysis*

Pyrolysis is the conversion of organic substance into another by means of heat or by heat in the presence of a catalyst. Vegetable oil, animal fat, algae oil, natural fatty acids or methyl esters of fatty acids can be pyrolyzed [111]. Although this method is not very cheap, however, fuel can be produced without extraction of lipids or hydrocarbons. More uniform product can be obtained and ideally increases yields over transesterification with this method [115]. Products are chemically similar derived from petroleum products, which are to gasoline and diesel fuel derived [28]. Also with pyrolysis some low value materials and sometimes more gasoline than diesel fuel are produced [116]. In comparison between pyrolysis and the other cracking processes, pyrolysis is seen more simple, pollution free and effective [33]. Sharma et al. reported that pyrolysis of the vegetable oil can produce a product which has high cetane number, low viscosity, acceptable amounts of sulfur, water and sediments contents, acceptable copper corrosion values [117].

*3.7 4. Transesterification*

Transesterification of the oil is the most promising solution to the high viscosity problem [114]. In this process, triglycerides are converted to diglycerides, then the diglycerides are converted to monoglycerides, and the monoglycerides are converted to esters (biodiesel) and glycerol (by-products) [118]. There are three common kinds of catalysts used in transesterification process such as lipase catalysts, acid catalysts and alkali catalysts. Each catalyst has advantages and disadvantages [113].

In the acid-catalytic transesterification, the reaction can be catalyzed by sulfuric, phosphoric, hydrochloric and organic sulfonic acids. Very high yields can be obtained by using this catalyst. These reactions need the use of high alcohol-to-oil molar ratios in order to obtain good product yields in practical reaction times. But ester yields do not proportionally increase with molar ratio and the reaction time is very long (3–48 h) [114, 119, 120]. Xu et al. studied the acidic transesterification of microalgae (Heterotrophic C. Protothecoides) oil. They used methanol for alcohol and they achieved 80% of FAME yield [121].

Johnson made a study on *Schizochytrium limacinum* microalgae species. He converted this algal oil to biodiesel with acidic transesterification and he achieved 82.6% of biodiesel yield [122].

In the alkali-catalytic transesterification, the reaction can be catalyzed by alkaline metal alkoxides, and hydroxides, as well as sodium or potassium carbonates. Sodium methoxide is the most widely used biodiesel catalyst. This reaction is faster than acid-catalytic transesterification and reactions can occur in low temperatures with a small amount for catalyst and

with little or no darkening of colour of the oil [114]. High quality can be obtained however this process is very sensitive to the presence of water and free fatty acids and needs lots of methanol. If the raw materials have a high percentage of free fatty acids or water, the alkali catalyst reacts with the free fatty acids to form soaps [113]. There are some studies on microalgae oil to produce biodiesel by using alkali transesterification. Velasquez-Orta et al. studied on biodiesel production from *Chlorella vulgaris*. In that study, alkali transesterification was used for conversion and they achieved 71% of FAME yield [123]. Ferrentino et al. studied on biodiesel production from microalgae too. They used Chlorella sp. oil and their production method was alkali transesterification. They have obtained high yield from their experiment [124]. In another study, Carvalho et al. used alkali transesterification for biodiesel production from algae oil. In their study, they used *Chlorella emersonii* oil and they have obtained 93% conversion yield [124].

It can be seen that there are some problems such as recovery of glycerol or removing catalysts from product and need of wastewater treatment in acid or alkali-catalytic transesterification. Enzymatic catalysts like lipases are able to catalyze the transesterification of triglycerides effectively. With this process glycerol can be easily recovered however enzymatic catalysts are often more expensive than chemical catalysts. The high cost of enzyme production is the main obstacle to the commercialization of enzyme-catalyzed processes. But using solvent-tolerant lipases and immobilized lipases can be a solution for this. Lipase-catalyzed transesterification is considered to be one of the most effective reactions for production of biodiesel [114]. In another study Tran et al. used microalgae oil (*Chlorella vulgaris* ESP-31) for producing biodiesel. Their method was enzyme-catalyzed transesterification and they used lipase in this process. In the result, they reported that they achieved 94.78 % of FAME yield [126]. Table 2 presents the transesterification studies for biodiesel production from microalgae oil.

Supercritical process, microwave-assisted method and ultrasonic-assisted process are novel methods used in biodiesel production area. Since these methods are novel methods and also algae are new materials for biofuel area, there is a few studies biodiesel production from algae oil with these novel methods, these studies were reviewed and presented below.

With *supercritical process* biodiesel production can be easily achieved without catalysts. Supercritical fluid is a substance whose temperature and pressure is above the critical point. These fluids are environmentally friendly and economic. Usually water, carbon dioxide and alcohol is used for supercritical fluid. In biodiesel production generally supercritical methanol and supercritical ethanol is used. Advantages of this process are being easier for purification, shorter the reaction time and more effective reaction [130]. In the study of Patil et al., using supercritical methanol produced biodiesel. The wet algae were used and the ratio of alcohol/ oil was chosen as 9:1. The temperature of the reaction occurred at 255 and 1200 psi and resulted in 90% of FAME yield [131].

*Microwaves* activate differences in small degrees of polar molecules and ions, because the molecular friction and chemical reactions start. Molecules have not the enough time to relax and heat generation occurs in a short time because energy interacts with molecules very quickly. Transesterification reaction is carried out with microwave in a short time and mi-

crowave results in an efficient manner. As a result in a short time separation and pure products with high yield is obtained. Thus, production costs and the formation of by-product are reduced [130]. Patil et al., made a study on biodiesel production from dry microalgae by using microwave-assisted process. KOH was used as catalyst in the study and microwave condition is set to 800 W. The performance of the study is around 80% [132]. The other study with macroalgae for microwave-assisted algal biodiesel was showed that methanol to macroalgae ratio of 1:15 was the best condition. In the study, sodium hydroxide concentration was 2 wt % and reaction time of 3 min for the best condition [133]. Koberg et al. was reported the study used *Nannochloropsis* for algal biodiesel production with microwave-assisted method. The higher biodiesel yield was observed which was around 37.1% with microwave technique. The same conditions for sonication technique resulted in lower yield [134].

| Algae strain | Method | Alcohol | Alcohol / oil molar ratio | Temp. | Time | Results | Ref. |
|---|---|---|---|---|---|---|---|
| *Heterotrophic C. Protothecoides (microalga)* | Acidic trasesterification | Methanol | 56:1 | 30 °C | 4 h | 80% (FAME Yield | [121] |
| *Chlorella vulgaris ESP-31 (microalga)* | Enzymatic transesterification (Lipase) | Methanol | 98.81 | 25-40 °C | 48 h | 94.78% (FAME Yield) | [126] |
| *Chlorella vulgaris (microalga)* | in situ alkaline transesterification | Methanol | 600:1 | 60 °C | 75 min | 71% (FAME Yield) | [123] |
| *Nannochloropsis oculata (microalga)* | heterogeneous transesterification | Methanol | 30:1 | 50 °C | 4 h | 97.5% (FAME Yield) | [127] |
| *Chlorella (microalga)* | In-situ acidic transesterification | Methanol | 315:1 | 23 and 30 °C | 15 min-2 h | 70-92% (FAME Yield) | [17] |
| *Chlorella sp. (microalga)* | Alkali Transesterification | Methanol | - | 100 °C | 25 h | 90 (Fluorometric Reading) | [124] |
| *Schizochytrium limacinum (microalga)* | Acidic Transesterification | Methanol | - | 90 °C | 40 min. | 82.6% (biodiesel Yield) | [122] |
| *Chlorella emersonii* | Alkali trasesterification | Methanol | 5:1 | 60 °C | 2 h | 93% conversion | [125] |
| *Fucus spiralis (macroalga)* | Alkali Transesterification | Methanol | 6:1 | 60 °C | 4 h | 1.6-11.5% (Process Yield) | [128] |
| *Commercially refined macroalga (Kelp)* | McGyan process | Methanol | 32:1 | 360 °C | 30 s | 94.7% (FAME Yield) | [129] |

**Table 2.** The transesterification studies for biodiesel production from microalgae oil

Recent years, *ultrasonic-assisted process* is widely used in biodiesel production. Mixing is very important factor for biodiesel yield in transesterification reactions. It is an effective mixing method in liquid-liquid mass transfer to provide better mixing. Powerful mixing creates smaller droplets than the conventional mixing and increases the contact areas between the oil phases. Also it provides the activation energy, which needs for initiating transesterification reactions [130]. In the study of Eihaze et al., they are focused on the in situ transesterification of microalgae by ultrasound technique. The reaction takes 1 h with the use of methanol/oil ratio to 315:1. The result was 0.295 ± 0.003 g biodiesel/g dry *Chlorella* which shows that this is higher than mechanically stirred in situ technique [135].

### 3.8. Design of algae and biodiesel production

In this section of study, algae production stages that cover the algae strain and location selection, algae cultivation, harvesting, oil extraction, and biodiesel production process from microalgae are presented by using ChemCad design program. All stages are given in this process flow diagram (pfd) and equipment table in detail. As it is seen in a process flow diagram (pfd), the streams between 1-8 are the area of the process where algae growth occurs. The algae bodies contain a lipid, which can be extracted and converted into a type of biofuel. The area where between stream1-8 has several large ponds to grow algae containing large amounts of lipid in preparation for lipid extraction. Once a pond is harvested, it is re-inoculated for another crop of algae (stream 11-13). Once the algae reach maturity in the growth ponds and have the desired lipid content, the cells are harvested in the area where stream 9-10. This area at a concentration of 1g-algae/L water. The algae collected will be dewatered, and the usable lipid is extracted for the reaction process where stream 9,10,14-16. The remaining algal biomass will be sent to algal pulp tank, it may be evaluated for biogas production in digesters. Lipids, catalysts and alcohol are sent for fuel conversion to heat-jacketed transesterification reactor. Once the lipid is harvested from the algae cells, the usable triglycerides are converted to biofuel in streams 16-18. Then products sent to the separator to separate biodiesel and byproduct glycerol in stream 21-27. The byproduct of this reaction is glycerol, which is removed and treated as waste. The biofuel is then ready to be used in modern farm equipment, or as a fuel supplement for diesel. All the equipments, tanks and ponds are labeled in the Figure 1.

## 4. Conclusion

Nowadays, demands on energy are caused to reduction of sources and environmental problems let the world to use alternative fuels. Microalgae have important potential as an alternative energy source. A lot of valuable products can be produced from microalgae such as biodiesel, biogas, bioethanol, medicines and nutraceuticals. Biodiesel is one of the most important alternative fuels. Microalgal biodiesel production is very new technology. In this study, microalgae and their classifications, important steps of biodiesel production from microalgae have been mentioned. In production sections, steps are explained briefly and easily understandable. Also advantages and disadvantages in the production are mainly dis-

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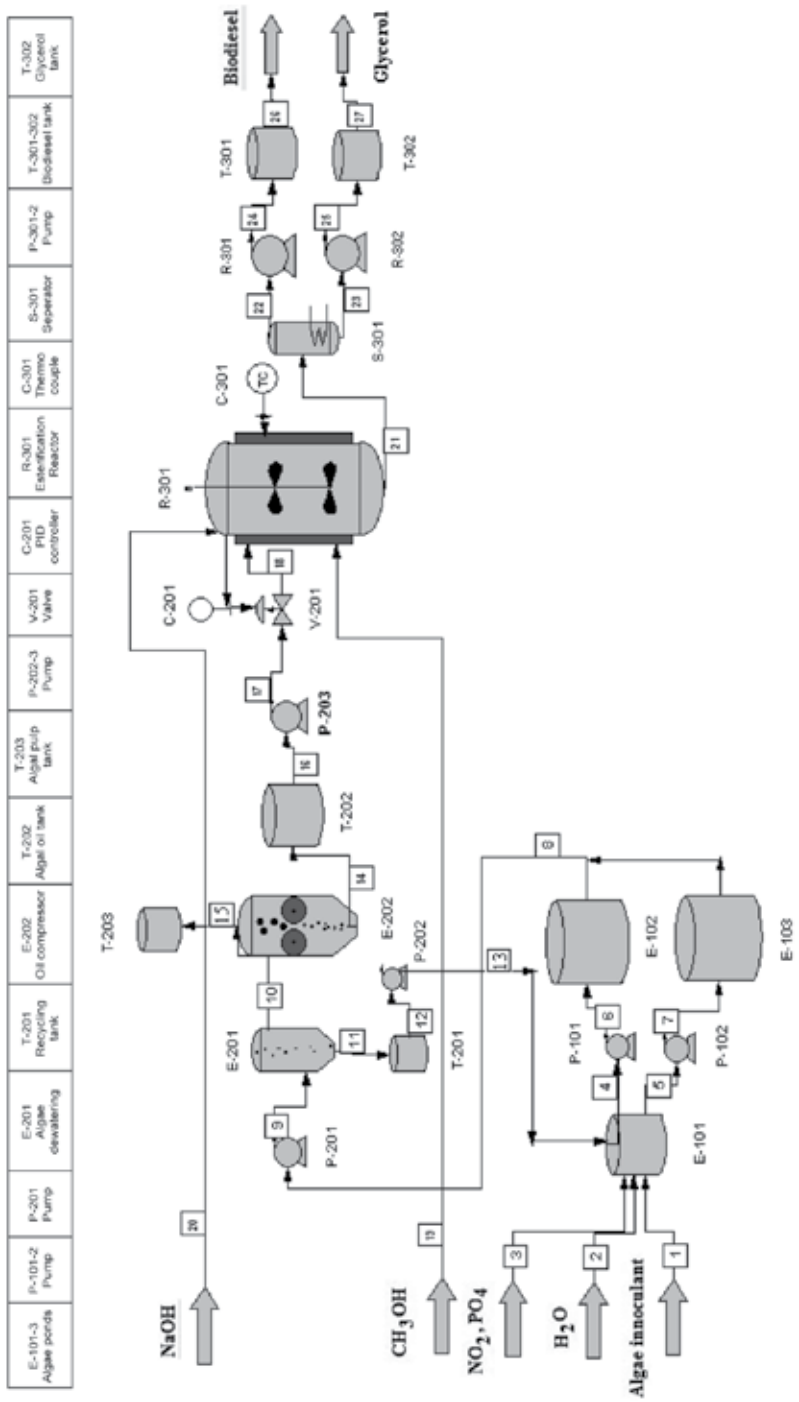


**Figure 1.** The process flow diagram of biodiesel production process from microalgae by ChemCAD.

cussed. At the end of this chapter, a biodiesel production from microalgae is designed by ChemCadprogram, which shows a simple process flow diagram for who desires to produce biodiesel from microalgae. Recently, microalgae are not economically viable. The main problems are the cost of capital cost. The rate of return is not short as it is expected. The operation cost is also affecting the total cost significantly. The main part, which makes the process expensive due to operation and capital costs, are algae growth, harvesting, dewatering, and fuel conversion. Beyond these, oil extraction step significantly increases the cost. If the oil could be extracted easily and at higher rates, the cost would be much lower. However, there are needs to innovate new ways to make the process economically feasible. Regardless, microalgae are seen as important resources for the future and there will be a lot of improvements on recent technology.

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Chapter 3

# Major Diseases of the Biofuel Plant, Physic Nut (*Jatropha curcas*)

Alexandre Reis Machado and Olinto Liparini Pereira

Additional information is available at the end of the chapter

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## 1. Introduction

Worldwide, concern over the consequences of global warming has resulted in intensified searches for potential plants that couldsupply raw materials for producing renewable fuels. Therein, physic nut (*Jatropha curcas* L.) has gained attention as a perennial culture that produces seeds with high oil content and excellent properties. In addition to these attributes, many studies have describedphysic nut as a culture resistant to pests and disease. However, in recent years, the expansion of areas under cultivation has been accompanied by the appearance of various diseases. Thus, this chapter aims to provide information about the main diseases that occur in physic nut and their diagnosis and to encourage further research on disease control.

The existing literature contains various descriptions of the pathogens occurring in culture, most of which are caused by fungi, and of which we address the following: *Glomerella cingulata* (Ston.) Spauld. et Schrenk.;*Psathyrella subcorticalis* Speg.;*Schizophyllum alneum* L.;*Aecidium cnidoscoli* P. Henn.; *Ramulariopsis cnidoscoli* Speg.;*Uromyces jatrophicola* P. Henn. (Viégas 1961);*Pestalotiopsis versicolor* Speg.(Phillips 1975);*Colletotrichum gloeosporioides* (Penz.)Sacc.; *Colletotrichum capsici* (Syd.) Butl.e Bisby.;*Passalora ajrekari* (Syd.) U. Braun (Freire & Parente 2006);*Phakopsora arthuriana* Buriticá & J.F. Hennen (Hennen et al. 2005);*Cochliobolus spicifer* Nelson (Mendes et al. 1998);*Cercospora jatrophicola* (Speg.) Chupp;*Cercospora jatrophigena*U. Braun; Pseudocercospora jatrophae-curcas (J.M. Yen) Deighton; Pseudocercospora jatrophae; Pseudocercospora jatropharum (Speg.) U. Braun (Crous & Braun 2003); and Elsinoë jatrophae Bitanc. & Jenkins (Bitancourt & Jenkins 1951). Existing reports on pathogens include research on collar and root rot Nectria haematococca Berk. & Br. [Haematonectria haematococca (Berk. & Broome) Samuels & Nirenberg], and its anamorph Fusarium solani (Martius) Appel & Wollenweber (Yue-kai et al. 2011), as well as Lasiodiplodia theobromae (Pat.) Grif-

fon & Maubl (Latha et al. 2009; Pereira et al. 2009), Phytophthora palmivora var. palmivora (E.J. Butler) E.J. Butler (Erwin & Ribeiro 1996) and*Clitocybe tabescens* (Scop, ex Fr.) Bres (USDA 1960).

## 2. Diseases

Although several descriptions of fungi exist, this chapter will discuss the most common and damaging diseases that affect physic nut, and draws on the following descriptions:

### 2.1. Anthracnose (figure 1)

*Colletotrichum gloeosporioides* (Penz.)Sacc.

*Colletotrichum capsici* (Syd.)Butl.and Bisby

This disease was first described in physic nut by the USDA (1960) in the USA, in Brazil by Viégas (1961), and later by Freire & Parente (2006) and Sá et al. (2011).Currently, the disease is present in all areas where physic nut is cultivated.

The most commonly observed symptoms are brown to black necrotic lesions that are irregularly shaped and appear on the edges and center of the leaf and which may contain a yellow halo. The lesions appear in the form of small, isolated points that coalesce and subsequently cause the complete destruction of the leaves. The fruit can also become infected, which leads to the appearance of dark brown lesions.

In addition to these symptoms, research in Mexico has indicated that the fungus *Colletotrichum capsici* caused stem canker and apical death of seedlings (Torres-Calzada et al. 2011).


**Figure 1.** Anthracnose in *Jatropha curcas*. Symptoms on leaf (A). Curved conidia, dense conidiophores and septate setae of *Colletotrichum capsici* (B).

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*Colletotrichum* is a fungus anamorph of the phylum Ascomycota and telemorph genus *Glomerella*. The species of this genus have the following characteristics: conidiomata that are acervular, subcuticular or epidermal, and may contain setae; conidiophores that are hyaline to brown; conidiogenous cells that are enteroblastic, phialidic and hyaline; conidia that arehyaline, aseptate (except prior to germination), straight or falcate, smooth and thin-walled; and appressoria that are brown, entirely or with crenate to irregular margins produced with germination of conidia (Sutton 1980).

*Colletotrichum* spp. is known to infect a large range of hosts and to cause various symptoms, the most common of which is anthracnose. This fungus can survive in seeds, crop residues, infected plants, and in soil as saprophytes. Although the disease occurs in various regions of the world, it is more severe in regions with a hot and humid climate (Agrios 2005).

So far, there are no recommendations for controlling this disease. Because of the damage it can cause to physic nut, this disease should be studied further.

### 2.2. Passalora leaf spot

*Passalora ajrekari* (Syd.) U. Braun

*Passalora jatrophigena* U. Braun & F.O. Freire

This disease was first described in Brazil by Braun & Freire (2004), and later by Freire & Parente (2006) in leaves of *Jatropha curcas* and *Jatropha podagrica*, and in others countries by Crous & Braun (2003).

The primary symptoms of this disease are rounded leaf lesions that are creamy to light brown in color, with a narrow dark brown halo, and later become limited by leaf veins and darken. Lesions measure 1-2 cm in diameter and rarely coalesce (Freire & Parente 2006).

The genus *Passalora* is a cercosporoid fungus, previously included in the genus *Cercospora* that has as its teleomorph the *Mycosphaerella.* Species share taxonomic characteristics such as branched, septate, smooth, hyaline to pigmentedhyphae; absent to well-developed stromata; solitary or fasciculate to synnematous conidiomataconidiophores, arising from stromata or hyphae, internal or superficial, pluriseptate, subhyaline to pigmented; conspicuous conidiogenous loci, with scars that are somewhat thickened and darkened; conidia that are solitary to catenate in simple or branched chains, amerosporous to scolecosporous, aseptate to pluriseptate, and pale to distinctly pigmented and hila that are somewhat thickened and darkened (Crous & Braun 2003).

Although it has been reported in several countries, to date this disease has not presented risk to physic nut cultivation.

### 2.3. Cercospora/Pseudocercosporaleaf spot (figure 2-3)

*Cercospora jatrophicola (*Speg.) Chupp,

*Cercospora jatrophigena* U. Braun

*Pseudocercospora jatrophae-curcas* (J.M. Yen) Deighton

*Pseudocercospora jatrophae* (*G.F. Atk*.) *A.K. Das & Chattopadh*.

*Pseudocercospora jatropharum* (Speg.) U. Braun

This disease manifests in the form of leaf spots that consist of well-delimited brown irregular necrotic spots (Dianese et al. 2010).

The genera mentioned above have the following taxonomic characteristics:

The genus *Cercospora* groups anamorphs of *Mycosphaerella* with hyphae that are colorless or near-colorous to pigmented, branched, septate, and smooth to faintly rough-walls. Stromata are lacking to well-developed, subhyaline to usually pigmented. Conidiophores are solitary to fasciculate, arising from internal hyphae or stromata, erect, subhyaline to pigmented. Conidiogenous loci (scars) are conspicuous, thickened and darkened. Conidia are solitary, scolecosporous, cylindrical-filiform, hyaline or subhyaline, mostly pluriseptate, and smooth and hila are thickened and darkened (Crous & Brown 2003).

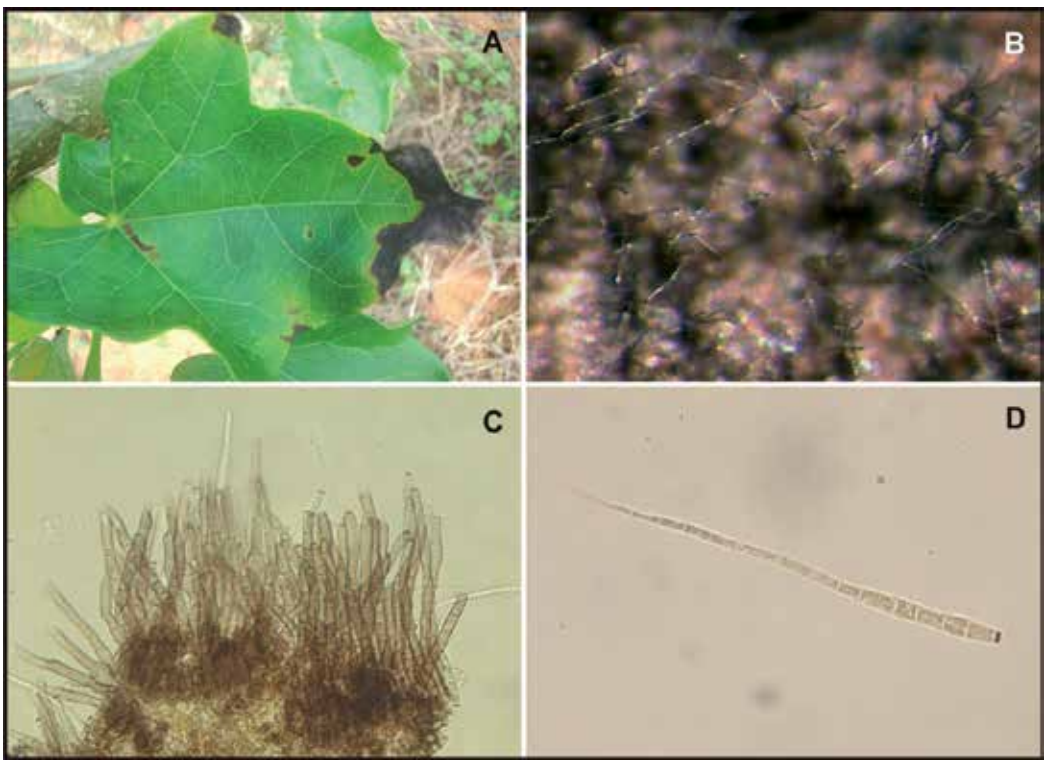


**Figure 2.** Cercospora leaf spot on *Jatropha curcas*. Necrotic symptoms on leaf (A); Fungal structures on leaf lesions (B); Pigmented conidiophores with conspicuous scars (C); Filiform conidia with conspicuous pigmented hilum (D).

The genus *Pseudocercospora*groups anamorphs of *Mycosphaerella* with basically pigmented conidiophores and inconspicuous, unthickened, not darkened conidiogenous loci; solitary,

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or catenulateconidia, aseptate to pluriseptate with conidial scars that are inconspicuous and not thickened (Crous & Brown 2003).

Crous & Braun (2003) cite the occurrence of five species of cercosporoid, indicated above, in the culture of physic nut. However, few studies have examined fungi cercosporoid in this crop. As a result, there is no information about favorable conditions, symptoms or disease control. To date, this disease has not presented risk to the cultivation of physic nut.

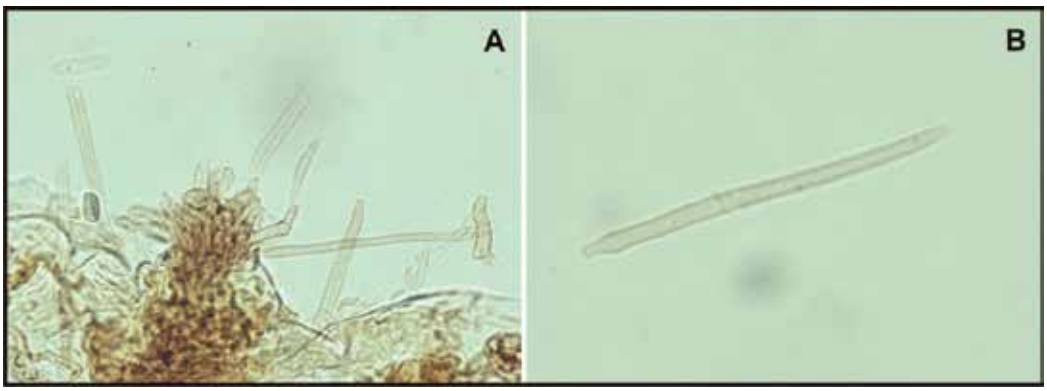


**Figure 3.** Pseudocercospora leaf spot on *Jatropha curcas*. Pigmented conidiophores with inconspicuous scars on conidiogenous cells (C) and filiform pigmented conidia with inconspicuous hilum (D).

### 2.4. Powdery mildew (figure 4)

*Pseudoidium jatrophae* (Hosag., Siddappa, Vijay. & Udaiyan) U. Braun & R.T.A. Cook

The powdery mildew caused by the fungus *Pseudoidium jatrophae* (Braun & Cook 2012) was previously described as *Oidium heveae* Steim by Viégas (1961) in Braziland *Oidium jatrophae* Hosag., Siddappa, Vijay. & Udaiyan (Braun & Cook 2012)in India. This disease occurs commonly in physic nut plantations and it has been frequently observed in various regions of Brazil and the rest of world.

The most common symptoms of the disease are the production of abundant white or gray mycelia in leaves, petioles, stems, flowers and fruits (Dianese & Cargnin 2008). With the evolution of the disease, infected plants may show necrotic lesions, which cause leaf fall, underdevelopment, death of buds and young fruit deformation (Bedendo 2011).

The fungus that causes this disease is a typical biotrophic pathogen of the phylum Ascomycota, order Erysiphales. This pathogen may be characterized by white or grayish colonies, septate and branched mycelia; conidiophores that are erect or ascending, cylindrical, hyaline, septate and forming conidia singly; conidia that are usually large in proportion to the diameter of the conidiophores, simple, smooth, ellipsoid-ovoid doliiform, hyaline, single-celled (Braun & Cook 2012).

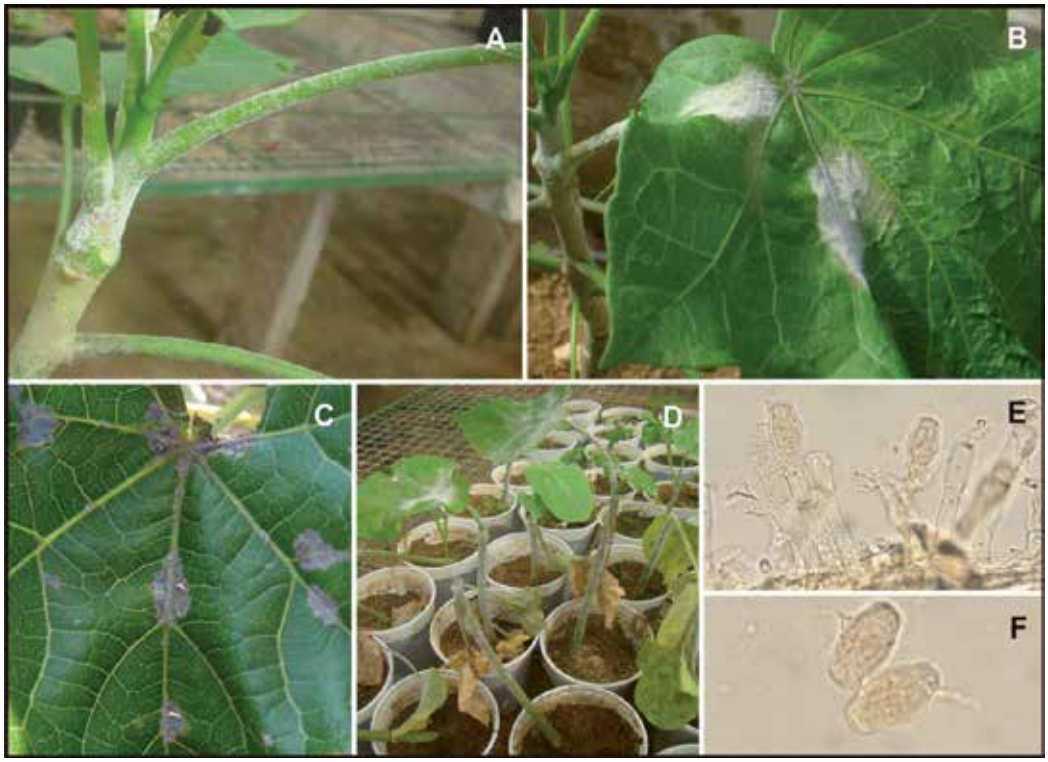


**Figure 4.** Powdery mildew on *Jatropha curcas*. Symptoms on petiole and stem (A);Symptoms on leaf (B) Leaf lesions on old infections (C); Symptoms on seedlings (D);Conidiophores (E); Conidia (F).

The disease generally favors warm temperatures, humidity of 75-80% and reduced light. Heavy rains are generally unfavorable to the pathogen (Furtado &Trindade 2005). In Brazil, the disease usually occurs in the dry season, apparently without causing extensive losses, because its occurrence coincides with the plants' period of natural defoliation (Saturnino et al. 2005).

Currently, there are no fungicides recommended for culture, but some studies cite that spraying sulfur fungicides works to control this fungus. Another measure is to control alternative hosts, especially plants of the family Euphorbiaceae (Furtado & Trindade 2005; Saturnino et al. 2005; Dias et al. 2007).

### 2.5. Rust (figure 5)

*Phakopsora arthuriana* Buriticá & Hennen

The first report of this disease in *Jatropha curcas*, described its cause as *Uredo jatrophicola* Arthur(Arthur 1915). In Brazil, this disease was first found in 1936 in São Paulo (Viégas 1945). Currently, it is widely distributed throughout Brazil (Dias et al. 2007) and several other countries.

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The fungus that causes this disease was previously classified as *Phakopsora jatrophicola* (Arthur) Cummins; however, it was reclassified as *Phakopsora arthuriana* Buriticá & Hennen (Hennen et al. 2005).

The symptoms manifest in the leaves, initially in the form of small chlorotic points on the upper surface, which correspond to the underside of the leaf, and then small protruding pustules, which after breaking, release a powdery mass of uredospores of orange color, giving a ferruginous aspect. In severe infections, pustules coalesce to form necrotic spots, which are reddish brown and irregularly shaped and can destroy the leaf (Dias et al. 2007; Carneiro et al. 2009).

The *Phakopsora arthuriana* belongs to the phylum Basidiomycota, class Pucciniomycetes. It is characterized by uredinia hypophyllous, occasionally epiphyllous, in small groups opening by a pore, surrounded by numerous not septate paraphyses that project outside the host; urediniospores, ellipsoid, to obovoid, sessile, closely and finely echinulate, germ pores obscure; telia hypophyllous, suberpidermal in origin, closely around the uredinia; teliospores irregularly arranged, cuboid, ellipsoid to polygonal (Hennen et al. 2005).

Currently there are no fungicides recommended for this culture. However, according to Dias et al. (2007), protective copper fungicides can control this disease.

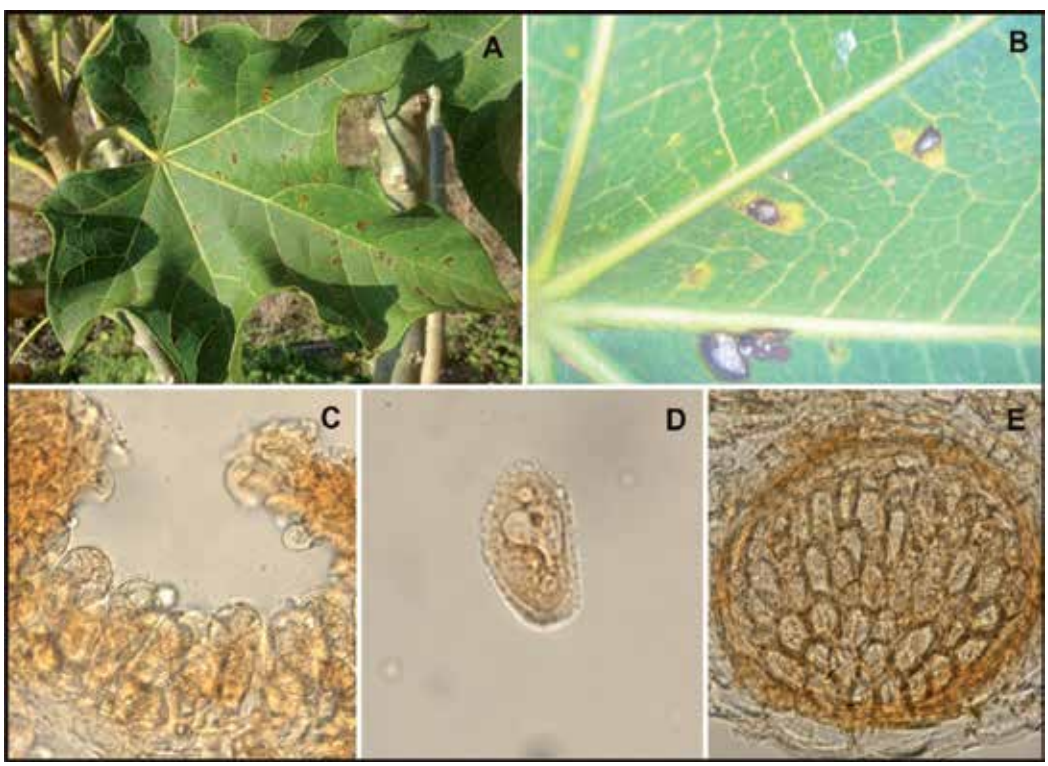


**Figure 5.** Rust disease on *Jatropha curcas*. Symptoms on adaxial leaf surfaces (A-B);Uredinia (C);Urediniospores (D); Telia with teliospores (E).

### 2.6. Stem canker and dieback (figure 6)

*Lasiodiplodia theobromae*(Pat.) Griffon & Maubl

The first report of this disease in Brazil was made byFreire & Parente (2006) and in Malaysia by Sulaiman & Thanarajoo (2012).

The disease manifests in the form of dieback that can progress until it takes over the trunk of the plant. Stem cankers have also been observed, causing necrotic lesions on branches and vascular discoloration. In Malaysia, disease incidence can be as high as 80% of a plantation (Freire &Parente 2006; Sulaiman & Thanarajoo 2012).

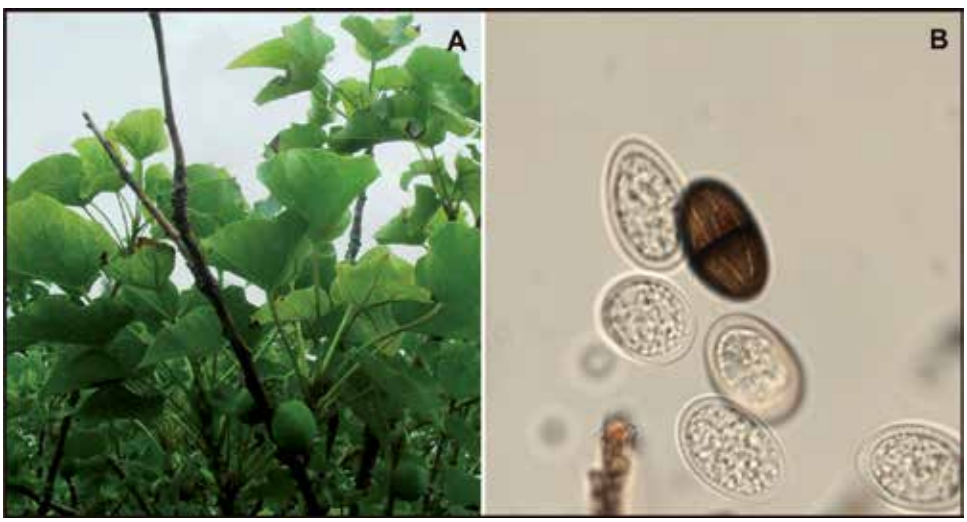


**Figure 6.** Dieback on *Jatropha curcas*. Symptoms observed in the field (A); Hyaline and pigmented conidia of *Lasiodiplodia theobromae* (B).

Characteristics of the *Lasiodiplodia* species commonly include the presence of paraphyses within the conidiomata pycnidial and conidia that are initially hyaline and aseptate. But in maturity, one median septum is formed, and the walls become dark brown with the formation of longitudinal striations due the deposition of melanin granules on the inner surface of the wall.

The identification of the *Lasiodiplodia* species based solely on morphological characteristics is not easy. Currently, it is known that what was initially identified as *Lasiodiplodia theobromae* is in fact a species complex (Alves et al. 2008). Thus, molecular studies are needed to correctlyidentify the pathogen, as was done by Thanarajoo & Sulaiman (2012).

*Lasiodiplodia* spp. is a fungus of the phylum Ascomycota, family Botryosphaeriaceae. Fungi in this family are known to survive as endophytes and demonstrate symptoms when plants are under some stress (Slippers & Wingfield 2007). Thus, many researchers see them as opportunistic pathogens.

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Control of this disease can be achieved by pruning and destroying affected branches. Later plants should be brushed with copper fungicides or thiophanate methyl for injuries (Furtado & Trindade 2005). Additionally, balanced fertilization, soil analysis and sufficient levels of irrigation in regions with long periods of drought can aid in disease control.

### 2.7. Collar and root rot (figure 7)

*Fusarium solani*(Martius) Appel & Wollenweber

*Lasiodiplodia theobromae*(Pat.) Griffon & Maubl

*Neoscytalidium dimidiatum*(*Penz*.) *Crous & Slippers*

*Macrophomina phaseolina*(Tassi) Goid.

The first report of this disease in Brazil was made by Pereira et al. (2009), who identified it as being caused by *Lasiodiplodia theobromae*. In India, this same pathogen was reported by Latha et al. (2009), and *Macrophomina phaseolina*was reported by Patel et al. (2008). In China, Yue-Kai et al. (2011) identified the fungus *Fusarium solani*, and Machado et al. (in press) made the first description of *Neoscytalidium dimidiatum(Penz.). Crous & Slippers associated this pathogen with collar and root rot in physic nut in Brazil.*

*All the pathogens mentioned above are typical soil fungi. They occur in a wide range of hosts, can be spread by seeds, and survive as parasites, saprophytes, endophytes or resistant structures, such as chlamydospores in Fusarium and Neoscytalidium or sclerotia in Macrophomina.*

This disease has acquired great importance, because it can reduce productivity by causing the sudden death of plants and making cultivation areas unviable. The symptoms most commonly observed are wilting, leaf yellowing with subsequent fall, and cracks in the collar region. In the collar region, the appearance of black fungal structures in the bark of the plant has been consistently observed. Upon being removed from the soil, plant roots rot and the vascular system is affected by necrotic symptoms, ranging from light brown to black. Due to loss of support, the plants have often already fallen due to the wind.

The genus *Fusarium*has the following general characteristics: bright aerial mycelium, hyphae septate, conidiophores variable, single or grouped in sporodochia; conidia hyaline variable, principally of two kinds -multicellularmacroconidia, slightly curved or bent at the pointed ends and typically canoe-shaped; unicellular, ovoid or oblongmicroconidia, borne singly or in chains and also grouped in false heads, formed in mono or polyphialides; thick-walled chlamydospores are common in some species (Barnett & Hunter 1998).

The common characteristics of *Lasiodiplodia* species include the presence of paraphyses within the conidiomata pycnidial and initially hyaline and aseptateconidia. However, in maturity, one median septum is formed, and the walls become dark brown with the formation of longitudinal striations, due the deposition of melanin granules on the inner surface of the wall.

The genus *Neoscytalidium*is a group of fungi that produces synanamorph *Scytalidium*-like with septate and oblong to globosearthroconidia formed from aerial mycelia. Initially hyaline, with age, the arthroconidia become brown and with a thick wall. Commonly observed

are pycnidia that are dark and globose immersed or superficially in a stroma that produces *Fusicoccum*-like conidia that are hyaline and ellipsoid to nearly fusiform. Dark septate conidia can also be observed.

Characteristics of the *Macrophomina* spp. generally include the formation of dark mycelia and abundant production of sclerotia in PDA. Eventually, the formation of conidiomata pycnidial can be observed, with the release of hyaline conidia with apical mucoid appendages.

In areas prone to prolonged dry seasons, a higher incidence of collar and root rot has been observed. Therefore, it is believed that the water stress is the main factor that predisposes plants to this disease.

The above-mentioned pathogens are difficult to control, due to the fact that they survive in soil. Therefore, to reduce disease incidence, it is first necessary to provide water and fertilizer balanced for proper plant development. When transplanting seedlings to the field, all forms of injury should be avoided. Another control measure would be to use healthy propagative material as well as seed treatments.

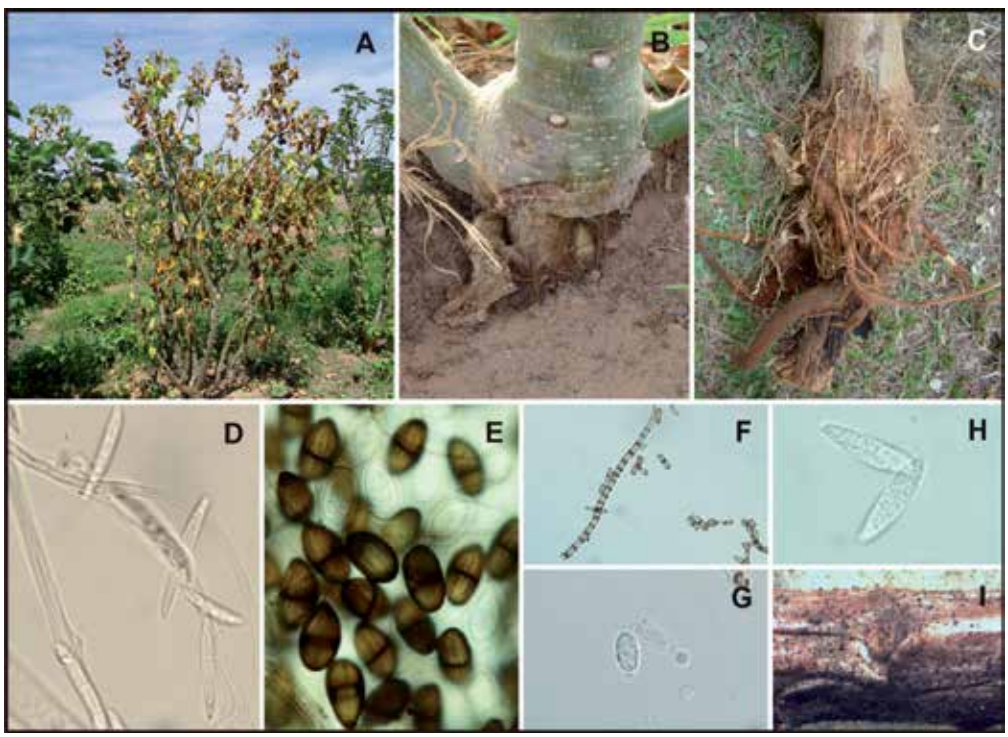


**Figure 7.** Collar and root rot on *Jatropha curcas*. Wilting symptoms observed in the field (**A**);Detail of thecollar rot (**B**);Detail ofroot rot (**C**);Macroconidia of *Fusariumsolani*(**D**); Pigmented and hyaline conidia of *Lasiodiplodia theobromae*(**E**);Arthroconidia of *Neoscytalidium dimidiatum*(**F**);*Fusicoccum*-like conidia of *Neoscytalidium dimidiatum*(**G**); Conidia of *Macrophomina phaseolina*(**H**). Sclerotia of *Macrophomina phaseolina* produced on sterilized Pine twigs in culture (**I**).

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### 2.8. Yellow mosaic

In addition to the several fungal diseases mentioned, there is also yellow mosaic, a disease caused by a strain of the virus *Indian Cassava Mosaic Virus* (Gao et al. 2010). This disease, detected in physic nut plantations in India, causes mosaic, reduced leaf size, leaf distortion, blistering and stunting of diseased plants. The disease is transmitted by the vector *Bemisia tabaci* in a non-persistent manner, but not through mechanical inoculation or seeds (Narayanaet al. 2006).

## 3. Seed associated fungi

Seeds propagate the majority of cultures worldwide. These cultures are vulnerable to infection by several pathogens that can survive in seeds. These pathogens may cause reduction of seed germination, as well as deformation, discoloration, reductions in size and weight, and deterioration during storage. They can further contribute to rotting roots, damping-off, necrosis in leaves, and the spread of diseases across long distances. Consequently, these diseases cause losses valued at billions of dollars (Neergaard 1977; Agarwal & Sinclair 1997). To date, few studies have addressed the seed pathology of physic nut, and there is no information available about the losses that seed pathogens cause in this culture. But, follows below the major pathogens and saprophytic fungi associated with seeds.

*Macrophomina phaseolina* (Figure 8)

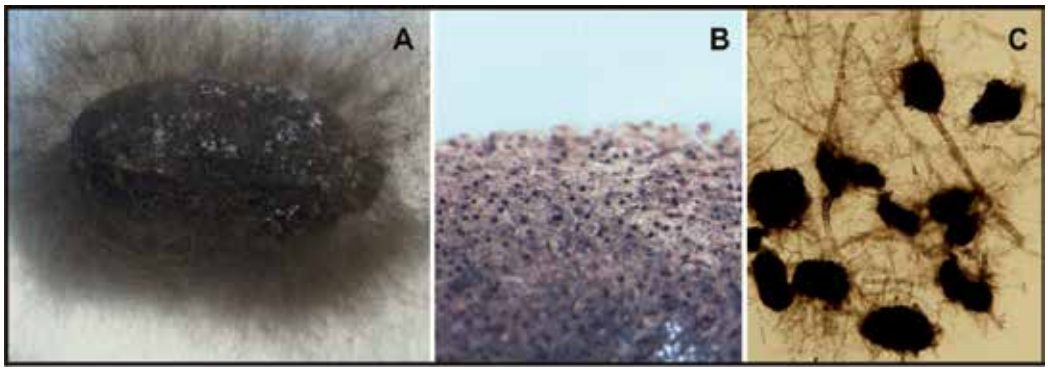


**Figure 8.** *Macrophomina phaseolina*on *Jatropha curcas* seed. Seed covered by mycelium (**A**); Detail of sclerotia on seed (**B**);Black sclerotia (**C**).

*Colletotrichum capsici* (Figure 9)

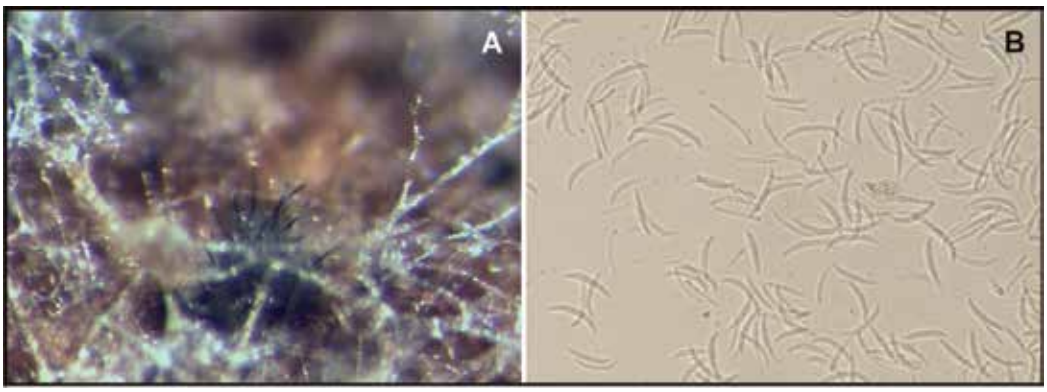


**Figure 9.** *Colletotrichum capsici* on *Jatropha curcas* seed. Conidiomata with black setae on seed surface (**A**). Curved asseptate conidia (**B**).

*Fusarium* sp. (Figure 10)


**Figure 10.** *Fusarium* sp. on *Jatropha curcas* seed. Seed covered by hyaline mycelium (**A**); Radicle with necrotic lesion (**B**); Macroconidia (**C**).

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*Lasiodiplodia theobromae* (Figure 11)

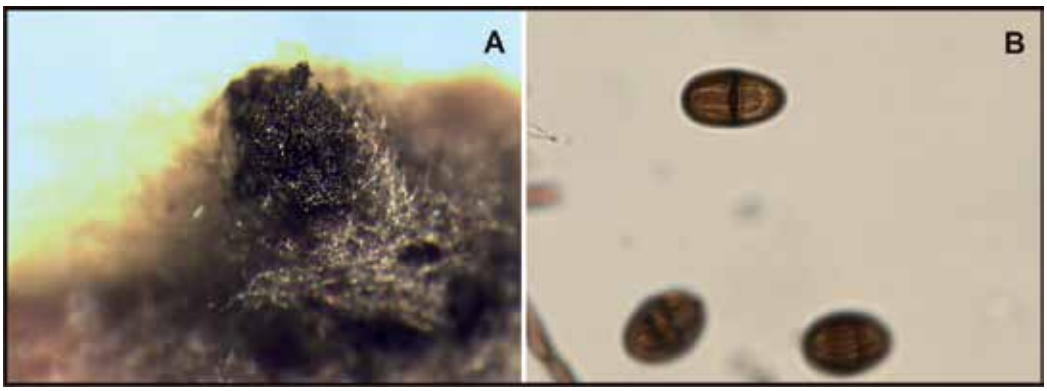


**Figure 11.** *Lasiodiplodia theobromae* on *Jatropha curcas* seed. Conidiomata producing a black cirrus of conidia on seed surfasse (**A**); Detail of mature conidia (**B**).

*Curvularia* sp. (Figure 12)

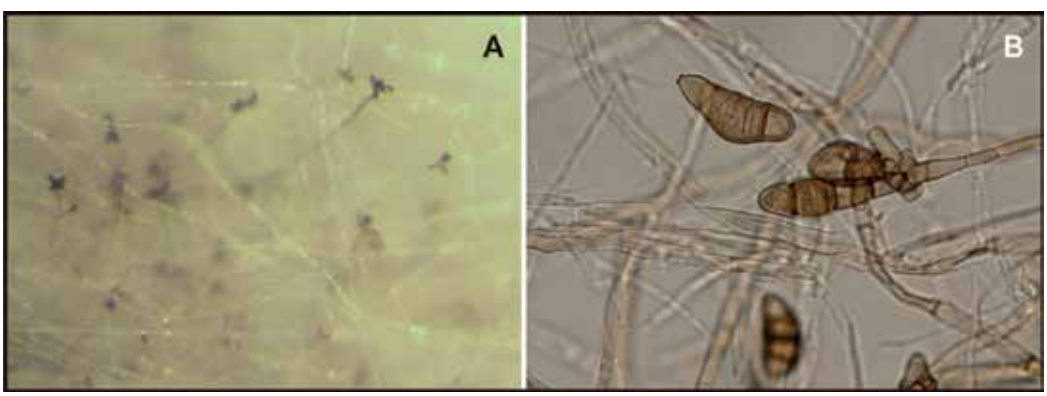


**Figure 12.** *Curvularia* sp. on *Jatropha curcas* seed. Mycelium and conidiophores producing conidia on seed surface (**A**); Dark septate conidia (**B**).

Other fungi commonly associated with Jatropha curcas seeds (Figure 13)

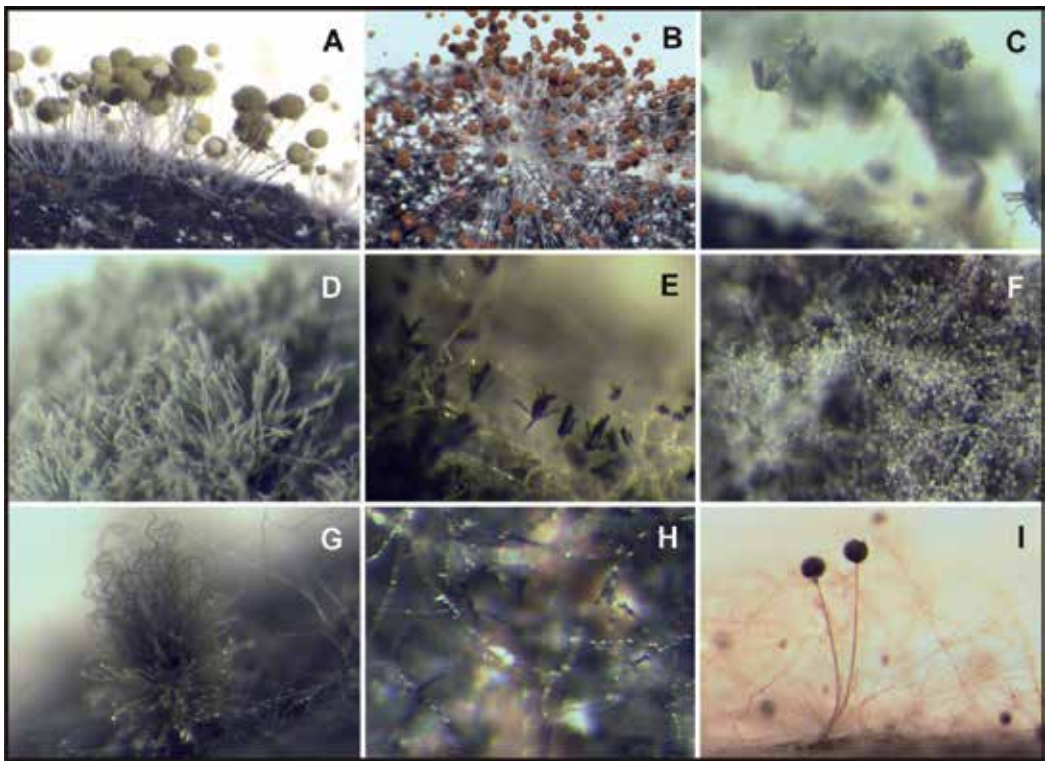


**Figure 13.** Genera of fungi often observed on Jatropha curcas seeds: Aspergillus (**A-C**); Penicillium (**D**);Stachybotrys (**E**); Acremonium (**F**); Chaetomium (**G**);Alternaria (**H**); Rhizopus (**I**).

Although there are no recommendations for fungicide useon physic nut, treatments can be administered by soaking seeds for 20 minutes in a solution of 1 liter of formaldehyde 40% diluted in 240 liters of water (Massola and Bedendo, 2005). This treatment is indicated for the seeds of *Ricinus communis* L., but it also works well for physic nut.

## 4. Conclusion

Despite the fact that most literature considered physic nut as resistant to pests and diseases, this review emphasizes the diversity of pathogens associated with this plant and the damage that they may cause. Most of these diseases may become a serious problem for Brazilian farmers, due to its severity and the lack of registered chemical products for these pathogens. Studies should be carried out in order to know the environmental conditions that favor to these diseases on *J. curcas*, as well as the development of control strategies and resistant varieties.

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Chapter 4

# Biodiesel Feedstock and Production Technologies: Successes, Challenges and Prospects

Y.M. Sani, W.M.A.W. Daud and A.R. Abdul Aziz

Additional information is available at the end of the chapter

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## 1. Introduction

In order to achieve the biodiesel central policy of protecting the environment, replacing petroleum diesel and protecting and/or creating jobs, a good understanding of biodiesel history is essential. This is because consumers always tend to buy cheap rather than "green" fuels. Moreover, it is more difficult for a new technology to dislodge one that has reached societal standard. The more the popular technology is used, the more it improves; becoming less expensive due to wider market potentials. Petrodiesel has become the "life-blood" of our economy. It would be almost impossible to find a commercial product today that does not consume diesel fuel during its production and distribution [1-4]. Therefore, the aim of this chapter is to provide an overview on the history and motivation, successes, challenges and prospects of biodiesel as source of energy. This will provide a global outlook in making biodiesel an economical and eco-friendly alternative to petroleum diesel.

The historical developments of the biofuel industry in general and biodiesel in particular, is unlike many industries. This is because the driving factors for its advances are more of economics and politics than technological [5]. As early as 1853, transesterification was conducted on vegetable oil in the search for a cheap method to produce glycerine for producing explosives during World War II by E. Duffy and J. Patrick [6-8]. In 1937, G. Chavanne, Belgian scientist patented the "Procedure for the transformation of vegetable oils for their uses as fuels". ''Biodiesel" as a concept was thus established [9]. It is a simple process where alkoxy group of an ester compound (oil or fat) is exchanged with an alcohol. However, it was not until 1977 that first patent on commercial biodiesel production process was applied for by Expedito Parente; a Brazilian scientist [10].

Prior to the discovery of and boom in fossil fuels, power was mainly generated from steam. However, the use of hydro-energy consumes large resources coupled with the inefficiencies

of the steam engine where only about 10 to 12% efficiency is derived from new power generation plant. A patent for an efficient thermal engine which was to be operated on peanut oil was filed in 1892 by Rudolph Diesel in Germany. By 1893, Diesel's invention was demonstrated in an exhibition in Paris. Within five years of its invention, Diesel's engine ran on its own power with 75% efficiency against its initial 26% efficiency [11]. In 1912, Diesel published two articles [12,13] in which he reflected:

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*"The fact that fat oils from vegetable sources can be used may seem insignificant to-day, but such oils may perhaps become in course of time of the same importance as some natural mineral oils and the tar products are now. (…) In any case, they make it certain that motor power can still be produced from the heat of the sun, which is always available for agricultural purposes, even when all our natural stores of solid and liquid fuels are exhausted."*

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The demand for biofuels began to increase in America from the 1890's to 1920's. These were attributed to the pioneering efforts on the diesel engine by Adolphus Busch and Clessie L. Cummins along with other engine manufacturers. However, the biofuel industry was faced with a major challenge of cheap and readily available feedstock. Unfortunately for the biofuel industry, at this same period, the petroleum industries found out more advanced technologies for improving the properties of the "black gold". The discoveries of large reservoirs and developments created new markets for this "black gold". Therefore, by 1940, diesel engines were altered to enable them use petroleum-based fuels which have lower viscosities. Thereafter, the sales of biodiesel were weakened and the production structure was pushed to the background. Therefore, no significant efforts were made to increase the public awareness on its potentials. This period witnessed increased demands for automobiles which were propelled by petroleum fuels. The availability of public funds, and new transportation infrastructure such as interstate and highway systems helped in this regard [14].

The early post-WWII fossil fuel demand and supply was influenced by the commencement of offshore oil and gas production in 1945 at the Gulf of Mexico and the invention of jet aircraft [14]. However in the 1970s, speculations regarding the finite nature of the fossil oil reserves became an issue worth pondering over. In 1973 and 1978, OPEC reduced oil supplies and increased the prices to meet with the shortages of the petroleum crisis of that time. This marked the reemergence of the potentials of biofuels in the public consciousness. Thus in 1979, South Africa started the commercial development of biodiesel. Sunflower oil was transesterified and refined to a standard similar to petroleum diesel fuel [15]. The outcome was the discovery of several sources and technologies that improved engine performance with reduced environmental impacts. Experiences from past were used in achieving improved efficiencies, while reducing costs by developing the renewable energy marketing advantage.

The procedure for the production, quality and engine-testing for biodiesel was finalized and published internationally in 1983. The South African technology was obtained by Gaskoks; an Austrian company. Gaskos established the first pilot plant for biodiesel production in 1987.

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By April of 1989, the firm set up the first commercial-scale plant producing 20 million gallon per year (MGPY). However during this period, biodiesel was only being produced on a noncommercial scale in the United States. The growth in producing biodiesel in Europe began in 1991 because of the need to reduce environmental impacts from emissions of greenhouse gases (GHG). Three years later, the first commercial biodiesel production was started in America. By 2000, the Commodity Credit Corporation started subsidizing value-added agriculture towards biodiesel production. The past decade (2002 to 2012) witnessed an unprecedented production of biodiesel. Incentives from policy makers such as tax exemptions, tax credits and renewable fuel standards aided the biodiesel growth. However, some properties of biodiesel also contributed to the unprecedented growth we are witnessing in the biodiesel industry [16-18].

The increasing interests on biodiesel is fueled by the need to find a sustainable diesel fuel alternative. This is mainly because of environmental issues, apprehensions over energy independence and skyrocketing prices. Several processing options are available for the biodiesel production. The various feedstocks and processing conditions provide several processing technologies. The choice of a particular technology is dependent on catalyst and the source, type and quality of feedstock. Others include postproduction steps such as product separation and purification and catalyst and alcohol recovery. The dominant factor in the production process is the cost of feedstock while capital costs contribute only about 7%. It is therefore essential to utilize cheap feedstock to reduce the overall production costs. In the same regards, some technologies are designed to handle variety of feedstocks.

## 2. Past achievments

Non-fossil fuel alternatives are favored because of their common availability, renewability, sustainability, biodegrablability, job creation, regional development and reduced environmental impacts. Table 1 summarizes some of the major successes of biodiesel.

### 2.1. Feedstocks

Numerous feedstocks have been experimented in biodiesel production. Advancements from such experimentations led to establishment of waste-to-wealth biodiesel production. Cheap and readily available raw materials such as used cooking oil and yellow grease are used for producing biodiesel. These efforts helped in reducing the environmental impacts associated with dumping in landfills as well as saves the cost of paying for such dumping. Another notable success is the use of Jatropha or the "miracle plant" in many developing countries. The fact that it can be cultivated almost anywhere with minimal irrigation and less intensive care, made it suitable for peasant farmers. Sustained high yields were obtained throughout its average life cycle of 30–50 years. Castor plantation are also intercropped with jatropha to improve the econmic viability of jatropha within the first 2 to 3 years [19]. Another oil crop that is used to improve soil quality is the nitrogen-fixing *Pongamia pinnata*. It produces seeds with significant oil contents.

## 2.2. Technologies

Biodiesel is one of the most thoroughly tested alternative fuel in the market today. Studies by many researchers have confirmed similar engine performance of biodiesel to petroleum diesel. Transesterification produce oil with similar brake power as obtained with diesel fuel. Minimal carbon deposits were noticed inside the engine except the intake valve deposits which were slightly higher. The level of injector coking was also reduced significantly lower than that observed with D2 fuel [7,17]. An important breakthrough in transesterification is the Mcgyan Process®, which can utilize various inexpensive, non-food-grade and free fatty acids (FFAs) containing feedstocks (Figure 1). The process can be small in physical size and it utilizes heterogeneous catalysts to produce biodiesel within 4 s [20,21]. The easy fatty acid removal or EFAR system ensures that no wastes are produced from the process. It eliminates post production costs such as the washing and neutralization steps. To achieve 100% conversion, it recycles all unreacted feedstock and excess alcohol back into the reactor. Energy efficiency is also achieved through heat transfer mechanism; in-coming cold reactants are preheated by the out-going hot products [20,21].

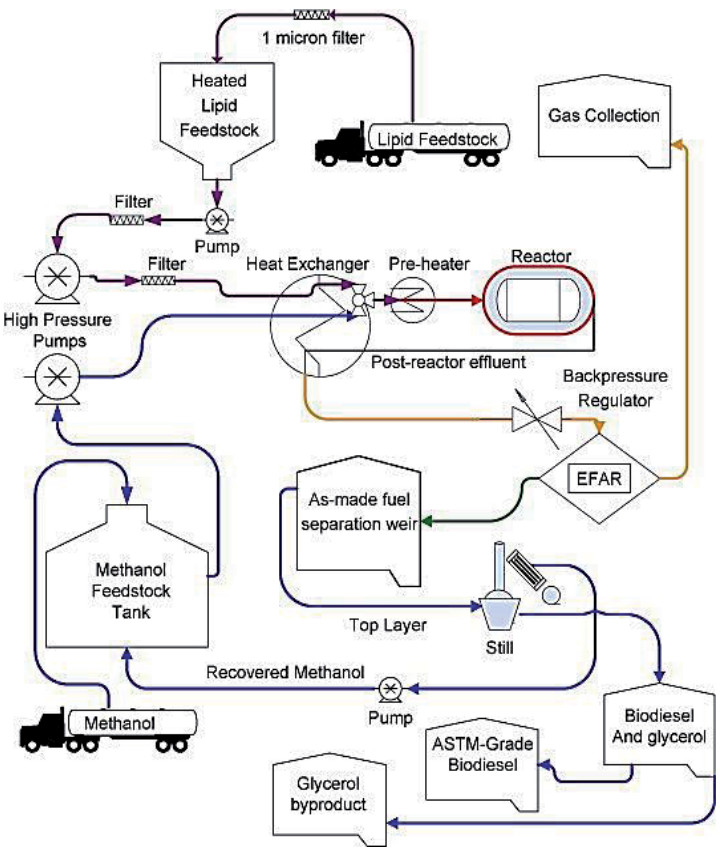


**Figure 1.** Process flow diafram of a biodiesel plant based on the Mcgyan proces[21].

| Economic & social impact | Environment impact | Energy security |
|---|---|---|
| Sustainability; made from agricultural or waste resources | Reduced 78% GHG emissions | Reduced dependence on fossil fuels |
| Fuel diversity & improved fuel efficiency & economy | Reduced air pollution | Domestic targets |
| Improved rural economy | Biodegradability | Supply reliability |
| Increased income tax & trade balances | Improved land & water use | Readily available |
| International competitiveness | Carbon sequestration | Renewability |
| Increased investments on feedstocks & equipment | Lower sulfur content | Domestic distribution |
| Technological developments (R & D) | Lower aromatic content | Improved fuel economy |
| Higher cetane number (52 vs. 48), lubricity & flash point | Lesser toxicity | Comparable energy content (92.19%) |
| Knowledge development & diffusion | Safer handling & storage | Strict quality requirements are met |
| Strong growth in demand & market formation | | Viscosity 1.3 to 1.6 times that of D2 fuel |
| Improved engine performance | | Good energy balance (3.24:1 vs. 0.88:1) |
| Reduces the need for maintenance & prolongs engine life | | |
| Compatible with all conventional diesel engines | | |
| Offers the same engine durability & performance | | |
| Has the potential of displacing petroleum diesel fuel | | |
| Comparable start-up, torque range & haulage rates | | |

**Table 1.** Major achievements of biodiesel [16,23-27]

### 2.3. Environmental impacts and health effects

A 78% reduction in GHG emission was reported by the U.S. Departments of Agriculture and Energy with biodiesel usage. Essentially, biodiesel is non-aromatic and sulphur-free as compared with petrodiesel which contains 20 to 40 wt.% aromatic compounds and 500 ppm

$SO_2$ [7]. The potential of pure biodiesel to form ozone (smog) from hydrocarbons is 50% less. Also, sulfates and oxides of sulfur (major constituents of acid rain) are essentially eliminated from the exhaust emissions compared to petrodiesel. These help in curbing the increasing global warming problems. Average decrease of 22.5% for smoke density, 17.1% for CO and 14% for $CO_2$ have been reported when biodiesel was used [22]. Human life expectancy is thereby enhanced because of improved air quality.

*2.3.1. Energy independence*

Biodiesel reduces the excessive reliance on fossil fuels. This enhances the global energy security [17]. It also has the potential to replace oil importation since it is produced domestically, thereby providing additional market for agricultural products. It supports the rural communities where it is cultivated by protecting and generating jobs. Producing biofuels equivalent to 1% of automobile fuel consumption in the EU protected and/or created approximately 75,000 jobs [16]. Approximately, for every unit of fossil energy used in biodiesel production, 4.5 units of energy is gained. Moreover, lesser energy is required for biodiesel production than the energy derived from the final product [16].

## 3. Different feedstocks used in the production of biodiesel

More than 350 oil-bearing crops have been identified as potential sources for producing biodiesel. However, only palm, jatropha, rapeseed, soybean, sunflower, cottonseed, safflower, and peanut oils are considered as viable feedstocks for commercial production [28].

### 3.1. Edible feedstocks

Depending on availability, different edible oils are utilized as feedstocks for biodiesel production by different countries. Palm oil and coconut oil are commonly used in Malaysia and Indonesia. Soybean oil is majorly used in U.S. [30].

### 3.2. Non-edible feedstocks

In order to reduce production costs and to avoid the *food-for-fuel* conflict, inedible oils are used as the major sources for biodiesel production. Compared to edible oils, inedible oils are affordable and readily available. They are obtained from *Jatropha curcas* (jatropha or ratanjyote or seemaikattamankku), *Pongamia pinnata* (karanja or honge), *Calophyllum inophyllum* (nagchampa), *Hevca brasiliensis* (rubber seed tree), *Azadirachta indica* (neem), *Madhuca indica and Madhuca longifolia* (mahua), *Ceiba pentandra* (silk cotton tree), *Simmondsia chinensis* (jojoba), *Euphorbia tirucalli,* babassu tree, microalgae, *etc*. [31]. Among the 75 plant species which have more than 29% oil in their seed/kernel; palm, *Jatropha curcas*, and *Pongamia pinnata* (Karanja) were found to be the most suitable for biodiesel production [32]. Many European countries utilize rapeseed [29]. During World War II, oil from *Jatropha* seeds was used as blends with and substituted for diesel [33,34]. It has been reported that biodiesel produced from palm and

Jatropha have physical properties in the right balance; conferring it with adequate oxidation stability and cold performance [35]. Most of the strict requirements set by the American and European biodiesel standards for biodiesel have been achieved [36]. The major oils used for producing biodiesel are presented in Table 2.

| Group | Source of oil |
|---|---|
| Major oils | Coconut (copra), corn (maize), cottonseed, canola (a variety of rapeseed), olive, peanut (groundnut), safflower, sesame, soybean, and sunflower. |
| Nut oils | Almond, cashew, hazelnut, macadamia, pecan, pistachio and walnut. |
| Other edible oils | Amaranth, apricot, argan, artichoke, avocado, babassu, bay laurel, beech nut, ben, Borneo tallow nut, carob pod (algaroba), cohune, coriander seed, false flax, grape seed, hemp, kapok seed, lallemantia, lemon seed, macauba fruit (Acrocomia sclerocarpa), meadowfoam seed, mustard, okra seed (hibiscus seed), perilla seed, pequi, (Caryocar brasiliensis seed), pine nut, poppy seed, prune kernel, quinoa, ramtil (Guizotia abyssinica seed or Niger pea), rice bran, tallow, tea (camellia), thistle (Silybum marianum seed), and wheat germ. |
| Inedible oils | Algae, babassu tree, copaiba, honge, jatropha or ratanjyote, jojoba, karanja or honge, mahua, milk bush, nagchampa, neem, petroleum nut, rubber seed tree, silk cotton tree, and tall. |
| Other oils | Castor, radish, and tung. |

**Table 2.** Major oil species for biodiesel production [37]

### *3.2.1. Algae oil*

Currently, algae-based biodiesel is the focus of many research interests because they have the potential to provide sufficient oil for global consumption. It has the potential to produce biodiesel yields >100 times those attainable per hectare from plant feedstock (Table 3). Besides their high lipid contents and fast growth rate, microalgae have the potential to mitigate the competitions for land-use and food-for-fuel conflicts. They are also able to reduce the GHG effect via $CO_2$ sequestration [38]. Microalgae can be cultivated in habitats which are not favorable for energy crops. Compared with oilseeds, the harvesting and transportation costs of microalgae are relatively low. *Nannochloropsis*, members of the marine green algae are considered the most suitable candidates for biodiesel production. These strains have shown high lipid content and biomass productivity. However, research in this area especially algal oil extraction is still limited and in early stages.

### *3.2.2. Other feedstocks*

Used vegetable oils (UCO), yellow grease (8-12 wt% FFA), brown grease (>35 wt% FFA), and soapstock (by-product of refining vegetable oils) are potential feedstocks for biodiesel production. Their low costs and availability make them suitable for reducing the production

costs of biodiesel. To achieve this however, the problems associated with high FFA which are common to these feedstocks, particularly when alkaline catalysts are employed need attention. Solid acid catalysts are currently receiving great attention because they are suitable for feedstocks containing FFAs [39-41]. Another process that has the potential of processing these feedstocks is supercritical transesterification. The pretreatment step, soap and catalyst removal common to alkaline catalysis are eliminated since the process requires no catalyst [42,43]. The process has fast reaction rate which significantly reduces the reaction time [44]. The process is insensitive to water and FFAs [43,45]. However, this method is not economical because it requires high reaction temperature, pressure and higher molar ratio of alcohol to feedstock [42,43,46]. Another interesting feedstock is *Salicornia bigelovii* (Halophytessuch). It can produce equal biodiesel yields obtained from soybeans and other oilseeds. They grow in saltwater of coastal areas unsuitable for energy crops.

| Microalgae/Plant | Oil yield (L/ha/year) | Oil content (% wt in biomass) | Required land (M $ha^{-1a}$) | Biodiesel productivity (kg biodiesel/ha/ year) |
|---|---|---|---|---|
| Microalgae[b] (high oil content) | 136 900 | 70 | 2 | 121 104 |
| Microalgae[c] (low to low oil content) | 58 700 to 97 800 | 30 to 50 | 4.5 | 51 927-85 515 |
| Oil palm (*Elaeis guineensis*) | 5 950 | 30 to 60 | 45 | 4747 |
| *Jatropha* (*Jatropha curcas* L.) | 1 892 | Kernel: 50 to 60<br>Seed: 35 to 40 | 140 | 656 |
| Canola/Rapeseed (*Brassica napus* L.) | 1 190 | 38 to 46 | 223 | 862 |
| Soybean (*Glycine max* L.) | 446 | 15 to 20 | 594 | 562 |
| Corn/Maize (Germ) (*Zea mays* L.) | 172 | 44 to 48 | 1540 | 152 |

**Table 3.** Estimated oil content, yields and land requirement for various biodiesel feedstocks.[36,47,48]

## 4. Methods of oil extraction

The three common methods used in extracting oil are: (i) Mechanical extraction, (ii) solvent extraction and (iii) enzymatic extraction.

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### 4.1. Mechanical extraction method

This method is used by smaller production firms for processing less than 100,000 kg/day. Usually, an engine driven screw press or a manual ram press is used to extract 68–80% or 60–65% of the available oil respectively. Pretreatment such as dehulling and cooking increase oil yields to 89% and 91% after single and dual pass respectively [48,49]. However, most of the mechanical presses are designed for particular seeds which affect yields with other seeds. Also, extra treatments such as degumming and filtration are required for oil extracted by this technique.

### 4.2. Chemical (solvent) extraction method

The commonly used chemical methods are: (1) soxhlet extraction, (2) Ultrasonication technique and (3) hot water extraction [48,49]. Solvent extraction (or leaching) is typically used for processing more than 300,000 kg/day [50]. Yields are affected by particle size, solvent type and concentration, temperature and agitation. To increase the exposure of the oil to the solvent, the oilseeds are usually flaked. After extraction, the oil-solvent mixture or *miscella*, is filtered while heat is used to vaporize the solvent from the miscella. Steam is injected to remove any solvent remaining from the oil. The immiscibility of the solvent and steam vapors is used to separate them in a settling tank after condensation. The highest oil yields are obtained with n-hexane. However, the process requires higher energy and longer time compared to other methods. Furthermore, the human health and environmental impacts associated with toxic solvents, waste water generation and emissions of volatile organic compounds are challenges facing this method.

### 4.3. Enzymatic extraction method

Oilseeds are reduced to small particles and the oil is extracted by suitable enzymes. Volatile organic compounds are not produced by this method which makes it environmentally friendly when compared to the other methods. However, it has the disadvantage of long processing time and high cost of purchasing enzymes [51].

## 5. Technologies used in biodiesel production

Several researches were carried out to overcome or minimize the problems associated with producing biodiesel. The methods that have been used for minimizing the viscosity of vegetable oils for practical application in internal combustion engines include: pyrolysis, microemulsification, blending (diluting) and transesterification. Dilution and microemulsification are not production processes and are therefore not discussed in this chapter. A summary of vegetable oils and animal fats and the major biodiesel production technologies are presented in Table 4.

### 5.1. Pyrolysis or catalytic cracking

Pyrolysis is the heating of organic matter in the absence of air to produce gas, a liquid and a solid [52]. Heat or a combination of heat and catalyst is used to break vegetable oils or animal fats into smaller constituents. Olefins and paraffins are thus obtained with similar properties to petrodiesel where such products derived the name "*diesel-like-fuel*" [53]. Studies on effects of rapeseed particle size showed that the product yield is independent of the oilseed particle size [52]. The maximum temperature range for conversion of bio-oil is 400°C to 450°C [54]. Rapid devolatilization of cellulose and hemicellulose occur at this temperature. Heating rate and temperature have significant effects on bio-oil yields, char and gas released from olive [55]. The viscosity, flash and pour points and equivalent calorific values of the oil are lower than diesel fuel. Though the pyrolyzate has increased cetane number, it is however lower than that of diesel oil. Apart from reducing the viscosity of the vegetable oil, pyrolysis enables de-coupling of the unit operation equipment in shorter time, place and scale. It produces clean liquids which needs no additional washing, drying or filtering. Product of pyrolysis consists of heterogeneous molecules such as water, particulate matter, sulfur, alkanes, alkenes and carboxylic acids [39,56]. Consequently, it is difficult to characterize fuel obtained from pyrolysis [52]. This process is energy consuming and needs expensive distillation unit. Moreover, the sulfur and ash contents make it less eco-friendly [57].

### 5.2. Transesterification (alcoholysis)

Transesterification is the most widely employed process for commercial production of biodiesel. It involves heating the oil to a designated temperature with alcohol and a catalyst, thereby restructuring its chemical structure. This conversion reduces the high viscosity of the oils and fats. For the transesterification of triglyceride (TG) molecule, three consecutive reactions are needed. In these reactions, FFA is neutralized by the TG from the alcohol. One mole of glycerol and three moles of alkyl esters are produced (for each mole of TG converted) at the completion of the net reaction. These separate into three layers, with glycerol at the bottom, a middle layer of soapy substance, and biodiesel on top [57]. Transesterification is a reversible reaction. To obtain reasonable conversion rates therefore, it requires a catalyst. The reaction conditions, feedstock compositional limits and post-separation requirements are predetermined by the nature of the catalyst. Table 5 presents a genaral overview of the several transesterification techniques for biodiesel production.

#### *5.2.1. Homogeneous alkali-catalyzed transesterification*

Alkali catalysts such as NaOH and KOH were preferred over other catalysts because of their ability to enhance faster reaction rates [63]. This is because they are readily available at affordable prices and enable fast reaction rates [24]. Detailed review on base-catalyzed transesterification of vegetable oils can be found in ref [64]. However, homogeneous catalysis has been faced with the been faced with the problems saponification, highly sensitive to FFAs, expensive separation requirement, waste water generation and high energy consumption.

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### *5.2.2. Homogeneous acid-catalyzed transesterification*

Though the performance of this method is not strongly affected by FFAs in the feedstock, the process is not as popular as the base-catalyzed process. This is because the use of strong acids such as $H_2SO_4$ [65,66], HCl, $BF_3$, $H_3PO_4$, and organic sulfonic acids [67], is associated with higher costs and environmental impacts. Moreover, the technique is about 4000 times slower than the homogeneous base-catalyzed reaction. The mechanism of the acid-catalyzed transesterification can be found in ref [68].

### *5.2.3. Heterogeneous acid and base-catalyzed transesterification*

Solid acid can simultaneously catalyze the esterification and transesterification without the need for pretreating feedstocks with high FFAs. Thus, this technique has the potential of reducing the high cost of biodiesel production by directly producing biodiesel from readily available and low-cost feedstocks [67].
Solid basic catalysts also have the potential of reducing the cost of biodiesel production because of lesser catalyst consumption, reuse and regeneration. However, these catalysts have some disadvantages which hinder their wide acceptability. These include mass transfer (diffusion) problem which reduces the rate of reaction as a result of the formation of three phases with alcohol and oil. Other problems associated with base catalyzed transesterification are loss of catalyst activity in the presence of water and post-production costs such as product separation, purification and polishing.

### *5.2.4. Enzymatic transesterification*

Some of the problems associated with homogeneous catalysts such as expensive product separation, wastewater generation, and the presence of side reactions are avoided with enzymatic transesterification [69]. Enzyme immobilization is usually done to enhance the product quality, increase the number of times the catalyst is reused and to reduce cost [28,70]. However, several technical difficulties such as high cost of purchasing enzymes, product contamination, and residual enzymatic activity are limiting the applicability of this technique.

### *5.2.5. Supercritical alcohol transesterification*

Unlike the conventional transesterification of two heterogeneous liquid phases involving alcohol (polar molecule) and non-polar molecules (TGs), supercritical transesterification is done in single homogeneous phase. Subjecting solvents containing hydroxyl groups (such as water and alcohol) to conditions in excess of their critical points make them to act as superacids. Under supercritical conditions, alcohol serves a dual purpose of acid catalyst and a reactant [46,71]. The absence of interphase solves the mass transfer limitations which gives the possibility of completing the reaction in minutes rather than several hours. In fact, the Mcgyan Process® was used to produce biodiesel under 4 s [19,20]. However, this process is not economical especially for commercial production as it requires expensive reacting equipment due to high temperature and pressure [72]. Studies are currently being undertaken in order to reduce these high reacting conditions.

| Direct use | Dilution with vegetable oils | Microemulsion of oils | Pyrolysis and catalytic cracking | Transesterification of oils and fats | |
|---|---|---|---|---|---|
| **Advantages** | **Advantages** | **Advantages** | **Advantages** | **Catalytic** | **Non-catalytic** |
| Simple process | Simple process and non-polluting | Simple process and non-polluting | Simple process & non-polluting<br>no additional washing, drying or filtering required | | |
| Disadvantages | Disadvantages | Disadvantages | Disadvantages | Acid-catalyzed | BIOX cosolvent process |
| Highly viscous | Highly viscous | Incomplete combustion | Contains heterogeneous molecules | Alkali catalytic | Supercritical alcohol |
| Highly unstable | Highly unstable | Injector needle sticking | Low purity | Enzyme-catalyzed | Microwave and ultrasound assisted |
| Low volatility | Low volatility | Carbon deposits | Requires high temperature | Catalytic supercritical alcohol | |
| Not suitable for commercial production | Not suitable for commercial production | Not suitable for commercial production | Requires expensive equipment | See Table 5 for advantages and disadvantages | |

**Table 4.** Use of vegetable oils and animal fats and major biodiesel production processes.

## 5.3. Technologies

### *5.3.1. Microwave assisted transesterification*

The microwave irradiation as energy stimulant has been attracting the attention of many researchers. This is because the reaction process fast (within minutes), it employs a lower alcohol-oil ratio and it reduces by-products quantities. It uses a continuously changing electrical and magnetic fields to activate the smallest degree of variance of the reacting molecules. These rapidly rotating charged ions interact easily with minimal diffusion limitation [73]. However, this process also has commercial scale-up problem because of high operating conditions and safety aspects [74]. An even more daunting challenge is in increasing the irradiation penetration depth beyond a few centimeters into the reacting molecules.

### *5.3.2. Ultrasound assisted transesterification*

This process utilizes sound energy at a frequency beyond human hearing. It stretches and compresses the reacting molecules in an alternating manner. Application of high negative

pressure gradient beyond the critical molecular distance forms cavitation bubbles. Some of the bubbles expand suddenly to unstable sizes and collapse violently. This causes emulsification and fast reaction rates with high yields since the phase boundary has been disrupted [75-77].

| **Chemical catalysed** | | | | **Chemical catalysed (Modified)** | | | **Biochemical catalysed** | **Noncatalysed** |
|---|---|---|---|---|---|---|---|---|
| **Homogeneous acid** | **Homogeneous base** | **Heterogeneous acid** | **Heterogeneous base** | **Microwave irradiation** | **Ultrasound (sonication)** | **Oscillatory flow reactor** | **Enzyme** | **Supercritical methanol** |
| *Merits* | *Merits* | *Merits* | *Merits* | *Merits* | *Merits* | *Merits* | *Merits* | *Merits* |
| Employs feedstocks with high FFAs ("/>2 wt %) | Reaction is 4000 times faster than homogeneous acid catalysed reactions | High possibility of reusing and regenerating catalyst many times | High possibility of reusing and regenerating catalyst many times | Speeds up rate of reaction (from hours to minutes) | Increases FAME production from seedcakes | Increases mixing of reactants | Operates at milder reaction conditions | Simultaneous transesterification of TGs and esterification of FA |
| No pretreatment required | Operates at mild temperature (50 to 80 °C) | Simultaneous transesterification of TGs and esterification of FA | Saves cost of purchasing catalyst | Improves catalyst activity and selectivity | *In situ* extraction transesterification | Efficient heat and mass transfer | Cleaner biodiesel and glycerol are produced | High biodiesel yield |
| | Lower alcohol-to-oil (5:1) molar ratio | Simpler and less energy intensive | Simpler and less energy intensive | Minimizes energy consumption | High FAME yields | Higher yield in shorter time compared to batch-type | Energy consumption is minimized | Simultaneous transesterification of TGs and esterification of FA |
| | High biodiesel yield | Does not require feedstock pretreatment | Easy separation of products | | Eliminates of saponification | Reduces reactor length-to-diameter ratio | Waste generation is minimized | Requires no catalyst |
| | Catalysts are cheap and readily available | Mild reaction conditions & less prone to leaching | Mild reaction conditions | | Low reaction time | Reduces costs | | Relatively fast reaction rate |
| | | Waste generation is minimized | | | Mild reaction conditions | | | Short reaction time (<30 minutes) |
| | | Relatively fast reaction rates | | | Enhances mass transfer | | | |
| | | Easy product separation | | | | | | |
| | | Saves cost of purchasing catalysts | | | | | | |
| | | Minimizes solvation of active sites by action of water | | | | | | |
| | | Eliminates of saponification | | | | | | |

| Chemical catalysed | | | | Chemical catalysed (Modified) | | | Biochemical catalysed | Noncatalysed |
|---|---|---|---|---|---|---|---|---|
| **Homogeneous acid** | **Homogeneous base** | **Heterogeneous acid** | **Heterogeneous base** | **Microwave irradiation** | **Ultrasound (sonication)** | **Oscillatory flow reactor** | **Enzyme** | **Supercritical methanol** |
| | | Reduces size & cost of reaction vessel | | | | | | |
| | | Very attractive commercially | | | | | | |
| *Challenges* | *Challenges* | *Challenges* | *Challenges* | *Challenges* | *Challenges* | *Challenges* | *Challenges* | *Challenges* |
| Very slow reaction rate & mineral acids used are corrosive to the equipment | Highly sensitive to water and FFAs in the oil | Availability of specific catalysts at low cost. Researches are ongoing to find low cost precursors. | Requires feedstock pretreatment & catalyst get poisoned with prolong exposure to ambient air | Difficulties in process scale-up from laboratory scale to large-scale | Difficulties in process scale-up from laboratory scale to large-scale | Difficulties in process scale-up from laboratory scale to large-scale | High cost of enzymes | Energy intensive |
| Catalyst required in large quantities | Requires refined feedstock (0.5 % FFA; 0.06% $H_2O$) | Limitation due to diffusion problems. This is solved by designing catalysts with large interconnected pores with high concentration of acid sites | High cost of reacting vessels | Depth of radiation is limited to a few cm. | Requires advanced technology | Requires advanced technology | High production cost | Very expensive |
| Requires high alcohol-to-oil molar ratio | Water saponifies the esters and FFAs reacts with the catalyst | | Two-step reaction of esterification and transesterification | Requires advanced technology | Safety issues in equipment handling | Safety issues in equipment handling | Enzymes easily denatured | Not commercially profitable |
| Higher temperature | Requires methanol-to-oil of 6:1 (or higher) molar ratio instead of the stoichiometric 3:1 ratio | | Product contamination from leaching of active catalytic sites | Safety issues in equipment handling. | | | Not commercially profitable | Safety issues |
| Undesirable etherification reaction (dialkyl or glycerol ethers) | Soap formation (FFA"/>2%) | | Water saponifies the esters and FFAs reacts with the catalyst making purification difficult | | | | Very slow reaction rates (slower than homogeneous acid catalysed) | High temperature and pressure |

| Chemical catalysed | | | | Chemical catalysed (Modified) | | | Biochemical catalysed | Noncatalysed |
|---|---|---|---|---|---|---|---|---|
| **Homogeneous acid** | **Homogeneous base** | **Heterogeneous acid** | **Heterogeneous base** | **Microwave irradiation** | **Ultrasound (sonication)** | **Oscillatory flow reactor** | **Enzyme** | **Supercritical methanol** |
| Separation and purification of glycerol | Loss of catalyst | | Catalyst leaching leads to product contamination | | | | Sensitive to methanol | Energy intensive |
| Not commercially profitable | Reduces biodiesel yield & generates wastewater | | Purification decreases biodiesel yield | | | | | |

**Table 5.** Merits and challenges surrounding transesterification processes[78]

## 6. Current challenges and future prospects

In order to make biodiesel profitable, several technical challenges need to be resolved. The most important challenge is in reducing the high cost of feedstock. Low-cost feedstocks such as algal oils, used cooking oils and animal fats are utilized to increase biodiesel profitability. However, presence of higher amounts of water and FFAs in these feedstocks poses the problems of saponification and extra pretreatment and purification costs with alkali catalysts. The challenge facing researchers currently is developing efficient heterogeneous acid catalysts that would alleviate these problems. Also, diversifying the by-product of biodiesel production processes is critical to ensuring its economic, social and environmental sustainability.

### 6.1. Vegatable oil as feedstock for biodiesel

Currently, biodiesel production costs are higher than those of petroleum diesel. Subsidies such as tax exempt and excise duty reductions are essential to make biodiesel price-competitive. It is not certain whether these political supports will be sustained in the future. It is therefore crucial for the biofuel industry to establish readily available and affordable feedstocks and efficient production systems to sustain its market growth.

### 6.2. Non-food crops

Early studies have indicated relative differences in the cultivation patterns and oil production management of the non-food feedstocks compared to food crops. These are still under investigation [79]. Therefore, more data is needed to evaluate the sustainability index to estimate the real global impact of these feedstocks. Microalgae are promising in solving most of the problems associated with energy crops. However, the cultivation and extraction technologies are still at their infancy and need major advancements for sustainable commercial production [1]. The oil extraction methods currently in use for algal

oil are expensive. Efficient mixing from pumps or motionless mixers is required to ensure homogeneity and to reduce mass transfer limitations. However, this increases the dispersion of glycerol into the FAME phase and the time required for separation. Techniques that utilize motionless mixing requires higher temperature and pressure to achieve shorter residence time. This increases energy consumption and cost implications. This aspects of biodiesel production technology is still being developed.

### 6.3. Effects of moisture and FFA

The key parameters that determine the viability of most feedstocks is FFA and moisture content. Pretreatments to less than 0.05% FFA is required for homogeneous alkali catalysts [80,81]. Prolonged storage in the presence of water and air leads to microbial growth and fuel degradation. This contributes to deposit formation on fuel injectors and engine damage. Heterogeneous acid catalysts are utilized to avoid the pretreatment and post production costs and storage problems.

### 6.4. Pyrolysis

Pyrolysis generates aromatic toxins. The bio-oil produced is corrosive due to high acidity, water content and other impurities such as solids and salts. These and other problems such as variable viscosity make it unstable and unsuitable for direct use [82]. It has 40% less energy density compared to diesel fuel because of the high oxygen content [83]. Depending on the feedstock and reacting conditions used, bio-oil is 10 to 100% more expensive than petroleum diesel. There is also the need to establish standards for product quality, use and distribution [1]. In order to stabilize the composition of the bio-oil and reduce water and oxygen content, processes such as steam reforming, hydro-treatment, hydro-cracking and emulsification with mineral diesel for direct use are employed [84-87].

### 6.5. Alcohol

Methanol is toxic, highly flammable and contributes to global warming. Gaskets and rubber seals made from natural rubber get easily deteriorated when biodiesel containing a high level of alcohol is used [80]. Therefore, control or replacement of the alcohol content is required. The biodiesel produced with methanol from fossil sources has approximately 94 to 96% biogenic content. In order to produce a 100% renewable biodiesel (fatty acid ethyl ester; FAEE), bioethanol is currently experimented as a substitute for methanol [88]. However, it is expensive to purify and recover ethanol because it forms an *azeotrope* with water. Additionally, chemical grade ethanol is usually denatured with poisonous substances to prevent it from being abused. Therefore, it is difficult to obtain pure chemical grade ethanol.

### 6.6. Supercritical alcohol process

The residence time for this process is within 4 s to 10 min because of efficient mixing [71,72]. However, due to higher reacting conditions of temperature and pressure, the process is faced with some limitations. Process scale-up for commercial production is the major one amongst

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them. The process requires more energy at extra cost and higher molar ratio of alcohol-to-oil (42:1). Also, there is the need to quench the reaction in a rapid manner. This prevents the biodiesel from decomposing as a result of the high temperature and pressure. To reduce the high operating conditions and increase product yield, some researchers employ co-solvents, such as hexane, $CO_2$, and CaO [72]. Oil and alcohol are sparingly soluble in each other. However, small amount of hexane (2.5 wt%) added increased the biodiesel yield from 67.7% to 85.5% under supercritical conditions [72]. This was made possible because the co-solvent increased the homogeneity of the reactants. Supercritical $CO_2$ is a facile substance that can obtained at affordable cost. It is also environmentally friendly and can effectively used in the reaction and safely recovered via depressurization. A process that combines co-solvents in superctical conditions is promising in increasing product yield, reducing process time and overall production costs.

### 6.7. Biodiesel/glycerol separation and FAME quality

The slightly soluble nature of FAMEs and glycerol makes product separation a necessary step. The product is usually allowed to settle for some hours into the different phases. However, the solubility of glycerol in ester and vice versa is increased in the presence of excess unreacted methanol which acts as solvent. This solvent action by the methanol increases the post production costs. Besides, it is also essential to remove all traces of TGs which form emulsion layer between the two phases. The presence of this layer further makes separation difficult and expensive. On the other hand, the storage, transportation, distribution and retail infrastructure used for petroleum diesel can be used for biodiesel even in its neat form. This will reduce construction costs for establishing new insfrasctures for biodiesel. However, biodiesel degrades after long period of storage. In order to prevent this from occuring, advances in storage and distribution logistics have to be developed. Also, similar logistics employed by the petroleum industry could be adapted.

### 6.8. Use of cosolvents

A technique developed to overcome mass transfer limitations and to increase the rate of reaction is the use of cosolvents such as methyl tert-butyl ether (MTBE) and tetrahydrofuran (THF). High quality FAMEs is obtained at moderate conditions (30 °C) within 10 minutes. However, the process requires larger and special “leak proof” reacting vessels and complete removal of the cosolvent from the product.

### 6.9. $NO_x$ emissions

Despite the favorable environmental impacts in terms of overall reduced GHG emissions, biodiesel has the potential to increase $NO_x$ emissions. Approximately 3 to 4%, 4 to 6% and 6 to 9% over petroleum diesel is emitted from B20, B40 and B100 respectively [89]. Adjustments in combustion temperatures and injection timing [90], use of antioxidants [91] and catalytic conversion techniques were successful in reducing these emissions [90].

### 6.10. Economic analysis

As discussed in the introductory section, vegetable oils have other important uses. Recently, dielectric oils and synthetic lubricants used for electric transformers have joined the market competition for these raw materials. This will impact negatively towards the cost of raw materials for the biodiesel industry [92]. About 15% of lubricants used in vehicles in some European countries are from vegetable oil derivatives [93]. Additionally, the heating value of biodiesel is 10% lower than that of petroleum diesel. This is because of the substantial amount of oxygen in the fuel. Moreover, it also has a higher specific gravity of 0.88 when compared to 0.85 of petroleum diesel. Therefore, its overall energy content per unit volume is having an impact which is approximately 5% lower than that obtained from petroluem diesel [94]. This results in higher specific fuel consumption values of the biodiesel. Another problem encountered when switching from petroleum diesel to biodiesel in the same fuel system is the clogging of the fuel filters. This is because biodiesel acts as a solvent which dissolves sediments in diesel fuel tanks [95]. On a positive note, sales of purified glycerol (glycerine) saved 6.5% of the operational cost [97-99] while 25% saving was reported in ref [96] from the utilization of waste soapstock with respect to virgin soybean oil. However, it is necessary to compensate the negative cost implications from commercial production of biodiesel from such low value feedstocks before valid conclusions can be derived.

## 7. Conclusions

Some of the major challenges faced by the biodiesel industry include readily available and affordable feedstocks, competition from a popular and cheaper energy source, technological advancements and acceptability. Those challenges requiring immediate attention are product stability under long storage, lower energy content, cold flow properties, catalyst leaching, microalgal oil extraction and NOx emissions. Despite these challenges however, the historical development of biodiesel is intriguing. Biodiesel has successfully remained an energy source to be reckoned with even after being relegated to the background for so many years. Concerns over diminishing oil reserves, increasing crude oil prices and associated environmental impacts aided the reemergence of biodiesel; making it the fastest growing industry worldwide. Several technologies were developed while more advances are in the process of being established. Other successes associated with the biodiesel industry include reduction in environmental impacts, job creation, energy security and waste-utilization. Biodiesel is regarded as a viable alternative or additive to petrodiesel because of its good properties such as nontoxicity, clean-burning, renewability and acceptability. Consequently, the prospects of the biodiesel industry are numerous. The biodiesel production process is shifting from other sources to algal oil and heterogeneous acid catalysts. Algal oil is a more reliable and efficient source. It has the potential of producing yields of more than 100 times those attainable per hectare from oilseeds. Affordable and readily available non-food feedstocks such as microalgae have been produced in commercial scale without competing with arable land or causing deforestation. Additionally, the use of heterogeneous acid catalyst produces cleaner and higher yields.

It employs cheaper and readily available feedstocks and minimizes pre- and post-product costs. These and other factors such as waste-utilization and cleaner emissions will help ensure biodiesel as a cheaper energy source with greater economic benefits and healthier environments.

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Chapter 5

# Prospects and Potential of Green Fuel from some Non Traditional Seed Oils Used as Biodiesel

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Additional information is available at the end of the chapter

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## 1. Introduction

Today's diesel engines require a clean-burning, stable fuel that performs well under a variety of operating conditions. Biodiesel is the only alternative fuel that can be used directly in any existing, unmodified diesel engine. Because it has similar properties to petroleum diesel fuel, biodiesel can be blended in any ratio with petroleum diesel fuel. Many federal and state fleet vehicles in USA are already using biodiesel blends in their existing diesel engines (Harwood, 1981). The low emissions of biodiesel make it an ideal fuel for use in marine areas, national parks and forests, and heavily polluted cities. Biodiesel has many advantages as a transport fuel. For example, biodiesel can be produced from domestically grown oilseed plants. Producing biodiesel from domestic crops reduces the dependence on foreign petroleum, increases agricultural revenue, and creates jobs.

Presently world's energy needs are met through non-renewable resources such as petrochemicals, natural gas and coal. Since the demand and cost of petroleum based fuel is growing rapidly, and if present pattern of consumption continues, these resources will be depleted in near future. It is the need of time to explore alternative sources of fuel energy. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable and easily available. Fatty acid methyl esters derived from renewable sources such as vegetable oils has gained importance as an alternative fuel for diesel engines. The edible oils such as soybean oil in USA, rapeseed oil in Europe and palm oil in countries with tropical climate such as Malaysia are being used for the production of biodiesel (Knothe, 2002).

### 1.1. Historical background

Biodiesel, an alternative diesel fuel, is made from renewable biological sources such as vegetable oils and animal fats. It is biodegradable and nontoxic, has low emission profiles and so far is environmentally beneficial (Krawczyk, 1996). Bio-diesel production is not something new, because the concept of using vegetable oil as fuel dates back to 1895. Rudolf Diesel developed the first diesel engine which was run with vegetable oil in 1900. The first engine was run using groundnut oil as fuel (Bijalwan *et al*., 2006). In 1911, Rudolf Diesel stated that the diesel engine can be fed with vegetable oil and would help considerably in the agricultural development of the countries which use it. In 1912, Rudolf Diesel said, the use of vegetable oils for engine fuels may seem insignificant today. But such oils may become in course of time as important as petroleum and the coal tar products of the present time (Babu and Devaradjany, 2003). After eight decades, the awareness about environment rose among the people to search for an alternative fuel that could burn with less pollution. Rudolf Diesel's prediction is becoming true today with more and more bio-diesel being used all over the world. With the advent of cheap petroleum, appropriate crude oil fractions were refined to serve as fuel and diesel fuels and diesel engines evolved together. In the 1930s and 1940s vegetable oils were used as diesel fuels from time to time, but usually only in emergency situations. Recently, because of increases in crude oil prices, limited resources of fossil oil and environmental concerns there has been a renewed focus on vegetable oils and animal fats to make biodiesel fuels. Continued and increasing use of petroleum will intensify local air pollution and magnify the global warming problems caused by $CO_2$ (Shay, 1993). In a particular case, such as the emission of pollutants in the closed environments of underground mines, biodiesel fuel has the potential to reduce the level of pollutants and the level of potential or probable carcinogens (Krawczyk, 1996).

Considerable research has been done on vegetable oils as diesel fuel. That research included palm oil, soybean oil, sunflower oil, coconut oil, rapeseed oil and tung oil. Animal fats, although mentioned frequently, have not been studied to the same extent as vegetable oils. Some methods applicable to vegetable oils are not applicable to animal fats because of natural property differences. Oil from algae, bacteria and fungi also has been investigated (Shay, 1993). Terpenes and latexes also were studied as diesel fuels. Microalgae have been examined as a source of methyl ester diesel fuel (Nagel and Lemke, 1990).

### 1.2. Sources of biodiesel

Alternative diesel fuels are made from natural, renewable sources such as vegetable oil and fats (Ratledge *et al*., 1985; Lee *et al*., 1995). There are more than 350 oil-bearing crops identified, among which only soybean, palm, sunflower, safflower,cottonseed, rapeseed and peanut oils are considered as potential alternative fuels for diesel engines (Pryor *et al*., 1982).

Vegetable oils are promising feedstocks for biodiesel production since they are renewable in nature, and can be produced on a large scale and environmentally friendly (Patil & Deng, 2009). Vegetable oils include edible and non-edible oils. More than 95% of biodiesel production feed stocks come from edible oils since they are mainly produced in many regions and the properties of biodiesel produced from these oils are much suitable to be used as diesel fuel

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substitute (Gui *et al*., 2008). However, it may cause some problems such as the competition with the edible oil market, which increases both the cost of edible oils and biodiesel (Kansedo *et al*., 2009).

In order to overcome these disadvantages, many researchers are interested in non-edible oils which are not suitable for human consumption because of the presence of some toxic components in the oil. Non edible oil crops can be grown in waste lands that are not suitable for food crops and the cost of cultivation is much lower because these crops can still sustain reasonably high yield without intensive care (Kumar *et al*., 2007; Gui *et al*., 2008)

Animal fats contain higher level of saturated fatty acids therefore they are solid at room temperature that may cause problems in the production process. Its cost is also higher than vegetable oils (Singh, 2009). The source of Biodiesel usually depends on the crops amenable to the regional climate. In the United States, soybean oil is the most commonly Biodiesel feedstock, whereas the rapeseed (canola) oil and palm oil are the most common source for Biodiesel, in Europe, and in tropical countries respectively (Knothe, 2002). A suitable source to produce Biodiesel should not competent with other applications that rise prices, for example pharmaceutical raw materials. But the demand for pharmaceutical raw material is lower than for fuel sources. As much as possible the Biodiesel source should fulfill two requirements: low production costs and large production scale. Refined oils have high production costs, but low production scale; on the other side, non-edible seeds, algae and sewerage have low production costs and are more available than refined or recycled oils. The oil percentage and the yield per hectare are important parameters to consider as Biodiesel source.

Algae can grow practically in every place where there is enough sunshine. Some algae can grow in saline water. The most significant difference of algal oil is in the yield and hence its biodiesel yield. According to some estimates, the yield (per acre) of oil from algae is over 200 times the yield from the best-performing plant/vegetable oils (Sheehan *et al*., 1998b).

### 1.3. Biodiesel production

The seed oils usually contain free fatty acids, phospholipids, sterols, water, odorants and other impurities. Because of these the oil cannot be used as fuel directly. To overcome these problem the oil requires slight chemical modification mainly pyrolysis, microemulsion, dilution and transesterification. Pyrolysis is a method of conversion of one substance into another by mean of heat or by heat with the aid of the catalyst in the absence of air or oxygen (Sonntag, 1979). The process is simple, waste less, pollution free and effective compared with other cracking processes.

The vegetable oil is diluted with petroleum diesel to run the engine. Caterpillar Brazil, in 1980, used pre-combustion chamber engines with the mixture of 10% vegetable oil to maintain total power without any alteration or adjustment to the engine. At that point it was not practical to substitute 100% vegetable oil for diesel fuel, but a blend of 20% vegetable oil and 80% diesel fuel was successful. Some short-term experiments used up to a 50/50 ratio.

A micro emulsion define as a colloidal equilibrium dispersion of optically isotropic fluid microstructure with dimensions generally into 1–150 range formed spontaneously from two

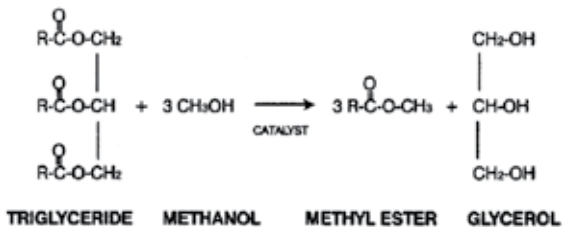


**Figure 1.** Transestrification Reaction

normally immiscible liquids and one and more ionic or more ionic amphiphiles (Schwab *et al*., 1988). They can improve spray characteristics by explosive vaporization of the low boiling constituents in micelles (Pryde, 1984). The engine performances were the same for a microemultion of 53% sunflower oil and the 25% blend of sunflower oil in diesel (Ziejewski *et al*., 1983). A microemulsion prepared by blending soyabean oil, methanol, and 2-octanol and cetane improver in ratio of 52.7:13.3:33.3:1.0 also passed the 200 h EMA test (Goering, 1984).

Transesterification or alcoholysis is the displacement of alcohol from an ester by another in a process similar to hydrolysis, except than alcohol is used instead of water. This process has been widely used to reduce the high viscosity of triglycerides. A catalyst is usually used to improve the reaction rate and yield. Excess alcohol is used to shift the equilibrium toward the product because of reversible nature of reaction. For this purpose primary and secondary monohybrid aliphatic alcohols having 1-8 carbon atoms are used (Sprules and Price, 1950).

The main factors affecting transesterification are the alcohol to oil molar ratio, catalyst concentration, reaction temperature and reaction time. The methanol to oil ratio needs to be higher than stoichiometric ratio to drive the equilibrium to a maximum ester yields. The molar ratio is associated with the type of vegetable oil used. Ikwuagwu *et al*., 2000 stated that molar ratio was 6: 1 for rubber seed oil. It was also undertaken sunflower oil was used (Vicente *et al*., 2005). Catalysts are classified as alkali, acid and alkali-alcoholic. Transesterification of jojoba oil catalysed with sodium metoxide (Canoira *et al*., 2006). Sodium hydroxide was also choosen to catalyse the transesterification of rubber seed oil beacause it is cheaper. Different homogeneous catalysts were used to transesterify sunflower oil to obtain 100% biodiesel yield by using sodium methoxide catalyst (Vicente *et al*., 2005).

Transesterification consist of a number of consecutive, reversible reactions. It is usually reaction of vegetable or waste oil respectively with a low molecular weight alcohol, such as ethanol and methanol. During this process, the triglyceride molecule from vegetable oil is removed in the form of glycerin. The triglycerides are broken step wise into diglycerides, monoglyceride and finally converted into methyl esters and glycerol (Fig: 1).

There are various types of transesterification that includes based, acid and lipase catalyzed. The petroleum and other fossil fuels contain sulfur, ring molecules & aromatics while the biodiesel molecules are very simple hydrocarbon chains, containing no sulfur, ring molecules or aromatics. Biodiesel is thus essentially free of sulfur and aromatics. Biodiesel is made up of almost 10% oxygen, making it a naturally "oxygenated" fuel (Noureddini & Zhu).

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**Figure 2.** Electric Oil Expeller

**Figure 3.** Mechanical Oil Expeller

**Figure 4.** Preparation of Methoxide catalyst

**Figure 5.** Mixing of catalyst

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**Figure 6.** Separation of glycerin from Biodiesel

**Figure 7.** Biodiesel Filtration

**Figure 8.** Milk Thistle Flower

**Figure 9.** Milk Thistel Seeds

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### 1.4. Biodiesel Scenario at Global Level

Use of bio-diesel is catching up all over the world especially in developed countries.

- In **Malaysia**, the tropical climate encourages production of bio-diesel from palm oil (Meher et al., 2006; Lam and Lee, 2011).
- The **United States** is contributing 25% of the world green house gases: i.e., oil and coal. We also need to reorganize its 70% of oil consumption is in transportation. The cost of bio-diesel is $3.00 a gallon. With the tax subsidy available in the law now, it could be sold for about $1.80. It is clearly known that the future depends on bio-fuels as replacement for fossil fuels. At present, USA uses 50 million gallons and **European countries** use 350 million gallons of bio-diesel annually. It is mixed with 20% of bio-diesel in fossil diesel.
- **France** is the country which uses 50% of bio-diesel mixed with diesel fuel.
- In **Zimbabwe**, 4 million jatropha has been planted in 2000 ha by the end of 1997.
- In **Nicaragua**, one million Jatropha curcas has been planted in 1000 ha. The harvest of pods reached 333000 tonnes in the 5th year with a seed of 5000 tonnes and the oil extracted was approximately 1600 tonnes per annum.
- In **Nepal**, 22.5 ha of area are planted with 40,000 rooted cuttings of Jatropha curcas. The rural women co-operative have been trained to extract oil, produce soap and use 30:70 mix (oil/kerosene) of oil and kerosene in stove without smoke (Bijalwan *et al*., 2006; Paramathama *et al*., 2007).

### 1.5. Non traditional seed oils

Various plant resources either edible or non-edible are used for biodiesel production. While in this study three oil seed plants i.e. wild safflower, safflower and milk thistle belonging to family Asteraceae were selected as non edible oil feed stocks for biodiesel production. These oil seeds are considered as nontraditional energy crops as these are un commonly cultivated and mainly found as weed. The raw material (crude oil) from these oil seeds can be used as a feed stock for biodiesel production.

***Carthamus oxyacantha* M. Bieb.**

Carthamus oxyacantha M. B. (Figure 9) is a spiny-leaved annual herb up to 1.5 m tall commonly known as Wild Safflower. It is a hardy and xerophytic noxious weed of winter crops. Like other spiny plants in the genus Carthamus, this species is not eaten by livestock, enabling it to spread on grazing lands. It also competes with and reduces the yield of cereal crops. It is a valuable source of non edible and drying oil (28-29% oil content) from waste lands (Deshpande, 1952). However, it was almost eradicated through regular campaigns due to noxious weedy nature. Fruit an achene, obovate or elliptic, 3–5.5 mm long, 2–3.5 mm wide, 1.5–2 mm thick, truncate at apex, marginal notch at base, cross sectional outline broadly elliptic to slightly 4-sided. Glabrous, smooth and glossy, bone-white to ivory, less frequently beige, with densely distributed blotches and speckles in shades of brown. Scar subbasal, an outlined, diamond-shaped cavity containing a rough, vertical ridge. Pappus early deciduous, absent. Apex a

round, rough, flat to uneven area, surrounded by irregularly edged black ring; style base deciduous. Embryo spatulate, cotyledons broad; endosperm absent. It is widely distributed in Afghanistan, Azerbaijan, India, Iran, Iraq, Kyrgyzstan, Pakistan, Tajikistan, Turkmenistan. *Carthamus oxycantha* seeds yields two types of oils: oleic oil and linoleic oil. Fatty acid oil composition of oleic oil is, palmitic acid 5-6%, stearic acid 1.5 -2%, oleic acid 74-80%, linoleic acid 13-18% and traces of linoleic acid and longer chain fatty acids. The fatty acid composition of linoleic oil, palmitic acid 5-8%, stearic acid 2-3%, oleic acid 8-30%, linoleic acid 67-89% and also traces of linoleic acid and longer chain fatty acids. *Carthamus oxycantha* fruit also contains proteins 20-25%, hull 60%, residual fat 2-15%. Flowers of *Carthamus oxycantha* contain two major pigments, the water soluble, yellow carthamidin and the formally important dye carthamin, flavonone which is orange red (Fernandez-Martinez *et al.,* 1993; Anjani, 2005). Flowers also contain 0.3-0.6% carthamin. Flavonoids, glycosides, sterols and serotonin derivatives have been identified from flowers and seeds (Figure 10) (Firestone, 1999). Two new glycosides, 2-O-methylglucopyranosyl-carthamoside and beta-D-fructofuranosyl carthamoside, along with the known compound 3', 4', 5, 7-tetrahydroxyflavanone have been isolated from *Carthamus oxyacantha* using recycling preparative HPLC. The structures of these compounds were established by mass spectrometric and extensive spectroscopic analysis (Hassan *et al.,* 2010). This oil seed plant is commonly found as noxious weed after harvesting of cash crop wheat. Throughout the world due to its weedy nature it is generally burnt after wheat harvesting, while in this study it is targeted as energy crop for biodiesel production.

**Figure 10.** Wild Safflower

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**Figure 11.** Wild Safflower seeds

### *Carthamus tinctorious* L. (Safflower)

Safflower is cultivated nontraditional seed oil crop which contains a higher percentage of essential unsaturated fatty acids and a lower percentage of saturated fatty acids than other vegetable seed oils. The oil, light colored and easily clarified, is used in liqueurs, candles, and as a drying oil in paints, linoleum, varnishes, and wax cloths. The flowers (Figure 11) have been the source of yellow and red dyes, largely replaced by synthetics, but still used in rouge. Annual thistle-like herb, branching above with a strong central stem to 1-2 m tall; leaves spiny, oblong or lanceolate, the upper ones clasping, minutely spinose-toothed; flowers in 1-6 heads per plant, 3-4.5 cm across, each head developing 20-60 seeds; corollas yellow, orange, white or red, surrounded by a cluster of leafy spiny bracts, which pass over gradually into the bracts of the involucre; achenes (fruits or seeds) (Figure 12) white, 7-9 mm long, shining. Many cultivars have been developed differing in flower color, degree of spininess, head size, oil content, resistance to disease and ease of harvest. Most common varieties have yellow or orange flowers, but red and white flowered varieties are known. Reported from the Central Asian and Near Eastern Center of Diversity, safflower thereof is reported to tolerate bacteria, disease, drought, frost, fungus, high pH, phage, salt, sand, rust, virus, wind, and wild. Wu and Jain (1977) discuss germplasm diversity in the World Collections of Safflower. ($2n$ = 24, 32). Believed to have originated in southern Asia and is known to have been cultivated in China, India, Pakistan, Persia and Egypt almost from prehistoric times. During Middle Ages it was cultivated in Italy, France, and Spain, and soon after discovery of America, the Spanish took it to Mexico and then to Venezuela and Colombia. It was introduced into United States in 1925

from the Mediterranean region and is now grown in all parts west of 100th meridian. Safflower grows in the temperate zone in areas where wheat and barley do well, and grows slowly during periods of cool short days in early part of season. Seedlings can withstand temperatures lower than many species; however, varieties differ greatly in their tolerance to frost; in general, frost damages budding and flowering thus reducing yields and quality. It thrives in heavy clays with good waterholding capacity, but will grow satisfactorily in deep sandy or clay loams with good drainage, and needs soil moisture from planting through flowering, Soils approaching neutral pH are best (Duke, 1978, 1979). Propagation is by seed, which are usually pretreated with insecticides and fungicides. Same machinery used for small grains may be used for planting, cultivation and harvesting. Seed should be planted in a soil prepared and completely free of seeds, when the soil temperature is about 4.4°C and the upper 10 cm of soil is moist. Seed germinates quickly at 15.5°C. Safflower matures in from 110-160 days from planting to harvest as a spring crop, as most of it is grown, and from 200 or more days as fall crop. It should be harvested when the plant is thoroughly dried. Since the seeds do not shatter easily, it may be harvested by direct combining. The crop is allowed to dry in the fields before threshing. Its average yields are 1,900 kg/ha, but yields above 4,500 kg/ha are not uncommon; in the Great Plains yields run about 850 kg/ha (C.S.I.R., 1948-1976). The world low production yield was 244 kg/ha in Israel, the international production yield was 789 kg/ha, and the world high production yield was 1,900 kg/ha in U.S.A. Yields higher than 4,000 kg/ha have been attained. Oil yields approach 50%, leaving a meal with ca 21% protein, 35% fiber, and 1-3% fat. Safflower is self-pollinated with some cross-pollination. Pollen and nectaries are abundant with insect working the flowers. Safflower is attacked by many fungi: *Alternaria carthami* (leaf spot and bud rot), *A. zinniae, Bremia lactucae, Cercospora carthami, Cercosporella carthami, Carthamus tinctorious* is only cultivated species of genus *Carthamus* commonly known as Safflower. It is cultivated since ancient times for not only the dye obtained from its flowers and medicinal uses but also for its seed oil and ornamental purposes.

***Silybum marianum* (L.) Gaertner**

Milk thistle (*Silybum marianum* Gaertn.) (Figure 7) is a winter annual or a biennial. Its current distribution includes most temperate areas of the world. It is a broad-leaved species belonging to Asteraceae that reaches a height of 200–250 cm. Milk thistle is grown commercially as a medicinal plant in Europe, Egypt, China, and Argentina but it has been reported as a noxious weed in many other countries. Stems glabrous or slightly tomentose. Leaves: basal wing-petioled, blades 15–60 cm, margins coarsely lobed; cauline leaves clasping, progressively smaller and less divided, bases spiny, coiled, auriculate. Phyllary appendages spreading, ovate, 1–4 cm including long-tapered spine tips. Corollas 26–35 mm; tubes 13–25 mm, throats campanulate, 2–3 mm, lobes 5–9 mm. Cypselae brown and black spotted, 6–8 mm; pappus scales 15–20 mm. $2n = 34$. *Silybum marianum* is sometimes cultivated as an ornamental, a minor vegetable, or as a medicinal herb. Young shoots can be boiled and eaten like cabbage and young leaves can be added to salads. The seeds (Figure 8) can be used as a coffee substitute. Extracts of *S. marianum* are used as an herbal treatment for liver ailments. Milk thistle is toxic to livestock when consumed in large quantities, and it forms dense stands in pastures and rangelands. California reports up to 4 tons per acre in heavily infested areas. The leaves are very distinctive,

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**Figure 12.** Safflower

**Figure 13.** Safflower seeds

with white marbling on the shiny green leaves. An annual or biennial, found in rough pasture, on grassy banks, in hedgerows and on waste ground. It is locally well-established and persistent, especially in coastal habitats in S. England, but is also a widespread casual. Lowland. Native of the Mediterranean region; naturalised or casual throughout much of

Europe and in N. America and Australia. The plant grows wild in Egypt on canal banks and in wet ground regions in the Nile Valley. The soil supporting this plant is fine-textured and moist. It occurs in two types, the most abundant has purple flowers while the least abundant has white flowers (Ahmad et al., 2008). Milk thistle is commonly found as a noxious weed in waste land and in along with cultivated filed of traditional crops. In this project this energy crop was first time reported as a feed stock for biodiesel production at global perspectives.

In this project these species were selected for biodiesel potential at global interest as renewable energy because of their oil which is non edible and species found as weeds on waste and marginal lands. The study conducted with aims to extract the seed oils from these resources for production of biodiesel through base catalyzed transesterification. Study may also confined to quality standards of biodiesel obtained from these species according to ASTM standards.

## 2. Methodology

The oil from these three resources was extracted by two methods;

1. Chemical method (Soxhlet Apparatus)
2. Mechanical method (Electric oil expeller) (Figure 1-2)

The oil seeds were oven-dried at 40°C over night and then ground with blender. 250ml of petroleum ether was poured into round bottom flask. Five gram of the sample was placed in the thimble and inserted in the centre of the extractor. The Soxhlet was heated at 60°C. When the solvent was boiling, the vapour rises through the vertical tube into the condenser at the top. The condensed liquid drips into the filter paper thimble in the centre, which contains the solid sample to be extracted. The extract seeps through the pores of the thimble and fills the siphon tube, where it flows back down into the round bottom flask. This process was allowed to continue for 3-4 hrs. Distinct layers of oil and petroleum ether appeared in round bottom flask. In this process of oil extraction, the solvent was recovered and reused. The resulting mixture containing oil was heated to evaporate solvent and weighed again to determine the amount of oil extracted. (AOAC, 1990). While in electric oil expelling method oil was extracted mechanically from seeds by using electric oil expeller (KEK P0015, 10127) and crude oil was collected in beakers for further processing. After an average of 5-6 turns, the oil is fully extracted from seeds.

### 2.1. Determination of free fatty acid number of seed oils

Free fatty acid content of oil seeds was determined by aqueous acid-base titration (Trajkovic *et al*., 1983). Two types of titration were performed i.e. blank titration and sample titration. In case of blank titration 0.14 g KOH was dissolved in 100 ml of distilled water to prepare 0.025M KOH solution and this solution was poured in burette. 10 ml of isopropyl alcohol and 2-3 drops of phenolphthalein were mixed in a conical flask and titrate it against 0.025 M KOH from burette until the color of solution became pink. Note the volume of KOH used. This was repeated three times to calculate mean volume of KOH used for blank titration. While in sample titration 9 ml

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isopropyl alcohol, 1 ml of wild safflower oil and 2-3 drops of phenolphathalein were taken in conical flask and titrate against 0.025M KOH from birette until end point i.e pink color appeared. Note the volume of KOH used and three readings were taken by repeating the same experiment to calculate the mean volume of KOH used to titrate the sample.

Acid number = (A-B) x C/D

A = Volume used in Sample/Actual titration, B = Volume used in Blank titration

C = Mass of Catalyst in g/l, D = Volume of oil used

### 2.2. Biodiesel synthesis

The method used for synthesis of fatty acid methyl esters (Biodiesel) from crude oil was alkali catalyzed transesterificaton (Ahmad *et al*., 2010) (Figure 3,4,5 &6). There are numerous transesterification citations in the scientific and patent literature (Bradshaw and Meuly, 1944; Freedman *et al*., 1984; Freedman *et al*., 1986; Schwab *et al*., 1987; Allen *et al*., 1945; Trent, 1945; Tanaka *et al*., 1981; Wimmer, 1992b; Ma *et al*., 1998a; Ma *et al* 1998b; and Ma *et al*. 1999). Crude oil contains impurties which could affect the quality, yield and process of transesterification. The filtration of crude oil was done by using whattmann paper NO: 42 (See Plate 1). The filtered oil was heated up to 125 $^{o}$C on hot plate (VELP Scientifica F20520166) in order to decompose triglycerides into monoglycerides and diglycerides. Transesterification of one liter oil (Plate 2) was carried out for the production of methyl esters by using different alkali catalysts (Ahmad *et al*., 2011). Sodium hydroxide (NaOH) and potassium hydroxide (KOH) were used as catalyst. A specific amount of each alkali hydroxide (6.3 g for one liter oil) was added to methanol (200 ml) to make alkali methoxide which was used as a catalyst in reaction. The prepared methoxide was added to oil at 65$^{o}$C and stirred for 35-40 min at 600 rpm.

After stirring the reaction mixture was kept overnight at room temperature to settle down distinct layers i.e. upper thin layer of soap, middle layer of FAME (fatty acid methyl ester) and the bottom dense layer of glycerin. These layers were then separated through separating glass funnel. Biodiesel washing is done with ordinary tap water in order to remove impurities and suspended particles. 3-4 washings were performed for complete clearance of biodiesel. Few drops of acetic acid were also added. The residual water was eliminated by treatment with anhydrous sodium sulphate ($Na_2SO_{4)}$ followed by filtration.

## 3. Results and discussion

| Catalyst | Wild Safflower | Safflower | Milk Thistle |
|---|---|---|---|
| NaOH | 2.74 | 1.75 | 2.32 |
| KOH | 2.81 | 1.82 | 2.46 |

**Table 1.** Determination of FFA (%) contents through aqueous acid base titration

| Catalyst | Catalyst concentration (g) | Wild Safflower (%) | | | Safflower (%) | | | Milk Thistle (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Biodiesel | Glycerin | Soap | Biodiesel | Glycerin | Soap | Biodiesel | Glycerin | Soap |
| **NaOH** | 6.3 | 88 | 12 | 0 | 82 | 17 | 1 | 84 | 15 | 1 |
| **KOH** | 6.3 | 80 | 20 | 0 | 78 | 20 | 2 | 82 | 18 | 0 |

**Table 2.** yield of biodiesel and by-products by using various catalysts

| Fuel Properties | Method | Wild Safflower | Safflower | Milk Thistle | HSD |
|---|---|---|---|---|---|
| Color | ASTM D-1500 | 2 | 2 | 2 | 2.0 |
| Density @40°C Kg/L | ASTM D-1298 | 0.8980 | 0.8623 | 0.8990 | 0.8343 |
| Kinematic Viscosity @ 40°C c St | ASTM D-445 | 6.45 | 6.13 | 6.23 | 4.223 |
| Sulphur % wt | ASTM D-4294 | 0.1103 | 0.00041 | 0.0123 | 0.05 |
| Total Acid No. mg KOH/gm | ASTM D-664 | 0.14 | 0.63 | 0.92 | 0.8 |
| Flash Point °C (PMCC) | ASTM D-93 | 110 | 80 | 92 | 60-80 |
| Pour Point °C | ASTM D-97 | -12 | -9 | -6 | -35 t0 -15 |
| Distillation @ 90% recovery °C | ASTM D-86 | 358 | 352 | 354 | 360.4 |
| Cloud Point °C | ASTM-2500 | +15 | +9 | +7 | -15 to 5 |
| Calorific Value BTU/LB | ASTM-240 | 16977 | 16566 | 16472 | 20,400 |
| Cetane Index | ASTM-976 | 50 | 52 | 51 | 46 |
| Phosphorus % wt. | ASTM D-6728 | - | - | - | - |

**Table 3.** Fuel Properties of Biodiesel (B100) in comparison with HSD

The major share of all energy consumed worldwide comes from fossil sources (petroleum, coal and natural gas). However these sources are limited, and will be exhausted by the near future. Thus looking for alternative sources of new and renewable energy such as biomass is of vital importance. Alternative and renewable fuels have the potential to solve many of the current social problems and concerns, from air pollution to global warming to other environmental improvements and sustainability issues (MacLeana and Laveb, 2003).

During the decade of 1930s and 1940s, neat vegetable oils were used in diesel engines under an emergency situation (Ma and Hanna, 1999). Currently, most of the biodiesel is produced from the edible or vegetable oils using methanol and an alkaline catalyst such as sunflower

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(Vicente *et al*., 2004), canola (Singh *et al*., 2006), palm (Darnoko and Cheryman, 2000; Cheng *et al*., 2004), soybean oil (Encinar *et al*., 2005) and waste vegetable oils (Felizardo *et al*., 2006; Dorado *et al*., 2002; Cetinkaya and Karaosmanolu, 2004). However, large amount of non-edible oils and fats are available such as safflower (Meka *et al*., 2007), Pongame (Ahmad *et al*., 2009), Sesame (Ahmad *et al*., 2011), and tigernut oil (Ugheoke *et al*., 2007) have been intensively investigated as potential low priced biodiesel sources. This study supports the production of biodiesel from non edible seed oils i.e. (wild safflower, safflower and milk thistle oil biodiesl as a viable sources of alternative to the diesel fuel.

### 3.1. FAMEs production

The percentage conversion of oil to biodiesel with NaOH and KOH at $65^0$C is given in table 2. The results illustrated the greater oil to FAMEs conversion with NaOH as compared to KOH. The most common way to produce biodiesel is transesterification reaction in which triglycerides react with an alcohol to produce fatty acid mono-alkyl esters (Biodiesel) and glycerol. Methanol is the most common alcohol because of its low price compared to other alcohols. This reaction is referred as methanolysis. Generally transesterification is catalysed by a basic or an acid catalyst. However, the basic catalysts are the most commonly used in industry, because the process proves faster and the reaction conditions are moderated (Freedman *et al*., 1984; Reid, 1911). In this project biodiesel was synthesized from wild safflower, safflower and milk thistle oil by base (NaOH and KOH) catalyzed transesterification with methanol. Most studies of the basic-catalysed transesterification of vegetable oils involve the calculations of the triglyceride conversion rate and the changes in product composition during reaction (Feuge and Gros, 1949; Freedman *et al*., 1984, 1986; Schwab *et al*., 1987; Peterson et al., 1991; Mittelbach and Trathnigg, 1990; Chang *et al*., 1996; Mittelbach, 1996; Coteron *et al*., 1997; Boocock *et al*., 1998; Noureddini *et al*., 1998; Vicente *et al*., 1998; Darnoko and Cheryan, 2000).

### 3.2. Fuel properties

Biodiesel is characterized by their viscosity, density, cetane number, cloud and pour points, calorific value, distillation range, flash point, ash content, sulfur content, acid value, and phosphorus contents. These parameters are specified through the ASTM (American Standard Testing Methods) standards. This standard identifies the parameters the pure biodiesel (B100) must meet before being used as a pure fuel or being blended with petroleum-based diesel fuel. The properties of these oils methyl esters (B100) are given in table 3. These values are in the close range and comparable with high speed diesel (HSD).

The viscosity difference forms the basis of an analytical method, i.e. viscometry, applied to determine the conversion of vegetable oil to methyl ester. The viscosity difference between the componential triacylglycerols of vegetable oils and their corresponding methyl esters resulting from transesterification is approximately one digit (Knothe, 2001). Kinematic viscosity has been included in biodiesel standards (1.9-6.0 mm2/s in ASTM D6751 and 3.5-5.0 mm2/s in EN 14214) (Knothe, 2005). The viscosity of these oil biodiesel were near to ASTM standards. Biodiesels have a viscosity close to that of diesel fuels. As the oil temperature increases its viscosity decreases (Sarin & Sharma, 2007). The lower the

viscosity of the biodiesel, the easier it is to pump and atomize and achieve finer droplets (Goodrum, 2007). The calorific value of edible and non-edible methyl ester was lower than that of diesel because of their oxygen content. The presence of oxygen in the biodiesel helps for a complete combustion of the fuels in the engine (Pramanik, 2003). Calorific value of these oil biodiesel were comparable to ASTM standard. The cetane number is one of the most commonly cited indicators of diesel fuel quality. It measures the readiness of the fuel to auto-ignite when injected into the engine. It is generally dependent on the composition of the fuel and can impact the engine's startability, noise level, and exhaust emissions. Cetane index of these three species were also in accordance with ASTM standards. The higher the cetane number, the more efficient the ignition is. Because of the higher oxygen content, biodiesel has a higher cetane number as compared to petroleum diesel. (Arjun *et al*., 2008). Flash point is the important temperature specified for safety during transport, storage, and handling (Krisnangkura, 1992). Flash point of these oil methyl esters were found to be higher as compared to HSD. The flash point of bio-diesel is higher than the petro-diesel, which is safe for transport purpose. The ASTM standard for total acid number for pure biodiesel is 0.8 mg KOH/g. The TAN or acid value is the total amount of potassium hydroxide necessary to neutralize the free acids in biodiesel sample (Arjun *et al*., 2008). Higher acid number could also cause degradation of rubber parts in older engines resulting in filter clogging The test result for the total acid number of these oil biodiesel were found to be ideal (Guo & Leung, 2003). Two important parameters for low-temperature applications of a fuel are cloud point (CP) and pour point (PP). The CP is the temperature at which wax first becomes visible when the fuel is cooled. The cloud point of methyl ester produced from these oils were found to be in accordance with ASTM standards. The PP is the temperature at which the amount of wax from solution is sufficient to gel the fuel; thus it is the lowest temperature at which the fuel can flow. The pour point of methyl ester produced from these oil were near to HSD. Biodiesel has a higher CP and PP compared to conventional diesel. The cloud points were affected by the presence of monoglycerides, however, the pour points were not affected. Moreover, the *cis* double bond present in the erucic acid of rapeseed oil hampered the lowering of the pour point of esters. The type of fatty acid branched chain available in the original oil has an impact on the pour point (Lee *et al*., 1995). Biodiesel contains virtually trace amount of sulfur, so $SO_2$ emissions are reduced in direct proportion to the petrodiesel replacement (Demirbas, 2007). Sulpur contents in these three oil yielding plants were very low as compared to HSD.

## 4. Conclusion and recommendations

Based on above findings these three species of family Asteraceae have higher potential as a raw material source for biodiesel production at global interest and application. Following are some key recommendations which might be useful for production of raw material availability, production and consumption of biodiesel at global perspective;

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1. In all developed countries, research and development has always played a vital role in profitable development of industry. In developed and some developing countries more and more R & D activities are being sponsored by the private sector and their Governments are assisting them and taking part in these activities by way of tax incentives and award schemes.

2. It is recommended that policies should be designed and incentives be offered by government to develop biodiesel companies and industries in the country.

3. Serious consideration should be given to establish a mega tree plantation for production of oil seeds in biodiesel application.

4. It is recommended that production of biodiesel to final use by consumer, quality should be given priority. Number of strategies should be given importance such as collection of seeds, extractions, processing, handling, storage and marketing. Therefore positive inspection system for all these sectors including agriculture, private sector and farming system.

5. In view of the present study as presented in this issue about the economic importance of national plants resources used for biodiesel production, research, development and cultivation efforts should be focused on these plants and identified other resources.

6. These three species are fast growing but cultivated on a small scale by rural farmers, could be produced on large scale for consumption and to be used as fuel. These species are more economical and need minimal quantity of water, fertilizer and pesticides. Such type of study on plant resources will make their data readily available for identifying promising species for future consideration for cultivation of biodiesel yielding crops.

7. It is proposed to further extend the project of bio-diesel. There is need to establish pilot projects to commercialize bio-diesel and set up its supply chain. The project may be extended step wise like conversion of vehicle fleets of designated departments on bio-diesel.

## List of abbreviations

ASTM = American Society for Testing and Materials

FA = Fatty Acid

FAME = Fatty Acid Methyl Esters

FFA = Free Fatty Acid

EM = Engine Manufacturing Association

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Section 2

# Biodiesel Production

Chapter 6

# Biodiesel: Production, Characterization, Metallic Corrosion and Analytical Methods for Contaminants

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Additional information is available at the end of the chapter

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## 1. Introduction

In face of recent changes in the edaphoclimatic conditions (climate and soil) occurring worldwide, it has been necessary reflections on the need to exploit natural resources in a sustainable manner. Sustainability is a systemic concept, relating to the continuity of economic, social, cultural and environmental aspects of human society. It proposes to be a means of configuring the civilization and human activity so that the society, its members and its economies can fulfill its needs and express its greatest potential at present, while preserving biodiversity and natural ecosystems, planning and acting to achieve pro-efficiency in maintaining undefined these ideals [1].

According to the International Energy Outlook -2011 (IEO 2011), the energy consumption in the world was 505 quadrillion of British thermal units (Btu) in 2008, while in 2006 this consumption was 472 quadrillion Btu. According to 2011 bulletin, the outlook for energy consumption in 2020 will be 619 quadrillion Btu, and 770 quadrillion Btu in 2035 for the countries of the Organization for Economic Cooperation and Development (OECD) [2]. This gradual increase in worldwide energy consumption increases the search for renewable energy, once the existing conventional sources are exhaustible ones, such as for example the oil. In this sense, biofuels have appeared, such as ethanol (bioethanol) and biodiesel, which emerged very strongly due to large government incentives.

Within the energy issues, Brazil, for its favorable natural conditions, presents a huge potential for the production of biofuels, especially ethanol and biodiesel, a fact which makes it a strategic country in relation to the sustainability of such market. There is an effort to consolidate energy

from renewable sources, as well as the use of byproducts from these industries, with the creation of programs and incentives by the federal government, such as Proálcool [3].

This text reports a brief historic background of biodiesel in Brazil, methods for biodiesel production indicating the main raw materials, and the regulated physical-chemical properties for the quality control of biodiesel discussing the consequences of cases of non-conformity and their regulated methods by European, American and Brazilian norms. A special topic is dedicated to metallic corrosion which is closely related to storage stability of biodiesel, and finally a comprehensive review of analytical methods developed for monitoring contaminants (glycerol and trace metal) in biodiesel is presented.

### 1.1. Historic background of biodiesel in Brazil

The first use of vegetable oil in diesel engine was tested at the request of the French government with the intention of stimulating energy self-sufficiency in its colonies in Africa, minimizing the costs relating to imports of coal and liquid fuels. The oil selected for the tests was from peanut, whose culture was abundant in tropical countries. The diesel engine produced by the French company Otto, powered by peanut oil, was presented at the Paris Exhibition in 1900. Other experiments conducted by Rudolph Diesel were held in St. Petersburg with locomotives powered by castor oil and animal oils. In both cases the results were very good and the engines showed good performance.[3] In chapter "Liquid Fuel" from Diesel's book "Die Entstehung des Dieselmotors" (The Emergence of Diesel Engines)[4], it mentions:

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For completeness, it is important that, back in 1900, vegetable oils were already being used successfully in diesel engines. During the Paris Exhibition of 1900, the French company Otto demonstrated the operation of a small diesel engine with peanut oil. This experiment was so successful that only some of the people present realized the circumstances in which it was conducted. The engine, which had been built to consume oil, was operated with vegetable oil without any modification. It was also observed that the consumption of vegetable oil resulted in a use of heat literally identical to the oil.

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Vegetable oils were also used as emergency fuel, among other applications, during the Second World War. For example, Brazil has banned the export of cottonseed oil because this product could be used to replace imports of diesel oil. Reductions in imports of liquid fuels were also reported in Argentina, which required greater commercial exploitation of vegetable oils. China produced "diesel", lubricating oils, "gasoline" and "kerosene", the last two by cracking processes, from tung oil and other raw material oilseeds. However, the requirements of war forced installation of cracking units of unusual technology base. Quickly, research activities with new oil sources were expanded, but with the subsequent decline in the price of crude oil barrel (post World War II), reaching more affordable price, these researches were abandoned, as happened in India [5].

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The use of vegetable oil as an alternative renewable fuel to compete with diesel oil was proposed in the early 1980. The more advanced study with the sunflower oil happened in South Africa because of diesel oil embargo, and the first International Conference on Plants and Vegetable Oils was held in Fargo, North Dakota, in August 1982 [6].

In Brazil, since the 1930s, efforts have been made to incorporate renewable fuels in the energy matrix, which are mainly accomplished by government authorities, universities and research institutes. In addition to the PRO-ALCOHOL (created in 1975, this program was designed to ensure the supply of ethanol from sugarcane in the process of replacing gasoline, as government initiative to tackle the successive increases in oil prices), it was established the Production Plan of Vegetable Oils for Energy Purposes (PRO-OIL), which from the 1980s was named as the National Program of Vegetable Oils for Energy Purposes. This program was designed to generate a significant surplus of vegetable oils, able to make their production costs competitive with mineral oil. It was envisaged by legislation a mixture of 30% of vegetable oil in diesel oil, with prospects for full replacement in the long term. At this time, it was proposed as a technological alternative the transesterification or alcoholysis of vegetable oils, highlighting the studies conducted at Federal University of Ceará, using different sources of vegetable oils such as soybean, babassu, peanut, cottonseed and sunflower, among others. Unfortunately, this program was abandoned by the government in 1986, when the oil price fell again in the international market along with the high cost of production and the crushing of oilseeds, which were decisive factors for the slowdown of the program. However, even after the end of PRO-OIL, there was a considerable progress in researches on the production and use of biodiesel in Brazil, which were conducted in different universities and research centers, particularly the registration of the first Brazilian patent deposited by Chemical Engineer Expedito José de Sá Parente [7]. In accordance with Parente he did not developed a new method: *The transesterification process has been known for many years. What I have patented was the production of esters for use as fuel in diesel cycle engines, which is entirely different from what Rudolf Diesel did. Modern engines could not run for a long time using a vegetable oil under the conditions tested by Diesel*. Table 1 shows the evolution of fuels in Brazil since the 1970s.

In Brazil, especially from the year 2005, when there was the beginning of the National Biodiesel Program, these surveys were intensified due to growing importance in using this material, and three years later, in January became mandatory the addition of 2% (v/v) of biodiesel to commercial diesel, whose mixture was called B2 (B for blend). In March 2008, the National Energy Research Council (CNPE) determined the mandatory addition of 3% (v/v) from July. Since January 1, 2010, the diesel oil sold in Brazil contains 5% biodiesel. This rule was established by Resolution number 6/2009 of the National Energy Policy Council (CNPE), published in the Official Gazette (DOU) in October 26, 2009, which increased from 4% to 5% the mandatory blend percentage of biodiesel to diesel oil. Until mid-2011, two more increases were conducted over, and at that time the addition was 5% (v/v) of biodiesel to diesel, and such addition was put forward to year 2011, previously proposed for the year 2013, due mainly to the increase in diesel consumption and the consequent increase of the fleet of cars using this fuel.

| Year | Event |
|---|---|
| 1973 | First Oil Shock |
| 1974 | Creation of the Pro-Alcohol |
| 1977 | Addition of 4.5% Ethanol to Gasoline |
| 1979 | Addition of 15% Ethanol to Gasoline |
| 1980 | Second Oil Shock |
| 1983 | Alcohol cars account for over 90% of total sales |
| 1985 | Percentage of Alcohol added to gasoline reaches 22% |
| 1989 | Oil prices fall and gasoline equates with alcohol |
| 1992 | Rio 92: Signing of the milestone about climate change |
| 90's | Alcohol comes to represent 20-25% of Gasoline |
| 2005 | Founded PNPB |
| 2007 | Third Oil Shock |
| January 2008 | Beginning of mandatory B2 |
| March 2008 | CNPE determines the mandatory use of B3 from July 2008 |
| April 2008 | Alcohol Consumption equates to Gasoline |
| July 2009 | Validity of B4 |
| January 2010 | Validity of B5 |

**Table 1.** Evolution of fuels in Brazil since the 1970s.

This same law, published on January 13, 2005, introduced the biodiesel in the Brazilian energy matrix and expanded the administrative competence of the National Agency of Petroleum and Natural Gas (ANP), which became, since then, the National Agency of Petroleum, Natural Gas and Biofuels. Since the publication of the abovementioned law, ANP took over the assignment to regulate and supervise the activities related to production, quality control, distribution, sale and marketing of biodiesel and diesel-biodiesel blend [8]. The continued rise in the percentage of biodiesel added to diesel demonstrates the success of the National Program for Production and Use of Biodiesel and the experience accumulated by Brazil in production and large-scale use of biofuels, especially the biodiesel.

### 1.2. Aspects of the biodiesel market in Brazil

According to the Monthly Bulletin of Renewable Fuels of the Ministry of Mines and Energy (MME), based on deliveries of auctions promoted by ANP, it shows that the estimated production in May 2012 was 173,000 $m^3$. The total of the year up to this month, the cumulative production was 958,000 $m^3$. The installed production capacity, in May 2012, stood at 6.092 million $m^3$/year (507,000 $m^3$/month) of which 88% is related to companies holding the social seal [9]. The Social Fuel Seal is a component of identification created from the Decree No.

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5297 of December 6, 2004, awarded by MDA to biodiesel producer who meets the criteria described in Instruction No. 01 of February 19, 2009. The Seal gives to the possessor the character of promoter for social inclusion of family farmers classified in the National Program of Family Agriculture (PRONAF).

## 2. Raw materials for biodiesel production

In general, biodiesel can be produced from any source having oil, either of animal or vegetable source; however, to ensure the quality of the final product, some factors must be observed, as, perennial crops, oil content and preferably with no potential for the food industry, as occurs primarily for soybean in Brazil. Furthermore, it should be noted the productivity per unit area, the agronomic balance and other aspects related to the life cycle of the plant (seasonal). These raw materials, along with the production processes, depend on the region concerned. The economic, environmental and social diversities have given distinct regional motivations for the production and consumption. Due to the favorable conditions of climate and soil, Brazil presents numerous possibilities for use as raw material for the biodiesel industry. It can be highlighted the use of soybean, castor, palm, cottonseed, sunflower, macauba, rapeseed, jatropha, animal fat (tallow) and residual oils, among which, the latter presents itself as an excellent alternative, because it is a material whose reuse at industrial level was insignificant until its application in the biodiesel industry. Oil contents of some of the oil seeds used in Brazil are shown in Figure 1.

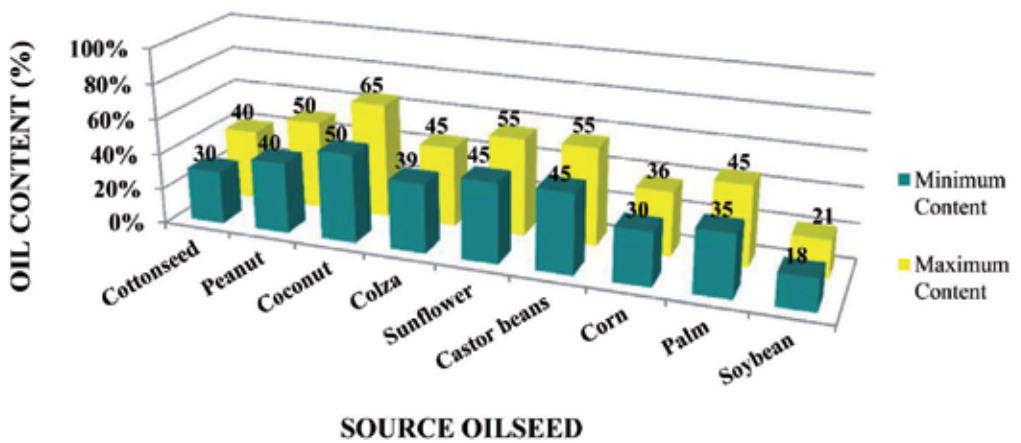


**Figure 1.** Oil content of some of the seeds used in Brazil.

In Brazil the most relevant is the use of soybean for biodiesel production, followed by beef tallow and cottonseed oil as described by Monthly Bulletin of Biodiesel (published by ANP - reference month May 2012) as shown in Figure 2.

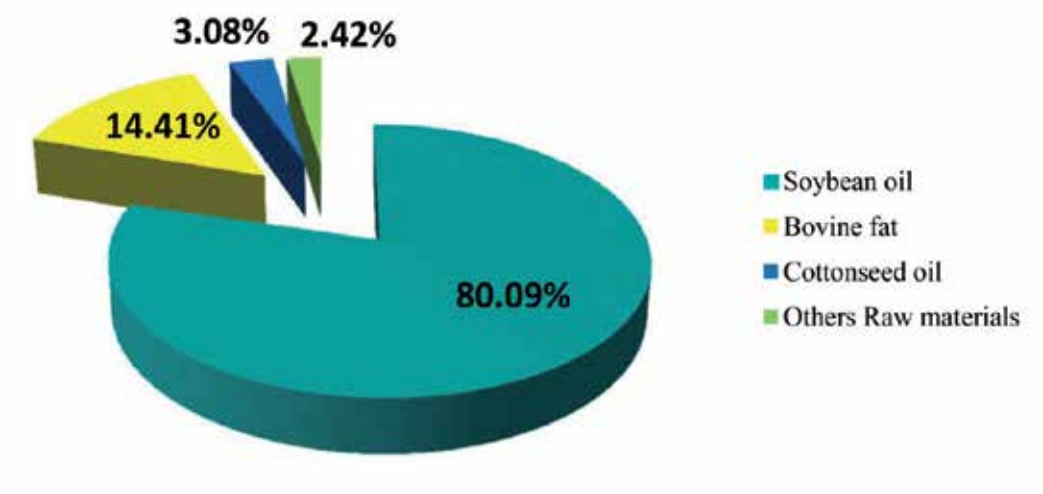


**Figure 2.** Oil sources used for biodiesel production in Brazil.

In Brazil, other species of oilseeds are cultivated and they are distributed by region as shown in Table 2.

| Region | Raw materials that are available |
|---|---|
| Southeast | Soybean, castor, cottonseed, sunflower, jatropha and macauba |
| South | Soybean, corn, rapeseed, sunflower, jatropha and cottonseed |
| Centralwest | Soybean, castor, cottonseed, sunflower, jatropha, macauba, palm and animal fat |
| North | Palm, babassu and soybean |
| Northeast | Babassu, soybean, castor, palm, jatropha, cottonseed and coconut |

**Table 2.** Distribution of oilseeds in Brazilian regions. Adapted from ref. [7].

Despite the many advantages in the use of biodiesel, there are two serious problems from the viewpoint of production of vegetable oils which can retard or impede the use of derivatives of vegetable oils as fuel. The first problem is the amount of oil produced. Some of the key raw materials used in Brazil and the corresponding harvest months and oil yields (t/ha) are listed in Table 3. The second problem is the quality of the extracted oil. Oils with high concentrations of polyunsaturated fatty acids are undesirable for biodiesel production, for decreasing their stability to oxidation [12]. Generally, the more unsaturated acids, such as linoleic and linolenic acids, respectively, with two and three unsaturations, are more susceptible to oxidation. In addition, these oils induce a higher carbon deposit than oils with high content of monounsaturated or saturated fatty acids as occurs to the palm oil.

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| Name | Scientific name | Harvest months/ years | Yield (ton/ha) |
|---|---|---|---|
| Peanut | *Arachis hypogea L.* | 3 | 0.6-0.8 |
| Cottonseed | *Gossipum Hirsutum L.* | 3 | 0.1-0.2 |
| Babassu | *Orbiguya phalenta Mart.* | 12 | 0.1-0.3 |
| Rapeseed | *Brassica napus L.* | 3 | 0.5-0.9 |
| Coconut | *Cocos mucifera L.* | 12 | 1.3-1.9 |
| Palm | *Elaesis guineenses L.* | 12 | 3-6 |
| Sunflower | *Helianthus annus L.* | 3 | 0.5-1.9 |
| Castor beans | *Ricinus communis L.* | 3 | 0.5-0.9 |
| Jatropha | *Jatropha Curcas* | 12 | 0.5-2.5* |
| Soybean | *Glycine Max (L.) Merril* | 3 | 0.2-0.4 |

**Table 3.** Name, Scientific name, harvests months and yields of raw materials for biodiesel production in Brazil. Adapted from refs. [10] and [11]*.

### 2.1. Cottonseed

The cotton is currently produced by more than 60 countries. China, India, U.S.A., Pakistan and Brazil emerge as major producers. Together, they account for 80% of world production of around 23.3 million tons. Only these five countries have more than 22 million hectares of planted areas, out of 35 million on average that are planted in different points of the planet [13]. The cottonseed oil shows 13-44% oleic acid (18:1) and 33-59% linoleic acid (18:2) [14], and gossypol, the main byproduct of the cotton industry, is a polyphenolic compound, yellow color, toxic, non-interfering in the biodiesel production process [15].

### 2.2. Palm

Over 70% of the world's oils come from 4 plant species: soybean, palm, sunflower and rapeseed, and currently, the palm oil (known in Brazil as "dendê") is the most traded oil in the world. It is used in the food industry in margarine, ice cream, biscuits, pies, and others. It is also used in the industry of hygiene and cleanliness in the composition of soaps, detergents and cosmetics. In the chemical industry, it is part of the lubricant composition and can also be used as biofuel [16].

In Brazil, the rise of the use of this oilseed is so great that the Federal Government launched in 2010, the Program for Sustainable Production of Oil Palm, which aims to regulate the expansion of oil production and offer tools to ensure a production in sustainable environmental and social bases [16].

This oilseed has the largest oil content among the raw materials for the biodiesel production reaching the yield of 6000 kg/hectare/year. This yield is about 15 times larger than the main raw material in Brazil, the soybean.

The palm oil presents approximately 35-47% palmitic acid (16:0) and 36-47% oleic acid (18:1) [14], and the main problem in using it as fuel is its high viscosity (≈ 20 Cst), about 10 times greater than oil diesel (≈ 2 Cts) (ASTM D445).

### 2.3. Rapeseed

The rapeseed (*Brassica napus*) is a winter oilseed of Brassica genus, from Cruciferae family and it has 39-45% oil with excellent quality for fatty acid composition. It is the main oil used for biodiesel production in Europe, and is usually known as "colza". It started in Canada in 1974 and with plant breeding developed a seed without erucic acid, a substance which limited its use for human consumption [14]. It represents, in percentage, 15.32% of world production of vegetable oils, considering the 2011/2012 crop, behind only the production of palm oil (33.14%) and soybean (27.28%) [17]. The rapeseed oil contains mostly oleic acid (18:1), 62% followed by 32% linoleic acid (18:2) [18].

### 2.4. Jatropha

The jatropha (*Jatropha curcas*) is a drought-resistant plant, multipurpose, having a large amount of oil compared to other inedible plants [19]. The highest incidence and the center of diversity of this genus are the Central America and the Caribbean Islands. The genus has over 210 species growing in different regions of the globe [20-21]. This oleaginous has a different composition when it comes to saturated fatty acids [22-23], due to the presence of 16.4% palmitic acid (16:0) against 2.3% soybean and 7% corn [24]. The jatropha contains mostly 40.3% linoleic acid (18:1), 37% linolenic acid (18:2) which indicates a good quality of biodiesel to be produced from this source because the lower the amount of raw material unsaturation, the better is the stability to oxidation [25].

### 2.5. Soybean

In 2010/2011 harvest, Brazil produced about 75 million tons of soybeans, 65% of this production was exported, equivalent to 48.7 million tons (29.1 million tons of grain, 13.7 million tons of bran and 1.6 million tons of oil). China is the main destination of Brazilian exports of grain (66%) and oil (60%) and the European Union is responsible for the majority of imported bran (70%) [26]. In 2011/12 harvest, the soybean had a stake in percentage terms in the world production of vegetable oils of 27.28% and is the second after only to oil palm, with 33.14% [17].

In Brazil, in May 2012, the soybean had a participation of 80.09% in the production of biodiesel. Taking into account that the biodiesel production in Brazil in that same month was 188,367 x $10^3$ $m^3$ (ANP) then about 150,863 x $10^3$ $m^3$ of the biodiesel produced was derived from soybean oil. This fact is due to the growth of agricultural productivity of soybean and also the technical-industrial feasibility in this oilseed cultivation.

The soybean has about 19.0-30.0% of oleic acid (18:1) and 44.0-62.0% of linoleic acid (18:2) [14].

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One aspect widely discussed in relation to this oilseed is the competition between the production for the biofuel market and food industry. In 2011/12 harvest, Brazil exported approximately 44.9% of the soybeans produced [10], due mainly to the increased buying power of emerging countries such as China and India and a small part (about 8%) was for national biodiesel production. China in this season was the largest buyer of Brazilian soybeans (66%) and processed refined oil (60%).

Regarding to soybean oil, Brazil in 2010/11 harvest produced about 7,434 x $10^3$ tons, and 5,495 x $10^3$ tons (74.85%) were used for domestic market and 1,758 x $10^3$ tons (23.95%) for export [26]. In Brazil, this situation has fluctuated over the months, due to the offseason of soybeans and also because many other oilseeds arose that can be intended for biodiesel production, such as jatropha, cottonseed, moringa, among other.

### 2.6. Waste cooking oil

This raw material represents currently a potential use for biodiesel production worldwide since it is a residue from cooking process of industries, restaurants, bars, among other outlets. The high cost of biodiesel in the national and international market is mainly due to the soaring cost of industrialized vegetable oils and virgin oils [27,28].

The biodiesel made from these raw materials is most often cheaper than from sources such as soybean, rapeseed and sunflower, but the quality of the oil used must be monitored to obtain a high quality biodiesel. The free fatty acid (FFA) content and water content (moisture content) are the main parameters to be analyzed. For a reaction catalyzed by alkali, a FFA content of less than 3% is required, since the use of oil with a high FFA content produces the hydrolysis of triglycerides at high temperatures during the frying process [29]. The residual oil has as striking characteristic a high acidity index and it is previously necessary a transesterification reaction, an acid catalysis using a strong acid (generally $H_2SO_4$) to promote the esterification of fatty acids and thereby reduction of FFA to less than 1% [27,28]. The residual cooking oil has mostly in its composition, oleic acid (18:1) and linoleic acid (18:2) [30].

The use of recycled cooking oil for Biodiesel production has been relevant in recent times, and the flight KL705, originated from Amsterdam with destination to Rio de Janeiro, was carried out in part with this raw material, fueling a Boeing 777-200 [31].

### 2.7. Moringa oleifera

The *Moringa oleifera* belongs to the family Moringaceae, consisting of only one genus (Moringa) and fourteen known species, native to northern India. This species is found in natural conditions in India, Africa, Asia, Arabia, South America and the Pacific and Caribbean Islands [32]. Moringa is a multipurpose plant from the leaves to the seeds, showing different properties. The Moringa leaves are the source of a diet rich in protein for both humans and animals. Moreover, oil extraction from its seed enables the utilization of this raw material to produce biodiesel having a remarkable oxidation resistance, with shelf time between 4 and 5 years [33]. The seeds of *Moringa Oleifera* contain between 33 and 41% m/m oil [33]. This val-

ue is considered a good natural emollient for cosmetic, based on tactile properties, a lower natural occurrence of color and odor besides a high concentration of oleic acid (>73%) [34]. Recently studies showing the potential of Moringa oil extracted from seeds of India and Pakistan for the biodiesel production were published [35,36]. The Moringa has in its composition about 7% of palmitic acid (16:1) and 78% of oleic acid (18:1) [32]. Variations in oil content within countries and species are attributed to possible changes in environmental and geological conditions of the regions [37].

## 3. Methods for biodiesel production

Vegetable oils and animal fats contain, in addition to triacylglycerols, free fatty acids, phospholipids, sterols, water and other impurities. These compounds give special properties to these raw materials which prevent their use directly as a fuel, as, for example, the blockage point and the high viscosity [38]. These problems can be overcome with adjustments to the compression engines; however, such possibility makes the process very expensive and often impractical. The adaptation of the fuel becomes more interesting and it can be used directly to the existing fleet, without adjusting existing technologies by those that cause chemical modifications (cracking, esterification and transesterification). In the last decade some reviews on the different methods for biodiesel production were reported in the literature [39-47].

The transesterification (also called alcoholysis) is generally a term used to describe the important class of organic reactions in which a triacylglycerol reacts with alcohol in catalytic environment and becomes ester and glycerol [39]. The overall process is a sequence of three consecutive and reversible reactions, in which the mono-and diacylglycerols are formed as intermediates of reaction. In the transesterification reaction three moles of alcohol are needed for each mol of triacylglycerol. In practice, it is used an excess of alcohol in order to increase the ester productivity (shifting the reaction towards the product side) and to allow the separation of the obtained glycerol (the impure fraction containing glycerol is called glycerin).

Glycerin makes the oil more dense and viscous, therefore, during the transesterification process, the glycerin is removed resulting in a product of lower viscosity. In the transesterification, the reaction of biodiesel production creates alkyl esters and glycerol, and the glycerol layer is denser than the ester one and it deposits at the bottom of the reactor [39]. The process is based on the stoichiometric reaction of the alkyl glycerol with alcohol, which in most cases has short organic chain in the presence of a catalyst [39-42]. Alcohols such as methanol, ethanol, propanol or butanol can be used in the transesterification reaction and the (produced) monoesters are known as methyl, ethyl, propyl and butyl esters, respectively. The raw materials used as triacylglycerol source for biodiesel production were discussed previously in the text (Section 2).

The technology for the biodiesel production prevailing in the world is methyl transesterification, where, vegetable oils or animal fat are mixed with methanol that, along with a cata-

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lyst, produce the biodiesel. This option occurs mainly because of high ethanol cost and operational facilities. The advantages of biodiesel production using methanol include: ester phase separation (biodiesel) of glycerol that occurs instantaneously; the alcohol recovery that is completed and can be returned to the process; the obtaining of glycerol which is feedstock to the chemical industry; the synthesis is more attractive under the industrial viewpoint, because it is faster and cheaper than the others.

In Brazil, the enterprises that are in operation adopt the methylic route; however, due to ethanol production in Brazil is established beyond favorable environmental factors, there are enterprises that adopt the ethylic route (at industrial level). The ethyl route is recognized as more ecologically friendly, because ethanol is a renewable source. Production of ethyl ester is slightly more complex and requires more steps and the use of specific centrifugal pump sand optimized for a good separation of glycerol from esters. The more alcohol is added to the oil, the faster is the conversion to ester, however, an excess can stabilize the emulsion and complicate the separation of glycerol.

The catalysis used for biodiesel production can be chemical or biological, homogeneous or heterogeneous, acidic or basic. The process for producing biodiesel by basic catalysis is faster than the process of acid catalysis, the biodiesel produced presents less corrosivity, [43,44] and the most used catalysis are the potassium hydroxides, sodium hydroxide, potassium methoxide and methoxide sodium. In Brazil, KOH is more expensive than NaOH, however, there is less soap formation using KOH [45]. The base catalysis is the preferred procedure when there are oils with low water content and low acidity index. On the other hand, if oils and fats have a high content of free fatty acids, it is recommended a pre-treatment or the acid catalysis, followed by an alkaline transesterification [46].

In general, the transesterification of oils or fats can be affected by several factors such as [48]: presence of free fatty acids, moisture [50], type of alcohol used, the molar ratio of alcohol/oil, concentration and type of catalyst, time, temperature of reaction [49] and the intensity of shaking [41].

The content of free fatty acids and moisture are important parameters to determine the feasibility of the basic process of transesterification of vegetable oils and animal fats, requiring low levels of free fatty acids and moisture in the raw material for higher conversion efficiency. Studies have shown [43] that the transesterification of beef tallow catalyzed by NaOH in the presence of free fatty acids (FFA) and water has its yield compromised. Therefore, the raw material that has a high content of free fatty acids can be purified by saponification or using acid catalysis for previous esterification of these acids [51].

The production of ethyl esters via basic catalysis becomes more difficult compared with the production of methyl esters, due to the undesired formation of stable emulsion during the reaction. Methanol and ethanol are immiscible in triglyceride at room temperature, and the reaction media are commonly kept under mechanical stirring to increase mass transfer. In methanolysis, these emulsions easily form two layers: a bottom one rich in glycerol and a top one rich in esters; in ethanolysis the phases are more stable complicating the separation and purification of esters [39,50].

The molar ratio of alcohol/oil is one of the most important variables affecting the yield of esters in the transesterification reaction. The transesterification is a reaction in equilibrium, which requires an excess of alcohol to drive the reaction in the formation of esters. For a maximum conversion of esters, the molar ratio should be greater than or equal to 6:1 [39], but a very high molar ratio of alcohol/oil interferes with the separation of glycerol since its solubility increase takes place. When glycerin remains in solution, it favors the reaction equilibrium for the reactants and then decreasing the yield of esters [52]. Some researchers [42,53] have studied the molar ratios between 3:1 and 15:1 with ethanol, and they have observed arise in the yield of esters with molar ratio increase up to a value 12:1. The best results were between 9:1 and 12:1 [48].

The catalyst concentration is another factor extremely important for the alkaline transesterification [53] since the excessive addition of catalyst can promote an acidity reduction, but, on the other hand, it leads to the formation of soap, hampering the separation of glycerol from esters and consequently decreasing the yield of the reaction.

The catalysts most used during transesterification are the alkoxides [54,55], hydroxides [56] and carbonates of sodium or potassium. The alkoxides of alkali cations such as potassium methoxide ($CH_3ONa$) are the most reactive catalysts, since they exhibit high yields (> 98%) in a short reaction time (30 min), even at low molar concentrations (0.5 mol $L^{-1}$). The greater efficiency of the catalyst $CH_3ONa$ relative to NaOH is described by Freedman and colleagues [48,54] which had a similar conversion of oil at concentrations of 1% NaOH and 0.5% $CH_3ONa$, while in the work developed by Ma and colleagues [45], NaOH showed better performance in ester than $CH_3ONa$in the transesterification of beef tallow. Vincent and colleagues [57] reported good yields obtained with methoxide catalyst, but higher conversion rate was obtained with NaOH and lower with $CH_3OK$ at 65° C, in methanol/oil ratio of 6:1 and catalyst concentration of 1%. Hydroxides of alkaline cations (KOH and NaOH) are more accessible in price than the respective alkoxides, but are less reactive.

Stirring of the reaction medium is an important factor in the transesterification process. Stirring should be intense to transfer amounts of mass of triglycerides from oil phase to the interface with methanol, as the reaction mixture is heterogeneous, consisting of two phases. In this case, the greater the stirring the higher is the mass transesterification [58]. Ma and colleagues [45] added NaOH and MeOH to the tallow beef melted in a reactor and found that after a certain reaction time without stirring anything occurred, suggesting the need of stirring for the reaction be initiated.

Some oils and fats, which may be used as raw materials for the production of biodiesel, have high levels of free fatty acids. The presence of free fatty acids impairs the synthesis of biodiesel via homogeneous basic catalysis [59]. In this sense, heterogeneous acid catalysts, which simultaneously promote reactions of alcoholysis of triglycerides and esterification of free fatty acids, present themselves as promising substitutes of homogeneous basic catalysts [60]. Moreover, such catalysts have the advantages inherent to heterogeneous catalysis, such as significantly reducing the number of purification steps of products as well as the possibility of reuse and enable the production of biofuel by a continuous process with fixed bed reactors [60].

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For the production of biodiesel there are other technological routes besides the transesterification, as for example, the esterification catalyzed by an acid, preferably the sulphonic or sulfuric acids. The obtained yield is very high (99%), but the reaction is slow, requiring high temperatures (above 100°C) over 3 hours to reach the mentioned output [45,46,61,62]. Furthermore, it is necessary to use a large excess of alcohol to ensure a high yield reaction. The acid catalysis is suitable for oils with high content of free fatty acids and moisture. In this case the process is the esterification of free acids and not the transesterification of triacylglycerol.

The enzymatic catalysis (biological) allows the simple recovery of glycerol, the transesterification of triglycerols with high content of fatty acids, the total esterification of free fatty acids, and the use of mild conditions in the process, with yields of at least 90%, making it a commercially viable alternative. In this type of catalyst there are no side reactions that result in by products, which reduce the expenditure of further purification. Some enzymes require cofactors, metal ions or organic compounds (coenzymes). These co-factors will influence the activity of biological catalyst [47]. The advantages of the process are: lack of aqueous alkaline waste, lower production of other contaminants, greater selectivity and good yields. The main drawbacks of this methodology are the high cost of pure enzymes, the high cost of extraction and purification process of macromolecules and their instability in solution, which represent an obstacle to the recovery of the biocatalyst after its use [47]. On the other hand, the immobilization of enzymes allows their reuse, reducing the process cost. In the case of biocatalysis in nonaqueous media, immobilization also results in improvement in enzyme activity. Thus, many processes of transesterification using immobilized lipases have been developed [51,63,64].

Another possible and effective reaction is the supercritical transesterification using methanol, which allows a conversion of 60-90% in only 1 minute and more than 95% in 4 minutes. The better reaction conditions are: temperature of 350°C, pressure of 30 MPa and the volumetric ratio between methanol and oil of 42:1 for 240 seconds. The supercritical treatment of lipids with suitable solvent such as methanol depends on the relationship between temperature, pressure and thermo physical properties such as dielectric constant, viscosity, specific mass (density) and polarity [65,66]. The process is attractive since it overcomes problems such as oil/fat waste that is rich in free fatty acids and also the problem of the presence of water that often favors the formation of soap. However, side reactions involving the unsaturated esters occur when the reaction temperature exceeds 300°C, resulting in loss of material. There is also a critical residence time value in high temperature above which the efficiency decreases [51,67].

The H-BIO process was developed to introduce the processing of renewable raw materials in the scheme of petroleum refining and allow the use of existing facilities. The vegetable or animal oil is blended with fractions of petroleum diesel to be converted to units of Hydrotreating (HDT), which are employed in refineries, especially for reducing the sulfur content and improving the diesel oil quality, adjusting the fuel characteristics to the legal specifications. It was already conducted tests with up to 30% vegetable oil in the HDT load, mixed with diesel fractions, generating a product that has the same characteristics as petroleum

diesel, but the use of such a high proportion of vegetable oil, in existing industrial HDT units meet operational constraints and limitations of some equipment that were not rated for such task in its original design. A patent of this method was registered (INPI PI0900789-0 A2) and a summary of this method is available at the webpage of Petrobras (Brazilian Oil Company) [68].

## 4. Physical-chemical properties of biodiesel

Because of the importance of biodiesel and regulations for its use in the country, the concern of the ANP (National Agency of Petroleum and Natural Gas and Biofuels) is to ensure a quality fuel in any situation, through the establishment of quality standards for biodiesel. The federal law 11.097/2005 establishes in the country the introduction of minimum percentages of mixture of biodiesel to diesel, and also the monitoring of such insertion. The Brazilian specification of biodiesel is similar to European and American ones, with some flexibility to meet the characteristics of domestic raw materials. This specification is issued by ANP decree. This agency has developed standards in recent years with respect to biodiesel, among them there are the resolutions 15, 41 and 42. Resolution No. 42 of ANP [69] provides a specification for biodiesel (B100) according to the provisions contained in the Technical Regulation No. 4/2004, part that composes such Resolution. B100 can be added to diesel oil in proportions defined by volume, sold by various economic agents authorized throughout the national territory. Any vegetable oil can be used as fuel for diesel engines, but some oils have better performance in terms of their thermodynamic properties [70].

Visual observation of biodiesel formed which should be presented clear and free of impurities, either in suspension, precipitated material or any other. This parameter is adopted only by ANP and it is a simple but important test which is performed in a tube without graduation because this parameter is a qualitative indication of quality.

Tables 4 and 5 present the specifications for biodiesel in accordance with Brazilian, European and American standards and the corresponding analytical methods, respectively.

The specific mass (density) is connected with the molecular structure, i.e. the longer the carbon chain of the ester alkyl, the greater is the density which is, however, reduced by the presence of unsaturations. It is an important parameter for the vehicle injection system. Biodiesel has a specific mass greater than the diesel. This parameter is variable according to the raw material, alcohol excess, among others; very high values may indicate contamination with soap and/or vegetable oil, and alcohol excess causes a decrease in density. The European standard presents as specification limit the values of 860-900 kg $m^{-3}$, according to the manual method EN ISO 3675 using glass hydrometers and the automatic method EN ISO 12 185 using digital densimeters, and the later with better repeatability. ASTM D6751 norm does not consider the specific mass as a measure of quality of biodiesels. ANP provides the specification range with values between 850 to 900 kg $m^{-3}$,

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which adopts the European standard methods, in addition to ASTM D1298 (manual) and ASTM D4052 (automatic), corresponding to NBR 7148 and NBR 14065, respectively. The Brazilian specification differs from European only for this parameter. However, as the reference temperature in Brazil is 20ºC and assuming that the lower the temperature of the test, the higher is the density, it can be concluded that the European specification is more restrictive.

The kinematic viscosity (measurement of internal resistance of liquid flow) is an important parameter for the vehicle injection system and fuel pumping system. It depends on the efficiency of the process (reduction of viscosity of raw material). The viscosity reaches high levels with polymerization processes and/or thermal or oxidative degradation. The EN 14214 standard provides an acceptable range from 3.5 to 5.0 $mm^2/s$ (EN ISO 3104 method), while the ASTM D6751 standard allows a broader range from 1.9 to 6.0 $mm^2/s$ (ASTM D445 method). The Brazilian standard adopts, besides the methods already mentioned, also the ABNT NBR 10441 method, with the allowed viscosity range from 3.0 to 6.0 $mm^2/s$. The bottleneck for this parameter is the upper limit of the specification which is the value of 6.0 $mm^2/s$ for ANP and ASTM, while the standard EN 14214 has a limit 5.0 $mm^2/s$, which may restrict the use of some raw materials as for example the biodiesel from castor beans.

The flash point corresponds to the lowest temperature at which the product generates steam enough to ignite when a flame is applied under controlled conditions. This analysis measures the power of self-igniting the fuel and is essential for safety in stocking, handling, transport and storage of fuel. Biodiesel has a flash point much higher than diesel, and low flash point is commonly linked to the presence of alcohol residue in the process. ANP states that the flash point presents at least 100°C and adopts the EN ISO 3679 method, the same of European standard, ASTM D93, the same used by ASTM D6751, and also recommends ABTN NBR 14598.

Water and sediment in biodiesel are usually higher than in diesel, as biodiesel is hygroscopic. The water may generate an unwanted reaction, producing free fatty acids, growth of microorganisms, corrosion and malfunctions of the engine [71]. ASTM D6751 standard adopted this parameter as a quality control for biodiesel using ASTM D2709 method, while ANP and European standard recommends coulometric method (Karl Fischer) EN ISO 12937, and the Brazilian standard also recommends ASTM D6304 method. Comparing the methods, it seems that the coulometric method has greater sensitivity, higher repeatability and lower response time compared to the volumetric method (ASTM D2709).

The total contamination is mainly originated from the raw material, from soaps formed during the process and from unsaponifiables such as wax, hydrocarbons, carotenoids, vitamins and cholesterol (animal origin and used oils). The unsaponifiables present higher boiling point and create waste and soaps in engines and which result in sulphated ashes and finally in abrasion. ASTM standard has not adopted this parameter that is indicated by the European and Brazilian standards, which recommend the same method of analysis described by EN 12662, with maximum specification limit of mg $kg^{-1}$.

| Property | Unit | Limits | | |
|---|---|---|---|---|
| | | ANP 07/2008 | EN 14214 | ASTM D6751 |
| Aspect | --- | Limpid and without impurities | --- | --- |
| Density | $kg/m^3$ | 850-900 (20°C) | 860-900 (15°C) | --- |
| Kinematic viscosity (40°C) | $mm^2/s$ | 3.0-6.0 | 3.5-5.0 | 1.9-6.0 |
| Water and sediment, max. | %vol. | --- | --- | 0.050 |
| Flash point, min. | °C | 100 | 101 | 130 |
| Distillation, 90% recovered vol., max. | °C | --- | --- | 360 |
| Carbon residue, max. | % mass | 0.050 (100% of the sample) | 0.3 (10% Distillation residual) | 0.05 (100% of the sample) |
| Sulfated ash, max. | % mass | 0.020 | 0.02 | 0.020 |
| Sulfur content, max. | mg/kg | 10 | 10 | 15 |
| Copper strip corrosion, 3h at 50°C, max. | Rating | 1 | 1 | 3 |
| Cetane number | --- | Note | 51 (min.) | 47 (min.) |
| Cold soak filterability | °C | By region | By region | --- |
| Pour point | °C | --- | By region | --- |
| Cloud point | °C | --- | --- | Note |
| Sodium and potassium, max. | mg/kg | 5 | 5 | 5 |
| Calcium and magnesium, max. | mg/kg | 5 | 5 | --- |
| Phosphorus content, max. | mg/kg | 10 | 4.0 | 10 |
| Total contamination, max. | mg/kg | 24 | 24 | --- |
| Ester content, min. | % mass | 96.5% | 96.5% | --- |
| Acid value, max. | mg KOH/g | 0.50 | 0.5 | 0.5 |
| Free glycerol, max. | % mass | 0.02 | 0.02 | 0.020 |
| Total glycerol, max. | % mass | 0.25 | 0.25 | 0.240 |
| Monoglycerides | % mass | 0.80 (max.) | 0.80 (max.) | --- |
| Diglycerides | % mass | 0.20 (max.) | 0.20 (max.) | --- |
| Triglycerides | % mass | 0.20 (max.) | 0.20 (max.) | --- |
| Methanol or Ethanol, max. | % mass | 0.20 | 0.20 | 0.20 |
| Iodine value | g $I_2$/100 g | Note | 120 (max.) | --- |
| Oxidation stability at 110 °C, min. | H | 6 | 6 | 3 |
| Water content, max. | mg/kg | 380 | 500 | --- |
| Linolenic acid | % mass | --- | 12 (max.) | --- |
| Polyunsaturated methyl esters (with more than four double bonds) | % mass | --- | 1 (max.) | --- |

**Table 4.** Comparison of limits for the quality control of biodiesel in accordance with Brazilian, European and American standards.

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The ester content is the main property as it indicates the degree of purity of the biodiesel produced and the efficiency of the production process used. It depends on the amount of unsaponifiables in the raw material and process variables (time, temperature, agitation speed, molar ratio, catalyst concentration, type of catalyst, water, free fatty acids, and alcohol). The low content of esters indicates a low yield of the transesterification reaction, i.e., most of the triacylglycerols has not reacted, which can cause difficulties in combustion and carbonization of engine cylinders. This parameter is required by the ANP and EN 14214 standards, whose minimum percentage is 96.5 % wt. using the EN ISO 14103 method. Brazilian standard also recommends ABNT NBR 15342 method for biodiesel from animal origin or for blends in which there is the presence of biodiesel from castor beans.

The carbon residue indicates the tendency of a fuel to form carbon deposits in engines. These residues are deposited in the nozzles and other parts of the engine, reducing their useful life. They correspond to the amount of triacylglycerols, soaps, leftover of catalyst and unsaponifiables, which are present in the final biodiesel. American and Brazilian standards adopt the same ASTM D4530 method, also having the same specification limit, no more than 0.050 % wt. Now the European standard indicates EN ISO 10370 method in which the maximum allowed is 0.3 % wt.

Sulphated ashes cause saturation of filters and wear on various parts of the engine and may be present in the form of abrasive solids, soluble metal soaps and catalyst residues. The maximum content of sulphated ashes in biodiesel is 0.020 % wt. set by the EN 14214 standard (EN ISO 3987 method), ANP (EN ISO 3987, ABNT NBR 6294 and ASTM D874 methods) and by ASTM D6751 (ASTM D874 method).

The sulfur content derived from the raw material generates toxic emissions affecting the performance of vehicle emission control system. Brazilian standard adopts methods of analysis described by ASTM D5453 (molecular fluorescence), EN ISO 20 846 (also by molecular fluorescence) and EN ISO 20 884 (dispersive X-ray fluorescence), with the maximum acceptable limit of 50 mg $kg^{-1}$. As to ASTM D6751, the maximum limit is 15 mg $kg^{-1}$ (ASTM D5453 method). The most restrictive is the European standard which recommends the methods given by EN ISO 20846 and EN ISO 20884, with the maximum value of 10 mg $kg^{-1}$.

Group I (Na + K) and group II (Ca + Mg) metal ions cause the formation of deposits of insoluble soaps, as well as catalyze polymerization reactions. They derive from catalysts employed in the production of biodiesel, as KOH, NaOH and / or $CH_3ONa$ or $CH_3OK$. Calcium and magnesium may also be present as impurities in the NaOH or KOH used.

Phosphorus can damage catalytic converters used in emission control systems of the engine. They comes mainly from the raw material and, eventually, from residues of the phosphoric acid used in the neutralization. Phosphorus is determined in biodiesel via optical emission spectroscopy with inductively coupled plasma. Both the European standard (maximum 4.0 mg $kg^{-1}$) as the Brazilian (maximum 10.0 mg $kg^{-1}$) recommends the analytical method EN ISO 14107. The Brazilian standard also recommends the method ABNT NBR 15553 and ASTM D4951. The latter is also indicated in the American standard (maximum 10.0 mg $kg^{-1}$).

| Property | Methods | | | | |
|---|---|---|---|---|---|
| | ANP 07/2008 | | | EN 14214 | ASTM D6751 |
| | ABNT NBR | ASTM D | EN/ISO | | |
| Aspect | --- | --- | --- | --- | --- |
| Density | 7148 14065 | 1298 4052 | EN ISO 3675 EN ISO 12185 | EN ISO 3675 EN ISO 12185 | --- |
| Kinematic viscosity (40°C) | 10441 | 445 | EN ISO 3104 | EN ISO 3104 | D445 |
| Water and sediment | --- | --- | --- | --- | D2709 |
| Flash point | 14598 | 93 | EN ISO 3679 | EN ISO 2719 EN ISO 3679 | D93 |
| Distillation, 90% recovered vol. | --- | --- | --- | --- | D1160 |
| Carbon residue | --- | 4530 | --- | EN ISO 10370 | D4530 |
| Sulfated ash | 6294 | 874 | EN ISO 3987 | EN ISO 3987 | D874 |
| Sulfur content | 15867 | 5453 | EN ISO 20846 EN ISO 20884 | EN ISO 20846 EN ISO 20884 | D5453 |
| Copper strip corrosion, 3h at 50°C | 14359 | 130 | EN ISO 2160 | EN ISO 2160 | D130 |
| Cetane number | --- | 613 6890 | EN ISO 5165 | EN ISO 5165 | D613 |
| Cold soak filterability | 14747 | 6371 | EN 116 | --- | D7501 |
| Pour point | --- | --- | --- | --- | --- |
| Cloud point | --- | --- | --- | --- | D2500 |
| Sodium and potassium | 15553 15554 15555 15556 | --- | EN 14108 EN 14109 EN 14538 | EN 14108 EN 14109 EN 14538 | EN 14538 |
| Calcium and magnesium | 15553 15556 | --- | EN 14538 | EN 14538 | --- |
| Phosphorus content. | 15553 | 4951 | EN 14107 | EN 14107 | D4951 |
| Total contamination | --- | --- | EN 12662 | EN 12662 | --- |
| Ester content | 15342 | --- | EN 14103 | EN 14103 | --- |
| Acid value | 14448 | 664 | EN 14104 | EN 14104 | D664 |
| Free glycerol | 15341 | 6584 | EN 14105 EN 14106 | EN 14105 EN 14106 | D6584 |
| Total glycerol | 15344 | 6584 | EN 14105 | EN 14105 | D6584 |
| Monoglycerides | 15342 15344 15908 | 6584 | EN 14105 | EN 14105 | --- |
| Diglycerides | 15342 15344 15908 | 6584 | EN 14105 | EN 14105 | --- |
| Triglycerides | 15342 15344 15908 | 6584 | EN 14105 | EN 14105 | --- |
| Methanol or Ethanol | 15343 | --- | EN 14110 | EN 14110 | EN 14110 |
| Iodine value | --- | --- | EN 14111 | EN 14111 | --- |
| Oxidation stability at 110 °C | --- | --- | EN 14112 | EN 15751 EN 14112 | EN 15751 |
| Water content | --- | 6304 | EN ISO 12937 | EN ISO 12937 | --- |
| Linolenic acid | --- | --- | --- | EN 14103 | --- |

**Table 5.** Established methods for the quality control of biodiesel in accordance with Brazilian, European and American standards.

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Corrosivity to copper (copper strip corrosion) due to sulfur compounds, as well as due to free fatty acids, can lead to problems of corrosion in storage tanks and some engine parts. As acids are included in this parameter, it keeps a relationship with the acid index. The maximum acceptable values are degree 1, for standard EN 14214 and ANP, and degree 3 for ASTM D6751 (the degree number corresponds to a visual comparison with the copper strip established by ASTM D4951).

The acid number can increase or accelerate corrosion of the engine. It also measures the presence of free fatty acids and other acids and is related to the quality of the process. In injection systems that work at higher temperatures, faster biodiesel degradation may occur increasing the level of acidity and causing problems in filters. All standards have adopted as the specification limit for that parameter the value of 0.5 mg de KOH/g. The ANP standard recommended the methods ABNT NBR 14448, ASTM D664 and EN ISO 14104, and these last two methods are also adopted by European standard and American standard, respectively.

The cetane number measures the ignition quality of fuel. A low cetane number indicates a poorer ignition, which can form deposits and wear on pistons as well as provide greater fuel consumption. It depends upon the feedstock besides the oxygenate content in biodiesel. The number of cetane is measured with the aid of a special motor and the cetane index is calculated. The cetane index is a useful tool to estimate the number of cetanes according to ASTM standard [51].

The content of total glycerol and free glycerol as well as monoglyceride, diglyceride and triglyceride, reflects the quality of biodiesel. A high content of these can cause problems ranging from the formation of crystals, crusts inside the fuel storage tank, contributing to the formation or deposit of waste on pistons, injectors, valves, rings (of segments), filters, up to clogging the nozzles, decreasing the engine life. These are intermediate products of the process that ended up not reacting. The free glycerin (a byproduct) depends on the efficiency of separation of esters/glycerin.

The presence of residual alcohol in the biodiesel may cause corrosion on items of aluminum and zinc, and also can influence the flash point, reduce the cetane number and decrease the lubricity of the engine [51]. The alcohol content is determined by the chromatographic method EN ISO 14110 indicated by the standards EN 14214, ASTM D6751 and ANP. The Brazilian standard also recommends the method ABNT NBR 15343.

The iodine value is related to viscosity and cetane number, and indicates the quantitative degree of unsaturation of esters that form the biodiesel. The method suggested by the Brazilian and European standards is the same EN ISO 14111, though the latter is the only one to provide a limit to the parameter of 120 g $I_2$/100 g.

The oxidation stability determines the degradation of biodiesel, and mixtures thereof. It is related to the time required to degrade the biodiesel under controlled heating and in the presence of oxygen. The method adopted by the ANP standard and also EN 14214 is given in EN 14112, where the specification limit for both is 6 h at least (induction time by Rancimat method). For ASTM D6751 standard this limit is 3 hours and the recommended method is EN 15751.

In addition to the mentioned properties, other parameters may also be important and relate directly to the raw material used in the production of biodiesel: the soap content, the boiling range of esters, the peroxide index (that express the oil oxidation degree), the filterability (that express the difficulty to filter the oil before injecting into the engine) and the gum content, which expresses the amount of gums formed by polymerization of unsaturated oil components during the combustion [51].

In Brazil, the National Program for Production and Use of Biodiesel in one of its aspects provides the Program for Engine Test and Experiment, which is coordinated by the Ministry of Science and Technology (MCT). This program establishes test performances on vehicles and stationary engines and the gradual use increase of the blend biodiesel/diesel and evaluating its consequent technical feasibility. Currently in Brazil, B5 blend is used and it is extremely important to carry out tests to ensure commercial guarantees to vehicles moved with this blend and the quality of the blend commercialized. Currently in Brazil, B5 blend is used and test performing is extremely important to ensure commercial guarantees to vehicles moved with this blend and the quality of the blend commercialized.

At low temperatures, biodiesel tends to partially solidify or increase its viscosity (fluidity loss) inhibiting or even stopping the flow of fuel with the consequent clogging of filters, damaging the starting system of the engine. In tropical countries like Brazil, where most states have high temperatures throughout the year, the effects of these engines operating at low temperatures are minimized, and such problems are mainly present in countries in North America and Europe. Some properties analyzed in such conditions are: cloud point (ASTM D2500), which is the temperature at which, in a process of fuel cooling, it is observed the formation of first crystals, cold filter plugging point (EN 116), which is the temperature where the fuel ceases to be filtered when a cooling occurs and pour point (EN 3016) which is the temperature that the fuel loses its fluidity at established test conditions.

Thus, a major problem is the biodiesels from raw materials that have a high content of saturated compounds in its chain, as occurs for the tallow and the palm oil [72]. Biodiesels from raw materials of animal source when compared to those of vegetable origin have higher pour point, cold filter plugging point and cloud point.

Despite having standards for performing tests, there are no fixed and established values for these parameters. In Europe, they are measured for each country in terms of its climate. In U.S.A., they are dependent on the seasons and climate and in Brazil they depend on the states and months of the year, as shown in Table 6.

Despite the problems that can arise with the use of this fuel in cold seasons and countries with low average temperature, the biodiesel has many advantages over petroleum diesel as, it is a renewable fuel with lower emissions of particulate matters, polycyclic aromatic hydrocarbons, and sulfur compounds; storage and handling of this material is safer, since it has a flash point much higher than petroleum diesel, and also a higher flammability point which ensures a greater safety in loading/unloading and handling of this material by the drivers and operators [72].

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| Brazilian Federation Units | Maximum limit, ºC | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | JAN | FEB | MAR | APR | MAY | JUN | JUL | AUG | SEP | OCT | NOV | DEC |
| SP - MG – MS | 14 | 14 | 14 | 12 | 8 | 8 | 8 | 8 | 8 | 12 | 14 | 14 |
| GO - DF - MT - ES – RJ | 14 | 14 | 14 | 14 | 10 | 10 | 10 | 10 | 10 | 14 | 14 | 14 |
| PR - SC - RS | 14 | 14 | 14 | 10 | 5 | 5 | 5 | 5 | 5 | 10 | 14 | 14 |

**Table 6.** Maximum limits of cold filter plugging point for the Brazilian Federation states according to the months of the year in accordance with the ANP resolution number 14 (May 2012). For the other federation states (not included in the table), the value remains in 19ºC.

## 5. Metallic corrosion in biodiesel

Although biodiesel has many properties that assist in good yield as a fuel as a relatively high flash point and good lubricating properties compared to diesel, some of these properties facilitate its self-oxidation, and oxidation of the metallic materials which they are in contact. The metallic corrosion becomes extremely important since many of the engine parts are composed of variety metals such as aluminum, copper, stainless steel and alloys. The percent of aluminum in engine parts includes piston (100%), cylinder heads (70%), and engine blocks (19%). Pumps and injectors are composed of copper and its alloys. Parts composed of stainless steel include fuel filter, valve bodies, nozzle and pump ring [73-75]. The metallic corrosion may occur due to the following factors:

a) biodiesel is an ester so makes hydrogen bonds with water; then it becomes much more hygroscopic compared to diesel which is composed by hydrocarbons. Water acts on the corrosion of metallic materials, or it causes the hydrolysis of biodiesel, resulting in fatty acids and glycerol which increase metallic corrosion, or it promotes microbial growth and thereby microbial corrosion [73,75-77].

b) the presence of impurities like water, methanol, free glycerol, free fatty acid, catalyst residues (Na and K) due to incomplete conversion or inadequate purification can also result in metallic corrosion [73,75].

c) due to its good lubricity, biodiesel dissolves more metallic parts than diesel, and these trace metals in solution enhance biodiesel degradation and promote metallic corrosion [73,75].

d) metals into biodiesel like brass, copper and aluminum act as catalysts for biodiesel oxidation. Therefore, the acid number of biodiesel increases proportionally with the corrosion rate for different metals [78-81].

Generally, the corrosion tests are performed by immersion tests with metallic coupons in biodiesel [79,80,82,83]. After immersion, the weight of the coupons was measured and the corrosion was analyzed by measurement of corrosion rate, according Equation 1:

$$Corrosion\ rate = \frac{W\ x\ 534}{D\ x\ T\ x\ A} \quad (1)$$

Where:

*Corrosion rate* = mpy (stands for mils (0.001 inch) per year);

W = weight loss (mg);

D = density (g $cm^{-3}$);

A = exposed surface area (square inch);

T = exposure time (h).

Kaul et al. [80] studied the corrosivity of biodiesel from different oil sources by static immersion tests using metallic (aluminum alloy) piston for 300 days at 15 to 40 ° C. In this study the authors observed that the corrosion rate varied with the chemical composition of each oleaginous. The corrosion tests were performed with *Jatropha curcas*, *Karanja*, *Madhuca indica* and *Salvadora* biodiesels. Corrosion rates values were 0.0117, 0.0058, 0.0058, and 0.1236 mpy, respectively. The *Salvadora oleoides* biodiesel presented the highest rate due to its higher content of total sulfur (1200 ppm), while other biodiesels presented lower concentrations of total sulfur (around 1 ppm), except *Madhuca indica* biodiesel (164.8 ppm). *Jatropha curcas* biodiesel was slightly more corrosive than *Karanja* and *Madhuca indica* biodiesels because of its elevated concentration of C18:2 (19–41%) fatty acid, which is more prone to oxidation due to presence of two double bonds.

The metal corrosivity also depends on the nature of the metal exposed to the biofuel. Haseeb et al. [82] performed static immersion tests with coupons of copper and leaded bronze (87% Cu, 6% Sn, 6% Pb) in palm biodiesel at room temperature (25-30 °C) for 840 h; the corrosion rates for copper and bronze were 0.042 and 0.018 mpy, respectively. Additionally, at 60 °C in 2640 h, the corrosion rates of both metals were relatively higher, 0.053 mpy for copper and 0.023 mpy for bronze. The corrosion resistance of bronze was believed to be related to the presence of alloying elements such as tin (Sn) in the alloy. These results clearly show that copper is more prone to corrosion by biodiesel.

Similar behavior was observed by Fazal et al. [83], which performed static immersion tests and evaluated the corrosion rates of copper, brass (Cu: 58.5 %; Zn:41.5), aluminum, cast iron (C:3 %; Si:1.84 %; Mn: 0.82 %; P:0.098%; S:0.089; Fe: balance) at room temperature (25-27 °C) and for 120 days in palm biodiesel. The authors verified that copper presented higher corrosion rates (0.39278 mpy) followed by brass (0.209898 mpy), which contained zinc its composition that probably reduced its corrosion. Aluminum presented higher corrosion rates (0.173055 mpy) than cast iron (0.112232 mpy). This is accordance with Geller et al. [84], which reported that copper alloys are more prone to corrosion in biodiesel than ferrous alloys.

Recently, Hu et al. [85] compared the corrosion rates of several metals in rapeseed biodiesel and proposed corrosion mechanisms through static immersion tests at 43 °C for 60 days. The obtained corrosion rates of copper, carbon steel, aluminum, and stainless steel were 0.02334,

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0.01819, 0.00324, and 0.00087 mmy, respectively (which correspond to 0.9336, 0.7276, 0.1296, and 0.0348 mpy, respectively). This study indicates that copper and carbon steel presented higher corrosion rates than aluminum and stainless steel.

Using scanning electron microscope with energy dispersive X-ray analysis and X-ray photoelectron spectroscopy to analyze the effects of biodiesel on the corrosion of different metallic materials, Hu et al. [85] reported that the corrosion process of metal surfaces in biodiesel was mainly attributed to the chemical corrosion and the products after corrosion were primarily fatty acid salts or metal oxides, depending on the studied metal. Elements of copper and iron are catalysts for the decomposition of biodiesel because they enabled various chemical reactions to easily take place. According to Hu et al. [85], metals were oxidized by oxygen and active oxygen atom dissolved in biodiesel, resulting in the formation of metal oxides (CuO, $Cu_2O$, $Fe_2O_3$, etc.). Copper and carbon steel were easily oxidized but aluminum and stainless steel were protected by films of metal oxide and then their corrosion rates were lower. The protective metal oxide layer prevented the metal surface from contact with the oxygen and atom oxygen as well as from contact with the oil sample.

Fazal et al. [86] has also found that the oxygen concentration increases with increasing temperatures, which may explain the high corrosion rates at higher temperatures. However, water and fatty acids are also responsible for metallic corrosion and need to be considered [80].

Similarly to Hu et al. [85] which carried out static immersion tests at 80 °C for 600 h in rapeseed biodiesel, Norouzi et al. [78] verified corrosion rates of 0.9 mpy for copper and 0.35 mpy for aluminum and confirmed that increase in temperature enhanced corrosion rates.

Another limiting factor that should be considered is the stirring. Fazal et al. [79] obtained corrosion rates of 0.586, 0.202, and 0.015 mpy for copper, aluminum, stainless steel, respectively, in immersion tests at 80 °C for 50 days under 250 rpm stirring in palm biodiesel. The corrosion rates presented superior values than those obtained in static immersion tests. Table 7 presents corrosion rates of different metallic materials in various biodiesels.

Analyzing the data in Table 7, palm biodiesel is less corrosive than rapeseed biodiesel. This different behavior can be correlated by the chemical composition of each biodiesel; rapeseed biodiesel presents 68.821 % oleic acid (C18:1) and 19.5927 % linoleic acid (C18:2) [78], whilst palm biodiesel presents 41.8 % oleic acid (C18:1) and 9.10 % linoleic acid (C18:2) [87].

The content of metal released to biodiesel during corrosion can be quantified. In the static immersion tests in rapeseed biodiesel performed by Hu et al. [85], 41.088 mg $L^{-1}$ Cu (copper coupon), 3.544 mg $L^{-1}$ Fe (carbon steel coupon) and 2.756 mg $L^{-1}$ Fe and 9.02 mg $L^{-1}$ Cr (stainless steel coupon) were obtained. On the other hand, Haseeb et al. [80] quantified lower amounts of metals in palm biodiesel (copper: 5 ppm Cu, bronze: 5 ppm Cu, 4 ppm Pb and 10 ppm Zn) because this biodiesel is less corrosive. According to McCormick et al. [88] the high concentration of metals in biodiesel generates higher oxidation of the biofuel. Biodiesel containing more than 6 ppm of metal exhibits very short OSI (oil stability index) induction time [88]. The negative effect of the presence of metal contaminants on the biodiesel oxidation

stability was also reported by other authors who performed experiments adding organometallic standards or powdered metals into different biodiesels [25,89-94].

| Operation | Biodiesel | METALS (mpy) | | | | | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | Aluminum | Copper | Bronze | Carbon steel | Stainless steel | Brass | Cast iron | |
| 300 days 15-40 °C static | *Jatropha curcas* | 0.0117 | - | - | - | - | - | - | [80] |
| | *Karanja*, | 0.0058 | - | - | - | - | - | - | [80] |
| | *Madhuca* | 0.0058 | - | - | - | - | - | - | [80] |
| | *Salvadora* | 0.1236 | - | - | - | - | - | - | [80] |
| 60 days 43 °C static | Rapeesed | 0.1296 | 0.9336 | - | 0.7276 | 0.0348 | - | - | [85] |
| 25 days 80 °C static | Rapeesed | ~ 0.35 | ~ 0.9 | - | - | - | - | - | [78] |
| 50 days 80 °C 250 rpm | Palm | 0.202 | 0.586 | - | - | 0.015 | - | - | [78] |
| 120 days 25-27 °C static | Palm | 0.173055 | 0.39278 | - | - | - | 0.209898 | 0.112232 | [83] |
| 35 days 23-30 °C static | Palm | - | 0.042 | 0.018 | - | - | - | - | [82] |
| | | - | 0.053 | 0.023 | - | - | - | - | [82] |

**Table 7.** Corrosion rate of some metals in different biodiesels.

Fazal et al. [79] and Hu et al. [85] have reported that biodiesel is more corrosive than diesel oil (based on the corrosion rates in different metallic materials). Figure 3 displays data from both reports [79,85] on the corrosion rate of copper, aluminum, stainless steel, and carbon steel in biodiesel and diesel. See that the first work only evaluated the corrosion of copper, aluminum, and stainless steel [79], while the second work also evaluated all the previous materials and in addition carbon steel [85]. The higher corrosion rates were also confirmed by the metal release verified in diesel and biodiesel, especially copper and iron [85]. Figure 3 also indicates that biodiesel presents more corrosive behavior than diesel oil and copper is not compatible with biodiesel.

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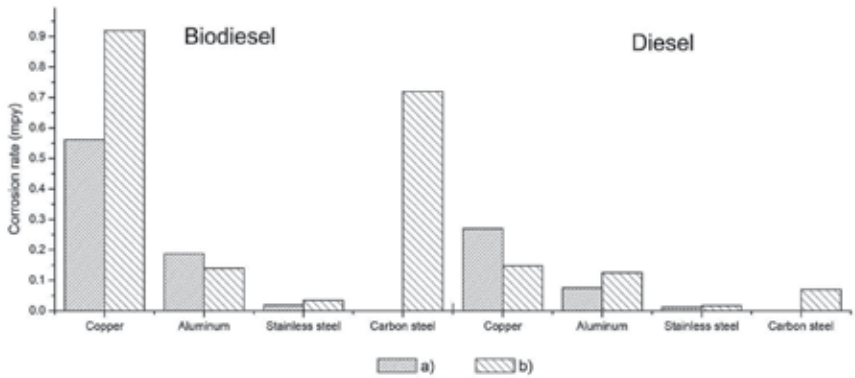


**Figure 3.** Corrosion rate of metals in biodiesel and diesel oil: a) adapted from Ref. [79]; b) adapted from ref. [85].

An alternative way to reduce the corrosive behavior of biodiesel is to use it as blends with diesel oil. According to Norouzi et al. [78], the greater the amount of diesel in blends, the lower corrosion rate as well as the lower total acid number (TAN). Figure 4 shows the variation of (a) corrosion rate of aluminum and copper coupons and of (b) TAN number of biodiesels exposed to these metallic materials through static immersion tests.

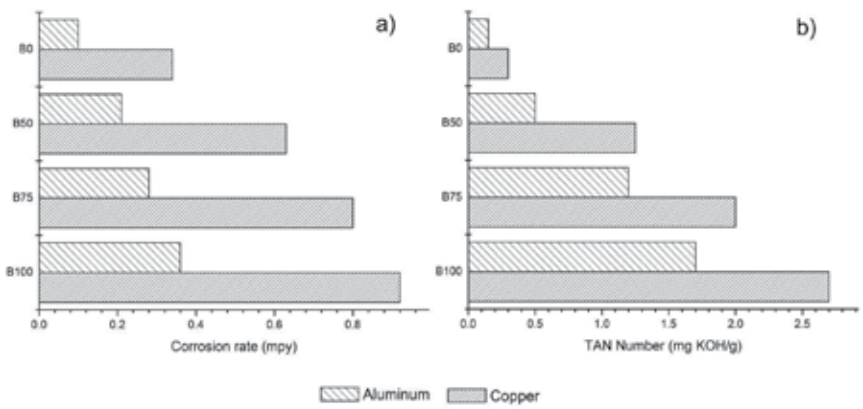


**Figure 4.** a) Corrosion rate of the Al and Cu and b) TAN of the rapeseed biodiesel-diesel blends in contact with both metals (adapted from ref. [78]).

There is a clear relationship between corrosion and TAN of biodiesel; the higher the corrosion rates the higher the TAN numbers. These results show that copper was more corrosive than aluminum and that as long the biodiesel-diesel blend was richer in biodiesel, higher corrosion rates were verified and consequently higher TAN numbers.

Some studies reported that the corrosiveness of biodiesel can be reduced by using corrosion inhibitors or antioxidants. Corrosion inhibitors act by the formation of adsorbed monolayer

films at the metal-solution interface. The common corrosion inhibitors in oil are imidazolines, primary amines, diamines, amino-amines, oxyalkylated amines, naphthaneic acid, phosphate esters, dodecyl benzene sulfonic acids, etc [95]. Amine inhibitors can reduce the dissolution of metal oxide layers into biodiesel by forming stable oxide layers on the metal surface [95]. Fazal et al. [95] reported the inhibition effect on the corrosion of grey cast iron (C: 3%, Si: 1.84%, Mn: 0.82%, P: 0.098%, S: 0.089%, Fe: balance) immersed (static) in biodiesel containing 100 ppm of ethylenediamine (EDA), n-butylamine (nBA), tert-butylamine (TBA) at room temperature for 1200 h. The corrosion rates in each case are shown in Figure 5. The results indicate that EDA is the most powerful corrosion inhibitor under the experimental conditions. However, the analysis of fuel properties revealed that the biodiesel containing EDA was more degraded.

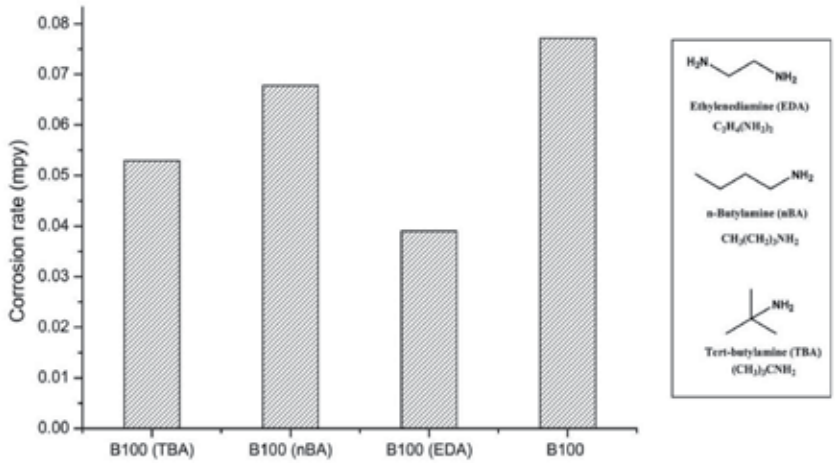


**Figure 5.** Corrosion rate of cast iron (duplicate and respective standard deviation) in palm biodiesel with and without corrosion inhibitors (EDA, nBA and TBA) and their respective chemical structures (adapted from ref. [95]).

Antioxidants can be classified into two groups: chain breakers and hydroperoxide decomposers. The chain breakers have two most common types of antioxidants: phenolic and amine-types. These antioxidants present a highly labile hydrogen that is able to abstract a peroxy radical and thus stops oxidation reactions in the ester chain [96]. The chain breakers antioxidants can be re-classified into natural antioxidants (tocopherols present in vegetables oils) and synthetic antioxidants such as butyl-hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), butyl-hydroxyanisol (BHA), pyrogallol (PY), and propyl gallate (PG) [96].

Liang et al. [97] have reported that synthethic antioxidants presented superior antioxidant activity in palm biodiesel than natural antioxidants. Other authors investigated BHT, BHA, TBHQ, and natural antioxidants as potential antioxidants in soybean oil biodiesel and TBHQ presented a superior antioxidant activity [98,99]. Jain and Sharma compared the efficiency of eight synthetic antioxidants in different biodiesels and they concluded that only three antioxidants significantly increased the stability of biodiesel in the order of TBHQ > PY

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> PG [96]. The addition of antioxidants can also overcome the low oxidation stability of biodiesels promoted by metal contamination (experiments performed by adding organometallic standards) in order to re-establish the required 6-hour induction time [25,90-94]. This statement is especially essential considering the presence of metals in biodiesel due to corrosion of containers and engine components.

Almeida et al. [100] evaluated the effect of the synthethic antioxidant TBHQ on the corrosive character of biodiesel against copper coupon through static immersion tests. Due to the strong catalytic effect of copper towards biodiesel oxidation, the oxidation stability of biodiesel decreased tremendiously after 24 h of exposure even in the presence of TBHQ. The copper content in biodiesel continuously increased with the exposure time and the metal concentration was much higher in the non-stabilized biodiesel. Then, the presence of TBHQ decreased the corrosion rate of the copper coupon and the authors claimed that the antioxidant may have acted as a corrosion inhibitor through the formation af a protective layer on the metallic surface. Performing mass-spectrometry (MS) and MS-MS analysis of deteriorated biodiesel containing TBHQ (after the metal corrosion for 24 h and 168 h), the authors have also identified the formation of new molecules of high molecular weight formed by the association between oxidized antioxidants molecules and free radicals of long-chain molecules (fatty acid derivatives). These results give light to possible side-reactions between phenolic antioxidants and esters molecules of biodiesel under oxidative conditions.

## 6. Analytical methods for contaminants in biodiesel

### 6.1. Free and total glycerol

The determination of the content of total glycerol and free glycerol in biodiesel is required by the different regulatory agencies and upper limits are established as listed in Table 4. Glycerol is the major by-product in the biodiesel production and its removal is necessary. A high content of total glycerol (sum glycerol, mono-, di- and triacylglycerols) and free glycerol can cause problems ranging from the formation of deposits in injectors, sediments inside the fuel storage tank, reducing the engine life. The recommended method for the determination of total and free glycerol in biodiesel is gas chromatography (GC) (Table 5). Both ASTM and EN methods require a derivatization step (silylation reaction) using N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) with a few differences in the standard solution concentrations and column temperature conditions. Table 8 and 9 present a comprehensive list of analytical methods developed for the determination of free glycerol and total glycerol, respectively. Generally, all analytical methods listed in Tables 8 and 9 required a sample preparation step. GC methods often required a derivatization step (modification of a functional group of the analyte such as the silylation reaction) which improves the chromatographic performance. Derivatization reagents (when required) are cited and in a separate column other sample preparation steps (analyte extraction procedures) are detailed in accordance with information contained in the literature.

| Method | Derivatization reagents | Analyte extraction procedures | Detection limit | Ref. |
|---|---|---|---|---|
| Enzymatic assay | No | 2 g sample + 6.0 mL of 0.1 mol $L^{-1}$ HCl | Not cited | [101] |
| GC-FID/ MS | BSTFA | No | $10^{-4}$-$10^{-5}$ % wt | [102] |
| HPSEC | No | No | Not cited | [103] |
| GC-FID | MSTFA/ pyridine | No | No cited | [104] |
| GC-FID/ MS | BSTFA | No | $10^{-4}$ % wt | [105] |
| HPLC-PAD | No | 4 g sample + 45 g $H_2O$ + 50 mL hexane stirred for 30 min at 40°C, 2 h standing | 1 µg g-1 | [106] |
| GPC-RI | No | PTFE membrane filtration | Not cited | [107] |
| Greenhill enzymatic assay | No | Not cited | 5 ppm | [108] |
| UV-Vis | $NaIO_4$, acetylacetone | 1 g sample + 4 mL hexane + 2 mL distilled water + 2 mL ethanol; 5 min vortex-stirring, 15 min centrifugation at 2000 rpm | Not cited | [109] |
| HPSEC | No | No | Not cited | [110] |
| HPLC-RI | No | 4-20 g sample + 4.5 mL distilled water; 30 min stirring, 2 h standing | $4x10^{-4}$ % wt | [111] |
| GC-FID | MSTFA/ pyridine | No | Not cited | [112-114] |
| CE-UV-Vis-DAD | $NaIO_4$ | 200 mg sample + 800 mg water + 200 µL chloroform; 10 min vortex-stirring, 15 min centrifugation at 2000 rpm | 4.3 mg $L^{-1}$ | [115] |
| SFC-MS-UV-ELSD | No | Methanol dissolution | Not cited | [116] |
| IC-PAD | No | 5 g sample + 45 g distilled water; 5 min shaking, 5 min standing | $7x10^{-5}$ % wt | [117] |
| UV-Vis-DAD | $NaIO_4$, acetylacetone | Aqueous extraction, 30 min heating | 0.011 % wt | [118] |
| Cyclic voltammetry | No | 2 g sample + 6.0 mL water; 5 min vortex, 10 min centrifugation; C18 filtration | 2.3 mg $L^{-1}$ | [119] |
| FIA-UV-Vis | $KIO_4$, acetylacetone | 1 g sample + 4 mL distilled water; 30 min shaking, 5 min centrifugation at 3000 rpm | $4x10^{-4}$ % wt | [120] |

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| Method | Derivatization reagents | Analyte extraction procedures | Detection limit | Ref. |
|---|---|---|---|---|
| Enzymatic assay with amperometry | No | 400 µL sample + 800 µL distilled water + 800 µL ethanol + 1600 µL heptane; 2 min vortex-stirring and centrifugation | $1x10^{-5}$ % wt | [121] |
| Enzymatic assay with amperometry | No | 400 µL sample + 800 µL distilled water + 800 µL ethanol + 1600 µL heptane; 2 min vortex-stirring and centrifugation | 0.013 % wt | [122] |
| Titration | $NaIO_4$, $C_2H_4(OH)_2$ | Aqueous Extraction | $9x10^{-4}$ % wt | [123] |
| GC-MS | BSTFA, TMCS, MSTFA | No | 0.04 µg $mL^{-1}$ | [124] |
| HPAE-PAD | No | Aqueous extraction | 0.5 µg kg-1 | [125] |
| UV-Vis | $NaIO_4$, acetylacetone | Aqueous extraction | 0.5 mg $L^{-1}$ | [126] |
| UV-Vis | No | Solid phase extraction | 0.004 % wt | [127] |
| FIA-Amperometry | No | 250 mg sample + 5.0 mL water; 5 min vortex, 10 min centrifugation | 5 mg $kg^{-1}$ | [128] |
| Enzymatic assay with colorimetric detection | No | 400 µL sample + 800 µL ethanol + 800 µL distilled water + 1600 µL heptane; 1 min vortex-mixing, 2 min centrifugation | $7.1x10^{-6}$ % wt | [129] |
| FIA-PAD | No | 1.0 g sample + 4.0 mL water; 5 min vortex-stirring and centrifugation | 44.2 µg $L^{-1}$ | [130] |
| Cyclic voltammetry | No | Not cited | 33 µmol $L^{-1}$ | [131] |
| GC-FID-MS | MSTFA | No | 0.053 % wt | [132] |
| GC-FID | MSTFA | No | 0.02-0.09 % wt | [133] |

CE: Capillary Electrophoresis; DAD: Diode Array Detector; ELSD: Evaporative Light Scattering Detector; FIA: Flow Injection Analysis; FID: Flame Ionization Detector; GC: Gas Chromatography; GPC: Gel Permeation Chromatography; HPAE: High Performance Anion Exchange Chromatography; HPLC: High Performance Liquid Chromatography; HPSEC: High Performance Size Exclusion Chromatography; IC: Ion Chromatography; IR: Infrared Spectrophotometry; PAD: Pulsed Amperometric Detection; RI: Differential Refractive Index Detector; SEC: Size Exclusion Chromatography; SFC: Supercritical Fluid Chromatography; UV-Vis: Ultraviolet Spectrophotometry; BSTFA: N, O-bis (trimethylsilyl)-trifluoroacetamide; MSTFA: N-methyl-N-(trimethylsilyl)-trifluoroacetamide; PTFE: Polytetrafluoroethylene; TMCS: Trimethylchlorosilane.

**Table 8.** Methods for free glycerol determination in biodiesel.

| Method | Derivatization reagents | Sample preparation | Detection limit | Ref. |
|---|---|---|---|---|
| Enzymatic assay | No | Saponification; Solid phase extraction with C8 | Not cited | [101] |
| Greenhill enzymatic assay | No | Saponification | 75 ppm | [108] |
| GC-FID | MSTFA | No | Not cited | [114] |
| IC-PAD | No | Saponification; 5 g sample + 45 g distilled water; 5 min shaking, 5 min standing | $7x10^{-5}$ % wt | [117] |
| UV-Vis-DAD | $NaIO_4$, acetylacetone | Saponification; Aqueous extraction, 30 min heating | 0.064 % wt | [118] |
| Enzymatic assay with amperometry | No | Transesterification; 0.1 g sample + 3.9 mL distilled water + 5.0 mL heptane; 2 min vortex-stirring and centrifugation | $1x10^{-5}$ % wt | [121] |
| Titration | $CH_3NaO$, $NaIO_4$, $C_2H_4(OH)_2$ | Aqueous Extraction | 0.0046 % wt | [123] |
| HPLC-UV-Vis | 9,9-dimetoxifluorene | No | 0.05 % wt | [134] |
| HPSEC-PAD | No | Saponification | 0.5 μg $kg^{-1}$ | [125] |
| UV-Vis | $NaIO_4$, acetylacetone | Saponification; Aqueous extraction | 1.4 mg $L^{-1}$ | [126] |
| Enzymatic assay with colorimetric detection | | Transesterification; 0.5 g sample + 2.0 mL distilled water + 2.0 mL heptane; 1 min vortex-mixing, 2 min centrifugation | $7.1x10^{-6}$ % wt | [129] |
| GC-FID-MS | MSTFA | No | 2.458 % w/w | [132] |
| GC-FID | MSTFA | No | 0.15-0.69 % wt. | [133] |

**Table 9.** Methods for total glycerol determination in biodiesel.

Several GC methods coupled with flame ionization (FID) or mass spectrometric (MS) detectors are listed in Tables 8 and 9 including works published since the early Nineties. The most recent contribution reported a new GC method with reduced sample preparation and analysis time (25 min elution) that can be applied for a wide range of oilseed-derived biodiesels including biodiesel from tallow, babassu, and palm kernel, which contain shorter chain fatty acids and then cannot be accurately analyzed by the EN 14105 method [133]. The EN14105 (and also the ASTM 6584 method) is based on the GC-FID method developed by

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Plank and Lorbeer [104], who reported the simultaneous determination of glycerol, mono-, di-, and triacylglycerides in C18 methylic esters produced from vegetable oils by the silylation of the free hydroxyl groups using MSTFA. Analytical methods employing different modes of liquid chromatography coupled with varied detectors were also reported but in less extension. The first HPLC method employed pulsed-amperometric detection and thus it was not necessary the derivatization step [106]. Additionally, this HPLC method allows the determination of residual alcohol (methanol or ethanol) [106]. A high performance size exclusion chromatography method was developed for the simultaneous determination of the total amounts of mono-, di-, and triacylglycerides, fatty acid methyl esters, free glycerol and methanol [110]. The method is simple, robust, relatively fast, and required minimal sample treatment (no derivatization steps); however, the SEC columns are quite expensive. Preliminary results using a supercritical fluid chromatography coupled with three different detectors (MS, UV, and ELSD) were reported [116]. Separation of fatty acid methyl esters, free fatty acids, and glycerol was obtained in less than 5 min [116].

The oxidation reaction of glycerol with periodate is the basis of the first spectrophotometric method developed for glycerol determination in biodiesel [109]. The oxidation of glycerol results in the formation of formaldehyde, which is reacted with acetylacetone leading to the formation of 3,5-diacetyl-1,4-dihydrolutidine that can be measured at 410 nm [109]. Other spectrophotometric methods using a similar approach were reported in the literature [118,120,126,127]. The great advantage of the spectrophometric methods is their low-cost, rapidness, accuracy and moderate sensitivity. A capillary electrophoresis method was developed based on the UV detection of iodate generated by the oxidation of glycerol by periodate [115]. Similarly, glycerol in biodiesel was determined by alkaline titration of formic acid generated by the oxidation of glycerol by periodate, which can be considered a very simple method easily assessed by local producers [123].

Electrochemical approaches (cyclic voltammetric, amperometric and pulsed-amperometric detectors) were also developed and coupled with chromatographic techniques, flow-injection methods, and enzymatic reactions. Electrochemical methods present high sensitivity, selectivity, can be easily miniaturized and require portable commercially-available instrumentation. Additionally, glycerol can be electrochemically detected without the use of derivatization reagents. Amperometric detection was often applied due to the easiness of its association with flow techniques such as chromatography or flow-injection methods [106,117,125,128,130]. The selection of amperometry or pulsed-amperometry is more related to the electrochemical oxidation of glycerol at different working electrode materials. Enzymatic assays employed enzymes which specifically convert glycerol to dihydroxyacetone phosphate generating $H_2O_2$ and consuming oxygen. Then, $H_2O$ generation or oxygen consumption can be easily monitored by electrochemical techniques (similarly to portable glucose-sensors) as well as using colorimetric assays (the commercial-available Greenhill assay provides the formation of a quinoneimine dye that shows maximum absorbance at 540 nm) [108]. The development of such kits for fast and low-cost monitoring of glycerol in biodiesel is an alternative to GC methods which employ bulky instrumentation and organic solvents during the derivatization step. However, enzymes require special condition of storage and

limited shelf-life time. To overcome such a drawback, the development of (electro)chemical sensors based on the direct detection of glycerol is promising.

Analytical methods developed for the determination of total glycerol in biodiesel (Table 9) typically reported the conversion of mono-, di-, and triglycerides into glycerol by saponification or transesterification reactions except GC or HPLC methods which separate and directly quantify each component present in biodiesel. Other analytical methods developed for the determination of free glycerol (listed in Table 8) can also be readily adapted to perform the determination of total glycerol.

### 6.2. Trace metals

The presence of metals in biodiesel can be arisen from catalyst residues (Na, K, Ca, Mg) and due to corrosion of storage tanks and automotive engine parts (e.g. Al, Cu, Cr, Fe, Mn, Pb, Zn, etc.). Metal ions cause the formation of deposits of insoluble soaps, as well as catalyze polymerization reactions of biodiesel degradation. In this way the different regulatory agencies establishes upper limits as listed in Table 4 for group I (Na and K) and group II (Ca and Mg) cations. However, no limits are established for transition metals which are strong catalysts for biodiesel oxidation even at trace concentrations as previous works have reported [89-94]. Therefore, analytical methods capable of monitoring trace metals in biodiesel are required and this information can be correlated with biodiesel oxidation stability. Table 10 presents a list of analytical methods developed for the determination of metals in biodiesel including sample preparation steps when required and detection limits.

Atomic absorption spectrometry (AAS) and inductively coupled plasma optical emission spectrometry (ICP OES) are the recommended technique by Brazilian and European standards (Table 5). The first analytical methods reported for the determination not only of Na, K, Ca and Mg as well as other metals were the spectrometric methods such as the recommended techniques AAS and ICP OES. AAS coupled to graphite furnace (electrothermal atomization) provides higher sensitivity and for this reason it has been applied for metal determination in biodiesels, although relatively higher cost and longer analysis time are verified. The main advantage of ICP OES is the lower chemical and spectral interferences and multi-element determination; dozens of elements can be analyzed at once after simple sample dilution, including phosphorus and sulfur which are also regulated by European, American and Brazilian norms (Table 4 and 5). However, the overall analysis costs can be so elevated that this method is not available to all analytical laboratories of quality control. ICP MS provides higher sensitivity than ICP OES if sub-ppb levels are required. Most of the spectrometric methods require very simple preparation steps such as sample dilution or sample microemulsion with surfactants, which is an elegant strategy to avoid sample digestions using of concentrated acids. Pre-concentration steps can be applied for AAS with flame atomization which is a low-cost spectrometer but does not provide low detection limits generally required for trace metal determinations. Then pre-concentration steps supplies the low sensitivity of flame atomic absorption (or emission) spectrometers. More details on the use of spectrometric methods to determine metals and metalloids in automotive fuels (including biodiesel) can be found in a recent review [156].

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| Method | Analyte | Sample preparation | Detection limit | Ref. |
|---|---|---|---|---|
| Inductively coupled plasma optical emission spectrometry | Ca, K, Mg, Na | Sample dilution in kerosene | 0.4 – 0.9 mg $kg^{-1}$ | [135] |
| Inductively coupled plasma mass spectrometry | 31 elements | Sample dilution in kerosene | 0.0109 – 22.7 µg $kg^{-1}$ | [136] |
| Inductively coupled plasma optical emission spectrometry (axial viewing) | Ca, Mg, K | Sample dilution in ethanol | 0.005 – 0.1 µg $g^{-1}$ | [137] |
| Inductively coupled plasma optical emission spectrometry (axial viewing) | Ca, Cu, Fe, Mg, Mn, Na | Sample emulsion with Triton X-100 and water | 0.007 – 0.165 µg $g^{-1}$ | [138] |
| Flame atomic absorption spectrometry | Na, K | Sample microemulsion with n-pentanol and Triton X-100 | 0.1 and 0.06 µg $g^{-1}$ | [139] |
| Flame atomic emission spectrometry | Na, K | Sample microemulsion with n-pentanol and aqueous acid solution | 0.1 µg $g^{-1}$ | [140] |
| Graphite-furnace atomic spectrometry | As | Sample microemulsion with n-pentanol and aqueous acid solution | 0.3 mg $kg^{-1}$ | [141] |
| Graphite-furnace atomic spectrometry | Cu, Pb, Ni, Cd | Focused microwave wet digestion | Not cited | [142] |
| Cold-vapor atomic fluorescence spectrometry | Hg | Sample microemulsion with n-pentanol and Triton X-100 | 0.2 µg $kg^{-1}$ | [143] |
| Inductively coupled plasma optical emission spectrometry | 28 elements | Microwave acid digestion | 0.1 – 136.5 µg $g^{-1}$ | [144] |
| Inductively coupled plasma optical emission spectrometry and mass spectrometry | 19 elements | Microwave acid digestion | 0.001 – 0.4 µg $g^{-1}$ | [145] |
| Graphite-furnace atomic spectrometry | Ni, Cd | Sample microemulsion with Triton X-100 and acid aqueous solution | 0.9 and 0.1 µg $L^{-1}$ | [146] |
| Flame atomic emission spectrometry | Cu, Ni, Zn | Pre-concentration by adsorption on chitosan microspheres | Not cited | [147] |
| Flame atomic emission spectrometry | Na, K | Sample dilution in ethanol | 2.16 and 2.00 mg $kg^{-1}$ | [148] |
| Inductively coupled plasma mass spectrometry | 32 elements | Microwave acid digestion | $10^{-6}$ mg $kg^{-1}$ | [149] |
| Potentiometry | K | Not required | 0.01 ppm | [150] |
| Potentiometry | K | Liquid-liquid extraction | $1.9 \times 10^{-5}$ mol $L^{-1}$ | [151] |
| Ion chromatography | Na, K, Ca, Mg | Liquid-liquid extraction, heating, sonication | 0.11 – 0.42 mg $kg^{-1}$ | [152] |
| Ion chromatography | Na, K | Liquid-liquid extraction | Not cited | [153] |
| Capillary electrophoresis | Ca, K, Mg, Na | Liquid-liquid extraction | 0.07 – 0.14 mg $L^{-1}$ | [154] |
| Square-wave stripping voltammetry | Sn | Dry-ashing decomposition | 0.14 µg $L^{-1}$ | [155] |
| Potentiometric stripping analysis | Cu | Sample dilution in hydroethanolic electrolyte | 200 ng $g^{-1}$ | [81] |

**Table 10.** Methods for trace metal determination in biodiesel.

Separation techniques such as ion chromatography and capillary electrophoresis were applied for simultaneous determination of cations in biodiesel after a simple liquid-liquid extraction [152-154]. The capillary electrophoretic (CE) method presented faster separation of ions, employed very low sample volumes and capillaries presents much lower cost than IC columns. Moreover, the CE method can also be used for glycerol determination after its chemical conversion by periodate as a previous method described [115].

Electroanalytical methods for metal determination in biofuels were recently reviewed [157,158]. Potentiometry is a well-known technique which allow sensitive detection of K in aqueous solutions and were also applied for biodiesel analysis after a liquid-liquid extraction [151]. An ion-selective electrode sensor associated with cellophane semi-permeable membrane was applied for K determination in biodiesel without a sample preparation step [150]. The elimination of the sample preparation step is a tendency in modern analytical methods since this step may provide sample contamination, analyte losses, and high analysis time, whose characteristics are avoided when developing an analytical method for routine analyses. Electroanalytical potentiometric stripping analysis was applied for Cu determination in biodiesel after its dilution in hydroethanolic electrolyte [81]. This is the first report on the use of electroanalysis for metal determination in biodiesel. Electroanalytical methods provide real advantages for routine analysis such as high sensitivity and selectivity employing a low-cost portable instrumentation. Similar technology applied for gluco-sensors (using disposable sensors) can be extended to on-site analysis of biodiesel, aiming not only the determination of trace metals but also other species in the biofuel [157,158].

### 6.3. Other trace contaminants

Other trace contaminants can be found in biodiesel such as water (moisture), residual alcohol (typically methanol and ethanol), sterols, phosphorus and sulfur. Table 4 shows the upper limits established for water and residual alcohol and Table 5 lists the recommended methods for the analysis of each parameter. The GC method developed Mittelbach et al [105] can also be applied for the determination of alcohol residues in biodiesel as well as the HPLC-PAD method [106]. A flow analysis method coupled to a membrane extraction was developed for methanol determination in biodiesel [159]. Methanol was detected by spectrophotometry (at 240 nm) after reaction with alcohol oxidase in aqueous solution [159].

Sterols are minor components found in animal fats and vegetable oils and that occur in biodiesel due to their solubility in the biofuel. Sterol glycosides can accelerate precipitate formation in biodiesel even at room temperature and block fuel filters [160-162]. HPLC methods [160,161], mass spectrometry [161], and a GC MS method [162] have been exploited for the determination of sterol glycosides. More information about the use of chromatographic techniques for biodiesel and biodiesel blends (not only including the analysis of sterols) can be found in a recent review [163].

The contents of phosphorus and sulfur in biodiesel are additional parameters regulated by European, American and Brazilian norms. Some ICP OES methods reported for metal deter-

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minations in biodiesel (Table 10) were also applied for the determination of phosphorus and sulfur [136,144,145,149]. A graphite-furnace AAS method was developed for the direct determination of phosphorus in biodiesel using a solid sampling accessory [164]. Simpler methodologies using spectrophotometry were developed [165,166]. In the first work biodiesel samples were mineralized (dry ashing) and their residue containing phosphate was reacted with 1-amino-2-naphthol-4-sulfonic acid to form a blue molybdenum complex [165]. In the second work biodiesel samples were digested using an acid mixture and the obtained solution containing phosphate was mixed with ammonium molybdate and potassium and antimony tartrate ion to form phosphomolybdic acid (yellow) [166]. An electroanalytical method was developed for phosphorus determination in biodiesel using a phosphomolybdic modified electrode [167]. X-ray fluorescence [168], and improvements on ICP OES [169] and ICP MS [170] methods for sulfur determination in biodiesel have been reported.

Analytical methods for monitoring of the transesterification reaction and for the determination of fatty mono-alkyl esters in biodiesel-diesel blends or in pure biodiesel as well as other parameters such as biodiesel oxidation and thermal stability are well-addressed in the review by Monteiro et al. [171] and is not discussed in this text.

## 7. Conclusion and perspective

Much effort has been dedicated to the development of new methods for biodiesel production and improvement of the traditional ones. A large variety of raw materials have been investigated for biodiesel production in the world, and Brazil has favourable environment and climate conditions in this scenario for the development of biodiesels from new oilseeds although the large Brazilian soybean biodiesel production. The production of biodiesels of new oilseeds has economic impact since small local producers can contribute for biodiesel production and the special characteristics of these biodiesels may improve the physical-chemical properties (e.g. acidity, oxidation stability, viscosity, etc.) of other biodiesels by using blends of biodiesels. Microalgae for biodiesel production is a promising sustainable source which has received incresing interest from researchers worldwide. Metallic corrosion is a real problem in storage stability of biodiesels and inside diesel engines and thus requires constant investigation. The monitoring of trace metals in the biofuel may provide information on how trace metals really affect biodiesel oxidation stability. Moreover, the presence of antioxidants and corrosion inhibitors in biodiesel plays key role on the metallic corrosion and deeper investigations would indicate the real need of additives in the biofuel and their required concentration. Modern analytical methods developed for monitoring contaminants in biodiesel have been reported and the creation of portable devices for the quality control of biofuels is a promising tendency. In the future, any local producer would have access to fast and reliable technologies to certificate the quality of its biodiesel during the production process. Additionaly, the real-time and on-site analysis of biofuels is desirable at local gas stations which receive large volumes of biodiesel and need to quickly check the real quality of the biofuel.

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Chapter 7

# Biodiesel Current Technology: Ultrasonic Process a Realistic Industrial Application

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Additional information is available at the end of the chapter

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## 1. Introduction

Biodiesel is briefly defined as a renewable fuel derived from vegetable oils or animal fats. Similarly, the American Society of Testing and Materials (ASTM) defines biodiesel as mono-alkyl long-chain fatty acids esters derived from fatty renewable inputs, such as vegetable oils or animal fats. The term "bio" refers to its origin from biomass related resources, in contrast to the traditional fosil-derived diesel, while the term "diesel" refers to its use on engines; as a fuel, biodiesel is typically used as a blend with regular diesel. To date, biodiesel is well recognized as the best fuel substitute in diesel engines because its raw materials are renewable, and it is biodegradable and more environmentally friendly; biodiesel probably has better efficiency than gasoline and exhibits great potential for compression-ignition engines.

Biodiesel was mainly produced from soybean, rapeseed and palm oils, although social and economic considerations have turned attention to second generation biomass raw materials such as *Jatropha curcas*oil [1]. It is well known that biodiesel competitiveness has to be improved, as to compare to curcas oil diesel, to spread out its consumption. Two routes are suggested to overcome this problem; one is related to get cheap raw materials (i.e., triglycerides, nonedible vegetable oils, animal fats and wasted oils), and other one is to reduce processing cost; notoriously both issues are interrelated [1]. The raw material origin is of great relevance because it determines the final biodiesel properties and also the type of process to be used. It is importance to notice that low-cost raw materials usually contain significant

amounts of free fatty acids (FFA), which lead to a complex and more expensive final process, e.g. the catalyst depletion is accelerated, the purification costs are increase, and the yield in alkali-catalyzed transesterification is decreased.In the other hand, processing costs could be reduced through simplified operations and eliminating decreased. On the other hand, waste streams. There are several current biodiesel technologies that tried to overcome the issues just indicated. For instance, some plants in Europe produce biodiesel by transesterification using supercritical methanol without any catalyst. In this case, the reaction is very fast (less than 5 min) and the catalyst absence decreases downstream purification costs. However, the reaction requires very high temperature (350–400 ºC) and pressure (100–250 atm) which, in turn, increases the capital and safety costs. Another suggested alternative is the use of heterogeneous catalysts that can be separated more easily from reaction products, and required less harsh reaction conditions than the supercritical methanol process. However, these technologies are still far to produce low cost biodiesel, even if they overcome some problems of the conventional process. In this scenario, new technologies are still required for the transformation of second and third generation biomass raw materials, as well as residual biomass, in sustainable production of biodiesel.

To this respect, recently, an increasing number of applications of ultrasonic processes(US) in chemical transformations have made sonochemistry an attractive area of research and development [13]. The main benefit of US is to enhance chemical reactivity by providing enough energy through out the cavitation phenomenon. The bubble implosions generated in this phenomenon provide sufficient energy to break chemical bonds. Thus, the application of US can completely change the reaction pathways as well as the reaction yield and selectivity. Importantly, the main benefits that can be pointed out from the application of US are the reaction rate increase and the use of less severe operating conditions, as well as shorten induction periods and reduction of reagents amount. An interesting extension of US is the possibility to apply it for the transesterification of vegetable oils to produce biodiesel. Typically, this reaction is kinetically slow and shows mass transfer limitations. Thus, cavitation phenomenon of the US could provide the activation energy required in the reaction as well as the conditions (i.e., mechanical energy) to improve the reaction mixing. In this way, US could provide technical an economic advantage for biodiesel production, as compared to conventional transesterification processes.

In this chapter we report the advantageous application of US for biodiesel lab scale production from *Jatropha curcas* oil (JCO). This proposal is in agreement with the search of optimized, sustainable biodiesel production. The chapter briefly describes the basics of current biodiesel technologies and, in more detail, the fundamentals and benefits provided by sonochemistry to alkaline transesterification process ("sonotransesterification" process). In addition, the experimental setup used for sonotranseterification and the main results to date are also discussed. In general, sonotranseterification shows a significant improve when applied to biodiesel production from JCO; when using a 4.5:1 molar ratio of alcohol/JOC, 25 ºC, atmospheric pressure and 60% of amplitude, yields up to 98% are obtained. Finally, these results are compared to more conventional processes such as supercritical methanol and heterogeneous catalysis for the same raw material. Results are discussed in terms of the ad-

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vantages/disadvantages of reaction operating conditions, energy demand and process time. Notoriously, sonotransesterification shows significant benefits as compare to conventional technologies, which could be further improved as the process be optimized.

## 2. Biodiesel

Biodiesel is obtained by transesterification reaction, also known as alcoholysis; in this reaction, vegetable oils (preferably non-edible oils) or animal fats are reacted with a significant excess of alcohol (methanol or ethanol), in the presence of a catalyst (homogeneous, heterogeneous or enzymatic), to form fatty acid alkyl esters (FAME) and glycerol, a valuable by-product for industry [2]. In a conventional biodiesel process (CBP), the alcohol-FAME phase is separated and the alcohol excess is recycled (Figure 1). Next, esters undergo a purification process, consisting of water washing, dry vacuum and subsequent filtering. In this process, importantly, the oil used as raw material must be cleaned and its FFA content must belower than 0.5wt%; otherwise, a pretreatment of the raw material must be carried out. Then, the oil is typically mixed with the alcohol in a 6:1 molar ratio, and 1 to 3% homogeneous catalyst (KOH or NaOH) is added to the reaction mixture. Reactants, including the catalyst, must be anhydrous to avoid soap formation. The reaction is then stirred for 40 to 60 minutes, at temperature between 50 and 60 °C, afterward the reaction is completed [2].

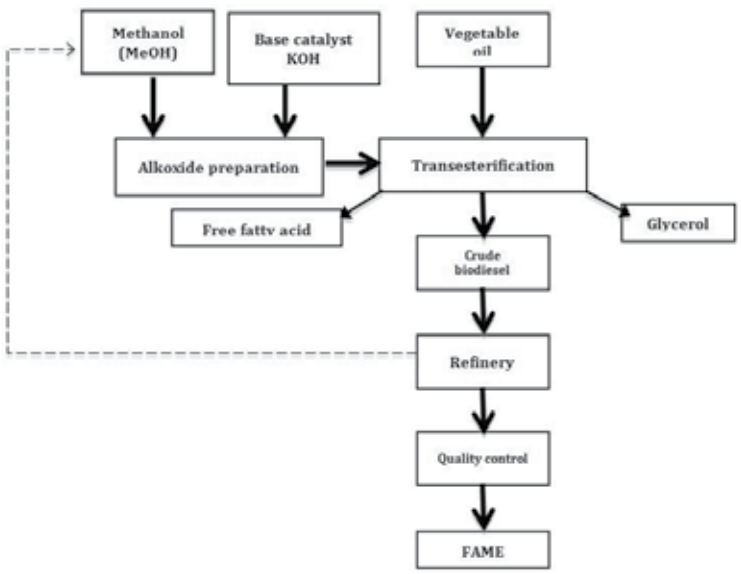


**Figure 1.** Flow diagram of conventional alkaline homogeneous process for biodiesel production [2].

The overall transesterification chemistry involves an exchange between the alcohol groups (i.e., methanol or ethanol) and glycerol, at given reaction conditions, to produce methyl or ethyl fatty acidsesters (Figure 2). Each fatty acid molecule has the same chemistry configura-

tion [3] and it only differs from other molecules for the carbon chain length or its unsaturation number, which leads to produce FAME with different properties that, in turn, impact the final biodiesel characteristics such as melting point, oxidation stability, etc. This is the reason why the raw material quality is suggested to be the key point for the biodiesel process. Figure 2 also shows the well-accepte dreaction pathway. From the thermodynamic point of view, triglycerides and methanol are well-accepted reaction pathway unable to react at room temperature and atmospheric pressure (i.e., 25ºC and 1 atm, respectively) because of the extremely low solubility of the alcohol into the oil; for this reason, catalysis plays an important role for the alcoholysis reaction to take place.

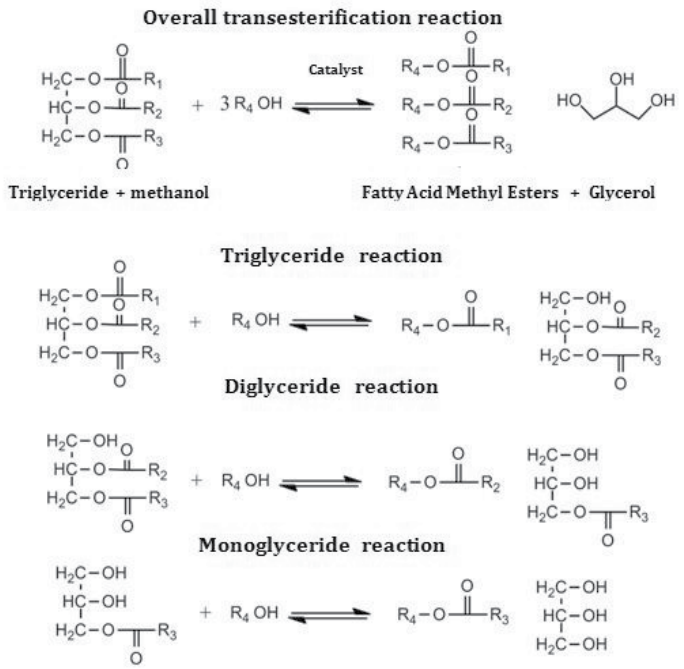


**Figure 2.** Well-generalized transesterification pathway [3].

Literature [1-4] describes that the first reaction step is the formation of an alkoxide ion ($RO^-$) through proton transfer from the alcohol. Actually, when homogeneous Brönsted basic catalysts (i.e., NaOH, KOH, $Na_2CO_3$) are interacts with the alcohol, the following reaction occurs:

$$KOH + CH_3OH \Rightarrow CH_3O^- + [K(OH)H]^+ \quad (1)$$

This alkoxide group then attacks the carbonyl carbon atom of the triglyceride molecule to form a tetrahedral intermediate ion (step 2); therefore, an alkoxide ($NaOCH_3$, $KOCH_3$) is often directly used as catalyst. This intermediate ion rearranges to generate a diglyceride ion

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and alkyl ester molecule (step 3). Next, the diglyceride ion reacts with the protonated base catalyst, which generates a diglyceride molecule and returns the base catalyst to its initial state (step 4). The resulting diglyceride is then ready to react with another alcohol molecule, there by maintaining the catalytic cycle until all the glyceride molecules have been complete converted to biodiesel at 60-80 ºC (Figure 2).

The conventional process (based on homogenous catalysts) has associated several problems, which makes it more expensive when compared to fosil-derived diesel, e.g. raw materials pretreatment and process and cost issues. If raw materials are taken into account, fat and oils cannot directly be used when large amounts of FFA are present. As previously indicated, when alkaline homogeneous catalysts are used, FFA should be less than 0.5 w/w% to avoid high soap formation. Moreover, expensive refinery steps are associated to separate the catalyst and the methanol/biodiesel/glycerol mixture. Generally, water is used to remove alkaline catalysts but this stage makes the overall process less important from environmental point of view. Other relevant issues such as reaction time, mass transfer limitations, optimized set of operating conditions (temperature, pressure, alcohol: oil ratio), determine the economic success of biodiesel production. Regarding the reactor technology, continuous biodiesel process (CBP), especially when equipped with tubular reactors, are always preferred as compared to batch processes. Obviously, this is due to the fact that CBP allow the processing of higher amounts of raw material. However, always that CBP is selected it should be considered the need to incorporate a centrifuge process for glycerol/biodiesel separation, which has a considerable increase in the processing cost. Therefore, an optimum conventional biodiesel process should be conducted at room temperature, atmosphere pressure, avoid water for homogeneous catalysts recovering, and use a low cost glycerol/biodiesel coalescence unit; importantly, the process should reached oil yields over 98%.

Table 1 shows some a comparison of current biodiesel technologies; it is evident from this table that there is a direct connection between complexity and process cost and the quality of final product. As outlined above, conventional process capital cost is low, but processing cost is high because of long reaction time, and separation and purification issues, among others. Regarding the supercritical methanol process, it seems simple and delivers high purity product but, also, capital and operating cost are too high because they are related to severe process conditions. With respect to the use of heterogeneous catalyst, it certainly improves the products separation and purification but, again, this technology is still far to be asuitable economic option because of the high temperature and long reaction time still required for the process. Moreover, another issue to overcome is the design of solid catalysts with appropriate acid sites configuration to improve yield and selectivity, and to decrease catalyst deactivation in hydrous conditions. On the other hand, the US process is less extended and its advantages have not totally documented. However, it could be postulated that the thousands of bubbles formed during the cavitation phenomenon of the US facilitates the formation of a methanol-KOH/oil microemulsion at high temperature, which drastically decreases mass transfer limitations. In this scenario, the transesterification reaction could be carried out within a few seconds, at room temperature (at the "bulk") and atmos-

pheric pressure, thus helping to decrease the process cost. The next section of this chapter describes the basic principles of ultrasound applied to transesterification reaction.

| Variable | Homogeneous Catalysis | Heterogeneous Catalysis | Enzymatic Catalysis | Non Catalytic SMP[2] |
|---|---|---|---|---|
| **Reaction time** | 0.5-4h | 0.5-5.5h | 1-8h | 120-240s |
| **Operation conditions** | 0.1 MPa, 30-65 ºC | 0.1-5 MPa, 30-200 ºC | 0.1 Mpa, 35-40 ºC | >25Mpa, >239.4 ºC |
| **Catalyst** | Acid/base | Metal oxides o carbonates | Lipase | Non |
| **Free fatty acid** | Soap formation | Esters | Esters | Esters |
| **Water** | Interfere | No interfere | No interfere | Act as catalyst to the process |
| **Yield** | Normal | Low to normal | Low to normal | High |
| **Purification** | Difficult | Easy | Easy | Very easy |
| **Downstream** | Water | Non | Non | Non |
| **Glycerol purity** | Low | Low to normal | Normal | High |
| **Process** | Complex | Normal | Simple | Simple |
| **Capital cost** | Low | Medium | High | Very high |
| **Operation cost** | High | High | Normal | High |

**Table 1.** Comparison of current biodisel technologies for processing biodisel[1] . Source: [9]. [2] SMP: Supercritical methanol process

## 3. Principle of Ultrasonic Process

Traditionally, sound is a subject studied in physics and it is not a well-met topic in a chemistry course and, so, is somewhat unfamiliar to practicing chemists. However, sonochemistry, which is defined as the use of sound to promote or enhance chemical reactions, has recently received much attention in several chemical reactions concerning sustainability process [5].

It is known that an acoustic wave is a propagation of pressure oscillation in a given medium (gas, liquid or solid), with the velocity of sound producing both the rarefication and compression phases. Figure 3 shows that sound waves are often disclosed as a series of vertical lines or shaded colors, where line separation or color depth represent the intensity or amplitude of the sine wave; the pitch of the sound depends upon the frequency of the wave. According to the sound spectrum, an ultrasonic wave is an acoustic wave whose frequency is above 20 kHz, which is not audible to human. Hence, when a liquid is irradiated by a strong ultrasonic wave, the pressure at some regions in the liquid becomes

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negative (expansion) because the acoustic amplitude of the wave is larger than the ambient pressure. Therefore, if the pressure wave propagating through a liquid has enough intensity, formation of vapor bubbles may occur because the gas dissolved in the liquid can no longer be kept dissolved, because the gas solubility is proportional to the pressure; this is known as the *cavitation phenomenon* [11].

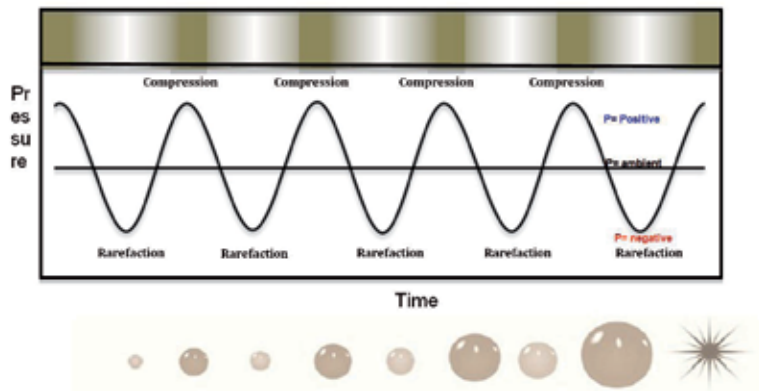


**Figure 3.** Sound waves interaction with a liquid medium [13]. The bubble growth due to the expansion-compression cycles resulting in the formation of localized "hot spots".

The bubbles formed in the cavitation phenomenon grow from nuclei, over many acoustic cycles, through an elastic process [10]. During the expansion cycle an inflow occurs into the bubble, due to the gradient in gas concentration of the fluid shell surrounding the bubble. As the gas diffusion rate into the bubble is proportional to the concentration gradient of dissolved gas, the net inflow of gas into the bubble is essentially higher during the expansion process. Then, when acoustic bubbles reach a critical size range they undergo a violent collapse. There are three at least theories to explain the chemical effects arising from the collapse of cavitation bubbles:

1. electrical theory,
2. plasma discharge theory and
3. super-critical theory.

Another approach is the "hot spot" theory. This theory suggests that bubbles growth is almost adiabatic up to the collapse. At this point, the gas in the bubble core is rapidly compressed (life time in the order of nanoseconds); hence, temperature of thousands of degrees and pressure of more than hundreds of atmospheres can be locally generated; this is the "hot spot" condition. It is noteworthy that, in addition to the extreme conditions of the "hot spot", a secondary region formed by a thin layer of the liquid surrounding the collapsed bubble, it is also transiently heated, although to a lesser extent; this thin layer is about 200 nm in thickness and may reach a temperature of 1726 ºC [11], see a simplified scheme of the "hot spot" model is shown in Figure 4.

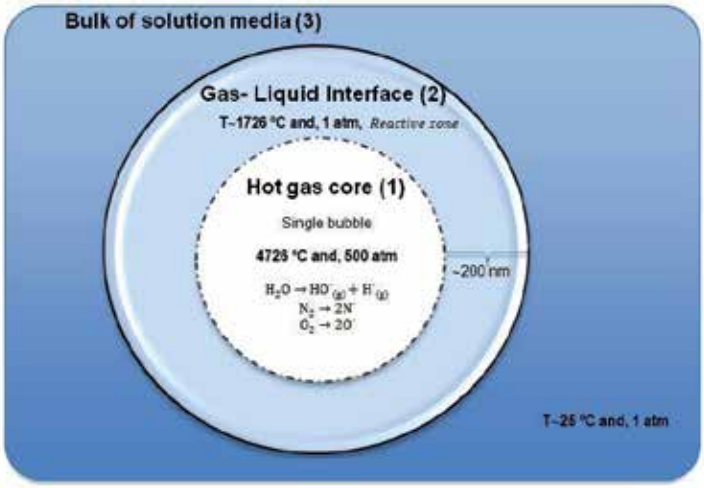


**Figure 4.** Hot-Spot model in the cavitation process [11].

The physicochemical properties of the solvent and solute, and also the gas in the bubble, have notorious effects on the cavitation phenomenon. Therefore, the sonochemical process is very complicated; it is more frequently influenced by the solvent because cavities are spontaneously formed with solvents having high vapor pressure, low viscosity, and low surface tension. Consequently, as liquid must overcome intermolecular forces to form bubbles, poor cavitation efficiency is obtained when solvents with low vapor pressure, high viscosity, surface tension and density are used. Nevertheless, these kinds of solvents have higher threshold for cavitation but more harsh conditions once cavitation begins; this might help in some chemical reactions [12]. On the other hand, there are several gas phase properties that affect sonochemical cavities, Adewuyi [13] recently reported that heat capacity ratio (also known as polytropic ratio, $\gamma$), thermal conductivity and solubility are the most important gas properties. $\gamma$ is involved with the amount of heat released and, hence, affect the final temperature and pressure produced in the adiabatic compression, according to the following equations [14, 15]:

$$T_{max} = T_0\left[\frac{P_a(\gamma - 1)}{P_v}\right] \tag{2}$$

$$P_{max} = P_v\left[\frac{P_a(\gamma - 1)}{P_v}\right]^{\left[\gamma/\gamma-1\right]} \tag{3}$$

Where $T_0$= bulk medium temperature, $P_v$= pressure in the bubble when bubble size is maximum or vapor pressure of the solution, $P_a$= acoustic pressure in the bubble at the moment of collapse.

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Thus, a gas with high thermal conductivity improves the heat transfer from collapsed bubbles to the liquid; this means that it reduces the temperature achieved in an implosion. The solubility of the gas in the liquid is also relevant. The more soluble the gas, the more likely it is to diffuse into the cavitation bubble. Soluble gases should originate the formation of larger number of cavitation nuclei and extensive bubble collapse, because these gases are readily forced back to the liquid phase. Therefore, a decrease of the bulk liquid temperature increases the rate of sonochemical reaction, unlike most chemical reaction systems. This is reasonable because the amount of dissolved gas increases and the vapor pressure of the liquid decreases and, then, less vapor diffuses into the bubble thus cushioning the cavitational collapse; in this condition the implosion more violent.

## 4. Sonochemical transesterification reaction

There are many aspects that make different the sonochemical and conventional chemical reactions. As already mentioned, the "hot spot" is a suitable concept to explain experimental results in many environmental sonochemistry reactions. This theory considers that reactive species and huge heat are produced from bubble cavitation; each bubble created from the interaction of the ultrasonic wave with the liquid is assumed to be a well-defined microrreactor [13]. Actually, according to the "hot spot" model there are three reactive zones:

1. a huge hot gas core,
2. a gas-liquid interface of approximately 200 nm, and
3. the bulk of the liquid media.

This model is frequently used in aqueous reactions, where solvent or substrate suffer homolytic (symmetrically) bond breakage to produce reactive species, and it assumes that free radicals may be in all the reactive zones. However, this model does not necessarily correspond to the thermodynamic reality of the transesterification reaction, because the system is constituted mainly of triglycerides (TG), small amount of FFA, KOH and methanol. Again, the methanol/oil phase is immiscible creating very large mass diffusional problems. But, in general, the energy generated by the US process produces free radicals, which are very reactive, and a significant amount of heat that improves mass transfer among phases [4]. This combined effect of very reactive species and intimate contact between phases could certainly improve the transesterification reaction rate. In this section, some ideas that further explain experimental results obtained in our laboratories are also discussed.

Figure 5 shows the sono transesterification model, which is an adaptation of Adewuyi´s model [13], constrained as follows.

1. Water hydrolysis is not considered as reactive species source because anhydrous conditions should be achieved for biodiesel processing; from our experience with JCO (FFA <1.5-5%), soap formation is not promoted.

2. Relative humidity of air is also dismissed; so, air is dissolved in the methanol and oil phases.

3. The supercritical theory recently proposed by Hua et al. [16] regarding to the transient supercritical water (373 ºC, 22.1 MPa) at the bubble-solution interface is also discarded, because under these conductions the interphase would be considered as a supercritical methanol microrreactor, and then the use of catalyst would become censer.

However, from our lab experience the sonication of methanol/oil mixture without alkaline catalysts does not produce FAME.

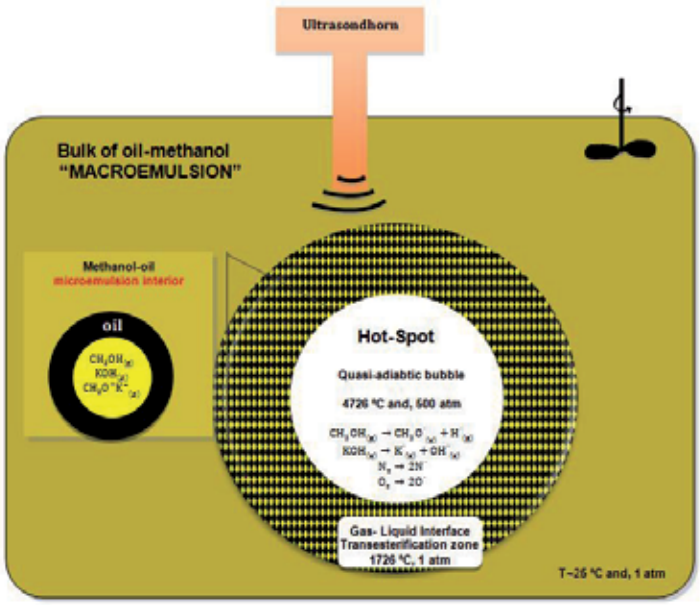


**Figure 5.** Transesterification cavitation model

The model depicted in Figure 5 assumes that a homogeneous methanol/oil macroemulsion is formed by mechanical mixing. It is very important to note that prior to the sonolysis, the methoxide ion produced, unreacted KOH, and methanol coexist inside the microemulsion. Once that the sound-macroemulsion interaction begins, cavitation is performed with vapor of methanol-KOH and air gas inside the bubbles, carrying out the dissociation reactions of the vapor and gas constituents; then, after several cycles of rarefication and compression, the implosion takes a place involving a significant rate of heat and mass transfer. The surrounding liquid quickly quenches a short-lived, localized entity exposed to high temperature (4226-4726 ºC) and pressure (over 1000 atm). Quenching occurs in few microseconds [17] and very fast cooling rates (about $10^{10}$ºC$^{-1}$). This process has a profound influence on the physical properties of interface (microemulsion), where the transesterification reaction is spontaneously carried out, at local temperatures *ca.* 2000 K, without any diffusional problems.

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As already mentioned, TG transesterification by basic catalysis consists of three consecutive, reversible reactions (Figure 2). In the reaction sequence, TG is converted stepwise to diglyceride, to monoglyceride and, finally, to glycerol, accompanied with the liberation of an ester at each step. The reaction mechanism of TG transesterification shown in Figure 6 indicates that in the catalyst-TG interaction the key step is the nucleophilic attack of the alkoxide ion, originating a different reaction chemical. The conventional transesterification process has been associated to a mass-transfer controlled regime occurring at the beginning of reaction. In addition, as the reaction proceeds and ester products act as emulsifiers, two rate-limiting steps change over time. One step is kinetically controlled and it is characterized by a sudden surge in product formation; the second step is reached once equilibrium is found near the reaction completion [19]. Importantly, in the sono transesterification model showed in Figure 5, neither mass transfer nor kinetic reaction are rate-limiting steps, but rather the chemical equilibrium.

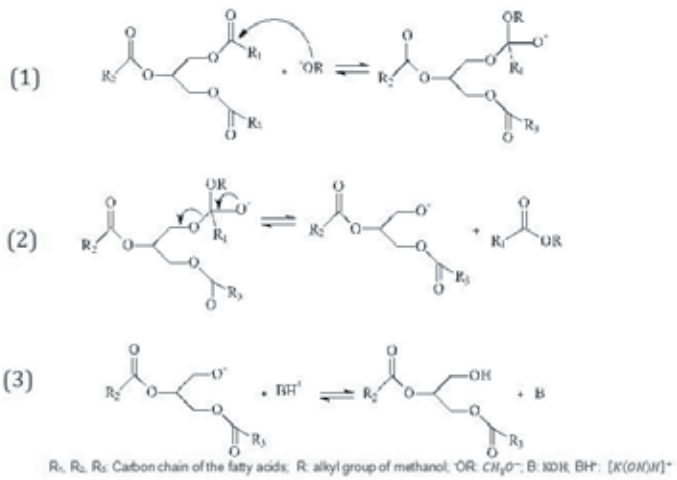


**Figure 6.** Homogeneous based-catalyzed reaction mechanism for triglyceride (TG) transesterification: methoxide ion form by dissociation of potassium hydroxide into methanol and it is encapsulated into TG-methoxidemicroemulsion, then: (1) $CH_3O^-$attacks nucleophilically to carbonyl group on TG, which leads to the tetrahedral intermediate formation; (2) intermediate breakdown; (3) regeneration of $CH_3O^-$ active species. These steps are repeated twice to complete TG transesterification, according to Loreto et al. [18].

## 5. Comparison of experimental biodiesel processing technologies from *Jatropha curcas*

### 5.1. Why *Jatropha curcas*?

Current feedstock for biodiesel production plants derive from a great biomass variety, including first generation biomass raw materials such as vegetable oils (e.g., soybean, cottonseed,

palm, peanut, rapeseed/canola, sunflower, safflower and coconut oils), animal fats (usually tallow) as well as spent or waste oils (e.g., used frying oils). But, given the fact that the use of vegetable oils has been strongly questioned, the use of second- and third-generation biomass feedstock is continuously growing. Among the raw materials coming from nonedible crops for humans, a key issue is their availability near to the biodiesel production plant. In this scenario, our research group is interested to use *Jatropha curcas* oil as feedstock. *Jatropha curcas L. (JC)* is a stress-tolerant ruderal, drought-resistant, oil-bearing small tree, which is well adapted to tropical, semi-arid regions and marginal sites. *JC* propagates easily and can be established quickly in a wide variety of soils with different agroclimatic conditions and does not put pressure on fertile agricultural land or natural ecosystems. In addition, *JC* is characterized for a short gestation period, low seed cost and, importantly, for the multiple uses that may have different parts of the plant [20, 21]. *JC* has received a lot of attention as a source of renewable energy, because its seeds contain 27–40% nonedible oil with a high quality of fatty acid profile (Table 2), which can be easily converted into biodiesel that meets American and European Standards (Table 3).

| Fatty acid | Systematic name | Structure | wt % |
|---|---|---|---|
| Lauric acid | Dodecanoic acid | C12 | - |
| Mysteric acid | Tetradcanoic acid | C14 | 0-0.1 |
| Palmitic acid | Hexadecanoic acid | C16 | 14.1- 15.3 |
| Palmitolileic acid | Cis-9-hexadecanoic acid | C16:1 | 0-1.3 |
| Stearic acid | Octadecanoic acid | C18 | 3.7-9.8 |
| Oleic acid | Cis-9-Octadecanic acid | C18:1 | 34.3-45.8 |
| Linoleic acid | Cis-9-cis-12-Octadecanoic acid | C18:2 | 29-44.2 |
| Linolenic acid | Cis-6-cis-9-cis-12-Octadecanoic acid | C18:3 | 0-0.3 |
| Arachidice acid | Ecosanoic acid | C20 | 0-0.3 |
| Behenic acid | Docosanoic acid | C22 | 0-0.22 |
| Gadoleic acid | | C24 | 14 |
| **Saturated** | **-** | **-** | **21.1** |
| **Unsaturated** | **-** | **-** | **78.9** |

**Table 2.** Fatty acids profile of *Jatropha curcas* oil [22]

In terms of availability, JC easily grows in Norwest Mexico, where our lab is located. For this reason, we set a research project to evaluate the potential of the local JC variety as source of renewable energy (i.e., biodiesel production). Notoriously, we also seek the utilization of valuable byproducts or residues of the conversion of *JC* oil to biodiesel; for instance, fruit husk and seed shell may lead to production of energetic pellets, part of the harvested seed shell may be used to produce humic acid, a biofertilizer; from the seed kernel, not only oil and subsequently biodiesel may be produced, but also protein flour for poultry, sheep,

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shrimp and tilapia (Figure 7). Once that biodiesel is produced a significant amount of glycerol become available, and we look for the production of high-added value chemical derive from glycerol catalytic conversion. Results presented hereby concern to biodiesel production, in particular the development of alternative strategies to improve the efficiency of the transesterification reaction and to decrease the overall processing cost. In this way, the proposed integrated approach clearly contributes for the development of sustainable biomass conversion processes.

| Parameter | JC Biodiesel | Diesel | USA ASTM | Europe EN |
|---|---|---|---|---|
| Density at 15ºC (g $mL^{-1}$) | 0.91 | 0.85 | 0.88 | 0.8-0.9 |
| Kinematicviscosityat 40°C ($mm^2s^{-1}$) | 3.43 | 2-8 | 1.9 - 6 | 3.5 - 5 |
| Cetane number | 52 | 47.5 | "/47 | >51 |
| FAME content (%) | >99 | 0 | - | > 96.5 |
| Sulfur (ppm) | 0 | <5 | <15 | < 10 |
| Flash point (ºC) | 186 | >61.5 | >93 | >101 |
| Acid number (mgKOH$g^{-1}$) | Depend of process | | ≤ 0.5 | ≤ 0.5 |

**Table 3.** USA and Europe international standards for biodiesel [23].


**Figure 7.** Productive chain for non-toxic *JC research p*roject in Norwest Mexico [23].

## 5.2. Materials and methods

### *5.2.1. Physical chemical JC oil characterization*

The study used *JC* from selected elite germoplsms and cultivated in three zones of Sinaloa, Mexico. The approach used to obtain *Jatropha curcas* oil (JCO) was the well-established cold pressing followed by solvent oil extraction. The JCO physicochemical properties studied in this work included: fatty acid profile, acid index (AI), saponification index (SI), peroxide index (PI), and iodine index (II), which were obtained following the methodologies suggested by the Association of Analytical Communities, AOAC.

The quality criteria for the production of biodiesel are specified in EN 14214. In particular, method EN 14103 specifies the FAME content, which is used to profile the vegetable or animal oil feedstock used in biodiesel production. EN 14103 requires calibration of all FAME components by relative response to a single compound, methyl heptadecanoate. This requires the measurement of accurate weights for each sample and the addition of an internal standard. The FAME range for which the method is intended lies between C14:0 and C24:1. A modified EN 14103 chromatographic method was used. In this method, FAME analysis was carried out in a 6890N Agilent Gas Chromatograph (GC), equipped with a capillary split/splitless injector and a selective 5973 Agilent mass spectrometer detector. A 1 $\mu$L split injection (split ratio 50:1) was made to a Supelco omega wax column (bonded polyethylene glycol), using 1 $mlmin^{-1}$of helium into the column as carrier. Samples were injected via an auto sampler series 7683 also from Agilent technologies. A good resolution and peak shape was obtained when using the following oven temperature program: The initial temperature, 100 °C was kept for 2 min; then a heating rate of 4 °C $min^{-1}$ was used to increase the temperature to 240 °C and, finally, this temperature was kept for 10 min. For identification and calibration of the individual FAME, the Supelco standard "37 Component FAME Mix" was used. The response and retention time of each component was experimentally determined. Then, the calibration was verified by both, the analysis of a calibration-check standard and the database of mass spectrum reported by the National Institute of Standards and Technology (NIST). Results of analyses were then compared with the certificate of analysis, verifying the quality of the calibration. The standard preparation for this technique consisted of the dilution of the FAME standard into 4 mL of n-heptane. The sample preparation was also quite simple with 100 μL of biodiesel feedstock into 4 mL of n-heptane. Finally, concentration reports were based on the area percentage rather than a mass percentage, to simplify the calculations.

On the other hand, quantitative determination of free and total glycerin in biodiesel (B100) was also carried out by gas chromatography, followed by a modified methodology proposed by the ASTM D6584-10aε[1].The same Agilent GC system was also used for this analysis, the only difference being the use of a MS detector. ADB-5 ms column from Agilent Technologies was used for free and total glycerin analysis, which is equivalent in chromatographic efficiency and selectivity to that of the MET-Biodiesel capillary column of Sigma Aldrich.

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*5.2.2. Transesterification procedure*

*5.2.2.1. Conventional process*

Conventional alkaline transesterification was conducted in a 2-necked glass reactor (100 mL, Aldrich). A homogeneous reaction mixture was obtained by using plate stirrers, and a constant reaction temperature was kept by using isolated bath vessels equipped with a stainless steel coils. The reaction temperature was fixed by using of a heater/cooler recirculation isothermal bath (Fisher Scientific 3016). Figure 8 shows that each reactor was connected to-cooled straight glass condenser to avoid alcohol leaks; water at 5ºC from another isothermal bath (Fisher Scientific 3028) was used as cooling fluid.

**Figure 8.** Transesterification reaction system for the conventional process

Anhydrous methanol (Sigma-Aldrich, 99.8 %,) and KOH reagent grade (Sigma-Aldrich, 90%) were used for all experiment of this study. The stirrer was fixed at 600 rpm, and the temperature at 40, 60, 70 or 90 C), a methanol: JCO molar ratio was 3:1 or 6:1. Previously to each reaction, methanol and KOH solutions were prepared according to the proposed molar ratio. Then, the reaction volume was fixed to 50 mL of JCO. After the desired reaction temperature was reached, a preheated methanol-catalyst solution was added to start the reaction. Reaction mixture was sampled after 15, 45, 60, 90, and 120 min. These samples were quenched by a sudden immersion of the sample to a plastic container at 0 C, for 15 min. Then, reaction products were purified according to the methodology suggested by Cervantes [24](Figure 9), and the biodiesel yield was determined by means of the following equation:

$$Yield = \frac{wt\ B_{100}}{wt\ oil} x100 \tag{4}$$

*5.2.2.2. Heterogeneous process*

As reported elsewhere [25], JCO transesterification was also conducted by using ZnO, $Al_2O_3$ and ZnO-$Al_2O_3$ mixed oxide powders as catalysts. The objective was to compare the heterogeneous catalytic conversion of the same JCO. In this case, the catalytic activity was measured in a Parr 4560 stirred tank reactor, operated at 1000 rpm, 250ºC, P= 14.7 atm. A methanol: JCO molar ratio of 6:1 and a 3 wt% of catalysts (based on JCO weight) were used. Previously to the reaction, the reactor was uploaded with the 50 ml of JCO, the required methanol to achieve the 6:1 molar ratio, and 1.36 g of catalyst. Then, the reactor was purged with nitrogen (Praxair, reagent grade) for 3 min to avoid JCO burning. The reaction time was 1 h and then the reactor mixture was suddenly cooled to room temperature. The product separation included the following steps.

1. Catalysts removal by means of vacuum filtration.
2. Methanol recovery by using a rotary evaporator at the same condition indicated in Figure 9.
3. Glycerol and biodiesel separation by centrifugation, using the same condition indicated in Figure 9.

At the end, the biodiesel yield was calculated by using equation 4

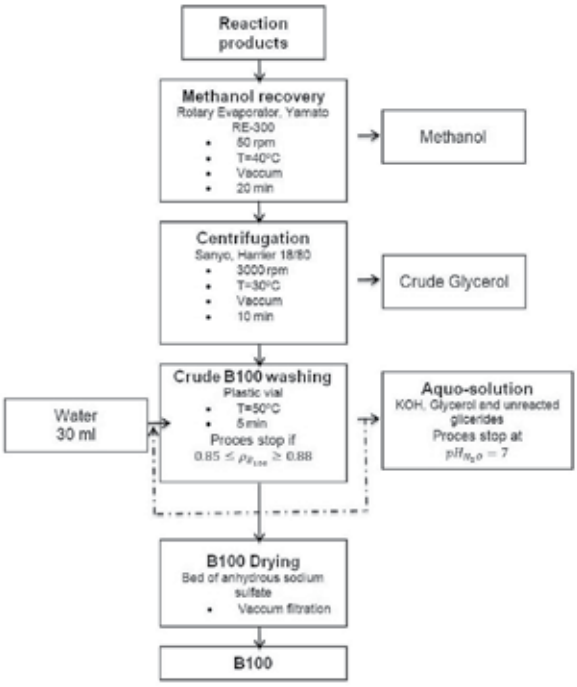


**Figure 9.** Biodiesel purification process for a conventional alkaline transesterification process.

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*5.2.2.3. Supercritical methanol process*

Non-catalytic transesterification process was evaluated by means of the supercritical methanol reaction. This process was also carried out using the 4560 Parr stirred tank reactor. The effect of both, methanol: JOC molar ratio (40:1 and 60:1) and temperature (250, 300, and 350ºC) was evaluated using nitrogen as co-solvent. Once the required reagents amounts were charged to the reactor, the air was vented with nitrogen and the stirrer was fixed at 1000 rpm. Next, the temperature was increased until the desired set point; in this process the pressure increased but not enough to reach the methanol supercritical point. Therefore, additional nitrogen was loaded to ensure 14 MPa. As an alternative to decrease the drastic operation conditions $N_2$was used as co-solvent. The reaction took place over 30 min, sampling the mixture every 5 min through the liquid reactor valve. After the reaction was finished, the reactor was suddenly cooled to room temperature. The product separation included the following steps.

**1.** Methanol recovery by using a rotary evaporator at the same condition indicated in Figure 9.

**2.** Glycerol and biodiesel separation by decantation.

At the end, the biodiesel yield was calculated by using equation 4.

*5.2.2.4. Ultrasonic process*

The sonotransesterification of JCO was conducted by using a highly efficient Hielscher Ultrasonic processor, model UP200 HS. This equipment was used to generate mechanical vibrations by means of the reversed piezoelectric effect (electric excitation), with frequency of 24 kHz, and a control range of 1 kHz. The vibrations were amplified by the S14 sonotrode fitted to the horn and formed as a $\lambda/2$ vibrators, and transferred via its end face to the JCO. To optimize the sonotransesterification reaction, the effect acoustic power density (N), sonication time (or reaction time), and methanol: JCOmolar ratio (MR) were evaluated at room temperature (25ºC) and ambient pressure (1 atm). Reaction temperature was controlled by using an isothermal bath (Fisher Scientific 3016). The continuous sonication of the reaction mixture was conducted using N=105 $Wcm^{-2}$ and a molar ratio of 6:1, following the approach described in Figure 9. Reaction time was fixed at 1, 2, 4, 6, 8, 10, 15, 20, 25 or 30 min. Next, the methanol: JCO molar ratio was evaluated varied to 3:1, 4:1 and 6:1. For the smaller reaction time and molar ratio, the acoustic power density effect was evaluated at 42, 63, 73.5, 84, 94.5, and 105 $Wcm^{-2}$. When the best set of parameters was found, an experiment was conducted again to determine the biodiesel quality.

## 5.3. Results and discussions

### *5.3.1. Physical chemical JC oil characterization*

The *Jatropha curcas* oil obtained from non-toxic, harvested seed in Northwest Mexico, seems to be an excellent candidate for biodiesel production due to its high quality. Table 4 includes the basic JCO physicochemical characteristics that back up this quality. The iodine index is a measurement of the oils unsaturation degree; a higher iodine index corresponds to higher degree of unsaturation [26], and probably leads to oxidation and viscosity problems. The JCO iodine index was 28.75 cg $I_2g^{-1}$, which is well below the maximum specified value (120 cg $I_2$ $g^{-1}$) for biodiesel as indicated in the EN14214 specification. The limitation of unsaturated fatty acids is convenient because heating higher unsaturated fatty acids results in polymerization of glycerides, leading to the formation of deposits or to deterioration of the lubricant [27]. Fuels with this characteristic (e.g Sunflower, soybean and safflower oil) are also likely candidates to produce thick sludge's in the sump of the engine, when fuel seeps down the sides of the cylinder into crankcase [26]. The JCO iodine index could was caused by the high content of unsaturation fatty acid such as oleic and linoleic acid (Table 5).

| Test | Parameter[1] |
|---|---|
| Appearance | Yellowish transparent |
| Free fatty acid (%) | 1.51 ± 0.10 |
| Density at 15ºC ($gml^{-1}$) | 0.92 ± 0.01 |
| Acid index (mg KOH $g^{-1}$) | 3.07 ± 0.12 |
| Saponification index (mg KOH $g^{-1}$) | 180.92 ± 2 |
| Iodine index (cg $I_2$ $g^{-1}$) | 28.75 ± 0.1 |
| Peroxide index (meq $O_2$ $Kg^{-1}$) | 18.5 ± 0.7 |

**Table 4.** Physical chemical properties of *Jatropha curcas* Oil. [1] Standard desviation measured from triplicate determinations.

In on another hand, JCO peroxide index was 18.5 $meqg^{-1}$, that is higher than the index recently reported in the literature for crude seed Jatropha oil, 1.93 $meqg^{-1}$ [26] and 2.5 $meqg^{-1}$[28]. Despite this high peroxides index, JCO upholds the good quality of biodiesel purposes. The JCO saponification index was 181 mg KOH $g^{-1}$, which suggested that JCO was mostly normal triglycerides, and very useful in biodiesel production due to its low FFA content (1.15wt%). The content of FFA was assessed from the acid index (AI) measurement, taking into account the composition showed in Table 5. The acid index of 3.07 mg KOH $g^{-1}$reported in Table 3 was lower than the values reported by other authors (10 - 14 mg KOH $g^{-1}$) for crude JCO [29, 30]; this could be attributed to the change of local environmental conditions where by the *Jatropha curcas* plant was grown. Therefore, acid index becomes a very important parameter to determine the most convenient

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processing route of a given FAME; this means that oils can undergo a pretreatment or direct transesterification as a function of FFA amount.

| Compound | Estructure | wt % |
|---|---|---|
| Palmitic | 16:00 | 23.992 |
| Stearic | 18:00 | 7.224 |
| Oleic | 18:01 | 41.368 |
| Linoleic | 18:02 | 27.186 |

**Table 5.** Faty acid composition de *Jatropha curcas* Oil determine by MS-CG.

The properties of triglyceride and biodiesel are determined by the amounts of each fatty acid present in the molecules. Chain length and number of double bonds determine the physical characteristics of both fatty acids and triglycerides [3]. Nevertheless, transesterification does not alter the fatty acid composition of the feedstocks, and this composition plays an important role in some critical parameters of the biodiesel, as cetane number and cold flow properties. Therefore, measuring fatty acid profile of JCO was another important target of this study. These results are shown in Table 5.

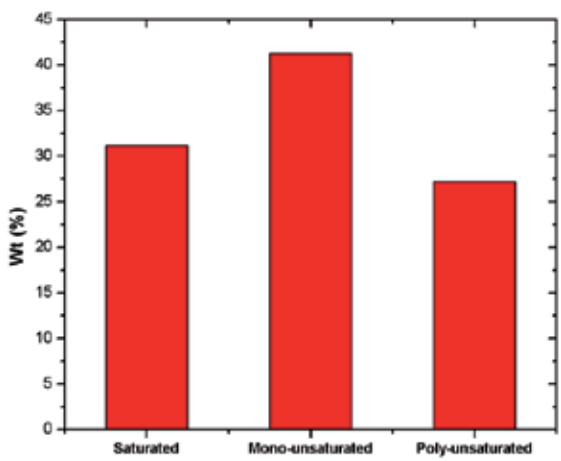


**Figure 10.** Type of fatty acids in *Jatropha curcas* oil from the Norwest of México

There are three main types of fatty acids that can be present in a triglyceride which is saturated (Cn:0), monounsaturated (Cn:1) and polyunsaturated with two or three double bonds (Cn:2,3). Ideally, the vegetable oil should have low saturation and low polyunsaturation,

that is, be high in monounsaturated fatty acids, as shown in Figure 10. Vegetable oils rich in polyunsaturated (linoleic and linolenic) acids, such as soybean and sunflower oils [26], usually produce methyl ester fuels with poor oxidation stability. In the other hand, vegetable oils with high degree of unsaturation (Cn:2,3) lead to a product with high freezing point, poor flow characteristics and may become solid (e.g palm oil) at low temperatures, although they may perform satisfactorily in hot climates. The main fatty acids in the JCO used in this study were the oleic, linoleic, palmitic and the stearic fatty acids. The predominant acids were monounsaturated (41.36%), polyunsaturated (27.18%) and saturated fatty acid (31.21%) (Figure 10). This result was in agreement with the reported by Akbar [26], although it was slightly different in terms of saturated and polyunsaturated compounds for the JCO from Malaysia. Thus, JCO can be classified as oleic–linoleic oil. Compared to others vegetable oil JCO had highest oleic acid contain than palm oil, palm kernel, sunflower, coconut, and soybean oil.

*5.3.1. Jatropha curcas oil transesterification*

Three current biodiesel technologies were evaluated and compared with the conventional homogeneous transesterification, using the JCO characterized above. The main objective was to evaluate the potential advantages of sonotransesterification in terms of operating conditions, transesterification rate and processing steps and costs.

*Conventional alkaline transesterification*

According to the overall transesterification pathway shown in Figure 1, stoichiometrically, JCO methanolysis requires three moles of methanol for each mole of oil. Since the transesterification of triglycerides is a reversible reaction, excess methanol shifts the equilibrium towards the direction of ester formation. As it is evident from Figure 11, the maximum yield for the conventional alkaline transesterification process (84%) was reached after 15 min reaction time; afterwards no signification variations were observed. In addition, when the methanol: JCO molar ratio was increased from 3:1 to 6:1, no major differences were found within the first 15 min; however, a higher biodiesel yield was observed in the experiment with a 6:1 molar ratio toward the end of the reaction. On the other hand, results shown in table 5 indicate that temperature effect is not important. These results correspond to the biodiesel yield evaluated after 15 min. Thus, the higher biodiesel yield was found at 40 °C, and then it decreased to around 73 – 75 % for temperatures between 60 and 90 °C.

Current results of the conventional process disclosed in Figure 11 and Table 6 suggested a significant improve to the conventional alkaline transesterification process, because the reaction yield was enhanced at a shorter reaction time (40 min as compared 60 min) and temperature (40 °C as compared to 60ºC) for industrial application [3]. A shorter reaction time can be translated to a continuous process with a shorter resident time and then, the possibility to reduce costs at the reaction stage. However, a higher JCO conversion is needed to ensure a sustainable process. Moreover, the biodiesel purification process is still a problem because it implies long times and it is energy demanding.

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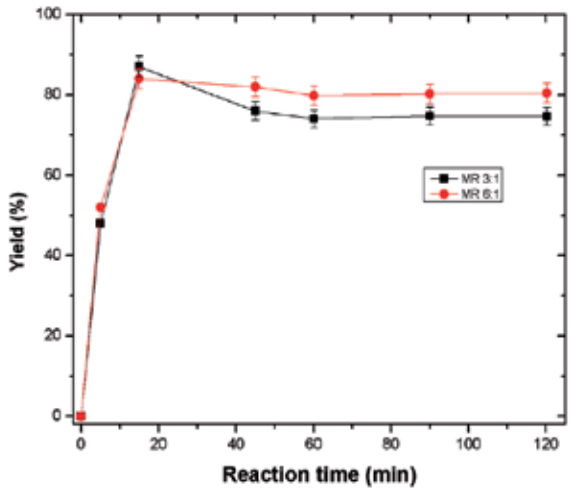


**Figure 11.** Progress of transterification reaction as function of methanol:JCO ratio at 40ºC

| Temperature, ºC | Yield, % (At 15 min of reaction time) |
|---|---|
| 40 | 84.0 |
| 60 | 73.06 |
| 70 | 73.60 |
| 90 | 75.30 |

**Table 6.** The effect of temperature on the performance of alkaline transesterification of JCO by conventional process.

*Supercritical methanol process*

Thus, as an alternative of the problems indicated above, the supercritical methanol process (SMP), using nitrogen as co-solvent, was conducted. Figure 12 shows that the best set of operating conditions for this non-catalytic process were: methanol: JCO mol ratio of 40:1 and 350ºC. Under these conditions, a biodiesel yield *ca*. 60% was obtained. From Figure 13, it can be observed that after 20 min the equilibrium was reached for the transesterification reaction for both molar ratios studied: 40:1and 60:1. This is a very promising result if it is compared with reported for palm [31] and soybean oils [32], where biodiesel yields up to 84% were obtained under very high pressure, 40 and 35Mpa, respectively.

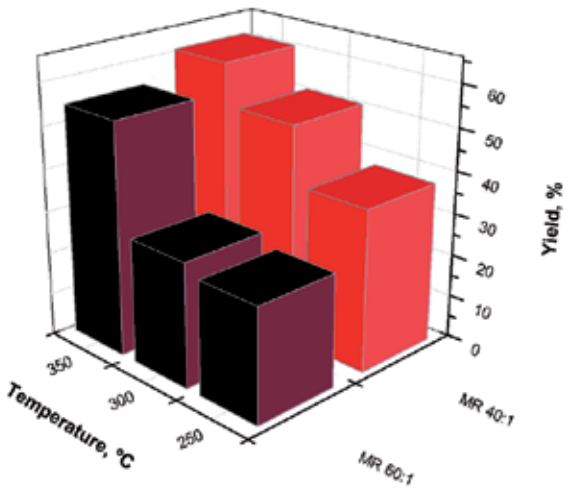


**Figure 12.** Effect of temperature and methanol: JCO molar ratio on the yield of Biodiesel obtained by supercritical methanol process at 14Mpa and 30 min.

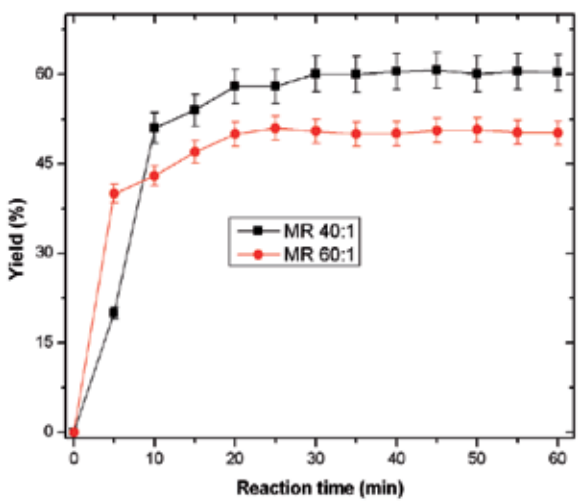


**Figure 13.** Progress of transterification reaction as function of methanol: JCO ratio at T= 350ºC for supercritical methanol process

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Importantly, supercritical methanolysis did not require any kind of catalyst, and no pretreatment to remove water or FFA was used in this work. A very simple separation processes – evaporation and layer separation – were used for biodiesel purification. Our findings agree with the literature that supercritical process is simpler and faster than conventional alkaline transesterification for biodiesel production. In addition, since wastewater was not introduced by pretreatment or washing processes, the supercritical process is environmental friendly. However, to date, high investment and energy cost are still required due to high temperature and pressure of the supercritical state. Another issue with economic implications is the large methanol needed to enhance the forward reaction without catalyst. It could expected that these costs are comparable to those of the pretreatment and separation process of the conventional alkaline transesterification process. Clearly, as the methanol demand be decreased, and the operating conditions be more moderate, the economic feasibility of supercritical methanol process would be possible.

*Heterogeneous process*

As indicated in the previous section, three heterogeneous powder catalysts, ZnO, $Al_2O_3$ and ZnO- $Al_2O_3$ mixed oxides supported on SBA-15 were evaluated for transesterification reaction. Figure 14 shows our best results to date, when experiments were conducted with a methanol: JCO molar ratio of 6:1, 250ºC, and 3 wt % of catalyst. Results were collected after 1 h of reaction time. Under these conditions, the equilibrium biodiesel yield (83%) was reached for the supported $Al_2O_3$ catalysts. Importantly, no catalysts deactivation was observed for at least 10 runs (without regeneration treatment). It is noteworthy that $Al_2O_3$is traditionally used as support instead of active phase due to its poor catalytic activity for transesterification [33]. In fact, in our experiments $Al_2O_3$ itself showed no more than 5% of FAME yield, but the it showed a totally different catalytic performance when it was well dispersed on SBA-15. On the other hand, several supported basic catalysts have also been reported in the literature -sodium [33] or potassium [34] loaded on a support (normally alumina), using several precursors and treated at high calcination temperatures (500–600ºC). The catalysts showed good activities (80-90 % biodiesel yield) at low temperatures (70-90ºC), but no data were reported about their stability. $K_2CO_3$ supported on both MgO and $Al_2O_3$ provided good results for rapeseed oil transesterification with methanol at 60–63 ºC, but $K_2CO_3$ leached into the solution.

Meantime, pure ZnO and ZnO supported on $Al_2O_3$ have also been reported as good transesterification catalyst. In experiments performed in a packed-bed reactor at 225-230 ºC, 91.4% and 94.3% of FAME yields were obtained, for 1 and 7 h, respectively [35]; in this case, no Zn leaching was practically observed (5 ppm). In addition, no data about catalysts has been reported. In our case, experiments conducted with ZnO and ZnO/$Al_2O_3$ showed biodiesel yields below 75 %. The most promising results found for the $Al_2O_3$/SBA15 have to be studied in detail to optimize the catalyst formulation.

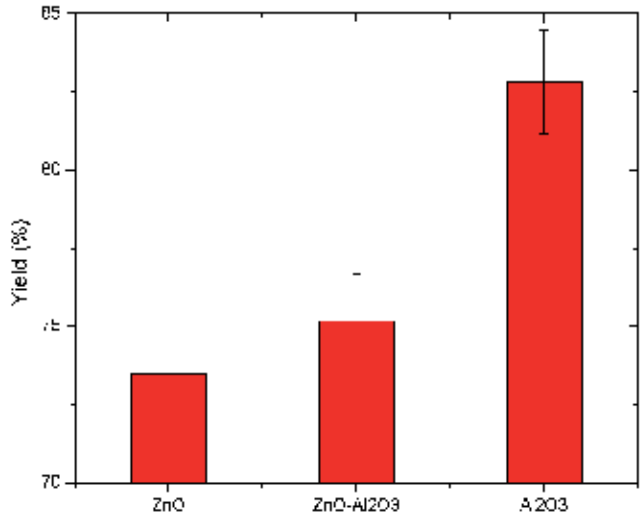


**Figure 14.** Yield of biodiesel of transesterification of JCO with MR=6, and 250ºC, 1 h and 3 wt.% of each heterogeneous catalyst.

*Sonotransesterification*

Experimental results of JCO sonotransesterification are shown in Figure 15. The first issue that became evident was that sonotransesterification was much faster than the conventional alkaline transesterification. Thus, in just 1 minute of reaction time the maximum FAME yield (*ca*. 65%) was reached for the experiment conducted with a methanol: JCO molar ratio of 6:1, an acoustic power density (N) of 105 $Wcm^{-2}$ and temperature of 25ºC. Moreover, Figure 16 shows that for 1 min of reaction time, a reduction of the methanol: JCO molar ratio from 6:1 to 4:1 increased the biodiesel yield. Under these conditions, a 71 % biodiesel yield was obtained. Notoriously, the later molar ratio is closer to the stoichiometric one, thus helping to decrease the excess of alcohol required by the other biodiesel technologies under comparison in this study. These results clearly showed the following advantages for the sonotransesterification process: a shorter processing time is required, a lower amount of alcohol is required (almost the stoichiometric amount), and the experiment is conducted at room temperature and atmospheric pressure.

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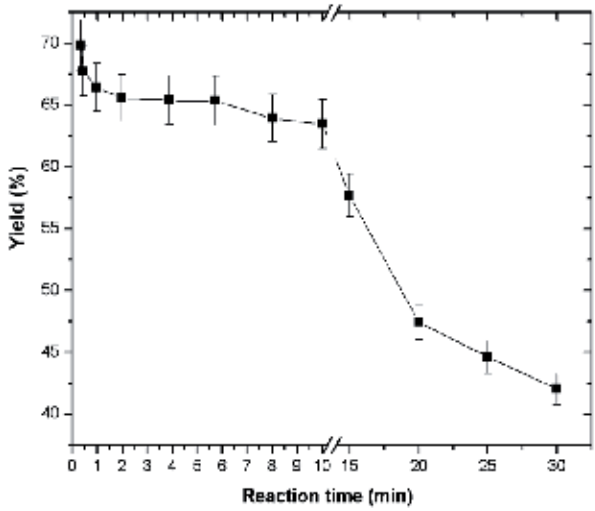


**Figure 15.** Effect of the sonication time on the yield of biodiesel by sonotransterification reaction with MR of 6:1, room temperature, and acoustic power density of 105 $Wcm^{-2}$.

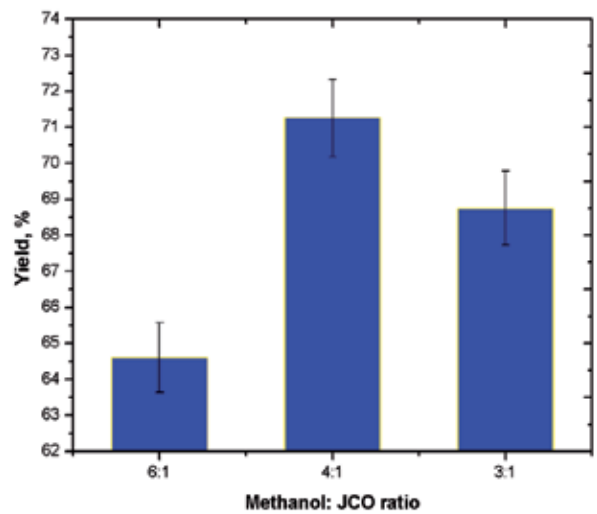


**Figure 16.** Effect of the methanol:JCO molar ratio on the yield of biodiesel by sonotransterification reaction at room temperature,1 min of reaction time, and acoustic power density of 105 $Wcm^{-2}$.

Despite of the important advantages initially found for the sono transesterification process in this work, the biodiesel yield had to be increased to make it atractive from the industrial point of view. To this respect, a more detail study of the acoustic power effect was conducted. Figure 17 shows that acoustic sonocation power had a significant effect on yield. For an N of 64 $Wcm^{-2}$, coupled with the best set of parameters used in previous experiments, a FAME yield up to 96% was reached at room temperature. The reason why a higher transesterification rate was obtained with the ultrasonic process was already outlined in the previous sections. Briefly, the huge local temperature generated in the "hot spot" formed during the cavitation phenomenon favors the formation of highly reactive species and promotes mass transfer. These issues are the key to improve the transesterification reaction rate because under the experimental ultrasonic conditions the process is not affected by mass transfer or by kinetic limitations, but rather by the equilibrium condition.

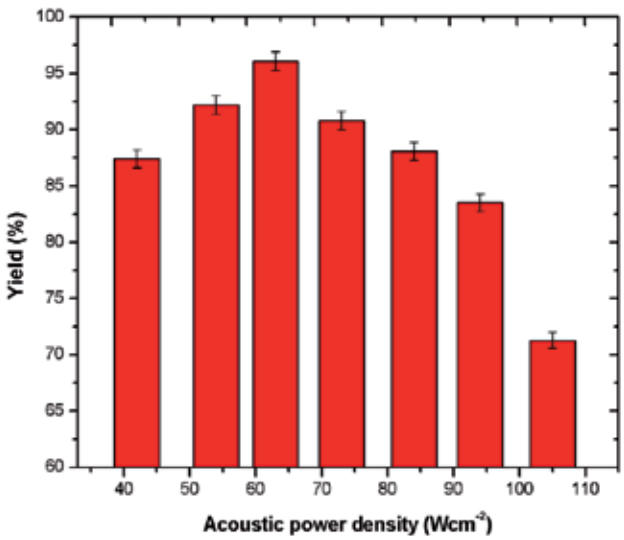


**Figure 17.** Effect of the acoustic power density on the yield of biodiesel by sonotransterification reaction with MR of 4:1, room temperature, and one minute of reaction time.

In this scenario, sono transesterification becomes a very attractive process to be implemented in a continuous industrial process. Thus, results found in Figure 17 were used to configure a continuous US process with a tubular sonorreactor, using a resident time of 1 min. In this case, a constant yield of 96% was reached. Importantly, the quality of the biodiesel obtained in this experiment, overcame the quality of biodiesel with international standards (Table 7).

| Parameter | Value |
|---|---|
| FAME content[a] (%) | 98 |
| Density at 15ºC ($gml^{-1}$) | 0.84 |
| Acid index ($mgKOHg^{-1}$) | 0.5 |
| Total glycerin (wt. %) | <0.2 |
| Free glycerin (wt. %) | <0.02 |

**Table 7.** Physical chemical properties of biodiesel obtained by sonotransesterification under continuous process with optimized conditions operated at room temperature. [a] after purification process

## 6. Conclusions

Nowadays, the conversion of non-edible and residual biomass feedstock into biofuels is already considered a suitable alternative for the generation of alternative energy sources. In particular, transesterification of oils and fats is a well-known technology, and the production of biodiesel is continuously growing using second- and third-generation biomass raw materials. The main technology used in the industrial production of biodiesel is based on the alkaline transesterification of vegetable oils with methanol. However, the problems related with this technology (mainly in operating conditions and product purification) are the driving force for research in the field of heterogeneous catalysis for biodiesel production and for the development of non-catalytic process under supercritical fluids. The use of heterogeneous catalysts and supercritical methanol process for transesterification reaction seem to be attractive for industrial application because theses simpler processes have a beneficial impact in the process economy. In particular, industry is making great research efforts to find the optimum catalyst formulation and to decrease the drastic operating conditions of supercritical methanol process. However, these technologies are still far to be economically attractive. A more recent alternative is the new ultrasound-assisted method for biodiesel production, which has to be tested and optimization for this particular application.

In this work, we evaluated and compared the performance of four technologies for the transesterification of JCO obtained from JC grown in Northwest Mexico: conventional alkaline catalyst (KOH), heterogeneous powder catalysts (ZnO, $ZnO/Al_2O_3$ and $Al_2O_3$/SBA 15), supercritical methanol and sonotransesterification. Results showed that the ultrasonic method has significant advantages as compared to the other three methods. Notoriously, ultrasonication reduced the transesterification reaction time to 1 min at room temperature and atmospheric pressure, as compared to 1-6 h in conventional processing under more drastic operating conditions. We suggest that this result could be explained with the proposed sonotransesterification cavitational model where by diffusional problems are eliminated. Our results demonstrated that acoustic power density and methanol: JOCmolratio are the most sensitive parameters to increase FAME yield for JCO; at the best set of experimental conditions, the biodiesel yield is higher than that obtained by conventional methods. Importantly, the ultrasound-assisted method was also effectively used for continuous production of bio-

diesel by using a plug flow reactor; the physicochemical properties of the biodiesel produced, such as acid value, density, FAME content, total and free glycerin were within the limits of ASTM and EN standards.

In summary, sonotransesterification is faster and easier to handle than conventional transesterification processes. The sonoreactor is significantly cheaper, and the process works under safer and less energy demanding conditions (e.g room temperature and ambient pressure). The major advantages of the current ultrasonic system include operational simplicity, short reaction time, high conversion and reusability. In summary, ultrasonic irradiation is a faster alternative that leads to higher product yield, and with the real possibility to benefit the process economy. Thus, the ultrasonic process discussed in this work established the basis for the development a sustainable process for biodiesel production, although some issues are still to be solved; for instance, if water is avoided in the purification process,the overall process would be even more environmentally friendly.

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Chapter 8

# Lipase Applications in Biodiesel Production

Sevil Yücel, Pınar Terzioğlu and Didem Özçimen

Additional information is available at the end of the chapter

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## 1. Introduction

Because of the global warming and depletion of fossil fuels, in recent years, intensive investigations are carried on for providing the greater use of sustainable biofuels instead of fossil fuels. Biomass, which various biofuels are produced from, has an important role among other alternative energy sources including wind energy, solar energy, geothermal energy, etc.

Biodiesel is one of the important biofuels and a clean energy source as an alternative to petroleum-based diesel fuels. Biodiesel has some advantages and disadvantages. Transportability, high combustion efficiency, low sulphur and aromatic content, high cetane number and biodegradability are advantages of the biodiesel [1]. Disadvantages of biodiesel are high viscosity, lower energy content, high cloud and pour point, high nitrogen oxide emission, lower engine speed and power, injector cooking, high price and engine erosion [2].

The flash point of biodiesel is higher than diesel fuel. This feature is important for fuel storage and transportation in the way of safety. Cetane number of biodiesel (~50) is higher than diesel fuel [3]. Biodiesel does not include aromatic and sulphur content and contains oxygen at the rate of 10-11% by mass [4]. Cetane number is an important factor to determine the quality of diesel fuel, especially ignition quality of diesel fuel. In other words, it determines the ignition tendency of fuel when being injected into engine. Ignition quality of biodiesel is determined by the structure of methyl ester [5].Viscosity is also an important factor for biodiesel. Viscosity affects mostly fuel injection equipment and the increase of fuel viscosity changes the viscosity at low temperatures. High viscosity has an negative effect on fuel spray atomization [6]. Amounts of elements and compounds in biodiesel and diesel fuel are present in Table 1 [7]. Biodiesel has more polar structure than diesel fuel because of the oxygen, which is an electronegative element present in its structure, and therefore biodiesel has higher viscosity comparing with diesel fuel. In addition, elemental oxygen content is responsible for lower heating value of biodiesel when compared with diesel fuel. [7-9]. Biodiesel

can be used in its pure form or when mixed with diesel fuel in certain proportions. Most common biodiesel blends are B2 (2 % biodiesel, 98 % diesel), B5 (5 % biodiesel, 95% diesel), B20 (20 % biodiesel, 80 % diesel) [10].

| | Biodiesel Content (%) | Diesel Content (%) |
|---|---|---|
| Carbon | 79.6 | 86.4 |
| Hydrogen | 10.5 | 13.6 |
| Oxygen | 8.6 | - |
| Nitrogen | 1.3 | - |
| C/H | 7.6 | 6.5 |
| n-Aliphatics | 15.2 | 67.4 |
| Olephenics | 84.7 | 3.4 |
| Aromatics | - | 20.1 |
| Naphtens | - | 9.1 |

**Table 1.** The comparison of elemental and chemical content of diesel and biodiesel [7]

The transesterification reaction can be influenced by several factors including molar ratio of alcohol, catalyst, presence of water, free fatty acid in oil samples, temperature, time and agitation speed. In this context, an understanding of the factors affecting the process is very important to make economically and environmentally biodiesel production [11].

To accelerate reaction rate, transesterification process is carried out in the presence of catalysts. So, biodiesel production is made by using chemical or enzymatic catalysts. Compared to chemical, enzymatic reaction is more attractive because of ability of make a high quality product, simplify the separation of products, mild reaction conditions, the reuse of the catalyst and especially environmental impact, although high conversion and reaction rate are obtained with chemical catalysts [11-14]. Lipase is important enzyme catalyst that catalyzes esterification and transesterification reaction to produce methyl esters (biodiesel). Figure 1 presents the enzymatic transesterification reaction [15].

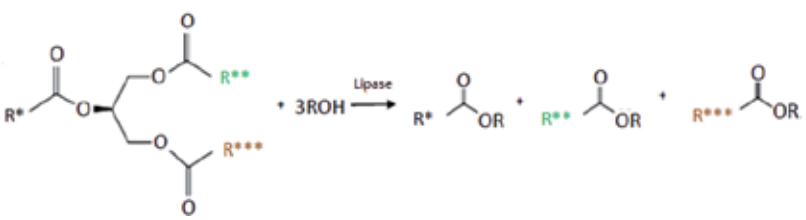


**Figure 1.** Enzymatic transesterification reaction [15].

In this study, enzymatic approach for biodiesel production was reviewed, and especially the usage of lipases in biodiesel production and factors affecting the effectiveness of lipase in reaction were explained in detail.

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## 2. Lipases in biodiesel production

Biocatalyst based biotechnological applications are receiving increasing attention. Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are the important biocatalysts because of their excellent biochemical and physiological properties. Lipases are the hydrolytic enzymes that can be used in various industrial applications for alcoholysis, acidolysis, amynolysis and hydrolysis reactions. Biodiesel production is one of the stunning applications of lipase. Lipase catalyzed biodiesel production was reported first by Mittelbach [16]. Lipase-catalyzed transesterification takes place in two steps, which involves hydrolysis of the ester bond and esterification with the second substrate [15]. A ping-ping bi bi mechanism generally used for kinetic studies of enzyme catalyzed transesterification.

Lipases can be isolated from many species of plants (papaya latex, oat seed lipase, and castor seed lipase), animals (pig's and human pancreatic lipases), bacteria, filamentous fungi and yeast [17-19]. For industrial enzyme production generally microorganisms are preferred because of their shortest generation time [20]. The other advantages of microorganisms can be listed as high yield of conversion of substrate into product, great versatility to environmental conditions and, simplicity in genetic manipulation and in cultivation conditions [20]. Although lipases from different sources are able to catalyze the same reaction, bacterial and fungal lipases are mostly used in biodiesel production such as *Aspergillus niger,Candida antarctica, Candida rugosa, Chromobacterium Viscosum, Mucor miehei, Pseudomonas cepacia, Pseudomonas fluorescens, Photobacterium lipolyticum, Rhizopus oryzae, Streptomyces sp., and Thermomyces lanuginose* [21]. *Candida rugosa*, obtained from yeast, is the most used microorganism for lipase production [22]. Recently, *Streptomyces sp.* was investigated as a potent lipase producing microbe for biodiesel production and found applicable in the field of biodiesel [23].

Specificity of lipases has a great importance in the selection of the usage area of lipases. Lipases can be divided into three groups due to their specificity as 1,3-specific lipases, fatty acid-specific lipases and nonspecific lipases. Especially, 1,3-specific lipases which release fatty acids from positions 1 and 3 of a glyceride and hydrolyze ester bonds in these positions such as *Aspergillus niger, Rhizopus oryzae and Mucor miehei* catalyze transesterification reactions efficiently [20,24]. The study of Du et al. [25], showed that higher yield (90%) was achieved for biodiesel production by using a sn-1,3-specific lipase, *Thermomyces lanuginosa* immobilized on silica gel (Lipozyme TL IM). Thus, the use of sn-1,3- specific lipases can give rise to biodiesel yield of above 90% under appropriate conditions [24]. Substrate specificity of lipases is also a crucial factor towards the biodiesel production which acts on the choice of the proper enzyme based on the composition of raw materials by consisting in the capability of distinguishing structural features of acyl chains [20,24]. Lipases from *Pseudomonas fluorescens, Pseudomonas cepacia, Candida rugosa, Candida antarctica and Candida cylindracea* are suitable for transesterification reaction by displaying both wide substrate specificity and regiospecificity [24].

### 2.1. Immobilization of lipases

The immobilization of enzymes, which is attracting worldwide attention, was firstly reported in 1971 at Enzyme Engineering Conference [26]. During the past decade, chemical modification, physical modification, and gene expression techniques have been developed to obtain more economic, active, selective, or stable lipases. Immobilization is a modification method that can be defined as attaching the enzyme onto an insoluble solid support material [18]. By immobilization more operational and temperature stable lipases can be obtained and also lipases can be reused in the reactions. In addition, reusability of lipases will be a possible solution to the high cost of the enzymes and make them suitable for applications in industrial scale. The comparison of free enzymes and immobilized enzymes is given in Table 2. Methods for enzyme immobilization can be classified as adsorption, covalent bonding, entrapment, and cross-linking. The selection of method and support material is a prominent factor for obtaining an efficient lipase. The results of comparative studies revealed that the same lipase molecule can show very different catalytic activities after immobilization onto different supports [27].

| Characteristics | Free Enzyme | Immobilized Enzyme |
|---|---|---|
| Price | High | Low |
| Efficiency | Low | High |
| Activity | Unstable | Stable |
| Reusability and recovery | Not possible | Possible |
| Tolerance to temperature, pH, etc. | Low | High |
| To separate from the substrate | Difficult | Easy |
| To separate from the product | Difficult | Easy |

**Table 2.** The comparison of free enzyme and immobilized enzyme [19]

#### *2.1.1. Adsorption technique*

Adsorption is the adhesion of lipase on the surface of the adsorbent by weak forces, such as van der Walls, ionic and hydrophobic interactions, or dispersion forces [28]. Immobilization via adsorption method is the simply mixing of an aqueous solution of enzyme with the carrier material for a period and washing away the excess enzyme from the immobilized enzyme on the carrier after a time [29]. The level of adsorption is strictly related to the pH, temperature and ionic strength. Adsorption is the most widely employed method besides

other methods because of its special commercial advantages and simplicity. Adsorption is the only reversible enzyme immobilization method. The advantages of adsorption is mild and easy preparing conditions, low cost, no need for chemical additives, the carrier can be recovered for repeated use, and high activity [30].

Various types of carriers used in immobilization of lipases. Acrylic resin, celite, polypropylene and textile membrane are broadly used carriers. Some of the reported results of adsorption technique based immobilized enzymes used in biodiesel production are summarized in Table 3. As can be seen from table generally the biodiesel yields using the enzymes obtained by adsorption method are higher than 85%. Novozym 435 is a commercial lipase, which is obtained by immobilization of *Candida antartica* lipase on acrylic resin and is a good catalyst that provides biodiesel yield higher than 90% with vegetable oil or waste cooking oil as feedstock [31]. The other commercialized lipase is known as *Candida sp.* 99–125 lipase immobilized on textile membrane, which can catalyze lard, waste oil and vegetable oils with higher yields that is more than 87% [31]. Besides many advantages of immobilization by adsorption method, the main disadvantage is that desorption of the lipase from the carrier occurs because of the weak interactions between the enzyme and support.

### *2.1.2. Covalent binding technique*

Another approach is covalent binding technique, which is the formation of covalent bonds between the aldehyde groups of support surface and active amino acid residues on the surface of the enzyme [29]. A variety of supports have been used such inorganic materials, natural polymers (agarose, chitin and chitosan), synthetic polymers (hydrophobic polypeptides,nylon fibers) and Eupergit® (made by copolymerization of N,N′-methylene-bis-(methacrylamide), glycidyl methacrylate, allyl glycidyl ether and methacrylamide) for immobilization of lipases by covalent binding [56].The main advantage of covalent binding method is obtaining thermal and operational stable enzymes because of strong interactions between the lipase and the carrier [31]. The comparison of biodiesel production performance using immobilized lipase via covalent binding method is summarized in Table 4. Chitosan is a promising carrier as a natural polymer due to its membrane forming and adhesion ability, high mechanical strength and facility of forming insoluble in water thermally and chemically inert films [57]. Xie and Wang [58], reported a technique for immobilization of *Candida rugosa* lipase on magnetic chitosan microspheres for transesterification of soybean oil.The immobilized enzyme was determined as an effective biocatalyst for the transesterification reaction due to giving a good conversion of soybean oil and retaining its activity during the four cycles [58].

Using two immobilized lipases with complementary position specificity instead of one lipase is a new approach to produce a cost effective biodiesel [19]. Lipase from *Rhizopus orizae* and *Candida rugosa* was covalently bound to the silica, which was used to produce biodiesel from crude canola oil. Under optimum conditions, the conversion rate of degummed crude canola oil to fatty acid methyl esters was 88.9%, which is higher than the conversion obtained by free enzyme mixture (84.25%) [59].

| Lipase Source | Carrier | Acid/Oil Source | Alcohol | Maximum Performance (%) | Reference |
|---|---|---|---|---|---|
| *Burkholderia sp. C20* | Alkyl-functionalized $Fe_3O_4$–$SiO_2$ | Olive | Methanol | 92 (conversion) | [32] |
| *Candida antartica* | Acrylic resin | Soybean | Methanol | 92(yield) | [33] |
| *Candida antartica* | Acrylic resin | Soybean and rapeseed | Methanol | 98.4 (conversion) | [34] |
| *Candida antartica* | Acrylic resin | Soybean and rapeseed | Methanol | "/>95 (conversion) | [35] |
| Candida antarctica B | Granular activated carbon | Palm | Isobutanol | 100 (conversion) | [36] |
| *Candida sp. 99–125* | Textile membrane | Lard | Methanol | 87.4 (yield) | [37] |
| *Candida sp. 99–125* | Textile (cotton) membrane | Salad | Methanol | 96 (conversion) | [38] |
| *Candida sp. 99–125* | Textile membrane | Crude rice bran | Methanol | 87.4 ( yield) | [39] |
| *Candida rugosa and Pseudomonas fluorescens* | Acurel | Palm | Ethanol | 89 (yield) | [40] |
| *Chromoacterlum viscosum* | Celite-545 | Jatropha | Ethanol | 92 (yield) | [41] |
| *Geobacillus thermocatenulatus* | Poly-hydroxybutyrate beads | Babassu | Ethanol | 100 (yield) | [42] |
| *Pseudomonas aeroginosa* | Celite | Soybean | Methanol | 80(yield) | [43] |
| *Pseudomonas cepacia* | Celite | Jatropha | Ethanol | 98 (yield) | [44] |
| *Pseudomonas cepacia* | Electrospun polyacrylonitrile fibers | Rapeseed | n-butanol | 94 (conversion) | [45] |
| *Pseudomonas cepacia* | Polystyrene | Sapium sebiferum | Methanol | 96.22 (yield) | [46] |
| *Pseudomonas cepacia* | Ceramic beads | Waste cooking | Methanol | 40 (yield) | [47] |
| *Pseudomonas fluorescens* | Porous kaolinite particle | Triglyceride triolein | 1-propanol | "/>90 (conversion) | [48] |
| Pseudomonas fluorescens and Pseudomonas cepacia | Polypropylene powder | Soybean | Methanol | 58 37 ( yield) | [49] |
| *Penicillium expansum* | Resin D4020 | Waste | Methanol | 92.8 (yield) | [50] |
| *Rhizomucor miehei* | Hydrophilic resins | Olive husk | Ethanol | - | [51] |
| *Rhizomucor miehei* | Silica | Waste cooking | Methanol | 91.08 (yield) | [52] |
| *Rhizopus oryzae* | Macroporous resin HPD-400 | Pistacia chinensis bge seed | Methanol | 94 (yield) | [53] |
| *Saccharomyces cerevisiae* | Mg–Al hydrotalcite | Rape | Methanol | 96 (conversion) | [5454] |
| *Thermomyces lanuginosus (Lipozyme TL IM)* | Hydrotalcite | Waste cooking | Methanol | 95 (yield) | [55] |

**Table 3.** Comparison of biodiesel production performance using immobilized lipase via adsorption method

| Lipase Source | Carrier | Acid/Oil Source | Alcohol | Maximum Performance (%) | Reference |
|---|---|---|---|---|---|
| *Burkholderia cepacia* | Niobium Oxide ($Nb_2O_5$) | Babassu | Ethanol | 74.13 (yield) | [60] |
| *Burkholderia cepacia* | Polysiloxane–Polyvinyl Alcohol ($SiO_2$–Pva) | Babassu<br>Beef Tallow | Ethanol | 100<br>89.7 (yield) | [60] |
| *Candida rugosa* | Chitosan Microspheres | Soybean | Methanol | 87 (conversion) | [58] |
| *Candida rugosa* | Chitosan Powder | Rapeseed Soapstock | Methanol | 95 (conversion) | [61] |
| *Enterobacter aerogenes* | Silica | Jatropha | Methanol | 94 (yield) | [62] |
| *Porcine pancreatic* | Chitosan Beads | Salicornia | Methanol | 55 (conversion) | [63] |
| *Pseudomonas fluorescens* | Toyopearl Af-Amino-650m Resin | Babassu | Ethanol | 94.9 (yield) | [64] |
| *Rhizopus oryzae* | Resin Amberlite Ira-93 | Pistacia Chinensis Bge Seed | Methanol | 92 (yield) | [63] |
| *Rhizopus oryzae* | Polystyrene Polymer(Amberlite Ira-93) | Soybean | Methanol | 90.05 (yield) | [65] |
| *Rhizopus Orizae +Candida rugosa* | Silica | - | Methanol | "/>98 (conversion) | [66] |
| Rhizopus orizae +Candida rugosa | Silica | Crude Canola | Methanol | 88.9 (conversion) | [59] |
| *Thermomyces lanuginosus* | Olive Pomace | Pomace | Methanol | 93 (yield) | [67] |
| *Thermomyces lanuginosus* | Polyglutaraldehyde Activated Styrene-Divinylbenzene Copolymer | Canola | Methanol | 97 (yield) | [68] |
| *Thermomyces lanuginosus* | Toyopearl Af-Amino-650m Resin | Palm | Ethanol | 100 (yield) | [64] |
| *Thermomyces lanuginosus* | Polyurethane Foam | Canola | Methanol | 90 (yield) | [69] |
| *Thermomyces lanuginosus* | Aldehyde-Lewatit | Soybean | Ethanol | 100 (conversion) | [70] |
| *Thermomyces lanuginosus* | Magnetic $Fe_3O_4$ Nano-Particles | Soybean | Methanol | 90 (conversion) | [71] |

**Table 4.** Comparison of biodiesel production performance using immobilized lipase via covalent binding method

### *2.1.3. Entrapment technique*

Entrapment method is based on capturing of the lipase within a polymer network that retains the enzyme but allows the substrate and products to pass through [72]. This method can be simply defined as mixing an enzyme with a polymer solution and then crosslinking the polymer to form a lattice structure that captures the enzyme [29]. Entrapment is often used for industrial applications because the method is fast, cheap and can be carried out under mild conditions [73]. Entrapment can be divided into three categories such as gel or fiber entrapping and microencapsulation [74]. A number of supports have been investigated such as alginate, celite, carrageenan, resins, acrylic polymers etc. Some carriers used for entrapment and the biodiesel production yields obtained by these enzymes are displayed in Table 5. A disadvantage of entrapment method is the mass transfer problem due to the act of support as a barrier, so the lipase became effective only for low molecular weight substrates [19,75].

| **Lipase Source** | **Carrier** | **Acid/Oil Source** | **Alcohol** | **Maximum Performance (%)** | **Reference** |
|---|---|---|---|---|---|
| Burkholderia cepacia | *K*-Carrageenan | Palm | Methanol | 100 (conversion) | [76] |
| Burkholderia cepacia | Phyllosilicate Sol–Gel | Tallow and Grease | Ethanol | 94 (yield) | [77] |
| Burkholderia cepacia | Mtms-Based Silica Monolith Coated With Butyl-Substituted Silicates | Jatropha | Methanol | 95 (yield) | [78] |
| *Candida antarctica* | Celite® | Triolein | Methanol | 60 (conversion) | [79] |
| *Candida rugosa* | Calcium Alginate Matrix | Palm | Ethanol | 83 (yield) | [80] |
| *Candida rugosa* | Activated Carbon | Palm | Ethanol | 85 (conversion) | [81] |
| Pseudomonas cepacia | Hydrophobic Sol–Gel | Soybean | Methanol | 67 (conversion) | [82] |
| *Pseudomonas fluorescens* Mtcc 103 | Alginate | Jatropha | Methanol | 72 (yield) | [83] |
| Via Encapsulation Method | | | | | |
| Burkholderia cepacia | Silica Aerogels | Sunflower Seed | - | 56 (conversion) | [84] |
| Burkholderia cepacia | *K*-Carrageenan | Palm | Methanol | 100 (conversion) | [85] |
| Candida antartica | Silica Aerogels | Sunflower Seed | Methanol | 90 (conversion) | [86] |

**Table 5.** Comparison of biodiesel production performance using immobilized lipase via entrapment method

*2.1.4. Cross linking technique*

Cross-linking is another method for immobilization that can be defined as the interaction of a three dimensional network within enzyme, coupling reagent, and carrier [19]. The advantage of cross-linking is obtaining stable lipases due to the strong interaction between the lipase and the carrier. On the other hand, the cross-linking conditions are intense and the immobilized lipase shows lower activity [31].

The high free fatty acid content of waste cooking oil form water by esterification with alcohol which cause agglomeration of lipase and lowering biocatalysis efficiency [87]. Hence, free *Geotrichum sp.* lipase was not a suitable enzyme catalyst for transesterification of waste cooking oil. Yan et al. [87], report a modification procedure for preparation of cross-linked *Geotrichum sp.* The obtained lipase exhibited improved pH and thermostable stability compared to free lipase. The relative biodiesel yield was 85% for transesterification of waste cooking oil with methanol.

Kumari et al. [88] studied the preparation of *Pseudomonas cepacia* lipase cross-linked enzyme aggregates. It was shown that cross linked lipases has a greater stability than free enzymes to the denaturing conditions. The enzyme also used to catalyze madhuca indica oil, which's transesterification is difficult by chemical routes due to its high free fatty acid content. As a result, 92% conversion was obtained after 2.5 h.

Immobilization of *Candida rugosa* lipase on fine powder of Scirpus grossus L.f. by glutaraldehyde by cross linked technique for biodiesel production from palm oil, as already investigated by Kensingh et al. [89]. It was concluded that immobilized lipase yielded higher conversion of biodiesel than that of free lipase.

Lorena et al. [90] investigated the immobilization of the *Alcaligenes spp.* lipase on polyethylenimine agarose, glutaraldehyde agarose, octyl agarose, glyoxyl agarose, Sepabeads® by the aggregation and crosslinking method. The transesterification of canola oil was achieved with a yield 80% using a six-step addition of methanol and lipase immobilized on Sepabeads® by the aggregation method.

All these methods are shown schematically in Figure 2.

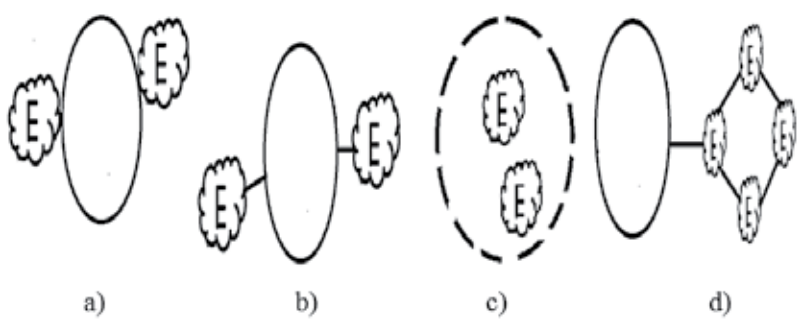


**Figure 2.** Schematic diagram of enzyme immobilization methods: a)Adsorption method b)Covalent binding method c)Entrapment method d) Crosslinking method

*2.1.5. Whole cell immobilization*

The applicability of lipases for the bulk production of fuels was limited significantly by the high cost of lipases [91]. Utilizing microbial cells such as fungi, bacteria, and yeasts cells containing intracellular lipase instead of extracellular lipases (free and immobilized lipase) is an easier and a cost effective way of enzymatic biodiesel production. Compared to conventional enzymatic processes, the use of whole cells provides excellent operational stability and avoids the complex procedures of isolation, purification and immobilization [91,92]. The general preparation steps for immobilized extracellular enzymes and whole cell enzymes showed in Figure 3. Biomass support particles have been used for immobilization of whole cells.

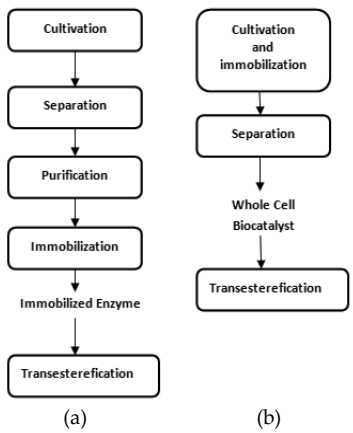


**Figure 3.** The preparation steps of a) immobilized extracellular lipase and b) whole cell biocatalyst

Aspergillus and Rhizopus have been most widely used as whole cell biocatalyst. Ban et al. [93], used first a whole cell biocatalyst, immobilized Rhizopus oryzae IFO4697 (a 1,3-positional specificity lipase) cells within biomass support particles, for the production of biodiesel and 91.1% methyl ester content was attained which was a similar result as that using the extracellular lipase. Many researchers have experimented on the use of whole cells to catalyze transesterification reaction summarized in Table 6.

A technique using glutaraldehyde cross-linking treatment on whole cell catalyst for methanolysis of soybean oil was developed by Sun et al. [94]. The glutaraldehyde cross linking treatment resulted in higher methanol tolerance and high catalytic activity (with the ratio of methanol to oil reaching 3). Also, a novel methanol addition strategy was proposed as stepwise addition of different amounts of methanol (1.0, 1.2, 1.5, and 2.0M equivalent of oil) every 24 h. It was found that the highest methyl ester yield could reach 94.1% after 24 h reaction by 1.2 mol, 1.5 M and 1.2 mol methanol additions at 0, 8, and 14 h. In general, the whole cell catalyzed process is slower than extracellular lipase catalyzed process. Sun et al. [94], also reported that the reaction time could be shortened by this way. It is clear that significant reduction in the cost of biodiesel production can be achieved by combining the whole cell biocatalyst process with stepwise addition of methanol.

| Lipase Source | Carrier | Acid/Oil Source | Alcohol | Maximum Performance (%) | Reference |
|---|---|---|---|---|---|
| Aspergillus niger | BSPs[a] | Waste Cooking | Methanol | 86.4 (yield) | [96] |
| *Aspergillus niger* | Polyurethane BSPs[a] | Palm | Methanol | >90 (yield) | [97] |
| Aspergillus niger | BSPs[a] | Palm | Methanol | 87 (yield) | [98] |
| Aspergillus oryzae NS4 | BSPs[a] | Soybean | Methanol | 98 (conversion) | [99] |
| *A. oryzae carrying* r-CALB[b] | BSPs[a] | Palm Soybean | Methanol | 85 90 (conversion) | [100] |
| *Aspergillus oryzae expressing r-FHL*[c] | BSPs[a] | Rapeseed | Methanol Ethanol 1-Propanol 1-Butanol | 96 (yield) 94 (yield) 96 (yield) 97 (yield) | [101] |
| *Escherichia coli* BL21 | - | Rapeseed | Methanol | 97.7 (conversion) | [102] |
| *Rhizopus chinensis* CCTCC M201021 | - | Soybean | Methanol | "/>86 (yield) | [103] |
| *Rhizomucor miehei displaying* Pichia pastoris | - | Soybean | Methanol | 83.14 (yield) | [104] |
| *Rhizopus oryzae IFO 4697* | BSPs[a] | Refined Rapeseed Crude Rapeseed, Acidified Rapeseed | Methanol | ~60(yield) ~60(yield) ~70(yield) | [105] |
| *Rhizopus oryzae IFO 4697* | BSPs[a] | Soybean | Methanol | ~90(yield) | [106] |
| *Rhizopus oryzae IFO 4697* | BSPs[a] | Soybean | Methanol | ~85(yield) | [107] |
| *Rhizopus oryzae IFO4697* | - | Soybean | Methanol | 71 (conversion) | [108] |
| *Rhizopus oryzae IFO 4697* and Aspergillus oryzae niaD300 (combined use) | BSPs[a] | Soybean | Methanol | ~100 (conversion) | [109] |

| Lipase Source | Carrier | Acid/Oil Source | Alcohol | Maximum Performance (%) | Reference |
|---|---|---|---|---|---|
| *Rhizopus oryzae ATCC 24563* | - | Soybean (Free Fatty Acid Content 5.5%) | Methanol | 97 (conversion) | [110] |
| *Rhizopus oryzae IFO 4697* | BSPs[a] | Soybean | Methanol | 72 (yield) | [111] |
| *Rhizopus oryzae* | Polyurethane foam BSPs[a] | Soybean | Methanol | 90 (conversion) | [112] |
| *Rhizopus oryzae* | BSPs[a] | Jatropha Curcas | Methanol | 80 (conversion) | [113] |
| *Rhizopus oryzae IFO 4697* | - | Soybean | Methanol | 86 (yield) | [114] |
| *Rhizopus oryzae* | BSPs[a] | Rapeseed | Methanol, Ethanol, 1-Propanol, 1-Butanol | 83, 79, 93, 69 (yield) | [101] |
| Serratia marcescens YXJ-1002 | - | Grease | Methanol | 97 (yield) | [115] |

[a]BSPs:Biomass support particles

[b]r-CALB: Candida antarctica lipase B

[c]r-FHL: Fusarium heterosporum lipase

**Table 6.** Comparison of biodiesel production performance using whole cell biocatalysts

Whole cell biocatalysts will be a way to industrialization of biodiesel production but the limited mass transfer efficiency of product and substrate is a hurdle to further investigations [95].

## 3. Feedstocks

The main aim of researches is to obtain a biodiesel, which will have a competitive price compared to other conventional sources of energy [116]. At this point, selecting the feedstock, represents more than 75-80% of the overall biodiesel production cost, is a vital step to ensure a cost effective biodiesel production. Different kinds of feedstock with varied range of edible and inedible vegetable oil, animal fats, waste oil, microbial oil and microalgae oil can be used for enzyme catalyzed transesterification [117].

### 3.1. Vegetable oils

Vegetable oils are candidates as alternative fuels for diesel engines with their high heat content [118]. But, direct use of vegetable oils is not possible because of the high kinematics viscosity of them which are varies in the range of 30–40 cSt at 38 °C and are about 10 times higher than of diesel fuel (Grade No. 2D) leads to many problems [118,119]. Therefore, modification of vegetable oil is necessary and the valuable product of this modification is named ''biodiesel''. The edible vegetable oils such as soybean [120,121], sunflower [122-124], palm [81,125], corn [126], cottonseed [127], canola [68,69,128] and olive [129,130] oils have been widely used in enzymatic transesterification. In developed countries, edible oils constitute more than 95% of biodiesel production feedstock because the produced biodiesel from these oils have properties very similar to petroleum-based diesel [131]. Also, the country and its climate, the oil percentage and the yield per hectare are effective parameters in selecting the potential renewable feedstock of fuel [118,132]. For example, while rapeseed oil prevailing the EU production, soybean oil prevailing the US and Latin American production, and palm oil mainly being used in Asia [133].

Inedible oils do not find a place in human consumption due to including toxic components. Therefore, inedible oils do not compete with food crops. Thus, inedible vegetable oils are an alternative feedstock for biodiesel production. Babassu (Orbinya martiana), Jatropha curcas (Linnaeus), neem (Azadiracta indica), polanga (Calophyllum inophyllum),karanja (Pongamia pinnata), rubber seed tree (Hevea brasiliensis), mahua (Madhuca indica and Madhuca longifolia), tobacco (Nicotina tabacum), silk cotton tree, etc. are promising inedible vegetable oil sources. Jatropha curcas is an attractive feedstock between various oil bearing seeds as it has been developed scientifically and found to give better biodiesel yield and productivity [134]. Crude Jatropha oil contains about 14% of free fatty acid that is too high for alkaline catalyzed biodiesel production [118]. However, high free acid content is not a problem in the production process of biodiesel via using enzyme catalysts. Besides Jatropha curcas, 26 species of fatty acid methyl ester of oils of including Azadirachta indica, Calophyllum inophyllum, and Pongamia pinnata were found most suitable for use as biodiesel, which adjust to the major specification of biodiesel standards of European Standard Organization, Germany, and USA [135]. Modi et al. reported conversion of crude oils of Pongamia pinnata (karanj), Jatropha curcas (jatropha) via immobilized Novozym 435 to biodiesel fuel with yield 90, and 92.7%, respectively [136].

### 3.2. Animal oils/fats

Animal fats are another group of feedstock for biodiesel production. Animal fats used to produce biodiesel via enzymatic route include lard [137], lamb meet [138] and beef tallow [139]. Animal fats are economically feasible feedstocks compared to vegetable oils. Animal fat methyl ester also has many favorably properties such as non-corrosive, high cetane number, and renewable [140,141]. However, animal fats saturated compounds lead to a tendency to oxidation and crystallization unacceptably at high temperatures [142].

### 3.3. Waste oils/fats

In general, around the world only half of the discharged edible oils recycled as animal feed or as raw material for lubricant and paint and the remainder is discharged into the environment [143]. Hence, the use of waste oils/fats for biodiesel production is very important to reduce and recycle the waste oil [143], to eliminate the environment and human health risk caused by waste oils [144] and to lower the biodiesel production cost. Waste cooking oil, animal fats, yellow grease, brown grease obtained from highly oxidized yellow grease or recovered waste grease from plumbing trap and waste sludge or soap-stock from the vegetable oil refining process were the major sources of waste oil have been used for biodiesel production [145]. The selection of a catalyst to be used for the production of biodiesel fuel is mostly influenced by the amount of free fatty acid content in various feedstocks [146]. The lipase-catalyzed reaction is a promising method for converting waste oils which contains high percentage of free fatty acids and high water content, into biodiesel with high yield [145]. It has been reported that Novozym 435 is capable of converting the used olive oils [129].

### 3.4. Algae oils

There is a considerable interest in the use of algae (micro and macro) oils for synthesis of biodiesel. Because these oils are cheap raw materials besides animal fats and have rapid growth rate and productivity when compared to conventional forestry, agricultural crops, high lipid content, tolerance for poor quality water, smaller land usage up to 49 or 132 times less when compared to rapeseed or soybean crops [142,147]. The smaller land usage brings the advantage of reducing the competition for arable soil with other crops, in particular for human consumption [147]. However, there are still some drawbacks for utilization of algae for biodiesel production. A considerable investment in technological development and technical expertise is needed to optimize the microalgae harvesting and oil extraction processes, to use cheap sources of $CO_2$ for culture enrichment [147]. Algae oils contain about 20-40% oil [148]. Several researchers have been experimented on microalgal oils as raw material for biodiesel production. Tran et al. [130], investigated the conversion of microalgal oil from Chlorella vulgaris ESP-31 to biodiesel by using immobilized Burkholderia lipase and a high fatty acid methyl esters conversion efficiency of 97.25 wt% oil (or 58.35 wt % biomass) was obtained for 48 h reaction. It is proposed that microalgal oil has good potential for application in the commercial production of biodiesel. The enzymatic conversion of microalgal oils to biodiesel in ionic liquids was firstly studied by Lai et al. [149]. Four microalgae two strains of Botryococcus braunii (BB763 and BB764), Chlorella vulgaris, and Chlorella pyrenoidosa have been catalyzed by two immobilized lipases, Penicillium expansum lipase and Candida antarctica lipase B (Novozym 435), in two solvent systems: an ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate, [BMIm][PF6]) and an organic solvent (tert-butanol). Penicillium expansum lipase was found more efficient for this application and the ionic liquid [BMIm] [PF6] showed a greater conversion yield (90.7% and 86.2%) obtained relative to the one obtained in the commonly used organic solvent tert-butanol (48.6% and 44.4%).

## 4. The effect of reaction parameterson enzymatic transesterification

### 4.1. The effect of temperature on enzymatic transesterification

Enzymatic transesterification takes place at low temperatures varying from 25 to 60°C. In general, initially the rate of reaction increases with rise in reaction temperature, because of an increase in rate constants with temperature and less mass transfer limitations [150,151]. Nevertheless, increased temperature after the optimum temperature promotes to denaturation and higher thermal deactivation of the enzyme, since it decreased the catalytic activity [152].

Various researches have been carried out to find out the effect of temperature on biodiesel production with immobilized enzymes. It is clear that immobilization provide more temperature resistance compared to free enzymes due to supplying a more rigid external backbone for lipase molecule [150,151]. However, optimum temperature is specific for each production. The studies about the effect of temperature for enzymatic transesterification are shown in Table 7.

| Lipase | Oil Source | Alcohol | Performed Temperatures In The Range (°C) | Optimum Temperature (°C) | Reference |
|---|---|---|---|---|---|
| Immobilized Aspergillus niger | Palm | Methanol | 25-50 | 40 | [153] |
| Immobilized Aspergillus niger | Waste Cooking | Methanol | 25-50 | 30 | [154] |
| Immobilized Burkholderia cepacia | Babassu | Ethanol | 39-56 | 39 | [155] |
| Candida antarctica | Cotton Seed | T-Butanol | 30-50 | 50 | [156] |
| Candida antarctica | Acid | Methanol | 30-50 | 30 | [157] |
| Immobilized *Candida* Sp. 99–125 | Salad | Methanol | 27-50 | 40 | [158] |
| Candida Sp. 99–125 | Waste Cooking | Methanol | 35-50 | 40-50 | [159] |
| Immobilized Enterobacter aerogenes | Jatropha | T-Butanol | 30-55 | 55 | [160] |
| Immobilized Enterobacter aerogenes | Crude Rapeseed | Ethanol | 25-50 | 35 | [161] |
| Lipozyme RM IM | Soybean | Butanol | 20-50 | 30 | [162] |
| Lipozyme RM IM | Soybean | Methanol and Ethanol | 40–60 | 50 | [163] |
| Lipozyme RM IM | Soybean Oil Deodorizer Distillate | Ethanol | 45-78 | 50 | [164] |
| Lipozyme TL IM | Rapeseed | N-Butanol | 30-60 | 40 | [165] |
| Lipozyme TL IM | Soybean | Ethanol | 20-50 | 35 | [162] |

| Lipase | Oil Source | Alcohol | Performed Temperatures In The Range (°C) | Optimum Temperature (°C) | Reference |
|---|---|---|---|---|---|
| Lipozyme TL IM | Palm | Ethanol | 30-78 | 50 | [166] |
| Novozyme 435 | Rapeseed | Methanol | 25-55 | 40 | [167] |
| Novozyme 435 | Tung and Palm | Methanol and Ethanol | 45-55 | 55 | [168] |
| Novozym 435 | Cottonseed | -(Dimethyl Carbonate As Organic Solvent) | 30-55 | 50 | [169] |
| Novozym 435 | Canalo | Methanol | 25-65 | 38 | [170] |
| Novozym 435 | Olive | Methanol | 30-70 | 40 | [129] |
| Novozym 435 | Soybean | T-Amyl | 30-60 | 40 | [171] |
| Novozym 435 | Sunflower | Methanol | 25-65 | 45 | [172] |
| Novozym 435 | Stillingia | Methanol | 30-60 | 40 | [173] |
| Novozym 435 | Cotton Seed | Methanol | 30-70 | 50 | [174] |
| Novozym 435, Lipozyme TL IM and Lipozyme RM IM | Soybean | Ethanol | 25-60 | 25 | [175] |
| Immobilized Penicillium expansum | Waste | T-Amyl | 25-55 | 35 | [176] |
| Immobilized Pseudomonas cepacia | Soybean | Methanol and Ethanol | 25–60 | 35 | [177] |
| Pseudomonas cepacia | Soybean | Methanol | 20–60 | 30 | [178] |
| Immobilized Pseudomonas fluorescens | Triolein | 1-Propanol | 40-70 | 60 | [48] |
| Pseudomonas fluorescens | Soybean | Methanol | 30-60 | 40 | [49] |
| Rhizopus chinensis CCTCC M201021 | Soybean | Methanol | 30-40 | 30 | [179] |
| Thermomyces lanuginosus | Canola | Methanol | 30-70 | 40 | [69] |

**Table 7.** Data on optimum temperature for enzymatic biodiesel production

### 4.2. The effect of water content on enzymatic transesterification

Water content is one of the key factors for enzymatic transesterification reaction that have a strong effect on lipase's active three-dimensional conformational state [21,180]. Biocatalysts, needs a small amount of water to retain their activities [181]. Lipase has an unique feature on the water-oil interface, and the lipase activity depends on this interface. The presence of an oil–water interface required because it provides a suitable environment for enzyme activation which occurs due to the unmasking and restructuring the active site through confor-

mational changes of the lipase molecule [182,183]. When the addition of water increased, the amount of water available for oil to form oil–water droplets also increases, hence increasing the available interfacial area [182]. Thus, enzymatic activity can not be possible in a water free media. However, excess water cause reverse reaction of hydrolysis. The amount of required water, to provide an optimum enzyme activity, differs according to the type of enzyme and reaction medium composition. Enzymes, substrates, organic solvent and also immobilized support have a crucial role on optimal water activity for lipase [184]. Optimum water content not only provides keeping the hydrolysis of ester linkages at the minimum level, but also ensures the highest degree of transesterification [24]. Thus, a better control of water content is very important for enzymatic process.

Water activity ($a_w$) is defined as free (boundness) water in the system, which is a ratio of vapor pressure over the given system versus that over pure water [24]. Thermodynamic water activity is the best predictor of reaction rate that can be determined in any phase by different kinds of sensors such as holographic sensor, Weiss LiCl humidity sensor [180,185]. Also, several methods have been developed for control of water activity, for example, equilibration with saturated salt solutions [186], addition of salt hydrate pairs [187,188] and introduction of air or nitrogen into the reactor [189]. Recently, Peterson et. al. developed a practical way for control of water activity in large-scale enzymatic reactions by using a programmable logic controller. On the other hand, percentage water content is another expression which is used widely in transesterification, generally assayed by Karl-Fischer coulometer.

In general, lipases show higher activity with higher water activities in solvent free systems instead of Candida antarctica lipase (Novozym 435) [184]. For Candida sp. 99–125 lipase, the optimum water content is 10–20% based on the oil weight to maintain the highest transesterification activity [31].

Salis et al., investigated production of oleic acid alkyl esters by using Pseudomonas cepacia and determined that $a_w$ in the range 0.4–0.6, 1-butanol:triolein 3:1 – were the best conditions to reach maximum enzymatic activity. It was also found that at the higher values of water activity, no hydrolysis reaction was occurred [190].

Noureddini and Philkana [82] tested immobilized Pseudomonas cepacia for the transesterification of soybean oil with methanol and ethanol and observed that increased addition of water provide a considerable increase in the ester yield. The optimal conditions were determined for processing 10 g of soybean oil by 475 mg lipase in 1 h as 1:7.5 oil/methanol molar ratio, 0.5 g water in the presence of methanol that resulted in 67 % yield and 1:15.2 oil/ethanol molar ratio, 0.3 g water in the presence of ethanol that resulted in 65% yield.

Al-Zuhair et al. studied the esterification of n-butyric acid with methanol in the presence of Mucor miehei lipase, and found similar results with literature [191] that higher water content, makes lipase more efficient [182].

Shah and Gupta used immobilized Pseudomonas cepacia lipase for ethanolysis of Jatropha oil and noted that the best yield 98% gained by in the presence of 4–5% (w/w) water in 8 h. The yield was only 70% in absence of water [44].

Kawakami et al. determined the effect of water content for transesterification of Jatropha oil and methanol to characterize Burkholderia cepacia lipase immobilized in an n-butyl-substituted hydrophobic silica monolith. The authors reported that biodiesel yield reached 90% with water content of 0.6% (w/w) after 12 h using a stoichiometric mixture of methanol and oil (3:1) [78].

Chen et al. investigated the effect of water content for production of biodiesel with oleic acid with methanol catalyzed by soluble lipase NS81020, produced by modification of Aspergillus oryzae microorganism, in the biphasic aqueous-oil systems and found that the esterification yield is low if the water was scant. The higher reaction rate and fatty acid methyl ester yield was obtained with 10 wt % water by oleic acid weight [192].

It is clear that during the past decade numerous investigations have been made to determine the optimal water content for transesterification. As a result, the necessary amount of water content is an important factor to create an interfacial surface between oil and water and to ensure optimal enzymatic activity. Also, water has a strong influence on structural integrity, active site polarity, and protein stability of lipase [21,193]. However, it differs from enzyme to reaction conditions.

### 4.3. The effect of acyl acceptors on enzymatic transesterification

Methanol, short chain alcohol, usually used as an acyl acceptor due to its low price and availability. Insoluble and a relatively high amount of methanol with respect to oil, have a negative influence on the stability of lipases and could be solved by a stepwise addition of the alcohols [15, 194]. To eliminate inhibitory effects of methanol some co-solvents are added to the reaction mixture. Tert-butanol is one of the important co-solvents which is added to enzymatic reaction. Usage of tert-butanol, a polar solvent, is also a possible solution for eliminating the inhibitory effects of methanol and glycerol (both of them soluble in tert-butanol) and suggested instead of using butanol [195]. Liu et al. [196], transesterified waste baked duck oil by three different commercial immobilized lipases (Novozym 435, Lipozyme TLIM and Lipozyme RMIM) with different monohydric alcohols (methanol, ethanol, propanol, isopropanol, isobutanol, isoamyl alcohol) and fusel oil-like alcohol mixture (containing 15% isobutanol, 80% isoamyl alcohol, 5% methanol) in solvent-free and tert-butanol systems. It was reported that each lipase presented a different kinetic pattern depending on the monohydric alcohols. The results showed that Lipozyme TL IM and Novozym 435 gave high conversion rate with isobutanol and isoamyl alcohol either in solvent-free or in tert-butanol system. Thus, the combined use of lipases, Novozym 435 and Lipozyme TLIM, as catalyst and fusel oil-like mixture as raw material for biodiesel synthesis was found effective in view of cost saving of biodiesel production [195].

Recently, novel acyl acceptors were investigated such as ethyl acetate, methyl acetate, butyl acetate, vinyl acetate [197], dimethyl carbonate [198]. Du and coworkers demonstrated the positive effect of methyl acetate, on enzymatic activity of Novozym 435 and found that lipase could be reused directly without any additional treatment [199]. The advantage of using methyl acetate is that the cost of the catalyst can be reduced dramatically due to the longer operational life and reusability of lipase. The byproduct of the system is triacetylgly-

cerol, which does not have any negative effect on the fuel property, and also no glycerol produced [200]. Hence, these advantages will provide industrial implementation of enzymatic biodiesel production. Dimethyl carbonate is another promising alternative acyl acceptor, which is eco-friendly, odorless, cheap, non-corrosive, and non-toxic [200]. The transesterification reaction is irreversible, because carbonic acid monoacyl ester, the intermediate compound, immediately decomposes to carbon dioxide and alcohol [200]. The fatty acid methyl ester yield is higher for lipase-catalyzed transesterification of vegetable oils with dimethyl carbonate besides conventional acyl acceptors (methanol and methyl acetate) [200]. Only, the higher price of acyl acceptor besides alcohols is a disadvantage [194].

### 4.4. Effects of the solvent on enzymatic transesterification reaction

In enzymatic transesterification reaction, excess of alcohol increases reaction efficiency, but if alcohol doesn't dissolve in reaction medium it can disrupt the enzyme activity. Methanol and vegetable oil in the values close to 1:1 molar ratio forms a solution in 40°C. Solvent is added into the reaction medium to increase the solubility of alcohol and thus it allows first step enzymatic transesterification by blocking degradation lipase catalytic activity [24]. To overcome deactivation of lipase activity and improve the lipase activity, various organic solvents have been used for enzymatic biodiesel synthesis. These solvents have been listed in Table 8. Cyclohexane, n-hexane, tert-butanol, petroleum ether, isooctane and 1,4-dioxane are mainly studied hydrophilic and hydrophobic organic solvents in enzymatic biodiesel production. In organic solvent medium, overall alcohol is added at the beginning of the reaction. In solvent free reaction medium, alcohol is added in several portions to prevent enzyme activity with high alcohol concentration [24].

Hexane is generally preferred because of its low cost and easily availability in the market. Some studies were performed in hexane solvent systems with soybean and tallow oil using monohydric alcohols [70,201, 202]. Nelson et al. performed transesterification of tallow with monohydric alcohols by Lipozyme IM 60 (M. miehei) and Novozyme SP435 (C. antarctica) in hexane and a solvent-free system. They compared the transesterification yields of two different systems. The yields with higher than 95% were obtained with methanol, ethanol and butanol with Lipozyme IM 60 lipase under hexane system (Table 8) while reaction yields under solvent-free system were 19% for methanol, 65.5% for ethanol, and 97.4% for isobutanol [201]. Similar results were found by Rodrigues et al. [70]. They compared the yields of transesterification of soybean with ethanol by Lipozyme TL IM. In the presence of n-hexane with 7.5:1 molar ratio of ethanol:soybean oil, the transesterification conversion was found to be as 100% while in solvent-free system the yield was 75%. At stoichiometric molar ratio, the yield was 70% conversion after 10 h of reaction in both systems. Transesterification conversion was obtained as 80% by three stepwise addition of ethanol, while a two step ethanolysis produced 100% conversion after 10 h of reaction in both solvent and solvent-free systems.

In enzyme catalyzed reaction, both alcohol amount and low glycerol solubility in biodiesel have negative effects on enzyme activity. Deposit of glycerol coating the immobilized catalyst is formed during the process, which reduces the enzymes activity [203]. The solubility of

methanol and glycerol in hydrophobic solvents is low. For this reason, this problem may occur in hydrophobic solvent system.

The enzymatic alcoholysis of triglyceride also was studied with petroleum ether, isooctane, cyclo hexane,1,4-dioxane (Table 4-2) [16,48,204]. Iso et al. [48], reported that when methanol and ethanol were used as alcohol in enzymatic transesterification, the reactions need an appropriate organic solvent [48]. On the other hand, the reaction could be performed without solvent when 1-propanol and 1-butanol was used. They also used, benzene, chloroform and tetrahydrofuran as solvent and immobilized P. fluorescens lipase as catalyst at 50°C to compare the results that of the 1,4 dioxane. The highest enzymatic activity was observed with 1,4-dioxane. The enzymatic activity increased with the high amount of 1,4-dioxane. But high conversion of oil (app.90%) to biodiesel was obtained with high proportion of 1,4 dioxane(90%). Although usage of high amount of solvents is not preferable in industry solvents can be recovered together with methanol after transesterifiation reaction.

Hydrophilic organic solvents can interact with water molecule in enzyme and this may affect the catalytic activity of enzyme. However, as shown in Table 8 high performance was ensured with hydrophilic solvents such as 1,4-dioxane and tert-butanol [48,156, 205-208]. Some studies were performed in the presence of t-butanol solvent because of positive effects on enzymatic catalyzed reaction. T-butanol has moderate polarity so methanol and glycerol are easily soluble in tertiary butanol. Solubility of methanol prevents enzyme inhibition and solubility of glycerol prevents accumulation on the enzyme carrier material. Another advantage of this solvent is sinteric hindrance. Due to this property, tert-butanol is not accepted by the lipase. High yield and conversions were obtained in the presence of t-butanol with various vegetable oils and immobilized lipases shown in Table 4-2. For example, Liu et al., [196] studied biodiesel synthesis by immobilized lipases in solvent-free and tert-butanol media. Each lipase showed a different conversion depending on the monohydric alcohols and immobilized lipase in solvent-free medium and tert-butanol system. For methanolysis, regardless of the lipase type, the conversion rate is higher in tert-butanol than that in solvent-free medium. Novozym 435 showed higher conversion rate with straight monoalcohols in tert-butanol medium. Lipozym RM IM and Lipozyme TL IM showed lower conversion with straight and branched monoalcohols (except methanol) in solvent free system. Similar results were obtained by Halim and Kamaruddin [208], in transesterification of waste cooking palm oil using various commercial lipases (Lipozyme RM IM, Lipozyme TL IM and Novozyme 435) in tert-butanol as reaction medium. Novozyme 435 was found to be more effective in catalyzing the transesterification with methanol in in-tert-butanol medium. It was also been demonstrated that even 3:1 methanol to oil molar ratio didn't inhibit the Novozyme 435 in tert-butanol system. Du et al. [209], showed that Lipozyme TL IM could be used without loss of lipase activity for 200 batches in tert-butanol system. Li et al. [210], used acetonitrileand tert-butanol mixture as co-solvent in transesterfication of stillingia oil with methanol. The highest biodiesel yield (90.57%) was obtained in co-solvent with 40% tert-butanol and 60% acetonitrile (v/v) with co-solvent. They also reported that co-solvent (as a mixture)enhance the tolerance of lipase to the methanol than the pure tert-butanol.

| Solvent | Oil | Alcohol | Lipase | Temp/ Time | Reaction mixture | Performance (%) | Ref. |
|---|---|---|---|---|---|---|---|
| Tert-butanol | Cotton seed | Methanol | Novozyme 435 *(Candida antarctica)* | 50 °C / 24h | 13.5% meth., 54% oil 32.5% tert-butanol, Lipase:1.7% (wt of oil) | 97 (yield) | [156] |
| Tert-butanol | Cotton seed | Methanol | Pancreatic lipase | 37 °C / 4 h | Methanol :oil mol ratio:1:15 Lipase:0.5% enzyme (wt of oil) water conc.5% (wt of oil) | 75–80 (conversion) | [205] |
| Tert-butanol | Rapeseed | Methanol | Novozyme435 & Lipozyme TL IM | 35 °C / 12 h | Methanol: oil mol. ratio 4:1 tert-butanol/oil vol. 1:1 Lipase: 3% Lipozyme TL IM 1% Novozym 435 (wt of oil) | 95 (conversion) | [206] |
| Tert-butanol | Soybean and deodorizer distillate | Methanol | Lipozyme TL IM Novozym 435 | 40°C/ 12 h | Methanol:oil molar ratio 3.6:1 Lipase :3% Lipozyme TL IM 2% Novozym 435 tert-butanol: 80% (wt of oil) | 84 ( yield) | [207] |
| Tert-butanol | Waste cooking palm | Methanol | Novozyme 435 | 40°C / 12 h | Methanol:oil mol. ratio 4:1, Lipase:4% (wt of oil) | 88(yield) | [208] |
| Tert-butanol | Waste baked duck | Methanol | Novozym 435 Lipozyme TL IM | 45 °C / 20 h | Methanol:oil mol. ratio 4:1, Lipase: 5 wt%(wt of oil) | 85.4, 78.5, (conversion) | [196] |
| Hexane | Tallow | Methanol Ethanol Propanol | Lipozyme IM 60 | 45 C/ 5 h | 0.34 M tallow in hexane (8 mL), Lipase: 10 (wt of oil) 200rpm | 94.8, 98.0, 98.5 (conversion ) | [201] |

| Solvent | Oil | Alcohol | Lipase | Temp/ Time | Reaction mixture | Performance (%) | Ref. |
|---|---|---|---|---|---|---|---|
| Hexane | Soybean | Methanol | Lipozyme IM 77 | 36.5°C/ 3h | Methanol :oil mol ratio:3.4:1 Lipase:0.9BAUN*of lipase; water 5.8% (wt% of oil) | 92.2 (yield) | [202] |
| Hexane | Soybean | Ethanol | Lipozyme TL IM | 30 °C/ 10 h | Ethanol:oil mol.ratio:7.5:1 Lipase: 15 %(wt of oil). 4% water | 100 (conversion) | [70] |
| Cyclo hexane | Sunflower | Methanol | Lipase AK Lipozyme TL IM Lipozyme RM IM | 40°C/ 24 h | Volume of organic solvent/ oil: 2 ml/0.2 mmol Lipase: 10% (wt of oil) | 65, 75, 35 (conversion) | [204] |
| Acetonitrile 60%and 40% t-butanol (v/v) | Stillingia | Methanol | Novozym 435 and Lipozyme TL IM | 40°C/ 24h | Methanol:oil mol ratio: 6.4:1 Lipase: 4% (w/w) of multiple-lipase (1.96% Novozym 435+2.04% Lipozyme TL IM) | 90.57 (yield) | [210] |
| Petroleum ether | Sunflower | Ethanol | Lipozyme IM Lipase AK | 45°C / 5h | Ethanol:oil mol. ratio:11:1 Lipase:20% (wt of oil) | 82, 99, (yield) | [16] |
| I-octane | Sunflower | Methanol | Lipase AK Lipozyme TL, IM Lipozyme RM,IM | 40 °C | Methanol: oil mol ratio::3:1 Vol. of organic solvent/oil: 2 ml/0.2 mmol | 80, 65, 60, (yield) | [204] |
| 1,4-dioxane | Triolein | Methanol | Lipase AK | 50°C / 80h | Methanol:oil mol. ratio: 3:1 90% solvent | ~70 (conversion) | [48] |

*BAUN:Batch Acidolysis Units Novo

**Table 8.** Effect of the solvent on the performance of enzymatic transesterification reaction

Although positive effects of the usage of the solvents on the transesterification reaction, some drawbacks has also been known) such as; extra reactor volume, solvent toxicity and emissions, solvent recovery and loss cost [133].

### 4.5. The effect of molar ratio of alcohol to oil on enzymatic transesterification

Biodiesel yield always increased due to the molar excess of alcohol over fatty acids in triglycerides in traditional transesterification system [15]. The transesterification reaction is reversible and so, an increase in the amount of one of the reactants will result in higher ester yield and minimally 3 molar equivalents of methanol are required for the complete conversion of methyl ester [174]. Conversely, for enzyme catalyzed transesterification, insoluble excess methanol which exists as fine droplets demonstrates negative effects on enzyme activity and also decrease the production yield [211]. The reaction medium is an important factor during the determination of the optimum molar of alcohol to oil. The inactivation of lipases occurs by contact with insoluble alcohol because the highly hydrophilic alcohol eliminates the layer of essential water from the enzymes [212]. Thus, stepwise addition of alcohol is a potential approach for ratio optimizing the molar ratio in solvent free systems [15]. Whilst, higher reaction rates could be obtained with a slight excess of alcohol in organic solvent systems [15].

The two-step reaction system was reported to avoid the inactivation of the lipase by addition of excess amounts of methanol in the first-step reaction, and by addition of vegetable oil and glycerol in the second-step reaction [213]. Watanabe et al. [213], used a two-step reaction system for methyl esterification of free fatty acids and methanolysis of triacylglycerols using immobilized Candida antarctica lipase. The first step reaction was methyl esterification of free fatty acids that was performed by treating a mixture of 66 wt % acid oil and 34 wt % methanol with 1 wt % immobilized lipase. The second step reaction was conducted to convert triacylglycerols to fatty acid methyl esters. In this step, a mixture of 52.3 wt % dehydrated first-step product, 42.2 wt% rapeseed oil, and 5.5 wt% methanol using 6 wt% immobilized lipase in the presence of additional 10 wt % glycerol was treated. The contents of fatty acid methyl esters was 91.1wt.% after the second step reaction was repeated by the use of immobilized lipase for 50 cycles using recovered glycerol.

Moreno-Pirajan and Giraldo [81], added different amounts of alcohol varied from 2.7 to 13.7 molar equivalents for methanol and from 5.7 to 26.7 molar equivalents for ethanol, based on the moles of triglycerides toward the transesterification of palm oil catalyzed by Candida rugosa lipase and 10.4 molar ratio for all alcohols to palm oil was determined as optimal alcohol requirement resulted in 85 mol% of methyl esters yield with n-butanol.

Lipase catalyzed esterification of palmitic acid with ethanol in the presence of Lipozyme IM 20 in a solvent free medium was investigated by Vieira et al. [212]. Different acid/alcohol molar ratios were tried as 0.16, 0.5, 1.0, 1.5, and 1.84. The best result was obtained with 0.5 acid/alcohol molar ratio.

Zaidi et al. [214], explained the correlation existing between the kinetic parameters and the chain-length of the substrates in esterification of oleic acid using nylon-immobilized lipase in n-hexane. It is observed that the inhibition coefficient of the alcohol increased from 0.034

to 0.42 mol $l^{-1}$, when the number of carbon atoms increased from 1(methanol) to 18 (oleyl alcohol), respectively.

Dizge and Keskinler [69], used immobilized Thermomyces lanuginosus lipase to produce biodiesel with canola oil with methanol and investigated the role of substrate molar ratio. The biodiesel production was conducted at 1:1, 1:2,1:3,1:4,1:5;1:6 and 1:10 oil/alcohol molar ratios at 40°C. The highest methyl ester yield (85.8%) was obtained at the oil/methanol molar ratio of 1:6. Two important result from this study can be concluded as (i) an increase in the number of moles of methanol resulted in an increase in the ester production, (ii) when the formation of esters reached a maximum level the further increases in the methanol concentrations cause a decrease in the formation of esters due to enzyme inactivation.

Thus, the actual amount of alcohol needed varies significantly depending on the origin of the lipase and fat.

## 5. Reactors for enzymatic transesterification

Through the industrialization of enzymatic biodiesel production, it is necessary to show the applicability of enzymes in reactor systems. Various reactors, including batch reactors, packed bed reactors and supercritical reactors have been investigated by researchers. Most of the investigations on enzymatic synthesis of biodiesel have been performed in batch reactors and packed bed reactors.

Batch reactors are simple designs used in the laboratory. In batch reactors, methanol shows a good dispersion in the oil phase. But the physical agitation caused by shear stress from the stirring would disrupt the enzyme carrier which shortens the enzymes life [31]. On the other hand, batch operation is labor intensive, and not suitable for automation [215]. Packed bed reactors are alternative of batch reactors which are substantially faster and more economical continuous reactors [216]. A packed-bed reactor system is most widely used in biotechnology, as it is easy to operate and scale up these systems. In addition, these systems have high bed volume. The most important advantage of these systems is that it is lowering shear stress on immobilized enzymes which leads to long-term enzyme stability [217]. Furthermore, stepwise addition of alcohol can be performed to reduce the inactivation of the enzyme caused by excess alcohol. One of the encountered problems with an immobilized lipase is the inhibition of the enzyme due to the cloggage of the catalyst by accumulation of the glycerol by-product inside the reactor [218]. Also, the separation of glycerol which remains in the bottom of the reactor can be achieved in a simple way by using more than one column. Recently, a packed-bed reactor system, in which a reactant solution is pumped through a column containing biomass support particles immobilized recombinant Aspergillus oryzae and the effluent from the column is recycled into the same column with a stepwise addition of methanol was developed by Yoshida et al. [219]. In this system, lipase retains its activity for five batch cycles and 96.1% methyl ester content was obtained with a residence time of 140 min per pass and stepwise addition of 4.25 molar equivalents of methanol to oil for 6 passes. The methanolysis of soybean oil in packed bed reactor system using

Rhizopus oryzae whole cell was studied by Hama et al. [112]. The final methyl ester content was over 90% at a flow rate of 25 l/h in the first cycle and also, after 10 cycles approximately 80% conversion was achieved. Wang et al. [216], developed Pseudomonas cepacia lipase – $Fe_3O_4$ nanoparticle biocomposite based packed bed reactors. A single-packed-bed reactor and the four-packed-bed reactor were used to produce biodiesel by using refined soybean oil. A high conversion rate (over 88%, 192 h) and great stability was achieved with the four-packed-bed reactor compared to single-packed-bed reactor. It is considered that the four-packed-bed reactor supplied a longer residence time of the reaction mixture in the reactor and lowered the inhibition of the lipase by products [216]. By this way, the reaction efficiency was improved. Additionally, the cost of biodiesel production can be reduced by the effective recycling of the enzyme catalysts [184].

Supercritical reactors also have been investigated by researchers for enzymatic biodiesel production. D. Oliveira and J. V. Oliveira [220], produced biodiesel from palm kernel oil in the presence of Novozym 435 and Lipozyme IM in supercritical carbon dioxide in the temperature range of 40–70 °C and from 60 to 200 bar using a water concentration of 0–10 wt % and oil/ethanol molar ratios from 1:3 to 1:10. Lipozyme IM showed better results and the highest reaction conversion was obtained as 77.5 %. It was observed that lipase structure changed at pressures beyond 200 bar. Madras et al. [221], synthesized biodiesel from sunflower oil in supercritical carbon dioxide catalyzed by Novozym. However, the obtained conversions, when the reaction was conducted in supercritical methanol and ethanol at the optimum conditions, were 23 and 27%, respectively [221]. Enzymatic transesterification of lamb meat fat in supercritical carbon dioxide was investigated by Taher et al. [222].The maximum conversion (49.2%) was obtained at 50°C, with 50% Novozym 435 loading, 4:1 molar ratio, within 25 h reaction. Supercritical reactors could not commercialized according to the low conversion rate and cost of the system.

Consequently, packed bed reactor systems seem to be a practical transesterification reactor system with high transesterification efficiency. These systems will bring industrial scale up enzymatic biodiesel production in an economic way.

## 6. Conclusion

Today, the growing energy necessity and environmental pollution problem requires the use of renewable alternative energy sources to become less dependent on fossil resources. As known, biodiesel is an important alternative energy resource and seems to be the fuel of future because it is an environmentally friendly, nontoxic, renewable, and biodegradable fuel.

Conventionally, biodiesel production is achieved by mainly alkaline or acid catalysts. The interest in the use of biocatalyst for biodiesel production has been an increasing trend due to its many advantages.

Biodiesel have been shown to be effectively produced by enzymatic catalyst and also, numerous researches have been performed to obtain highly active lipases and to optimize

process conditions for biodiesel production. Besides many advantages, to produce biodiesel by enzyme catalysts on an industrial scale, it is necessary to reduce the high cost of enzymes and obtain lipases with better features. The immobilization of lipases and genetic engineering methods seems to be an attractive way to obtain more active, stable, and reusable lipases in organic solvents and alcohols. Also, selection of alternative acyl-acceptors is an option for eliminating the negative effects of methanol on lipase activity.

It can be concluded that in enzyme catalyzed biodiesel production significant progresses have been made but further improvements such as novel reactor design should be addressed and emphasized in the future research in order to ensure industrial enzymatic biodiesel production. By making novel improvements, much attention will be focused on enzyme usage in biodiesel production, and especially lipase reactions will be applied much more in this area.

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Chapter 9

# Non-Catalytic Production of Ethyl Esters Using Supercritical Ethanol in Continuous Mode

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Additional information is available at the end of the chapter

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## 1. Introduction

Development of alternative renewable energy has become necessary because, among other factors, the possible shortage of fossil fuels and environmental problems. Among the renewable resources available for alternative fuel production, the conversion of fats and oils to biodiesel has been investigated and well documented in the literature [1-4].

The merits of biodiesel as an alternative to mineral diesel comprise a nontoxic, biodegradable, domestically produced, and renewable resource. Besides, biodiesel possesses a higher cetane number compared to diesel from petroleum and a favorable combustion emissions profile, such as reduced levels of particulate matter, carbon monoxide, and, under some conditions, nitrogen oxides [5,6]. Because of these environmental benefits, which means reduction of environmental investments, and also due to the relief from reliance on import needs, biodiesel fuel can be expected to become a good alternative to petroleum-based fuel.

The establishment of the Brazilian national program on biodiesel has prompted several studies on biodiesel production using different techniques and a variety of vegetable and animal sources. Methanol has been the most commonly used alcohol to perform transesterification reactions. However, in the Brazilian context, ethanol has been the natural choice since Brazil is one of the world's biggest ethanol producers, with a well-established technology of production and large industrial plant capacity installed throughout the country. Due to the fact that ethanol also comes from a renewable resource, thus, ethanol biodiesel appears as a 100% renewable alternative additionally enabling the replacement of traditionally used methanol by an innocuous reagent [7].

Typical raw materials investigated for the production of biodiesel, include soybean, sunflower, castor, corn, canola, cottonseed, palm, peanuts [1] and more recent studies highlight the use of *Jatropha curcas* oil [8,9]. A fact to be also considered to lower manufacturing costs and make biodiesel competitive, is the use of degummed oils that have lower cost than refined oils, besides the possibility of recycling the waste oils [10,11]. However, the choice of the oilseed to be used must consider the content in vegetable oil, yield and territorial adaptation.

Among other processes used for the production of biofuels from vegetable oils, such as pyrolysis and microemulsification, transesterification is the most common way to produce biodiesel [1,3]. Transesterification, also called alcoholysis, refers to the reaction of a triglyceride (from animal or vegetable source) with an alcohol in the presence or absence of catalyst to form fatty acid alkyl esters (i.e., biodiesel) and glycerol as a byproduct.

The complete transesterification is the reaction of one mole of triglyceride with three moles of alcohol, resulting in the production of 3 moles of esters and 1 mol of glycerol as shown in Figure 1. Transesterification is a reversible reaction which occurs in three steps with formation of intermediate products: diglycerides and monoglycerides.

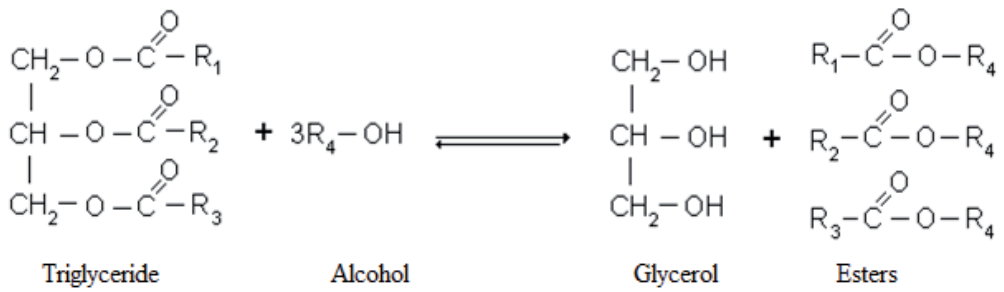


**Figure 1.** Transesterification reaction of a triglyceride with an alcohol.

The transesterification process reduces the average molar mass to approximately 1/3 compared to triglycerides, hence decreasing the viscosity and enhancing the mixture volatility. Unlike the original oil, biodiesel has similar properties and full compatibility with petroleum diesel, accordingly conventional diesel engines can be powered on biodiesel without requiring substantial mechanical modification [12]. After the reaction, the products consist of a mixture of fatty acid esters, glycerol, remainder alcohol, catalyst and a low percentage of tri-, di-and monoglycerides [13].

Among the factors affecting the yield of the transesterification reaction, one can cite: type and amount of catalyst, reaction time, temperature, molar ratio of oil to alcohol, content of free fatty acids and water in the substrates, agitation power, solubility between the phases and nature of the alcohol [10]. However, the extent of variables effect will necessarily depend on the method used [14].

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The homogeneous chemical catalysis (acid or basic) is the most used technique in the transesterification reaction at industrial scale, since it allows, in the case of alkaline catalysis, reaching high conversions at shorter reaction times [15-23].

The chemical method using homogeneous alkali catalysts, although simple, fast and with high yields, presents several drawbacks, such as costs of catalyst separation and difficulty of purification and separation of reaction products, which involves high production costs and energy consumption [24]. Because alkali catalyzed systems are very sensitive to both water and free fatty acids contents, the glycerides and alcohol must be substantially anhydrous. Water makes the reaction partially change to saponification, which produces soaps, thus consuming the catalyst and reducing the catalytic efficiency, as well as causing an increase in viscosity, formation of gels, and difficulty in separations [1,3,25]. As a consequence, the water and free fatty acids content should be less than 0.06% (w/w) and 0.5% (w/w) for transesterification reaction with alkali catalysts, respectively [1,26].

The transesterification reaction using homogeneous acid catalysts is preferred for the conversion of raw materials containing high levels of free fatty acids, because the acid catalyst can promote simultaneously the transesterification of the triglycerides and esterification of the free fatty acids to alkyl esters [27]. Although esterification of free fatty acids may proceed with a relatively high rate and high yields can be achieved, the kinetics of triglycerides transesterification is much slower, requiring high temperatures (above 373 K) and 24 hours of reaction for completion [12].

Thus obtaining of esters in two reaction steps for substrates with high acidity has been proposed, consisting of two approaches: (a) the acid esterification of free fatty acids and subsequent the alkaline transesterification of triglycerides [28-31] or (2) enzymatic hydrolysis of triglycerides, followed by the acid esterification of the fatty acids produced [32-34].

The use of heterogeneous chemical catalysts in alcoholysis of vegetable oils reduces the difficulties of separation of products and catalyst, resulting in the generation of lower effluents volume. The literature suggests the use of various acid and basic catalysts [35-37], with catalysts reuse in the process. However, heterogeneous chemical catalysis generally shows low yields compared to homogeneous alkaline catalysis.

The reaction catalyzed by enzymes (lipases) provides easy separation of catalyst from the reaction medium, catalyst reusability and higher purity of the reaction products. However, to date, the main disadvantages of this method refers to the long reaction times needed and the high cost of the enzymes [14], that progressively are deactivated during reaction course. The enzyme method can be conducted in the presence of organic solvents in order to minimize mass transfer limitations, immiscibility between phases and catalyst deactivation, requiring the use of higher ratios of solvent/vegetable oil (in the order of 40/1) to provide satisfactory reaction rates [38]. For the production of biodiesel in enzyme systems using pressurized solvents, smaller amounts of solvent can be used and the solvent can be easily separated from the reaction medium by system decompression [38-41]. High conversions have been reported for both systems but the use of high enzyme to substrates ratios has hindered large-scale implementation of such technique.

The efficiency of microwave irradiation [42-44] and the use of ultrasonic technology [45-47] in the transesterification of vegetable oils using different catalysts has been reported with the advantage of high reaction rates compared to conventional processes.

Recently, a catalyst-free technique for the transesterification of vegetable oils using an alcohol at supercritical conditions has been proposed, keeping the benefits of fuel quality and taking into account environmental concerns [48-53]. According to the current literature, catalyst-free alcoholysis reactions at high temperature and pressure conditions provide improved phase solubility, decreased mass-transfer limitations, afford higher reaction rates and simpler separation and purification steps [24]. Besides, it has been shown that the so-called supercritical method is more tolerant to the presence of water [54] and free fatty acids [54,55] than the conventional alkali-catalyzed technique, and hence more tolerant to various types of vegetable oils, even for fried and waste oils.

The reaction for biodiesel production at supercritical conditions requires high alcohol to oil molar ratios and the adoption of high temperatures and pressures for the reaction to present satisfactory conversion levels, leading to high processing costs and causing in many cases the degradation of the fatty acid esters formed [56-60] and reaction of glycerol formed with other components of the reaction medium [61-64], hence decreasing the reaction conversion [65-68,57,58]. Current literature shows some alternatives to reduce the expected high operating costs and product degradation, and such strategies usually involve: (i) addition of co-solvents [69-74]; (ii) two-step process with glycerol removal in the first step [75-77]; (iii) two-step process comprising hydrolysis of triglycerides in subcritical water and subsequent esterification of fatty acids [65,66,78]; (iv) use of microreator systems operating in continuous mode [74,79] and use of packed bed reactor [80].

The aim of this work is to provide a brief review on the continuous production of fatty acid ethyl esters (FAEE) by non-catalytic process using ethanol at supercritical conditions. These results are part of a broader project aimed at building a platform to allow the development of a new process for the production of biodiesel from vegetable oils. A section of this chapter will be dedicated to reviewing the characteristics of the supercritical method, comprising the research in the production of FAEE in continuous mode evaluating the role of process variables such as temperature, pressure, molar ratio of oil:ethanol and residence time. This review also focuses on the different configurations of reaction systems, like tubular reactor, microtube reactor, packed bed tubular reactor, as well as the experimental simulation of reactors in series and reactor with recycle. The effect of addition of co-solvent (carbon dioxide), water and free fatty acids to the reaction medium on the FAEE yield are evaluated and decomposition of FAEE produced and conversion of oil to FAEE are also considered.

## 2. Characteristics of the non-catalytic supercritical method for biodiesel production

The transesterification reaction using a solvent at pressurized conditions is one of the methods used for the synthesis of biodiesel [48]. This can be a secure way, without caus-

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ing environmental damage, and requires less investment in the overall process, since the equipment cost is offset by the high reaction rates, better efficiency and lower cost of products purification.

Glisic & Skala [81] reported the economic analysis of the processes for biodiesel production using homogeneous alkaline catalysis and supercritical method, noting that energy consumption is extremely similar in both cases. Since in the supercritical method the heating step involves high energy consumption, costs are compensated by the simpler purification step of the products (esters and glycerol), requiring lower power consumption, which leads to a high costs step of the conventional process. Deshpande et al. [82] reported an economic analysis of the proposed supercritical process and found that the biodiesel processing cost through the proposed technology could be half of that of the actual conventional methods.

The production costs of biodiesel can be minimized by the sale of by-products generated by the transesterification process, such as glycerin. However, when using the conventional method by alkaline catalysis, traces of catalyst can be found in the glycerin, which limits the use of this product. Thus subsequent purification steps are required [83,84], a fact that is not needed in the supercritical method, which proceeds with simple purification and separation of the biofuel produced and generates a high-pure glycerin [48,49,85].

Marchetti & Errazu [85] evaluated different processes for biodiesel production using vegetable oils with high content of free fatty acids, including the supercritical method and stated that the supercritical method is an attractive alternative from a technological point of view. Additionally, from the economic point of view, less wastewater is produced and a high quality glycerin is generated as a byproduct, however higher energy is required by the reaction step.

The reactivity in the supercritical state is higher than in the liquid or gas, which facilitates the transesterification reaction [86]. The supercritical point of ethanol and methanol are 514 K and 6.14 Mpa [27,51] and 513 K and 8.09 Mpa [48], respectively. The non-catalytic production of biodiesel with supercritical alcohol provides high reaction yields, since it promotes the simultaneous hydrolysis and transesterification of triglycerides and esterification of free fatty acids present in vegetable oil [50].

The supercritical method has the following advantages over other methods used for biodiesel production [67]:

**a.** Catalyst is not used in the reaction and purification procedures are much simpler, since the separation process of the catalyst and the saponified product is not required;

**b.** The supercritical reaction requires shorter reaction time than the traditional catalytic transesterification and the conversion rate is high. The catalytic transesterification requires, in some cases, hours to reach the reaction equilibrium, while supercritical method only minutes;

**c.** Low quality substrates of can be used in the supercritical method, since high levels of free fatty acids and water do not have a negative effect on the reaction.

The alcohol in the supercritical state solves or reduces the possible formation of two phases to form a single homogeneous phase, by decreasing the dielectric constant of alcohol in the supercritical state, which results in increased solubility of the oil [24]. Ma & Hanna [1] reported that the solubility of triglycerides in methanol increases at a rate of 2 to 3% (w/w) of 10 K increase in temperature.

Some disadvantages of supercritical method are nevertheless pointed out: high alcohol to oil ratios are required (in the order of 40:1), best results are obtained at temperatures above 573 K and high pressures, typically 20 MPa, which leads to high processing costs and energy consumption. In addition, the quality of biodiesel may be compromised by the low stability of certain fatty acid esters exposed to the drastic reaction conditions required. Thus, due to drastic increase in costs associated with the use of excess alcohol and equipment due to operation at high temperatures and pressures, improvements to the supercritical method for producing biodiesel are required [87].

Kiwjaroun et al. [88] investigated the biodiesel production processes by supercritical methanol combined with and alkaline catalyst and the impacts generated by each process on the environment, using LCA (life cycle analysis) as a tool. It was observed by these researchers that the supercritical method is advantageous compared to conventional method due to the less amount of wastewater generated, however, creates a high impact on the environment, mainly due to the large amount of alcohol used in the process, emphasizing the need for research regarding the reduction in operating conditions (temperature, pressure) and the amount of alcohol used in the process. Marulanda [89] evaluated the potential environmental impact assessment of the process for biodiesel production by non-catalyst supercritical method and conventional base-catalyzed process. The environmental assessment results indicated the supercritical process, even when working at a 42:1 molar ratio, has a lower impact than the conventional base-catalyzed process.

### 2.1. Decomposition

During supercritical transesterification, the high temperatures (above 573 K) employed and long reaction periods, a decrease in the conversion can be observed [7,57,65-68,73].

He et al. [67] evaluated the results obtained for the transesterification of soybean oil in supercritical methanol and concluded that the reason for the decrease in reaction yield is the decrease in the content of unsaturated esters, caused by isomerization, hydrogenation and thermal decomposition that would consume such esters, especially C18:2 (linoleic) and C18:3 (linolenate). Imahara et al. [56] evaluated the thermal stability of different samples of biodiesel and fatty acid esters in different conditions of temperature and pressure. The authors found that thermal degradation is more pronounced for the unsaturated esters above 573 K and 19 MPa and thermal stability of saturated esters is also affected. Kasim et al. [63] report that the percentage of trans isomers can reach levels up to 16% under certain reaction conditions (30 MPa, 573 K) for the transesterification of rice bran oil in methanol.

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At the supercritical reaction conditions, side reactions with the participation of the glycerol formed as byproduct can cause the degradation of other components present in the reaction medium. For instance, Anistescu et al. [61] performed the alcoholysis reactions using supercritical methanol at temperatures around 623-673 K and reported the absence of glycerol in the reaction products, the authors cogitated that reaction of glycerol with other compounds may have occurred. Aimaretti et al. [62] evaluated the reaction of refined soybean oil with supercritical methanol at different reaction conditions and at the conditions studied by the authors, glycerol was not formed. It is reported that glycerol is converted into lower molecular weight products and water at the beginning of the reaction and that water reacts with triglyceride to form free fatty acid, thus increasing the acidity of the product. In the course of the reaction, these fatty acids are converted into methyl esters. Also, the glycerol may react in different ways: (i) decomposition to produce products of lower molecular weight, such as acrolein, acetaldehyde, acetic acid, among others, (ii) polymerization to form polyglycerols, which occur at high temperature conditions and (iii) etherification with methanol to produce ethers of glycerol, thus consuming the alcohol in the reaction medium. Lee et al. [90], in the synthesis of biodiesel from waste canola oil, reported that side reaction was obtained by reacting glycerol and supercritical methanol at 543 K/10 MPa for 15, 30 and 45 minutes. The experimental results showed that these reactions could positively affect the overall biodiesel yield by providing oxygenated compounds such as 3-methoxy-1,2-propanediol, dimethoxymethane, and 2,2-dimethoxypropane as well methyl palmitate and methyl oleate.

In Vieitez et al. [57] a novel and simple GC method was proposed to evaluate de percentage of overall decomposition. Samples were treated with $BF_3$/MeOH [91] to derivatize all of the fatty acids (mono-, di-, and triglycerides, free fatty acids, and also ethyl esters) to the corresponding methyl esters, and then analyzed by GC. For the evaluation of the degradation percentage, palmitic acid was assumed not liable to degradation, considering its high stability, and was taken as reference (as an internal standard "native"). Thus, degradation was estimated as:

$$Decomposition\ (\%) = 100 \times \left[ 1 - \left( \frac{\sum P_i}{P_{16:0}} \right)_S \times \left( \frac{P_{16:0}}{\sum P_i} \right)_O \right]$$

where ΣPi was the summation of all fatty acid methyl ester percentages, P16:0 was the percentage of 16:0 ethyl ester, and subscripts “s” and “o” indicate that the expressions between brackets were evaluated considering the composition of the sample product and the original oil, respectively [57].

The use of the term "decomposition" of fatty acids referred to the decrease in its percentage (determined by gas chromatography) due to the formation of other compounds (not necessarily imply that they have "broken" but have suffered some type of alteration). Since there is no information about the determination of this parameter type, the method described below can be considered a new contribution to the area of the synthesis of biodiesel in supercritical alcohols.

### 2.2. Addition of co-solvent

A question to be considered is the addition of co-solvents to the reaction medium that can provide milder operation conditions, since the use of co-solvents reduces the limitations of mass transfer between phases involved [92] and increases the reaction rate offering an homogeneous reaction media [69,70].

As co-solvents in supercritical transesterification it can be used non-polar compressed gases, for example, carbon dioxide, methane, ethane, propane, n-butane and their mixtures [92]. Some studies have reported the use of heptane/hexane as co-solvent [93-95]. Among these the use of $CO_2$ at supercritical conditions has shown a promising future for environmentally friendly chemical processes, because it comprises a nonflammable solvent, nontoxic, inexpensive and readily available in high purity. Indeed, besides being a good solvent for extraction, carbon dioxide has also proved useful as solvent reaction medium [96]. However, a limiting factor for the use of carbon dioxide is low mutual solubility $CO_2$-triglycerides, which means that high pressures are required to solubilize the reagents [97].

The use of propane and n-butane as compressed solvent or even in the supercritical state seems to be a nice substitute for a variety of solvent in reactive systems. These gases offer as the main advantage the low pressure transitions systems found mainly in vegetable oils due to the higher solubility exhibited compared to that the use of $CO_2$ [97,98]. Pereda et al. [99] reported that the use of propane in the hydrogenation of triglycerides increases the miscibility of the components of the mixture, allowing the reaction to occur under conditions of a single homogeneous phase.

Yin et al. [72] reported that esters yield for the reaction using supercritical methanol increased when using carbon dioxide as cosolvent. Imahara et al. [93], in the alcoholysis of canola oil in methanol with the addition of supercritical $CO_2$, found that the addition of co-solvent increases the reaction yield, however, high molar percentage of $CO_2$ (above 0.1 $CO_2$/ methanol) led to a decrease in reaction conversion.

### 2.3. Two-step reaction

Based on the reports available in the literature it is suggested that the transesterification of vegetable oils at supercritical conditions can be conducted on alternative systems in order to reduce raw material costs and operating costs. There is a growing emphasis on the proposed system with a two-step reaction using reactors in series, with higher conversions to the system in one step [66] at mild operating temperatures and pressures and decreasing the amount of alcohol used in the process [87].

Kusdiana & Saka [65] and Minami & Saka [66] proposed the continuous synthesis of biodiesel from canola oil in two reaction steps, which consists primarily in the hydrolysis of triglycerides in pressurized water and subsequent esterification of fatty acids in supercritical methanol, with glycerol removed prior to FFA methyl esterification. This process is carried out under more moderate temperature and pressure compared to the process in one step.

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Busto et al. [100] reported that tubular reactors for supercritical transesterification must operate in order to minimize the axial dispersion, and as suggested by the authors, to satisfy this condition: reactions in a tubular reactor with separation step of unreacted products, with recycle the same or two or more reactors in series with intermediate separation of glycerol generated. One advantage of removing the glycerol formed in the reaction mixture is to allow the reaction to occur at lower ratios of alcohol to oil increasing the reaction rate for the production of biodiesel [87]. As cited by Aimaretti et al. [62], along the reaction, the alcohol used in the process is required by secondary reactions, which occur with glycerol.

D'Ippolito et al. [75] evaluated theoretically the non-catalytic process for producing biodiesel from experimental data and information available in the literature to determine an operating mode and operating conditions that reduce energy consumption and increase product quality. Results obtained suggest that the two-step process with intermediate removal of glycerol decreases the ratios of methanol to oil to about 10-15 times. Furthermore, not only the system pressure can be reduced as energy costs. In the process proposed by Crawford et al. [87], it is suggested that the obtained esteres by supercritical route can be made by transesterification of triglycerides with continuous removal of glycerol formed in the process, periodically or continuously, increasing the rate of ester formation. These authors argued that the reaction proceeded in this way can greatly decrease the amount of alcohol to be used in the process.

### 2.4. Intensification technologies in continuous biodiesel production

In the transesterification of vegetable oils, reaction rate can be limited by mass transfer between oil and alcohol because the very poor mutual miscibility. Hence, some process intensification technologies have been developed and applied to improve mixing and mass/heat transfer between the two liquid phases in recent years. Reaction rate is greatly enhanced and thus residence time may be reduced. Some of the technologies have been applied successfully in commercial production [101]. To reduce the limitations of mass and heat transfer in chemical reactions, literature indicates to conduct these reactions in microreactors [102-105] and in packed bed reactors [106-109].

In microreactors, mass and heat transfer increase due to the small size and large contact area [110] and the lowest internal diameters promote interaction with the reagents at the molecular level [111]. The internal diameter of microreactors, are typically10-300 μm [102,103]. Sun et al. [109] used reactors with 0.025 to 0.053 cm inner diameter and Guan et al. [112] used reactors with different inner diameters: 0.04, 0.06, 0.08 and 0.1 cm, calling them as microtube reactors. Furthermore, higher conversion and selectivity are obtained in a shorter reaction time as compared to batch system [102,113].

The rates of transesterification for biodiesel production are controlled by the rate of mass transfer between phases [112], being applied high rates of agitation for the batch system. Sun et al. [109] studied the production of biodiesel using alkaline catalysis with capillaries microreactors, and reported that the residence time is significantly reduced by the use of these reactors compared to the conventional process in batch

mode. Guan et al. [112] investigated the synthesis of biodiesel using microtube reactors for the alcoholysis of sunflower oil by basic catalysis, evaluating the influence of the length and internal diameter of the reactor. The conversion of the oil was strongly influenced by reactor geometry and the best results were obtained for the reactor with smaller diameter and greater length.

Although the phenomenon related to mass transfer is a key parameter to obtain better yields in biodiesel by the supercritical method and one approach suitable is the use of packed bed reactor. The packed bed system maximizes the interfacial surface area between the two phases (oil and alcohol) and the contact of the immiscible liquid-liquid two phases are improved towards achieving excellent mass transfer performance, which is obtained by extruding one phase into another, as the two phases flow through the particles openings, as commonly found in a packed bed reactor [106,114, 115].

Ataya et al. [106] reported the acid-catalyzed transesterification of canola oil with methanol using a packed bed reactor and showed that the mass-transfer limitations for two-phase experiments can be effectively overcome using a liquid-liquid packed bed reactor. Santacesaria et al. [108] performed the transesterification reactions in a simple tubular reactor filled with stainless steel spheres of different sizes and obtained that the reactions like methanol–soybean oil transesterification, mass transfer rate can greatly be increased also by favoring an intense local turbulence. The effects of packed bed reactor can be observed in other chemical reactions, for instance, Su et al. [115] evaluated the effect of packed microchannel reactors to perform the nitration of o-nitrotoluene with mixed acid and reported that the yield of this liquid-liquid multiphase reaction is increased by conducting the reaction using the packed reactor.

## 3. Configuration of reactors in continuous mode for supercritical ethanolysis

The following sections are dedicated to provide an overview of results obtained in supercritical ethanolysis in different reactor configurations. The schematic diagram of the experimental setup, developed by our research group, is shown in Figure 2. In these experiments, the residence time was simply computed dividing the volume of the reactor (mL) by the flow rate of substrates (mL/min) set in the liquid pump.

Results reported are in relation to content of esters in the sample determined by gas chromatography, following the European normative EN 14103 [116]. The data related to decomposition refer to derivatization of the samples with $BF_3$/methanol [91] to derivatize all of the fatty acids (mono-, di-, and triglycerydes, free fatty acids, and also ethyl esters) to the corresponding methyl esters and then analyzed by gas chromatography. For the evaluation of the decomposition percentage, palmitic acid was assumed not liable to degradation, considering its high stability [56,67]. These experimental procedures as well as analytical methods used are described in detail in the work of Vieitez et al. [57] and Silva et al. [79].

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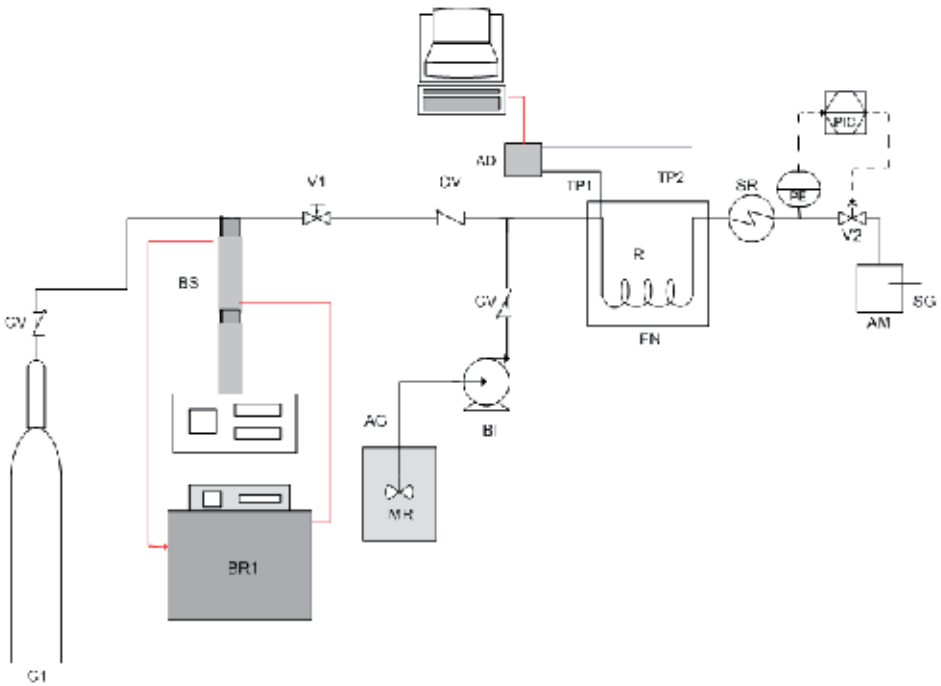


**Figure 2.** Schematic diagram of the experimental apparatus. RM - reactional mixture; MS - mechanical stirring device; LP - high-pressure liquid pump; CV - check-valve; A - solvent reservoir; B - thermostatic baths; SP - syringe pump; F - furnace; R - reactor; T1 - temperature indicator at the reactor inlet; T2 - temperature indicator at the reactor outlet; DA - data acquisition system; CS - cooling system; V1 - feed valve; PI - pressure indicator; PIC - controller; V2 - pressure control valve; S - glass collector; G - gas output. Taken with permission from Silva et al. [79].

### 3.1. Tubular reactor

The tubular reactor utilized was made of stainless steel tubing (316L 1/4 in. OD internal diameter of 3.2 mm HIP), being used in the work of Silva et al. [68], Vieitez et al. [57], Vieitez et al. [7], Bertoldi et al. [73], Vieitez et al. [117], Vieitez et al. [58], Vieitez et al. [118], Silva et al. [77], Vieitez et al. [119] and Vieitez et al. [120]. In these works, several approaches were made in order to optimize transesterification reactions for biodiesel production in supercritical ethanol in continuous tubular reactor and the better yields achieved for each study are presented in Table 1.

Silva et al. [68] investigated the effect of the variables temperature, pressure, oil to ethanol molar ratio and residence time on the yield of ethyl esters in the transesterification reaction of refined soybean oil. In that work, it was observed that an increase in temperature led to a sharp enhancement of reaction conversions and faster initial reaction rates. Also, as reaction time develops, a decline in the conversion reaction was observed for the temperature of 648 K. The reaction pressure had influence on the FAEE yields, with better yields obtained at 20 MPa. Regarding the effect of oil to ethanol molar ratio, results obtained by that study demonstrated that after a certain period of time higher values of molar ratio of ethanol to oil afford better con-

versions in shorter reaction times. This fact could be expected to a certain extent because in catalyst-free reactions an increase in the alcohol-to-oil molar ratio should provide greater contact between substrates, thus favoring reaction conversion. Besides, an excess of reactant could also shift the reaction to ethyl esters formation. In the experimental range investigated the authors reported ~80% in ethyl esters at the operating conditions shown in Table 1.

| Vegetable oil | Conditions and additional information | FAEE yield [%] | Decomposition [%] | Reference |
|---|---|---|---|---|
| Refined soybean oil | 1:40 oil to ethanol molar ratio; 623 K; 20 MPa; 35 min | ~80.0 | NR | [68] |
| Refined soybean oil | 1:40 oil to ethanol molar ratio; 623 K; 20 MPa; 28 min and water content of 2.5 wt% | 70.0 | ~ 14.0 | [57] |
| Refined soybean oil | 1:40 oil to ethanol molar ratio; 623 K; 20 MPa; 28 min and water content of 2.5 wt% | 70.0 | ~ 14.0 | |
| Refined soybean oil | 1:40 oil to ethanol molar ratio; 573 K; 20 MPa; 52.5 min and water content of 5 wt% | 70.0 | 3.0 | [7] |
| Degummed soybean oil | 1:40 oil to ethanol molar ratio; 623 K; 20 MPa; 28 min and water content of 10 wt% | 55.0 | NR | [117] |
| Castor oil | 1:40 oil to ethanol molar ratio; 573 K; 20 MPa; 28 min and water content of 5 wt% | 75.0 | ~11.0 | [58] |
| Sunflower oil | 1:40 oil to ethanol molar ratio; 623 K; 20 MPa; 42 min and water content of 5 wt% | ~69.0 | ~14.0 | [119] |
| High oleic sunflower oil | 1:40 oil to ethanol molar ratio; 623 K; 20 MPa; 42 min and water content of 5 wt% | ~75.0 | <5.0 | |
| Refined soybean oil | 1:40 oil to ethanol molar ratio; 573 K; 20 MPa; ~48 min and addition of 10% of free fatty acids to oil | 90.0 | <5.0 | [120] |
| High oleic sunflower oil | 1:40 oil to ethanol molar ratio; 623 K; 20 MPa; ~48 min and addition of 10% of free fatty acids to oil | 85.0 | ~8.0 | |
| Rice bran oil | 1:40 oil to ethanol; 573 K; 20 MPa; 26 min and addition of 10% of free fatty acids to oil | 82.0 | <5.0 | |
| Refined soybean oil | 1:40 oil to ethanol molar ratio; 598 K; 20 MPa; 110 min and $CO_2$ to substrates mass ratio of 0.05:1 | 76.0 | NR | [73] |
| Refined soybean oil | 1:1 oil to ethanol mass ratio; 598 K; 20 MPa; 30 min and operated with two reactors in series | 74.0 | ~5.0 | [77] |
| | 1:1 oil to ethanol mass ratio; 598 K; 20 MPa; 30 min and operated with recycle of 40 wt% | 75.0 | ~4.0 | |

NR = not reported

**Table 1.** Comparison of results obtained for transesterification reactions with supercritical ethanol in tubular reactor.

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Industrial scale synthesis of biodiesel generally relies on the transesterification of vegetable oils with a short-chain alcohol, mainly methanol, using chemical catalysts [12]. Because ethanol is readily available from fermentative processes using biomass from a varied source, ethanol biodiesel appears as a 100% renewable alternative, additionally enabling the replacement of traditionally used methanol by an innocuous reagent. Besides, in the Brazilian context, ethanol has been the natural choice because Brazil is one of the biggest ethanol producers in the world, with a well established technology of production and large industrial plant capacity installed throughout the country. However, the cost of ethanol is still higher than that of methanol, in particular where absolute (dry) ethanol is used in processes based on conventional catalytic methods [1,3].

Adopting the best experimental conditions (soybean oil to ethanol molar ratio of 1:40, 623 K and 20 MPa) reported by Silva et al. [68], Vieitez et al. [57] evaluated the effect of water content (2.5 wt% to 10 wt%) on the reaction yield. Results showed that the presence of water in the reaction medium seems to have a positive effect on the FAEE production. A significant increase in the ester content was observed for 598, 573, and 548 K for all residence time studied, suggesting that reaction conversions should be improved by the presence of water in the reaction medium. No relevant changes were observed corresponding to 623 K, probably due to the persistence of side degradation reactions. A moderate increase in the ester content also was found for the reaction performed at 523 K, which seemed to be the minimum temperature value that should be considered for conducting catalyst-free transesterification reactions under supercritical conditions. For all values of water content in the reaction medium, a point of maximum of ester yield was found within the residence time range investigated. The maximum FAEE concentration was found at 28 minutes of residence time for water content values of 0, 2.5, and 5%, while higher values of water content (7.5 and 10%) showed a maximum ester content for 42 minutes of residence time. The maximum point of ester content was positively affected by the presence of water in the reaction medium; i.e., at 300ºC and 52.5 min, an increase in water content from 0 to 5% led to an increase in FAEE concentration from 29.7 to 70.0%, respectively. Therefore, the presence of water in the reaction medium showed a favorable effect on the ester synthesis, due to its possible catalytic role for the transesterification process and reduction of fatty acids degradation [7].

As observed by Silva et al. [68], a decrease in reaction yield by increasing the reaction time was found. As shown in Figure 3 [57], significant differences were noticed between the fatty acid composition of the starting soybean oil compared to that of the original product, involving the reduction in the polyunsaturated fatty acid ethyl ester percentage (C18:1, C18:2 e C18:3) and the production of trans isomers, originally absent (Figure 3). Also, the authors reported high percentage of fatty acid decomposition in the temperature of study (623 K). For yields > 80% about 12 wt % of decomposition was observed and about 4.0% of triglycerides for system without addition of water.

Considering the occurrence of fatty acids decomposition at high residence times and the formation of isomers of ethyl esters formed, Vieitez et al. [7] reported the effect of temperature (523 K to 623 K) and water content (5 wt% to 10 wt%) on these factors and yield of esters. It was observed that temperature strongly affected the degree of degradation with values of

about 12 wt% and 28 wt% at 598 K and 623 K, respectively, for addition of 5 wt% of water in the reaction medium and high residence times. Moreover, the degradation phenomenon decreased as water concentration increased from 0 wt% to 10 wt%.

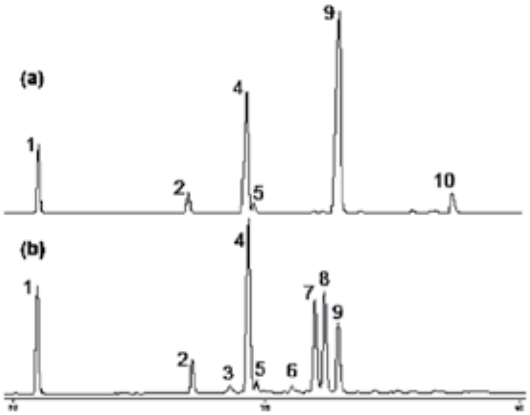


**Figure 3.** GC analysis of (a) soybean oil and (b) the product of the reaction (processed oil) performed at 350 °C, 20 MPa, 0% water, oil to ethanol molar ratio of 1:40 and 28 min of residence time. Peaks identification: C16:0 (1), C18:0 (2), *trans*-C18:1 (3), *cis*-9-C18:1 (4), *cis*-11-C18:1 (5), *trans*-6,12-C18:2 (6), *cis*-6,*trans*-12-C18:2 (7), *trans*-6,*cis*-12-C18:2 (8), *cis*-6,*cis*-12-C18:2 (9), and *cis*-9,12,15-C18:3 (10). Taken with permission from Vieitez et al. [57].

With respect to the effect of water concentration in the reaction medium on the degradation level, it was observed that the degradation phenomenon decreased as water concentration increased from 0 to 10 wt %. This reduction is in agreement with results showing that the addition of water may provide lower degradation levels and, accordingly, higher reaction conversions. Although no previous studies under similar conditions were found, these results are in agreement with some available references concerning the well-known favorable effect of the relatively low water activity on the oxidative stability of methyl linoleate or of vegetable oils. This phenomenon was attributed to different mechanisms, like the bonding of hydroperoxides, which decreases their reactivity, and an antioxidant effect due to hydration of traces of metals, which reduces their catalytic action [7].

The feedstock flexibility is the most important advantage to consider for biodiesel production methods because the resultant biodiesel price strongly depends on the feedstock price [121,122]. The cost of the raw materials currently represents about 70% of the total production cost [11]. The free fatty acids and water content in low grade feedstocks and hydrated ethanol pose a negative effect on the conventional homogeneous alkali-catalyzed process and heterogeneous catalytic methods, but can be successfully used in the transesterification reaction using an alcohol at its supercritical conditions. The evaluation of the effect of these variables on the efficiency of the transesterification reaction is highlighted. As shown in previous studies, the water content promotes the conversion of esters and decreases the degree of decomposition. Regarding the quality of the vegetable oil, studies concerning the effect of the vegetable oil type and free fatty acid content should be performed.

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While growth within the biodiesel sector can contribute to increase the price of soybean oil and other biodiesel source materials, the competitiveness of the sector can be adversely affected by these very same prices changes, as well as other economic factors. These emerging trends suggest that food and energy markets are likely to be more strongly linked in future - such that spikes and fluctuations in the prices of energy lead to corresponding changes in food prices [123]. Currently, the main resource for biodiesel production in Brazil is soybean oil, comprising about 80% of total feedstock [124], however, recently raw material price increases has motivated the use other raw materials towards a future global leadership of the country in biodiesel production and use of non-edible and waste oils with low-added value.

The ethanolysis of degummed soybean oil was reported by Vieitez et al. [117] to evaluate the use of alternative raw materials in order to reduce the production costs. The experiments were performed at 20 MPa, 623 K and oil to ethanol molar ratio of 1:40 and lower ester contents were obtained with degummed oil than from using refined oil. At 28 minutes of residence time about ~ 80% and 40% of esters were obtained for refined and degummed oil, respectively. Many possible reasons for these results are mentioned by the authors, like the possible adverse effect of some minor components with a higher concentration in the degummed oil, e.g. pigments or hydroperoxides, with a known pro-oxidant effect on the fatty acids.

In the same context, to in the search of low-cost raw materials, alternative to refined soybean oil, Vieitez et al. [58] evaluated the possibility of producing ethyl esters from castor oil, a plant considered interesting as a potential raw material for biodiesel production. The effect of temperature (523 K to 623 K) and water content (5 wt% to 10 wt%) was evaluated by keeping the pressure fixed at 20 MPa and oil to ethanol molar ratio of 1:40. The authors reported FAEE yields in the order of 75% at 573 K, 5 wt% of water content and 28 minutes of residence time. The authors emphasized that special care should be taken into account concerning reaction temperature, which could favor the occurrence of side reaction involving the consumption of high percentage do fatty acids when increased over 573K. For example, it was related >70.0 % of decomposition at 623 K for high residence times (> 28 minutes).

In a later study, Vieitez et al. [119] focused on the dependence of esters yield and decomposition as a function of vegetable oil composition (Table 2). The results obtained show a relation between the composition of vegetable oil and content of esters. Note that the content of esters, regardless of residence time considered, decreases in the following order: high oleic sunflower oil> sunflower oil> soybean oil> castor oil. This order, except by castor oil, is inversely with the degree of unsaturation of each oil, which confirms that the efficiency of the process dependency of the stability of the oil used. The castor oil has a high percentage of decomposition. This percentage increases in the following order for the vegetable oils studied: high oleic sunflower oil <sunflower oil <soybean oil << castor oil.

Considering that decomposition phenomenon may strongly affect the ester yield and that the chemical stability is mainly determined by the insaturation degree of the fatty material, it is of major interest to study the behavior of oils with different fatty acid compositions in this process. Table 2 shows the composition of the different oils studied and their corresponding iodine value (IV), which indicates concerning solely the fatty acid composition, HO-SFO should be the oil with the higher stability (lower IV), followed by SFO and SBO.

| Fatty acid | Soybean oil | Castor oil | Sunflower oil | High oleic sunflower oil |
|---|---|---|---|---|
| 16:0 | 10.9 | 1.0 | 6.2 | 3.5 |
| 18:0 | 3.5 | 0.9 | 3.3 | 2.5 |
| 18:1 | 26.0 | 3.4 | 32.0 | 87.4 |
| 18:2 | 52.7 | 4.6 | 56.3 | 4.7 |
| 18:3 | 5.0 | 0.4 | 0.4 | 0.2 |
| 18:1-OH | --- | 88.7 | --- | --- |
| IV[(a)] | 129.2 | --- | 128.5 | 85.3 |

**Table 2.** Fatty acid composition (wt%) of de vegetable oils studied. ((a)IV was calculated according method AOCS Cd 1c-85 [125])

The decomposition phenomenon was also studied in the work of Vieitez et al. [118], in which the stability of ethyl esters from soybean oil (SBOEE) exposed to high temperatures in supercritical ethanol was determined. In order to separately study the effect of such phenomenon, pure SBOEE were mixed with ethanol at a molar ratio 40:3 (ethanol:SBOEE) and exposed for different periods to supercritical conditions in a continuous system, at 20MPa and different temperatures. It was experimentally observed that the ester content of the processed samples were lower than that corresponding to the original SBOEE, indicating the occurrence of decomposition processes, which were more important as the temperature and residence time increased. The content of polyunsaturated esters of the treated SBOEE was lower than that of the starting mixture, showing that the decomposition rate was highly dependent on the nature and instauration degree of the alkyl chain. Therefore, results show that the exposure of the SBOEE to severe conditions required for efficiently performing the ethanolysis of vegetable oils by the supercritical method could cause the occurrence of important degradation processes of the lipid material. Such phenomenon could be identified as the main reason why the products from the supercritical transesterification of oils are less unsaturated than the raw materials. According to the results, the decomposition phenomenon is "selective" towards the polyunsaturated fatty esters, and there are no reasons to attribute such selectivity to the transesterification itself. Results also suggest that, in terms of the preservation of the integrity of the fatty acid chain, a supercritical transesterification process should not be performed at temperatures above 573 K, due to the high increase in the decomposition rate.

Recently, Vieitez et al. [120] evaluated the effect of the concentration of free fatty acids (FFAs) and type of vegetable oil on the yield of the reaction and decomposition of fatty acids. That work studied the effect of the addition of FFAs at various proportions to different vegetable oils (soybean oil, rice bran oil, and high oleic sunflower oil) on the efficiency of their conversion to ethyl esters by a continuous supercritical ethanolysis. When the reactor was operated at 573 K and 20 MPa with soybean oil using an alcohol/oil molar ratio of 40:1, an ester content of 53% was obtained. Under identical conditions but processing soybean oil with 10% of FFAs, the ester content rose to 91%. A similar favorable effect of the addition of

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FFAs on the efficiency of the process was observed when processing rice bran oil and high oleic sunflower oil. Processing oils from different origins may lead to different ester contents in the final product because of the occurrence of decomposition phenomenon at different extents depending upon oil composition and stability. Results showed that the addition of FFAs is a useful tool for favoring alcoholysis against decomposition, with the consequence of a substantial increase in process efficiency. Therefore, the addition of FFAs could be a useful for improving the supercritical transesterification of oils with a low initial acidity and low-quality fats, such as highly hydrolyzed RBO, which could be efficiently converted to biodiesel using this technology. Several favorable effects on the process can be attributed to the presence of high levels of FFAs in the raw material: a catalytic role in the transesterification of triacylglycerides, a high esterification rate of FFA themselves, and a dilutive effect on the glycerol in the reaction medium (thus avoiding several unwanted side reactions). The contribution of all of these factors permitted us to achieve high efficiencies even at milder reaction conditions, thus minimizing the decomposition phenomenon, which has been pointed out as one of the main drawbacks of the supercritical method [119].

As observed in the studies presented in Table 1, the high transesterification conversion requires high temperature, high pressure and high alcohol to oil molar ratio. Indeed, the high temperature and pressure require high initial investments (equipment costs) for the implementation of such process operated and safety management policy. As a result of the high alcohol to oil molar ratio greater energy consumption in the reactants pre-heating and recycling steps is unavoidable. Moreover, the high amount of alcohol in the biodiesel product retards the biodiesel-glycerol phase separation. Therefore, the use of those original parameters results in high capital costs, especially for the reactor and pump, being somewhat higher than the novel catalytic methods [126]. To increase the technical and economical feasibility of supercritical method, further studies are required to reduce the energy consumption and operating parameters of this process.

In an attempt to reduce the operating conditions of the transesterification reaction, Bertoldi et al. [73] proposed for the first time the addition of carbon dioxide as a co-solvent in the reaction medium for reactions in continuous mode. The experiments were performed in the temperature range of 573-623 K, from 7.5 to 20 MPa, oil to ethanol molar ratio of 1:10 to 1:40 and co-solvent to substrates mass ratio from 0:1 to 0.5:1. Results showed that the yield of ethyl esters decreased with increasing addition of carbon dioxide to the system. At 623 K; 20 MPa; oil to ethanol molar ratio of 1:40 and 35 min it was observed about 80% of esters yield for system without co-solvent [68] and about 40 % for addition of $CO_2$ to substrates mass ratio of 0.05:1. Phase equilibrium data for the binary system ethanol-$CO_2$ shows the existence of high mutual solubility for these compounds [127,129]. On the other hand, very poor solubility of carbon dioxide in soybean oil has been reported in the literature [97]. Thus, it is possible that the co-solvent is dragging some amount of ethanol from the oil phase, causing the occurrence of a two-phase flowing system, decreasing the content of ethanol in contact with the vegetable oil with a consequent reduction in reaction conversion.

Another proposal considered was the non-catalytic production of fatty acid ethyl esters from soybean oil in a two-step process with experimental simulation of two reactors operat-

ed in series and a reactor with recycle, reported by Silva et al. [77]. The justification of the authors refers to the reaction conducted in two steps with reactors in series and/or recycling the leaving stream with intermediate removal of glycerol can increase the yield of the reaction, since the reaction may take place at lower alcohol to oil ratios, increasing the reaction rates of ester production [87]. The reaction of glycerol formed during the process with other components of the reaction medium may lead to a decrease in ester yield [66] and the undesirable consumption of alcohol [62,90]. Another important point of conduction of reactions in two steps is that the non-reacted products, diglycerides and monoglycerides, and also the esters formed may act as co-solvents in the reaction medium, increasing the solubility between the phases [100,130]. For the reactor in series it was reported 74% in esters at 598 K, 20 MPa, oil to ethanol mass ratio of 1:1 and 30 minutes of residence time for the second reaction step. For the system with recycle of 40 wt% at similar conditions it was obtained 75%. In both cases the degree of decomposition was lower than 5.0%.

### 3.2. Microtube reactor

Microreactor systems designed for continuous production have been studied in recent years for the transesterification of vegetable oils [109,112]. In the microreactor system, mass and heat transfer could be greatly intensified due to its small space with a large surface area-to-volume ratio [112], providing high process yields in low reaction times [109].

In this context, Silva et al. [79] developed a microtube reactor of stainless steel tubing (316L 1/16 in. OD internal diameter of 0.76 mm HIP) to evaluate the effects of inner diameter on the FAEE yield and compare the results with those reported by Silva et al. [68] for the same conditions using a tubular reactor. At lowest temperature (523 K) only 3.12 % FAEE yield is obtained in the tubular reactor, while 19% is reached using the microtube reactor. At 598 K this yield is increased from 38% to 53% when changing from the tubular to the microtube reactor at the same residence time. Such results demonstrate that higher ethyl esters yields can be achieved at lower temperatures, short reaction times with a smaller reactor inner diameter, hence minimizing the total decomposition of fatty acids.

In the work of Silva et al. [79] it was evaluated the effect of process variables (temperature, pressure and oil:ethanol molar ratio) on the yield of esters and decomposition. It was found that this variable had a positive effect on FAEE yield. In that work, it was noticed that an increase pressure and lowest ratios of ethanol to oil led to higher degrees of decomposition. It was also observed higher decomposition rates for oil:ethanol molar ratio of 1:10 and pressure of 20 MPa. In the experimental range investigated, appreciable yields were obtained (70%) at 598 K, 20 MPa and oil to ethanol molar ratio of 1:20, with low total decomposition of fatty acid (<5.0 wt%).

Considering the increasing reaction rates and improved mass transfer between phases in the conduction of reactions in a microtube reactor and the results obtained by Bertodi et al. [73] when using a cosolvent for the continuous tubular reactor, Trentin et al. [74] evaluated the addition of carbon dioxide on the reaction medium of soybean oil transesterification carried out in a microtube reactor. Results showed that ethyl esters yields obtained increased with increasing addition of carbon dioxide to the system and the highest yields were obtained

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with addition of co-solvent to substrate mass ratio of 0.2:1 to the reaction medium. The authors reported that the differences found in relation the conduct of the reactions in the tubular reactor [73] can be attributed to the problems of mass transfer in the tubular reactor and due to the fact that the mass and heat transfer may be greatly enhanced due to the smaller internal space (which means higher fluid velocity at the same flow rate), and the higher surface area-to-volume ratio, leading to higher process yields.

Silva et al. [76], conducted reactions in two steps in a microtube reactor: two-series reactors and reactor with recycle, conducted. It was obtained about 78% of ethyl esters yields and <2.0 wt% of decomposition for 45 min in the simulation of two reactors operated in series at 573 K, 20 MPa, oil to ethanol mass ratio of 1:1 (for the one-step process the authors shows 40 % of ethyl esters in the same conditions at 25 min). These results are higher than those reported by Silva et al. [77] at lower temperature and lower decomposition degree, as was also observed for reactions with recycle. Furthermore, in that work, glycerol was obtained with ~90 wt% of purity (after evaporation of ethanol and simple decantation) for the system with recycle and this fact of course should be taken into account for the purpose of implementation of a cost-effective transesterification process.

### 3.3. Packed-bed reactor

Results presented for the transesterification in microtube reactors are undoubtedly significant. However, production capacities of the above microreactors are considerably lower than those of conventional reactors by reason of their specific structures. Fulfilling the volume requirements of small-fuel biodiesel processing plants for distributive applications seems difficult. It is thus a challenge to identify a method for maximizing high synthesis efficiency by mixing at the microscale as well as for increasing biodiesel production remarkably [107]. An alternative to these problems would be to conduct the reactions in packed-bed reactors filled with different materials in different diameters, such as stainless steel spheres [108], metal foams [107] and glass beads [106].

In the work of Andrade et al. [80] a packed-bed tubular reactor was developed, which was made of stainless steel tubing (316 L 1/4 in OD inner diameter 3.2 mm) and stainless steel tubing (304 L 30.5 mm OD inner diameter 13 mm HIP) packed with glass beads (4.5 mm diameter). The results obtained by authors demonstrate that much higher ethyl esters yields can be achieved with this configuration. It can be seen observed in the results that at 548 K only 11.5% FAEE yield was obtained in the tubular reactor (TR), while 35% is reached using packed-bed tubular reactor (PBTR). At 573 K this yield is increased from 16% to 55% from the use TR to the PBTR at the same residence time. Such results demonstrate that much higher ethyl esters yields can be achieved at lower temperatures, small reaction times, also minimizing the total decomposition of fatty acids with the use of packed-bed tubular reactor. The increased performance of the reaction in the PBTR may be possibly due to the maximized interfacial surface area between the two flowing phases.

Silva et al. [79] proposed the use of microtube reactor for continuous synthesis of FAEE and reported yields of about 53% at 598 K, 20MPa, oil to ethanol molar ratio of 1:20 and residence time of 25 min. At similar conditions with addition of carbon dioxide as co-solvent

($CO_2$ to substrate mass ratio of 0.2:1) in the microtube reactor, Trentin et al. [74] reported 58% of FAEE yield. At this same condition, the reaction conducted in the work of Andrade et al. (2012) in the PBTR, resulted in FAEE yields about 60%.

With the use of PBTR it can be obtained yields as high as ~ 83% at 598 K, 20 MPa, oil to ethanol molar ratio of 1:40 and 42 minutes of residence time. In such condition, it was observed 6.0 wt% of decomposition. In the evaluation of the effect of water content on the conversion, the authors reported 90% yield of ethyl esters and <5.0 wt% of decomposition at similar conditions with addition of 10 wt% of water to the reaction medium.

## 4. Conclusion

The non-catalytic transesterification at supercritical conditions is a promising method for esters production and has strong advantages, such as fast reaction time, feedstock flexibility, production efficiency and environmentally friendly benefits, but as observed in this manuscript the application of this methodology has some limitations, such as the operation conditions of elevated temperature and pressure and the use of higher amounts of alcohol in the reaction medium, which results in high energy costs for the process and degradation of the products generated. The analysis of these facts generate critical of the use of supercritical technology in transesterification reactions making them an open problem. Furthermore, prospective research is reducing the operating parameters and the decomposition of the reaction components are required to industrial scale application of the supercritical method.
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Section 3

# By-Products Applications

Chapter 10

# Approaches for the Detection of Toxic Compounds in Castor and Physic Nut Seeds and Cakes

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Olga Lima Tavares Machado

Additional information is available at the end of the chapter

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## 1. Introduction

The worldwide search for new fuel sources has grown during the last decades due to two main factors: the global concern about environmental issues and the high price of petroleum. Biodiesel is a type of biofuel that is already used in many countries, and its usage will most likely increase over the next few years. Biodiesel can be produced using different technologies and raw materials, such as vegetable oils, animal fats and microalgae oil. However, despite the wide range of oil sources for biodiesel production, vegetable oils are primarily used for this purpose. The choice of oilseed to be planted for biodiesel production depends on many factors, including the regional climate and soil conditions. The biodiesel industries in the US primarily use soybean oil, whereas in Europe, rapeseed is primarily used for biodiesel production. In tropical countries, biodiesel is produced from plants that grow in these tropical areas, such as palm, physic nut and castor bean.

In addition to biodiesel production using vegetable oils, the by-products generated at different steps during the production process have garnered increasing attention. Some of these by-products are generated in large amounts, making it both economically necessary and interesting to find a use for them. Currently, the residual cake, also known as the seed cake or press cake, has been shown to be a noteworthy by-product. The seed cake consists of the organic waste obtained during the oil extraction process by the pressing of seeds. Large amounts of residual cakes are generated during the oil extraction process. For example, for each ton of castor bean pressed for oil, a half-ton of cake is produced [1]. The residual cake can be used as fertiliser because of the macro- and microelements composition. Moreover, the protein content makes it useful as a component of animal feed.

Several countries from South and Central America and Asia are attempting to use new oilseed sources for biodiesel production. Two of the oilseeds that are expected to be used for this purpose are the castor bean (*Ricinus communis*) and physic nut (*Jatropha curcas*). The oil properties of these seeds are well known, and many processes have been developed to produce biodiesel from these seeds. However, the large amount of residual cakes that are produced during the biodiesel production process and how to dispose of or use these cakes remain a problem. Both the castor cakes (castor bean) and Jatropha cakes (physic nut) have great potential for use as fertilisers. Castor cakes are rich in macroelements, including N, P, K, Na, Mg and S, and were shown to supplement the nutritional requirements of plants, reduce the soil acidity by increasing the pH, increase the carbon content, reduce the presence of nematodes and promote overall soil health [2]. Jatropha cakes are already used as green manure, also because of the N, P and K content [3]. It is expected that the castor and Jatropha cakes can be used as animal feedstock. These oilseed cakes are high in protein; therefore, their use as an animal food supplement is highly desirable. However, the presence of toxic substances in the seeds of *R. communis* and *J. curcas* restrict the use of the residual cakes as feedstock. Many detoxification processes have been described to render castor and Jatropha cakes edible. However, there is currently no recognized standard and safe methodology that could be used in the industry. Most of the detoxification processes developed have some negative aspects, such as high prices that are limiting for use on an industrial scale or the validation method. This second problem is the most difficult to solve because it is necessary that the detoxified cakes be safe to use as animal feedstock. A flawed method to detect toxins in the cakes could be very dangerous because a non-detoxified residual cake could be used to feed animals and may lead to death. In addition to toxic components, it was shown that allergenic proteins are also present in the seeds of *R. communis*[4] and *J. curcas*[5], and many methods for the detoxification of residual cakes have been shown to efficiently eliminate the toxins but not the allergens. For example, during the 1960s, a detoxified castor cake was commercialised in Brazil as *Lex Proteic* [6]; however, despite the absence of toxins, the allergens remained present in the castor cake. In this chapter, different methods to detect toxins from *R. communis* and *J. curcas* will be described.

## 2. *Ricinus communis* toxins

Castor bean seeds have long been known for their toxicity. They are the source of the most potent phytotoxin known, the protein ricin. Moreover, the toxic alkaloid, ricinin, is also found in the castor bean; however, this compound is different from ricin in that it is not as toxic and can easily be removed from the castor cake.

### 2.1. Ricin

The toxin, ricin, has been known since ancient times because of its use in criminal practices. According to Olsnes [7], in 1887, Dixon had hypothesised that the *R. communis* toxin was a protein, and Kobert confirmed this hypothesis in 1913.

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Ricin is a type 2 ribosome-inactivating protein (RIP) that is found exclusively in the endosperm of castor bean seeds. As a type 2 RIP, ricin is a dimeric protein comprised of an A chain (32 kDa) and a B chain (34 kDa) linked by a disulfide bond [8]. The ricin A chain (RTA) is responsible for the enzymatic activity of the protein. This N-glycosidase enzymatic activity removes a specific adenine, depurination, ($A_{4324}$) residue from a region of rRNA known as the $\alpha$-sarcin/ricin loop (SRL) (Figure 1).

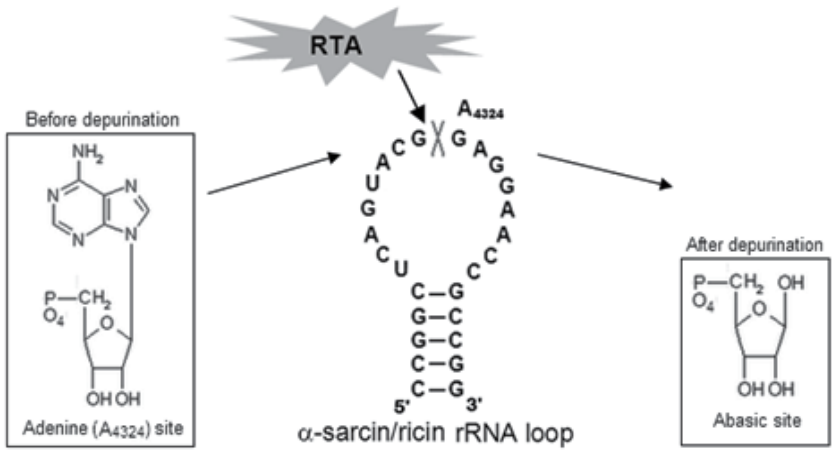


**Figure 1.** Theα-sarcin/ricin loop and the point of depurination by RTA N-glycosidase activity. The $A_{4324}$ site before depurination by RTA is shown on the left where the intact nucleotide is present. On the right an after depurinationabasic site (without the adenine) is shown.

The absence of this adenine residue inhibits binding of the elongation factor, thereby stopping protein synthesis [9]. The B chain (RTB) is a lectin that binds to glycoproteins and glycolipids on the cell surface and cytosol and mediates the internalisation and intracellular translocation of the toxin [10,11].

The ricin toxin is very efficient and a single molecule may inactivate 2,000 ribosomes per minute [12]. Because ricin can be used as a bioterrorism agent [13], many assays to detect ricin have been described. Some of these assays are highly accurate and can detect very low concentrations of the toxin. However, there is no standard methodology to use as a quality control for castor cake detoxification processes. Many methodologies to eliminate ricin toxicity from castor cakes have been described, and there are several promising processes when economic aspects are considered [14]. Therefore, to use castor cakes as animal feedstock, efficient methods to detect ricin toxicity after the detoxification process are needed to ensure quality control and safety before the material can be commercialised.

### *2.1.1. Detection of ricin*

Because ricin can be used as a bioterrorism agent, the search for fast and sensitive detection methods began soon after the first studies describing the mechanism of action of ricin. The earliest proposed detection method was the enzyme-linked immunosorbent assay (ELISA)

[15]. In this assay, rabbit anti-ricin antibodies (reduced IgG and Fab' fragments) conjugated withβ-D-galactosidase was used. Using the rabbit anti-ricin Fab'-β-D-galactosidase complex, it was possible to detect as little as 4 ng/mL of ricin with the sandwich ELISA technique. However, less sensitivity was observed when this method was utilised for determining the amount of ricin added to rabbit body fluids. In this case, the lowest concentration of ricin that could be assayed was 40 ng/mL. During the next two years, new methods based on radioimmunoassays were proposed [16, 17]. These radioimmunoassays were very sensitive and could detect 50-100 pg RTA and 500 pg RTB; however, the sensitivity was reduced to intact ricin. The matrix used for these assays consisted of 0.1% sodium azide and 0.1% bovine serum albumin (BSA) in 0.05 M sodium phosphate buffer. Limitations of these assays include the difficulties in handling radioisotopes and the long incubation period. Therefore, despite the high sensitivity of these assays, the drawbacks associated with radioimmunoassays make them less preferable than ELISA. Poli et al. [18] developed an enhanced colorimetric and chemiluminescent ELISA to detect ricin in biological fluids. This assay utilised an affinity-purified goat polyclonal antibody (pAb) to adsorb ricin from the solution. The same pAb was then used to form a sandwich, and avidin-linked alkaline phosphatase was used for colour development. Enhancement of the colourimetric assay was obtained because of the increased biotinylated antibody content and a reduction in the dilution ratio of the avidin-linked alkaline phosphatase. This assay could detect 100 pg/mL ricin in phosphate-buffered saline (PBS), human urine and human serum. This sandwich assay could also be used with a chemiluminescence detection reagent; however, the quantitation was limited to a range of 0.1–1 ng/mL and was subject to greater variability compared to the colourimetric assay. An ELISA using monoclonal antibodies (mAb) was performed to detect ricin in biological fluids [19]. This method was also based on the sandwich format using an anti-ricin B chain mAb to adsorb ricin from the solution and an anti-ricin A chain mAb conjugated to peroxidase as the second antibody that is then used to form a sandwich. The peroxidase allows for colour development and measurement of optical density at 450 nm. The sensitivity of this assay is 5 ng/mL and is lower than the sensitivity reported for the amplified and chemiluminescent immunoassays [18]. The ELISA is still used to detect ricin, and a commercial ELISA kit specific for ricin detection can be obtained [20]. However, ELISA has several disadvantages that prevent it from being the best method to detect of ricin. ELISAs consume too much time because of the washing steps involved and they also have limited throughput. ELISAs may also underestimate the actual ricin content in situations where antigen concentrations are high (hook effect) and specialised personnel are also required to perform the ELISAs.

To reduce the time necessary to assay for ricin, a method based on a fiber-optic sensor was developed [21, 22] and optimised [23]. A sandwich immunoassay scheme was used in which an anti-ricin IgG was immobilised onto the surface of an optical fiber. The limits of detection for ricin, as detected by laser-induced fluorescence, in a buffer solution and river water were 100 pg/mL and 1 ng/mL, respectively. The complete assay can be performed in 20 minutes.

The first immunochromatography assay to detect ricin was performed using antibody anti A-Chain mAb with two distinct specificities. An anti-RTB mAb (1G7) was immobilised to a

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defined detection zone on a porous nitrocellulose membrane, whereas an anti-RTA mAb (5E11) was conjugated to colloidal gold particles that worked as the detection agent [24]. The ricin-containing mixture was added to the membrane and allowed to react with the mAb 5E11-coated particles. This mixture moved across the porous membrane by capillary action until it reach the extremity containing the anti-RTB mAbs, which bound to the particles of ricin that were attached to the gold-labelled anti-RTA mAbs. The detection limit of this assay was 50 ng/mL ricin in phosphate-buffered saline (PBS). This sensitivity could be enhanced further to 100 pg/mL with the use of a silver enhancer. The advantages of these gold particles were their superior mobility, decreased aggregation and commercial availability. An immunochromatography assay was also used to shown differences in ricin content among different castor bean cultivars [25]. All the ricin isoforms were detected in the range of 1 to 2.5 ng/ mL in buffer.

In addition to using a better antibody for improved sensitivity, there was also a development regarding the technology of the solid phase surface of the immunoassay. The conventional microplate was exchanged for magnetic micro beads. Immunomagnetic (IM) assays to detect ricin were first used by Gatto-Menking et al. [26]. They used immunomagnetic electrochemiluminescence (IM-ECL) to detect ricin and other toxic agents, such as botulinus A, cholera β subunit, ricin and staphylococcal enterotoxoid B. Antibody-conjugated magnetic micro beads were used to capture the target toxins and ruthenium trisbipyridal chelate-labelled antibodies were used as the reporter. High sensitivity levels were obtained for all the tested toxins. All IM-ELC assays could be performed in a maximum combined incubation and assay time of approximately 40 minutes, and the sensitivity to ricin was 5 pg/mL. Some years later, an enhanced ECL assay had a detection limit of 0.5 pg/mL for ricin in PBS [27]. The same study demonstrated the detection of ricin by fluorogenic-chemiluninescence (FCL), and the sensitivity was 1 ng/mL. Advantages of these micro beads were due to their large surface area (Figure 2) that leads to enhanced sensitivity, to free moving microspheres coated with antibody that accelerates the reaction rates and reduces the assay time, and to easy detection using a simple magnetic field. Both the FCL and ECL had similar formats, except that the FCL used alkaline phosphatase as the label and detected the ricin through the measurement of fluorescence, whereas the ECL used ruthenium-trisbipyridal as the label and detected the ricin through photoemission. For a magnetoelastic surface sensor instead of microspheres, the detection technology was a sandwich immunoassay on the sensor surface. Biocatalytic precipitation was then used to cause a change in mass, which resulted in a change in the resonance frequency that allowed for quantitation of ricin at a detection limit of 5 ng/mL in aqueous media, such as water, blood or serum [28]. This magnetoelastic sensor had a sensitivity that was comparable to the ELISA; however, this assay had a much lower cost, was disposable and had a relatively quick analysis time.

The search for an assay to detect several toxins simultaneously led to the use of array systems. Three different toxins, ricin, SEB and *Yersinia pestis*toxin, were detected using a planar array immunosensor equipped with a charge-coupled device (CCD) [29]. This was a disposable and simple sensor array coated with different antibodies that were detected through the CCD. This planar array platform gave a detection limit of 25 ng/mL ricin, 5 ng/mL SEB

and 15 ng/mL *Y. pestis*, based on a goat anti-ricin antibody in PBS containing 0.05% (v/v) Tween-20. This detection method allowed for multiple sample analysis using a minimum amount of sample and simultaneous analysis that was inclusive of the controls. An antibody microarray biosensor for the rapid detection of both protein and bacterial analytes under flow conditions was developed using a micrometer-sized spot [30]. Using a non-contact microarray printer, biotinylated capture antibodies were immobilised at discrete locations on the surface of an avidin-coated glass microscope slide. The slide was fitted with a six-channel flow module that conducted analyte-containing solutions over the array of capture antibody microspots. Detection of the bound analyte was subsequently achieved using fluorescent tracer antibodies. The pattern of fluorescent complexes was interrogated using a scanning confocal microscope equipped with a 635-nm laser. The assays were completed in 15 minutes, and ricin detection was demonstrated at levels of 10 ng/mL.The detection limits for the other analytes were also relatively low. These assays were very fast compared to the previously published methods for measuring antibody-antigen interactions using microarrays (minutes versus hours). In addition, whereas other antibody microarray assays can detect specific proteins present in complex mixtures, this method could detect proteins and bacteria simultaneously. Recent improvements in the microarrays to detect ricin and other biological agents have been described. A method that used a bioanalytical platform that combined the specificity of covalently immobilised capture probes with dedicated instrumentation and immuno-based microarray analytics was able to detect ricin at 0.5 ng/mL in PBS and 1-5 ng/mL in milk [31]. However, despite the high sensitivity compared with the other array methods, this assay took approximately 90 minutes.

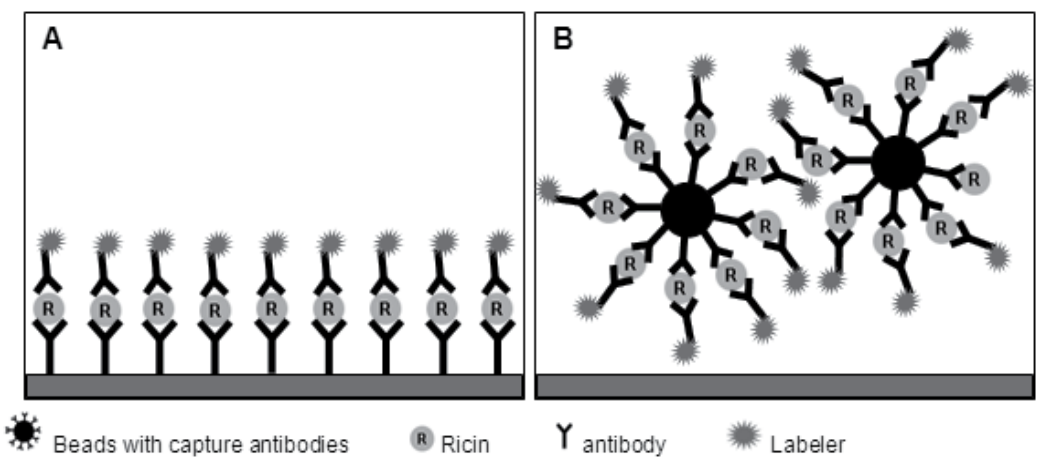


**Figure 2.** Comparative schematic of two immunoassays used to detect ricin. A) Sandwich ELISA. B) Microbeads immunoassay. The recorder antibody can be linked to different labeler molecules, as Ruthenium, alkaline phosphatase or horsehadish peroxidase.

Sano et al. [32] developed a method to detect antigens that combined the specificity of immunological analysis with the exponential amplification of PCR. This immuno-polymerase

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chain reaction (IPCR) was an interesting method to monitor the presence of ricin in samples [33]. A schematic representation of this method is shown in Figure 3.

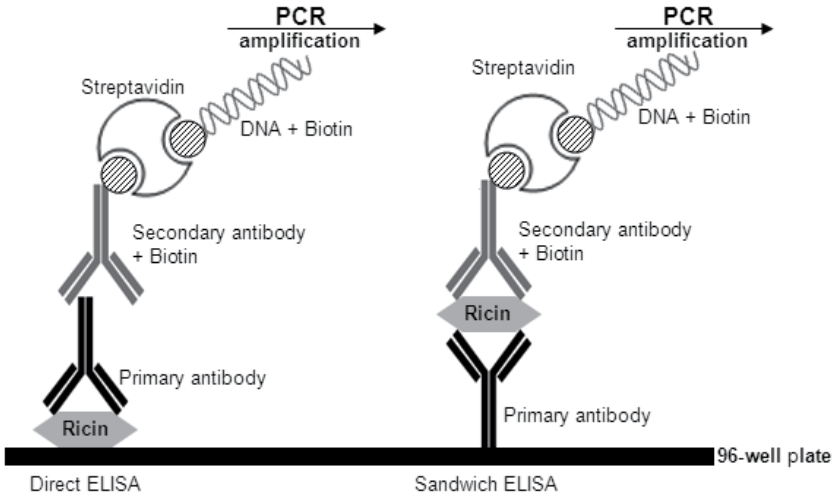


**Figure 3.** Schematic representation of the IPCR detection of ricin using both direct and sandwich ELISA to capture and report the toxin. The biotin-streptavidin interaction plays the bridge role between secondary antibody and the reporter DNA, which is amplified by PCR.

Ricin was dissolved at different concentrations in PBS, and detection was performed revealing a detection limit of 10 fg/mL. The assay was then performed with ricin dissolved in human serum revealing a detection limit of 0.5 fg/mL. The method has also been used for post-intoxication evaluation of the biological half-life of ricin. IPCR analysis of sera from mice fed ricin showed that the toxin was rapidly sequestered from the sera (30 minutes) with a half-life ($t_{1/2}^{\alpha}$) of 4 minutes [34]. The time required to complete the entire IPCR process is 9 hours. Compared with conventional immunological methods, IPCR requires a greater amount of time because of the PCR itself and the post-PCR analysis. Moreover, the use of more expensive reagents and the increased reagent consumption make this technique less attractive than conventional immunological methods. However, these limitations are counterbalanced by greater sensitivity (8 million times greater than conventional ELISA), enabling a broader range of applications.

In recent years, highly sophisticated mass-spectrometry (MS)-based methods for the detection and quantification of ricin have been developed. It was shown that ricin could be unequivocally identified by liquid chromatography-electrospray (LC-ES) MS/MS experiments with reduced, cysteine-derivatised, trypsin-digested material [35]. It was also shown that MALDI-MS could be used to detect intact ricin and to screen samples for ricin peptides. The amount of crude sample required was a few milligrams containing less than 5% ricin. According to the authors, the selection of a few marker peptides from the A and B chains can be used as a method to improve the sensitivity and efficiency of this method. A method

combining immunocapture and analysis by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) for ricin detection was also described [36]. Ricin samples were applied to magnetic spheres coated with a monoclonal anti-B-chain antibody. After acidic elution, tryptic peptides of the A and B chains were obtained by accelerated digestion with trypsin in the presence of acetonitrile. Three of the 20 peptides obtained were used for ricin detection by MALDI-TOF MS. This assay had a limit of detection estimated at 50 ng/mL, and the result could be obtained in approximately 5 hours. These results are not as exciting compared to other more sensitive and faster methodologies; however, an interesting feature is that MS detection provides increased specificity because of the simultaneous monitoring of several characteristic ricin-specific peptides. Furthermore, the possible miniaturisation of MALDI-TOF technology suggests that the assay could be adapted for use with a portable mass spectrometer. A recent study described the combination of a multiplex-immunoaffinity purification approach followed by MALDI-based detection for the simultaneous identification of different toxins, including ricin [37]. Selected antibodies against each toxic agent allowed for the specific and simultaneous capture of these toxins. The toxins were subsequently identified by MALDI-TOF MS following a tryptic digest, and after an assay time of 8 hours, the ricin could be detected at a minimum of 200 ng/mL. The time requirement and detection limit were not satisfactory for this assay; however, ricin could be detected in complex matrices, such as milk and juice.

Aptamers are artificial nucleic acid ligands that can be generated against amino acids, drugs, proteins and other molecules. They are isolated from complex libraries of synthetic nucleic acids by an iterative process of adsorption, recovery and reamplification. Because of their high thermostability when compared with antibodies, aptamers have potential applications in analytical devices, including biosensors, and as therapeutic agents [38]. Assays for protein identification and quantitation were developed and applied to ricin detection [39, 40]. A multiplex aptamer microarray was generated by printing an anti-ricin RNA aptamer onto either streptavidin (SA)- or neutravidin (NA)-coated glass slides. The limit of detection in a sandwich assay format after optimisation studies was 15 ng/mL in PBS. This assay was also used to detect other proteins and showed satisfactory results. Capillary electrophoresis (CE) has been shown to be a viable alternative to traditional immunoassays when coupled with laser-induced fluorescence detection. Haes et al. [41] demonstrated that capillary electrophoresis could be used to detect ricin by monitoring its interaction with a fluorescently tagged aptamer under non-equilibrium conditions. The quantitative response revealed a detection limit as low as 14 ng/mL. This study also revealed that the presence of nucleases in the sample leads to a slight decrease in the ability of the aptamer to detect ricin; however, it is still possible to detect the toxin at very low concentrations. This assay can be performed in less than 10 minutes, consumes minimum quantities of material, and generates a low amount of waste.

Liquid-crystal (LC) based sensors that can be used as rapid and effective detection technologies have attracted a significant amount of attention in recent years [42], and their utility regarding ricin detection has previously been demonstrated [43]. This method relied on the use of LCs 5CB to amplify and report the presence of ricin captured by an affinity ligand.

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One merit of this approach is that the ricin can be imaged on chemically functionalised surfaces and transduced into an optical signal. The optical signal caused by the orientational transition of the LCs could easily be identified with polarised light microscopy. However, despite the success of the LC-based sensor, which did not use complex instrumentations and did not involve any labelling steps, the limit of detection of 10μg/mL was not as good compared to other methods. Similar to other assays, this interesting technology must be improved to become among the most sensitive methods for ricin detection.

Despite the many methods to detect the presence of ricin, the detection of the toxin in castor cakes subjected to detoxification is not performed in a standard manner. Anandan et al. [44] used different physical and chemical treatments to detoxify castor cakes, and the ricin content was determined based on electrophoretic analysis. They reported that ricin bands did not appear in SDS-PAGE samples of autoclaved (15 psi, 60 minutes) and lime treated (40 g/kg) castor cakes. Solid-state fermentation by *Penicillium simplicissimum* also reduced the ricin content when fermented castor waste samples, which were not the cake but an extremely alkaline waste, were evaluated by electrophoresis [45]. However, this detection method has many disadvantages compared to the described techniques. The first disadvantage is the low sensitivity of the method. A lower ricin concentration that remains lethal cannot be detected; therefore, if electrophoresis is used as the detection method, another more sensitive assay needs to be performed to validate the detoxification process. Another problem is related to the long assay time and specialised personnel required to perform these analyses and the necessity of performing a Western blot assay to confirm the identity of ricin.

The greatest problem that affects not only electrophoresis, but also all the ricin detection methods described in this chapter, is the inability to detect the biological activity of the toxin. Each proposed assay can detect the presence of ricin at minimal concentrations and many of these are able to do so in a very sensitive and specific way; however, they cannot determine whether the toxin is biologically active. To validate the castor cake detoxification processes, it is important to be able to detect the biological activity of ricin. This is because some of the described toxin inactivation processes can be related to modifications in the active site of the enzyme, and although ricin may be present in processed cake, it may be not active and the product would be safe to use in animal feed.

### *2.1.2. Detection of ricin biological activity*

The first method of detecting ricin activity was based on measuring the inhibition of protein synthesis in a rabbit reticulocyte cell-free system mediated by toxic tryptic peptides from ricin [46]. The method was justified because of the long period of time required to observe intoxication symptoms in animals. It was reported that similar to the native protein, toxic ricin peptides could inhibit protein synthesis in a cell-free system. This information reinforces the necessity for assaying ricin biological activity after subjecting the castor cake to detoxification processes.

The ability of the RIPs in inhibit protein synthesis can be monitored with *in vitro* translation assays using the rabbit reticulocyte lysate system [47, 48]. One disadvantage of these assays is the use of a multistep procedure to determine the RIP activity by measuring the incorpo-

ration of radioactive amino acids after the addition of mRNA or polysomes to the system. Therefore, an *in vitro* transcription/translation single-step assay utilising the luciferase bioluminescence detection system was described to characterise mistletoe lectin I (ML-I) and ricin [48]. The *in vitro* translation assay couples the following reactions into one step: (1) DNA consisting of a coding sequence is transcribed into messenger RNA; and (2) RNA is then translated into proteins in a cell lysate (product of burst cells) that provides ribosomes and other necessary components. When the translated protein is luciferase, the fluorescence acts as a protein synthesis indicator, and the absence of fluorescence indicates that protein synthesis was inhibited (Figure 4). The inhibition of luciferase synthesis by ricin was achieved when the toxin was used at a minimum concentration of 30.2 pM (~800 pg/mL). The RIP specificity of this assay was proved using formycin 5'-monophosphate (FMP) as a specific inhibitor of RIP activity. The limit of detection is comparable to those obtained with other methodologies, and the assay also showed the toxic activity of ricin.

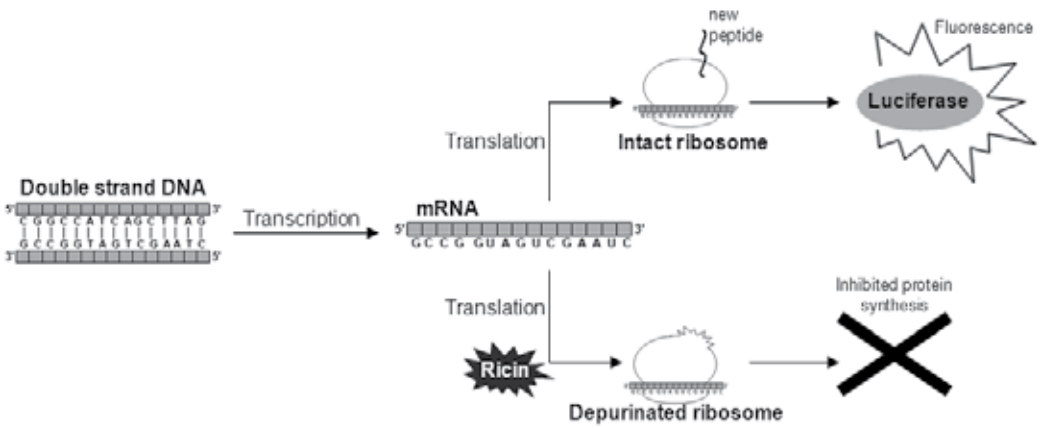


**Figure 4.** Translation and protein synthesis inhibition by ricin. The assays based on this activity detect the presence of an specific reporter protein. In the presence of the protein can not be synthesized. The luciferase is the best described example for this kind of assay.

The inhibition of protein synthesis was also the target of a method to detect ricin in a "well-in-well" device [49]. The miniaturised system presented a mechanism to supply nutrients continuously and remove by-products, leading to higher protein expression yields and larger detection signals. This method showed a detection limit of 0.3 ng/mL ricin. The nested-well device was also used for measuring the toxicity of ricin after physical or chemical treatment. The good results obtained with inactivated ricin make this method a good choice for use in castor cake detoxification processes.

The N-glycosidase activity removes an adenine residue from the $\alpha$-sarcin/ricin loop of rRNA. The removed adenine can be used as a positive indicator of biologically active ricin. The most common method for quantifying free adenine in a variety of applications is the detection of fluorescent-derivatised adenine by HPLC [50]. To detect ricin activity based on

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rRNAdepurination, a high-throughput, enzyme-based colorimetric adenine quantification assay was developed [51]. The key step of this assay is the conversion of adenine to AMP and concurrent release of pyrophosphate from PRPP. Pyrophosphate is then cleaved to phosphate by inorganic pyrophosphatase. To enhance the signal, the AMP formed is converted by 5′-nucleotidase to adenosine and inorganic phosphate, finally resulting in three phosphates for each adenine. Inorganic phosphate was quantified by a modified procedure with a commercially available kit. All four enzyme reactions of the assay, including colour development, occur simultaneously in approximately 15 minutes inside the same reaction tube, and the rate of adenine released by the commercially obtained RTA was determined to be 43 pmol adenine/pmol RTA per hour.

Recently, several methods using electrochemiluminescence (ECL) to detect ricin activity were also developed [52, 53]. First, a deadenylation assay using paramagnetic beads could detect ricin in crude extracts [52, 54]. Synthetic biotinylated RNA substrates were cleaved by the combined actions of the ricin holotoxin and a chemical agent, N,N′-dimethylethylenediamine. The annealing of the product with a ruthenylatedoligodeoxynucleotide resulted in the capture of ruthenium chelate onto magnetic beads, enabling the electrochemiluminescence (ECL)-based detection of RNA N-glycosidase activities of toxins. Compared to ECL immunoassays [26], the ECL activity assay presented lower sensitivity, reaching a detection limit of 100 pg/mL. The disadvantage of the ECL immunoassay compared to the ECL activity assay is that the antibodies recognise surface features of the proteins (epitopes) that may be unrelated to any enzymatic activity or other mechanism of toxicity. Therefore, it may be possible for inactive protein toxins to cause positive signals in these immunoassays resulting in an over-estimation of the threat. The plate-based assay unlike the bead-based assay, included wash steps that enabled the removal of food particles, thereby maximising the matrix effects and improving the limits of detection. The limits of detection for ricin in apple juice, vegetable juice, and citrate buffer using the bead-based assay were 0.4, 1, and 0.1 μg/mL, respectively. By contrast, the limits of detection for ricin using the plate-based assay were 0.04, 0.1, and 0.04 μg/mL in apple juice, vegetable juice, and citrate buffer, respectively. These data suggest that the plate-based assay is the best method for detecting ricin activity by ECL.

The ricin detection methods based on adenine liberation and direct infusion electron spray ionisation mass spectrometry have been shown to provide rapid, selective, and sensitive detection of various peptides and small nucleic acids, and these methods should provide a sensitive method for the real-time analysis of RIP enzymatic activity by monitoring adenine release. Therefore, high-performance liquid chromatography (HPLC) and selected ion monitoring mass spectrometry (MS) were used to develop a quantitative assay for adenine release from a synthetic RNA substrate by the ricin A chain [55]. The sensitivity of this MS assay made it possible to measure RIP activity at approximately 0.6- to 600 ng/mL. A more specific assay to detect ricin by MS was developed by Becher et al. [56] in which they used an anti-B chain mAb immobilised on magnetic beads to capture the toxin. Ricin toxicity was measured through quantification of the free adenine by HPLC-MS. The immunoaffinity step

combined with enzymatic activity detection led to a specific assay for the entire functional ricin protein with a lower limit of detection of 100 pg/mL.

When mass spectrometry was used to detect ricin activity, a combination of three techniques, all performed on the same sample, provided a sensitive and selective analysis of ricin isolated from a food or clinical sample and measured the activity of the toxin [57]. First, ricin was isolated from abundant proteins in a food or clinical sample, such as milk, apple juice, serum or saliva through immunoaffinity capture on antibody-coated beads. Second, the activity of ricin was examined through interaction of the toxin with a DNA substrate that simulated the *in vivo* target of the toxin. The DNA substrate was analysed by MALDI-TOF MS, allowing for sensitive and selective measurements of the depurination of the DNA substrate. Finally, in the third step, the ricin was subjected to tryptic digestion, and the resulting tryptic fragments were analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS), allowing for direct examination of the composition of the ricin protein based on the molecular weight change caused by the depurination activity. The limit of detection was approximately 300 ng/mL.

The mass spectrometry based methods for detecting ricin activity through monitoring adenine liberation have some disadvantages that make them not suitable for use in the validation of the detoxification processes of the castor cakes. These disadvantages include complications regarding the handling of mass spectrometers and the interpretation of results that requires highly specialised personnel. Another problem is that adenine liberation may not be the most efficient method to detect biologically active ricin because depurination activity is not a unique mechanism involved in ricin toxicity. It was previously shown that non-cytotoxic RTA mutants could depurinate ribosomes in yeast cells without the occurrence of cell death and apoptosis signals [58].

Toxicology assays to detect ricin based on the activity against animals could be the best way to evaluate the efficiency of castor cake detoxification processes because of the desire to use this by-product as animal feedstock. However, despite the ethical questions surrounding the use of *in vivo* models, there are also economic and infrastructure problems. Housing live animals to evaluate toxic activity requires physical space and maintenance. Therefore, an *in vitro* assay based on the cytotoxicity against Jurkat clone E6-1 cells was developed to detect ricin in different beverages, such as orange juice, coffee and soda, and food matrices, such as milk, milk baby formula and soy baby formula [59]. After incubating the cells in a 96-well plate with ricin, the culture was maintained overnight at 37ºC and 5% $CO_2$. Aliquots of each treated well were collected and assayed for lactate dehydrogenase (LDH) activity with a colorimetric assay. LDH was released from the cytosol upon cell damage and was positively correlated with cell death. Ricin was detected in each assayed matrix with a sensitivity of 10-100 pg/mL. It was also shown that ricin cytotoxicity could be inhibited by the administration of an anti-ricin neutralising antibody that works as a qualitative mechanism. Other cell culture assays were also recently developed. Sehgal et al. [60] used Vero cells (*Chlorocebus sabaeus* kidney cells) to evaluate the cytotoxicity of different ricin isoforms. They showed that the isoforms R-I, R-II and R-III were detected at a minimum concentration of 20 mg/mL, 10 ng/mL and 2 ng/mL, respectively. Subsequently, they showed that the cytotoxicity of the

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three isoforms is time dependent and that the R-III isoform is more glycosylated than the other two isoforms [61].

The possibility of using cell culture models to evaluate ricin toxicity by colorimetric assays, such as the LDH assay, seem to be a good idea for use as a biological test to determine the efficiency of the castor bean cake detoxification process. It was reported that solid-state fermentation (SSF) reduced the ricin levels in castor bean alkaline waste from Petrobras (the national petroleum company of Brazil) during the biodiesel production process [45, 62]. This was determined by molecular exclusion chromatography and electrophoresis. To verify the biological activity of ricin after SSF at different time intervals, an *in vitro* assay using the Vero cell line was performed [63]. Using this methodology, it was verified that after 24 and 48 hours of fermentation, the cell culture showed slight growth inhibition. The waste was completely detoxified after only 72 hours of fungal growth. The cell incubation period with the protein extract from the fermented waste was 24 hours, and cell death was determined by cell counting with an optical microscope and measurement of LDH activity using a colorimetric assay (Figure 5).

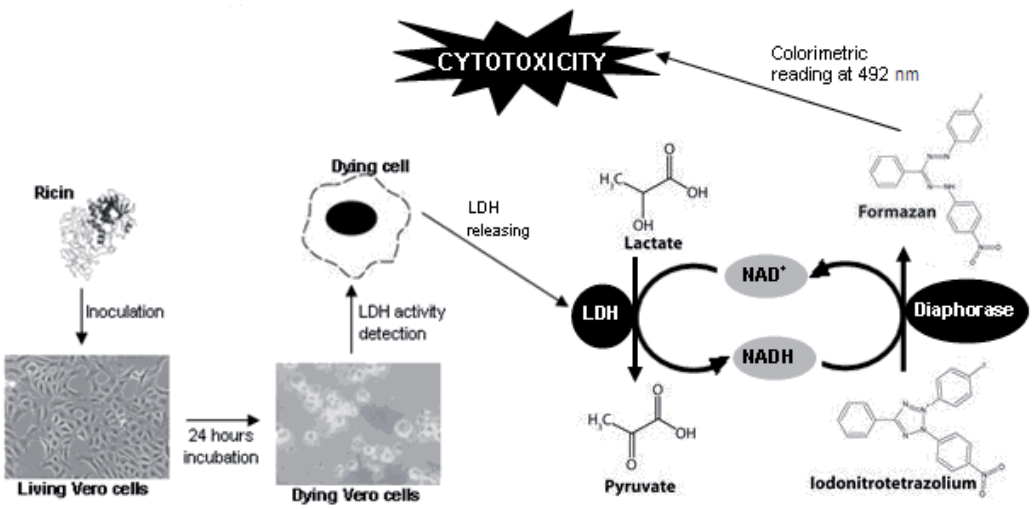


**Figure 5.** Cytotoxicity assay using Vero cells. The cell monolayer in a 24-well plate was incubated with ricin for 24 hours. An aliquot from each well was removed and mixed with the assay solution for LDH activity determination. The formazan formed a salt that caused the solution to turn red. The cytotoxicity is measured based on the intensity of this colouration.

When the cell counting and LDH assays were compared to determine the cytotoxicity of ricin against Vero cells, it was reported that both methods are efficient and detected ricin at a minimum concentration of 10 ng/mL [64]. After adjusting the method to detect the purified protein, they used the Vero cell cytotoxicity assay to evaluate the following two castor cake detoxification processes: SSF using *Aspergillus niger*and treatment with calcium compounds. The results with the Vero cells showed that both treatments were efficient in eliminating ricin toxicity from the castor cake.

## 3. *Jatropha curcas* toxins

Two main toxic components are present in the physic nut plant, the ribosome-inactivating protein, curcin, and phorbol esters. Among these toxins, the phorbol esters are the most dangerous toxic components in *J. curcas*and limit the use of Jatropha cake in animal feed.

### 3.1. Curcin

Curcin (28.2 kDa) is a type 1 RIP that is found in *Jatropha curcas* seeds [65] and leaves [66]. Curcin is different from ricin in that it is a monomeric protein with N-glycosidase activity but lacks a lectin chain [67]. Therefore, this protein is much less cytotoxic than ricin and other type 2 RIPs because it cannot enter cells by binding to sugar residues. Despite the fact that curcin is less toxic than phorbol esters, it has been reported to be toxic to some animals, including sheep, goats, chickens and calves and also to humans [68-72]. Because of the low toxicity of curcin, there are not many detection methods specifically for this toxin. The most common detection methods are the inhibition of translation in rabbit reticulocyte lysates and the measurement of N-glycosidase activity [67]. Although there are few publications describing the different methods to detect curcin, many of the assay methods for ricin could be applied to other RIPs, including curcin.

### 3.2. Phorbol esters

Phorbol esters (PE) are polycyclic compounds in which two hydroxyl groups in neighbouring carbons are esterified to fatty acids, and these substances are present in many different plants, including *J. curcas* [73]. The PE molecules are dependent on a tetracyclic diterpene carbonic structure termed tigliane. The different hydroxylation points of tigliane determine the different varieties of PE and their toxicity [74].

The PEs and their different derivatives are known for their tumour induction activity. They activate protein kinase C (PKC), which plays a critical role in signal transduction pathways and regulates cell proliferation [75]. By contrast, it was reported that some types of PEs could induce apoptosis [76].

Several detoxification processes used to eliminate PEs from Jatropha cake have been previously described [14], and some of the existing detection methods were used to confirm the effectiveness of these processes.

#### *3.2.1. Detection of phorbol esters*

Many of the phorbol ester detection methods are related to using Jatropha cake as animal feedstock, which are different from ricin containing cakes that can be used as bioterrorism agents. Therefore, there are few techniques for PE detection compared to ricin detection methods.

The determination of irritant activity caused by phorbol esters was first demonstrated by Adolf et al. [77]. The irritant activity of PE isolated from different Jatropha species was as-

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sayed in rat ears, and the irritant dose 50 for *J. curcas* PEs was 0.02 μg/ear. More than two decades later, *in vivo* studies of PE toxicity are still performed in rats and mice [78, 79]. Jatropha cake subjected to alkali and heat treatments to reduce the PE level was used to feed rats, and several clinical aspects and the mortality rate were compared with rats fed untreated cake [78]. Using these rodents to detect PE toxicity was effective because even the treated Jatropha cakes with low levels of PE (8.1 mg%) caused rat mortality after 11 days. The acute toxicity of PE was determined in Swiss Hauschika mice by intragastric administration [79]. The $LD_5$ and $LD_{95}$ were 18.87 and 39.62 mg/kg body mass, respectively. These toxicity assays efficiently detect PE toxicity; however, they are problematic because of maintaining and sacrificing many animals due to the large quantities of residual cake that is generated.

The most commonly used method to detect and quantify PE from *Jatropha curcas* is reverse phase - high-performance liquid chromatography (RP-HPLC). This method was standardised to detect PE in different provenances of *J. curcas*, and it was the first method to identify the absence of PE in seeds from Papantla, Mexico [80, 81]. The protocol established in this study has been optimized [82] to show the presence of PE. The limit of detection of PE by RP-HPLC analysis is approximately 4 μg, as described by Devappa et al. [83]. RP-HPLC detection has been used by many researchers to determine the efficiency of Jatropha cake detoxification processes, including hydrothermal processing techniques, solvent extraction, solvent extraction plus treatment with $NaHCO_3$, ionizing radiation, heating, bio-detoxification and surfactant solution extractions [84-88]. This technique can also be used to identify different PE species present in *J. curcas*, and the difference in PE composition among Jatropha seeds from different regions, cultivars and assessments [83, 89-91]. HPLC was also used to determine the PE content in oil extracted from the seeds [92-94].

Similar to ricin detection methods, the biological activity of phorbol esters must to be assayed to guarantee the efficiency of the Jatropha cake detoxification processes. Because Jatropha cake is used as feedstock, quality control of detoxification processes is often performed using live animals, such as rats [77, 79]), sheep [95], pigs [96] and fish [97, 98]. With a few exceptions, this type of biological activity control is usually preceded by RP-HPLC detection and quantification of PEs. Therefore, it remains necessary to continue using RP-HPLC and sacrificing animals to detect the presence and biological activity of PEs because toxicity evaluation using live animals is not the best method for use on a large scale. Other biological tests have previously been described for assaying PE toxicity, and some of these assays are very sensitive and simple to perform on a large scale.

Earlier reports regarding *J. curcas*have described molluscicidal activity of the seed extracts against *Oncomelania quadrasi* [99] and of the root extracts against *Bulinus truncatus* [100]. However, the most well-established molluscicidal test using snails was described by Liu et al. [101]. They tested several plant extracts, including *J. curcas* phorbol esters in methanol, against three schistosome vector snails: *Oncomelania hupensis*, *Biomphalaria glabrata*and *Bulinus globosus.* The 4-β-phorbol-13-decanoate was the most effective phorbol ester against the snails. It killed both species ($LC_{100}$) at a concentration of 10 mg/mL. One disadvantage of this method is the requirement of a large volume of the test substances because the assay must to be performed in 100 mL Petri dishes. However, the assay using snails continues to be used

and is sometimes combined with HPLC detection and quantification steps. Another species that was tested for PE toxicity was *Physa fontinalis,* which was sensitive to 0.1 mg/L (6.7% mortality) PE-rich extract, and the $LC_{100}$ was reported as 1 mg/mL [93, 102]). The variation in PE sensitivity among the snails may be related to species-specific PE sensitivity and/or different chemical properties of the PEs. In addition to testing for PE activity against host snails, the susceptibility of the parasite *Schistosoma mansoni*was also assayed [103]. This test had the advantage of requiring a small volume of test substance. The PE-rich methanol extract from *J. curcas* crude oil that was obtained by pressing the seeds was able to kill all the cercarie ($LC_{100}$) at a concentration of 25 mg/mL.

The efficacy of phorbol esters against insects has been shown recently. Termites (*Odontotermes obesus*) were used as a target to test PE toxicity [104]. Because it was necessary to use HPLC to isolate and quantify PE from *J. curcas* seeds, they tested different concentrations of PE (500-5 mg/mL) over a period of 1 to 72 hours. The $LC_{100}$ was determined after 72 hours of treatment using 5 mg/mL of PE. However, to decrease the assay time, it was necessary to use higher concentrations of PE. To obtain the $LC_{100}$ after 12 hours of treatment, they used 500 mg/mL of PE. Another study using insects was recently performed by Devappa et al. [105]. They tested a PE-enriched fraction (PEEF) against *Spodoptera frugiperda* and the mortality was evaluated 24 hours after treatment with different concentrations of PEEF. A minimum mortality (20%) was reached using 0.5 mg/mL PEEF and a maximum of 80% mortality was observed with 2 mg/mL PEEF. The sensitivity to PEs of both species (*O. obesus* and *S. frugiperda*) is not very different, and this assay showed that PEs can be used as an insecticide and that insects are good models for detecting the toxic activity of PEs.

Some crustaceans are widely used as toxicity indicators in bioassay systems. Phorbol ester toxicity has previously been assayed to *Artemia salina* and *Daphnia magna* [83]. The advantages of using *A. salina* in toxicological assays were demonstrated by Ruebhart et al. [106]. These advantages include wide commercial availability of the cysts, easy storage, maintenance and hatching of the cysts, the assay is cost effective, simple, rapid and sensitive, less test samples are required, the assays can be performed in 96-well microplates and meets the ethical animal treatment guidelines of many countries. The best PE induced mortality rate (72%) was observed using a concentration of 47 mg/mL [83]. Increasing the concentration did not effectively improve the mortality rate because 6000 mg/mL of PE was needed to reach 100% mortality. Different types of PEs were previously tested against *A. salina* [107] and there was variation in the mortality rates to each PE. This reinforces the role of the PE chemical structure and purity with regard to toxicity. The first toxicological assay of PEs from *J. curcas* using *Daphnia magna* showed that these crustaceans are more sensitive to PEs than *A. salina* [83]. The $LC_{100}$ was only 3 mg/mL, and the lowest effective concentration, which induced 26% mortality, was 0.5 mg/mL. Although snails were more sensitive to PEs than crustaceans, the use of *A. salina* and *D. magna* is preferred for assaying a large number of PE samples because the test can be performed in 96-well plates.

Similar to the molluscicidal, insecticidal and antiparasitic activity, PE toxicity against microorganisms was also reported. It was demonstrated that phorbol esters from *Sapium indicum* had antibacterial activity [108]. Six bacteria genera were recently tested for PE toxicity. The

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maximum concentration of PE-rich extract for each bacterium tested was 537 μg/mL for *Bacillus subtillis*, 250.7 μg/mL for *Pseudomonas putida*, 215 μg/mL for *Proteus mirabilis*, 394 μg/mL for *Staphylococcus aureus*, 215 μg/mL for *Streptococcus pyogenes* and 465.7 μg/mL for *Escherichia coli* [83]. Compared with the other biological assays presented here, the use of bacteria to detect the toxic activity of PEs is not very effective because the sensitivity is much higher than those reported for *D. magna*. The use of PEs as an antibacterial agent was also not as effective compared with the other compounds. The antifungal activity of *J. curcas*PEs extracted from residual cake has previously been tested [83, 109]. The toxicity of the PE-rich extract (from Jatropha cake) against *Fusarium oxysporum*, *Pythiumaphani dermatum*, *Lasiodiplodia theobromae*, *Curvularia lunata*, *Fusariums emitectum*, *Colletotrichum capsici* and *Colletotrichum gloeosporioides* was assayed and the concentrations that inhibited 100% of mycelial growth was 6, 3, 6, 5, 3, 4and 10 mg/L, respectively. Although a high concentration of PEs was required to reach 100% inhibition, they used 500 μg/mL PEsand reported minimum mycelial growth inhibition values for each species [109]. Another PE-rich extract toxicity study using fungi was recently reported [83]. In this study, it was demonstrated that of seven species of fungi, the most sensitive to PE toxicity were *Botrytis cinerea*, *Fusarium oxysporum* and *Fusarium moniliforme* and 100% inhibition was achieved at a concentration of 114.6 μg/mL. The other four species tested, *Aspergillus niger*, *Aspergillus flavus*, *Curvularia lunata* and *Penicillium notatum*, were less susceptible to PE toxicity and 100% inhibition was reached using 143.3 μg/mL. Antimicrobial tests using bacteria and fungi efficiently detect PE toxic activity and could be used for quality control to determine the effectiveness of Jatropha cake detoxification processes.

Because PEs are activators of protein kinase C (PKC), a biochemical assay to detect PEs based on this property was described [110]. In this method, PKC is incubated with Mg-ATP and a synthetic peptide which is labelled with a fluorescent dye. When a PKC activator is present, the active enzyme phosphorylates the peptide. When the reaction mixture is separated by electrophoresis, the phosphorylated peptide becomes negatively charged and migrates to the positive pole. The fluorescently labelled peptide can then be quantified by densitometric analysis. This assay was used by Wink et al. [110] to determine the activity of PEs sequestered by *Pachycoris klugii*. The positive control (12-O-tetradecanoylphorbol-13-acetate) was used at a concentration of ~6μg/mL and indicated that this activity assay is very sensitive. Because of the high sensitivity and availability of commercial PKC activity assay kits, this method could be used for the rapid and efficient detection of PEs in detoxified Jatropha cake.

Although many methods have been described to detect *Jatropha curcas* phorbol esters, these biological tests are not specific to PEs. In contrast to ricin detection assays that can combine biological assays with antibody recognition [59, 63, 64], PEs cannot be tested with this methodology. The best method to test for PEs is to continue using HPLC analysis followed by a biological test. The most well-established biological assay is the assay using snails, which has previously been used as a quality control for Jatropha cake detoxification [83, 103]. Although several of the *in vitro* assays, such as PKC activity and toxicity against microorganisms are more sensitive, they were not used for this purpose, and additional studies are necessary.

## 4. Conclusion

Currently, several processes to detoxify castor bean and Jatropha cakes have been developed however, it is essential to choose a method that is universally accepted to validate such processes of detoxification. The literature indicates that the method to be used to evaluate the toxicity of castor cake is different from what should be used for jatropha cake.

Among the different methods that can be used to assess the presence of ricin some are more suitable to control attacks bioterrorist. They are sensitive methods that detect the presence of ricin, but need not evaluate the biological activity.

In this review, methods based on Vero cell viability are best suited to validate the processes of castor cake detoxification. Vero cells, epithelial cell line isolated from African green monkey are indicated since these cells maintain cell organelles characteristics and stable structure when in contact with the cake detoxified. Evaluation procedures for Jatropha are still under development. The detection of phorbol esters by reverse phase chromatography, associated with toxicity tests on snails are recommended.

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Chapter 11

# Bio-Detoxification of Jatropha Seed Cake and Its Use in Animal Feed

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Additional information is available at the end of the chapter

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## 1. Introduction

Biodiesel production using the seed oil of *Jatropha curcas* L. (physic nut), as a raw material, results in large amounts of solid residue, called Jatropha seed cake. This seed cake contains lignocellulosic compounds, water, minerals and proteins [1-3]. However, it also contains toxic compounds and anti-nutritional factors [1-3]. The detoxification and reuse of this seed cake is very important for adding economic value, and also reduces potential environmental damage caused by improper disposal of this by-product.

The toxicity of Jatropha seed is mainly attributed to a group of diterpene esters called phorbolesters. These esters are present in high concentrations in toxic seed varieties but in lower concentrations in a non-toxic seed variety from Mexico [4]. Phorbol esters activate protein kinase C, a key signal transduction enzyme released in response to various hormones and developmental processes in most cells and tissues [5,6].

In addition to the phorbol esters, there is also a toxic protein called curcin in Jatropha seed cake. This protein has two polypeptide chains and is able to inhibit protein synthesis [7]. Curcin is a ribosome-inactivating protein and promotes mucosal irritation and gastrointestinal hemagglutinating action [8].

Phytic acid (myo-inositol hexaphosphoric acid) and tannins are considered anti-nutritional factors because they inhibit the absorption of proteins and minerals [9-11]. Phytic acid is a

compound formed during seed maturation [12]. The seed of *J. curcas* has a high concentration of phytic acid, up to 10% of its dry matter [2]. Tannins are polyphenols water-soluble and polar solvents [13]. The tannin content in the seeds of *J. curcas* is low, representing only 3% of its dry weight [13].

Detoxification of the Jatropha seed cake could allow its use as a protein-rich dietary supplement in the animal feed [1,14,15].

The use of residue or by-products in animal nutrition can minimize expenditures on the development of food sources, such as soybean, cotton and wheat meals, without causing undesirable effects on the overall production system. However, it is first necessary to know the nutritional value and effects of the by-product's inclusion in animal diets.

Some studies have used physical and chemical treatments to detoxify Jatropha seed [2,16,17]. These methods have been effective but require the use of chemicals that may result in other the presence of other residues. Conversely, bio-detoxification does not require the application of any chemical compounds. It may also reduce the concentrations of phorbol esters and anti-nutritional factors to non-toxic levels [18].

## 2. Methodology

### 2.1. Microorganism, fungal growth conditions and inoculum production (spawn)

The isolate Plo 6 of *P. ostreatus* used in this study belongs to a culture collection from the Department of Microbiology at the Federal University of Viçosa, MG, Brazil. *P. ostreatus* was grown in a Petri dish containing potato dextrose agar culture medium at pH 5.8 and incubated at 25 °C. After seven days, the mycelium was used for inoculum production (spawn) in a substrate made of rice grains [19]. The rice was cooked for 30 min in water with a ratio of 1:3 rice: water (w/w). After cooking, the rice was drained and supplemented with 0.35% $CaCO_3$ and 0.01% $CaSO_4$. Seventy grams of rice was packed into small glass jars and sterilized in an autoclave at 121 ºC for 1 h. After cooling, each jar was inoculated with 4 agar discs (each 5 mm in diameter) containing the mycelium. The jars were then incubated in the dark at room temperature for 15 d.

### 2.2. Substrate and inoculation

The *J. curcas* seed cake was obtained from an industry of biodiesel (Fuserman Biocombustíveis, Barbacena, Minas Gerais State, Brazil).

To select the most suitable substrates for lignocellulolytic enzyme production, we conducted preliminary experiments with Jatropha seed cake and various lignocellulosic residues. We tested *P. ostreatus* growing on Jatropha seed cake with different percentages of eucalyptus sawdust, eucalyptus bark, corncobs, and coffee husks [20]. The addition of these agroindustrial residues was necessary to balance the carbon and nitrogen ratio, which might benefit mycelial growth [21-23].

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The compositions selected for biological detoxification were based on the results of the above preliminary experiments (Table 1). The substrates were humidified with water to 75% of their retention capacity. Then, 1.5 kg of each substrate was placed in polypropylene bags and autoclaved at 121 °C for 2 h. After cooling, the substrates were inoculated with 75 g of spawn and incubated at 25 °C. Samples from non-inoculated autoclaved bags were kept as controls.

| Substrates | Mass substrates (kg) | |
|---|---|---|
| | Jc | Agroindustrial residue |
| Jatropha seed cake (Jc) | 20 | 0 |
| Jc + 10% eucalypt bark (JcEb10) | 18 | 2 |

**Table 1.** Substrate compositions used for *Pleurotus ostreatus* growth

### 2.3. Chemical composition of the substrates and enzymatic assays

The phorbol ester contents were analyzed by high performance liquid chromatography (HPLC), as previously described [2]. A standard curve was made using solutions of phorbol-12-myristate 13-acetate (Sigma Chemical, St. Louis, USA) at concentrations from 0.005 to 0.5 mg $mL^{-1}$.

To determinate the dry mass, 1.5 kg of the substrate was dried at 105 ºC until a constant weight was obtained.

The levels of tannins and phytic acid were quantified by a colorimetric method [24,25].

The laccase and manganese peroxidase activities were measured using 2,2'-azino-bis-3-etilbenzotiazol-6-sulfonic acid [26] and phenol red solution [27] as substrates, respectively. Xylanase and cellulase activity was calculated by measuring the levels of reducing sugars produced by the enzymatic reactions [28,29]. Phytase activity (myo-inositol hexakisphosphate phosphohydrolase) was determined using the Taussky-Schoor reagent [30].

The level of reducing sugars was determined by the dinitrosalicylic acid (DNS) method (99.5% dinitrosalicylic acid, 0.4% phenol and 0.14% sodium metabisulfite).A standard curve was made with D-glucose, with concentrations from 0.5 to 1.5 g $L^{-1}$ [31].

### 2.4. Digestibility of Jatropha seed cake and ammonium production in rumen liquid measured *in vitro*

To analyze the suitability of the chosen substrates (Table 1) in animal feed, we determined their levels of dry matter (DM), organic matter (OM), crude protein (CP), mineral matter (MM), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), non-fiber carbohydrates(NFC), hemicellulose (HEM), cellulose (CEL) and lignin according to previously described methodology [32,33].

The *in vitro* dry matter digestibility (IVDMD) was determined according to a previous method [34], with some modifications. One liter of rumen fluid was collected from fistulated cattle kept at the Department of Animal Science, Federal University of Viçosa, about two hours after feeding. The animals' diet consisted primarily of grass and corn silage. The rumen digesta was filtered through 4 layers of gauze and the liquid fraction was stored in capped plastic flasks and refrigerated. Ruminal fluid was incubated at 39 °C for 30 min to suspend feed particles and precipitate protozoa, which allowed ruminal bacteria to be collected anaerobically from the middle of the flasks. The IVDMD assay was performed in two steps. In the first step, 350 mg of each substrate sample, harvested before and after colonization by *P. ostreatus*, was incubated with a mixture of 4 mL of ruminal fluid and 32 mL of McDougall buffer. This procedure was performed in anaerobic bottles with a continuous flow of carbon dioxide ($CO_2$). Bottles were then sealed with rubber stoppers and aluminum closures and incubated for 48 h at 39 °C at 120 rpm. In the second step, after incubation, the filtered material was placed inpre-dried and weighed porcelain filters and washed with hot water four times or until complete removal of all McDougall solution. Next, we added 70 mL of a detergent solution,and the samples were autoclaved for 15 minutes at 121 °C. After heat treatment, the filters were washed again with hot water until complete removal of the detergent solution, and then washed using 10 mL of pure acetone. The filters were heated to 105 °C for 16 h or overnight. After that, the filters were placed in a desiccator, and the dried mass was measured on an analytical balance.

To analyze the production of ammonia, the samples were incubated under the same conditions as described for the IVDMD process. These samples were placed into two flasks containing buffer, rumen fluid and the substrates samples (Table 1) harvested before and after fungal colonization. Ammonia quantification was determined using ammonium chloride as an indicator and absorbance was measured in a spectrophotometer (Spectronic 20D) at 630 nm [35].

### 2.5. Animal assay

The experiment was conducted in theGoat Experimental Section from the Department of Animal Science at the Federal University of Viçosa - MG, BRAZIL. Twenty-four healthy female Alpine goats weighing 20 ±1.5 kg, with a mean age of five months, were used. This experiment was performed after the Jatropha seed cake had been bio-detoxified by *P.ostreatus*Plo6 [20].

#### *2.5.1. Experimental design*

The experimental trial lasted 72 days. During the first12 days, animals were allowed to adapt to the experimental diet. The data were collected during the following 60days.

The animals were kept in individual confinement stables (1.5x2.0m) equipped with food and water systems. The stables had fully slatted floors adapted for the total collection of feces and urine. Water was provided *ad libitum*. The daily food consumption was quantified by subtracting the total offered feed.

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The diets were formulated to meet the nutritional requirements of goats with a starting ody weight of 20 kg and a daily weight gain of 100g [37]. The feed contained an average of 12% crude protein.

The treatments consisted ofthe detoxified substrates at four levels: 0, 7, 14 and 20% (based on total dry matter) inforagehayTifton-85 (*Cynodon* spp). The ratio of forage: concentrate was 30:70(Table 2).

| Ingredient | Bio-detoxified jatropha seed cake (% dry mass) | | | |
|---|---|---|---|---|
| | 0 | 7 | 14 | 20 |
| Forage hay Tifton-85 | 33.18 | 31.26 | 31.26 | 31.25 |
| Jatropha seed cake bio-detoxified | 0.00 | 6.97 | 13.93 | 19.90 |
| Maize flour | 57.20 | 55.98 | 51.84 | 47.11 |
| Soybean meal | 8.37 | 4.57 | 1.76 | 0.53 |
| Sodium chloride | 0.20 | 0.20 | 0.20 | 0.20 |
| Calcareous | 0.95 | 0.92 | 0.90 | 0.90 |
| ADE vitamins | 0.08 | 0.08 | 0.08 | 0.08 |
| Micromineral mixture* | 0.03 | 0.03 | 0.03 | 0.03 |
| Sodium bicarbonate | 0.40 | 0.40 | 0.40 | 0.40 |
| **Chemical composition (%)** | | | | |
| Dry mass (DM) | 84.65 | 84.75 | 84.80 | 84.81 |
| Crude protein (CP) | 12.84 | 12.05 | 11.73 | 11.89 |
| Ether extract (EE) | 3.38 | 3.25 | 3.06 | 2.87 |
| Neutral detergent fiber (NDF) | 41.00 | 42.46 | 44.42 | 45.94 |
| Lignin | 2.55 | 4.04 | 5.67 | 7.06 |
| Calcium | 0.19 | 0.22 | 0.26 | 0.30 |
| Phosphorus | 0.27 | 0.28 | 0.30 | 0.32 |
| Net energy (NE, Mcal/kg) | 1.87 | 1.72 | 1.63 | 1.54 |

**Table 2.** The chemical composition and ingredient proportions of the diet

The feed was supplied twice a day as a complete mixture to allow intake of approximately 10% of the offered amount. The amount was based on the intake of the previous day.

To determine *in vivo* digestibility and nitrogen balance, we collected total feces and urine for five days. Ten percent of the total excretion was sampled. Urine was stored in plastic bags containing 20 mL of sulfuric acid (40% v:v).

The fecal metabolic nitrogen level (Nmet.fecal) was calculated according to previously established methods [37]. The amount of undigested nitrogen (Nund) was calculated as the difference between fecal nitrogen (Nfecal) and Nmet.fecal. To determine the fraction of urinary nitrogen of endogenous origin (Nuend), a previously published equation was used [38]. From the difference between the urinary nitrogen and endogenous urinary nitrogen (Nuend) levels, we calculated the exogenous urinary nitrogen (Nuexo). Nitrogen balance (NB) was estimated with the following equation: NB = N ingested - [Nund + Nuexo]. The biological value of protein was calculated according to previous methods [39].

The chemical compositions of feeds, orts and feces as a percentage of DM, MM, OM, CP, EE, NDF, ADL and NFC were determined according to previous methodology [32, 33, 40]. The total protein level in the bio-detoxified Jatropha seed cake was calculated from the total nitrogen content by applying the correction factor 4.38. The net energy (NE) was obtained by a previously reported equation [41].

The blood samples were collected in the morning, before supplying the feed, by jugular puncture and vacuum tubes. Blood was stored with and without the anticoagulant EDTA. After this procedure, the tubes were refrigerated and sent to a laboratory for blood biochemical analysis to determine the hemogram compounds. This analysis included the numbers of erythrocytes, hemoglobin, hematocrit and leukocytes. In the Blood serum was analyzed creatinine, alkaline phosphatase, urea and total protein.

### 2.6. Statistical analyses

The experiments on phorbol ester degradation, anti-nutritional factors, *in vitro* digestibility of Jatropha seed cake, and production of liquid ammonia in the rumen were of a randomized design with 5 replicates each. The resulting data were subjected to analysis of variance (ANOVA), and the mean values were compared by Tukey's test ($p < 0.05$) using Saeg software (version 9.1, Federal University of Viçosa).

The experiments on animals were distributed in a completely randomized design with six replicates per diet condition. The resulting data were subjected to analysis of variance (ANOVA) and regression analysis ($p < 0.05$). Regression models (linear, quadratic or cubic) were fitted to the observed significance (5% level of probability) using the REG procedure (SAS 9.0).

## 3. Results

After 15 days of inoculation, *P. ostreatus* Plo 6 completely colonized the substrates (Figure 1). This illustrates the ability of this fungus to grow in the presence of both phorbol esters and anti-nutritional factors.

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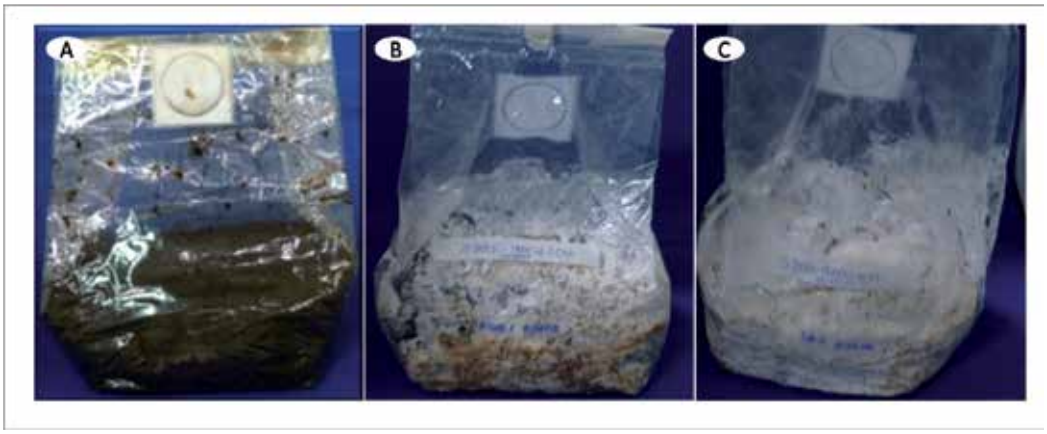


**Figure 1.** Mycelial growth of *P. ostreatus* Plo 6 in substrate containing varying percentages of Jatropha seed cake. Before the inoculation(A) and 15 days after inoculation: (B) Jatropha seed cake (Jc) and (C)Jc + 10% eucalyptus bark (JcEb10).

### 3.1. Phorbol ester degradation

Autoclaving the substrates (at 121 °C) reduced the phorbol ester content by an average of 20% (Figure 2). However, these compounds were not degraded at 160 °C for 30 min [17]. Moreover, the addition of sodium hydroxide and sodium hypochlorite combined with heat treatment was able to reduce only 25% of the phorbol concentration [42].

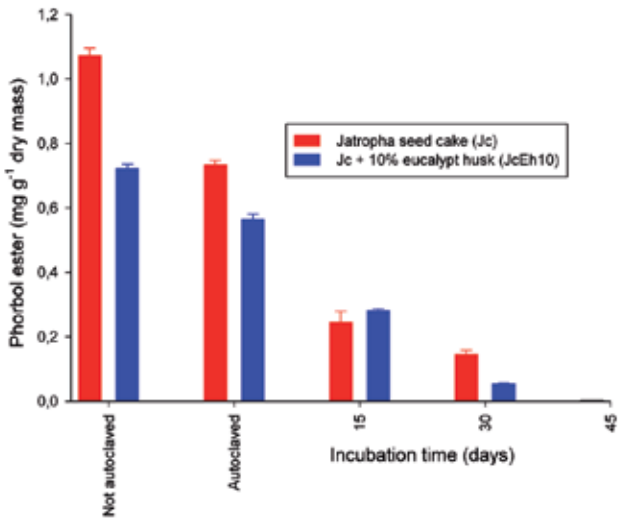


**Figure 2.** Phorbol ester degradation by *Pleurotus ostreatus* Plo 6 in substrates with varying proportions of Jatropha seed cake (Table 1).

In this study, *P. ostreatus* degraded 99% of the phorbol ester after a 45-day incubation (Figure 2). This rate of degradation was higher than rates observed when chemical deodorization, de-acidification, or bleaching agents were applied to *J.curcas* oil and seed cake [43]. With the exception of bleaching, none of the above chemical processes were effective in reducing the amount of phorbol esters in *J. curcas* seed [44].

The ability of *P. ostreatus* to depolymerize lignin (Figure 3) explains the observed phorbol ester degradation (Figure 2). The degradation of other organic compounds such as chlorophenols and aromatic hydrocarbons also occurs due to depolymerization by laccase and MnP activity [45,46]. The activities of these enzymes of *Phanerochaete* sp [47] and *P. ostreatus* [48] have also been reported to cause dye discoloration in the textile industry and the elimination of pollutants. However, other enzymes may have also influenced degradation of thetoxic compounds. Higher cellulase and xylanase activities (Figure 3) were observed between the 15$^{th}$ and 30$^{th}$ incubation days, as indicated by a 58% and 85% degradation of phorbol ester, respectively. However, on the 15$^{th}$ day of incubation we observed lower phorbol ester degradation and lower ligninase activity in the substrate containing eucalyptus bark (Figures 3). This result supports the hypothesis that phorbol ester degradation occurs because of co-metabolism by the enzymes responsible for lignin depolymerization.

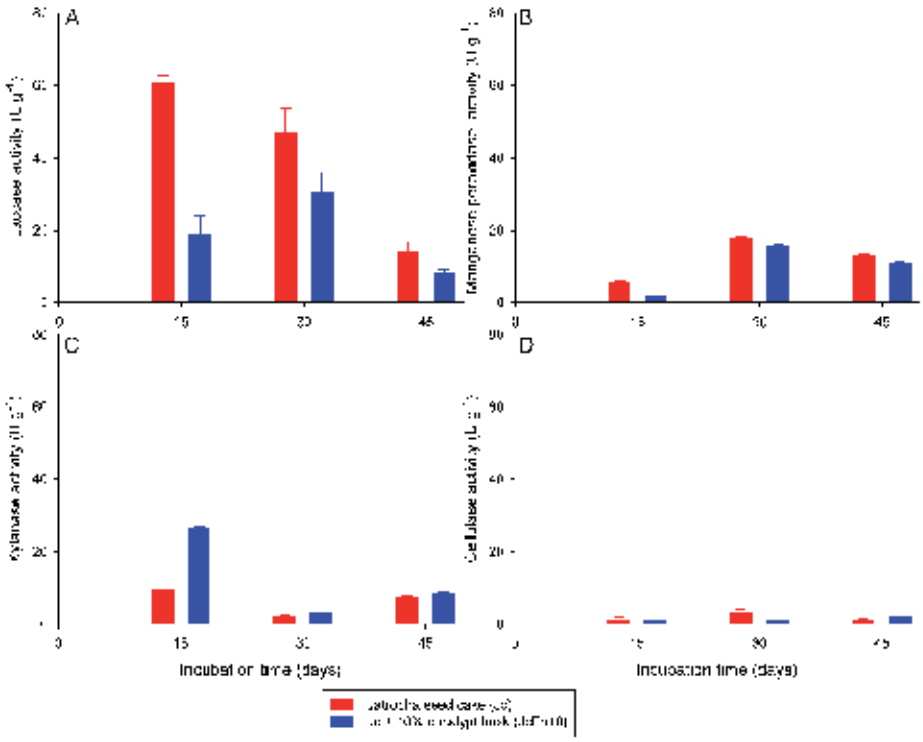


**Figure 3.** Lignocellulolytic enzymes activity of *Pleurotus ostreatus* Plo 6 in substrates with varying proportions of Jatropha seed cake (Table 1).

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After 45 d of substrate incubation with *P. ostreatus*, the residual average phorbol ester concentration was1.8 x $10^{-3}$ mg $g^{-1}$ dry mass (Figure 2). This concentration is much lower than the 0.09 mg $g^{-1}$of phorbol esters found in the non-toxic variety of *J. curcas* [17].

### 3.2. Degradation of anti-nutritional factors

Tannin concentrations observed in the seed cake (Figure 4) are similar to those previously reported in the fruit peel of *J. curcas* [4]. The greatest concentration of this compound was observed in the eucalyptus bark substrate (Figure 4). This may have been due to the prior presence of tannins in the eucalyptus bark [49].

The thermal treatment of the substrates decreased the tannin concentration by 46% (Figure 4). This result was similar to that observed in vegetables after cooking or autoclaving at 121 °C and 128 °C for different periods of time [10].

Regardless of the substrate, tannin degradation by *P. ostreatus* Plo 6 increased as a function of the incubation time. The highest observed rate was between 15 and 30 d in the substrate with eucalyptus bark (Figure 4). A high tannin degradation rate was also observed in *Pleurotus* sp. cultivated in coffee husk for 60 d [50].The degradation of tannin is related to tannase activity (tannin acyl hydrolase). This enzyme's activity in polyphenol degradation has been reported in *Aspergillus* and *Penicillium* [51]. Thus, *P. ostreatus* can degrade the tannins in Jatropha seed cake.

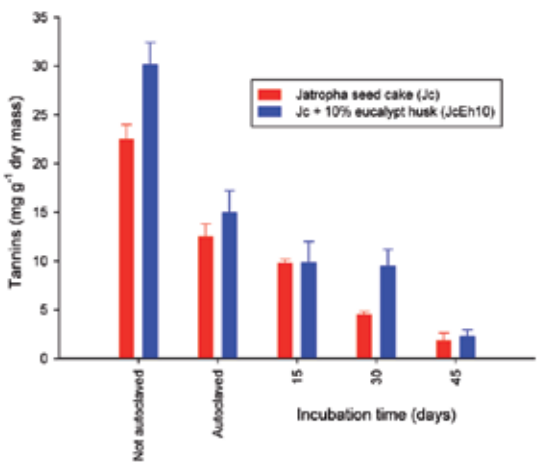


**Figure 4.** Tannin degradation by *Pleurotus ostreatus* Plo 6 in substrates with different proportions of Jatropha seed cake.

Although phytic acid is considered to be heat-stable [52], the amount of phytic acid decreased by 20% after sterilization of the substrates, (Figure 5). A degradation of 50% of this anti-nutritional factor was also been observed in legumes subjected to autoclaving at 121 ºC for 90 min [10].

Phytase activity by *P. ostreatus* caused a 95% decrease of phytic acid in the substrates (Figure 5). A high degradation rate of this anti-nutritional factor by microbial phytase has previously observed in culture medium containing rapeseed meal that has phytic acid content between 2% to 4 % of the dry mass [53]. The presence of this enzyme has also been observed in *Aspergillus* sp [9], *Agaricus* sp, *Lentinula* sp and *Pleurotus* sp [54]. Thus, *P. ostreatus* degrades the phytic acid that is present in Jatropha seed cake and thereby increases its potential for use in animal feed.

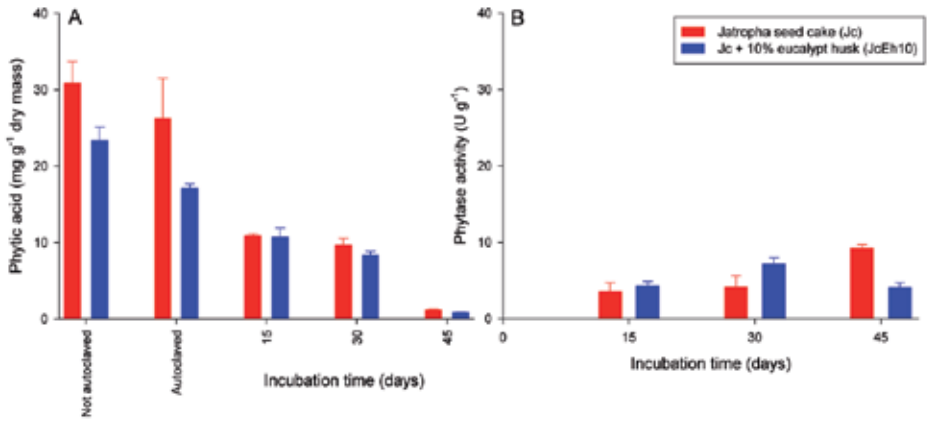


**Figure 5.** Phytic acid degradation (A) and phytase activity (B) by *Pleurotus ostreatus* Plo 6 in substrates with varying proportions of Jatropha seed cake.

### 3.3. Digestibility of Jatropha seed cake and ammonium production in rumen liquid *in vitro*

Many agro-industrial residues contain a higher content of fibers, of low digestibility, than proteins, vitamins and minerals. The colonization or fermentation of these by-products by microorganisms, especially lignocellulosic fungi, can efficiently and affordably increase their digestibility and nutritional value [55]. This procedure has been used successfully in cotton waste [56] by colonization with *Brachiaria* sp [57].

Before fungal colonization, we observed higher levels of CP, lignin, ADF and EE in the Jatropha seed cake (Table 3). These data show the importance of adding eucalyptus bark to balance carbon and nitrogen and decrease the fat content, thus resulting in improved fungal growth. Furthermore, these data confirm the potential of using the bio-detoxified seed cake as a source of protein and lipids in ruminant diets [58]. The use of foods rich in these nutrients in animal diets is important because (a) the proteins are the main source of nitrogen and amino acids, and (b) lipids can reduce the production of methane by the rumen [59]. For every 1% increase in the amount of fat added to the diet, there is a 6% reduction in methane

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emissions by ruminant animals. This reduction in methane production may be due to a negative effect on the lipid protozoa and methanogenic archaea [60].

In the ruminant diet, proteins and amino acids supply nitrogen for microbial protein production. Proteins synthesized by microorganisms of the rumen have a higher nutritional value than dietary protein. According to Alemawor et al. [61], the low level of protein in the skin of cocoa limits its use as animal feed. In this context, increasing the CP in Jatropha seed cake by colonization with *P. ostreatus* (Table 3) increases its potential for use in animal feed.

| Components (g 100g$^{-1}$) | Jatropha seed cake (Jc) | | Jc + 10% eucalypt bark (JcEb10) | |
|---|---|---|---|---|
| | 0 (control) | 45 days | 0 (control) | 45 days |
| Dry mass (DM) | 95.027$^{aB}$ | 96.243$^{A}$ | 95.028$^{aB}$ | 96.076$^{A}$ |
| Organic matter (OM) | 93.304$^{aA}$ | 91.136$^{B}$ | 92.738$^{bA}$ | 91.972$^{B}$ |
| Crude protein (CP) | 11.438$^{aB}$ | 13.158$^{A}$ | 11.075$^{bA}$ | 11.264$^{A}$ |
| Ether extract (EE) | 17.929$^{aA}$ | 7.563$^{B}$ | 16.214$^{bA}$ | 7.097$^{B}$ |
| Non-fiber carbohydrates (NFC) | 63.937$^{bB}$ | 70.915$^{A}$ | 65.259$^{aB}$ | 73.800$^{A}$ |
| Neutral detergent fibre (NDF) | 49.217$^{aB}$ | 53.920$^{A}$ | 49.445$^{aB}$ | 54.129$^{A}$ |
| Acid detergent fibre (ADF) | 37.549$^{aA}$ | 35.243$^{B}$ | 34.442$^{bB}$ | 37.363$^{A}$ |
| Lignin | 20.890$^{aA}$ | 16.558$^{B}$ | 16.902$^{bA}$ | 12.246$^{B}$ |
| Hemicellulose | 21.669$^{bA}$ | 14.279$^{B}$ | 25.331$^{aA}$ | 17.022$^{B}$ |
| Cellulose | 25.661$^{bA}$ | 23.837$^{B}$ | 32.058$^{aA}$ | 22.030$^{B}$ |
| Ash | 6.696$^{bB}$ | 8.864$^{B}$ | 7.262$^{aB}$ | 8.028$^{A}$ |
| *In vitro* digestibility | 54.902$^{bB}$ | 77.918$^{A}$ | 60.306$^{aB}$ | 83.899$^{A}$ |

**Table 3.** The chemical composition of different proportions of Jatropha seed cake and agro-industrial residues colonized for 45 days by *P. ostreatus*

Ether extract content in substrates also decreased after incubation with *P. ostreatus*(Table 3). The reduction was independent of substrate and averaged57%, suggesting that the fungus may have used lipids as a nutrient source. This reduction also contributes to the use of Jatropha residue in ruminant diets because it is typically recommended that EE represent less than10% of a diet's dry matter.

After inoculation with *P. ostreatus* Plo 6, the substrates showed an increase of DM and CP and a reduction of organic material (Table 3). This is similar to observations made in cocoa husks fermented with *P. ostreatus* [61] and in *Jatropha curcas* kernel cake fermented with *Aspergillus niger* and *Tricholoma longibrachiatum* [62]. These data suggest two biological processes: (a) the uptake or absorption of organic matter by the fungus, resulting in the production of proteins and mycelial growth (increased dry weight); and (b) the mineralization or degradation of organic matter resulting in an increase in mineral content (ash) and NFC (Table 3). Assimilation of organic material resulting in an increase in crude protein was also found in

*P. sajor-caju* grown in cotton waste [56]. Degradation or mineralization of organic matter was also observed in a culture of *P. ostreatus* on eucalyptus bark [63].

We observed an increase in carbohydrates after inoculation with *P. ostreatus* (Table 3). This increase confirms the degradation of more complex compounds such as lignin, cellulose and hemicellulose by *P. ostreatus* (Table 3). Degradation of these compounds in the Jatropha seed cake substrate contributed to an increase in dry matter digestibility (Table 3). An increase in digestibility after lignin degradation has also been shown after cultivation of *Phanerochaete chrysosporium* on cotton stalks [64], *P sajor-caju* in agroindustrial residue [57] and *P. ostreatus* on eucalyptus bark [63].

Therefore, colonization of Jatropha seed cake by *P. ostreatus*, with or without addition of eucalyptus bark, was shown to increase the nutritional value and *in vitro* digestibility of this by-product from the biodiesel production chain.

*3.3.1. Ammonia production by microorganismsin the ruminal liquid*

The ruminant's microorganisms are large and genetically diverse, consisting of bacteria, fungi, protozoa and viruses [65]. These microorganisms contribute to the fermentation of substrates that have low solubility (e.g., plant material rich in fiber) in organic acids, methane, ammonia, acetate, lactate, formate, ethanol, propionate, $CO_2$ and $H_2$ [66].

The ammonia production observed in this study can be considered low (Figure 6). The production of this compound by rumen ammonia-producing bacteria may vary from 33 to 159% of the dry mass depending on the bacterial species and protein content of the diet [67]. Rumen microorganisms are capable of incorporating a large portion of the produced ammonia by deamination of amino acids and hydrolysis of nitrogen compounds. However, when the rate of deamination exceeds the rate of assimilation, protein catabolism results in the undesirable and inefficient process of high ammonia production and low retention of nitrogen [68]. This undesirable process can be characterized by the loss of protein through the excretion of nitrogen as urea in the animal's urine [69]. According to previous studies, the rate of dietary protein degradation is directly proportional to the ammonia production and protein nitrogen loss. Therefore, low ammonia production by microorganisms in the rumen fluid demonstrates that the substrates colonized with *P. ostreatus* exhibit good digestibility and that the rate of ammonia uptake is not exceeded (Figure 6).

The highest ammonia production was observed in substrates colonized by *P. ostreatus* (Figure 6). The increased production of ammonia may be due to the higher amount of crude protein in these substrates (Table 3). This shows that the colonized substrates have a greater capacity to be degraded by rumen microorganisms than those that were not colonized. This higher capacity can be a result of the following: (a) the presence of toxic compounds which inhibit microbial growth in substrates without fungal colonization or (b) a reduction in lignocellulosic compounds by enzymatic action resulting in compounds (e.g., NFC, CP and NDF) contributing to the growth and metabolism of microorganisms (Table 3). This degradation/mineralization of lignocellulosic compounds is also reflected in the increase in dry matter and ash content (Table 3).

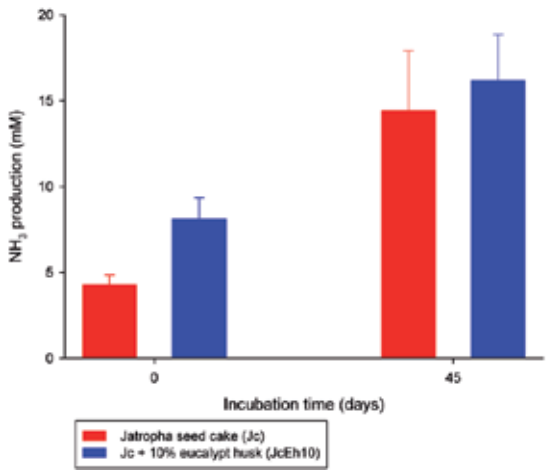


**Figure 6.** Ammonia concentration in batch cultures containing ruminal liquid and the substrates evaluated (Table 1). Substrates were harvested before and after 45 days of colonization by *Pleurotus ostreatus* Plo 6.

Finally, it is important to note that *P. ostreatus* Plo 6's reduction in levels of phorbol esters (99%) and ammonia (Figure 6) did not inhibit the development of rumen bacteria. This result confirms the detoxification of Jatropha seed cake by *P. ostreatus* and again highlights the importance of fungal colonization in the preparation of the cake for use in animal feed.

### 3.4. Animal assay

#### *3.4.1. Food intake, digestibility and nitrogen balance*

The intake of dry matter and nutrients was influenced by the different amounts of detoxified Jatropha seed cake in the diet (Table 4). The DM intake (% BW) and NDF showed a quadratic response ($P < 0.05$), and there was a positive linear effect ($P < 0.05$) on DM intake (g/kg BW0.75). The DM, OM and CP increased linearly ($P < 0.05$) and no changes were observed in either the EE and NFC consumption by animals (Table 4).

The increase in DM intake may be attributed to a reduction of the energy values in the experimental diets (Table 2). In this sense, the animals ate more DM to reach their energy requirements. Thus, we can infer that the consumption and palatability of the diets was not restricted by the inclusion of detoxified Jatropha seed cake, although it increased DM intake by the animals. In prior experimental animals, the replacement of soybean meal by Jatropha seed cake resulted in a decrease in DM ingestion, which was attributed to the presence of anti-nutritional factors [70]. In this study, the maximum intake of DM and NDF was 3.68 and 1.67% of BW, respectively. This was not enough to promote the rumen fill effect. In diets with a low energy level, animals tend to exceed the consumption limit of 1.2% of BW, offsetting any food energy deficiency [66].

The increase in the DM intake resulted in increases in intakes of OM and CP. However, this increase had no effect on the overall consumption of EE and NFC. These results support the theory of compensation in DM intake by animals on diets with a low concentration of energy.

| Intake (g $d^{-1}$) | Jatropha seed cake (% dry mass) | | | | Regression | $R^2$ | CV (%) |
|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 20 | | | |
| Dry mass (DM) | 701.34 | 719.90 | 826.27 | 891.35 | Y = 681.609+10.058x | 0.94 | 13.67 |
| DM (%BW) | 2.91 | 2.84 | 3.19 | 3.68 | $Y = 2.901-0.030x+0.003x^2$ | 0.99 | 10.33 |
| DM (g/kg$BW^{0.75}$) | 64.41 | 63.61 | 71.89 | 81.65 | Y = 61.327+0.883x | 0.83 | 10.42 |
| Neutral detergent fiber(%BW) | 1.17 | 1.19 | 1.38 | 1.67 | $Y = 1.170-0.009x+0.001x^2$ | 0.99 | 9.83 |
| Organic matter | 673.35 | 696.55 | 795.80 | 854.01 | Y = 657.092+9.545x | 0.95 | 13.62 |
| Crude protein | 91.40 | 87.55 | 97.29 | 106.09 | Y = 87.469+0.791x | 0.72 | 14.10 |
| Ether extract | 23.54 | 24.10 | 26.10 | 26.06 | ns | -- | 14.07 |
| Non-fiber carbohydrates | 272.74 | 274.36 | 304.92 | 307.44 | ns | -- | 16.10 |
| Digestibility (%) | | | | | | | |
| Dry mass | 74.16 | 68.10 | 65.40 | 62.46 | Y = 73.328-0.565x | 0.97 | 7.24 |
| Organic matter | 74.88 | 69.02 | 65.90 | 63.06 | Y = 74.136-0.577x | 0.97 | 6.83 |
| Crude protein | 67.92 | 54.10 | 52.14 | 46.70 | Y = 65.305-0.984x | 0.89 | 10.53 |
| Ether extract | 80.40 | 77.62 | 78.61 | 76.92 | ns | -- | 5.18 |
| Neutral detergent fiber | 69.32 | 64.19 | 60.55 | 58.05 | Y = 68.774-0.560x | 0.98 | 7.44 |
| Non-fiber carbohydrates | 82.46 | 78.50 | 75.75 | 73.75 | Y = 82.050-0.432x | 0.98 | 7.99 |

**Table 4.** Consumption and apparent total tract digestibility of dry matter and nutrients in goats fed with biodetoxified Jatropha seed cake

The inclusion of increasing levels of detoxified Jatropha seed cake promoted a linear reduction ($P < 0.05$) in the digestibility of DM, OM, CP, NDF and NFC diets tested (Table 4). The exception was EE digestibility, which did not show significant variation and had average values of 78.39%. This reduction in dry matter digestibility can be attributed to an increase in passage rate as a function of consumption, resulting in the shorter digestion time of nutrients in the gastrointestinal tract [66]. This effect is associated with the highest possible lignin concentration of the experimental diets.

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In relation to nitrogen metabolism, significant effects on Nuendo, nitrogen balance and the biological value of protein from the level of detoxified Jatropha seed cake added were not observed (Table 5). The intake of nitrogen, excretion of Nfecal, Nmet.fecal, Nundig, Nuexo and urinary nitrogen were influenced in a linear manner at the levels studied (Table 5). Losses of nitrogen in the urine and feces were 28.63 and 40.20% of the consumed nitrogen, respectively.

| Variable (g $d^{-1}$) | Jatropha seed cake (% dry mass) | | | | Regression | $R^2$ | CV (%) |
|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 20 | | | |
| Consumed nitrogen | 14.62 | 14.01 | 15.57 | 16.98 | Y = 13.993+0.126x | 0.72 | 14.10 |
| Nfecal | 4.67 | 5.22 | 6.79 | 7.95 | Y = 4.416+0.169x | 0.96 | 20.44 |
| Nmet. fecal | 0.39 | 0.37 | 0.42 | 0.45 | Y = 0.372+0.003x | 0.72 | 14.14 |
| Nund | 4.28 | 4.84 | 6.37 | 7.50 | Y = 4.042+1.166x | 0.97 | 21.64 |
| Urinary nitrogen | 4.56 | 3.49 | 4.29 | 5.19 | $Y= 4.495-0.194x+0.011x^2$ | 0.70 | 25.38 |
| NUend | 1.80 | 1.86 | 1.90 | 1.80 | Ns | -- | 8.07 |
| Nuexo | 2.77 | 1.63 | 2.39 | 3.39 | $Y = 2.703-0.213x+0.012x^2$ | 0.75 | 43.50 |
| NB | 7.58 | 7.53 | 6.80 | 6.09 | ns | -- | 36.41 |
| BVP (%) | 72.54 | 77.91 | 72.70 | 63.86 | ns | -- | 21.18 |

**Table 5.** Nitrogen consumption, excretion, balance and retention by goats fed with bio-detoxified Jatropha seed cake

Generally, urea concentration is correlated with ammonia content in ruminants because digestive microorganisms using nitrogen require energy for the synthesis of bacterial proteins. Most likely, there was excess of ruminal ammonia, which increased the excretion of nitrogen in the urine; thus, levels of 12% CP in the diet of growing goats can promote higher levels in waste nitrogen. Valadares et al. [71] also found an increase in nitrogen excretion in urine when they provided a similar amount of protein to zebu cattle.

Nitrogen balance (NB) and biological value did not differ between the evaluated diets. However, the positive observed values of NB suggest its use in the synthesis of tissue.

### *3.4.2. Blood parameters*

The experimental diets did not significantly alter the blood parameters of the animals (Table 6). The resulting values were similar to those of normal goats [72]. The hemoglobin concentration was similar to that observed in goats fed with Jatropha seed cake [62].

From the leukocyte values observed in this study, it could be inferred that animals did not experience inflammation after ingesting bio-detoxified Jatropha seed cake (Table 6).

The absence of significant effects in the content of creatinine, alkaline phosphatase and total protein by the different levels of bio-detoxified Jatropha seed cake (Table6) shows that liver function was not altered in animals fed the experimental diets.

| Variable | Jatropha seed cake (% dry mass) | | | | Regression | CV (%) | Reference* |
|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 20 | | | |
| Hematological profile | | | | | | | |
| Erythrocytes (x $10^6$ $mm^3$) | 4.93 | 5.16 | 4.81 | 5.01 | ns | 11.88 | 8 a 18 |
| Hemoglobin (g $dL^{-1}$) | 11.61 | 11.55 | 11.60 | 11.82 | ns | 5.93 | 8 a 12 |
| Hematocrit (%) | 31.75 | 31.83 | 31.68 | 32.33 | ns | 6.18 | 22 a 38 |
| Leukocytes (n $\mu L^{-1}$) | 12179 | 12405 | 10533 | 11583 | ns | 14 | 4000 a 13000 |
| Biochemical profile | | | | | | | |
| Creatinine (mg $dL^{-1}$) | 0.76 | 0.82 | 0.76 | 0.75 | ns | 7.48 | 1 a 1.82 |
| Alkaline phosphatase (U $l^{-1}$) | 173.95 | 154.90 | 186.62 | 163.84 | ns | 18.95 | 93 a 387 |
| Urea (mg $dL^{-1}$) | 20.76 | 21.53 | 18.09 | 20.53 | ns | 14.47 | 21.4 a 42.8 |
| Total proteins (g $dL^{-1}$) | 6.90 | 6.66 | 6.73 | 6.57 | ns | 4.71 | 6.4 a 7 |

**Table 6.** Hematological and biochemical blood profiles of goats fed with bio-detoxified Jatropha and of normal control goats

Urea levels in the blood can increase in response to diets with low energy [73]. However, we did not observe this effect.

Thus, inclusion of Jatropha seed cake bio-detoxified by *P. ostreatus* shows promise as an animal feed supplement because it did not result in changes to blood parameters or clinical symptoms of poisoning. This included goats fed with up to 20% of the treated residue. Conversely, ingestion of Jatropha residue treated with organic solvents (ethanol and hexane) caused diarrhea and other side effects in swine [74]. Similarly, diarrhea and death resulted in goats fed with Jatropha seed cake colonized by *Aspergillus*, *Penicillium* or *Trichoderma* [62].

## 4. Conclusions

The residue of *J. curcas* increases with increased biodiesel production, so it is necessary to find an appropriate usefor these residues. In this study we demonstrate the potential to

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transform the residue of biodiesel containing lignocelluloses, toxic compounds, and anti-nutritional factors into animal feed. This process adds economic value to biodiesel production and avoids theimproper disposal of its by-products in the environment.

Thebio-detoxification of Jatropha seed cake promotes the reduction of phorbol ester levels and increases the nutritional value of this residue. The resulting alternative food can be included in amounts up to20% (DM)in the diet of growing goats.

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Chapter 12

# Biomethanol from Glycerol

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Additional information is available at the end of the chapter

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## 1. Introduction

Methanol is an important bulk chemical in the chemical industry. The global methanol demand was approximately 32 million metric tons in 2004 and is expected to grow [1]. Methanol is used mainly for the production of formaldehyde, acetic acid, and application products including polymers and paints. Furthermore, methanol can be used as a clean and renewable energy carrier [1]. Methanol is mainly produced from syngas, a mixture of $H_2$, CO, and minor quantities of $CO_2$ and $CH_4$. Syngas is commonly produced from fossil resources like natural gas or coal. Biomass, however, can also be used as resource for syngas and allows the synthesis of green methanol. Green methanol not only has environmental benefits, but may also lead to considerable variable cost reductions if the biomass resource has a low or even negative value.

### 1.1. Renewable methanol

Methanol synthesis from biomass was already proposed during the first oil crisis in the 1970s [1]. In the 1980s a comprehensive review was published on the production of methanol from syngas derived from wood. Different gasification technologies were proposed and demonstration projects of these technologies were discussed [2, 3]. In the mid 1990s several projects on methanol synthesis from biomass were initiated such as the Hynol project in the USA and the BLGMF (black liquor gasification with motor fuels production) process in Sweden [4-6]. Schwarze Pumpe, Germany developed a process to convert coal and waste, including sewage sludge, to methanol (capacity ± 150 ML/y) [7]. Unfortunately no experimental data of these processes are available in open literature.

Several initiatives were started in the 2000s. At a scale of 4 t/d, Chemrec in Sweden produces methanol and dimethyl ether (DME) since 2011. Syngas is obtained by entrained flow gasifi-

cation of black liquor [8]. The production of methanol from glycerol is demonstrated on industrial scale by BioMCN in The Netherlands [8]. At BioMCN, the natural gas reforming unit has been modified to enable steam reforming of glycerol. The syngas is converted to methanol in their conventional packed bed methanol synthesis reactors, with a capacity for methanol production of 250 ML/y [8].

The amount of published experimental results on the production of methanol from biomass is rather limited. Most publications on methanol production from biomass are desk-top studies and data comparison is difficult [5, 6, 9-13]. These studies often combine biomass gasification and conventional methanol synthesis with, in some cases, electricity production [5, 6, 11, 13]. Xu *et al.* conducted an experimental study and demonstrated methanol production from biomass by reforming pyrolysis liquids into $H_2$ and $CO_2$ followed by catalytic syngas conditioning to convert part of the $CO_2$ into CO [14]. Methanol synthesis was conducted in a packed bed reactor, with an overall carbon conversion of around 23% (corresponding with a methanol production rate of 1.3 kg methanol/kg catalyst/h).

An interesting concept of using biomass to produce methanol is the co-processing of biomass and fossil resources, e.g. co-gasification of biomass with coal or natural gas [9, 10, 12]. The advantage of co-feeding natural gas is that the syngas derived will become more suitable for methanol synthesis as syngas from biomass is deficient in $H_2$ and syngas from natural gas in CO or $CO_2$.

The concepts involved in the current processes for the synthesis of methanol from biomass generally involve an initial gasification step at elevated temperatures and pressures. The approach demonstrated in this chapter is syngas production through a hydrothermal process, viz. conversion of a wet biomass stream to syngas by reforming in supercritical water (RSCW), followed by high pressure methanol synthesis.

An interesting wet biomass resource is glycerol, the by-product from the biodiesel industry. In Europe, the share of transportation fuel to be derived from renewable resources in 2020 is targeted at 10% [15]. It is expected that biodiesel and ethanol will make up the largest share and consequently Europe's biodiesel production increased significantly in the 2000s (see Figure 1) [16]. In the last few years, though, economics of biodiesel production deteriorated as the income from the sales of glycerol decreased, while the costs of feedstock increased.

As for the production of every (metric) ton of biodiesel, roughly 100 kg of methanol is required and a similar quantity of glycerol is produced, both methanol demand and glycerol production increased. An interesting option addressing the surplus of glycerol and the demand for methanol is to produce methanol from the glycerol. If this process is conducted by the biodiesel producer he will become less dependent on the methanol spot price, there is a (partial) security of supply of methanol, and by-products can be used as a green and sustainable feed product. However, the scale of traditional methanol synthesis (> 2000 t/d) is much larger than the scale of methanol synthesis explored in the Supermethanol project.

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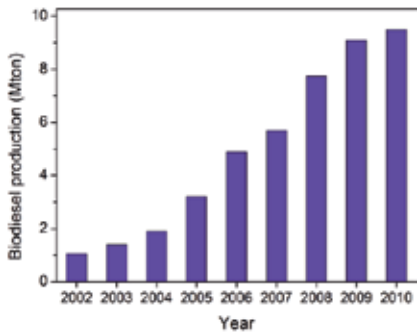


**Figure 1.** Biodiesel production in Europe. Adapted from reference [16].

The Supermethanol initiative focuses on a small/medium scale biodiesel plant (30,000 – 100,000 t/y) and the aim of the project is to develop a methanol synthesis process using glycerol as feed at a capacity matching the biodiesel production. The glycerol intake for the production of syngas will be in the range of 3000 up to 10,000 t/y [17]. The scope of the Supermethanol concept is schematically outlined in Figure 2.

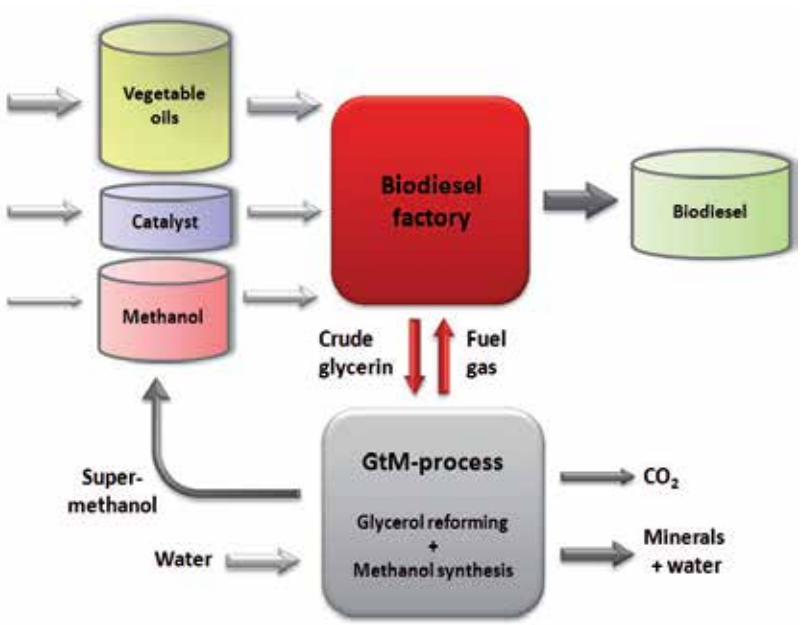


**Figure 2.** Outline of the Supermethanol concept. The glycerol-to-methanol (GtM-) process is the process under investigation in the Supermethanol project.

The biodiesel factory is the core of the process in which vegetable oils react with methanol in the presence of a catalyst to produce biodiesel and by-product glycerol. The glycerol can be converted into methanol in the glycerol-to-methanol (GtM-) process. This process is an inte-

gration of two separate processes, viz. the reforming in supercritical water (RSCW) of glycerol to syngas, followed by the conversion of this syngas into methanol. Additional fuel gas is produced, which can be used to generate heat for the biodiesel production or in the GtM-process. A more detailed overview on the GtM-concept is given in the next section.

## 2. The glycerol-to-methanol concept

### 2.1. Theoretical considerations

The most attractive syngas for methanol synthesis has a stoichiometric number ($S_N$), defined in Eq. 1, of approximately 2, which corresponds to the stoichiometric ratio for methanol synthesis.

$$S_N = \frac{\left(H_2 - CO_2\right)}{\left(CO + CO_2\right)} = 2 \tag{1}$$

When glycerol decomposes solely into $H_2$, CO, and $CO_2$ the maximum $S_N$ is 1.33. This is illustrated by Eqs. 2 and 3. In Eq. 2, glycerol decomposition into syngas including the reversible water-gas shift (WGS) reaction is given. The syngas composition at equilibrium (neglecting methanation), expressed in terms of x, is a function of the temperature and the water concentration.

$$C_3H_8O_3 \xrightarrow{xH_2O} \left(3\text{-}x\right)\ CO + \left(4\text{+}x\right)\ H_2 + x\ CO_2 \tag{2}$$

Application of the definition for $S_N$ and introduction of gas phase compositions in terms of x (see Eq. 3) confirms that the $S_N$ value is 1.33 at most and independent of the progress of the WGS reaction:

$$S_N = \frac{\left(H_2 - CO_2\right)}{\left(CO + CO_2\right)} = \frac{\left(4 + x - x\right)}{\left(3 - x + x\right)} = \frac{4}{3} \tag{3}$$

The $S_N$ value, though, can be increased by the addition of $H_2$ to, or removal of $CO_2$ from, the syngas. To obtain the highest methanol yield per kg glycerol, glycerol reforming followed by syngas conversion should proceed, for example, according to Eq. 4, where glycerol is selectively converted into $H_2$, CO, and $CO_2$. Subsequently, all $H_2$ and CO react to methanol, while the $CO_2$ remains.

$$3\ C_3H_8O_3 + 2\ H_2O \rightarrow 7\ CO + 14\ H_2 + 2\ CO_2 \rightarrow 7\ CH_3OH + 2\ CO_2 \tag{4}$$

As a theoretical maximum, 2.33 mol carbon/mol glycerol end up in methanol (77.8% on carbon basis or 0.81 kg methanol/kg glycerol on weight basis). Actual yields, however, will be lower as both processes, glycerol reforming and methanol synthesis, involve equilibrium reactions and the occurrence of other reactions like the formation of higher hydrocarbons, higher alcohols (HA), and methanation.

### 2.2. Description of the continuous integrated GtM-bench scale unit

The integrated unit consists of a reformer section and a methanol synthesis section. A schematic flow sheet of the unit is given in Figure 3. An extensive description of the reformer section is published elsewhere [18]. The reformer section was operated in continuous mode with a throughput of 1 L aqueous feed/h. Glycerol and water were introduced to the system from feed containers F1 or F2 through a pump and subsequently reformed in five reforming reactors (R1 – R5) in series. The temperature in each reactor can be adjusted individually.

During operation *in situ* separation of the water and gas phase after the reformer section was performed in a high pressure separator (HPS). The liquid phase in the HPS, can either be depressurized and transferred to a low pressure separator (LPS) or recycled via a recycle pump. In the former operating mode (using the LPS), the gases dissolved in the aqueous phase are released, quantified (Gallus G1.6 gas meter), and analyzed (gas chromatography, GC). In the latter operation mode (recycle mode) the gases remain dissolved and fresh glycerol feed is injected in the recycle stream before the first reforming reactor (R1). If required all reforming reactors can easily be filled with catalyst. The gas phase from the HPS was directly fed to the methanol synthesis section without upgrading or selective removal of components.

The methanol section contains three tubular packed bed reactors (P1 – P3, each $L$ = 500 mm, $ID$ = 10 mm) surrounded by heating jackets. A heating/cooling medium was flown through the heating jackets to control the temperature in the reactors. Temperatures were recorded at 4 positions inside packed bed P2 (at locations 2 to 30 cm from the entrance) and at the exit of packed bed reactors P2 and P3. Two or three of the tubular reactors were filled with catalyst particles ($1 < d_p < 3$ mm). The mixture of methanol, water, and unconverted gases leaving the last packed bed (P3) was cooled (cooler C2) using tap water, depressurized and cooled (cooler C3) to temperatures below 263 K to trap all condensables. Liquid samples were collected in a vessel and the unconverted gas was quantified (Gallus G1.6 gas meter) and analyzed by GC. The methanol synthesis reactors were operated at temperatures of the heating medium between 473 – 523 K and at similar pressure as the reformer section. Several process parameters were logged during operations and the locations, where they were measured, are indicated with letters in bold in Figure 3.

The process pressure was the average of $\mathbf{A}_{1\text{-}3}$, the temperature of reactor R5 ($T_{R5}$) was measured at **B** at the end of reactor R5, the glycerol feed flow at **C**, the gas flow of the HPS and LPS at $\mathbf{D}_1$ and $\mathbf{D}_2$ respectively, the temperature at the end of the methanol synthesis bed at **E**, the amount of liquid product at **F**, and the unconverted gas flow at **G**.

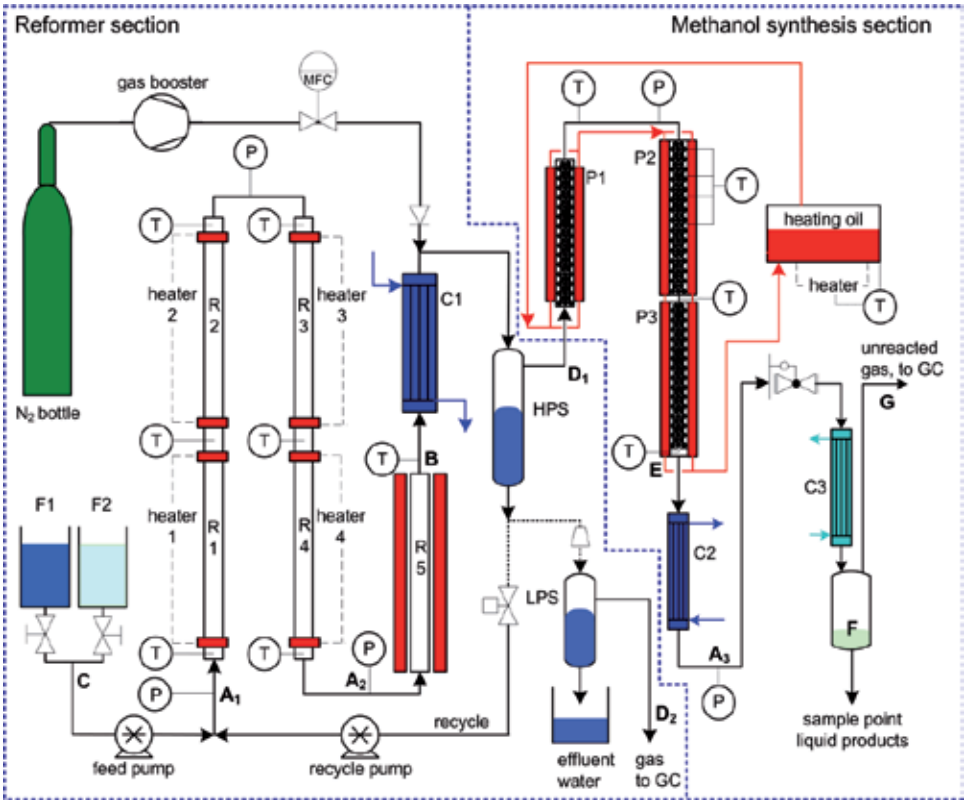


**Figure 3.** Flow sheet of the integrated GtM-bench scale unit. HPS and LPS refer to high pressure separator and low pressure separator respectively. F = feed container, C = cooler, P = packed bed reactor, R = reforming reactor. The bold capital letters correspond to the locations where relevant process parameters were measured [8].

### 2.3. Analyses

The composition of the off-gas from the reforming section and methanol synthesis section was analyzed using an online dual-column gas chromatograph (GC 955, Syntech Spectras) equipped with thermal conductivity detectors. CO was analyzed and quantified using a molsieve 5 Å column ($L$ = 1.6 m) with helium as carrier gas. $CH_4$, $CO_2$, and $C_{2+}$ were analyzed on a Chromosorb 102 column ($L$ = 1.6 m) with helium as carrier gas. $H_2$ was analyzed on the molsieve column using argon as carrier gas. The total organic carbon (TOC) content of the effluent water from the RSCW process was analyzed using a TOC analyzer (TOC-$V_{CSN}$, Shimadzu). The water content of the methanol was determined by Karl Fischer-titration. The composition of the organics in the liquid phase after the methanol synthesis reactor was determined with a GC (HP 5890 series II) equipped with a flame ionization detector (FID) over a Restek RTX-1701 column ($L$ = 60 m, $ID$ = 0.25 mm) coupled with a mass spectrometer (MS, HP 5972 series). The FID was used for the quantification of the components and the MS for the identification of the components. The FID was calibrated for the main

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constituents of the organic fraction: methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 1-pentanol, 2-methyl-1-propanol, 2-methyl-1-butanol.

### 2.4. Definitions

The carbon conversion of glycerol ($\zeta_{gly}$) in the reformer section is defined as the difference between the molar carbon flow of glycerol in the feed and the effluent ($\phi_{C,gly}$ - $\phi_{C,effluent}$) over the molar carbon flow of glycerol in the feed ($\phi_{C,gly}$):

$$\zeta_{gly} = \frac{\phi_{C,gly} - \phi_{C,effluent}}{\phi_{C,gly}} \cdot 100\% \tag{5}$$

The overall conversion of carbon in glycerol to carbon in methanol ($\zeta_C$) in the integrated unit is the molar carbon flow in methanol ($\phi_{c,MeOH}$) over the molar carbon flow of glycerol in the feed.

$$\zeta_C = \frac{\phi_{C,MeOH}}{\phi_{C,gly}} \cdot 100\% \tag{6}$$

The methanol yield ($\eta$) is the mass flow of methanol ($\phi_{MeOH}$) produced over the mass flow of glycerol ($\phi_{gly}$) fed.

$$\eta = \frac{\phi_{MeOH}}{\phi_{gly}} \tag{7}$$

The conversion of gas component i ($\zeta_i$) in methanol synthesis is defined as the molar conversion rate ($\phi_{i,in}$ - $\phi_{i,off}$) over the molar flow of component i originally present ($\phi_{i,in}$) after glycerol reforming.

$$\zeta_i = \frac{\phi_{i,in} - \phi_{i,off}}{\phi_{i,in}} \cdot 100\% \tag{8}$$

The carbon selectivity towards product i ($\sigma_i$) is defined as the molar carbon flow of product i ($\phi_{C,\,i,\,off}$) over the molar carbon flow of glycerol in the feed.

$$\sigma_i = \frac{\phi_{C,i,off}}{\phi_{C,gly}} \cdot 100\% \tag{9}$$

### 2.5. Research strategy

The integration of syngas production in an RSCW-process and syngas conversion in methanol synthesis is the core of the GtM-process. In the RSCW of glycerol a high pressure syngas is produced. The use of this high pressure syngas has distinct advantages for methanol synthesis, which will be dealt with in Section 4. However, before successful integration both processes need to be optimized separately, which was done at the laboratories of the Biomass Technology Group (BTG) in The Netherlands. A unit was available to investigate both process separately before integraton. Results obtained for each process were used to optimize the overall process and maximize the overall carbon conversion ($\zeta_C$), which was the main focus of the research study on the integrated process.

## 3. Glycerol reforming in supercritical water

### 3.1. Introduction to reforming

Water becomes supercritical at conditions above its critical temperature ($T_c$ = 647 K) and critical pressure ($P_c$ = 22.1 MPa). In the phase diagram in Figure 4 the square area in the upper right corner represents the supercritical area of water [19].

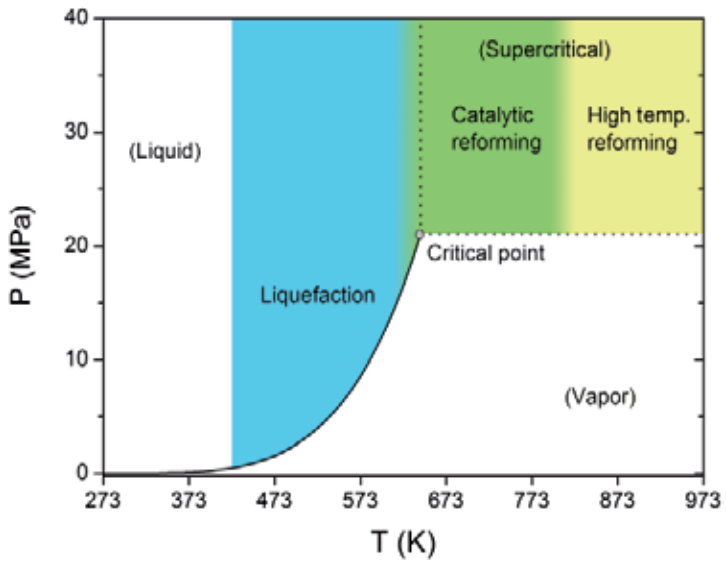


**Figure 4.** Phase diagram of water. Phases are indicated in parenthesis. Hydrothermal processes with their typical conditions are indicated in the colored areas. Adapted from reference [19].

Typical process conditions for three processes considered for wet biomass valorization are indicated here, viz. liquefaction, catalytic reforming, and high temperature reforming. Hydrothermal liquefaction is conducted at temperatures below and pressures above the critical

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point, where biomass is degraded to yield mainly bio-crude (a viscous water-insoluble liquid), char, water-soluble substances, and gas [20]. RSCW of biomass is aimed at gas production and is carried out at conditions beyond the critical point. Here, water acts both as reaction medium and reactant. RSCW of biomass can be subdivided into catalytic reforming and noncatalytic reforming. Catalytic reforming is predominantly carried out at the lower temperature range, while noncatalytic or high temperature reforming is conducted at the higher temperatures (see Figure 4 [19]).

RSCW is characterized by the occurrence of many reactions, proceeding both in series and in parallel. The overall reaction of an actual (biomass) feed to liquid and gas phase products is shown in Eq. 10.

$$C_xH_yO_z + a\ H_2O \rightarrow \alpha\ CO + \beta\ H_2 + \gamma\ CO_2 + \delta\ H_2O + \varepsilon\ CH_4 + \eta\ C_{x'}H_{y'}O_{z'} \quad (10)$$

By-products ($C_{x'}H_{y'}O_{z'}$) are low molecular weight organic compounds, polymerized products, higher hydrocarbons ($x' \geq 2$, $z' = 0$), or elemental carbon ($y' = z' = 0$). Some of the low molecular weight organics can react further to gas phase components. Subsequent reactions of the gas phase components may also occur. The following gas phase reactions may occur, depending on process conditions [21]:

$$CO + H_2O \leftrightarrow CO_2 + H_2 \quad (11)$$

$$CO + 3\ H_2 \leftrightarrow CH_4 + H_2O \quad (12)$$

$$CO_2 + 4\ H_2 \leftrightarrow CH_4 + 2\ H_2O \quad (13)$$

$$2\ CO \leftrightarrow CO_2 + C \quad (14)$$

$$CH_4 \leftrightarrow C + 2\ H_2 \quad (15)$$

$$CO + H_2 \leftrightarrow C + H_2O \quad (16)$$

The individual reaction rates depend on operating conditions and the presence of catalysts. A number of parameters affect the carbon conversion in RSCW, such as feedstock type, feed concentration, operating conditions, presence of catalysts or catalytic surfaces, and interaction between different components. The state of the art of RSCW in the 2000s has been reviewed extensively in several publications [19, 22-28].

### 3.2. Reforming of pure glycerol and crude glycerin

The reforming experiments were carried out using only the reforming section of the unit depicted in Figure 3. Typical conditions were temperatures of 723 – 923 K, residence times between 6 – 45 s, and feed concentrations of 3 – 20 wt%. The pressure was around 25 MPa. Two different types of glycerol were used, viz. pure glycerol and crude glycerin. Crude glycerin is glycerol derived from biodiesel production. The crude glycerin used in this research study contains approximately 5 wt% NaCl. The presence of alkali (in this case $Na^+$) influences mainly the WGS reaction (see Eq. 11). The main gas products for pure glycerol and crude glycerin were: $H_2$, CO, $CO_2$, $CH_4$, and $C_2H_6$. At complete conversion, roughly 2 mol of carbon in glycerol are converted to carbon oxides while 1 mol of carbon ends up as a hydrocarbon. The gas composition/yield appeared to be a function of the conversion and independent of the feed concentration. The conversion is a function of the process severity, a combination of the residence time and operating temperature. In Figure 5 the gas yields (mol gas/mol glycerol) of the two types of glycerol are depicted. The trend lines shown are fitted to experimental data points [18]. The differences between pure glycerol and crude glycerin can be mainly attributed to the extent of the progress of the WGS reaction [18].

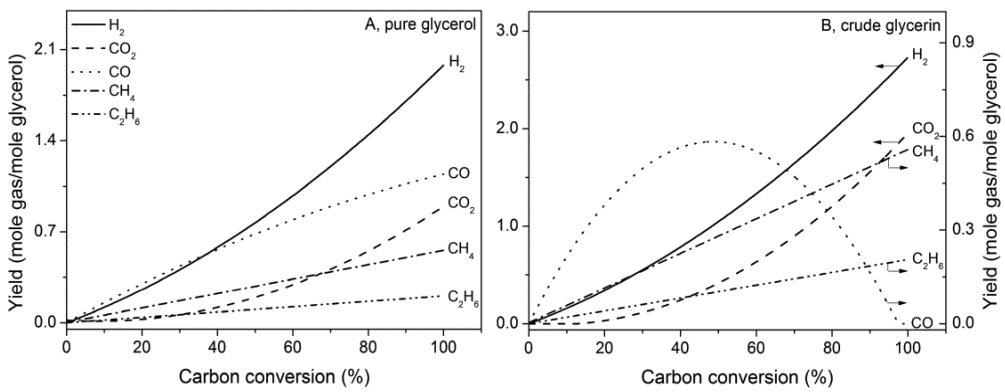


**Figure 5.** Relations between the conversion and the gas yield for pure glycerol (A) and crude glycerin (B) [29].

From reforming studies with methanol as model compound it was concluded that the hydrocarbons present in the gas mixture in case of glycerol reforming appear to be primary gas phase products. In the methanol reforming experiments in the same unit, gas mixtures with similar $H_2$, CO, and $CO_2$ ratios were obtained, but hydrocarbons were hardly observed. Thus, gas phase reactions producing hydrocarbons, e.g. methanation, hardly proceed in the system, which indicates that hydrocarbons are primary gas phase products formed upon glycerol decomposition and not or only to a small extent by gas phase reactions. In glycerol reforming the reactions depicted in Eqs. 10 and 11 proceed, while Eqs. 12 and 13 barely take place. Coke formation was not observed at all and most probably the reactions depicted in Eqs. 14-16 do not proceed.

Based on the experimental results, a simplified reaction scheme for the decomposition of glycerol with a focus on gas production was established and is given in Figure 6. More information on the selection of primary and secondary gas phase products can be found in literature [18].

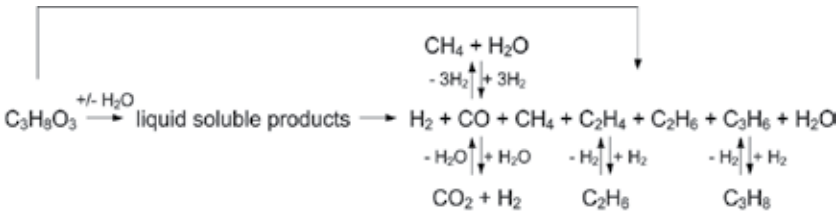


**Figure 6.** Decomposition pathways for glycerol in SCW to gaseous products including possible follow-up reactions [18].

In this scheme, $CH_4$ is shown as a primary product, but can also be formed as a secondary product by methanation. Furthermore, water is produced and the WGS reaction and alkene hydrogenation are included. It is suggested that glycerol can either decompose into liquid soluble products that react further to gas products or that glycerol can directly decompose into gas products. In practice probably both reaction pathways occur. The overall mechanism at complete conversion of glycerol decomposition proceeds through the dehydration of 1 mol $H_2O$/mol feed [18].

An important quality indicator for the gas composition is the $S_N$ value as defined in Eq. 1. A $S_N$ as close as possible to 2 is desired for methanol synthesis, but as was shown in Eq. 3 a $S_N$ value of 1.33 is the maximum for gas derived in glycerol reforming. The experimental $S_N$ as function of the glycerol conversion is depicted in Figure 7. It can be seen that for both types of glycerol the $S_N$ decreases with increasing conversion. The values are almost equal up to 60%, but differ considerably at the higher conversion. The most attractive $S_N$'s are obtained at the lower conversion although they remain below 1. The $S_N$ can be improved by suppressing the formation of hydrocarbons, which is a challenge as hydrocarbons are formed as primary gas phase products.

### 3.3. Catalytic reforming

Catalytic reforming was investigated to improve the quality of the syngas obtained in the noncatalytic reforming experiments for subsequent methanol synthesis. An extensive description of catalytic reforming using five different catalysts is given in literature [29]. The catalytic experiments were conducted at temperatures between 648 to 973 K at pressures between 25.5 - 27.0 MPa. The feed concentration was 10 wt%, and the residence time varied from 8 to 87 s. The experiments were conducted using only three reactors (R2, R3, and R4 in Figure 3), with only the latter two reactors containing catalyst. The catalysts clearly promote the glycerol decomposition rate and higher conversion were measured compared to noncatalytic reforming. A typical figure with the gas concentration as a function of the tempera-

ture for a Ni based catalyst is given in Figure 8. The equilibrium curves were calculated using a model described in literature [18].

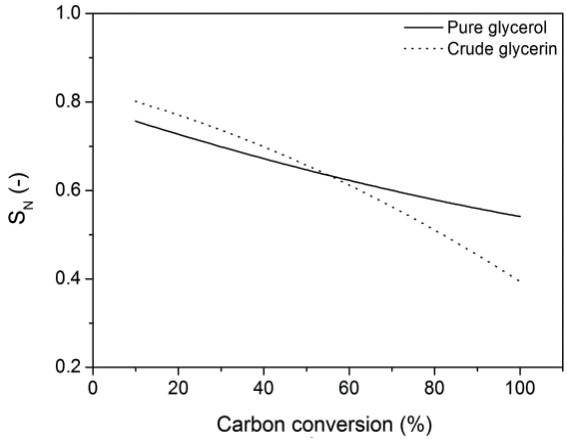


**Figure 7.** $S_N$ for pure glycerol and crude glycerin as a function of the carbon conversion.

The gas composition is a function of the temperature and in this case equilibria are reached at temperatures exceeding 780 K. This catalyst strongly promotes methanation, as the $CH_4$ concentration is much higher than in noncatalytic reforming. At the higher temperatures the $CH_4$ concentration goes down according to thermodynamics. When a Ni based catalyst is used the WGS reaction (Eq. 11) is at equilibrium and almost all CO is converted into $CO_2$. After the reforming experiments traces of coke were visually observed at the catalyst surface and the reactor wall.

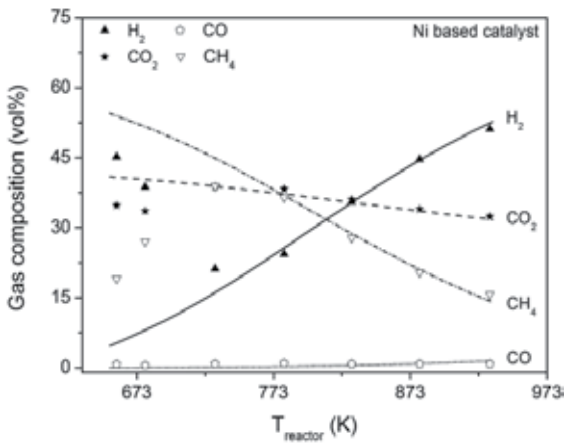


**Figure 8.** Gas concentration as a function of the temperature for a Ni based catalyst. $P$ = 25.5 – 27.0 MPa, [glycerol] = 10 wt%. The curves represent equilibrium compositions, the symbols are experimental points [29].

The performance of this catalyst expressed in $S_N$, hydrocarbon content, and conversion is given in Figure 9. The conversion is almost complete over the whole temperature range. The concentration of hydrocarbons reaches a maximum as a function of the temperature and decreases at the higher temperatures. The $S_N$ has the inverse profile and increases with higher temperatures, but still remains below 1.

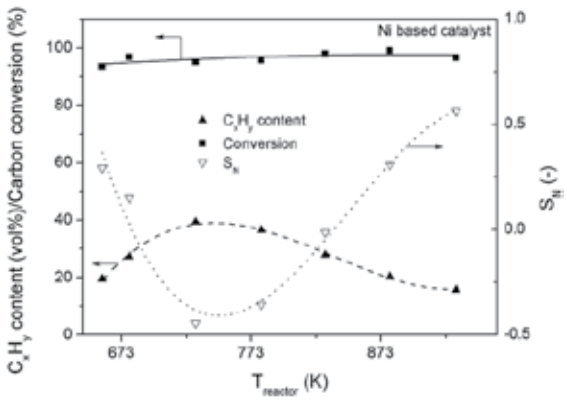


**Figure 9.** Performance indicators. $P$ = 25.5 – 27.0 MPa, [glycerol] = 10 wt%, $\phi_{gly}$ = ± 100 g/h. The lines are trend lines and for illustrative purposes only.

### 3.4. Consequences of the reforming process for the integrated concept

The gas composition in noncatalytic reforming appeared to be not very attractive for methanol synthesis mainly due to the formation of hydrocarbons. Over a Ni based catalyst, $CH_4$ was the only hydrocarbon present in the gas phase. Its maximum concentration was close to 40 vol% at 730 K. A temperature increase resulted in a decreasing $CH_4$ concentration. A further decrease can be realized at higher temperatures and lower feed concentrations. Reduction of the hydrocarbon content has a positive effect on the $S_N$. At feed concentrations around 4 wt% and temperatures of approximately 1000 K, a $S_N$ above 1 can be obtained. These conditions are the most attractive from a gas composition point of view (see also Section 5).

## 4. Methanol synthesis

### 4.1. Introduction to methanol synthesis

Methanol synthesis is conducted generally in catalytic gas-solid packed bed reactors. Three equilibrium reactions, taking place at the catalyst surface, are important: (i) the hydrogenation of CO (Eq. 17), (ii) the hydrogenation of $CO_2$ (Eq. 18), and (iii) the WGS reaction (Eq. 11):

$$CO + 2\,H_2 \leftrightarrow CH_3OH \tag{17}$$

$$CO_2 + 3\,H_2 \leftrightarrow CH_3OH + H_2O \tag{18}$$

All reactions are exothermic. The conversion of $CO+CO_2$ at chemical equilibrium is a function of pressure, temperature, and gas composition (see Figure 10).

Methanol synthesis at industrial scale was initiated by BASF in the 1920s. The operating temperatures were high (573 – 633 K) because of the low catalyst activity [30, 31]. High pressures (15 – 25 MPa) were needed to obtain reasonable conversions. When more active Cu based catalysts and better syngas purification techniques became available, the operating temperature and pressure could be reduced. This development led to the so-called low pressure methanol synthesis process (5 – 10 MPa, 490 – 570 K) which was developed by ICI in the 1960s. Since then, most high pressure units have been converted to low pressure systems [31, 32]. Both synthesis processes require large recycle streams of unconverted syngas due to the limited conversion per reactor pass as is shown in Figure 10 [32]. The reactor temperatures can be lowered further (to 463 – 520 K) due to the recent development of more active catalysts.

In the research study described in this chapter, a combination of low temperatures (468 – 545 K) and high pressures (15 – 25 MPa) is investigated. At this combination of pressure and temperature the Equilibria conversions towards methanol are high (see Figure 10).

### 4.2. Methanol condensation

The methanol conversion in conventional methanol synthesis is restricted by the chemical equilibrium as shown in Figure 10. There are several opportunities to circumvent the limitations imposed by thermodynamic equilibria and they mainly involve *in situ* removal of methanol. This can be done, for example, by methanol adsorption on fine alumina powder or dissolving methanol in tetraethylene glycol, n-butanol, or n-hexane [33-35]. Another method involves *in situ* condensation at a cooler inside the reactor [36]. With all the different methods mentioned higher syngas conversions were obtained but at the same time all methods have drawbacks including the use of other chemicals, complicated operation procedures, or low yields. Conversions beyond the chemical equilibrium can be obtained with *in situ* condensation of methanol and water without adsorbents or coolers. Condensation occurs at a combination of high operating pressures and low temperatures. Condensation has only been shown indirectly in literature by experimental observations of conversions beyond equilibrium or theoretical models [37-40]. We've demonstrated *in situ* methanol condensation visually in a view cell reactor. In this reactor a propeller-shaped stirrer was equipped with catalyst pellets. The view cell was operated semi-batch wise. Methanol synthesis started when syngas ($H_2/CO/CO_2$ = 70/28/2 vol%) was fed to the reactor. The most striking observation was *in situ* condensation at 20.0 MPa and 473 K (Figure 11). Liquid formation was also observed at 17.5 MPa and 473 K and for other gas compositions [41].

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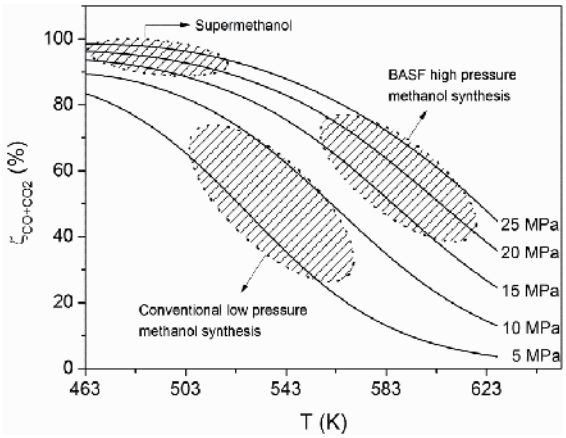


**Figure 10.** Equilibria in methanol synthesis. Approximate conditions are given for (i) conventional processes, (ii) BASF's high pressure process, and (iii) Supermethanol (this work). Syngas: $H_2/CO/CO_2/CH_4$ = 67/24/4/5 vol% [8].

The liquid accumulated in the view cell upon prolonged reaction times. Part of the catalyst became immersed and even after complete immersion methanol synthesis from syngas bubbling through the liquid went on.


**Figure 11.** Liquid formation in a view cell. $P$ = 20.0 MPa, $T$ = 473 K, Syngas: $H_2/CO/CO_2$ = 70/28/2 vol%.

The liquid phase consists of mainly methanol and water. The exact composition is a function of conversion, process conditions, and syngas composition. Condensation may have positive effects on methanol synthesis as will be demonstrated later on in this chapter. Conversions higher than the chemical equilibrium are achieved and almost complete conversion of the limiting component(s) can be obtained at appropriate conditions. As a consequence, recycle and purge streams are not necessary, the limitations on the $S_N$ become less strict, and methanol yields may be increased for a given reactor volume. Most probably the reaction rates in methanol synthesis will be higher at high pressure than at conventional conditions due to

higher partial pressures of the reactants, however, experimental validation is required to validate this hypothesis.

### 4.3. Modelling simultaneous phase and chemical equilibria

A solution model to calculate equilibrium conversions in methanol synthesis including condensation was developed. The effects of process conditions (pressure, temperature, gas composition) can be assessed with the model. Dew points were calculated using Eq. 19 for a given pressure and temperature for each component [42, 43]. A modification of the Soave-Redlich-Kwong equation of state (for polar components) was used to calculate the fugacities of each phase.

$$f_i^V = f_i^L \tag{19}$$

Where, $f_i$ is the fugacity of component i, V and L denote the vapor and the liquid phase respectively. The simultaneous chemical and phase equilibria were calculated using Eq. 19 and theoretical equilibrium constants [44]. The model is described and explained in more detail in the literature [45].

A typical equilibrium diagram for $H_2/CO/CO_2/CH_4$ = 70/5/20/5 vol% is given in Figure 12. The equilibrium diagram for this gas composition illustrates clearly the influence of condensation on the equilibrium conversion.

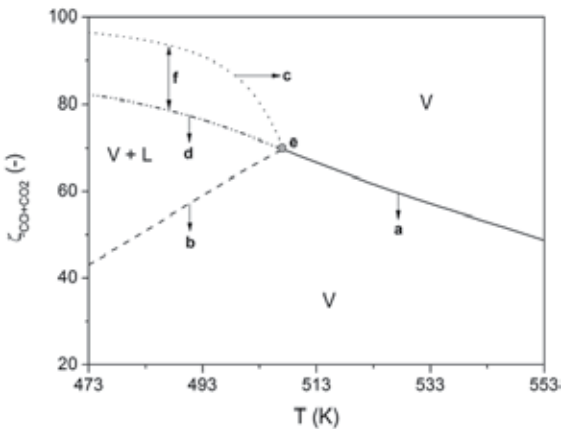


**Figure 12.** Example of an equilibrium conversion diagram including chemical and phase equilibria. Gas phase equilibrium curve (**a**), dew point curve (**b**), equilibrium curve including liquid formation (**c**), extrapolation of the gas phase equilibrium curve (**d**), point where all equilibrium curves merge (**e**), difference between extrapolated gas phase equilibrium and equilibrium including liquid formation (**f**). $P$ = 20.3 MPa, syngas: $H_2/CO/CO_2/CH_4$ = 70/5/20/5 vol% [41].

In the diagram, 4 curves are shown. Curve **a** (solid curve) is the gas phase equilibrium curve. Curve **b (**dashed curve) is the conversion at which a dew point occurs. Curve **c (**dotted curve) is the equilibrium curve including condensation and curve **d** (dashed-dotted

curve) is an extrapolation of the gas phase equilibrium curve. The 4 curves come together in point **e.** In this point ($T$ = 507 K), the gas composition is at equilibrium and the dew point temperature of the mixture equals the reactor temperature. Curve **d**, the extrapolation of curve **a,** is the equilibrium conversion when condensation is neglected. When condensation occurs, the equilibrium conversion is much higher which is indicated by the arrow marked **f** (difference between curve **c** and **d)** in Figure 12. The value for **f** amounts to 13.9% at 473 K ($\zeta_{CO+CO2}$ = 82.6% vs. $\zeta_{CO+CO2}$ = 96.5%) for this particular gas composition.

### 4.4. High pressure methanol synthesis in a packed bed reactor

Methanol synthesis experiments were conducted in the packed bed reactor using 3 different syngases (see Table 1) and pressures of about 20 MPa. Gas 1 and 2 represent typical methanol synthesis gases ($S_N$ = 2.0 – 2.3), with gas 1 rich in CO and gas 2 rich in $CO_2$. The composition of gas 3 resembles a typical syngas obtained in the reforming of glycerol or biomass in general. For this gas $S_N$ < 2 and $H_2$ is the limiting component. The experiments were performed with a large amount of catalyst to approach the equilibrium conversion.

To check the assumption of equilibrium at the reactor outlet and that the experiments were not conducted in the kinetic regime experiments with different flow rates were conducted for gas 1. For the experiments, methanation and the formation of higher hydrocarbons were negligible. For gas 1, the CO+$CO_2$ conversion at 468 K and 20.7 MPa was 99.5%, which is 7.7% higher than the equilibrium conversion calculated at 7.5 MPa. For gas 2, the difference between methanol synthesis at 20 and 7.5 MPa is more pronounced. The CO+$CO_2$ conversion at 484 K was 92.5% which is 46.9% higher than the equilibrium conversion predicted at 7.5 MPa.

| Gas | $H_2$ | CO | $CO_2$ | $CH_4$ | $C_2H_6$ | $S_N$ | Remarks |
|---|---|---|---|---|---|---|---|
| | (vol%) | (vol%) | (vol%) | (vol%) | (vol%) | (-) | |
| 1 | 67.0 | 24.4 | 3.5 | 5.1 | - | 2.3 | Industrial gas |
| 2 | 69.9 | 5.0 | 20.0 | 5.1 | - | 2.0 | $CO_2$ rich gas |
| 3 | 54.2 | 28.9 | 10.9 | 4.0 | 2.0 | 1.1 | Simulated RSCW gas |

**Table 1.** Compositions of the different gases used in methanol synthesis.

The experimental and predicted equilibrium conversions for gas 1 and gas 2 are shown in Figure 13. At temperatures above 495 K for gas 1 and 507 K for gas 2 only a gas phase is present, while at lower temperatures condensation occurs. Methanol production continues in the two phase system until phase equilibrium and chemical equilibrium are reached. At the lowest temperatures in the range, the CO+$CO_2$ conversion is nearly complete for gas 1 (Figure 13A). The conversion of CO+$CO_2$ decreased with increasing temperature as dictated by thermodynamics. The experimental conversions coincide nicely with the conversion predicted by the model. The effect of condensation was more pronounced for gas 2 (Figure

13B). Here, the experimental conversion is even 12.7% higher than the extrapolated gas phase equilibrium curve (ζ = 79.8% vs. 92.5% at 484 K).

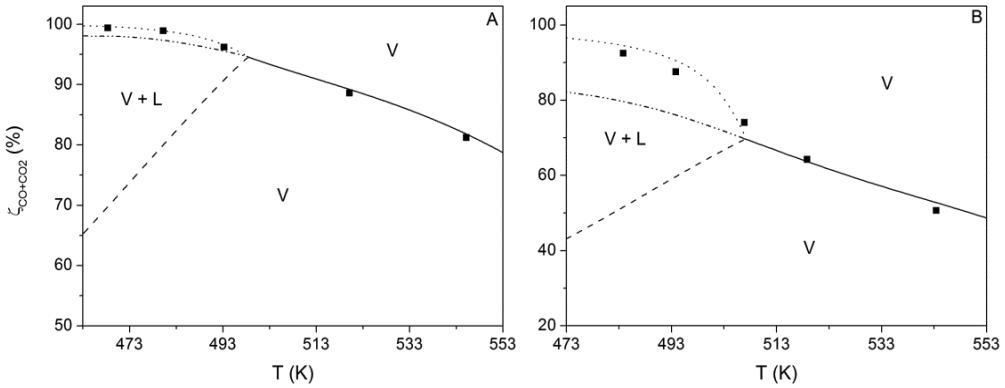


**Figure 13.** Equilibrium diagrams for methanol synthesis. Gas 1 (A), *P* = 19.7 MPa [41]. Gas 2 (B) [45], *P* = 20.3 MPa. Symbols: experimental data; curves: model results.

In Figure 14, both the CO+$CO_2$ conversion (Figure 14A) and the $H_2$ conversion (Figure 14B) are depicted for gas 3. At the two lower temperatures, the equilibrium predictions coincide nicely with the experimental data for, but this changes at the higher temperatures most probably due to the formation of HA. This is a common phenomenon for systems at higher temperatures with high CO partial pressures [46, 47]. HA formation is not included in the equilibrium model and this explains the deviations at higher temperatures.

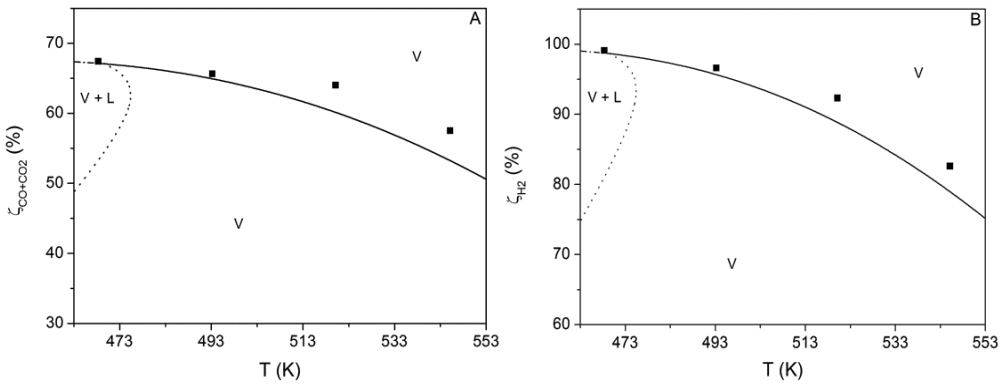


**Figure 14.** Equilibrium diagram for methanol synthesis from gas 3 including the dew point curve for $\zeta_{CO+CO2}$ (A) and $\zeta_{H2}$ (B). Symbols: experimental data; curves: model results. *P* = 19.4 MPa [45].

The concentration of the main HA is given in Figure 15. The methanol concentration clearly decreases over the temperature range in favor of the HA. Based on thermodynamics the formation of higher alcohols is expected as HA formation is favored over methanol [48]. The

main HA formed in the experiments were ethanol and 1-propanol followed by 1-butanol, 2-methyl-1-propanol, and 2-methyl-1-butanol.

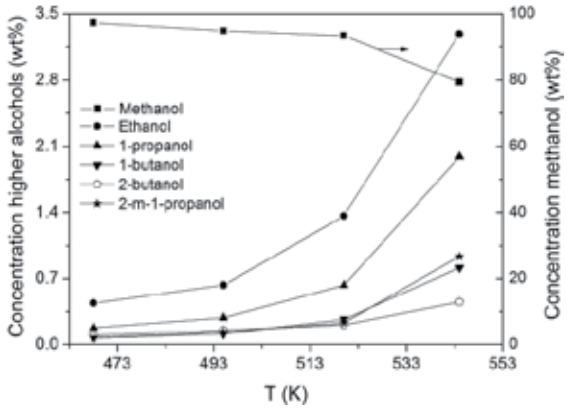


**Figure 15.** Methanol and HA concentrations as a function of the temperature for gas 3. $P$ = 19.9 MPa.

### 4.5. Consequences of methanol synthesis for the integrated concept

The main result from high pressure methanol synthesis experiments is the observation that high conversions of the limiting component are attainable. These conversions are higher than calculated on the basis of chemical gas phase equilibria and are due to condensation. When the HA concentration was low, the experimental conversion corresponded nicely with the equilibria predicted. In conclusion, when a combination of high pressure and moderate temperature ($463 \leq T \leq 500$ K) is used, high conversions for glycerol derived syngases are expected.

## 5. Demonstration of the integrated concept

To demonstrate the integrated concept for methanol synthesis, experiments were conducted in the integrated continuous unit depicted in Figure 3 [8]. Both, process conditions and recycle options of the reforming section were investigated to maximize methanol yields. This requires proper operating conditions for each reactor section (reforming, methanol synthesis) to limit by-product formation (e.g. $CH_4$, higher hydrocarbons, and HA) and to allow operation at high equilibrium conversions in methanol synthesis. In this section, both the results for the overall integrated process will be discussed as well as the result for the reformer section in these experiments. Four different cases are considered with different feed concentrations and operating conditions. An overview of the experiments is presented in Table 2 and a short resume is given below.

The first case is considered the base case. The experiment of the base case was conducted using the LPS without recycling the effluent water (see Figure 3). Part of the gas produced is

lost as it dissolves in the aqueous phase that leaves the process. In case 2, 3, and 4 the effluent water from the reformer is recycled after the HPS. Operating with a recycle stream at high pressure in RSCW is a unique feature. As a consequence of the recycle stream no gas is lost in the reformer section through the LPS and additionally, the water consumption of the process is reduced significantly. In these experiments the glycerol reforming is carried out catalytically by using the Ni based catalyst in combination with higher temperatures. All $C_{2+}$ hydrocarbons are then reformed and the $CH_4$ equilibrium concentration decreases (with higher temperatures) yielding a more attractive gas composition [29].

Finally, in case 4 an extra methanol synthesis packed bed (P1) was added to achieve equilibrium gas phase conditions at the outlet. The methanol synthesis section now consists of 3 packed bed reactors (P1 – P3) in series. The three packed beds were operated at different temperatures, viz. ± 518 K (P1), ± 503 K (P2), and 481 – 482 K (P3) by cooling the heating medium between the reactors. The reaction rate in methanol synthesis depends strongly on temperature. Higher temperatures lead to higher reaction rates. In the first reactor (P1) the reaction rate will be relatively high while the second (P2) and third (P3) are used to achieve equilibrium. Typical run times for the experiments were 6 – 10 h and steady state was reached in approximately 2 h. In case 4 the operating time exceeded 20 h of which 16 h in the integrated mode. This experiment is considered as the long duration experiment.

| Case | Catalyst[a] (g) | | | Recycle | Catalyst[b] (g) | | |
|---|---|---|---|---|---|---|---|
| | R3[d] | R4[d] | R5[d] | | P1[d] | P2[d] | P3[d] |
| 1 | - | - | - | | - | 50 | 51 |
| 2 | 10 | 10 | 3 | yes | - | 50 | 51 |
| 3 | 10 | 10 | 3 | yes | - | 50 | 51 |
| 4[c] | 10 | 10 | 3 | yes | 51 | 50 | 51 |

[a]Ni based catalyst.

[b]A commercial $Cu/ZnO/Al_2O_3$ catalyst.

[c]The catalyst in the reforming section was replaced by fresh catalyst.

[d]R = reformer, P = packed bed reactor for methanol synthesis.

**Table 2.** Overview of experiments in the integrated unit. R3-5 and P1-3 correspond with the reactors in Figure 3 [8].

### 5.1. Reformer performance

Typical conditions for the reformer section (see Figure 3) for these experiments were pressures from 24 to 27 MPa and temperatures between 948 and 998 K. At these conditions the residence times of the reformer section (R1 – R5) were in the range of 30 – 35 s. The composition and quantity of the off-gas were analyzed to determine the carbon balance. The hydrocarbon concentration in the off-gas is the summation of the concentrations of $CH_4$ and $C_2H_6$. The main results of the glycerol reformer section are summarized in Table 3.

Carbon balance closure for the reformer is very satisfactorily and was between 95 and 104%. The glycerol conversion was almost complete for all experiments, which is in line with previous work [18, 29]. The syngas produced had the following composition range: $H_2/CO/CO_2/C_xH_y$ = 44 – 67/1 – 21/16 – 34/2 – 18 vol%, $0.7 \leq S_N \leq 1.2$. The results for each case will be discussed separately in the following section.

### *5.1.1. Base case*

The base case experiment was conducted with a glycerol feed concentration of 10 wt% at 27 MPa and 948 K. The unit was operated without a catalyst in the reformer section and in a once-through mode. Part of the gas (ca. 13%) dissolved in the effluent stream from the LPS (see Figure 3, $D_2$) and is not used for the subsequent methanol synthesis. Due to the absence of catalysts, a significant amount of CO is present in the syngas. The product gas has a relatively low $S_N$ value of 0.7, which is mainly caused by the formation of hydrocarbons (18 vol % consisting for approximately ⅔ of $CH_4$ and ⅓ of $C_2H_6$).

| Case | $P_a$ | $T_{R5}$ | $\phi_{gly}$ | [Gly.] | $H_2$ | CO | $CO_2$ | $C_xH_y$ | $S_N$ | $\zeta_{gly}$ | $C_{bal}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | (MPa) | (K) | (g/h) | (wt%) | | | (vol%) | | (-) | (%) | (%) |
| 1 | 27 | 948 | 106 | 10.4 | 44 | 21 | 17 | 18 | 0.7 | 96[b] | 96 |
| 2 | 24 | 998 | 97 | ± 10 | 55 | 2 | 32 | 11 | 0.7 | 99.9[c] | 97 |
| 3 | 24 | 998 | 35 | ± 4 | 59 | 1 | 34 | 6 | 0.8 | 99.9[c] | 104 |
| 4 | 26 | 998 | 35 | ± 4 | 66 | 1 | 30 | 3 | 1.2 | 99.9[c] | 95 |
| Loc.[d] | $A_{1-3}$ | B | C | | $D_1$ | $D_1$ | $D_1$ | $D_1$ | | | |

[a]The pressure is an average pressure. The actual operating pressure is the indicated pressure ± 1 MPa.

[b]Based on carbon content in the effluent water.

[c]Experiment conducted in recycle mode. Glycerol conversion is estimated based on previous work [29].

[d]Locations where the parameters were measured (see Figure 3).

**Table 3.** Results of the reforming section (before methanol synthesis) [8].

### *5.1.2. Case 2 – 4*

The intention for case 2 was to aim for higher $S_N$ values. A Ni based catalyst was added to reactor R4 and R5 to reform the higher hydrocarbons. As a consequence the WGS reaction also reached equilibrium and almost all CO was converted into $CO_2$ [29]. The temperature of reactor R5 was increased with 50 K compared to the base case to aim for a more advantageous equilibrium composition (less $CH_4$). Furthermore, the effluent water was recycled at high pressure. Recycling the effluent water drastically reduces the water consumption of the process. The recycle flow was adjusted in such a way that the aqueous reactor inlet flow was comparable to the inlet flow in the base case. Compared to the base case no gas was lost through the effluent stream from the LPS. The gas composition obtained over this catalyst differed substantially from the

base case. The CO concentration was reduced from 21 vol% to 1 – 2 vol% and the concentration of the hydrocarbons was significantly lower and approached equilibrium ($C_2H_6 \approx 0$ vol% and $CH_4 \approx 11$ vol%). The $S_N$ value was similar to the base case.

For case 3 the glycerol feed concentration was reduced to approximately 4 wt%. The $H_2$ and $CO_2$ concentration increased compared to the base case, whereas the CO and hydrocarbon concentration were lower, resulting in a slightly more attractive $S_N$ value of 0.8. In the last case (4), a fresh reforming catalyst was used leading to the lowest hydrocarbon concentration and the highest $H_2$ concentration of all experiments. For instance, $C_2H_6$, which accounted for ⅓ of the hydrocarbon content in the base case, was not detected in the product gas. The $S_N$ increased to 1.2 which is close to the theoretical maximum of 1.33 (Eq. 3).

### 5.2. Performance of the integrated process

The results for the integrated process, including methanol synthesis, are presented in Table 4. The equilibria in methanol synthesis were calculated with the data from Table 3 as input and the equilibrium model described in Section 4.3. If applicable, condensation of methanol and water was accounted for in the equilibrium calculations [45]. The equilibrium data should be considered with some care, because the results are based on the assumption of constant gas composition and gas flow from the reformer section. As for the reforming experiments, all methanol synthesis experiments have good closures of the carbon balance (93 – 96%), particularly when regarding the complexity of the integrated process. A detailed summary of the experimental results of the integrated process is given below. As in section 5.1, case 1 is considered as base case and the results of the other experiments are compared to this experiment.

| Case | $T$ | $H_2$ | CO | $CO_2$ | $C_xH_y$ | $\eta^a$ | MeOH[b] | $H_2O$ | $\zeta_{CO+CO2}$ | $\zeta_C$ | $C_{bal}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | (K) | | | (vol%) | | | | (wt%) | | (%) | |
| 1 | 468 | 3 | 2 | 44 | 51 | 0.27 | 99 | 1 | 58 | 26 | 95 |
| equi | 468 | 5 | 2 | 44 | 49 | 0.28 | 99 | 1 | 59 | 28 | - |
| 2 | 498 | 42 | 0 | 40 | 18 | 0.29 | 67 | 33 | 35 | 27 | 96 |
| equi | 498 | 25 | 1 | 46 | 28 | 0.35 | 66 | 34 | 45 | 33 | - |
| 3 | 483 | 49 | 0 | 40 | 11 | 0.27 | 65 | 35 | 39 | 26 | 93 |
| equi | 483 | 15 | 1 | 61 | 23 | 0.50 | 65 | 35 | 54 | 48 | - |
| 4 | 481 | 20 | 0 | 60 | 20 | 0.62 | 65 | 35 | 71 | 60 | 94 |
| equi | 481 | 11 | 1 | 69 | 19 | 0.65 | 65 | 35 | 70 | 62 | - |
| Loc.[c] | **E** | **G** | **G** | **G** | **G** | **F** | - | - | - | - | - |

[a]Units = (kg methanol/kg glycerol).

[b]The liquid phase is assumed to consist of water and methanol. The methanol concentration here is calculated by 100 wt% – (water concentration). The exact composition of the organic phase is given in Table 5.

[c]Locations where the parameters were measured (see Figure 3).

**Table 4.** Results of methanol synthesis from glycerol derived syngas [8].

*5.2.1. Base case*

In the experiment in case 1, the methanol synthesis reactors were operated at 468 K. Hydrocarbons are inert in methanol synthesis and as a results their concentration increased strongly in the off-gas of the methanol synthesis reactor to over 50 vol%. The $H_2$ and CO concentration in the outlet of the methanol reactor were 3 and 2 vol%, respectively. The $CO_2$ concentration increased compared to the reforming gas, as mainly CO was converted to methanol. The gas composition and liquid yield at the exit of the methanol reactor were close to equilibrium, with the liquid yield slightly lower and experimental conversion slightly higher than predicted by equilibrium modelling. The overall carbon conversion was 26% which is equal to a methanol yield of 0.27 kg methanol/kg glycerol. The conversion of 26% is the highest conversion possible with such a syngas composition, because equilibrium was reached.

*5.2.2. Case 2 – 3*

In case 2, a different approach was followed. Due to the Ni based catalyst in the reforming section the hydrocarbon concentration decreased and almost all $C_2H_6$ was reformed. Furthermore, almost all CO was converted into $CO_2$, which therefore became the main carbon source of methanol. The temperature of the methanol synthesis reactors was increased compared to the base case (from 468 – 498 K), because methanol synthesis from mainly $CO_2$ proved to be slower than methanol synthesis from CO. The methanol yield was, with 0.29 kg methanol/kg glycerol, similar to the base case, but in case 2 equilibrium was not reached. Higher conversion are thus possible with longer residence times.

For case 3 the glycerol feed flow was reduced with a factor 3 to improve the gas composition, resulting in a reduction in feed gas flow. Therefore, to obtain higher conversion, the temperature of the methanol synthesis reactor was reduced to 483 K. Again, the carbon conversion and methanol yield (0.27 kg methanol/kg glycerol) were comparable to the base case, but remained far from equilibrium.

*5.2.3. Case 4: Long duration experiment in the integrated unit*

In case 4 (long duration run, 20 h) an extra methanol synthesis packed bed (P1) was filled with catalyst. The methanol synthesis reactors were operated at three different temperatures (± 518 K (P1), ± 503 K (P2), and 481 – 482 K (P3). The lowest hydrocarbon concentration in the gas phase after the reformer was observed due to the use of a fresh reforming catalyst. As a consequence the corresponding carbon conversion in the methanol synthesis unit increased to 60% ($\eta$ = 0.62 kg methanol/kg glycerol). Nevertheless, even higher methanol yields are possible as equilibria were not yet achieved. In the first 4 h of the long duration experiment, only the reformer section was operated. Methanol synthesis was carried out over a 16 h period and the hourly liquid methanol yields and volumetric flows at the exit of the methanol synthesis unit (point G in Figure 3) are shown in Figure 16.

Though some scattering in the methanol yield can be noted (due to some pressure fluctuation and uncontrolled release by the back pressure valve during the experiment), the integrated system was running steadily and the methanol yield was more or less constant. The

selectivity ($\sigma$) is depicted in Figure 16B. The methanol selectivity is equal to the carbon conversion and amounts to 60% on average. An average value of 8.7% of the carbon present in glycerol ends up as $CH_4$. The scattering pattern for the methanol selectivity is similar to the scattering in the methanol yield in Figure 16A. The selectivity towards $CH_4$ is also more or less constant. It seems that no deactivation or reduced activity for both the reformer section and the methanol synthesis section were observed during the course of the experiment.

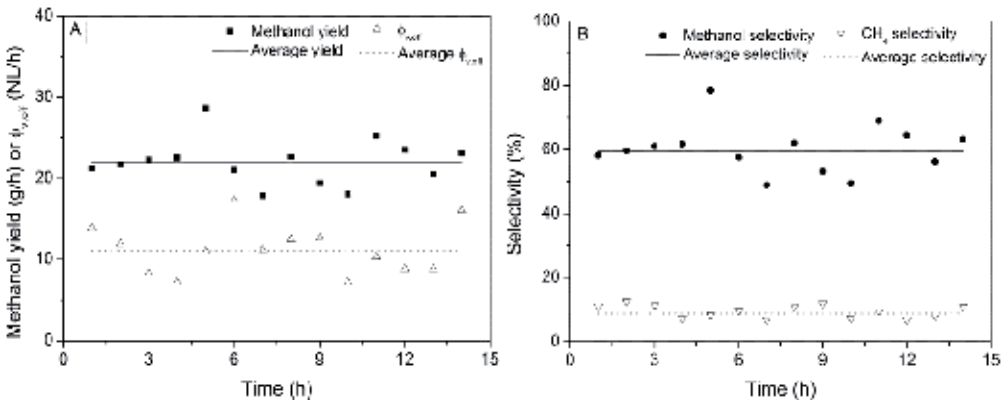


**Figure 16.** Methanol yields from glycerol and volumetric flow at the exit of the methanol reactor of the long duration experiment (A). Carbon selectivity towards methanol and $CH_4$ (B) [8].

### 5.3. Liquid composition after methanol synthesis reactor

The main constituents of the liquid products of the integrated experiments are given in Table 5. The liquid phase was analyzed on methanol, water, and the eight most common HA. In general, the concentration of HA was very low (< 0.23 wt% of the total and < 0.24 wt% of the organic fraction), probably due to the low temperatures of the methanol synthesis and the high $CO_2$ content of the feed gas, leading to the formation of water [46]. Ethanol was the most predominant among the HA, with a maximum concentration of 1.2 wt‰. Noticeably, when methanol was predominantly synthesized from $CO_2$ (case 2 – 4) the concentrations of HA were negligible. This is in agreement with literature data which show that the concentrations of HA decrease at higher $H_2$/CO ratios in the syngas feed [46, 47].

| Case | Liquid product | | | | Higher alcohols | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | MeOH | $H_2O$ | HA | Purity[a] | EtOH | 2-propanol | 1-butanol | 2-m-1-propanol | 2-m-1-butanol |
| | (wt%) | | | | (wt‰) | | | | |
| 1 | 97.8 | 1.1 | 0.23 | 99.8 | 1.2 | 0.7 | 0.2 | 0.2 | 0.1 |
| 2 | 66.5 | 33.0 | 0.00 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | 65.9 | 34.6 | 0.00 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 4 | 65.1 | 34.9 | 0.01 | 99.9 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |

[a]Methanol content of the organic fraction.

**Table 5.** Composition of the liquid phase [8].

### 5.4. Process analysis

The experiments conducted in the integrated unit were aimed at obtaining high carbon conversions and methanol yields when reforming aqueous glycerol solutions to syngas followed by methanol synthesis. The gas composition after reforming appeared to be the most critical factor and has a major effect on the final methanol yield. Particularly the formation of hydrocarbons should be avoided in the reformer section as hydrocarbons are inert in the subsequent methanol synthesis. Therefore, hydrocarbon reduction was the main objective in the experimental reformer program and was pursued by the application of catalysts, higher reforming temperature, and reduction of the feed concentration. Application of a suitable catalyst (Ni based) indeed led to a considerable reduction in the amount of hydrocarbons in the reformer off-gas, though as a consequence almost all CO was converted into $CO_2$. Further research will be required to identify reformer catalysts that promote glycerol decomposition rates and hydrocarbon reforming, but do not enhance the WGS reaction. In this respect, Ir-based catalysts are promising because of good performance in aqueous phase reforming [49].

The conversion of CO into $CO_2$ in the reformer section, as observed when using the Ni based catalyst, is not detrimental for the subsequent methanol synthesis. With the commercial methanol synthesis catalyst used in this study, $CO_2$ hydrogenation is possible, as was also proven here, though the overall reaction rates in methanol synthesis are lower than in case of CO hydrogenation [50]. An advantage, however, of $CO_2$ hydrogenation is the high purity of the organic fraction, as the formation of HA is suppressed by, most probably, the presence of water [46, 51].

In the demonstration of the integrated concept, pure glycerol was used as feedstock for the process. When crude glycerin is used salts are present and they have to be removed upfront. Continuous salt removal is possible and has been demonstrated in the literature [52].

## 6. Conclusion

A successful experimental demonstration of glycerol conversion to methanol was shown by the integration of two processes. Glycerol was reformed in supercritical water to syngas and the syngas was subsequently converted to methanol. Before integration of the two processes the processes were investigated individually. In glycerol reforming a gas containing mainly $H_2$, CO, $CO_2$, $CH_4$, and $C_2H_6$ was produced. When a Ni based catalyst was used the higher hydrocarbons were reformed and $CH_4$ approached its equilibrium concentration. In methanol synthesis *in situ* condensation was observed which positively influences the equilibrium conversion. At temperatures around 473 K and pressures above 20 MPa almost complete conversion of the limiting components was obtained.

The methanol yields of the integrated process depended mainly on the gas composition obtained in the glycerol reforming process, which appeared to be the most attractive for methanol synthesis at high temperature and low feed concentration in combination with a Ni based catalyst. The continuous unit was modified during the experimental program to increase the methanol yields. The effluent water of the reformer section was recycled at high pressure, to reduce the water consumption of the process. The highest methanol yield of 0.62 kg methanol/kg glycerol was obtained using the Ni based catalyst in the reformer section and recycling of the effluent water. In this particular experiment glycerol was converted to mainly $H_2$ and $CO_2$ and smaller amounts of $CH_4$ and CO. In this experiment, 60% of the carbon present in the glycerol ends up in methanol. These yields are close to the equilibrium yields. The integrated unit was operated smoothly for more than 16 h without catalyst deactivation.

The scope of the project is much broader than the production of methanol from glycerol for the reuse in biodiesel production. Due to the investigation of the individual processes more insights in reforming and methanol synthesis were obtained. Furthermore, the feedstock for the reforming process was glycerol in this case, but several types of biomass (preferably liquid) including aqueous phase fractions from pyrolysis oil upgrading, black liquor, etc. can be used for the reforming process. When these types of feedstocks are 'green' renewable methanol can be produced, which is a promising process for the (near) future.

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Chapter 13

# Utilization of Crude Glycerin from Biodiesel Production: A Field Test of a Crude Glycerin Recycling Process

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Kensuke Kurahashi and Takahiko Wakamatsu

Additional information is available at the end of the chapter

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## 1. Introduction

### 1.1. Background

Worldwide, increasing quantities of biodiesel fuel (BDF) are produced, along with bioethanol. The production of BDF generates glycerin (also known as glycerol) as a by-product. Because alkaline metal oxides or alkaline hydroxides are commonly used as catalysts for the transesterification of vegetable oils with methanol, the glycerin stream is strongly alkaline, and therefore must be neutralized, demineralized, rinsed with water, and dried before glycerin is combusted. This has been a barrier to the popularization of BDF [1-3].

Osaka Prefecture University (abbreviated as OPU) in Sakai, Japan, is promoting a "Campus Zero Emissions" project, intended to recycle the resources within the campus. We have a bench-scale methane fermentation plant and BDF production plant based on ultrasound that is capable of producing BDF and methane from waste cooking oil [4-7] and food waste.

Methane fermentation is one of the main processes used for food waste (nitrogen and carbon mixtures) stabilization. High nitrogen concentration and pH inhibits growth of bacteria because of toxicity caused by high levels of ammonia. Microorganisms require carbon and nitrogen for metabolism, and the relationship between their amounts in organic materials is represented by the C/N ratio. Optimum C/N ratios in anaerobic digesters are between 20 and 30. A high C/N ratio is an indication of rapid consumption of nitrogen by methanogens, and results in lower gas production. On the other hand, a lower C/N ratio causes ammonia accumulation and pH values exceeding 8.5, which is toxic to methanogenic bacteria. Optimum C/N ratios in digester contents can be achieved by mixing materials with high and low C/N ratios, such as the raw glycerin byproduct of BDF production.

One of the authors, Tokumoto, has filed three patent applications regarding a leading-edge technology that allows the fermentation of strongly alkaline waste glycerin using anaerobic microorganisms, without additional processes. This technology is expected to compete with fermentation technology that uses a microorganism with high glycerin degradation ability. However, the latter is, in general, based on the selection of the most favorable individually cultivated microorganism system from a wide variety of individually isolated fungi. The cost of an operation that utilizes such delicate microorganisms is high and this is one of the barriers to commercialization of the system (Figure 1) [8-12]. Using high-level experience in microbiological control, our project is working towards the establishment of a new process based on a low-cost, combined cultivation system.

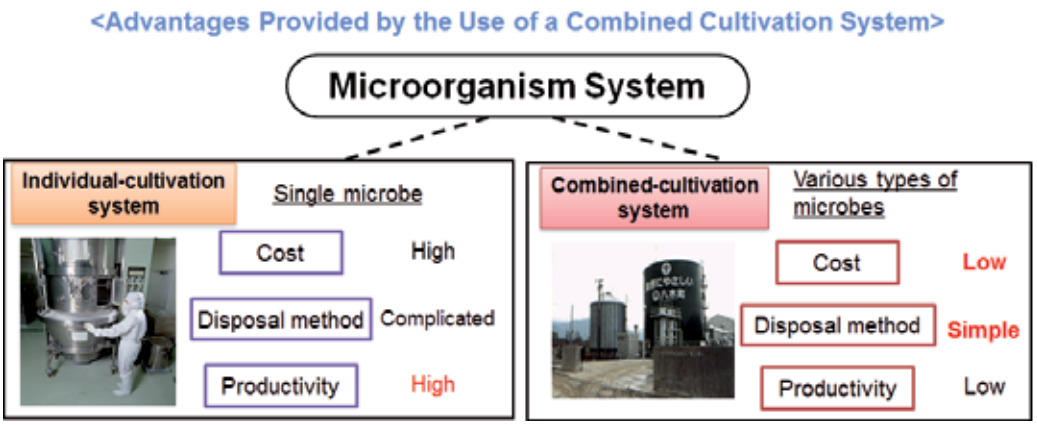


**Figure 1.** Comparison between individual- and combined-cultivation systems

### 1.2. Purpose

The campus zero-emission project is intended to establish the business model for OPU's resource recycling process using waste cooking oil. In this model, we produce BDF from waste cooking oil discharged from the dining halls on campus and generate methane from the glycerin by-product to supply fuel for motorcycles, vehicles, and electric power facilities [13-14]. This research activity will result in verification tests based on about 10,000 students and teaching/clerical staff members as monitors, and then develop the test results into a comprehensive recycling process for waste cooking oil.

Commercial-scale plants will be standardized at the end of this project, based on the operational data from the methane fermentation plant and utilization facilities (motorcycles, other vehicles, and electric power facilities) shown in the figure below.

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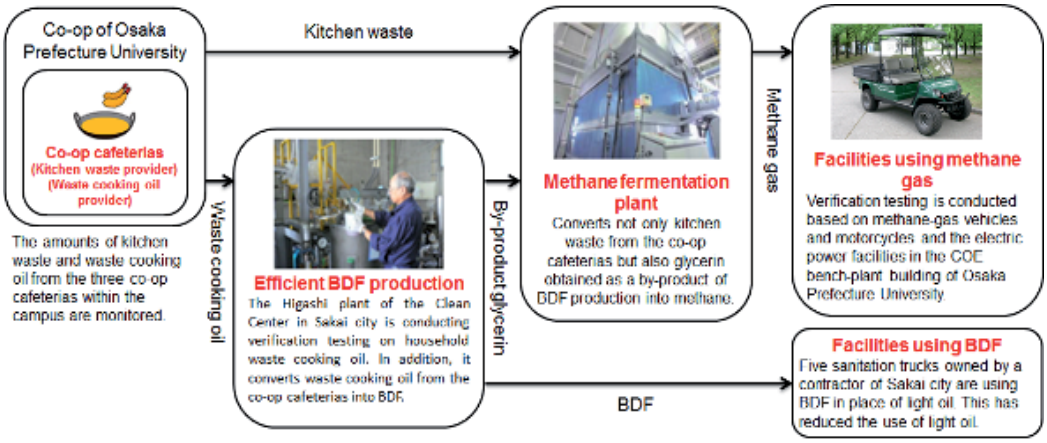


**Figure 2.** Entire experiment flow chart

## 2. Results and discussion

### 2.1. Methane fermentation reactor

The methane fermentation plant has only one fermenter, with a fermentation vessel volume of 10 $m^3$ and an operating capacity of 5 $m^3$. From startup, the plant used bean curd lees as a fermentation substrate (fermentation feedstock for breeding microorganisms and generating methane) to achieve stable operation. We then added another fermentation substrate – part of the kitchen waste from the OPU's co-op cafeteria. After stable operation was achieved, we then added waste glycerin, the by-product generated during BDF production.

### 2.2. Operational testing on the fermentation plant

*2.2.1. Alkali considerations of the microorganism fermentation plant*

The figure below shows the correlation between the amount of gas generated and the raw material disposal rate.

We purchased seed sludge from the methane fermentation plant of the Bioecology Center in Yagi Cho, Kyoto Prefecture and charged this into the fermenter in our methane fermentation plant. After the startup (neutralization) process, we used bean curd lees as a fermentation substrate, which resulted in favorable biogas production after about 10 days (Figure 3). In this case, we observed a positive correlation between the amounts of bean curd lees disposed of and the biogas generated. From May 12 to June 5, the amount of generated biogas per unit amount of bean curd lees was 0.68 $m^3$/kg, calculated by the least-squares method (Figure 3).

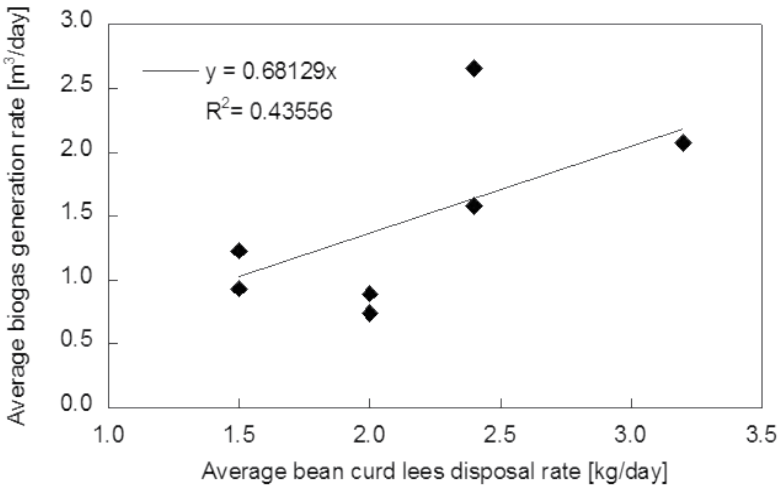


**Figure 3.** Correlation between the amounts of gas generated and bean curd lees disposal rate

*2.2.2. Biogas from co-op waste*

Temporal changes in the amounts of biogas produced after the kitchen waste from the co-op cafeteria was added are shown in Figure 4.

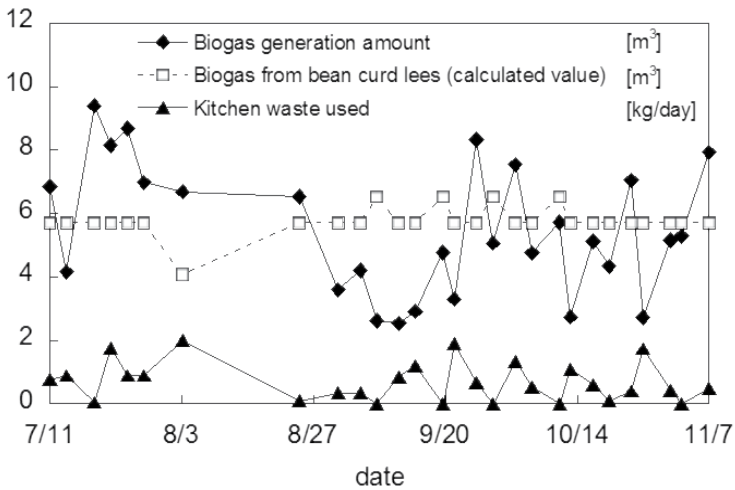


**Figure 4.** Amount of biogas generated from kitchen waste discharged from the co-op cafeteria

The amounts of biogas generated shown in Fig. 4 are actual measured values. The amounts of biogas from the bean curd lees were obtained by multiplying the biogas generation rates calculated in Figure 3 by the amount of bean curd lees. The subtraction of these values may

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result in negative values in some cases. For this reason, the figure does not indicate the amounts of biogas from the kitchen waste discharged from the co-op cafeteria, but only the amounts of waste added.

In producing these results, we successfully disposed of co-op waste without destabilizing the biogas production at the plant, which provided a favorable biogas production even using co-op kitchen waste. In this case, we successfully converted a maximum of 2 kg co-op kitchen waste into biogas per day.

The results, however, also indicated that the kitchen waste delivered a conversion efficiency lower than that for bean curd lees alone. Bean curd lees are vegetable-protein food and contain significant nitrogen and exhibit a low C/N value. The C/N value is usually regarded as an index of the favorability of substrates towards anaerobic fermentation [15], and the low value for bean curd lees suggests that the existence of the low C/N-value substrates, bean curd lees, in this system could decrease the reactivity of the microorganisms producing biogas. It should be noted, however, that in the long-term, increases in the use of co-op waste lead to gradual increases in the amount of biogas generated.

Figure 5 shows the monthly number of cafeteria users in 2011. A university-specific trend was observed, in that the number of users decreased during the long vacation period in August and September. A similar trend among the kitchen waste used and amount of biogas generated in Figure 4 and the number of the cafeteria users in Figure 5 indicates that the gas production amount is strongly correlated with the number of cafeteria users. Consequently, the largest influence is the decrease in the amount of organic substances contained in the kitchen waste. When the number of users was stable in September and October, the biogas production stabilized, meaning that the plant seems to provide stable operation as a whole. With these behaviors in mind, this research is characterized by the fact that investigating the amount of waste enables the estimation of the performance of the fermentation plant, based on the number of users of the dining facility or facilities in the business place.

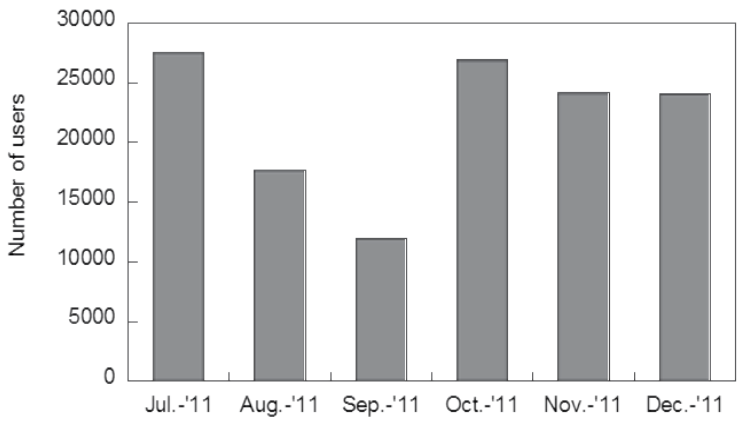


**Figure 5.** Number of co-op cafeteria users

*2.2.3. Converting waste glycerin into biogas*

The amounts of biogas achieved when bean curd lees, kitchen waste, and waste glycerin were used as substrates are shown in Figure 6.

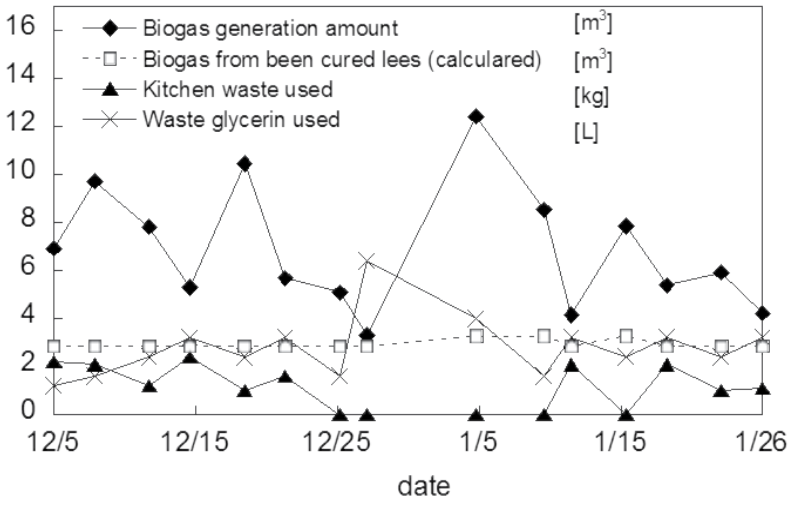


**Figure 6.** Amounts of biogas generated from bean curd lees, kitchen waste, and waste glycerin

The amounts of biogas shown are actual measured values. The amounts of biogas produced from the bean curd lees were obtained by multiplying the biogas generation rates calculated in Figure 3 by the amount of bean curd lees. For the kitchen waste and waste glycerin, the figure indicates the amounts used.

The amount of waste glycerin is correlated with the amount of biogas generated, whereby the addition of glycerin significantly increases the amount of biogas. Therefore the disposal of waste glycerin in this process has significant advantages. It is estimated that the biogas generation rate per unit amount of glycerin was 1.63 $m^3$/kg on average. This is about 2.5 times the biogas generation rate when bean curd lees alone were used. It was also observed that about 2 kg of co-op kitchen waste per day were steadily decomposed by fermentation, as seen above. The operation continued steadily for about a month. In addition, the amounts of bean curd lees, kitchen waste, and waste glycerin we used this time were small compared with the operating capacity of the fermentation vessel, and the full capacity has not yet been used.

*2.2.4. Estimated reduction in $CO_2$ emissions*

The disposal flow and the calculated reduction in $CO_2$ emissions per unit amount disposed are shown in Figure 7.

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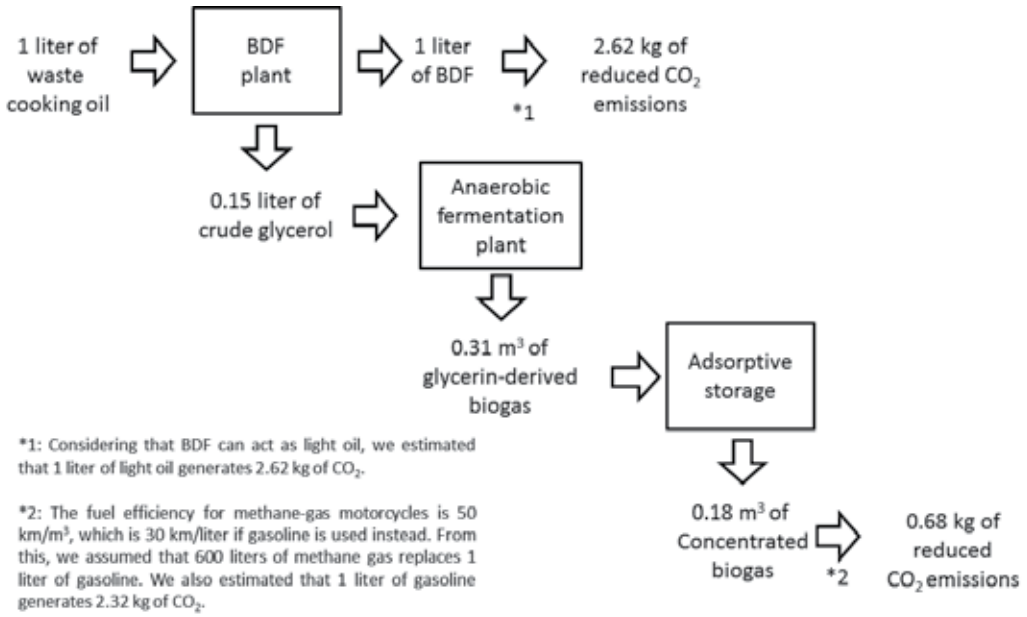


**Figure 7.** Disposal flow and reduced $CO_2$ emissions

Because waste cooking oil is a plant-derived organic substance, it is possible to estimate the reduction in $CO_2$ emissions based on carbon neutrality. For simplicity, we ignored the $CO_2$ emissions associated with machine operation. We also assumed that we could treat BDF equally as an alternative to light oil. Furthermore, we considered that 600 liters of biogas can replace one liter of gasoline, based on the fuel efficiency of motorcycles. The definitions of $CO_2$ emissions from fossil fuels were based on the guidelines from the Ministry of Environment [16].

The disposal of glycerin at the anaerobic fermentation plant effectively reduces $CO_2$ emissions by more than 30% compared with waste cooking oil wholly incinerated, without additional treatment. When glycerin is incinerated, a fossil fuel is normally used as a combustion aid. If this is taken into consideration, this method may have an even greater effect on reducing $CO_2$ emissions. Only part of the kitchen waste from the co-op cafeteria is currently disposed of. Disposal of all kitchen waste will further reduce $CO_2$ emissions.

While a long-term testing and verification period is required, it is expected that biogas generation from waste glycerin will further improve, providing a larger reduction in $CO_2$ emissions.

### 2.3. Biogas fuel conversion facilities

The effective use of the biogas generated in the methane fermentation vessel as a fuel requires a system for temporarily storing the biogas, a system for concentrating the methane contained in the biogas, and facilities that can make effective use of the concentrated (refined) biogas.

The concentrated methane gas can be used "as-is", if the required pressure is low. On the other hand, if it is used as a fuel for vehicles, then equipment for charging biogas into the vehicles is required. Figure 8 shows the biogas use flow chart. A description of the equipment we operated in this research is given below.

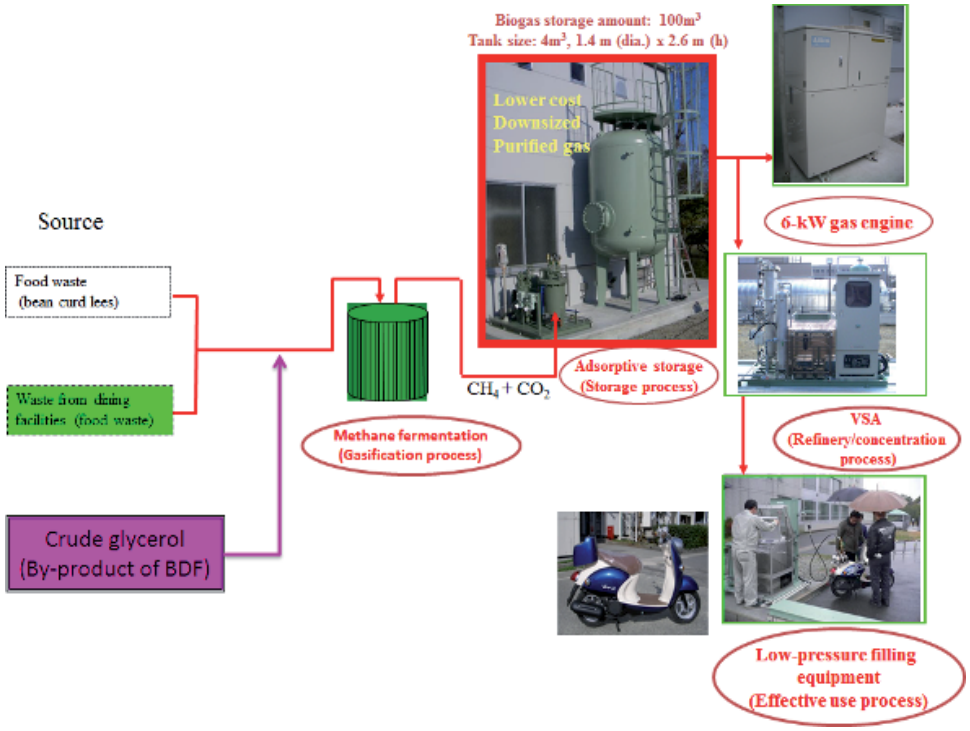


**Figure 8.** Biogas use flow chart

### *2.3.1. Adsorptive biogas storage system*

#### *2.3.1.1. Principle*

The effective use of the biogas generated by methane fermentation requires a buffer tank for storing gas in order to make 100% use of the biogas, because it is difficult to balance the amount generated with usage at any one time.

In most cases currently, biogas is stored under a low pressure of a few hundred mm of $H_2O$, which is the gas pressure inside the fermentation vessel. However, this method can store only the same amount of gas as a gas tank. The storage of a larger amount of gas involves problems associated with size and equipment cost. To solve these problems, a method has been considered that fills the gas tank with a microporous absorbent to enable adsorptive storage of gas, allowing the storage of a large amount of gas at room temperature and under a relatively low pressure [17].

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This method uses a phenomenon whereby methane, the major component of biogas, is physically absorbed in micropores of absorbents at a density close to that of its liquid state (Figure 9). This technology is expected to provide large-volume storage even under a relatively low pressure, because it even absorbs methane that is not liquefied by pressure. This new, attractive storage method, if commercialized, could provide safer storage of digestion gas with a lightly equipped device, according to purpose, and allow the transportation of biogas to other points of consumption, which is not common at the moment. In addition, it has the advantage that it can provide gas purification, which is required for effective use of biogas, through simultaneous adsorptive storage. Because biogas must be effectively stored in a limited area within the premises of the plant at this time, we used an adsorptive methane storage system.

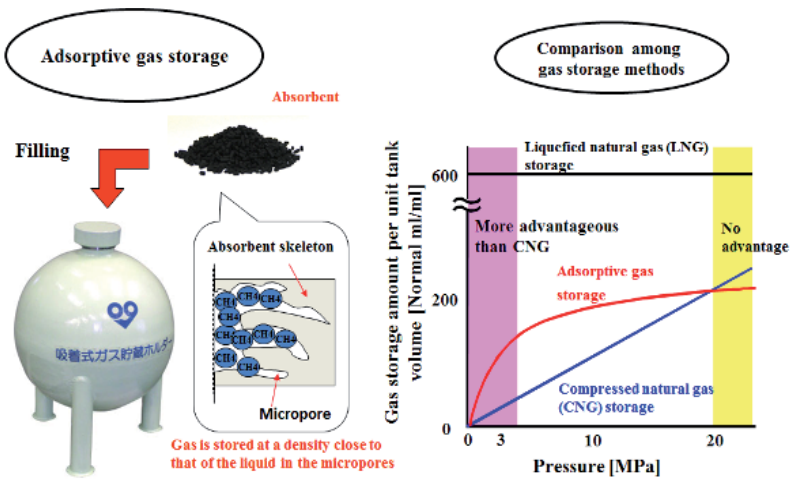


**Figure 9.** Principle of adsorptive storage

*2.3.1.2. Device specifications (stationary large-capacity type, Figure 10)*

- Type

  Biogas-absorbent-filling-type, vertical cylindrical pressure tank

- Applicable laws and regulations

  Construction Code for Pressure Vessels (second-class pressure vessels)

- Intended use

  Adsorptive storage of biogas

- Gas storage capacity

  Effective amount: approx. 100 $Nm^3$

  activated carbon storage capacity of about 4 $m^3$

- Dimensions

1,700 mm (dia.) x 2,600 mm (H) (Trunk: 1,900 mm)

- Fluid

  Refined biogas ($CH_4$: 60%, $CO_2$: 40%)
- Operating pressure

  0 – 0.75 MPa (G)
- Operating temperature

  10 – 50˚C
- Design temperature

  50˚C
- Design pressure

  0.8 MPa (G)
- Absorbent amount

  Approx. 2.1 tons (coconut shell activated carbon)
- Main unit

  SS400 (epoxy-coated inner surface)
- Ladder and handrail

  SS400 and SGP
- Hanging ring

  SS400
- Foundation bolts and nuts

  SS400

**Figure 10.** The adsorptive biogas storage system

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*2.3.1.3. Storage performance*

The storage performance depends significantly on the ambient air temperature, absorbent temperature, and the incoming and outgoing flow rates of the target gas, and is therefore difficult to measure accurately from the actual tank dimensions (Figure 10). For this reason, we used a small insulating container to measure the storage performance, similar to that of the actual tank, and evaluated the storage performance by rapidly filling it with 100% methane. We charged the gas at an absorbent temperature of 25°C. It was verified that, with the activated carbon charged 100%, the container can store 100 $m^3$ of methane, approximately 25 times the tank capacity, if it operates in the range of 0 – 0.6 MPa.

*2.3.2. Adsorptive, isolated methane concentrating device*

*2.3.2.1. Principle*

In general, biogas generated in the methane fermentation vessel contains $CH_4$, $CO_2$, saturated $H_2O$, $H_2S$, and trace quantities of organic components generated during the decomposition process. To make effective use of this gas, it is necessary to refine it (concentrate the methane, which will act as the fuel). If it is used as fuel for vehicles, the methane must be delivered at a purity of at least 95% and the water vapor must have a dew point of -55°C or less.

The methods for refining biogas include pressure swing adsorption using an absorbent, the separation membrane technique using polymeric separation membranes, and the absorption technique using (alkaline) water. In this research, we used pressure swing adsorption (PSA) because it is able to produce methane and remove water [18].

As Figure 11 shows, the device uses an absorbent with a controlled micropore diameter to selectively absorb and remove carbon dioxide based on the difference in their molecular sizes. It also concentrates the methane and removes impurities. This means that using an absorbent with micropore sizes between the molecular sizes of methane and carbon dioxide, the device can separate methane, the major component of biogas, as well as carbon dioxide, water and impurities. At the same time, it also absorbs and removes water, which has a smaller molecular diameter than carbon dioxide. Note that the molecular sizes follow the order methane > carbon dioxide > water.

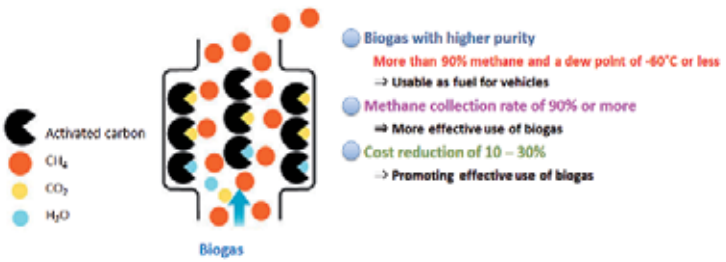


**Figure 11.** Principle of biogas purification

Using two adsorption towers filled with the absorbent described above, concentrated methane with a low dew point can be seamlessly obtained by alternately repeating the adsorption and regeneration processes.

*2.3.2.2. Device specifications (Figure 12)*

**i.** Entrance biogas conditions

**1.** Flow rate: 3.2 $Nm^3/h$

**2.** Pressure: 100 mm $H_2O$

**3.** Biogas composition

$CH_4$: 60%, $CO_2$: 40%, water: saturated

Trace quantities of ingredients (e.g., hydrogen sulfide and ammonia): 1 ppm or less

**ii.** Exit product gas conditions

**1.** Methane purity: at least 95%

**2.** Methane collection rate: at least 90%

**Figure 12.** The adsorptive, isolated methane concentrating device

*2.3.2.3. Characteristics of methane concentration*

The absorbent used for methane concentration is a carbon molecular sieve (CMS) with a micropore diameter adjusted to be approximately 0.3 to 0.35 nm [19]. If it is used to separate carbon dioxide and methane, then the difference in adsorption rate is used instead of the difference in equilibrium adsorption capacity. Table 1 and Figure 13 show the data for the equilibrium amount adsorbed and adsorption rate curve, respectively [20]. As Figure 13 indicates, the amount of carbon dioxide reached 90% of the equilibrium adsorption capacity within one minute, while almost no methane was absorbed. This principle can be used to perform adsorption separation.

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| | $CO_2$ | Methane |
|---|---|---|
| Adsorption amount (ml/g) | 55.2 | 26.9 |

**Table 1.** Equilibrium adsorption capacities (under one atmosphere pressure)

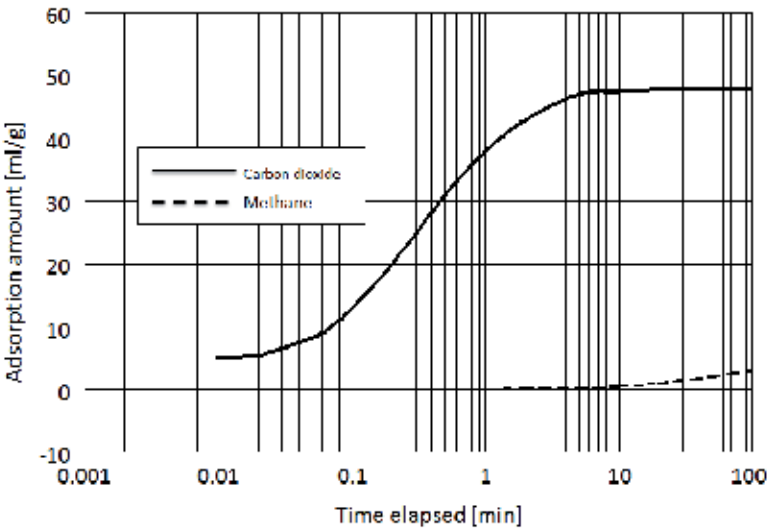


**Figure 13.** Adsorption rate curves for carbon dioxide and methane

*2.3.3. Operational testing for biogas*

**1.** Overview

Refined biogas can be effectively used as a fuel in vehicles and cooking appliances. In the plant, we verified the establishment of effective systems that allow biogas to be used as a fuel in light cars for food sales, service buggy cars used within the campus and for motorcycles, as well as a fuel for cooking in the cafeteria and other facilities.

Using a filler, we charged approximately 95% biogas refined through PSA into a typical natural-gas light car, on-campus service buggy cars, motorcycles equipped with a fuel canister filled with an absorbent, and adsorptive storage cylinders for transfer filled with an absorbent. The filling equipment used charges gas under a low filling pressure of 0.98 MPa or less, and is not, therefore, restricted by any laws or regulations in Japan.

As methane vehicles, we used on-campus light minivans, on-campus adsorptive service buggy cars, and adsorptive motorcycles. Large quantities of biogas must be stored under a high pressure. This requires adherence to the High Pressure Gas Safety Act and other laws and

regulations, causing the unit price of biogas to rise. The system verified in this research project eliminates the need to address this matter, providing safe transfer of large volumes of biogas.

Figure 14 shows the flow chart for extracting methane from a methane adsorptive storage tank for use as a fuel in vehicles. Using these systems, biogas is expected to be able to be used in a wider range of applications, including the consumer segment.

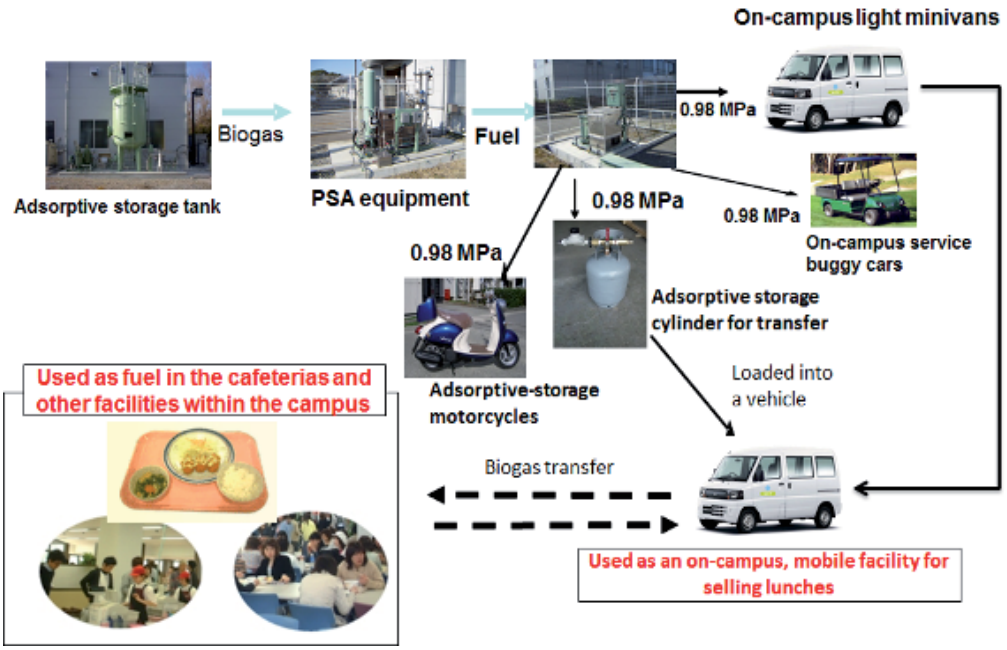


**Figure 14.** Flow chart for the use of biogas as a fuel in vehicles and cooking

**2.** Overviews of the devices

[Biogas filler] (Figure 15)

- Type

  Low-pressure filling equipment for biogas

- Discharge flow rate of the gas compressor

  1.6 $Nm^3/h$ or more

- Filling pressure

  0.98 MPa G or less

- Entrance biogas composition

  Methane: 95%, $CO_2$: 5%

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**Figure 15.** The biogas filler

[Light minivan] (Figure 16)

- Fuel tank
- Storage pressure

  0.98 MPa
- Storage capacity

  Approx. 0.5 $m^3$
- Travel range

  6 – 8 km

**Figure 16.** A light minivan

[On-campus adsorptive service buggy (Figure 17)

- Fuel tank
- Storage pressure

  0.98 MPa

- Storage capacity

  3.5 $m^3$

- Travel range

  70 – 90 km

**Figure 17.** An on-campus adsorptive service buggy

[Adsorptive Motorcycle] (Figure 18)

- Fuel tank
- Storage pressure

  0.98 MPa

- Storage capacity

  1 $m^3$

- Travel range

  50 km

**Figure 18.** An adsorptive motorcycle

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# 3. Conclusion

## 3.1. Consideration of business sizes

| | | Motorcycle | Buggy | Vehicle (within the premises) | Vehicle (outside the premises) |
|---|---|---|---|---|---|
| Biogas storage capacity [$m^3$] | | 1 | 3.5 | 0.5 | 12.5 |
| Mileage [km] | | 50 | 80 | 7 | 175 |
| Mileage per day [km] | | 5 | 10 | 7 | 50 |
| Annual number of operation days [days] | | 200 | 200 | 200 | 200 |
| Required monthly number of cafeteria users [people] | | 506 | 2212 | 2528 | 18057 |
| Annual disposal amount of glycerin [L] | | 9.74 | 42.6 | 48.7 | 348 |
| Annual reduction of $CO_2$ emissions [kg] | BDF | 170 | 744 | 850 | 6075 |
| | Biogas | 44.1 | 193 | 221 | 1577 |
| | Total | 214 | 937 | 1071 | 7651 |
| Use | | Delivery of mail, etc. | Travel within the premises | Material transportation within the premises | Material transportation from/to the premises |

**Table 2.** Utilizations of biogas and their effects on $CO_2$ emissions reduction

Based on numerical values regarding biogas uses and utilizations (from product catalog data), we considered the required business sizes.

In the model, we used the number of users of the co-op cafeteria and the amount of waste cooking oil generated. The co-op cafeteria of Osaka Prefecture University is used by 23,000 people per month on average and 3,000 liters of waste cooking oil are discharged annually. We considered the amount of glycerin derived from BDF production to be one quarter of the amount of waste cooking oil. Table 2 summarizes the uses and utilizations of the biogas along with their effects on $CO_2$ emissions reduction.

Based on the data in the table, we estimated that the business model proposed by this research can be applied to any business place that has a dining facility used by hundreds of people a month. With increases in the number of users, the form of use and utilization develops; if a business place has a dining facility or facilities used by more than 20,000 people a month, then it is expected that the business can expand the use range to include the utilization of business vehicles.

Brazil, an excellent exemplar for biomass energy power generation, has started to make efforts to reduce fossil fuel use by blending 10% BDF into light oil, similar to the use of bioethanol in the past. The process proposed can be applied to all vehicles, including natural-gas and diesel vehicles, as long as they use an internal combustion engine. The largest challenge is fuel storage. Because gas changes volume with temperature, it is important to increase the amount of biogas stored per unit volume. Our partner, Osaka Gas Engineering, owns leading-edge technology for biogas storage and its application, which is expected to popularize the business model.

### 3.2. Future challenges

Below are the future challenges associated with business projects that use biogas as a fuel and other uses:

**1.** Decreasing the fuel price per unit heating value to or below that of city gas

In many cases, the use of biogas as a fuel is compared with the use of city gas in terms of cost because they exhibit similar properties [21]. The comparison, however, normally indicates that biogas has no clear advantage.

On the other hand, to achieve sustainable development, it is important to use biogas – a recyclable, carbon-neutral fuel. For this reason, it is necessary to give a preferential tax rate according to its use and implement a system that facilitates subsidies for equipment installation, for example.

**2.** Developing a comprehensive plan covering the entire surrounding area when constructing a biogas generation facility

To use biogas, a waste-derived fuel, at low cost, it is imperative that the raw material (waste) can be collected intensively and that local facilities can use the generated biogas. This means that it is necessary to develop a comprehensive plan covering all neighboring areas when constructing a biogas generation facility.

Based on the characteristics of the university, this research covers all processes ranging from the generation of waste and the production of biogas and BDF to their uses, so it may provide an excellent case study for developing a regional plan.

**3.** Eliminating restrictions to the use of biogas through fuel transfer, based on an adsorptive storage technology

Currently, the sewage plants in Osaka city use sludge digestion to dispose of sludge. For the effective use of the biogas generated there, electric power generation and many other applications are being considered and implemented.

On the other hand, when sewage plants and other facilities make effective use of biogas, that use is subject to many laws and regulations (e.g., High Pressure Gas Safety Act, Gas Business Act, and Building Standards Act), depending on the installation site, and therefore is restricted in some cases. If electric power is generated within the premises of a sewer plant, the generation efficiency is lower than that of large electric power generation

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facilities, and the location where the collected hot water should be used must be considered.

The biogas transfer system based on the adsorptive storage technique is an effective solution to these problems.

Examples of useful applications may include a sewer plant or a methane fermentation facility that cannot make effective use of biogas because it is located in a non-industrial area; biogas generated there can be transferred to a large electric power generation facility using an adsorptive storage tank installed in an ISO-defined container for use as a fuel. In this case, the use of biogas is not subject to the various laws and regulations and it is possible to safely transfer large volumes of biogas. Biogas has similar properties to natural gas and can be used at power plants and other facilities that use natural gas as fuel. Therefore, its use is expected to grow.

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Section 4

# Biodiesel Applications in Engines

Chapter 14

# Application of Biodiesel in Automotive Diesel Engines

Yanfei Li, Guohong Tian and Hongming Xu

Additional information is available at the end of the chapter

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## 1. Introduction

### 1.1. background

Diesel engines due to the better fuel economy have been widely used in automotive area. However, the limited reserve of fossil fuel and deteriorating environment have made scientists seek to alternative fuels for diesel while keeping the high efficiency of diesel engine. Fuel consumption is expected to increase from 86 million barrels per day to 112 million barrels per day by 2035 according to the report published by US Energy Information Administration in 2011 [1]. The limited reserve cannot afford this usage. Another challenge is environmental deterioration and climate change. Excessive emissions of carbon dioxide ($CO_2$) to the atmosphere are regarded as the leading cause of global warming. In addition, other emissions, such as $NO_x$, $SO_2$, also have a close relationship with other forms of climate change, such as photochemical smog and acid rain. Due to these, the regulations on fuel economy and emission limits are increasingly stringent. Table 1 shows the EU emissions regulations for passenger cars came into force since 1992.

| Tier | Date | | | | | HC+$NO_x$ | |
|---|---|---|---|---|---|---|---|
| Euro 1† | Jul-92 | 2.72 (3.16) | - | - | - | 0.97 (1.13) | 0.14 (0.18) |
| Euro 2 | Jan-96 | 1 | - | - | - | 0.7 | 0.08 |
| Euro 3 | Jan-00 | 0.64 | - | - | 0.5 | 0.56 | 0.05 |
| Euro 4 | Jan-05 | 0.5 | - | - | 0.25 | 0.3 | 0.025 |
| Euro 5 | Sep-09 | 0.5 | - | - | 0.18 | 0.23 | 0.005 |
| Euro 6 (future) | Sep-14 | 0.5 | - | - | 0.08 | 0.17 | 0.005 |

**Table 1.** European emissions regulations for passenger cars (Category M*), g/km

However, it is not a long-term solution even though these measures can help alleviate or reduce the emissions and extend the lifetime of fossil fuel in industry, because one day fossil fuel would run out if the fuel consumption is kept at nowadays' rate. In addition, the decrease in fossil fuel reserve would lead to the increase of oil price. The rising fuel price raised the cost-competiveness of other energy sources, such as wind energy and solar energy. Hence some sectors, such as industrial and buildings, are driven towards other substitute energy sources when possible, whereas in transportation sector, liquid fuel is still the preferred choice. Consequently, the transportation share of the total liquid fuels increases in the projected period, accounting for 80% of the total increase in liquid fuel production [1].

Therefore, the efforts have been made to seek the alternative for fossil fuel, especially after the energy crisis in 1970s. People are trying to find a sustainable way to power the engines.

### 1.2. Biodiesel

Among the alternatives for fossil diesel, biodiesel has been widely investigated due to its renewability, comparable properties to fossil diesel and the reduction in main emission products.

Biodiesel is mainly comprised of mono-alkyl esters of long chain fatty acids and it was defined in standard ASTM D6751. Normally feedstock such as vegetable oil and animal fat is used to produce biodiesel through transesterification method.

With the on-going development of biodiesel, the categorization of biodiesel is developed. Generally biodiesel can be categorised by the readiness of feedstock and produce technologies. The biodiesel made from vegetable oil and animal fats using transesterification method is normally recognised as first-generation biodiesel. The second-generation biodiesel, Biomass to liquid (BTL) fuel is to turn cellulose into fuel components (enzyme fermentation or gasification through Fischer-Tropsch synthesis), and the feedstock theoretically can be any bio mass such as waste agriculture, wood chips etc. Some biodiesel from jatropha, algae, etc., despite being produced by transesterification method, is widely regarded as second-generation due to the technical challenge of feedstock planting and harvesting. Normally the second-generation biodiesel can supplement the drawbacks of the first-generation biodiesel particularly being non-competitive with food.

Different from above definition, another new fuel, Hydro-treated vegetable oil (HVO), using the same feedstock as 1st generation biodiesel, is viewed as second generation biodiesel, and BTL is third-generation [17]. The authors still categorize it into second-generation biodiesel because HVO shares the same feedstock with first-generation biodiesel even though it is made through different way and has better quality than first-generation biodiesel through transesterification.

#### *1.2.1. History*

Vegetable oil has been used in diesel engine long time ago. In 1900 after the invention of diesel engine,), Dr. Diesel used peanut oil to run one of his engines at the Paris Exposition of 1900. Vegetable oils were used in diesel engines until 1920s. The recent use of vegetable oil

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as the alternative for diesel starts from early 1980s due to the concern about the energy supply. But biodiesel is not commercialised until late 1990s.For the direct use of vegetable oil, several difficulties occur, including the high viscosity, acid composition, free fatty acid content, and gum formation due to oxidation and polymerization during storage and combustion, carbon deposition, and oil thickening (Ma and Hana, 1999). Therefore, the direct use of vegetable oil may not be satisfactory and practical. The technologies to improve the vegetable oil appeared.

*1.2.2. Production process*

There are several ways used to produce FAME through vegetable oil, pyrolysis, cracking and transesterification. The common method is transesterification. Figure 1 shows the chemical reaction of FAME production. Triglycerides, the main component in vegetable oil and animal fat, reacts with alcohol in a caustic environment and produce Fatty Acid Methyl Ester (FAME) or Fatty Acid Ethyl Ester (FAEE) and the byproduct glycerol. As a result, biodiesel is a mixture of esters, small amount of glycerol, free fatty acids, partially reacted acylglycerol, and residual raw materials. Normally methanol is used for the reaction for the higher reaction rate and lower price. The fuel qualities may be varied in terms of alcohol used. Methyl ester was better than ethyl ester from the point of engine performance: higher power and torque could be achieved.

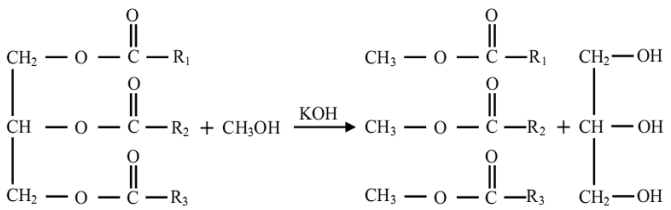


**Figure 1.** Transesterification reaction in caustic environment

Different from transesterification, Hydro-treating of vegetable oil or animal fat (HVO) have been developed by several companies, such as Neste Oil, Axens IFP, and Honeywell UOP. In the hydro-treating process, vegetable oil or animal fat is also the feedstock. Hydrogen is added into the plant to remove the oxygen content and saturate the C=C and the final products are paraffin, propane, water and $CO_2$. Propane is also a promising and valuable fuel product. Due to the excellent properties,

$$\text{Triglycerides} + H_2 \xrightarrow[\text{Catalyst}]{====>} \text{Green diesel} + H_2O/CO_2 + \text{Propane}$$

**Figure 2.** Product routes of transesterification and hydro-treated method

Fischer-Tropsch (FT) method is another way to produce synthetic fuel, using various lignocellulosic feedstock. BTL (biomass-mass-to-liquid) fuel, GTL (Gas-to-liquid) fuel, and CTL (Coal-to-liquid) fuel are produced with this method. GTL and BTL are not sustainable fuels for natural gas and coal are not renewable. However, in this chapter GTL is included later because it shares the similar production process and has similar physiochemical properties with BTL. Figure 3 shows the manufacturing process of FT synthetic fuel. The solid feedstock (coal and biomass ) are initially gasified, then the composition of the syngas and $CO_2$ and sulphur compounds are removed before the synthesis process. After the synthesis process, the products are refined and the refined products includes the synthetic diesel and gasoline blendstock.

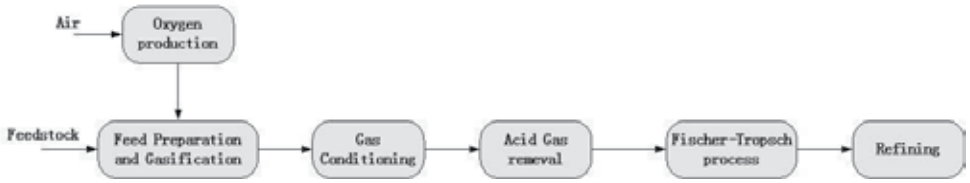


**Figure 3.** FT fuel manufacturing process

### 1.3. Biodiesel standards

Due to the difference in the feedstock and manufacturing process, the FAME products may vary very much. Table 2 lists several main standards used in the world, aiming at reach the satisfaction and the equipment compatibility.

| Bio-Diesel | Unit | Austrian Standard C1190 Feb. 91 | Australian Bio-diesel Standard | DIN 51606 (1997/9/1) | U.S. Quality Specification NBB/ASTM | Euro Standard EN 14214 |
|---|---|---|---|---|---|---|
| Density at 15°C | g/cm3 | 0.86 - 0.90 | 0.86 – 0.89 | 0.875 - 0.90 | / | 0.86 - 0.90 |
| Viscosity at 40°C | mm2/s | 6.5 - 9.0 (20°C) | 3.5 – 5.0 | 3.5 - 5.0 | 1.9 - 6.0 | 3.50 - 5.00 |
| Flash point | °C | Min. 55 | 120.0°C | Min. 110 | Min. 100 | Min. 120 |
| | (°F) | -131 | | -230 | -212 | -248 |
| CFPP | °C (°F) | | | | | |
| | summer | Max. 0 (32) | / | Max. 0 (32) | / | |
| | winter | Max. -8 (17.6) | | Max. -20 (-4) | | |
| Total sulphur | mg/kg | Max. 200 | Max. 50 mg/kg | Max. 100 | Max. 500 | Max. 10.0 |
| Conradson (CCR) at 100% at 10% | % mass | Max. 0.1 | Max 0.05 | Max. 0.05 | Max. 0.05 | / |
| | | / | Max 0.30 | / | / | Max. 0.30 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| Cetane number | - | Min. 48 | Min. 51 | Min. 49 | Min. 40 | Min. 51 |
| Sulfated ash content | % mass | Max. 0.02 | Max. 0.02 | Max. 0.03 | Max. 0.02 | Max. 0.02 |
| Water content | mg/kg | free of deposited water | | Max. 300 | / | Max. 500 |
| Water & sediment | vol. % | / | Max. 0.05 | / | Max. 0.05 | / |
| Total contamination | mg/kg | / | Max. 24 | Max. 20 | / | Max. 24 |
| Copper corrosion ( 3 hs, 50°C) | degree of Corrosion | / | < 10mg/kg sulphur – 1 "/ 10mg/kg sulphur – 3 max | 1 | No. 3b max. | 1 |
| Neutralisation value | mg | Max. 1 | / | Max. 0.5 | Max. 0.8 | Max. 0.50 |
| Oxidation stability | h | / | Min 6 @ 110°C | / | / | Min. 6.0 |
| Methanol content | % mass | Max. 0.30 | 0.2 | Max. 0.3 | Max. 0.2 | Max. 0.20 |
| Ester content | % mass | / | Min 96.5 | / | / | Min 96.5 |
| Monoglycerides | % mass | / | / | Max. 0.8 | / | Max. 0.80 |
| Diglycerides | % mass | / | / | Max. 0.4 | / | Max. 0.20 |
| Triglycerides | % mass | / | / | Max. 0.4 | / | Max. 0.20 |
| Free glycerine | % mass | Max. 0.03 | Max. 0.02 | Max. 0.02 | Max. 0.02 | Max. 0.02 |
| Total glycerine | % mass | Max. 0.25 | Max. 0.25 | Max. 0.25 | Max. 0.24 | Max. 0.25 |
| Iodine value | | / | / | Max. 115 | / | Max. 120 |
| Linolenic acid ME | % mass | / | / | / | / | Max. 12.0 |
| Polyunsaturated ("/ =4db) | % mass | / | / | / | / | Max. 1 |
| Phosphorus content | mg/kg | / | Max. 10 | Max. 10 | / | Max. 10.0 |
| Alkaline content (Na +K) | mg/kg | / | / | Max. 5 | / | Max. 5.0 |
| Alkaline earth metals (Ca + Mg) | mg/kg | / | / | / | / | Max. 5.0 |

**Table 2.** Biodiesel Standards

### 1.4. Pros and Cons

The following summarised the advantages of biodiesel:

- Renewable energy source in comparison with traditional fossil fuel
- Degradability
- Much less sulphur, leading to lower toxic substances in the exhaust
- Absence of PAHs and around 10% of oxygen help the reduction of HC and CO
- Various feedstock

The use of bio-diesel fuels cannot occur without adopting a series of precautions. Indeed, unless the proper precautions are taken, biodiesel fuels can cause a variety of engine performance problems including filter plugging, injector coking, piston ring sticking and breaking, seal swelling and hardening/cracking and severe lubricant degradation. Bio-diesel also requires special treatment at low temperatures to avoid an excessive rise in viscosity and loss of fluidity.

Long-term storage problems can be observed as result of the poor oxidation stability of biodiesel fuels. Thus additives may be needed to improve storage conditions. Furthermore, biodiesel is an excellent medium for microbial growth. As water accelerates microbial growth and is more prevalent in biodiesel than in petroleum based fuels, special care must be taken to remove water from fuel storage tanks to avoid operational problems such as sediment build-up, premature filter plugging or storage tank corrosion.

### 1.5. Security of supply

Another reason for the search of alternative fuel is the energy security. The economical growth can promote the demand for energy. Table 3 listed crude oil reliance on imported oil of US and China. The reliance of the two countries are up to 44.8% and 56.54%, respectively. The energy supply can be well alleviated if biodiesel can be produced and used in commercial scale. Further analyses are needed to understand the fuel difference and can help fuel design during the biodiesel production process. The Commission Green Paper (CEC, 2000) reported an ambitious EU programme on the usage of biodiesel that 20% alternative fuel substitution by 2020 in conventional fuel in the road transport sector is set. On another hand, the utilisation of biodiesel leads to concerns of land use, deforestation and negative effect on bio-diversity needs further exploration.

| | Year 2007 | Year 2008 | Year 2009 | Year 2010 | Year 2011 |
|---|---|---|---|---|---|
| US[1] | 58.2% | 57.0% | 51.5% | 49.2% | 44.8% |
| China | 47.2% | 49.8% | 52% | 54.8% | 56.5% |

**Table 3.** [1] US Department of Energy, Energy Information Administration, Monthly Energy Review, Washington, DC, March 2012, Table 3.3aCrude oil reliance of US and China from 2007 - 2011

## 2. Fuel properties

### 2.1. Fuel composition

Due to the various feedstocks for biodiesel, the fuel composition varies in a wide range. Generally the fats and oils contain 10 common types of fatty acid consisting of 12- to 22-carbon chain, and over 90% are between 16- and 18-carbon chains [11]. Table 4 shows the composition of some common FAME. Some of these are saturated, some are monounsaturated and others are poly-unsaturated. The composition of biodiesel determined the chemical and physical properties, such as the fuel viscosity, surface tension, cetane number (CN),

oxidation stability, low-temperature properties, as well as the following combustion and emission characteristics.

### 2.2. Viscosity

Viscosity is a measure of resistance to flow of a liquid due to internal friction and it is one of the most important parameters in evaluate the fuel quality. Viscosity affects engine working process very much. Higher viscosity would prohibit atomisation and instability of fuel droplets, and promote the formation of deposit. This also explains why neat vegetable oils have difficulty when used in diesel engines directly. The viscosity can be measured according to the standards such as ASTM D445 or ISO 3104. The viscosity of individual saturated fatty acid ester increases with carbon chain length and non-linearly decreases with the increase of number of double bonds [4]. In addition, the position of C=C double bond and the branching in the ester moiety has less effect on viscosity. Biodiesel has a higher viscosity than fossil diesel. At lower blend ratio, the viscosities of diesel and biodiesel/diesel blend are very close. As the blend ratio continues to increase, biodiesels show a much higher value. This can partly explain why biodiesel/diesel blends with lower blend ratio are widely used in diesel engines.

| | | Rapeseed (high erucic) | Rapeseed (low erucic) | Soybean | Sunflower | Coconut oil | Palm kernel oil | Palm oil (Africa) | Palm oil (Indonesia) | Beef tallow | Chicken fat | Fish oil |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Saturated fatty acids | < C10:0 | | | | | ~13 | ~7 | | | | | trace |
| | C12:0 | trace | trace | | trace | 41-46 | 41-45 | trace | trace-0.5 | trace | | trace |
| | C14:0 | trace | trace | <0.5 | trace-0.1 | 18-21 | 15-17 | 1-2 | ~1 | 2-4 | ~1 | 6-9 |
| | C16:0 | 2-4 | 3-6 | 8-12 | 5.5-8 | 9-12 | 7-10 | 41-46 | 41-47 | 23-29 | 20-24 | 11-20 |
| | C18:0 | 1-2 | 1-2.5 | 3-5 | 2.5-6.5 | 2-4 | 2-3 | 4-6.5 | 4-6 | 20-35 | 4-7 | 1-4 |
| | C20:0 | 0.5-1 | <1 | <0.5 | <0.5 | trace | trace-0.3 | ~0.5 | ~0.5 | <0.5 | | trace-1 |
| | C22:0 | 0.5-2.0 | trace-0.5 | trace | 0.5-1.0 | trace | trace-0.5 | | | trace | | trace |
| | C24:0 | 0.5 | trace-0.2 | | <0.5 | | | | | | | trace-1 |
| Unsaturated fatty acids | C14:1 | | | | | trace | | | | ~0.5 | | trace |
| | C16:1 | ~0.5 | 0.1-0.5 | trace | <0.5 | trace | | <0.5 | ~0.5 | 2-4 | ~7 | 6-11 |
| | C18:1 | 11-24 | 52-66 | 18-25 | 14-34 | 5-9 | 10-18 | 37-42 | 37-41 | 26-45 | 38-44 | 12-15 |
| | C18:2 | 10-22 | 17-25 | 49-57 | 55-73 | 0.5-3 | 1-3 | 8-12 | ~10 | 2-6 | 18-23 | 1-2 |
| | C18:3 | 7-13 | 8-11 | 6-11 | trace-0.4 | trace | trace-0.5 | trace-0.5 | trace-0.5 | ~1 | ~1 | 0.5-1 |
| | C20:1 | ~10 | 1.5-3.5 | <0.5 | <0.5 | trace | trace-0.5 | trace | trace | <0.5 | | 1-16 |
| | C20:x* | | trace-0.1 | | | | | | | trace | | 6-19 |
| | C22:1 | 41-52 | trace-2.5 | | trace-0.3 | | | | | | | 1-20 |
| | C22:x* | | | | | | | | | ~0.5 | 0.5-1 | 5-14 |
| | C24:1 | | trace | | | | | | | | | trace-1 |
| Other constituents | Phosphatides | 2.5 | | 1.1-3.2 | <1.5 | | | 0.05-0.1 | | <0.07 | | |
| | Tocopherols | 0.07-0.08 | | 0.09-0.12 | 0.07-0.1 | 0.003 | | 0.02-0.12 | | 0.001 | | |
| | Sterols | 0.5-1.1 | | 0.2-0.4 | 0.25-0.45 | 0.05-0.1 | 0.08-0.12 | 0.04-0.08 | | 0.08-0.14 | | ~0.3 |

* x > 1
trace ≤ 0.05%

http://www.dieselnet.com/tech/fuel_biodiesel_app.html

**Table 4.** Composition of common FAME.

### 2.3. Cetane number (CN)

CN is used to evaluate fuel ignition quality determined by the time between start of injection and start of combustion. Higher CN indicates shorter time after the injection. CN is mainly determined by the fuel composition and can affect engine startability, noise and

emission characteristics. Generally, biodiesel has a higher CN than mineral diesel. This can be attributed to the longer carbon chain length of biodiesel. Unsaturation and carbon chain length are the most two influential factors of CN [16, 12, 22]. Higher saturation degree and longer fatty acid chain length can lead to a lower CN. The positions of chemical group may also influence the CN. The CNis the highest when the carbonyl group is at the end of the carbon chain and lowest in the middle of the carbon chain. In addition, a higher level of hydroperoxides increases CN and a shorter chain length of the alcohol moiety may also increase CN [12, 22].

### 2.4. Low-temperature property

Diesel engines may encounter the start-up and performance problems at low temperatures. As ambient temperatures decrease towards the fuel saturation temperature, high-molecular-weight compound begin to nucleate and form was crystals. The existence of was crystals may affect the fuel supply and engine performance. Three parameters, cloud point (CP), pour point(PP), and cold filter plugging point (CFPP) are used to describe low-temperature properties. The temperature at which crystals become visible is called CP because the crystals lead to a cloudy suspension. if the temperature continues to decrease, the crystals would fuse together and form larger agglomerates. The temperature at which crystal agglomeration is large enough to prevent free pouring of fluid is called PP. A more complicated test procedure is involved in order to obtain CFPP. The test uses a vacuum to draw a 20 cc fuel sample through a 45 micron screen within a 60 seconds. The lowest temperature that the fuel can still flow through the filter is called CFPP.

This is an very concerning issue in application of biodiesel into diesel engines. Neat biodiesel has poorer low-temperature performance than conventional diesel. Therefore, when biodiesel is used in cold condition, the biodiesel crystafdl may block the fuel pipe and the fuel filter, and even abrade the high-pressure fuel pump, shorting the lifetime of vehicle engines. Research has shown that the cold flow property is associated with the saturated FAME in vegetable oil based biodiesel. The higher the proportion of saturated FAME and the longer chain FAME in saturated FAME, the poorer the cold temperature performance is [31].

Generally, the low-temperature properties can be improved by following methods:

- Blending with fossil diesel
- blending with additives
- Crystallization fractionation by decreasing the saturated alkyl ester content in the biodiesel.
- Employing branched esters

## 3. Application in Diesel Engines

The engine performance fuelled with biodiesel is crucial for the application of biodiesel. The mainly involved problems may include corrosion, material degradation, injector coking, fil-

ter plugging and piston ring sticking, engine deposits etc. therefore, in the following section, the studies focusing on these issues were introduced.

### 3.1. Fuel spray characteristics

Injection spray is the process that fuel is injected from nozzle, and it is associated with following fuel atomisation, interaction with surrounding gas, mixture formation and combustion. Regarding to a new fuel applied into the diesel engine, the spray process is different due to the different properties from diesel, and the control strategy should be changed accordingly in order to achieve the optimum performance. Viscosity, surface tension and density are the three main parameters, which influence fuel spray characteristics. Higher viscosity and surface tension will prohibit the atomisation and instability of fuel droplets. Due to the different biodiesels properties from diesel, studies on the spray characteristics are necessary.

#### *3.1.1. Near-field spray characteristics*

In the near-field of nozzle, the spray is dominated by the injection dynamics while the spray is affected by the ambient conditions in the far field. According to Hiroyasu's model, before the $t_{breakup}$, which represents the time for fuel jet breakup, the penetration length is proportional to the time after start of injection, namely ASOI. However, the non-linear phenomenon has been observed by a number of researchers. The acceleration process has been found to be different among fuels. Figure 4 compares the morphology of the spray process of the three tested fuels, ULSD, RME and GTL and Figure 5 shows the spray tip penetration length evolution after start of injection (ASOI) using an ultrahigh-speed CCD camera of up to 1 million shots per second. The initial non-linear penetration can be observed, indicating the acceleration period at the initial spray stage. GTL fuel has longer penetrating length than RME and diesel even though it was overtaken by RME 70 μs ASOI. Several publications have reported that GTL with lower density has a shorter penetration delay. However, these were based on the global fuel spray characteristics using a relatively low speed camera [21, 13]. The temporal resolution is not high enough to capture the near-field spray process.

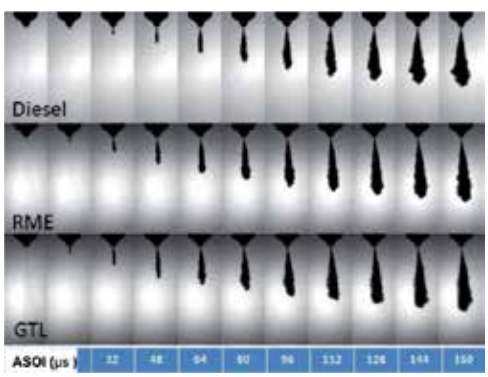



**Figure 4.** Sequence of spray images in a single time-resolved ULSD spray (Pinj=120 MPa, $P_{amb}$ =3.0 MPa and $t_{dur}$=1.5 ms)

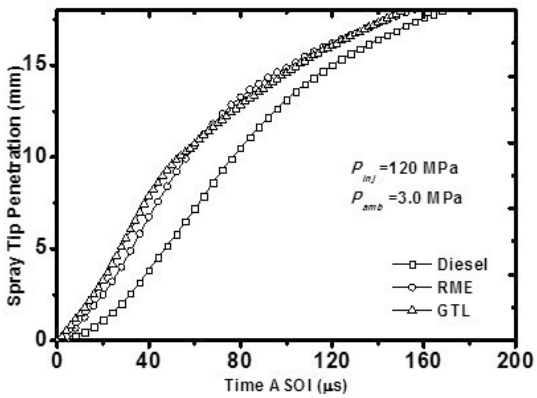



**Figure 5.** Spray tip penetration length evolution against time ASOI ($P_{inj}$=120 MPa, $P_{amb}$ =3.0 MPa and $t_{dur}$=1.5 ms)

*3.1.2. Macroscopic spray characteristics*

Normally, biodiesel shows a longer penetration and narrower spray angle than fossil fuel due to the higher viscosity, surface tension and density. The penetration length of biodiesel increases with the blend ratio, higher biodiesel content requires longer breakup time [15]. The difference between the two type fuels can be varied at different conditions. Senatore et al. [24] experimentally studied biodiesel spray characteristics at different ambient pressures. The authors showed that little difference can be observed at the ambient pressure of 1.2 MPa while the penetration length significantly increased in contrast to diesel spray at the ambient pressure of 5.0 MPa. In addition, biodiesel may have a lower penetration velocity due to the negative effect of fuel density on spray velocity [9].

*3.1.3. Sauter Mean diameter (SMD)*

SMD is one of the parameters to evaluate fuel atomisation quality and represents the ratio of total droplet volume to surface area. Smaller SMD indicates more small fuel droplets and the larger contact area with surrounding gas. Due to the high viscosity and surface tension, SMD of biodiesel is higher than fossil diesel. Allen et al. [3] conducted the comparative analysis on 15 biodiesels and a larger SMD, between 5%-40%, can be observed and concluded an empirical equation to estimate SMD:

$$SMD = 0.002103\mu + 0.000330\sigma \quad (1)$$

where μis fuel dynamic viscosity (Pa.s) and σis fuel surface tension (N/m).

Figure 6 compared diesel with neat RME and GTL at different injection pressure along the spray axis in terms of SMD. It can be seen that the injection pressure has a significant impact on droplet size. The SMD decreases dramatically when the injection pressure increases from 80 MPa to 120 MPa. GTL has the lowest SMD among all the three measured fuels at the giv-

http://dx.doi.org/10.5772/ 53222

en conditions while RME has the largest droplet size. The SMD evolution also decreases with the increase of the axial distance downstream of the nozzle even though there is a slightly increase from 40 mm to 50 mm at the 80 MPa condition. This may be caused by the droplet coalesce.

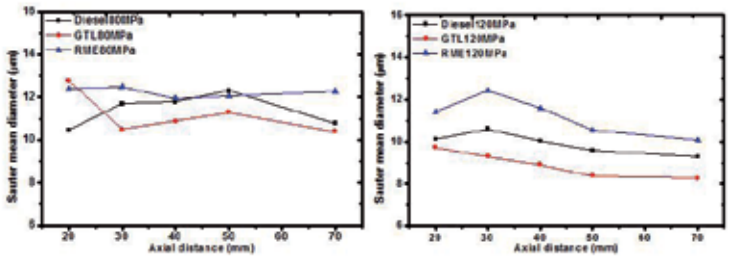



**Figure 6.** SMD distribution along the spray axis under injection pressure of 80 MPa (Left) and 120 MPa (Right)

*3.1.4. Wear Performance and Durance*

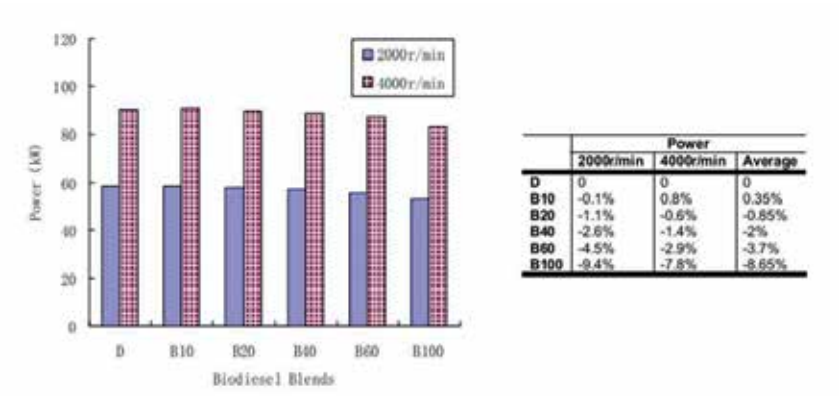
In diesel engines, the engine parts are lubricated by the fuel itself. In order to meet diesel engine emission standards, Ultra-low sulphur diesel (ULSD) are produced, which has a maximum sulphur content of 15 ppm. However, the relatively poor lubricity of ULSD may lead to the failure of engine parts, such as fuel pumps and injectors. The inherently greater lubricity of Biodiesel can offset the drawback of ULSD, and a small percentage of biodiesel can restore the lubricity of diesel [28].

It is also necessary to study the engine endurance in order to fully apply biodiesel into vehicle operation. Graboski et al. [14] reviewed previous studies and concluded that nitrile rubber, Nylon 6/6 and high-density polypropylene exposed to methyl soyester and D-2 blends exhibited changes in physical properties and fluorinated elastomer must be adopted for biodiesel application. Terry et al. [30] examined the durability of a set of five commonly used elastomers in automotive fuel systems in different biodiesel blends (B5 and B20) and the effect of a highly oxidized biodiesel blends on the elastomers was studied. The results demonstrated that it appeared to be compatible with these elastomers, for highly oxidized and unoxidized B5 and unoxidized B20, but B20 prepared from highly oxidized biodiesel shows the potential for significant problems.

### 3.2. Engine Output performance

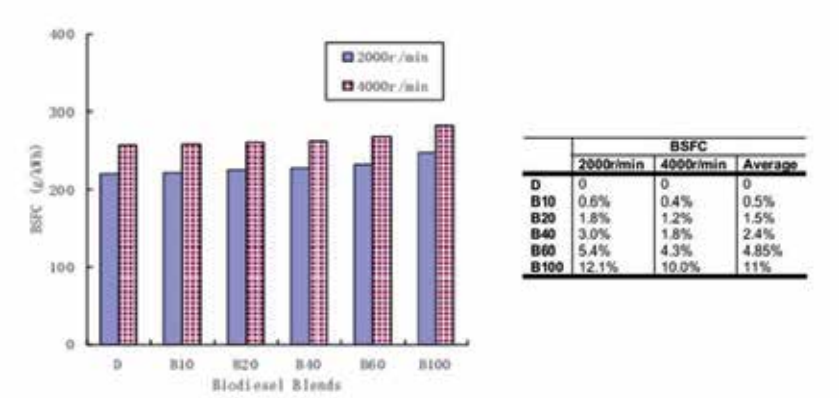
The adaptability of biodiesel in diesel engines has been well studied from low blend ratio to neat biodiesel. Due to the potential damage of biodiesel on vehicle, normally biodiesel blended with diesel were mostly studied. In general, typical heating value for biodiesel is lower than that of fossil diesel. A greater amount of fuel is subsequently required to maintain the same engine output. Greater fuel consumption of up to 13% with heavy-duty engines over the United States Federal Test Procedure (US-FTP) cycle was observed. Due to

the lower heating value, engine power loss is expected and the loss increases with the blend ratio of biodiesel in diesel [25, 33]. Figure 7 shows the output power of an 4-cylinder common-rail diesel engine with different biodiesel blends at two engine speeds. With the increase of biodiesel blend concentration, maximum out power was gradually reduced, especially in the higher blend ratio. Figure 8 presents the brake specific fuel consumption (BSFC) corresponding to the condition of Figure 7. The obvious increase in fuel consumption has been observed using higher biodiesel/diesel blends. From Figure 7 and Figure 8, it can be found that the output performance and fuel economy of biodiesel/diesel blends are very close to those of diesel when the blend ratio is under 20%. Therefore, biodiesel/diesel blends with lower blend ratio are preferred.




| | Power | | |
|---|---|---|---|
| | 2000r/min | 4000r/min | Average |
| D | 0 | 0 | 0 |
| B10 | -0.1% | 0.8% | 0.35% |
| B20 | -1.1% | -0.6% | -0.85% |
| B40 | -2.6% | -1.4% | -2% |
| B60 | -4.5% | -2.9% | -3.7% |
| B100 | -9.4% | -7.8% | -8.65% |

**Figure 7.** Output power of different biodiesel blends at two speeds [33]




| | BSFC | | |
|---|---|---|---|
| | 2000r/min | 4000r/min | Average |
| D | 0 | 0 | 0 |
| B10 | 0.6% | 0.4% | 0.5% |
| B20 | 1.8% | 1.2% | 1.5% |
| B40 | 3.0% | 1.8% | 2.4% |
| B60 | 5.4% | 4.3% | 4.85% |
| B100 | 12.1% | 10.0% | 11% |

**Figure 8.** BSFC of different biodiesel blends at two speeds [33]

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### 3.3. Combustion characteristics

In diesel engines, combustion is to release energy contained in fuel, then impart work on piston, and power the engine. Factors affecting combustion characteristics include fuel properties and in-cylinder conditions. Biodiesel has a higher CN and the effect of CN on combustion has been discussed in previous section. The average peak cylinder pressure increases when biodiesel or its blends are used. For the application of biodiesel into diesel engines, advanced injection timing and increased injection pressure have been normally used. This is due to their differences in density and bulk modulus of compressibility. Combustion and emissions characteristics have been investigated by Chuepeng et al. [7] using different RME blends from B0 to B50 in a single-cylinder diesel engine in terms of engine load, EGR (Exhaust gas recirculation), and injection timing. At the same engine load, the proportion of fuel burnt in the premixed phase increases and the start of combustion is advanced as the proportion of RME in ULSD increases. With the same operating conditions, increase in EGR rate of up to 20%, slightly reduces the peak pressure and increases ignition delay.

### 3.4. Emissions

#### *3.4.1. Regulated emissions*

A number of studies on the engine emissions of engines powered by biodiesel or blends have been carried out. Environmental Protection Agency (EPA) in the United States correlated the biodiesel ratio with the changes in pollutants using statistical regression analysis and also the average effect of biodiesel on heavy-duty diesel engines [10]. The $NO_x$ emissions increased with the concentration of biodiesel and the increase is by 10% at B100 while HC, CO and PM were greatly reduced. The significant reduction of emissions of HC, CO and PM can be attributed to the oxygen content in biodiesel.

It has been widely reported that $NO_x$ increases as biodiesel is used in diesel engines. A number of efforts have been made in order to understand the formation mechanism and eliminate this penalty. There are several main reasons have been suggested:

- Advanced injection timing
- Oxygen content in biodiesel
- Double bond
- Radiative heat transfer
- Higher adiabatic flame temperature

An advanced injection timing due to the higher bulk modulus of biodiesel in pump-in-line injection system leads to the earlier start of combustion, resulting in higher in-cylinder temperature, which can increase $NO_x$ emission [29, 2]. However, it is well understood that advanced injection timing increases $NO_x$ emission in diesel engine [20], and this seems not to be the main contribution to $NO_x$ increase as broad application of common rail injection system, which can well control the injection timing. Schmidt et al. [23] experimentally studied

the effect of concentration of oxygen in intake gas on $NO_x$ emission, and found that $NO_x$ emission increases with the oxygen content in mixture. However, the effect of oxygen content in air on combustion is different from that of oxygen content in biodiesel itself. The radiative heat transfer may also play a role in the $NO_x$ increase. Soot radiation is the primary way of heat loss from in-cylinder flame and biodiesel can reduce this heat loss and will increase the flame temperature and produce more $NO_x$ [6]. The double bond in biodiesel composition is another potential to increase $NO_x$ emission. The double bonds lead to higher adiabatic flame temperature, and the biodiesel with higher unsaturated ester percentage corresponded to higher $NO_x$ emission [27, 19]. Ban-Weiss et al. [5] also revealed that slight difference in the adiabatic flame temperature can lead to a measurable increase in $NO_x$. Mueller et al. [20] suggested that $NO_x$ increase in biodiesel-fuelled engine is the result of a number of mechanisms, and the relative importance of each mechanism may vary under different operating conditions and indicated that air/fuel mixture close to stoichiometric at ignition and in the standing premixed auto-ignition zone near flame lift-off length may be the key factors in explaining the $NO_x$ increase, whose effect could cause higher local and average in-cylinder temperature and lower radiative heat losses.

Therefore, three main strategies to alleviate the $NO_x$ emission can be proposed: one is to determine the biodiesel compound that can lower $NO_x$ emission or use a proper base fuel and additives, another is to design the combustion system to prohibit $NO_x$ production by lower the combustion temperature, and the third one is to recalibrate the engine by tuning the injection strategy.

### *3.4.2. Unregulated emissions*

For other unregulated emissions from an engine fuelled with biodiesel, polycyclic aromatic hydrocarbon (PAH) and nitro PAH compounds are substantially reduced, as well as the lower levels of some toxic and reactive HC species [26]. The PM composition (i.e. volatile material and elemental carbon) from the combustion of RME-based biodiesel blend (B30) in a turbo-charged engine with EGR operation was studied using thermo-gravimetric analysis (TGA) [8]. Generally, total PM mass from B30 combustion was lower than that for diesel in all engine operating conditions. Elemental carbon PM mass fractions were slightly lower for the B30. The volatile material portions of the B30 particulates are greater than those of diesel particulates irrespective of engine operating condition. For both fuels used in the test, volatile material was observed to be higher at idle speed and light load when exhaust emissions were at low temperature.

In previous regulations on PM, mass is the only concern. With the increasing concern on exhaust emissions, the PM size and number are to be limited by future emission regulations. [32] studied the particulate matter characteristics of RME10 and GTL10. It was found that the application of RME10 and GTL10 leads to a reduction in both total particle number and non-volatile part number over the test conditions. The obtained images from SEM (Scanned Electronic Microscopy) for the three test fuels are shown in Figure 9. The images show the morphology of PM at two magnifications. The authors found that PM from diesel combustion has more clusters than those from RME10 and GTL10 from Figure 10 (a), (c) and (e), indicating that primary particle size of the tested fuels is around 20 mm Figure 10. (b), (d) and (f).

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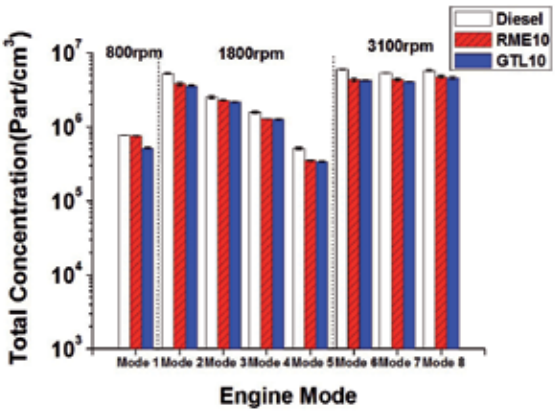


**Figure 9.** Exhaust particulate number concentration (total)

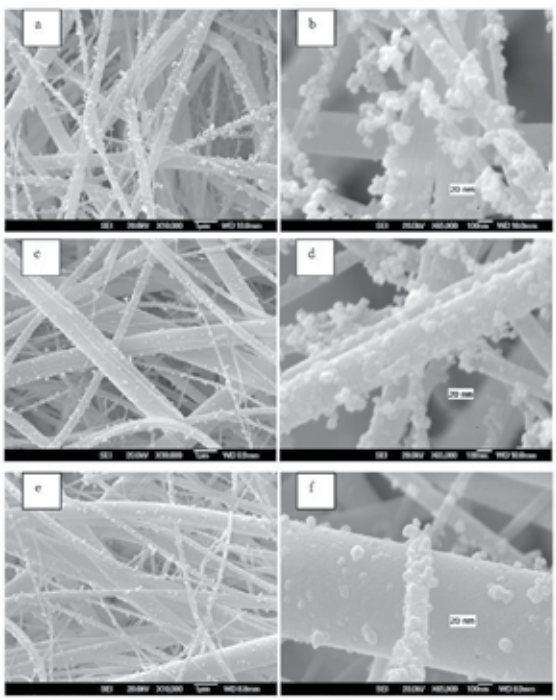


**Figure 10.** Particle morphology (captured under engine mode of 1800 rpm, 30 Nm): (a) Diesel magnification of 10000; (b) Diesel magnification of 65000; (c) RME 10 magnification of 10000; (d) RME magnification of 65000; (e) GTL10 magnification of 10000; (f) GTL10 magnification of 65000

### 3.5. Engine emission optimisation

Two popular methods have been used to reduce the engine out emission for biodiesel-fuelled engines: injection strategy and EGR. For the former, the combustion process can be controlled by injection timing and injection pressure. For the time being, the common rail injection system has been widely used and multiple injections up to of 5 times can be realised. Through this way, the fuel injection rate is controllable. The $NO_x$ can be reduced through pre-injection with small amount fuel; this prevents a long period of ignition delay and therefore leads to a lower peak pressure; for the latter, EGR is always an effective way to reduce $NO_x$ emission. Due to the induction of exhaust gas, the global in-cylinder temperature is reduced, avoiding the thermal conditions favoured by $NO_x$ formation. Ladommatos et al. [18] also revealed that the reduction in combustion temperature is a consequence of the reduced peak rate of the premixed phase combustion due to the lower oxygen availability.

## 4. Conclusions

Biodiesel is the most promising fuel in the near future as an alternative to fossil diesel. Despite of its advantages, it still has some disadvantages such as source for massive feedstock, relatively poor low-temperature properties, increase in $NO_x$ emissions, etc. These issues should be sorted out before biodiesel is applied into diesel engines in a large scale. Therefore, in-depth studies on the application of biodiesel into diesel engines are necessary. The research on alternative feedstocks is also an important area and the second-generation biodiesel is more promising made from algae and the genetic modification is a potential way to solve this problem of source of massive feedstock. The low-temperature fuel properties can be improved by additives or the production routine. In addition, diesel engines should also be optimised in order to achieve the optimal performance and emissions.

*Abbreviations:*

| | |
|---|---|
| ASOI | After start of injection |
| BTL | Biomass-to-liquid |
| BSFC | Brake specific fuel consumption |
| CCD | Charge-coupled device |
| CN | Cetane number |
| CO | Carbon monoxide |
| CTL | Coal-to-liquid |
| EPA | Environmental Protection Agency |
| FAME | Fatty acid methyl ester |
| GTL | Gas-to-liquid |

| | |
|---|---|
| HC | Hydrocarbon |
| HVO | Hydro-treated vegetable oil |
| NOx | Nitric oxide |
| PAH | Polycyclic aromatic hydrocarbon |
| RME | Rapeseed methyl ester |
| ULSD | Ultra-low sulphur diesel |

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Chapter 15

# Simulation of Biofuels Combustion in Diesel Engines

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Additional information is available at the end of the chapter

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## 1. Introduction

In the study of the working process, the development of new engine construction or modernization of an existing one is necessary to use simulation with mathematical models. Modeling of the processes inside the cylinder allows in a first approximation to evaluate engine performance, choose the rational value of adjustment or constructive parameter, to reduce material, labor and time required to conduct experimental research.

One of the most difficult process for simulation is the combustion process in diesel engines. This process is determined and accompanied by a number of other processes and phenomena. There is intense interaction between the motion of the fuel jets and air flow in the cylinder, heat transfer between the combustion chamber zones and walls, volume evaporation from the surface of liquid droplets. All this leads to the formation of the active nucleus of the fuel oxidation and its ignition, volumetric and then the diffusion combustion.

Currently, there are a number of hypotheses about the behavior of each of these processes and their interaction. For each hypothesis proposed mathematical description of a different degree of accuracy.

The most complex model implemented technology of Computational Fluid Dynamic (CFD) - three-dimensional simulation of gas flow and the injected fuel in the cylinders and manifolds of internal combustion engines [1-5]. The most popular programs are: KIVA (Los Alamos National Laboratory, Los Alamos, New Mexico); STAR-CD (CD-adapco, headquarter Melville, New York, USA); FIRE (AVL, headquarter in Gratz, Austria); VECTIS (Ricardo, headquarter Shoreham-by-Sea, England, United Kingdom).

For example, the software package AVL FIRE Engine includes over 20 different models of formation and spread of the jet, its decay, crushing drops, collisions between them, the

evaporation of fuel and its interaction with the wall of the combustion chamber [6]. The formation of liquid films, their distribution and evaporation, the interaction with the walls and the liquid fuel torches are also simulated. Several models describe the processes of ignition, combustion and the formation of harmful substances, taking into account detailed chemical kinetics of reacting systems.

A significant technical challenge of CFD models is the complexity of calculations and the need for powerful computers. Data preparation only for one simulation with highly skilled personal could takes a few days. Calculation time for one variant of the engine - a few hours and sometimes days. Implementation of these programs for optimization calculations is problematic because optimization process has to count thousands of design options.

Thermodynamic and phenomenological models that use the 0 - or 1-dimensional representations, require less time and resources. The most popular programs were GT-Power (Gamma Technologies, Inc, headquarter Westmont, Illinois, USA), BOOST (AVL,Gratz, Austria), WAVE (Ricardo, Shoreham-by-Sea, England, United Kingdom), DIESEL RK (Moscow State Technical University named after Bauman, Moscow, Russian Federation). These software products usually include a one-dimensional model of gas exchange. To calculate the mixing and combustion in a diesel engine used empirical or semi-empirical models [7-11].

The most sophisticated models of combustion used in thermodynamic models are models of H. Hiroyasu [9], as well as Razleytsev N.F. and Kuleshov A.S. models[7, 8]. In these models, the propagation of fuel jet is described by the criterial equations obtained on the basis of experimental data. It has been assumed in this modelsthat the main influence on the rate of heat generation rate has drops evaporation rate and the speed of the air penetrated in the combustion zone. Also, the effects of air swirl on the development of fuel sprays is considered. In models of mixing, combustion and evaporation using an average diameter of the droplet on the Sauter. The fuel jet is considered as a set of zones, each of which has a characteristic temperature, the volume, fuel-to-air ratio.

These models allow us to investigate the influence on the combustion of compression, timing and duration of the injection, hole diameter and the number of sprays in the fuel injector, characteristics of fuel injection, combustion chamber shape, correlate the direction of fuel jets with combustion chamber and swirl intensity, take into account the interaction of jet fuel with the walls and to each other and finally allow you to perform multi-factor multi-criteria optimization.

However, the use of this class of models requires detailed design information of the simulated engine, setting up empirical relations and coefficients to make a relatively labor-intensive verification.

Widespread empirical or semi-empirical models of combustion, which describe the geometric shape of the heat generation curve [10-15] (second group) are also presented. Such models are easy to describe and versatility of use. For example, in a model of prof. VibeI.I. [10], the rate of combustion and the proportion of burnt fuel are described by semi-empirical dependencies:

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$$\frac{dx}{d\varphi} = -C\frac{m+1}{\varphi_z}\varphi^m \exp(C\varphi^{-m+1}); \quad (1)$$

$$x = 1 - \exp(C\varphi^{-m+1}), \quad (2)$$

where $\bar{\varphi} = \varphi \Big/ \varphi_z$, $\varphi$, $\varphi_z$ - respectively, relative duration of combustion, the current duration of combustion from the start of combustion and combustion duration shown in angles of rotation of the crankshaft;

*C* - constant (for example, at the end of the combustion when $x = x_Z = 0.999$, C = ln (1-0,999) = -6,908);

*m*-index of combustion character.

Feature of empirical models is that all input values of the calculation formulas are constant values and are given by experimental data or chosen from the recommended by investigators ranges. For example, in first approximation, prof. Vibe I.I. recommends$0 \le m \le 0{,}7$for diesel engines, and in the work of scientists from Bauman Moscow state University(Moscow, Russian Federation) values of *m* range from -0.3 to 0.7.

Use of this class of models suitable for describing the combustion in a specific engine running on one mode of his work.When changing a constructive parameter and adjusting the engine or the conditions of his work empirical models stop producing an accurate result.

The drawback of empirical models of combustion is the complexity of their use in calculations of the harmful substances formation in diesel engines, in particular nitrogen oxides. NO output in accordance with the thermal theory of ZeldovichU.B. [16] is extremely sensitive to the magnitude of the temperature in the cylinder. Therefore, in these calculations, it is important to accurately determine the temperature and, consequently, the heat generation curve. This curve, calculated by the empirical models as a rule have one peak that does not comply with the combustion process in diesel engines for most modes of operation. Accordingly, the accuracy of the calculation output of harmful substances by using models of this class is relatively low.

Most of the problems that arise in the practice of design and research of various diesel engines can be solved using "intermediate" type models [11, 18-20] (third group). These models combine the advantages of computational methods from first and second groups.

A number of models describes the combustion process by using Vibe I.I. relationships (1) and (2) [19, 20], but unlike empirical models the indices of combustion duration $\varphi_z$ and combustion character *m* are functions of design parameters and operation modes.

The data obtained by processing the experimental indicator diagrams, confirm the correctness of this approach (Fig. 1)

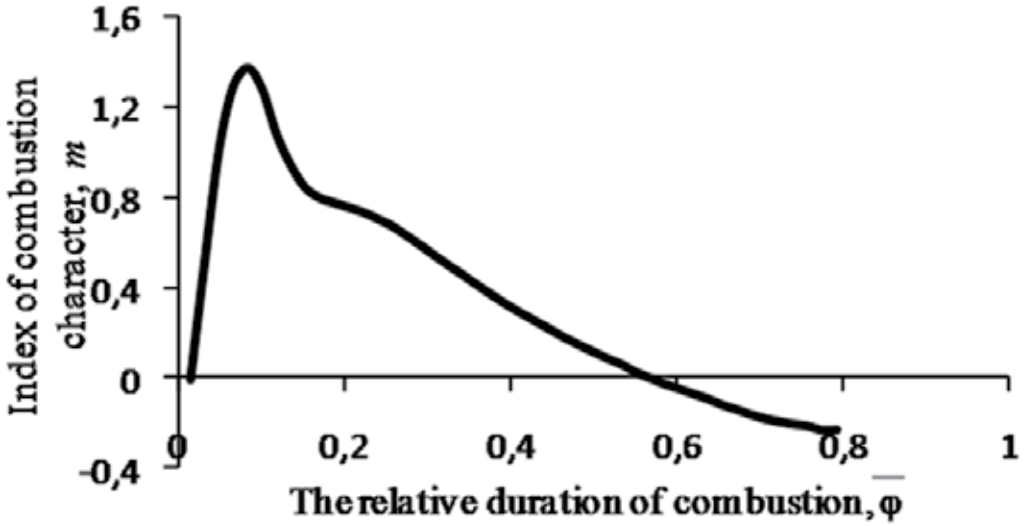


**Figure 1.** The change in the index of combustion character *m* during operation cycle in the 4 stroke autotractor diesel engine with turbocharger (SMD-23)

According to the variable nature of the index of combustion character*m* for the differentiation of equation (2) the next dependence was obtained which is different from equation (1):

$$\frac{dx}{d\varphi} = -C\exp(C\bar{\varphi}^{m+1})\frac{1}{\varphi_z}\left[(m+1)\bar{\varphi}^{m} + \bar{\varphi}^{m+1}\ln\bar{\varphi}\frac{dm}{d\bar{\varphi}}\right]$$

Filipkovsky A. I.proposed to determine the index of combustion character*m* and the duration of the combustion $\varphi_z$in Vibe I.I. dependencies (1) and (2) as a function of the parameters of the evaporation, diffusion and chemical kinetics of reaction [19]. The model takes into account the main factors that determine the combustion process:

- design features of the combustion chamber (chamber shape, the diameter of the cylinder and the neck chamber, swirl ratio);
- characteristics of the fuel injection and atomization (diameter and the effective cross section of nozzle holes, duration, and mean pressure of injection, amount the fuel during operation cycle, the physical characteristics of the fuel);
- thermo-and gas-dynamic parameters of the charge in cylinder (pressure and density of charge at the end of a conditional extended to top dead centre (TDC) compression, the tangential velocity of the charge in the combustion chamber);
- mode parameters of the engine (speed, excess air ratio).

The model assumed that the development of chain reactions begins with the start of fuel injection into the diesel cylinder, rather than the beginning of combustion, as in the model of prof. VibeI. I. The curve of heat generation rate, calculated by the model, has one peak.

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Calculations of heat curves by the model of Filipkovsky A.I. for medium-speed four-stroke diesel engines with turbocharging, 26 cm bore and stroke 34 cmand four-stroke diesel engines with turbocharging, 32 cm bore and stroke 32 cm with volume mixing processes have shown good agreement with experimental data. However, practical application of this model for high-speed automotive diesel with a volume-film-mixing processes did not produce positive results. The discrepancy between the calculated and experimental data is greatest in the partial modes, where the curve has a two-peak heat generation rate in nature (Fig. 2). Finally, this method gives a significant error in the calculations for biofuels because of significant differences in the physicochemical properties of diesel fuels and biofuels.

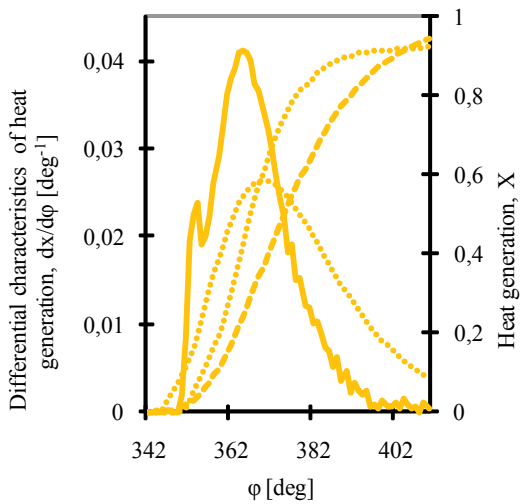


**Figure 2.** Comparison of experimental and calculated heat generation characteristics of the ethyl esters of rapeseed oil ..... – Calculation by the method of Dr. Filippovsky A.I.; ——— – Experimental data

Despite these problems, the Filipkovsky A.I. model, in our opinion, has the potential for further improvement. Obviously, it is necessary to adapt this model to integrate features of medium-speed diesel engines, physicochemical properties of biofuels, as well as operation modes of small and medium loads, where the heat generation rate has two-peak character.

Significant influence on the combustion process have physical and chemical properties of fuel. In present study, the features of the processes in the engine cylinder associated with the use of bio-fuels of plant-based origin, in particular mixtures of rapeseed oil (RO) with diesel fuel (DF) and the ethyl ester of rapeseed oil (EERO). In conducted by authors experimental studies have shown that the presence of oxygen in the molecule of biofuels will intensify the process of diffusion combustion, which should be considered when developing a mathematical model.

This chapter describes the results of experimental studies of biofuels in diesel engines, the mathematical model of combustion in the diesel enginecylinder and the results of verification.

## 2. Experimental studies of biofuels in diesel engines

Experimental studies are needed to obtain basic data for modeling, getting a number of empirical coefficients in the model equations and refinement of physical laws, comparison of experimental and calculated data.

### 2.1. Investigation of physicochemical properties of biofuels

Physicochemical properties of the investigated biofuels are presented in Table 1.

Analysis of the data in Table. 1 shows that the properties of plant-based fuels are significantly different from the properties of diesel fuel: PM and EEROafter comparison with DFhave respectively 14 and 13.5% less low heat values, for 10 and 8.1% higher density, for 14.1 and 21.9% higher surface tension, and for 22.8 and 8.5 times higher viscosity. For the combustion of 1 kg of RO and EERO required respectively 12.7 and 12.6% less air, which is associated with the presence of oxygen in the structures of their molecules.

It should be noted that the trial set of EERO contained unreacted raped oil, so the physical and chemical properties of ethyl differ are different from those given in the technical literature [21].

| Property | Diesel fuel (DF) | Rape oil (RO) | Ethyl ester of rapeseed oil (EERO) | Mixtures | | |
|---|---|---|---|---|---|---|
| | | | | DF: RO (3:1) | DF: RO (1:1) | DF: RO (1:3) |
| Elemental composition, %: | | | | | | |
| carbon (C) | 87 | 77,9 | 77,6 | 84,5 | 82 | 79,8 |
| hydrogen (H) | 12,6 | 11,9 | 12 | 12,3 | 12 | 11,8 |
| oxygen (O) | 0,4 | 10,2 | 10,4 | 3,2 | 5,9 | 8,5 |
| sulfur (S) | 0,04 | 0 | 0 | 0,03 | 0,02 | 0,01 |
| The amount of air for combustion per mass unit of fuel Lo, kg / kg | 14,4 | 12,7 | 12,7 | 13,9 | 13,5 | 12,9 |
| High heat value Qv, MJ / kg | 44,95 | 39,3 | 39,2 | 43,4 | 42,0 | 40,6 |
| Low heat value of Qn, MJ / kg | 42,2 | 36,8 | 36,9 | 40,7 | 39,3 | 38,0 |
| The density ρ, g/m3 (20 ˚ C) | 825 | 915 | 895 | 849 | 872 | 894 |
| The kinematic viscosity ν, mm 3 / s (20 ˚ C) | 3,8 | 87 | 32,48 | 26,3 | 47,6 | 67,8 |
| The surface tension σ * 103 N / m (20 ˚ C) | 28,9 | 33,3 | 36 | 30,1 | 31,2 | 32,3 |

**Table 1.** Physicochemical properties of biofuels.

The difference between the physical and chemical properties of biofuels on the properties of diesel fuel is the cause of changes in diesel working process and performance, which should be considered when simulating processes inside the cylinder.

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### 2.2. Studies of dispersion atomized biofuels

To clarify the empirical and criterial relationships that characterize the quality of atomization of biofuels, an experimental study was made of atomizationdispersion.

Single injections were made on glass plates coated with a layer of soot and kerosene and on a top side covered with a layer of magnesium oxide (which has a bright white color) for clarity of prints fuel droplets.

The studies were conducted on the following frequencies of high pressure fuel pump camshaft rotation: 900,700 and 770 rpm. Fuel rack setting was made for maximum fuel delivery.

Micrographswere obtained in the experimental study of dispersion of the atomization of various fuels and shown on Fig. 3.

Photomicrographs are processed according to the procedure [22]. The relative fuel atomization characteristics were obtained as the results (Figures 4-7): Differential (R0 - quantitative; R2 - surface; R3 - volume) and integral (S0 - quantitative; S2 - surface; S3 - volume).

The data obtained allowed to estimate the average diameter of fuel droplets of different composition.

The most commonly used parameter for calculating the evaporation of fuel is the average volume-surface droplet diameter (Sauter diameter):

$$d_{32} = E_{32} d_c M^{0.0733} / \left(\rho We\right)^{0.266}, \tag{3}$$

where $E_{32}$ is a constant factor depending on the design of the nozzle and the method of averaging the droplet size;

$d_c$-diameter of atomized holes;

$M$-criterion, which characterizes the ratio of surface tension and viscosity;

$W_e$- Weber criterion;

$\rho$- air density to fuel ratrio

Calculations of Sauter diameter of droplets for a four-stroke auto-tractor turbocharged diesel engine, which has a cylinder diameter 120 mm and 140 mm stroke running on standard diesel fuel using the standard fuel system show that the value of $d_{32}$on nominal power mode ranges from 26 to 29 microns. Greater droplet diameter values obtained in experimental studies (Fig. 4), due to the fact that the injection was made into the environment under atmospheric conditions. It is obvious that in a running diesel engine a high temperature of the charge in the cylinder causes a greater atomization and evaporation of fuel droplets.

Therefore, when refinement dependencies (3) for the case of biofuels, relative (not absolute) values of $d_{32}$(Table 2) was used. Analysis of the data in Table. 2 shows that the dependence (3) with appreciable error describes the variation of $d_{32}$ for plant-based fuels. Authors have pro-

posed an empirical correction for the dependence (3), depending on the viscosity of the fuel and allows to do more accurate calculation of the average volume-surface diameter of drops:

$$k_f(\nu) = -0,00010939 \cdot \nu^2 + 0,0052066 \cdot \nu + 0,98179447. \quad (4)$$

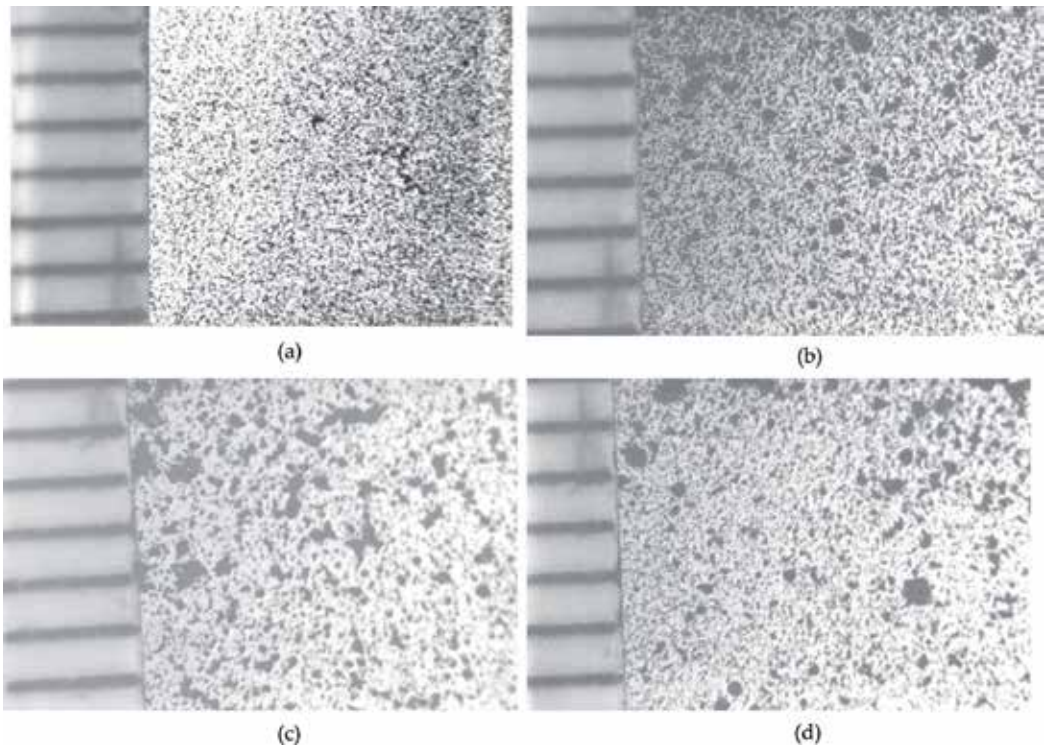


**Figure 3.** Micrograph of atomized fuel droplets (n = 900 rpm): a standard diesel fuel (a) a mixture of diesel fuel and rapeseed oil in the ratio 1:1 (b); pure rapeseed oil (c); ethyl ester of rapeseed oil (d)

| | Diesel Fuel (DF) | Mixture DF: RO (1:1) | Rape Oil (RO) | Ethyl ester of rapeseed oil(EERO) |
|---|---|---|---|---|
| $\bar{d}_{32}$(experiment) | 1,00 | 1,509 | 2,000 | 1,877 |
| $\bar{d}_{32}$(calculated by the formula (3)) | 1,00 | 1,537 | 3,176 | 1,488 |
| $\bar{d}_{32}$(adjusted value) | 1,00 | 1,509 | 1,927 | 1,541 |

**Table 2.** The relative diameters of the droplets of different fuels

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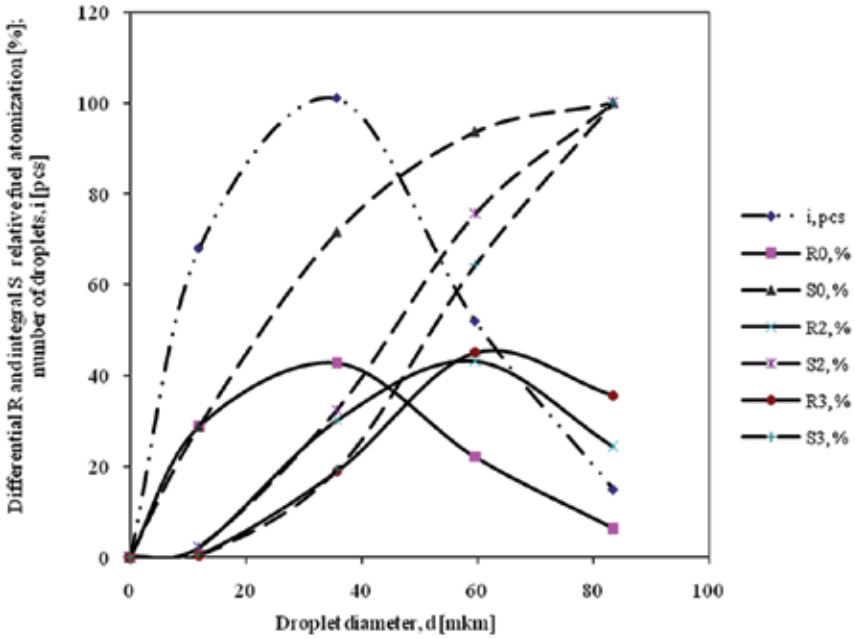


**Figure 4.** The relative atomization characteristics of diesel fuel (high pressure fuel pump camshaft rotation speed 900 rpm)

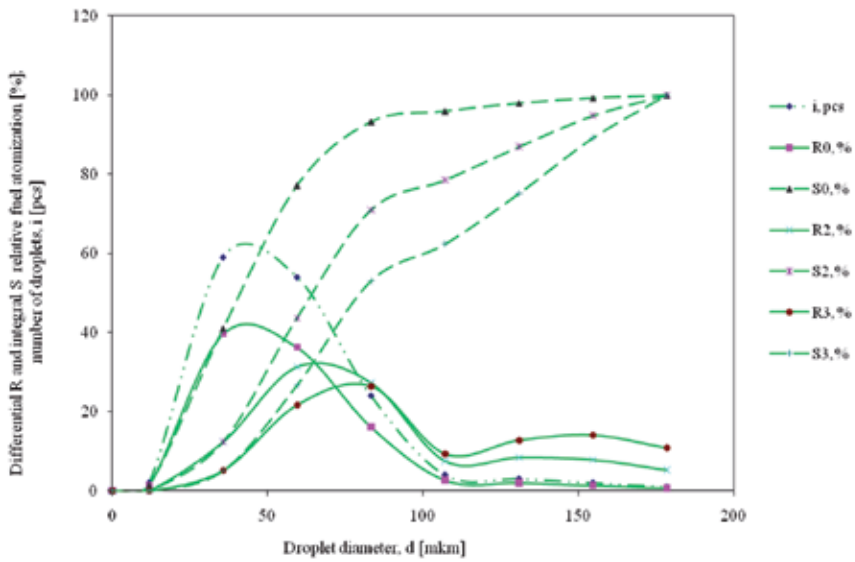


**Figure 5.** The relative characteristics of spray mixture DF and RO (1:1) (high pressure fuel pump camshaft rotation speed 900 rpm)

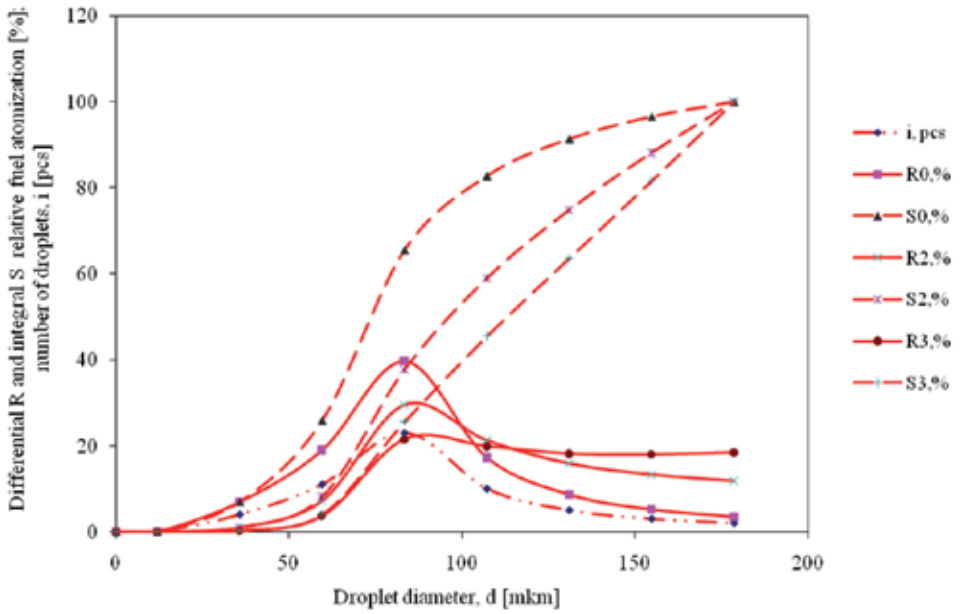


**Figure 6.** The relative atomization characteristics of RO (high pressure fuel pump camshaft rotation speed 900 rpm)

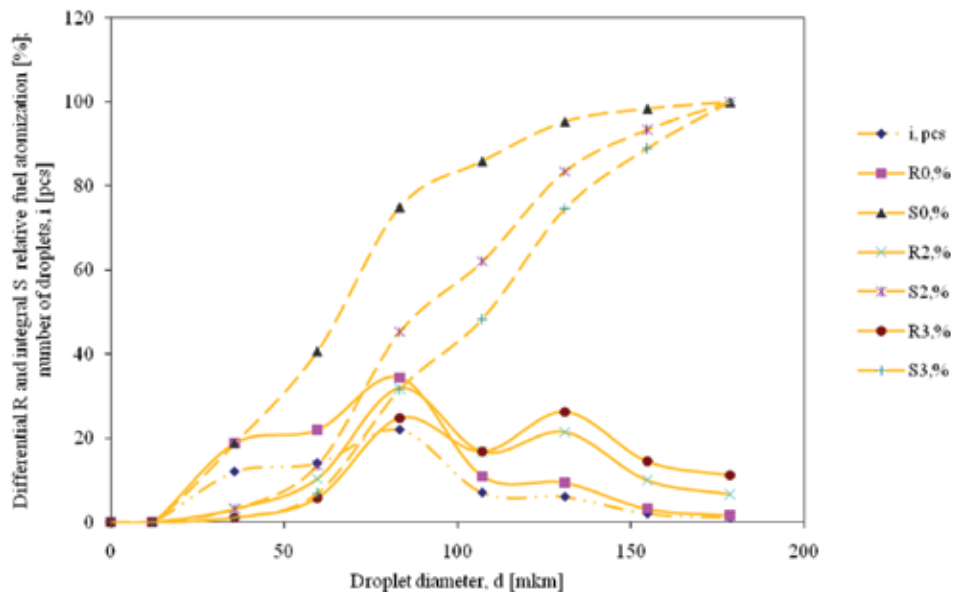


**Figure 7.** The relative atomization characteristics EERO (high pressure fuel pump camshaft rotation speed 900 rpm)

Dependence (3), corrected to (4) becomes:

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$$d_{32} = \frac{k_f \cdot d_c \cdot E_{32} \cdot M^{0.0733}}{\left(\rho \cdot We\right)^{0.266}}. \quad (5)$$

### 2.3. Experimental investigations of biofuels implementation in diesel

Experimental studies of the engine running on traditional and biofuels performed on a test bench with a diesel engine SMD-23, equipped with a turbocharged and intercooler system. Brief technical characteristics of a diesel engine SMD-23 is shown in Table. 3, the picture of experimental facility is shown in Fig. 8.

| Parameter | Value |
|---|---|
| Number of cylinders | 4 |
| Bore, mm | 120 |
| Stroke, mm | 140 |
| The geometric compression ratio | 15,5 |
| Rated power, kW | 120 |
| Rated speed rpm | 2000 |

**Table 3.** Summary of technical characteristics of a diesel engine SMD-23

**Figure 8.** Experimental stand

Engine tests were conducted on the modes of engine load characteristic related to rated power mode with engine speed 2000rpm and peak torque mode with engine speed 1500 rpm.

During the tests on each mode, the parameters of air and fuel delivery systems, exhaust gas, coolant and oil were measured. Engine speed and torque were also detected. Indexing, the definition of stroke in injector idle and measuring the pressure in the fuel injection pipe was carried out. Also emissions were measured and included $NO_2$, CO and smoke registration.

The values of injection timing angle and adjust fuel pump adjustments remained unchanged.

Main diesel indices that running on a different fuels are shown in Fig. 9.Lets consider the effect of physicochemical properties of bio-fuels on the performance of diesel.

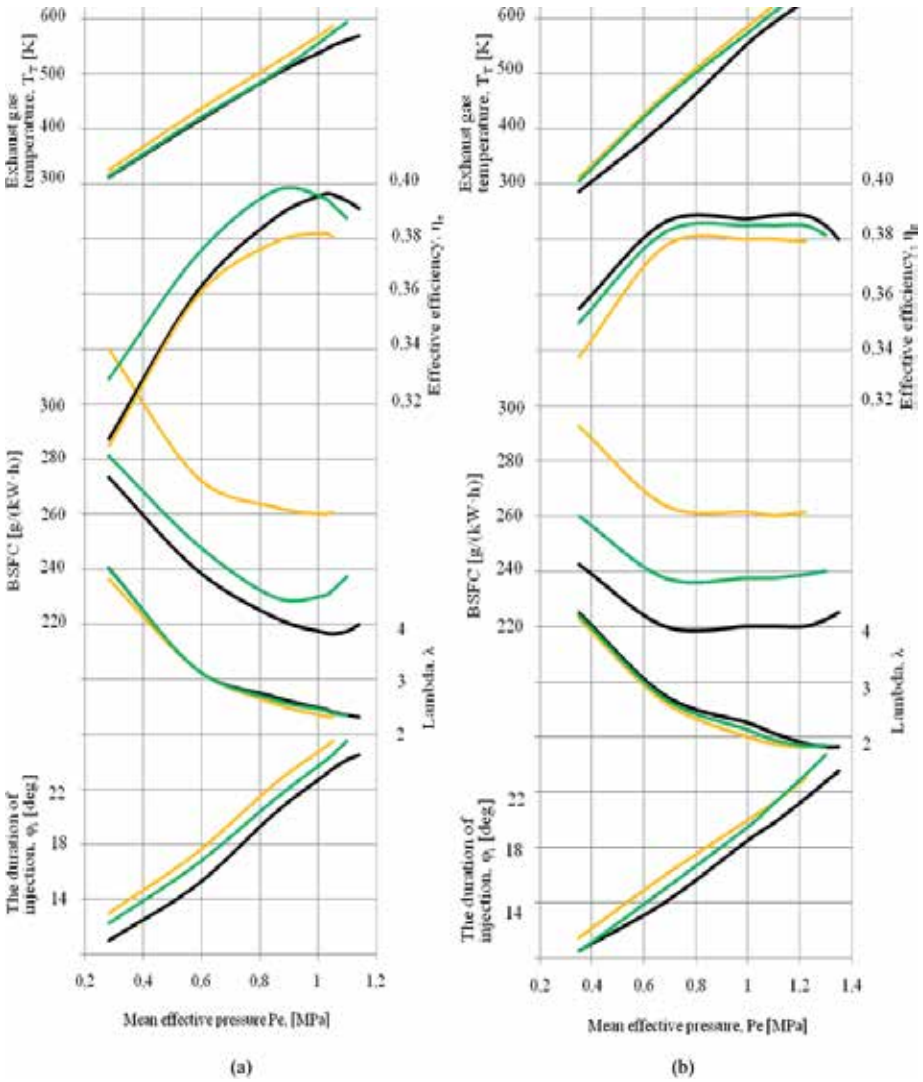


**Figure 9.** Effect of load on the performance of diesel exhaust gas SMD-23: engine speed 2000 rpm (a); engine speed 1500 rpm (b) ▬ -DF; ▬ - EERO; ▬ RO: DF (1:1)

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Injected fuel and air mixing. Injection of plant-based fuel into the combustion chamber is carried out with the higher maximum pressure than the injection of diesel fuel, which is explained by the influence of lower compressibility and higher viscosity of plant-based fuels.

Greater surface tension force and greater kinematic viscosity (see Table. 1) provide more later decay of injected plant-based fuel on the droplets and formation of smaller atomizing cone in comparison with diesel fuel. This dramatically increases the diameter of fuel droplets. As shown in [21, 23, 24], atomizing cone is reduced by 10% (using mthyl ester of rapeseed oil) and the average volume-surface droplet diameter d32 increases, respectively, in 1,5-1,877 times (when using pure RO, a mixture of RO and DF (1 to 1) and EERO).

Injection of plant-based fuels with the higher maximum pressures greater diameters of droplets in combination with a greater specific weight increase penetrating power and the range of fuel jet. The duration of injection of plant-based fuels$\varphi_i$increases slightly (1-2 crank angle) as a result of significant increase in injection pressure with a small increase in a fuel delivery.

The above factors lead to the fact that in the case of using plant-based fuels the volume fraction of mixing is reduced and the fraction of wall-film mixing is increase. The quality of volume mixing become lowered in this case.

The period of ignition delay. As a result of processing the experimental indicator diagrams, integral and differential characteristics of heat generation in the cylinder were obtained over the entire range of investigated fuel mixtures and regime characteristics of the engine. In Fig. 10 shows the relative heat generation characteristics when burned pure diesel fuel, and mixtures EERO,RO: DF (1:1) on high load (Pe = 1.25 MPa and Pe = 1.01 MPa) and medium (Pe = 0.57 MPa and 0 67 MPa) load at engine speed 1500 rpm and engine speed 2000 rpm. From the analysis of these characteristics is difficult to see any legitimate differences in the ignition delay period of plant-based fuels and diesel fuel.

Consequently, we can conclude that the flammability of plant-based fuels is almost unchanged in comparison with diesel fuel flammability.

The first peak of heat generation rate. As we can see from Fig. 10, on the most modes the maximum heat generation rate for plant-based fuel in this period is lower than for diesel fuel. In addition, the area under the first peak of the curve dx / dφsmaller, and hence smaller the amount of fuel burned out in this period.

This fact is obviously related to the deterioration of the mixing between the ignition delay when using mixtures of RO with the DF and EERO. Reduction in the angle of frame divergence, increasing the relative amount of fuel that enters the wall of the combustion chamber, a significant increase in the average diameter of droplets leads to a deterioration in mixing formation and reduce the relative amount of fuel vaporized during the period of ignition delay.

The second peak of heat generation rate. After burning the fuel, evaporated during the period of ignition delay, there is a diffusion combustion of the fuel droplets in the fuel torch, as well as the fuel evaporating from the walls of the combustion chamber after the contact of the torch and the wall. The nature of the combustion process in this period determines the indicator performance of the cycle [25].

In an experimental engine used a cylindrical combustion chamber, that implements volume-film mixture formation. Obtained data is contradictory ex facte (see Fig. 10). Increasing the amount of fuel reaching the wall, large diameter drops, the heterogeneity of atomization when using plant-based fuels should lead to a decrease in the rate of evaporation and combustion of fuel, especially in the modes of small loads, when the wall has a lower temperature. However, it is clear that almost on all modes there is an increase in the rate of combustion as compared to DF. The deterioration in the amount of mixing in this case does not lead to a decrease in combustion rate and it can be seen not only in the modes of high loads, but also in modes of low loads.

Intensification of the diffusion combustion of plant-basedfuels, can obviously be explained by the presence of oxygen in the structure of the molecule. When burning fuel droplets of biofuel, the oxygen is in the molecule of fuel. This oxygen is more active than molecular oxygen. That is why, even at low temperatures of plant-based fuel oxidation rate of its "own" oxygen is very high. All this probably leads to an increase in diffusion combustion rate in general.

The increase in the rate of combustion of plant-based fuels in the main period of combustion in most cases leads to a slight increase in average temperatures and pressures in the cylinder. In addition, the exhaust gas temperature rise in the exhaust manifold (see Fig. 9.).

The period of slow combustion. During this period there was burning of fuel in the cylinder. From Fig. 10 difficult to see the end of the combustion of different fuels. However, it is clear that the differences between the test fuel at the end of the combustion is low. Accelerated burning of plant-based fuels, during the second period of combustion, apparently compensate by slow combustion in the first period. So the total duration of combustion is practically unchanged.

Effective performance. As it can be seen from Fig. 9, the use of plant-based fuels leads to an increase in break specific fuel consumption because of reduction in their low heat value compared to diesel fuel. Changing in effective efficiency of the diesel engine is not so clear.

The increase in the rate of diffusion combustion, high quality film mixing on the high loads modes lead to an increase in the effective efficiency if we use plant-based fuels.

At low load modes mixture formation deteriorates in the volume of the combustion chamber. In addition, the share of plant – based fuel burning in the relatively cold wall surface areas of combustion chamber, which explains the decrease in the effective efficiency at low load modes.

The toxicity of exhaust gases. When using supplements of vegetable-based oils to diesel fuel, and if diesel engine operating on pure EERO on modes of high and medium loads the smokeemission reduced in 1.6-2 times and NO emissions increase for 5-15%. In most modes of low-load smoke and NO are reduced (in 1.2-2 times), or remain unchanged. CO emissions using different fuels are comparable.

Experimental studies have provided initial information on the physicochemical properties of plant-based fuels, low of flow injection, atomization, mixing and combustion in the cylinder, the data for mathematical modeling processesinside the cylinder.

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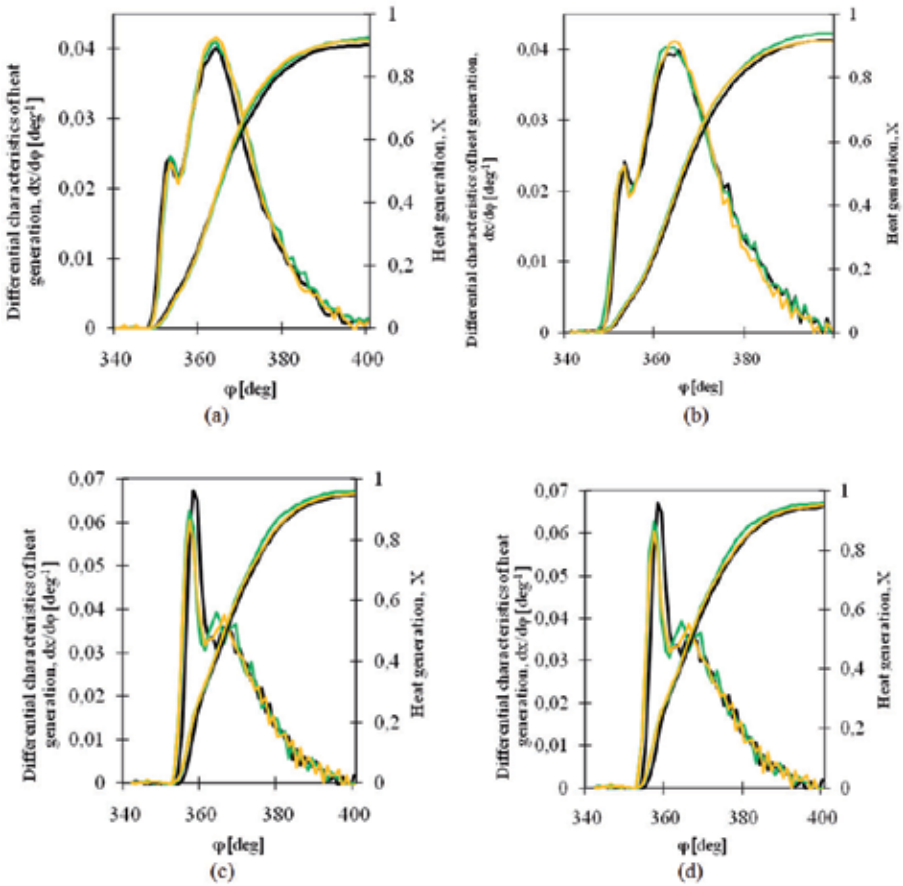


**Figure 10.** Heat generation characteristics in the diesel cylinder: engine speed 1500 rpm: Pe = 1.25 MPa (a) Pe = 0.67 MPa (b); engine speed 2000 rpm: *Pe* = 1,01 MPa (c), *Pe* = 0,57 MPa (d); ▬ -DF; ▬ - EERO; ▬ RO: DF (1:1)

These differences in the physicochemical properties of biofuels and their impact on flow processes in the cylinder of a diesel engine form the basis for the developed mathematical model of combustion.

## 3. Description of the proposed mathematical model of combustion

Lets consider the features of the proposed model of combustion.

Differential characteristic of heat generation proposed to describe by two curves corresponding to the periods of ignition (or "fast" combustion) and the diffusion combustion

$$\left(\frac{dx}{d\varphi}\right)_I = -A \cdot C \cdot \exp(C \cdot \overline{\varphi}_I^{m_I+1}) \frac{6n}{\varphi_{zI}} \left[ (m_I + 1)\overline{\varphi}_I^{m_I} + \overline{\varphi}_I^{m_I+1} \ln \overline{\varphi}_I \frac{dm_I}{d\varphi_I} \right] \quad (6)$$

$$\left(\frac{dx}{d\varphi}\right)_{II} = -C \cdot \xi_v \cdot S \cdot \exp(C\overline{\varphi}_{II}^{m_{II}+1}) \frac{6n}{\varphi_{z_{II}}} \left[ (m_{II} + 1)\overline{\varphi}_{II}^{m_{II}} + \overline{\varphi}_{II}^{m_{II}+1} \ln \overline{\varphi}_{II} \frac{dm_{II}}{d\varphi_{II}} \right] \quad (7)$$

where $A$ - coefficient taking into account the influence of the proportion of vaporized fuel during the ignition delay at the rate of fast combustion;

$C$ - coefficient taking into account the completeness of combustion;

ξv - the degree of efficient use of air charge;

$S$ - coefficient taking into account the share of fuel burned for the period of the ignition (linking the two periods).

The index «I» related to parameters that identified ignition, the index «II» - the process of diffusion combustion.

Dynamics indicators for the respective periods of combustion:

$$m_I = 4 \cdot \overline{\varphi}_{mI} \cdot \left(1 - \overline{\varphi}^{\overline{\varphi}_{mI}}\right); \quad (8)$$

$$m_{II} = 9 \cdot \overline{\varphi}_{mII} \cdot \left(1 - \overline{\varphi}^{\overline{\varphi}_{mII}}\right), \quad (9)$$

where -$\overline{\varphi}_{mI}$ and $\overline{\varphi}_{mII}$ relative moments of maximum heat generate rate;

$\overline{\varphi}_I$ and $\overline{\varphi}_{II}$ - the relative angles of the crankshaftrotation: $\overline{\varphi}_I = \varphi / \varphi_{ZI}$, $\overline{\varphi}_{II} = \varphi / \varphi_{ZII}$;

φ - the current angle of the crankshaft rotation from the start of combustion;

$\varphi_{ZI}$, $\varphi_{ZII}$ - respectively, the duration of fast and diffusive combustion.

At each calculated section the values $\left(\frac{dx}{d\varphi}\right)_I$ and $\left(\frac{dx}{d\varphi}\right)_{II}$ was comparing. The final calculated heat generation rate $\frac{dx}{d\varphi}$ took the value of greater meaning of two rates.

The total amount of burnt fuel are determined by integrating the function dx / dφ in the area of combustion

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$$x = \int_{\varphi_N}^{\varphi_k} \frac{dx}{d\varphi} d\varphi, \tag{10}$$

where $\varphi_N$, $\varphi_k$ - respectively the beginning and the end of combustion.

In formulas (6) - (9) there are the parameters $A$ and, $\varphi_{ZI}$ and $\varphi_{ZII}$, $\xi_v$, which, unlike the parameters of the known formulas Vibe I.I. accounted specific processes of fuel injection, mixing, evaporation, combustion, and the interaction of these processes with each other.

In generalizing the data obtained by processing the experimental indicator diagrams, empirical correlations was proposed to determine the relative moment of maximum heat generation rate during periods of combustion:

$$\bar{\varphi}_{mI} = 0,8 + \frac{0.03 \cdot b_{\grave{e}} \cdot \varphi_{ZI}}{6 \cdot n}; \bar{\varphi}_{mII} = 0,16 + \frac{0.03 \cdot b_{\grave{e}} \cdot \varphi_{ZII}}{6 \cdot n}, \tag{11}$$

where $b_u$- a constant of relative evaporation.

The relative constant of evaporation

$$b_u = K_u / d_{32}^2 \tag{12}$$

where $K_u$ - constant of evaporation, calculated for the average diameter $d_{32}$of the droplet under Sauter.

Prof. Razleytsev [8] estimated, that during the evaporation of fuel in a diesel engine cylinder the average evaporation constant:

$$K_{uT} = (10^6 p_c)^{-1} \tag{13}$$

where $p_c$ - the pressure in the cylinder at the end of a conditional extension to TDC compression.

Theoretical constant $K_{uT}$ does not include an increase in the rate of evaporation of droplets during combustion, the effect of size of drops, speed and frequency of turbulent vortices arising in the diesel cylinder. This dependence is in practical calculations can be taken into account by correction function $\Upsilon$:

$$Ku = Y \cdot K_{uT}. \tag{14}$$

In [8] proposed the following formula for determining the correction function:

$$Y = y\left(W_T d_{32}\right)^{0.75} p_c{}^{0.25}, \tag{15}$$

where $y$ - constant empirical coefficient depending on the design of the combustion chamber and taking into account the effect of unaccounted secondary factors.

$W_T$ - the tangential velocity of the charge in the combustion chamber;

$p_c$ - the calculated pressure at the end of a conditional extension to TDC compression.

It should pay particular attention to the coefficient of $y$. It is obvious that there are permanent factors that defined $y$ coefficient - engine design, adjustments and settings mode. On the other hand, while using different fuels - the value of this ratio will be determined by physical and chemical properties of fuels.

As shown in Section 2, an important property of plant-based fuelsthat have a material effect on the combustion process is the oxygen content in the molecule. Increasing the number of bound oxygen in the molecule leads to an increase in the rate of diffusion combustion. Accordingly, the simulation of combustion is expedient to increase the coefficient $y$ proportional to the share of the oxygen in the molecule of fuel.

In this study, a constant value $y$ was adjusted for each fuel type on the basis of providing the best agreement between calculated and experimental data. For all the calculations for one type of fuel $y$ constant has not changed.

In accordance with the original model [19] the duration of diffusion combustion:

$$\tau_{zII} = \varphi_i + \varphi_b, \tag{16}$$

where $\varphi_i$ - the duration of fuel injection;

$\varphi_b$- the duration of burn-out fuel after the injection.

The duration of burn-out fuel $\phi_b$ characterized by the time of evaporation and combustion of large droplets delivered in the diesel engine cylinder at the end of injection. This time depends on the fineness of atomization, the distribution of drops, the parameters of the working fluid in the cylinder, air-fuel ratio, etc. $\phi_b$ can be calculatedfrom the formula [8]:

$$\varphi_b = K_\alpha \cdot \varphi_e, \tag{17}$$

where $\phi_e$- the duration of the evaporation of large droplets of fuel;

$K_\alpha$- correction function which takes into account the time of fuel vapors burning.

The duration the large droplets evaporation of fuel:

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$$\varphi_e = \frac{d_K^2}{K_u} \tag{18}$$

where $d_K$ - an average diameter of large drops of fuel injected into cylinder by the end of the fuel delivery.

In [8] proposed to determine the diameter of the large drops by the formula:

$$d_K = B \cdot d_{32} \tag{19}$$

In this formula, the size factor is:

$$B = 1.5 + 0.018 \exp\left(\Delta p_{fi}^{0,272}\right), \tag{20}$$

where $\Delta p_{fi}$- the average pressure drop during injection, MPa.

Correction function at the time of burning-out of fuel vapors can be determined from the dependence [8] :

$$K_\lambda = 1 + {}^{A_3 K_u}\!/_{(\lambda - 1)} \tag{21}$$

where $\lambda$- is the ratio of actual air-to-fuel ratio to stoichiometry for a given mixture;

$A_3$ - coefficient, which is determined by identifying a number of experimental data for defined row of engines and can be taken equal to $2.5 \bullet 10^6$.

We proposed to determine the duration of the fast combustion as a function of the duration of the ignition delay period:

$$\tau_{zI} = \tau_i \cdot K_\lambda, \tag{22}$$

where $\tau_i$ - the period of ignition delay in seconds.

If to go to the crank angle, the duration of fast and diffusive combustion are determined by the following formulas:

$$\varphi_{ZI} = \tau_{ZI} 6n; \varphi_{ZII} = \theta_i + \varphi_b 6n, \tag{23}$$

where $\theta_i$ - injection advanced angle.

In developed model has been assumed that burn rate during the ignition mostly depends on the amount of fuel vaporized during the period of ignition delay. In turn, the calculation of the first peak heat generation rate coefficient taking into account the influence of the proportion of vaporized fuel during the ignition delay period.

$$A = K_I \cdot \sigma_I \tag{24}$$

where $\sigma_I$ - the relative amount of fuel injected during ignition delay period $\varphi_i$;

$K_I$ - coefficient of proportionality.

In [23] proposed the dynamics of heat generation during diffusion combustion (equation (7)) to adjust with the ratio ξv, which is a degree of efficient use of air charge in cylinder:

$$\xi_V = \frac{\lambda_\partial}{\lambda}, \tag{25}$$

where$\lambda_m$ - the average $\lambda$ coefficient in the combustion zone;

$\lambda$ – the estimated value of $\lambda$ in the cylinder for full combustion of the fuel injected into the cylinder.

The coefficient $\xi_V$ takes into account the interaction of fuel torch with the wall of the combustion chamber and other factors that reduce the amount of oxidant entering the combustion zone. In [23, 28] describes a method of determining this ratio.

The mathematical model of combustion is integrated into the thermodynamic model of the closed-loop workflow engine with a turbocharger.

## 4. Implementation of mathematical models for practical calculations

Comparison of calculated and experimental characteristics of heat generation and indicator diagrams using different fuels is shown in Fig. 11-13. It can be seen that the proposed mathematical model provides a satisfactory agreement between the calculated and experimental data in a wide range of biofuels, loads and engine speeds.

A precise description of the combustion process is important in modeling the formation of harmful substances in the cylinder. For example, the error in determining the temperature in

the cylinder 80-90K leads to a change in the calculated NO output by 30%, error in determining the temperature of 190 K change in the calculated NO output is 2.7 times [26]. Obviously, using the proposed mathematical model rather than empirical or semi empirical models provide a more accurate calculation of the formation of harmful substances in the diesel engine cylinder.

The adequacy of the developed mathematical model was tested also for its response to the changing influence of parameters - the compression ratio, injection duration and injection delay angle (Fig. 14). It is seen that the obtained numerical data trends and logical and do not conflict with similar data of other researchers [26-28].

We can conclude that the developed mathematical model allows us not only to describe the dynamics of heatgeneration with sufficient accuracy, but also to adequately respond to changes in design and adjustments in the parameters of diesel.

## 5. Conclusion

Actual and perspective task for modern engine – building has been introduced and solved in the chapter. This task included the development of mathematical model of alternative (biofuels) and fossil fuel (diesel) combustion calculation in the cylinder of diesel engine. It is shown that the physical-chemical properties of biofuels differ significantly from the properties of diesel fuel, which leads to changes in the processes of fuel injection, mixture formation and combustion. All this has a significant impact on the efficient and environmental performance of diesel engines.

Authors have proposed mathematical model that adequately describes the process of combustion of conventional diesel and bio-fuel in the cylinder of diesel engine. It was confirmed by the results of calculation and experimental studies.

The mathematical model proposed by the authors can be used to solve optimization tasks in internal combustion engines running on a diesel fuel, as in this model combustion processes are linked with parameters of engine design and engine working process parameters. The model is developed in a parametric form and reflects the change in design and adjustment parameters of the diesel engine.

An important characteristic of a new mathematical model is an adequate description of the first phase of the combustion process (first peak), which is associated with fuel burn-out, accumulated during the ignition delay, which allows more reliable to calculate the temperature of the working fluid in the cylinder of diesel engine and, consequently, with greater reliability to calculate by Zeldovich methoda number of nitrogen oxides that are formed in the cylinder of diesel engines.

The proposed model can be used in university training programs for professionals in the field of internal combustion engines, as well as in practice of firms participating in the modernization of existing and development of advanced diesel engines.

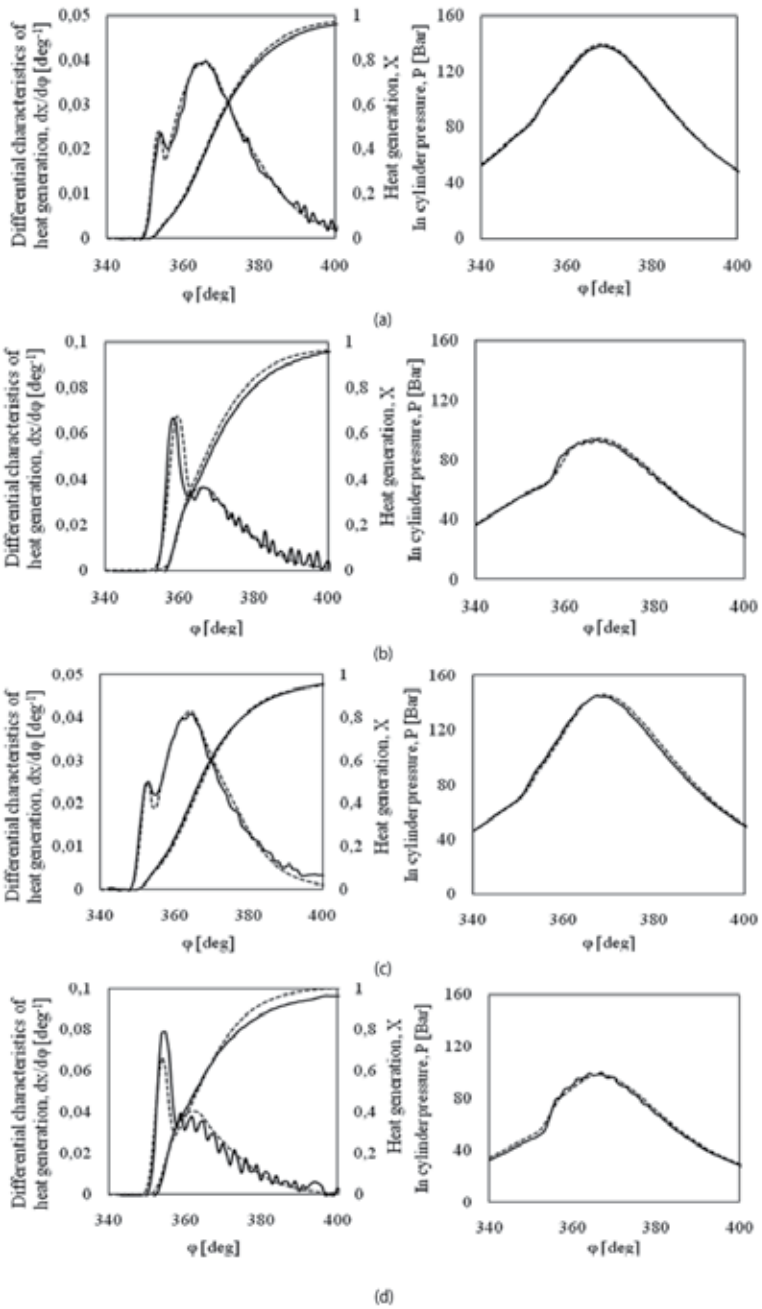


**Figure 11.** Verification of the model of calculation of heat generation process. Diesel: engine speed 2000 rpm,Pe = 1.1 MPa (a); engine speed 2000 rpm,Pe=0.56 MPa (b); engine speed 1500 rpm, Pe=1.35 MPa (c); engine speed 1500 rpm,pe=0.67 MPa (d); ——— Experiment; – – – – – Calculation of the refined model

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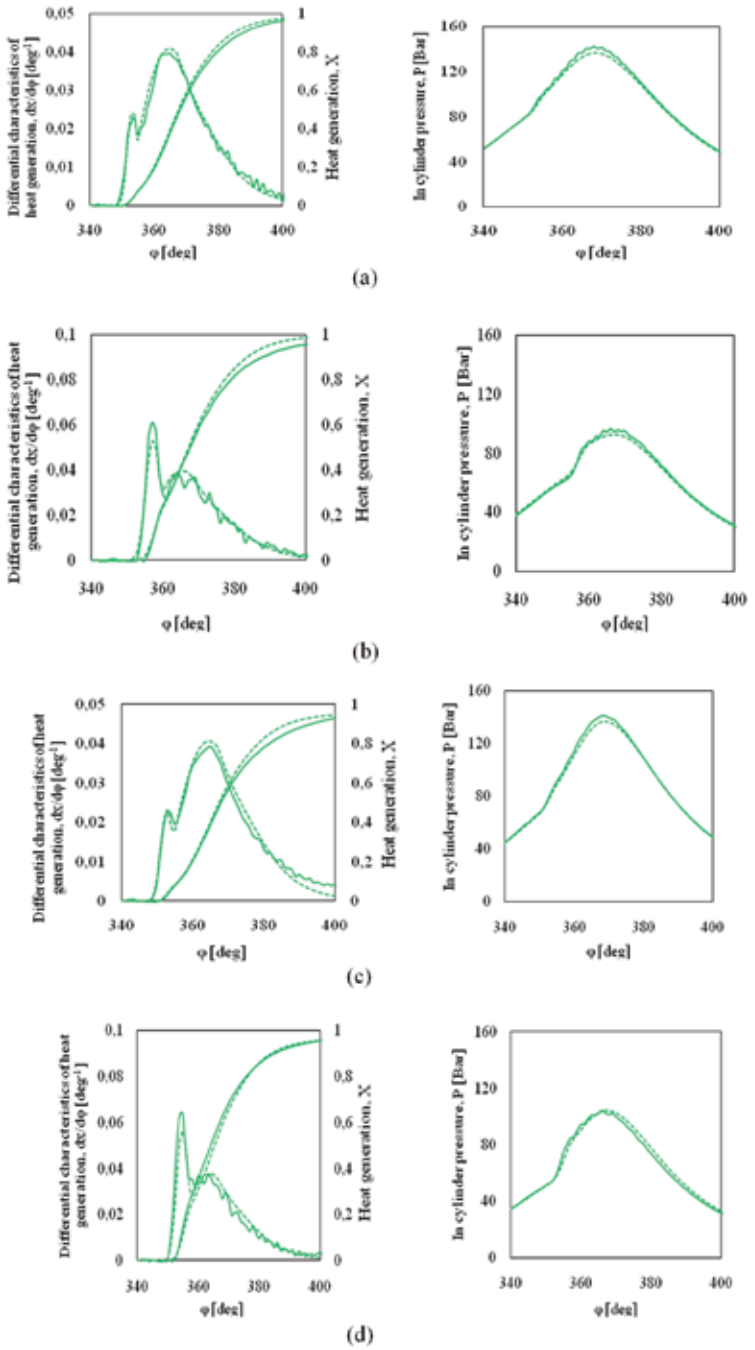


**Figure 12.** Verification of the model of calculation of heat generation process. A mixture of RO: DF (1:1): engine speed 2000 rpm, $p_e$ =1.1 MPa (a); engine speed 2000 rpm, $p_e$ =0.56 MPa (b); engine speed 1500 rpm, $p_e$ =1.35 MPa (c); engine speed 1500 rpm, $p_e$ =0.67 MPa (d); ——— Experiment; - - - - - Calculation on the refined model

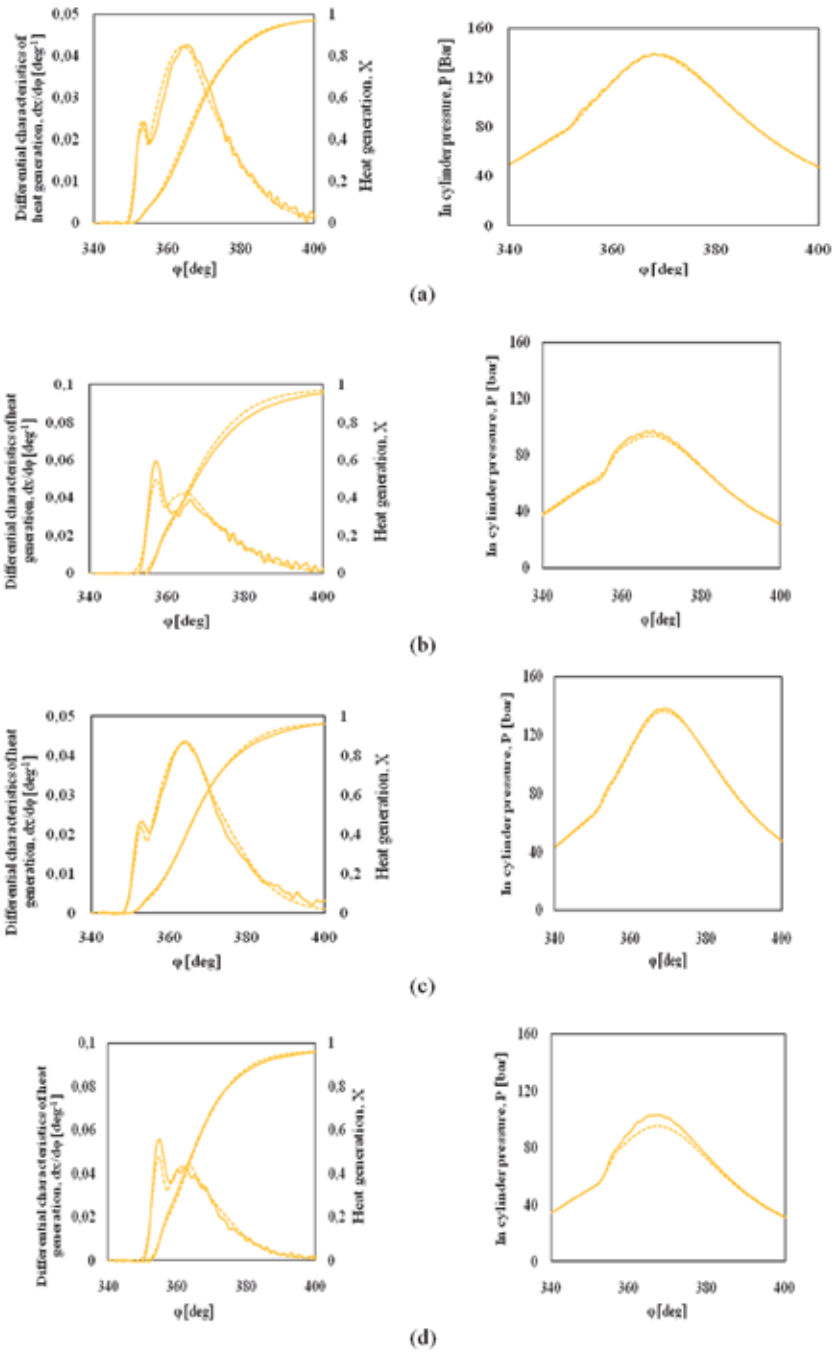


**Figure 13.** Verification of the model of calculation of heat generation process. EERO: engine speed 2000 rpm, $p_e$ =1.1 MPa (a); engine speed 2000 rpm, $p_e$ =0.56 MPa (b);engine speed 1500 rpm, $p_e$ =1.35 MPa (c);engine speed 1500 rpm, $p_e$ =0.67 MPa (d); ——— Experiment; - - - - - Calculation of the refined model

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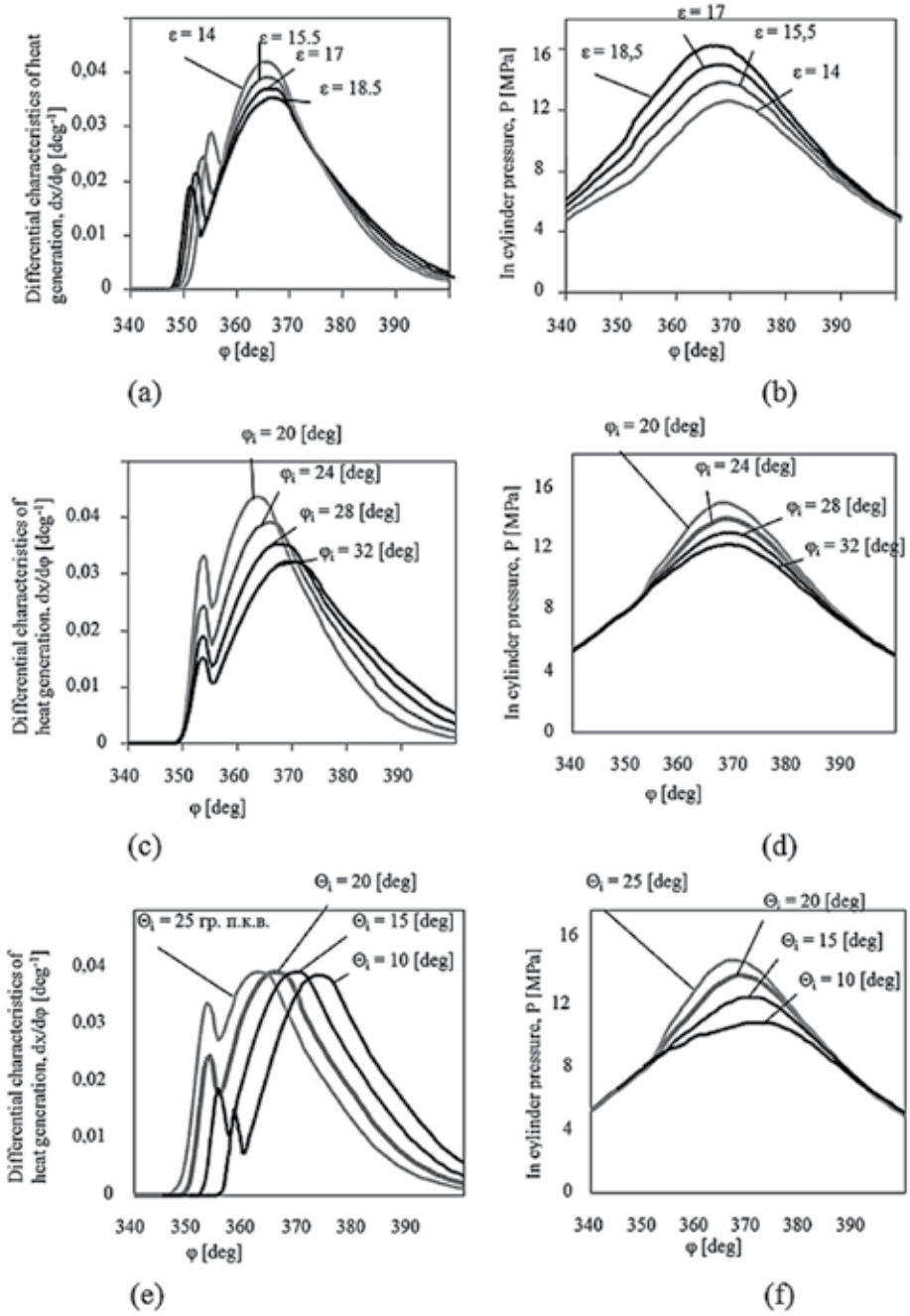


**Figure 14.** Effect of changing the compression ratio ε (a, b), the injection duration $\varphi_i$ (c, d) and injection delay angle $\Theta_i$ (e, f) at the rate of heat generation and pressure in the cylinder of diesel engine

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Chapter 16

# An Analysis of Physico-Chemical Properties of the Next Generation Biofuels and Their Correlation with the Requirements of Diesel Engine

Artur Malinowski, Joanna Czarnocka and Krzysztof Biernat

Additional information is available at the end of the chapter

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## 1. Introduction

There is a pressing need to haste developing advanced energy technologies to reduce dependency on crude oil and climate protection. Biofuels – liquid and gaseous fuels derived from organic matter – can play an important role in reducing of carbon dioxide ($CO_2$) emissions in the transport, and can raise the energy security. By 2050, biofuels could provide 27% of total transport fuel. The use of biofuels could avoid around 2.1 gigatonnes (Gt) of $CO_2$ emissions per year when produced sustainably. To meet this vision, most conventional biofuel technologies need to improve conversion efficiency, cost and overall sustainability. Conventional biofuel technologies include well-established that are already producing biofuels on a commercial scale. These biofuels, commonly referred to as first-generation, include sugar- and starch-based ethanol, oil-crop based biodiesel and straight vegetable oil, as well as biogas gained through anaerobic digestion. The International Energy Agency has undertaken an effort to develop a series of global technology road maps covering 19 advanced technologies, commonly referred to as second- or third-generation. This new technologies are still in the research and development (R&D), pilot or demonstration phase [1].Significant decrease of fossil fuels and lack of new ones becomes the basis for the Olduvai theory, published by R.C. Duncan [2]. The theory postulated that in the years 2012-2030, because of shortage of energy, the world would go through an economic crisis. This crisis would lead to collapse of industrial civilization. So, there is a need to look for alternative, renewable sources of raw materials.

According to the current EU Directive on promoting the use of energy from renewable sources [3], petrochemical companies are obliged to market fuel containing biocomponents (2009/28/EC). Biomass means the biodegradable fraction of products, waste and residues from biological origin, from agriculture, forestry and related industries including fisheries and aquaculture, as well as the biodegradable fraction of industrial and municipal wastes. Assuming biomass as the basic source of materials for the production of biofuels, two main material pathways and the suiting material processing technologies have been considered in the European definition. That is referred to as BtL (biomass-to-liquid) or, as an alternative, BtG (biomass-to-gas) and WtL (waste-to-liquid) or, as an alternative, WtG (waste-to-gas).

Biofuels are divided into groups according to their state of matter. According to Annex 1 to Communication from the Commission of the European Communities No. 34 of 2006, COM (2006)34 final, biofuels have been divided into liquid, gas, and others, with first and second generation biofuels having been introduced in this Communication for the first time. However, an idea of "synthetic biofuels" has been introduced and defined as "synthetic hydrocarbons or mixtures of synthetic hydrocarbons produced from biomass, for example SynGas produced from gasification of forestry biomass or SynDiesel."

## 2. Classification of biofuels

In the European classification, the following biofuels have been separated because of the state of matter:

**1.** Liquid biofuels:

- Bioethanol got from biomass or biodegradable waste fractions, possible for use as biofuel E5 of 5% ethanol and 95% petrol contents or as biofuel E85 of 85% ethanol and 15% petrol contents;
- Biodiesel containing methyl-esters [PME ("pure vegetable oils"), RME ("rapeseed methyl esters"), FAME ("fatty acid methyl esters")] produced from vegetable oil, animal oil or recycled (for example post-frying) fats and oils, meeting the requirements of relevant quality standards for B5 diesel oils of 5% ester and 95% petroleum-based diesel contents, B30 diesel oils of these proportions being 30% and 70%, respectively, and B100 exclusively consisting of pure esters of properties meeting the relevant standard specifications;
- Biomethanol produced from biomass, for use as biofuel or a fuel ingredient;
- BioETBE, that is Ethyl-tertio-butyl-ether produced from bioethanol, used as a petrol additive to increase the octane rating and to reduce knocking and added to petrol at a percentage rate of 47%;
- BioMTBE, that is Methyl-tertio-butyl-ether produced from biomethanol, used for the same purposes as those of the BioETBE and added to petrol at a percentage rate of 36%;
- BtL, that is Liquid fractions or mixtures of liquid fractions produced from biomass, for use as biofuels or fuel ingredients;

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- Pure vegetable oils (PVO) produced through pressing, extraction or similar, inclusive of refining, but chemically unmodified, which can be used as biofuel when compatible with the engine involved and when meeting the matching environmental protection requirements.

2. Gaseous biofuels:

- BioDME transport fuels gained from Renewable Energy Sources (RES), that is Dimethylether produced from biomass, for direct use as biofuel for compression-ignition engines;
- Biogas, that is Biofuel produced from biomass or the biodegradable fractions of waste, purified to natural gas quality;
- Biohydrogen as biofuel produced from biomass or the biodegradable fractions of waste.

3. Other renewable fuels, that is Biofuels not named above, originating from sources as defined in Directive 2001/77/EC and suitable to power transport.

This division resulted from the reasons discussed above, in particular from assessment of the usability of specific fuels in the present-day engine technologies, availability of the feedstock needed, and environmental impact of the fuels. The formal division of biofuels into specific generations has been published in the report titled "Biofueels in the European Union, a Vision for 2010 and Beyond". According to this report, biofuels have been divided into first generation biofuels, referred to as "conventional biofuels," and second generation biofuels, referred to as "advanced biofuels."

The first generation ("conventional") biofuels include:

- Bioethanol (BioEtOH, BioEt), understood as conventional ethanol got through hydrolysis and fermentation from raw materials such as cereals, sugar beets.;
- Pure vegetable oils, got through cold pressing and extraction from seeds of oil plants;
- Biodiesel, consisting of RME or FAME and fatty acid ethyl esters (FAEE) of higher fatty acids of other oily plants and gained as the result of cold pressing, extraction and transesterification;
- Biodiesel, consisting of methyl and ethyl esters and gained as the result of transesterification of post-frying oil;
- Biogas, got by purification of wet landfill or agricultural biogas;
- BioETBE, got by chemical processing of bioethanol.

The idea of second generation biofuels development is based on an assumption. Feedstock to be used for producing such fuels should equally include biomass, waste vegetable oils and animal fats, as well as any waste substances of organic origin that are useless in the food and forestry industries. The second generation ("advanced") biofuels includes:

- Bioethanol, biobutanol, and blends of higher alcohols and derivative compounds, got as the result of advanced of hydrolysis and fermentation of lignocellulosic biomass (excluding the feedstock for food production purposes);

- Synthetic biofuels, being products of biomass processing and gained by gasification and proper synthesis into liquid fuel ingredients (BtL) and products of process biodegradable industrial and municipal wastes, including carbon dioxide (WtL);
- Fuels for compression-ignition engines, got from biomass through Fischer-Tropsch, inclusive of synthetic biodiesels got by blending of lignocellulosic products;
- Biomethanol, got as the result of lignocellulose transformation, inclusive of Fischer-Tropsch synthesis, as well as with the use of waste carbon dioxide;
- Biodimethylether (bioDME), got by thermochemical processing of biomass, inclusive of biomethanol, biogas, and synthetic biogases being derivative products of biomass transformation;
- Biodiesel as biofuel or a fuel ingredient for compression-ignition engines, got by hydrorefining (hydrogenation) of vegetable oils and animal fats;
- Biodimethylfuran (bioDMF), obtained from sugar transformation, inclusive of transforming cellulose in to thermochemical and biochemical processes;
- Biogas as synthetic natural gas (SNG) or biomethane, obtained in result of lignocelluloses gasification, correct synthesis, or purification of agricultural, landfill, and sewage sludge biogas;
- Biohydrogen got in result of gasification of lignocellulose and synthesis of the gasification products or as the result of biochemical processes.

The European Commission Directorate-General for Energy and Transport proposed to separate third generation biofuels, defining them as those for which the technology of universal gain and introduction of such fuels may be developed in 2030s or even later, according to the estimates. Preliminarily, biohydrogen and biomethanol have been classified in this group. The third generation biofuels may be obtained by the methods similar to those used in the second generation biofuels, but from the feedstock (biomass) having been modified at the plant growing stage with the use of molecular biology techniques. The objective of such changes is to improve the conversion of biomass into biofuels (biohydrogen, biomethanol, biobutanol) by for example cultivation of trees of low lignin content, development of crops with enzymes incorporated as required, etc.

Separating a new, fourth generation of biofuels was proposed because of the need to close the carbon dioxide balance or to cut out the environmental impact of this compound. Therefore, the fourth generation biofuel technologies should be developed with considering the CCS ("Carbon Capture and Storage") at the raw material preparation and biofuel production stages. The raw materials used for production of such fuels should be the plants of increased $CO_2$ assimilation rates at the plant growing stage and the technologies applied must be devised considering the capture of carbon dioxide in proper geological formations by causing the carbonate stage to be reached or the storage in oil and gas exploitation cages.

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## 3. The main directions of advanced fuel technology's development

Within the planned perspective of the production and use of biofuels, the fuels are required: to be available in enough large quantities; to have acceptable technical and energy characteristics for being suitable for fueling engines or heating; to be inexpensive at both the production and sale stages; to cause smaller environmental hazard in comparison with the conventional fuels; to improve energy independence.

Based on the experience and on results of the research work carried out, we should strive in the nearest future to get biofuels as hydrocarbon blends produced by definite pathways. Such pathways will make it possible to get alternative fuels for IC engines with simultaneous closing of the $CO_2$ cycle. Therefore, the advanced biofuels should be:

- Synthetic biofuels made as blends of hydrocarbons produced in result of biomass gasification and pyrolysis [4] ( figures 1 and 2)

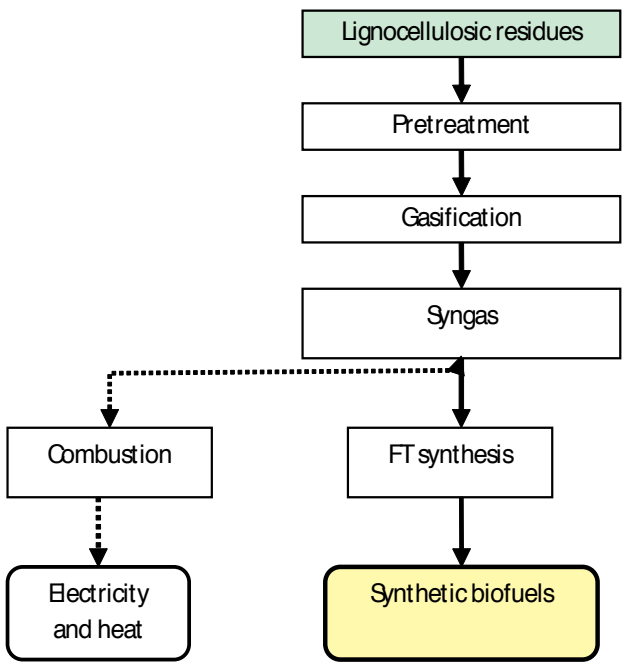


**Figure 1.** Schematic diagram of biomass to liquid process in Choren, Germany.

The main piece of biomass gasification technology is the patented Carbo-V process that allows to produce tar-free synthetic gas, a breakthrough for biomass to energy change. The gas consisting mainly of CO and $H_2$ can be used as a combustion gas for the generation of electricity, steam or heat, or for the make of transport fuels (BtL). Compared with fossil die-

sel, the combustion of BtL diesel reduces PM's (particulate matters) emissions by 30 to 50% and hydrocarbon emissions by up to 90 %. It achieves superior combustion characteristics while no engine adjustments are needed. But perhaps its most important feature is the ability to recycle atmospheric $CO_2$ into the fuel thus closing the sustainability cycle.

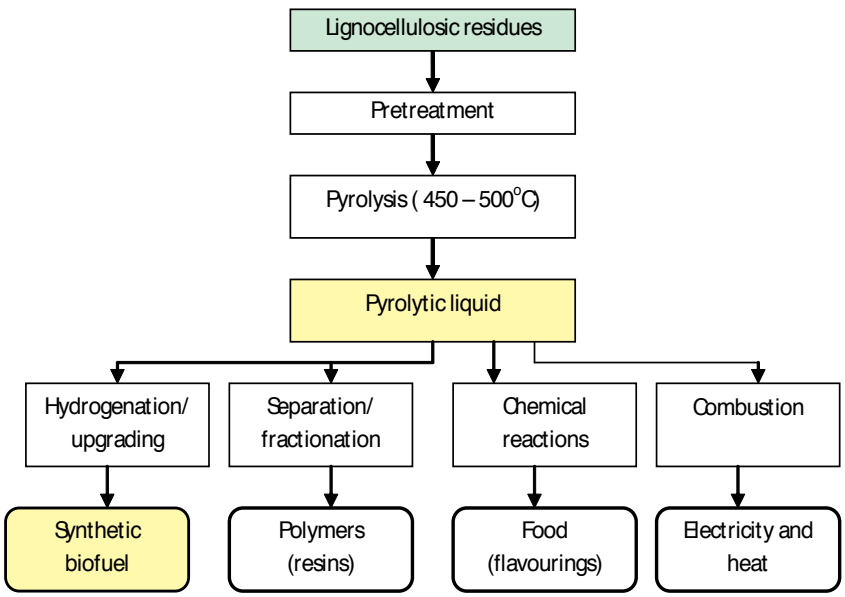


**Figure 2.** Schematic diagram of Rapid Thermal Processing (RTP) TM technology in Ontario, Canada (owner Ensyn). It is commercial installation.

Another promising technology is related to pyrolysis. Rapid Thermal Processing (RTP) is a fast thermal process where biomass is rapidly heated without oxygen. The biomass is vaporized and rapidly cooled to produce high yields of pyrolysis oil. The pyrolysis oil is fractionated into chemicals for engineered wood products (resins) and fuel for thermal applications. The resulting char and gases are used for energy. RTP™ typically yields 65 to 75wt% pyrolysis oil from dried woody biomass.

- Biofuels earned from biomass in result of other thermochemical processes, such as pyrolysis or processes of depolymerisation and hydrogenation of biomass decomposition products (hydrothermal upgrading-HTU processes);
- Fuel blends composed of hydrocarbons gained from biomass, including those directly or indirectly obtained from sugars in result of biological or chemical processes,
- Biofuels being other sugar derivatives;
- Biomethane and other gaseous fuels got from biomass gasification processes or agricultural, landfill, and sewage sludge treatment processes;

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- Bioethanol and higher alcohols -biobutanol and their derivatives, obtained from biomass in result of biochemical or catalyzed thermochemical processes (figure 3);

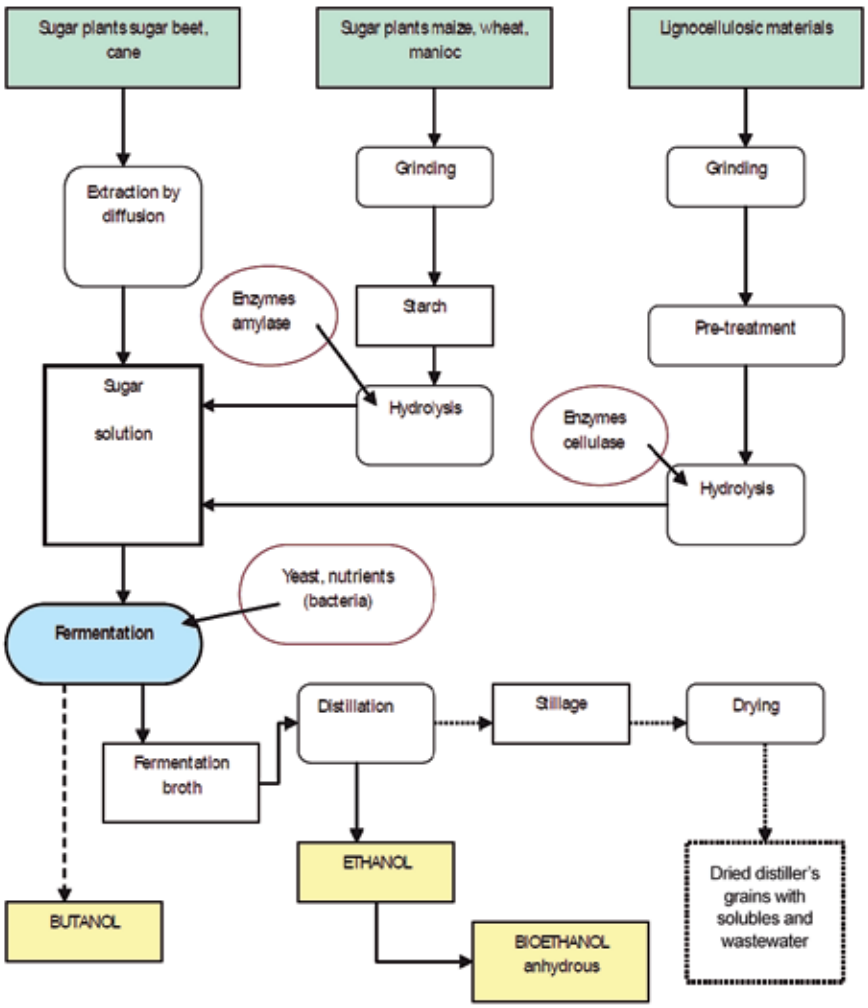


**Figure 3.** Processes of biochemical biomass conversion.

- Biofuels obtained by utilization of carbon dioxide for production of microorganisms or by direct or indirect synthesis of carbon dioxide of natural origin in thermochemical and biochemical;
- Biofuels obtained from synthetic gas produced as a product of direct or indirect (through methanol) conversion of biomass or GHG;
- Biofuels (HVO, hydrogenated vegetable oils) got by hydrogenation of waste vegetable and animal fats.

## 4. Application of selected types of biofuels of the first and second generation

Among the proposed alternative fuels, vegetable oils have received much attention in recent years for diesel engines owing to their advantages as renewable and domestically produced energy. The major disadvantage of pure vegetable oils is their inherently high viscosity, leading to poor fuel atomization, incomplete combustion, coking of fuel injectors, ring carbonization, and accumulation of vegetable oil in the lubricating oil. Several methods are consequently being used to reduce vegetable oil's viscosity. Blending of vegetable oils with an alcohol of lower viscosity is one of the methods [5, 6].

Main alcohols used as a fuels ingredient are: methanol, ethanol and n-butanol. These alcohols have different properties. Some of them are presented in Table 1. They are compared to conventional engine fuels.

| Fuel | Energy density | Heat of vaporization | Kinematic viscosity at 20°C |
|---|---|---|---|
| Diesel | 38.6 MJ/l | 0.47 MJ/kg | "/>3 cSt |
| Gasoline | 32.0 MJ/l | 0.36 MJ/kg | 0.4–0.8 cSt |
| Butanol | 29.2 MJ/l | 0.43 MJ/kg | 3.64 cSt |
| Ethanol | 19.6 MJ/l | 0.92 MJ/kg | 1.52 cSt |
| Methanol | 16.0 MJ/l | 1.20 MJ/kg | 0.64 cSt |

**Table 1.** The properties of different alcohols and engine fuels

It is interesting the butanol has similar energy density as petrol. Butanol is good solvent of heavy hydrocarbons (such diesel fuels). The mixture of these components is homogeneous and doesn't separate after several months. In contrast, ethanol is slightly soluble in diesel fuel. It is important the water is nearly insoluble in butanol, in contrast to ethanol which dissolves water in any proportion.

The old and new technology of butanol production is known as an ABE process (Acetone-Butanol-Ethanol) and the second generation process using lignocellulosic waste materials, respectively. The conventional ABE fermentation process is based on sugar's material (cane or beet) or starch (wheat, corn or rice) which is easily broken down into sugars. During the fermentation formed the three components: acetone, n-butanol and ethanol ( in ratio of 3:6:1) as main products. The process is performed by anaerobic gram- positive bacteria of the geneus clostridia ( mainly Clostridium acetobutylicum, but also C. Beijerinckii, C. butylicum and others). The ABE process is not profitable because of low productivity and poor selectivity. One of the courses covers metabolic engineering issues, that is modification of metabolic pathway to increase resisting clostridia bacteria to higher concentrations of fermentation products, and improve the efficiency and selectivity. Low yield of the fermentation of butanol synthesis requires research on butanol recovery techniques. There are

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many separation techniques of fermentation products, e. g. liquid-liquid extraction, perstraction, pervaporation (membrane separation with gaseous permeate discharge) combined with immobilization of bacterial cells, adsorption or reversed osmosis. It is estimated that effective solutions development can help to increase of profitability up to 40-50%.

The ideal feedstock for bioconversions could be waste biomass, for example straw, wood chips and paper pulp effluent. Also crops specially grown for their high biomass production rate (kenaf, miscanthus and short rotation woody crops). Such sources of raw materials can be described as "cellulosic biomass" because of their high cellulose and hemicellulose content. The feedstock used in fermentation determines the selection of strains and process conditions. The company Green Biologics is developing biobutanol production from glycerol and other wastes from industry and agriculture, using genetically modified thermophilic bacteria of the genus geobacillus and sells derived fuel named Butafuel (figure 4).

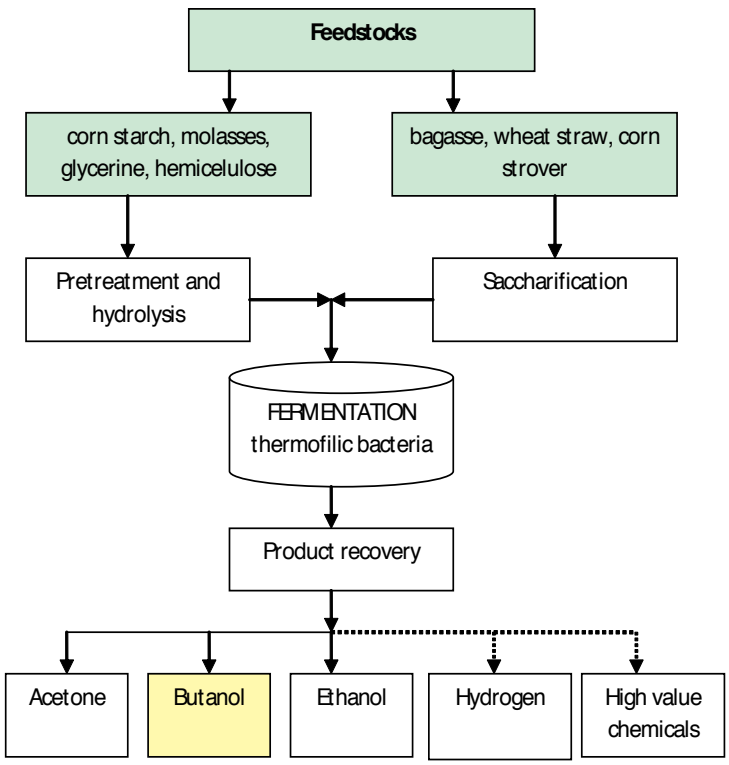


**Figure 4.** Schematic diagram of GBL's technology, Green Biologics Ltd. Biobutanol used to produce fuels, paints, coatings, resins, polymers and solvents.

Butanol, like ethanol, can blend well with gasoline. Biobutanol can replace gasoline in E85 fuel. Also, butanol could be a future for blending with diesel. Butanol contains more oxygen compared to the biodiesel, leading to further decline of soot. $NO_x$ emissions can also be reduced because of its higher heat of evaporation, which results in a lower combustion tem-

perature. The butanol has more advantages than the widely used ethanol and FAME. However, the main disadvantage of butanol is low production. Biobutanol is noncorrosive and can be shipped via pipeline.

| No. | Butanol isomers | Main application |
|---|---|---|
| 1 | 1-butanol | Gasoline additive, solvents, plasticizers, chemical intermediate, cosmetics |
| 2 | 2-butanol | Solvents, chemical intermediate, industrial cleaners, perfumes or in artificial flavors |
| 3 | iso-butanol | Gasoline additive, solvent and additive for paint, industrial cleaners, ink ingredient |
| 4 | tert-butanol | Gasoline additive for octane booster and oxygenate; intermediate for MTBE, ETBE, THBP; denaturant for ethanol; solvent |

**Table 2.** The main application of butanol isomers. Ref. [7]

This work presents a novel way of using alcohols and pure vegetable oil as fuels for a diesel engine. It was shown the possibility of use of higher alcohols as a solvent for straight vegetable oil (the mixture was named BM). Such a mixture, after getting the density similar to the density of diesel fuel, was mixed with diesel fuel (D) giving biomixdiesel (BMD). For BMD preparation was used the n-butanol and iso-amyl (by-product of ethanol fermentation) as an alcohol, rapeseed oil and conventional diesel fuel. Another biofuels as an example of second generation were obtained by nonoxidative thermal/pyrolytic cracking of straw (nearly 200 microns) followed by biooil hydrotreating. The last one- HVO diesel was obtained by catalytic hydroconversion of vegetable oil mixtures. Hydrotreated vegetable oils do not have the harmful effects of ester-type biodiesel fuels, like increased $NO_x$ emission, deposit formation, storage stability problems, more rapid aging of engine oil or poor cold properties. HVOs are straight chain paraffinic hydrocarbons that are free of aromatics, oxygen and sulfur and have high cetane numbers. All three biofuels were examined according to EN-590:2009 standard [8]

### 4.1. Experimental and results

#### *4.1.1. Assessment of the physico-chemical properties of the BMD biofuel*

To assess the quality of biofuel containing components such as higher alcohol and rapeseed oil were prepared two experimental blends based on previous works [9, 10]. Major scientific works regards diesel-biobutanol mixtures [11] and minor triple mixtures with vegetable oil. The main component of mixtures was conventional diesel (in 80% vol.), made up to 100% with two mentioned above biocomponets. Experiment was carried out with two higher alco-

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hols: n-butyl alcohol and iso-amyl alcohol. First were prepared blends consisting of selected alcohol and rapeseed oil in a ratio of 2:1 ( BioMix), and then received blend was introduced into diesel fuel (D). Prepared samples are marked with symbols BMD-1 (with n-butanol) and BMD-2 (with iso-amyl alcohol). Mixtures of BMD-1 and BMD-2 were clear, without haze and sediment. New biofuels stored for several days at room temperature showed no features of separation. Diesel fuel used to compose biofuels met all quality requirements according to EN-590. Table 3 shows the basic features of diesel, and Table 4 compares properties of n-butyl alcohol and iso-amyl.

| No. | Property | Result |
|---|---|---|
| 1. | Cetane number | 53,0 |
| 2. | Density at 15°C, kg/m$^3$ | 836,2 |
| 3. | Flash point, °C | 63 |
| 4. | Carbon residue (on 10% distillation residue), %(m/m) | <0,10 |
| 5. | Distillation<br>%(V/V) recovered at 250°C,<br>%(V/V) recovered at 350°C,<br>50%(V/V) recovered at , °C<br>95%(V/V) recovered at , °C<br>finish boiling point, °C | 39,5<br>94,9<br>266,7<br>350,5<br>362,4 |

**Table 3.** Basic physico-chemical properties of diesel fuel

| No. | Property | n-butyl alcohol | iso-amyl alcohol |
|---|---|---|---|
| 1. | Density at 20 °C, kg/m$^3$ | 810 | 814 |
| 2. | Boiling point °C | 117 | 138 |
| 3. | Flash point, °C | 30 | 43 |

**Table 4.** Properties of n-butyl alcohol and iso-amyl alcohol

Prepared biofuels samples were examined according with regulatory needs of the standard EN 590. The results got are presented in Table 5 and Table 6.

| No. | Property | Test method | Result | | Limits EN 590 |
|---|---|---|---|---|---|
| | | | BMD-1 | BMD-2 | |
| 1 | Cetane number | EN ISO 5165 | 44,4 | 45,0 | min 51,0 |
| 2 | Cetane index | EN ISO 4264 | 46,8 | 46,9 | min 46,0 |
| 3 | Density at 15ºC, kg/m$^3$ | EN ISO 12185 | 837,9 | 837,8 | 820,0 – 845,0 |
| 4 | Polycyclic aromatic hydrocarbons, % (m/m) | EN 12916 | 1,9 | 1,9 | max 11 |
| 5 | Sulfur content, mg/kg | EN ISO 20846 | 5,7 | 5,7 | max 10,0 |
| 6 | Flash point, ºC | EN ISO 2719 | < 40,0 | 45,0 | above 55 |
| 7 | Carbon residue (on 10% distillation residue), %(m/m) | EN ISO 10370 | 0,48 | 0,27 | max 0,30 |
| 8 | Ash content, %(m/m) | EN ISO 6245 | < 0,001 | < 0,001 | max 0,01 |
| 9 | Water content, mg/kg | EN ISO 12937 | 110 | 110 | max 200 |
| 10 | Total contamination, mg/kg | EN 12662 | <6,0 | 9,0 | max 24 |
| 11 | Copper strip corrosion (3 h at 50$^0$C) | EN ISO 2160 | class 1 | class 1 | class 1 |
| 12 | Lubricity, corrected wear scar diameter (wsd 1,4) at 60°C , µm | EN ISO 12156-1 | 281 | 339 | max 460 |
| 13 | Viscosity at 40ºC, mm$^2$/s | EN ISO 3104 | 2,710 | 2,827 | 2,00 – 4,50 |
| 14 | Distillation<br>%(V/V) recovered at 250ºC,<br>%(V/V) recovered at 350ºC,<br>50%(V/V) recovered at , ºC<br>95%(V/V) recovered at , ºC<br>Finish boiling point, ºC | EN ISO 3405 | 47,3<br>261,9<br>349,9 | 44,2<br>261,4<br>349,7 | < 65<br>min 85<br>max 360 |
| 15 | Fatty acid methyl ester content, FAME , %(V/V) | EN 14078 | < 1,6 | < 1,6 | max 7,0 |
| 16 | Oxidation stability, g/m$^3$ | EN-ISO 12205 | 66 | 39 | max 25 |

**Table 5.** Comparison of the results of biofuels BMD-1 and BMD-2 according to EN 590

| No. | Property | Test method | Result | | Climate-related requirements | | |
|---|---|---|---|---|---|---|---|
| | | | BMD-1 | BMD-2 | Summer | Spring and autumn | Winter |
| 1 | Cold filter plugging point, CFPP, ºC | EN 116 | -21 | -21 | max 0 | max -10 | max -20 |
| 2 | Cloud point, ºC | ISO 3015 | -6 | -6 | Limits only for arctic climate | | |

**Table 6.** Comparison of low-temperature properties of fuels BMD-1 and BMD-2 to the climatic requirements of EN 590

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Comparing the results of biofuels BMD-1 and BMD-2 with quality requirements for diesel fuel it is worth to note that most of the parameters meet these requirements; however, several features deviate from the normative requirements. Cetane number is similar on both biofuels and amounted 44.4 and 45.0 for BMD-1 and BMD-2, respectively and is lower than the required standard that is at least 51 units. This is because of sharing 20% biocomponents. Rapeseed oil has a cetane number about of 40-50 units and a small addition to the diesel fuel should not drastically reduce the cetane number. However alcohol is usually characterized by a high octane number, which is good in case of composing gasoline, added to the diesel fuel can degrade the diesel engine start-up parameters.

The process of starting engine and his operation is also influenced by fractional composition of fuel, particularly temperature distillation of 50% by volume of fuel, $T_{50}$. The lower the temperature $T_{50}$ the easier the start, but at too low temperature ignition characteristics fuel property is worsen - cetane number decreases.

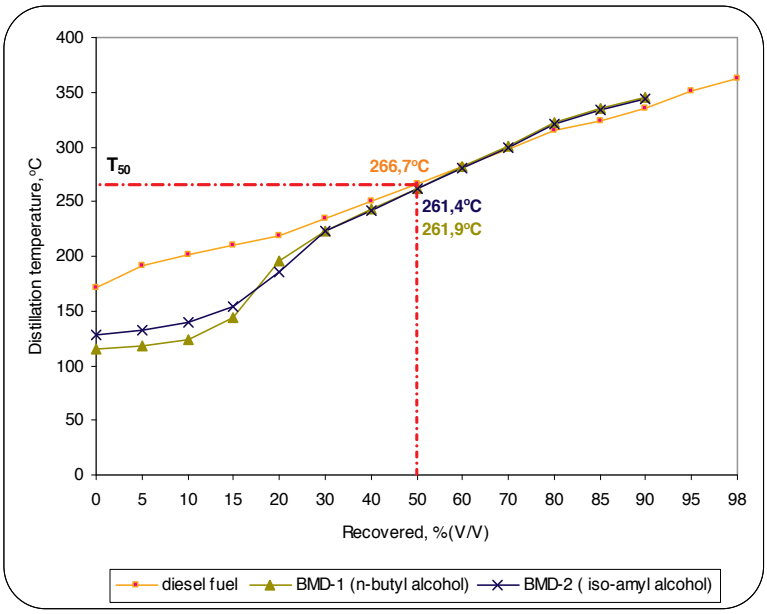


**Figure 5.** Distillation of diesel fuel, biofuels BMD-1 and BMD-2 comparison.

Figure 5 shows the distillation composed of biofuels compared to diesel. $T_{50}$ temperatures for the tested biofuels BMD-1 and BMD-2 are similar and amounted 261.9 and 261.4°C, respectively and slightly differs from the $T_{50}$ for diesel fuel - 266.7 °C. The temperature range from about 250 °C to 340 °C showed the curves of the distillation of biofuels and diesel fuels are similar. The beginning of the distillation is unusual for sample BMD-1, which begins to boil in temperature 114°C, and BMD-2 at a temperature of 127°C. Alcohol is distilled off first at the early stage of the distillation followed by hydrocarbons and rapeseed oil. Distillation points out the biofuel combustion in the engine can be irregular.

Contributing alcohol in biofuel can cause the decline of ignition temperature. Flash point of biofuel blend is determined by its flash point of the lightest ingredient, which n-butyl alcohol and iso-amyl alcohol was 37 °C, and 45 °C, respectively. Safety in transport and storage of diesel fuel requires the ignition temperature be higher than 55°C. Thus, the fuel with a lower flash point cannot be marketed and sold, could possibly be used as fuel for selected fleets.

The tested biofuel has a high tendency to form sludge and carbon deposits, which is determined by the remains of carbon residues. BMD-1 sample carbon residue value exceeds regulatory requirements by 60%, for the BMD-2 approaches the limit. Biofuel with such high carbon residues will cause form deposits in the combustion chamber, the valves, piston rings and injector parts. Sediments and carbon residues can change conditions of heat exchange, worsen the quality of fuel atomization and eventually can lead to immobilization of the vehicle.

Both samples of biofuels have good low temperature properties (Table 6). Tested cold filter blocking temperature for both samples amounted -21 °C, and the cloud temperature -6°C and slightly was different from the similar parameters of diesel. Low-temperature stability studies have shown that biofuel stored for several days at a temperature of about-10°C becomes cloudy, but segregation was not observed and had the liquid properties. Viscosity of biofuels is correct and for BMD-1 and BMD-2 were 2.710 $mm^2/s$ and 2.827 $mm^2/s$, respectively. Proper fuel viscosity is important, because directly influence on the quality of atomization and combustion. Other biofuels quality parameters measured; do not differ from the normative requirements. The sulfur and water content, polycyclic aromatic hydrocarbons, solids, ash residue, lubricity and density are within the limits. Please note that this biofuel may not be used for long-term storage, it is unsatisfactory because of their oxidation stability. The sludge after being marked with an accelerated aging process is large, about 2 times the standard requirements. It is therefore recommended product produced in small quantities, intended for fast using.

Tested biofuels BMD-1 and BMD-2 were assessed for regulatory quality requirements. It is difficult to clearly settle, which biofuel blends is better. Features such as low cetane number, low flash point, and atypical distillation limit the usefulness of both biofuels to power the diesel engines. Preliminary experiments should be continued for improvement to compose biofuels and carry out the procedure for selecting additives. It is necessary to increase the cetane number. The proper corrosion protection should be considered because of the presence of alcohol ingredient in biofuel. To introduce new BMD biofuels still needs much research and formula improvements.

### *4.1.2. Emission test for BMD fuel*

Based on the physicochemical properties of biofuels BMD-1 and-2 for further test was selected BMD mixture with n-butanol only. Prepared under laboratory conditions mixtures of n-butanol with diesel fuel were examined on the chassis dynamometer. In the first step the rapeseed oil was mixed with butanol as such parts to obtain a mixture having a density similar to the density of diesel fuel. This mixture is denoted as a BM (BioMix). In the second

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step this fuel (BM) was mixed with conventional diesel fuel (D) to get biomixdiesel (marked as a BMD). These fluids were mixed in the following parts:

- biomix (BM) 20 % v/v,
- diesel fuel (D) 80 % v/v,

giving fuel called as biomixdiesel (BMD20). In contrast to the mixture of ethanol with rape methyl ester and conventional diesel fuel, this mixture is homogeneous. The comparison of new fuel with requirements of the standard diesel fuel is presented in Table 7.

| No. | Property | Test method | Results BMD20 | Limits EN 590 |
|---|---|---|---|---|
| 1 | Cetane number | EN ISO 5165 | 44,4 | min 51,0 |
| 2 | Cetane index | EN ISO 4264 | 46,8 | min 46,0 |
| 3 | Density at 15ºC, kg/m$^3$ | EN ISO 12185 | 837,3 | 820,0 – 845,0 |
| 4 | Polycyclic aromatic hydrocarbons, %(m/m) | EN 12916 | 1,9 | max 11 |
| 5 | Sulfur content, mg/kg | EN ISO 20846 | 5,7 | max 10,0 |
| 6 | Flash point, °C | EN ISO 2719 | < 40,0 | above 55 |
| 7 | Carbon residue (on 10% distillation residue), % (m/m) | EN ISO 10370 | 0,48 | max 0,30 |
| 8 | Ash content, %(m/m) | EN ISO 6245 | < 0,001 | max 0,01 |
| 9 | Water content, mg/kg | EN ISO 12937 | 112 | max 200 |
| 10 | Total contamination, mg/kg | EN 12662 | <6,0 | max 24 |
| 11 | Copper strip corrosion (3 h at 50$^0$C) | EN ISO 2160 | class 1 | class 1 |
| 12 | Lubricity, corrected wear scar diameter (wsd 1,4) at 60°C , µm | EN ISO 12156-1 | 281 | max 460 |
| 13 | Viscosity at 40ºC, mm$^2$/s | EN ISO 3104 | 2,710 | 2,00 – 4,50 |
| 14 | Distillation<br>%(V/V) recovered at 250°C,<br>%(V/V) recovered at 350°C,<br>95%(V/V) recovered at , °C<br>Finish boiling point, °C | EN ISO 3405 | <br>47,3<br>349,9 | <br>< 65<br>min 85<br>max 360 |
| 15 | Fatty acid methyl ester content, FAME , %(V/V) | EN 14078 | < 1,6 | max 7,0 |
| 16 | Oxidation stability, g/m3 | ISO 12205 | 66 | max 25 |

**Table 7.** Properties of investigated fuel

The investigations of fuel properties under working conditions were carried out with a modern diesel engine on the chassis bed dynanomyter in the NEDC test (New European Driving Cycle). This test consists of two parts: UDC (Urban Driving Cycle) and EUDC (Extra Urban Driving Cycle). The first part represents urban driving, in which a vehicle is started in the morning (after being parked all-night) and driven in stop-and-go rush hour traffic. The maximum speed is 50 km/h. The second part represents extra-urban driving with a maximum speed of 120 km/h.

Main parameters of car engine (power, torque, specific fuel consumption) and the main exhaust gas ingredient (in this case CO, $CO_2$, $NO_x$, total hydrocarbons-THC, particulate matter-PM, THC+ $NO_x$) and fuel consumption is evaluated and explain here in g/km. In Table 8 obtained are presented results.

| Emission | Fuel | Pollutants, g/km | | | | | | Fuel consumption |
|---|---|---|---|---|---|---|---|---|
| | | THC | CO | $CO_2$ | $NO_x$ | THC+$NO_x$ | PM | g/km |
| UDC | BMD 20 | 0,1000 | 1,3900 | 163,6367 | 0,1933 | 0,2967 | 0,0042 | 6,2467 |
| UDC | Diesel | 0,0833 | 1,3400 | 162,1367 | 0,2000 | 0,2867 | 0,0053 | 6,1933 |
| EUDC | BMD 20 | 0,0100 | 0,0367 | 118,7567 | 0,2133 | 0,2267 | 0,0064 | 4,4667 |
| EUDC | Diesel | 0,0100 | 0,0467 | 114,5500 | 0,1600 | 0,1700 | 0,0078 | 4,3167 |
| NEDC | BMD 20 | 0,0467 | 0,5367 | 135,2933 | 0,2067 | 0,2533 | 0,0056 | 5,1233 |
| NEDC | Diesel | 0,0367 | 0,5233 | 132,1100 | 0,1767 | 0,2167 | 0,0069 | 5,0100 |

**Table 8.** Examples of investigations results on the car test chassis bed by NEDC test load and by fueling the engine with examined BMD20 and standard Diesel fuel

Supplying the car engine with different fuel leads to a diversity of parameters of the engine. But the differences are not so significant. Differences between the results got for the tested BMD20 fuel and diesel fuel are presented below. The results of investigations of pollutant emission are presented here as the results got by fueling the engine with the conventional diesel fuel. The results expressed in g/km are shown in Table 9.

| Test | Pollutants | | | | | | Fuel consumption |
|---|---|---|---|---|---|---|---|
| | THC | CO | $CO_2$ | $NO_x$ | THC+$NO_x$ | PM | |
| | g/km | | | | | | |
| UDC | 0,0167 | 0,0500 | 1,5000 | -0,0067 | 0,0100 | -0,0011 | 0,0533 |
| EUDC | 0,0000 | -0,0100 | 4,2067 | 0,0533 | 0,0567 | -0,0014 | 0,1500 |
| NEDC | 0,0100 | 0,0133 | 3,1833 | 0,0300 | 0,0367 | -0,0013 | 0,1133 |

**Table 9.** Relative changes of pollutants emission and fuel consumption by fueling the car engine with BMD20 and Diesel fuel

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The results are presented in graphical form as well on the Figure 6.

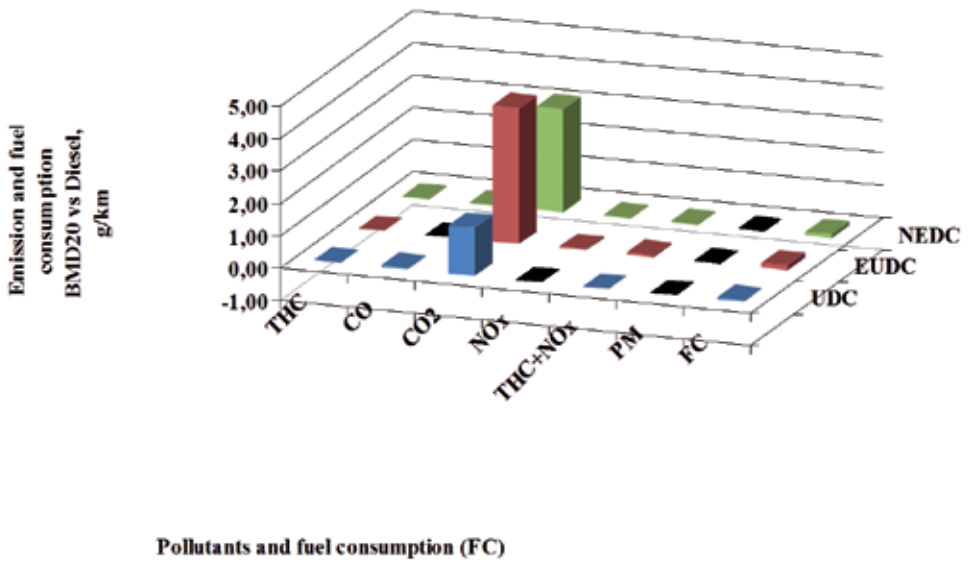


**Figure 6.** The differences in emission and fuel consumption during fueling the car engine with BMD20 and Diesel fuel recorded during the test bed investigation of the car

In all phases of the test, during fueling the engine with BMD20, the increase of the carbon dioxide ($CO_2$) is observed. Emissions of other toxic components and fuel consumption don't differ from those when the engine is fueled by conventional fuel.

Small differences in the results are becoming clear when relative changes are expressed in percentages. The results of this analysis are presented in Table 10 and pictured on Figure 6.

| Test | Pollutants | | | | | | Fuel consumption |
|---|---|---|---|---|---|---|---|
| | THC | CO | $CO_2$ | $NO_x$ | THC+$NO_x$ | PM | |
| | % | | | | | | |
| UDC | 20,00 | 3,73 | 0,93 | -3,33 | 3,49 | -20,75 | 0,86 |
| EUDC | 0,00 | -21,43 | 3,67 | 33,33 | 33,33 | -18,38 | 3,47 |
| NEDC | 27,27 | 2,55 | 2,41 | 16,98 | 16,92 | -18,93 | 2,26 |

**Table 10.** Relative changes of pollutants emission and fuel consumption during engine fueling with BMD20 and Diesel fuel

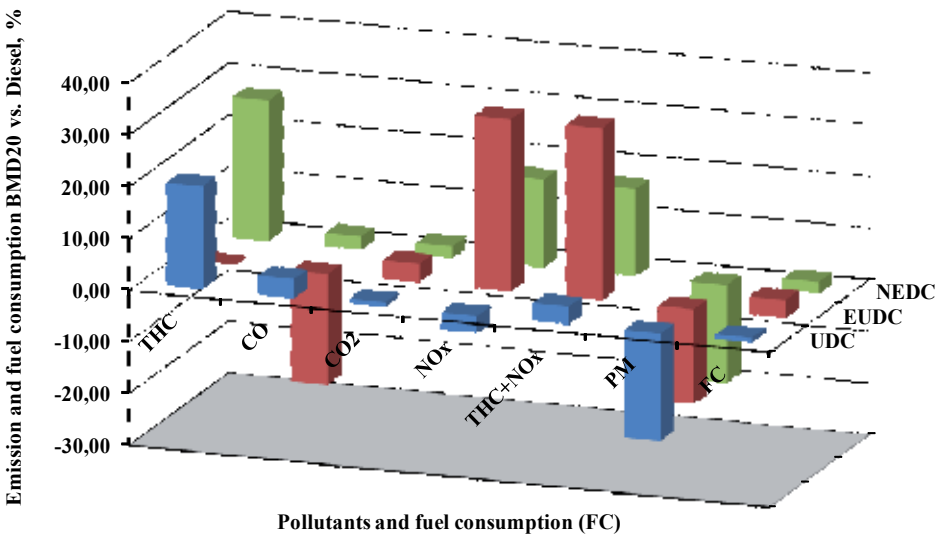


**Figure 7.** Relative changes of emissions and fuel consumption of the car engine fueled with BMD20 and diesel oil.

During fueling the car engine with the BMD20, the fuel consumption is not significantly different (Table 10) from that noted for Diesel fuels. The differences in emitting pollutants were dependant on the test phase (UDC or EUDC). For example the emission of THC in the EUDC phase is the same as that recorded for diesel oil, but the quantity of THC grows in UDC phase. Thus in full test (NEDC) the relative emission of THC grows. Other trend is observed for carbon monoxide (CO) emissions. In the UDC phase emission of CO slightly increases, in the EUDC phase significantly decreases (more then 21%) so, therefore in the NEDC test the emission of CO slightly grows. The emissions of $NO_x$ grows, first, in the EUDC phase. This is understandable if we take into consideration the engine load – the higher combustion temperatures (peaks) in this phase favor to form nitrogen oxides. In the same phase the emission of THC neither increases nor decreases, so in the entire test, the summary quantity of THC+$NO_x$ increases (the emission of THC increases in the UDC phase). It is important that, the PM emission decreases in all phases of test. The decrease is significant, about 21% in the UDC phase and more than 18% in the EUDC phase.

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Results were obtained without any change of engine control parameters (the engine control parameters were the same as during supplying the engine with conventional diesel fuel). It seems that after optimization of engine control features the results would be much better.

### 4.2. Assessment of the physico-chemical properties of the synthetic diesel fuel obtained by biomass depolymerization

Biomass depolymerization is a process for the reduction of complex organic materials. Under pressure and heat, long chain polymers of hydrogen, oxygen, and carbon decompose into biocrude oil. The depolymerization process for fuel production from organic materials takes two forms, thermal only or with assisted catalysts usually aluminum silicate type doped with non-precious metals for example Na, Ca. Although the thermal depolymerization has been understood for some time, human-designed processes were not efficient enough to serve as a practical source of fuel because more energy was required than was produced. Research breakthroughs in the 1980's led to efficient processes that were eventually commercialized [12, 13]. Green diesel was obtained by non-oxidative thermal/pyrolytic cracking of straw (around 200 micron) followed by biooil upgrading in hydrogen process. Fuel had a clear yellow color and has been tested in accordance with EN 590, one parameter not included in the specification standard (iodine number) was checked because of some doubts about non-saturated hydrocarbons content. It's known that if these hydrocarbons are present in significant quantity in the fuel, than it will cause polymerization and change the physico-chemical properties of the fuel.

| No. | Property | Test method | Results | Limits EN 590 |
|---|---|---|---|---|
| 1 | Cetane number | EN ISO 5165 | 58,6 | min 51,0 |
| 2 | Density at 15ºC, kg/m$^3$ | EN ISO 12185 | 815 | 820,0 – 845,0 |
| 3 | Polycyclic aromatic hydrocarbons, %(m/m) | EN 12916 | 0,8 | max 11 |
| 4 | Sulfur content, mg/kg | EN ISO 20846 | 120 | max 10,0 |
| 5 | Flash point, ºC | EN ISO 2719 | 43,5 | above 55 |
| 6 | Water content, mg/kg | EN ISO 12937 | 150 | max 200 |
| 7 | Viscosity at 40ºC, mm$^2$/s | EN ISO 3104 | 3,45 | 2,00 – 4,50 |
| 8 | Iodine number , gI/100g | | 8 | |

**Table 11.** The results of synthetic biofuel (Green diesel) according to EN 590.

Discussion of above parameters in Table 11 are presented below

- Cetane number - the standard EN 590 required a minimum cetane number- 51, fuels with higher cetane number have shorter ignition delays, providing more time for the fuel combustion process to be completed. Generally the engine is "soft", it's easier on the speed falls, helps to start the engine, slows pollution injector nozzles, limits the participation of PM in the exhausted gas and reduces engine noise. In the tested fuel cetane number was 58.8.
- The content of polycyclic aromatic hydrocarbons (PAHs) - in the test fuel PAH content amounted 0.8 and was significantly below the maximum value 11
- Density at 15 ºC - has been marked below the lower limit of the density of 820 kg/m$^3$ and amounted 818 kg/m$^3$ for tested fuel. It could be adjusted by distillation the light fraction.
- Water content - the test fuel contained 170 mg/kg of the water and the result was below the maximum value of 200 mg/kg.
- Flash point - It was found the ignition temperature of the test fuel is below the value of 55 ºC and was 43.5 ºC, which was caused by the presence of a light fraction of the fuel.

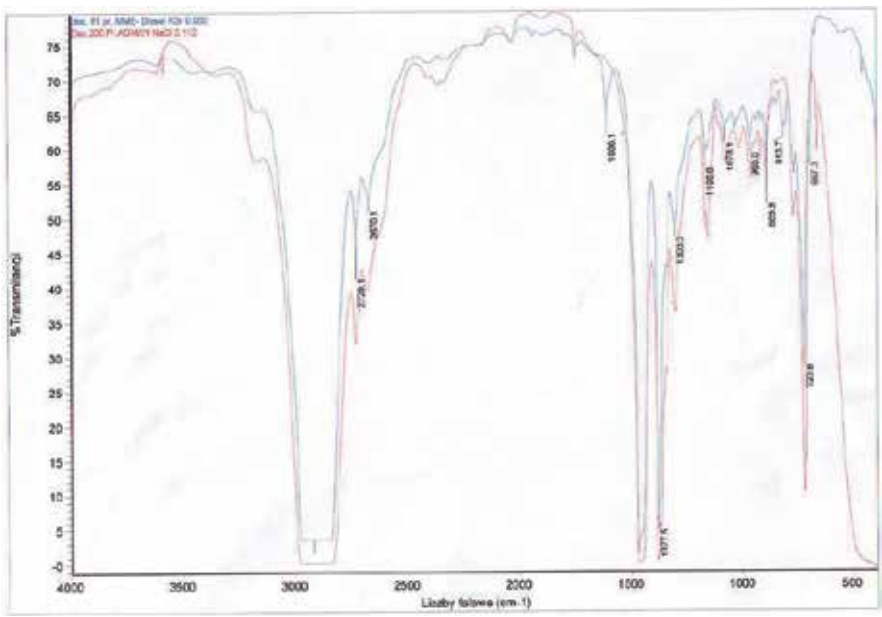


**Figure 8.** The comparison of IR spectrums of commercial diesel oil (red spectrum) and a sample of the green diesel (blue spectrum).

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The other limits of EN 590 were not tested as the fuel is still under technological development but above results are promising. Sulfur content in fuel after preliminary hydrodesulphurization process significantly exceeded specification value of 10 ppm Infrared spectrum of green diesel was compared with spectrum of commercial diesel oil (Figure 8).

IR spectrums of analyzed commercial diesel and green diesel were similar in appearance, what shows that both fuels contain similar chemical groups. The spectrums show little difference, that reveals the presence in the spectrum of green diesel peaks at wave number 1606 $cm^{-1}$ and around 814 $cm^{-1}$. These bands can be attributed to the unsaturated bonding (alkenes, aromatics). The spectrum has also characteristic bands of the groups - $CH_3$ and-$CH_2$ alkanes, including alkanes having carbon numbers greater than four, wave number band around 723 $cm^{-1}$. The lower band intensity in comparison with the spectrum of commercial diesel may point out a lower content of long-chain hydrocarbon sample. Analyzes mark the tested green diesel could meet the needs for diesel EN 590 if the level of sulfur below 10 ppm, increased the density and required temperature of ignition would be achieved.

### 4.3. Assessment of the physico-chemical properties of the synthetic diesel fuel – HVO diesel

Hydrotreating of vegetable oils or animal fats is an alternative to esterification for producing FAME. Hydrotreated vegetable oils do not have the harmful effects of ester-type biodiesel fuels, like increased $NO_x$ emission, storage stability problems and poor cold properties. HVOs are straight chain paraffinic hydrocarbons that are free of aromatics, oxygen and sulfur and have high cetane numbers. Examined HVO diesel oil was obtained at demonstration plant by catalytic hydroconversion of straight rapeseed oil. Selected features of HVO diesel are presented in Table 12.

Characteristics presented in Table 12 confirmed superior properties of HVO diesel compared with commercial diesel oil. It does not contain polynuclear aromatic and has low sulfur content. HVO diesel does not exceed the limit values for water. This product has a low tendency to foam, test parameters meet the requirements of EN 590 grade F. This means HVO diesel itself, without additives has excellent winter properties. Also the HVO diesel had a high resistance to oxidation which 2.5 times exceeds the needed normative values. During the HFRR test (High Frequency Reciprocating Rig, not presented here) was indicated the lubricant film produced by the sample is unstable, which is obvious at the early stage of the test. Typical diesel oil film thickness should exceed 90%, while in the HVO diesel case was only 78%. This shows the inadequate lubricating properties of the sample and that a lubricating additive is necessary. HVO is based on the hydrotreating, which could be used not only to convert plant-derived oils such as soybean, rapeseed, and palm, but also non-edible oils, such as Jatropha and algal oils as well as animal fats [14]. UOP and Eni have developed the Ecofining ™ based on conventional hydroprocessing technology to produce diesel-fuel ("green diesel") [15] or jet-fuel [16]. Similar technologies have been developed by Neste Oil (NExBTL Renewable Diesel) [17] and Petrobras (H-Bio process) [18].

| No. | Property | Test method | Results | Limits EN 590 |
|---|---|---|---|---|
| 1 | Cetane number | EN ISO 5165 | "/> 76 | min 51,0 |
| 2 | Cetane index | EN ISO 4264 | 91,8 | min 46,0 |
| 3 | Density at 15ºC, kg/m3 | EN ISO 12185 | 776,5 | 820,0 – 845,0 |
| 4 | Polycyclic aromatic hydrocarbons, | EN 12916 | | max 11 |
| | mono, %(m/m) | | < 6,0 | |
| | di+, %(m/m) | | < 1,0 | |
| | tri+, %(m/m) | | < 0,1 | |
| 5 | Sulfur content, mg/kg | EN ISO 20846 | < 3 | max 10,0 |
| 6 | Flash point, °C | EN ISO 2719 | 71 | above 55 |
| 7 | Carbon residue (on 10% distillation residue), %(m/m) | EN ISO 10370 | < 0,01 | max 0,30 |
| 8 | Ash content, %(m/m) | EN ISO 6245 | < 0,001 | max 0,01 |
| 9 | Water content, mg/kg | EN ISO 12937 | 20 | max 200 |
| 10 | Total contamination, mg/kg | EN 12662 | 1 | max 24 |
| 11 | Copper strip corrosion (3 h at 500C) | EN ISO 2160 | class 1 | class 1 |
| 12 | Lubricity, corrected wear scar diameter (wsd 1,4) at 60°C , µm | EN ISO 12156-1 | 313 | max 460 |
| 13 | Viscosity at 40ºC, mm2/s | EN ISO 3104 | 2,609 | 2,00 – 4,50 |
| 14 | Viscosity at 50ºC, mm2/s | EN ISO 3104 | 2,183 | --- |
| 15 | Distillation | EN ISO 3405 | | |
| | Initial boiling point, oC | | 190,4 | -- |
| | 5 %(V/V) recovered at , oC | | 225,4 | -- |
| | 10 %(V/V) recovered at , oC | | 242,6 | -- |
| | 20 %(V/V) recovered at , oC | | 259,0 | -- |
| | 30 %(V/V) recovered at , oC | | 267,3 | -- |
| | 40 %(V/V) recovered at , oC | | 271,8 | -- |
| | 50 %(V/V) recovered at , oC | | 274,9 | -- |
| | 60 %(V/V) recovered at , oC | | 277,6 | -- |
| | 70 %(V/V) recovered at , oC | | 280,3 | -- |
| | 80 %(V/V) recovered at , oC | | 283,8 | -- |
| | 90 %(V/V) recovered at , oC | | 288,2 | -- |
| | 95 %(V/V) recovered at , oC | | 291,9 | -- |
| | Finish boiling point, oC | | 306,1 | max 360 |
| | Recovery, %(V/V) | | 98,3 | -- |
| | Residue, %(V/V) | | 1,2 | -- |
| | %(V/V) recovered at 250oC | | 13,5 | < 65 |
| 16 | CFPP, oC | EN 116 | -19 | * |
| 17 | Cloud point, oC | ISO 3015 | -18 | * |
| 18 | Pour point, oC | ISO 3016 | -21 | -- |
| 19 | Oxidation stability, g/m3 | ISO 12205 | 10,5 | max 25 |

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| No. | Property | Test method | Results | Limits EN 590 |
|---|---|---|---|---|
| 20 | Heating value<br>- lower, MJ/kg<br>- higher, MJ/kg | PN-C-04062 | <br>43,7<br>46,8 | <br>--<br>-- |
| 21 | Composition:<br>- hydrogen, %(m/m)<br>- carbon, %(m/m)<br>- nitrogen, %(m/m) | ASTM D5291 | <br>14,7<br>85,5<br>0,1 | <br>--<br>--<br>-- |

*- depending on the climatic requirements

**Table 12.** Comparison of the results of synthetic biofuel (HVO diesel) with EN 590.

## 5. Conclusions

Blends of n-butanol, rapeseed oil and conventional diesel fuel showed promising results and what is most important the biofuel was prepared by simple blending of biocomponents with diesel oil. Nearly the same fuel consumption compared with diesel oil was noted and the emissions of main toxic compounds including PM decreased. The other two second generation Green and HVO goals met main requirements of standard specification EN 590. The use of biofuels in transport depends on few causes like: availability of raw materials, low cost production of biofuels, low selling price, the calorific value, high quality and compliance with the needs of fuels for automotive engines.

Synthetic hydrocarbon fuels, considered as the best solution replacements of fossil fuels, may be obtained by biomass gasification followed by FT process, biomass pyrolysis towards biocrude oil followed by catalytic upgrading, by novel hydrothermal upgrading (HTU) getting biocrude with low content of oxygen for further upgrading. Currently, because of promoting the use of biofuels, diesel is a mixture of petroleum hydrocarbon fractions, and fatty acid methyl esters (FAME). On the market there are the most common fuel oils with a content of 7 and 20% vol. FAME. The research results has shown that diesel fuel can be formulated using other biocomponents like higher alcohols such as biobutanol, pure vegetable oil. Biofuels should have a high cetane number, high calorific value, normal rheological properties and proper viscosity. Also important is the fuel spray and its evaporation in the engine. Biofuel quality with many of biocomponents should be thoroughly investigated, because the individual components may interact with fuel system materials. For example support of proper emulsifiers are needed in diesel fuel containing ethanol, because it allows to preserve of uniformity of fuel at low temperatures. Synthetic biohydrocarbons are chemical compounds with similar physico-chemical properties characteristic for middle distillates used in the production of petroleum diesel. By changing the conventional fuel formula one have to be aware of the requirements posed by modern fuels car engines, in this respect must be maintained full compliance requirements.

Biofuels considered in this work were compared with diesel oil from the point of view of various physico-chemical limits included in EN 590: 2009 standard and the new technical specification under development by CEN (European Committee for Standardization) under the title: Automotive fuels - Paraffinic diesel fuel from synthesis or hydrotreatment- requirements and test methods, and based on recommendations of the world's car companies members of the Committee for the Affairs of the Worldwide Fuel Charter (WWFC). Figure 9 summarized the technologies used for production biofuels discussed in this chapter. Some of them like HVO diesel (NExBTL) are already commercially available, and others are at demo stage.

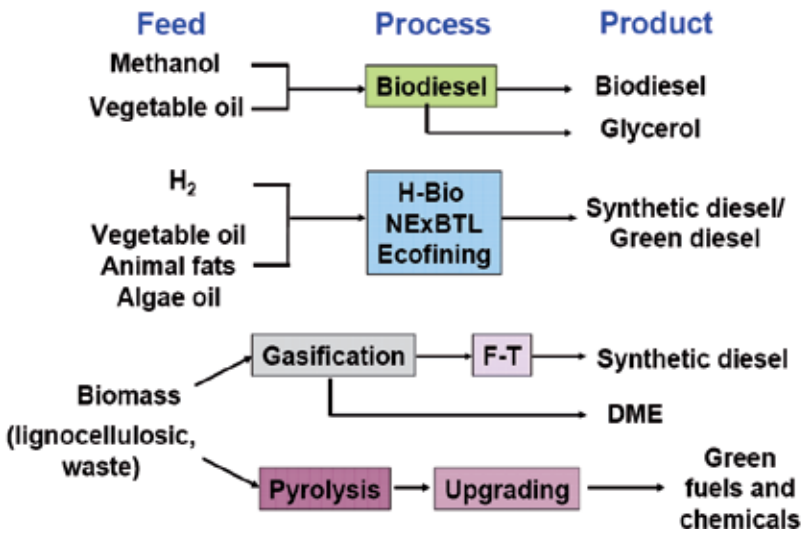


**Figure 9.** Current (green rectangle) and future (the blue and pink rectangles) most promising ways for producing of second generation biodiesel [19]

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Chapter 17

# Physico-Chemical Characteristics of Particulate Emissions from Diesel Engines Fuelled with Waste Cooking Oil Derived Biodiesel and Ultra Low Sulphur Diesel

Raghu Betha, Rajasekhar Balasubramanian and Guenter Engling

Additional information is available at the end of the chapter

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## 1. Introduction

A ground breaking invention in 1893 by Rudolf Diesel (Diesel Engine – named after him) made a mark in the world of internal combustion engines; his engine was the first one to prove that fuel could be ignited without a spark. Since this invention, diesel engines have been widely used in various applications such as automobiles, agriculture, ships, electricity generators, construction equipment etc., all over the world. Diesel engines were proved to be very efficient in terms of delivering the required energy levels for their use at very low operating and maintenance costs when compared to gasoline engines. However, diesel engines now pose a serious threat to human health and adversely impact the urban air quality (Sydbom et al., 2001). Diesel engine exhaust contains a host of harmful substances including airborne particulate matter (PM), carbon soot, toxic metals, polycyclic aromatic hydrocarbons (PAHs), nitrogen oxides which induce ozone formation, carbon monoxide, carbon dioxide, volatile organic compounds and other compounds such as formaldehyde and acrolein (EPA, 2002). Of these pollutants, PM from diesel exhaust is of great concern because of a number of reasons: (1) Diesel engines are known to be the largest source of PM from motor vehicles. Two thirds of PM emitted from mobile sources are from diesel vehicles (EPA, 2002); (2) Human exposure to diesel exhaust particles (DEP) is high as these particles are emitted at ground level unlike that of smoke stacks. United Stated of Environmental Protection Agency (USEPA) reported that 83% of people living in the USA are exposed to concentrated diesel emissions from sources such as highways, heavy industries, construction

sites, bus and truck depots etc (EPA, 1999); (3) The freshly emitted DEP includes ultra fine particles (UFPs, aerodynamic diameter (AED) < 100 nm). These particles can bypass the natural defense of respiratory tract and enter deep into the alveolar region of respiratory system from where they could enter into blood stream (Oberdorster, 2001); (4) The particulate-bound soluble organic compounds such as PAHs, nitro-PAHs and transition metals are considered mutagenic or carcinogenic. PM from diesel exhaust is listed as a ''likely Carcinogen,'' citing cancer risk in the range of one in 1000 to one in 100,000 people for each microgram of annual average exposure (EPA, 2002).

Apart from the health impacts, DEP also has potential environmental impacts. Black carbon or soot from diesels affects cloud cover and is a significant contributor to atmospheric warming (Hansen et al., 2000). In view of these concerns and also to meet the current and future regulation standards imposed by local environmental protection agencies, a large number of researchers have conducted research to reduce the diesel particulate levels by various methods such as introducing new engine designs (Guerrassi and Dupraz 1998; Park, et al., 2004), development of particle trap systems and after-treatment devices (Stamatelos 1997), improving the fuel quality (Kaufmann, et al., 1999). Despite these developments over many decades which improved the emission quality of diesels without any doubt, diesel engines still represent a significant source of PM. Holmen and Ayala (2002) reported that the use of particle trap systems reduces total particle number concentration by 10 to 100 times from diesel exhaust, but also found that the use of particle trap-equipped diesel engines may sometimes result in elevated nanoparticle (diameter < 50 nm) emissions. Also, there is an ongoing debate on whether the diesel engine can be modified and improved continuously down to meet the future regulation standards without economic impact. It is believed that developments on engine design needed to meet upcoming regulations would increase the costs and eventually gasoline vehicles could take the place of diesel vehicles (Pischinger, 1996).

As an alternative strategy to improve emission quality of diesels, the option of replacing diesel or petroleum based fuels by renewable bio-fuels is gaining popularity in recent years. Biodiesel (also known as fatty acid alkyl esters), an alternative fuel derived through transesterification process from vegetable oils or animal fats, has received much attention as a result of renewed interest in renewable energy sources for reducing particulate and greenhouse gas (GHG) emissions from diesel engines. In addition, it also helps in alleviating the depletion of fossil fuel reserves (Pahl, 2008; Janauan and Ellis, 2010). Biodiesel is reported to be carbon neutral because of the lower net carbon dioxide production (Ferella et al., 2010; Gunvachi et al., 2007; Carraretto et al., 2004), making it an important fuel source in the era of climate change. Another main driving force for biodiesel is the lower emissions of PM, CO, hydrocarbon, aromatic and polycyclicaromatic compounds (Xue et al., 2011, Atadashi et al., 2010). In view of these advantages, usage of biodiesel is increasing rapidly. This is reflected in government policies such as partial detaxation, investment in production and research of biodiesel by many countries that include USA, Brazil, European Union, South East Asian and other countries all over the world. With these increased awareness and governmental policies of different countries, annual production of biodiesel nearly tripled globally be-

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tween 2000 and 2005. According to National Biodiesel Board (NBB) in USA alone 460 million gallons of biodiesel were sold in 2007, 700 million gallons in 2008, and 802 million gallons in 2011, showing a tremendous raise from 2 million gallons sold in 2000. This move from fossil fuels to bio-fuels as a power source is also caused by the economic consequences due to stringent regulations ((EN-590, 2004) in Europe and (ASTM-D-975, 2006) in USA) imposed on the fuels used in transportation. The notable restriction includes reducing sulfur content in the fuel which increased the investment cost of oil companies and the final fuel price which drive the nations to develop their own reserves indigenously and decrease their dependence on Middle East countries for fuel.

Biodiesel is compatible with conventional petroleum based-diesel and can be completely blended with diesel in any proportion. The chemical composition and several properties of biodiesel make it an attractive option over traditional diesel. Biodiesel has higher cetane number, lubricity, combustion efficiency, biodegradability and lower sulfur and aromatic content (Fazal et al., 2011; Demirbas, 2008). In contrast, there are also unfavorable properties in biodiesel such as being more prone to oxidation, lower heating value, and higher cloud and pour points (Szulczyk and McCarl, 2010). Majority of biodiesel is being produced from soybean, rapeseed, and palm oils. Even though most of the biodiesel feedstock is renewable, competition with food supply has become a serious concern recently because certain feedstocks appear to be edible oils (Januan and Ellis, 2010; Mercer-Blackman et al., 2007). Therefore, alternative feedstocks such as non-edible oils, algae oils, and waste oils have arisen to prominence in recent years. Biodiesel produced from transesterification of waste cooking oil (WCO) is one of the most attractive automotive fuels to be used in place of petroleum diesel because of the added advantages over other types of biodiesel. WCO reuse eliminates the need for disposal, thus alleviating the environment and human health issues associated with waste oil disposal (Giracol et al., 2011). The lower cost of WCO feedstock can also help to make biodiesel competitive in price with conventional diesel (Meng et al., 2008). Many studies have been initiated to investigate the impacts of biodiesel made from several feedstocks including WCO on particulate emissions as compared to diesel fuel (Chung et al., 2008; Lin et al., 2011a; Lapuerta et al., 2008, Turrio-Baldassari et al., 2004; Durbin et al., 2007). An apparent decrease in PM emissions with the biodiesel content can be considered as an almost unanimous trend (Lapuerta et al.,2008). Most of the research on emissions from biodiesel is targeted towards physical properties of PM such as particulate mass, number concentrations and their size distributions. Apparently very little information is available pertaining to the health and environmental impacts of particulate emissions of biodiesel due to paucity of data on their chemical composition.

## 2. Phyiscal characterization of PM emitted from WCOB and ULSD

Conventional regulatory procedure involving dilution tunnel sampling and filter collection proved to be satisfactory for collection of PM from diesel engines a decade ago. However, in the current scenario, the low emission rate (~1mg/km) of PM from modern-era diesel engines places difficulties in sampling through traditional procedures because of their high de-

tection limits. Nor is this traditional method agreeable to recent regulations aimed at in-use emissions monitoring or to the vast variety of off road applications. A workshop was organized by Coordinating Research Council (CRC) in 2002 to discuss possible changes to measurement of DPM, and it is proposed that particle number-based methods potentially allow detection at very low levels with consistency provided formation of nucleation mode particles can be avoided (CRC, 2002). However, it is a difficult task to establish particle number-based standards and a standard methodology for measurement because of the sensitivity in detection, and great variability of nano-sized particles in engine exhaust. The European PMP (Particle measurement Program) and many other organizations including USEPA are working towards improving the methodology for measurement of solid particles to supplement the traditional mass method. A well-designed dilution tunnel satisfying the above requirements and reducing losses is the first step for this purpose.

Typically, DEP are agglomerates of many primary spherical particles of about 15-40 nm diameter. Airborne particles differ in size, composition, solubility and therefore also in their toxicological properties. It is a well-established fact that the current standards on diesel engine emissions not only improved the engine technology but also the fuel quality. These modern day engines emit particles of very low diameter (Su et al., 2004). Most of the particle mass exists in the accumulation mode in the diameter range of 0.1-0.3 μm (Kittelson, 1998). A large part of UFPs go unnoticed when only mass concentration is used as a metric. UFPs contribute a small fraction to the mass concentration of ambient aerosol particles, but may contribute disproportionately to their toxicity because of their high number concentration and surface area, high deposition efficiency in the pulmonary region, and high propensity to penetrate the epithelium (Donaldson et al., 2000).

### 2.1. Design of dilution tunnel

A dilution tunnel is a closed and controlled chamber where hot exhaust from engines, industrial stacks etc is mixed with dilution gas (usually ambient air) prior to sampling. Dilution sampling was originally used to characterize fine particle emissions from combustion sources because it simulates the rapid cooling and dilution that occurs as exhaust mixes with the atmosphere. Several researchers have developed dilution tunnels over decades for this purpose; one of the popular dilution sampling systems to simulate atmospheric conditions is CALTECH design (Hildemann et al., 1989). However dilution tunnels can also be used to freeze the size distribution by proper design criteria to avoid unwanted nucleation, condensation and coagulation. These dilution tunnels are developed for consistent measurement of particle number concentrations (PNC) from diesel engines.

The design of the sampling and dilution system determines largely what is measured later. Burtscher (2005) suggested in his review paper that the solid nanoparticles from the exhaust are closely related to health impacts and thus solid fractions of the exhaust should be separated from the volatile fraction and be studied for better understanding of health effects. Also, Kittelson (1998) identified that nucleation and coagulation of volatile fraction changes dramatically during dilution and sampling making it difficult to design a standard. Thus, one of the major issues in dilution sampling of engine exhaust is to decrease or eliminate

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nucleation processes. Typical dilution tunnels for particualte sampling from engines are designed to meet the following requirements.

**a.** To reduce the particle concentration in raw exhaust to a concentration that can be handled by the measurement system;

**b.** To reduce the temperatures to an adequate value usually close to ambient temperature;

**c.** To control the nucleation/condensation processes;

**d.** To reduce the losses of particulate matter during dilution and sampling.

**a. Reducing the PM concentration**

The PM concentration in raw exhaust varies with engine model, design, applied load and other parameters. A rough estimate of the total number concentrations of diesel engines is in the range of $10^8$~$10^9$ $cm^{-3}$. The present day sampling equipment and monitoring devices are designed to capture/monitor the particles in nanoscale that are abundantly present in today's diesel exhaust. These sampling instruments are highly sensitive, fragile and are reliable. Heavy loading of PM can easily disrupt the configuration, damage crucial systems leading to either inaccurate data, or render the equipment to be useless. So, it is required that the concentrations be brought down well below the instrument's maximum capacity.

**b. Reduction in exhaust temperature**

A typical temperature of raw exhaust is in the range of 200~400$^0$C, and such high temperatures can damage the charger columns and sensitive electrode plates used for detecting nanoparticles. Also, collection of PM on filter media from hot raw exhaust can alter the chemical composition of PM by inducing chemical reaction between the collected particles and filter media. Current regulation, for example the one used by the USEPA, requires that, PM be collected on filter media after the exhaust has been diluted and cooled to a temperature below 52$^0$C (CFR, 2001).

**c. Reduction in nucleation /condensation and coagulation**

Engine exhaust contains both gaseous phase and particle phase pollutants. Because of lower volatility and saturation coefficient some organic compounds and other precursor gases such as sulfur dioxide either condense onto the pre-existing particle surface altering their size (diameters), or nucleate to form new particles affecting number concentration. Also, particles can coagulate during dilution changing both the diameter and number of particles. Impact of these processes is very uncertain as they change dramatically during dilution and sampling (Kittelson, 1998) and thus makes comparisons from different sources difficult. It is therefore recommended that the dilution tunnels be designed to minimize or eliminate nucleation, condensation and coagulation (Burtscher, 2005).

**d. Reducing losses during sampling**

Particles are lost during transfer of exhaust from a tail pipe to sampling instruments and during dilution due to particle – wall interactions. These losses include mechanical losses such as inertial impaction, gravitational settling, electrostatic deposition, and due to diffu-

sion (Kittelson and Johnson 1991). Apart from mechanical losses particles are also lost due to thermophoretic deposition. These losses can impact the particle number concentration and size distribution.

The primary design objective of the dilution tunnel is to make sure that what is released at tail pipe is measured at sampling instruments. In other words, it is designed to minimize/ eliminate dilution artifacts, reduce losses, avoid nucleation to preserve the number and size distribution of particles as it is emitted from tail pipe. Before describing the actual design, it is useful to understand the theoretical basis underlying the design. The following sections 2.1.1 and 2.1.2 describe the important mechanisms that play a key role in altering the physical and chemical properties of particles during dilution and the measures taken to prevent such changes. Section 2.1.3 presents actual design of the dilution tunnel.

*2.1.1. Nucleation, condensation an coagulation*

Concentration of an inert species ($C_i$) when diluted is given by (Kerminen and Wexler 1995).

$$C_i - C_{i,A} = f(C_{i,E} - C_{i,A}) \tag{1}$$

where, $C_{i,E}$and $C_{i,A}$ are concentrations in the exhaust and ambient air respectively, and $f$ is dilution factor. Dilution factor simply means when the exhaust dilutes, a small parcel of air contains a certain fraction of the original exhaust and the remaining fraction is ambient air. That fraction of original exhaust in the air parcel is the dilution factor (*f*).

Temperature of an air parcel also changes in similar manner.

$$T - T_A = f(T_E - T_A) \tag{2}$$

where, $T_E$ and $T_A$ are the exhaust and ambient temperature, respectively.

**a. Nucleation**

Nucleation and condensation go hand in hand. Nucleation of nanoparticles from the diesel exhaust takes place as the exhaust cools during the dilution process (Abdul-Khalek et al., 1999). When partial pressure is much higher than vapor pressure of nucleating species, they undergo phase transformation. At the same time, due to their low volatility and the existence of large surface area of particles, sulfuric acid and many organic compounds can also condense quickly on the particles. However, Zhang and Wexler (2002) reported that nucleation favors compounds with both low volatility and low-molar volume in the condensed phase. High carbon number organics which usually possess very low vapor pressure, but significantly large condensed-phase molar volume are less likely to be the nucleating species than sulfuric acid (Zhang and Wexler 2004). Although it is still not clear about the nucleation mechanism from $SO_2$, it is widely believed that nucleation occurs through binary nucleation of water-sulfuric acid system (Kulmala et al., 1990) or ternary nucleation of Water-

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$H_2SO_4$-$NH_3$ system (Korhonen et al., 1999). Precursor gas, sulfur dioxide, is oxidized to form trioxide which gets converted to sulfate with water vapor.

$$SO_2(g) + ½\, O_2(g) \rightarrow SO_3(g) \tag{3}$$

$$SO_3(g) + H_2O\ (g) \rightarrow H_2SO_4(g) \tag{4}$$

The critical value ($C_{crit}$) for the gas phase concentration of sulfuric acid required for binary nucleation to take place is given by the following formula (Jackervoirol and Mirabel, 1989).

$$C_{crit} = 0.16 \exp(0.1T - 3.5rh - 27.7) \tag{5}$$

where $C_{crit}$ is in μg/m$^3$, T is Temperature in Kelvin, rh is scaled between 0-1. When the critical ratio $H_2SO_4(g)/C_{crit}$ becomes greater than 1, nucleation (gas-particle conversion) occurs instantaneously, giving birth to fresh nuclei in another log-normal distribution; these are called dilution-induced nuclei mode particles. From eq (5), it is clear that critical concentration is a function of temperature. When the temperature of diluted exhaust is high, the critical concentration required for nucleation tends to increase exponentially. Thus, it is recommended to dilute the hot exhaust with heated air rather than the conventional way of diluting the exhaust with air at ambient temperature. Also, Zhang and Wexler (2004) studied the viability of sulfuric acid induced nucleation and the coupling effect of condensation and nucleation by simulating various cases and reported two important findings: (1) As surface area of combustion induced particles increases, the critical ratio drops and in extreme cases nucleation is totally quenched. This is not to say that supersaturated mixture is not formed, but the sulfuric acid is condensed onto the surface area of particles; (2) Extremely rapid dilution leads to super-saturation of nucleating species and thus nucleation. The possibility of nucleation becomes lower if the dilution is smooth.

**b. Condensation**

The vapor pressure for volatile compounds is proportional to its temperature. Vapor pressure is related to dilution factor by (Zhang and Wexler 2004)

$$p^0 \alpha \exp\left(-\frac{\Delta H}{Rf\, T_E}\right) \tag{6}$$

Close to tail pipe, the temperature of exhaust far exceeds the ambient temperature, and therefore equation (2) reduces to (T $\alpha$ f). As a result, a decrease in temperature leads to an exponential decrease in vapor pressures, and the highly super-saturated vapors could make the time scale of condensation very short as 0.1 sec. Another factor important for condensation is particle surface area; by reducing the available particle surface area, condensation can be quenched.

**c. Coagulation**

Coagulation of particles during dilution occurs either due to turbulent shear, or due to Brownian coagulation. Zhang and Wexler (2004) evaluated coagulation through turbulent shear which has a time scale of $\tau_{ts} = \rho_p / \left( 48 \hat{m}_p \sqrt{\varepsilon_k / 120} v \right.$ and found that $\tau_{ts}$ for turbulent shear is approximately $10^{18}$ sec, because these tiny particles have such small cross sectional area that shear is insufficient to bring them together. The only mechanism is through Brownian coagulation and it is very insignificant when compared to other mechanisms.

*2.1.2. Particle-wall interaction*

When the engine exhaust is sampled and passed through dilution tunnel and sampling tubes, particles are lost due to deposition on sampling surfaces. Particles are deposited through several ways which include mechanical (inertial, gravitational, electrostatic, diffusion) and thermal (thermophoretic losses). In their review of variability in particle emission measurements during heavy duty transient tests, Kittelson and Johnson (1991) discussed the impact of exhaust system temperatures on particle measurement and provided recommendations to minimize the effects on aerosol sampling. They calculated losses during the heavy-duty transient test for a typical test facility and found that the majority of particle loss (5%) is due to thermophoresis, whereas inertial, gravitational, electrostatic, and diffusion depositions put together resulted in a loss of 0.2%. Thermophoresis is a physical phenomenon in which particles, subjected to a temperature gradient, move from high- to low temperature zones. A temperature gradient is established during sampling and the dilution between exhaust and the sampling surfaces due to difference in their temperatures. This gradient results in thermophoretic deposition of particles on sampling and dilution system surfaces. Eventually, these deposits are reentrained in the exhaust stream, or cause fouling of sampling surfaces. Reentrainment is unpredictable, and increases variability in mass measurements because of the increase in the number of coarse particles. These particles are not necessarily representative of diesel aerosol and make aerosol size distribution measurements more difficult. To avoid large differences in temperature between lines and exhaust gas, sampling lines should be fully insulated and kept to optimum size to reduce the residence time of exhaust in the sampling lines. Short sampling lines also reduce gravitational and diffusional losses.

*2.1.3. Dilution tunnel*

Figure 1 shows the schematic of a typical dilution tunnel used for particulate sampling from diesel engines. The engine exhaust was sampled through a sampling probe inserted into the main exhaust stream. The exhaust was then directed into a dilution tunnel, where the hot exhaust was mixed with a stream of pressurized, particle free, dry air in two stages using. In the first stage, the exhaust was transferred into the primary dilution tunnel, where the hot exhaust was mixed with a stream of pressurized particle free dry air preheated to a temperature of close to the temperature of the exhaust, to avoid particle nucleation, condensation, etc. The primary dilution tunnel was also heated to a temperature close to engine exhaust

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temperature to avoid thermophoretic deposition of particles onto the walls of the dilution tunnel. In stage 2, some portion of the diluted exhaust from the primary dilution tunnel was transferred to the secondary dilution tunnel. During the primary dilution, vapor pressures of volatile compounds decreased, allowing the secondary dilution with cold dilution air without condensing the volatile components. At this stage, the exhaust was mixed with dilution air at ambient temperature to bring down the temperature of the hot exhaust to ambient temperature for sampling. The completely diluted exhaust was then directed to particle measuring instruments. The dilution ratio was calculated by measuring the CO2 concentrations in the raw exhaust, dilution air and diluted exhaust.

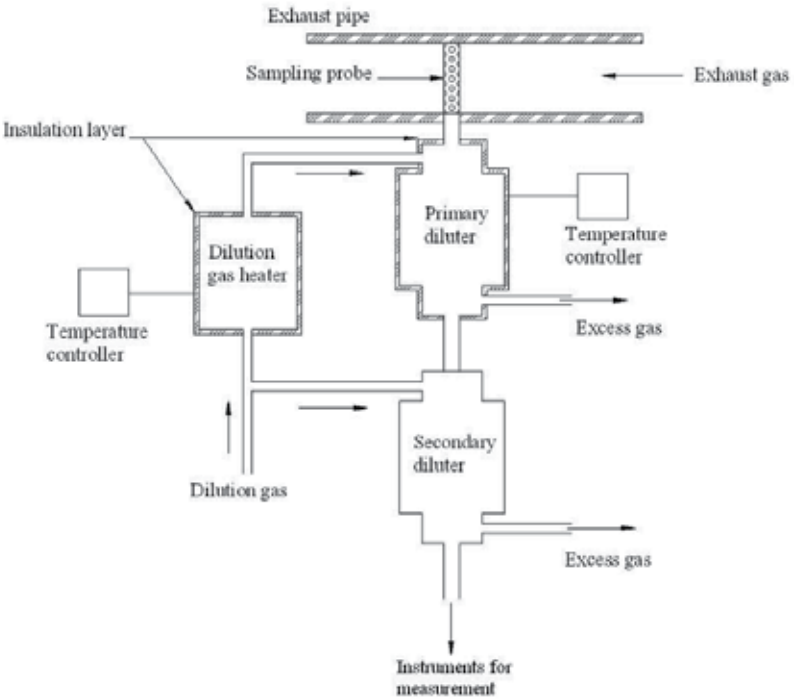


**Figure 1.** Schematic of a typical dilution tunnel.

A two stage dilution is adopted, firstly to prevent rapid dilution, a dilution is not achieved instantaneously but gradually and secondly, to dilute the exhaust in two different environments to reduce nucleation/condensation. In the primary dilution tunnel, since the exhaust is mixed with pre-heated air, time required for condensation is high comparable to the residence time of the exhaust in dilution tunnel (1~2 sec). In the secondary dilution tunnel, since a small sample from the already diluted exhaust is drawn, the available surface area for condensation is reduced by many times thus condensation is avoided in both stages of dilution.

### 2.2. Particulate mass concentration

Biodiesel (BD) and blends of ultra low sulfur diesel (ULSD) – BD had a significant effect on particulate emissions. Studies were conducted extensively on PM mass emissions from engine fueled with BD. Although some authors have reported an increase in PM emissions relative to diesel (Durbin et al., 2000; Munack et al., 2001; Alfuso et al., 1993), a large number of studies have confirmed a noticeable decrease in particulate mass while using BD (Lapeurta et al., 2008). However, the reported reductions varied very much depending upon engine conditions, experimental set up, fuel used, engine fuel system and other factors. Several studies reported reductions in the range of around 40 to 50% (Krahl et al., 1996, Lapeurta et al., 2002; Bagley et al., 1998). However, reductions as high as 70 to 90% were also reported by few studies (Canakci and Van Gerpen, 2001; Camden Australia 2005; Kado, 2003; Kalligeros et al., 2003). $PM_{2.5}$ (AED ≤ 2.5 μm) concentrations for ULSD and WCOB blends were reported by Betha and Balasubramanian, 2011a. They observed that with an increase in percentage of biodiesel in the fuel mixture, particle mass was reduced for B50, and for WCOB (B100) for all loading conditions of engine. For a particular fuel blend, the PM mass increased with load. Lapuerta et al. (2008) reported that many previous studies observed PM reductions in the range of 40-70% when biodiesel was used. The percentage reduction of $PM_{2.5}$ reported by Betha and Balasubramanian, 2011a (~35% at full load) was slightly lower than the range reported by Lapuerta et al. (2008). This is mainly because most of the studies, as reported by Lapuerta et al. (2008) in their review paper, compared biodiesel to conventional low sulfur diesel (sulfur content < 500 ppm) whereas in the study conducted by Betha an Balasubramanian 2011a the comparison of $PM_{2.5}$ emissions was made with ULSD (sulfur content < 15 ppm). The reduction in PM emissions when using BD can be attributed to the following reasons: (1) the absence of aromatics (Knothe et al., 2006), which are considered as soot precursors, in biodiesel reduces the amount of PM formed during combustion; (2) the higher oxygen content in BD tends to enhance the combustion process resulting in lower particulate emissions; and (3) Finally, the presence of unsaturated fatty acids in BD leads to more complete combustion processes. Unsaturated fatty acids have lower boiling points than diesel, and they can evaporate faster in the combustion chamber than diesel (Song and Zhang, 2008). In addition, the higher viscosity and density of BD compared to ULSD can lead to an increase in the injection pressure. Likewise, higher bulk modulus of compressibility of vegetable oils and their methyl esters can lead to advanced injection timing (Boehman et al., 2004) while using BD. As a result, the BD fuel enters the combustion chamber relatively quicker compared to ULSD (Lapuerta et al., (2008). This advancement in combustion process while using BD increases the residence time of soot particles in the combustion chamber, and thus they undergo further oxidation (Cardone et al., 2002) leading to reduction in PM emissions.

### 2.3. Particle number concentrations and size distributions

New engine designs and emission control devices reduced particulate mass emission drastically allowing the engines to operate below the emission level standards. However, the concerns about UFPs and nanoparticles (AED < 50 nm) which can contribute to human

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health effects (Oberdiester et al., 2001, Nel, 2005) significantly raised a serious concern to develop new ambient standards in terms of particle number rather than mass (Burtscher, 2004). Therefore, particulate number concentrations and size distributions are increasingly studied in comparative studies of particulate emissions from diesel and BD (Kittelson, 1998; Lapeurta et al., 2008; Zhu et al., 2010, Di et al., 2009a and 2009b; Di et al., 2008; Burtscher, 2004). In the literature, both an increase and decrease in the total particle number concentrations were reported when using BD. Di et al. (2009b) observed that the total particle number increased 1.5 – 2.5 times when using WCOB compared to diesel depending on the engine load. Similar increments (1.35 – 2.4 times) were observed by them in another study using a direct injection diesel engine. On the other hand, Lapuerta et al. (2007) tested two differently stressed WCOB and observed a reduction (~3 times) in total particle number concentrations (PNC) compared to diesel. Although both of them used WCO in their study, contradictory trends were reported. Studies on other types of BD have also reported contradictory results. A summary of PNC of ULSD and WCOB emissions during different engine operating conditions is provided in Table 1 (Betha et al., 2011a) and their size distributions are shown in Figure 2 (Betha et al., 2011a). In contrast to PM mass emissions, PNC decreased with an increase in load for all the fuels (shown in Table 1). At higher WCOB-ULSD blend ratios, the percentage decrease in PNC with increasing load was relatively small compared to ULSD. For ULSD, total PNC decreased by 26% at full load when compared to idle, or no load conditions whereas, for biodiesel, it decreased by only around 9%.

| | Total particle number concentration(# $cm^{-3}$ ) | | | |
|---|---|---|---|---|
| **Engine Load (%)** | **ULSD** | **B20** | **B50** | **B100** |
| 0 | $1.14 \times 10^7$ | $1.05 \times 10^7$ | $9.57 \times 10^6$ | $8.98 \times 10^6$ |
| 30% | $9.82 \times 10^6$ | $8.99 \times 10^6$ | $9.19 \times 10^6$ | $8.58 \times 10^6$ |
| 70% | $9.00 \times 10^6$ | $8.91 \times 10^6$ | $8.87 \times 10^6$ | $8.40 \times 10^6$ |
| 100% | $8.46 \times 10^6$ | $8.34 \times 10^6$ | $8.31 \times 10^6$ | $8.15 \times 10^6$ |

**Table 1.** Total PNC for ULSD and WCOB (B100) blends at various loads (Betha et al., 2011a)

It is expected that in diesel engines, particle counts would increase with an increase in load. However, a decrease in PNC with increasing load was observed in this study. Chung et al. (2008) also observed a reduction in PNC at higher loads in their study using a Yanmar backup generator similar to the one used in this study. This observed decrease in PNC was probably due to the transfer of particles from nucleation to accumulation or coarser mode at higher loads. It was observed that at higher loads, nucleation mode particles which contribute to a major fraction of total number decreased and accumulation mode particles increased. As a result, there was an overall decrease in total PNC. This shift in particles from the nucleation mode to the accumulation mode was evident from the PSD shown in Figure 2 (Betha et al., 2011a).

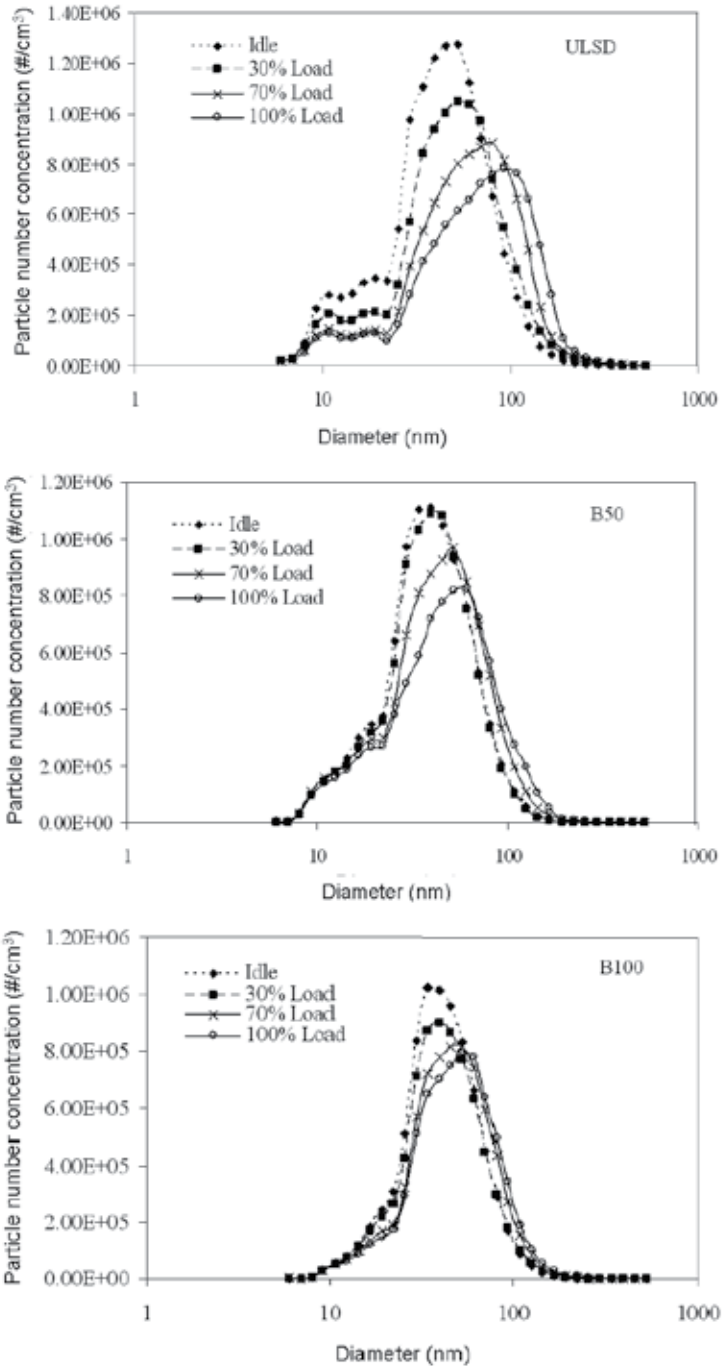


**Figure 2.** Particle size distributions of emissions at different loads for ULSD, B50, WCOB (B100) (Betha et al., 2011a)

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From the PSD shown in Figure 2, it can be observed that with the increase in load, particle peak diameters increased for all fuels. The magnitude of the increase in particle peak diameters at higher loads was greater for the ULSD than the biodiesel. For ULSD, the peak diameter increased from 52.3 nm at idle mode to 93 nm at full load and for biodiesel (B100) peak diameter increased from 34 nm at idle to 52.3 nm at full load. However, in their study on particle emissions from stationary diesel engines, Di et al.(2010) and Zhu et al. (2010) reported an increase in total PNC when engine load was increased. Although there is no definitive explanation for the difference in particle emission trends observed by the above-mentioned studies and trends shown in Figure 2 as well as the study by Chung et al. (2008), a possible reason can be the differences in engine capacities and operating conditions. Both Di et al. (2010) and Zhu et al. (2010) conducted their studies on diesel engines which had much larger capacity and power (~4000 cc and 88 kW). However, the stationary diesel generator used for the the shown results (Table 2 and Figure 2) (296 cc and 4.5 kW) and the one used by Chung et al. (2008) (4.8 kW) have lower capacity and power.

The relative number percentage of nucleation (diameter < 50 nm), accumulation (50-100 nm) and fine particles (diameter > 100 nm) emitted from diesel and biodiesel (B100) fuels at various loads is shown in Figure 3. Biodiesel had relatively a higher fraction of nucleation mode particles ranging from 55% to 70 % at different loads when compared to diesel (35% – 60%). A decrease in the nuclei mode particles (diameter < 50 nm) and an increase in accumulation and fine particles (diameter > 100 nm) were observed with an increase in load for both the fuels. For the stationary engine running with ULSD at full load, nucleation (32%), accumulation (40%) and fine particles (28%) shared almost a similar fraction of particles to the total PNC. However, for BD, nucleation (51%) and accumulation mode particles (43%) were major contributors to the total number concentrations. The fraction of accumulation mode particles increased from 30% during idle mode to 40% at full load in the case of ULSD, and a similar increase was observed for biodiesel as well (from 30% at idle mode to 43% at full load). This observation implies that diesel engines emit more accumulation particles at higher loads. At higher loads, more fuel is injected into the combustion chamber to generate additional torque needed and also the residence time for the particles in the combustion chamber decreases relatively. Therefore, the oxidation of particulate soot tends to be reduced, leading to the release of a large fraction of accumulation and fine particles. In the case of biodiesel, the inherent oxygen in the fuel improves the oxidation of soot. Therefore, the percentage increase of fine particles is relatively less for BD.

## 3. Chemical properties of particulate emissions

Since diesel engines are one of the most significant air pollutant sources in urban areas (Cass, 1998), chemical composition of diesel exhaust has been widely investigated. The chemical profile of PM plays a crucial role in health and environmental impacts. Variations in the chemical composition of aerosols alter their hygroscopicity and can lead to changes in the cloud-active fraction of the aerosols, or cloud condensation nuclei (CCN) number concentration (Ward et al., 2010). Some carcinogenic and toxic chemical compounds present in DEP when biologically

available can affect human health. Diesel engine emissions consist of a wide range of organic and inorganic compounds in gaseous as well as particulate phases (Bünger et al., 2006). Concentrations of most particle-bound chemical constituents depend on the type of engine, engine load, fuel and lubrication oil properties (Dwivedi et al., 2006). Large surface area of DEP enables adsorption of organics and inorganic compounds from the combustion process and/or the adsorption of additional compounds during transport in the ambient air. DEP consists mainly of elemental carbon (EC) (75%), organic carbon (OC) (19%) and small amounts of sulfates, nitrates (1%) and metals & Elements (4%) (Figure 4) (EPA, 2002a).

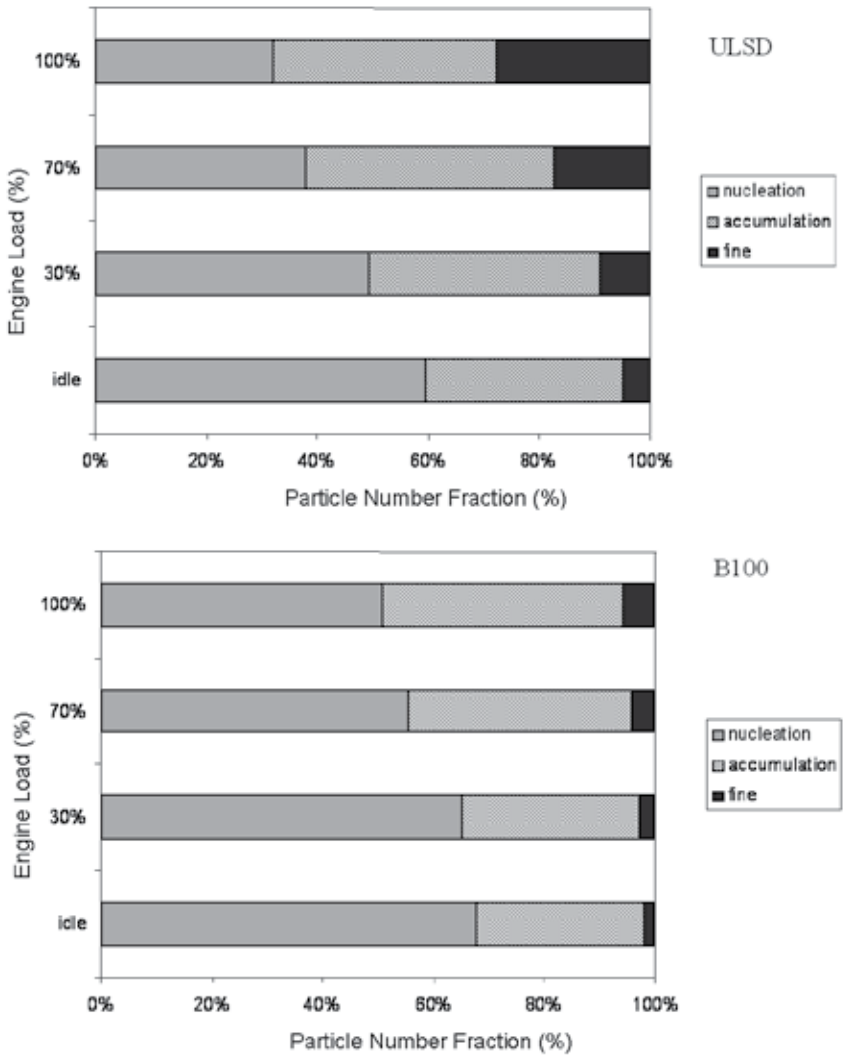


**Figure 3.** Fractionation of particle emitted from ULSD WCOB (B100) for various loads (Betha et al., 2011a).

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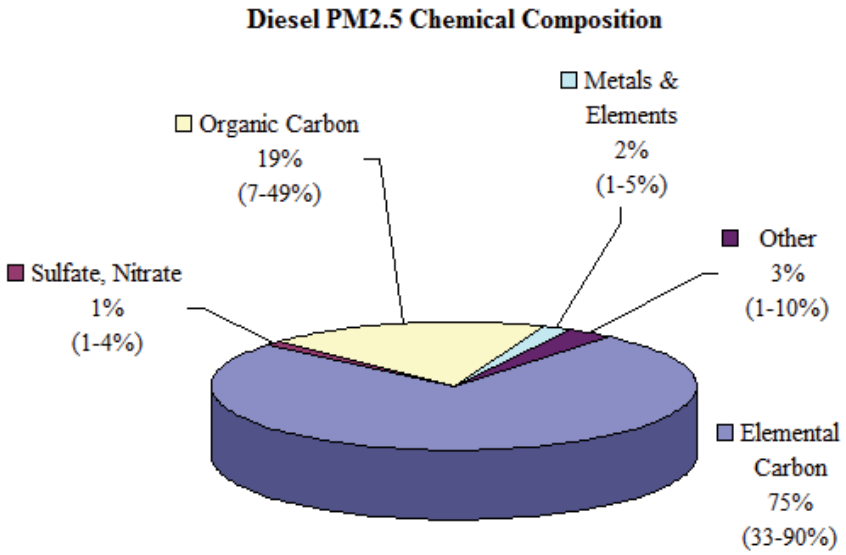


**Figure 4.** Typical chemical compositions for diesel particulate matter ($PM_{2.5}$) (EPA, 2002a)

### 3.1. Particle-bound polycyclic aromatic hydrocarbons (PAHs)

Particulate-bound organic compounds, especially PAHs, are highly carcinogenic. PAHs and their derivatives (nitro-PAHs) together comprise less than 1% of the mass of DEP (EPA, 2002a). The emissions of these compounds are comprehensively studied for diesel engines fuelled with diesel and BD (Jung et al., 2006; Bagley et al., 1998; Correa et al., 2006; Karavalakis et al., 2009; Turro-Baldassari et al., 2004; Zou and Atkinson, 2008; Karavalakis et al., 2010). A majority of studies have found a significant decrease in PAHs emissions with BD compared to that with diesel (Bagley et al., 1998; Correa et al., 2006; Karavalakis et al., 2009). However, a couple of studies have indicated only statistically insignificant reduction in PAHs (Turro-Baldassari et al., 2004; Zou and Atkinson, 2008) when BD is used. The reduction in PAH emission may be attributed to the presence of excess oxygen in BD and the absence of aromatic and polycyclic aromatic compounds in the fuel. One study (Karavalakis et al., 2010) was found in literature reporting higher PAHs emissions when using BD. Karavalakis et al. (2010) tested BD made from soybean oil and used frying oil. They found lower PAH emissions with BD made from soybean oil. However, BD made from used frying oil emitted more PAH compounds compared to those from diesel. They attributed the increase in PAHs to dimers, trimers, polymerization products, and cyclic acids present in the biodiesel made from used frying oil.

### 3.2. Particulate-bound elements and trace metals

Studies on particulate-bound metals emitted from BD combustion are not as comprehensive as PAHs, despite a strong correlation between human health risk and particulate-bound

metals (Hu et al., 2008; Verma et al., 2010). Very few studies (Dwivedi et al., 2006; Cheung et al., 2011) were found in literature investigating particulate-bound metals in BD. Dwivedi et al. (2006) conducted a comparative assessment and characterization of particulate-bound trace elemental emissions from diesel and rice bran oil derived BD. Elements such as Cr, Ni, Pb, Cd, Na, Al, Mg, and Fe were investigated. The authors observed that concentration of metals such as Cr, Fe, Al, Zn, Mg increased while others (Pb, Cd, Na, Ni) were reduced with the usage of B20 (20% BD). Cheung et al. (2011) also observed higher concentrations of Fe, Zn, Mg when using BD. However, Cr, Al, Pb, Cd, Na, Ni were lower in BD emission compared to diesel. In both the studies, the particulate-bound elemental concentrations were mainly attributed to the fuel and lubricating oil composition apart from engine wear. Metals and elements that were found higher in fuel were also found higher in the emissions. Since the particulate-bound elements largely depend on fuel quality and composition apart from engine wear, their concentration in the exhaust is expected to vary with feedstock of BD. In another study Betha et al. (2011b) investigated the particulate bound elements from WCOB (B100), ULSD and their blend (B50). They observed that particulate emissions were reduced with the usage of WCOB. However, most of the elements which are known to be toxic such as Zn, Cr, Ni were very high in the WCOB exhaust compared to ULSD. Elements such as As, Co, Al, Mn were found to be in higher levels in ULSD. Elements such as Cr, Cu, Fe, Ba, Zn, Mg, Ni, and K were found in higher concentrations in B100 compared to ULSD. Similar findings were reported by Dwivedi et al. (2006). They found that concentrations of Zn, Fe, and Cr were higher in biodiesel compared to those in diesel. The higher concentrations of elements, especially Cu, Fe, Zn, in B100 used in this study can be attributed to the raw material from which biodiesel was prepared. BD used in this study was derived from WCO generated in restaurants and food courts. The oil has been used for cooking and frying of various food products. Elements such as Cu, Fe, Zn and Mn were found in vegetables, (Kawashima and Soares, 2003) Cu, Fe, and Zn in meat (Lombardi-Boccia et al., 2005) and Cu, Zn and Cd in fish (Atta et al., 1997). These elements might have been released into the oil during cooking and therefore, the concentrations of Fe, Zn, Cu, Mn in BD were found to be significantly high. In addition, these elements can also be leached out from the cooking utensils due to heating (Kuligowski and Halperin, 1992).

## 4. Estimation of health risk due to particulate emissions

Human health risk assessment was conducted based on the mean concentrations of particulate-bound elements determined through the experimental study. Health risk assessment is especially useful in understanding the health hazard associated with inhalation exposure to PM emitted from B100 compared to that of ULSD. The details and steps involved in health risk assessment are described in detail elsewhere (See and Balasubramanian, 2006). Briefly, it involves four important steps (NRC, 1983) as described below.

Hazard identification – elements which have known toxicity values are considered. Al, Cr, and Mn induce non-carcinogenic effects while As, Cd, Cr, Ni and Co induce carcinogenic health effects.

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Exposure assessment –This involves estimation of chronic daily intake (CDI) of these elements calculated from the following equations.

$$\text{CDI}(\text{mg kg}^{-1}\text{day}^{-1}) = \frac{\text{Total dose (TD,mg m}^{-3}\text{) x inhalation rate (IR, m}^3\ \text{day}^{-1}\text{)}}{\text{Body weight (BW,kg)}} \quad (7)$$

$$TD = C \times E \quad (8)$$

where C is concentration of pollutant and E is deposition fraction of particles by size given by (Volckens and Leith, 2003),

$$E = -0.081 + 0.23\ln (D_p)^2 + 0.23\ \text{sqrt}\ (D_p) \quad (9)$$

where Dp is the diameter of particles. In this study, $PM_{2.5}$ (Aerodynamic diameter ≤ 2.5 μm) was used i.e. Dp is 2.5 μm. IR is typically assumed to be 20 $m^3\ day^{-1}$ and BW to be 70 kg for adults. As for children, the IR and BW are assumed to be 10 $m^3\ day^{-1}$ and 15 kg, respectively.

(3) Dose-response assessment- It is the probability of health effects according to the dose of pollutant of concern. Assuming only inhalation as the major exposure route, the reference dose (RfD, mg $kg^{-1}$, $day^{-1}$) for toxic elements that are non-carcinogenic was calculated from reference concentrations (RfC, $mg/m^3$) provided by USEPA. Likewise, for carcinogenic elements the inhalation slope factor (SF, $mg^{-1}$ kg day) was calculated from inhalation unit risk values (IUR, $mg^{-1}\ m^3$) provided by USEPA.

(4) Risk characterization or estimation of health risk - was calculated based on the exposure and dose–response assessments. For non-carcinogenic metals, it is indicated by (United States Department of Energy, 1999):

$$\text{Hazard Quotient}\ (\text{HQ}) = \text{CDI/RfD} \quad (10)$$

For carcinogenic metals, total carcinogenic risk is estimated in terms of excess life time cancer risk (ELCR) given by: (United States Department of Energy, 1999).

$$\text{ELCR} = \text{CDI} \times \text{SF} \quad (11)$$

The human health risk assessment was carried out to quantify the risk associated with the particulate-bound metals emitted from ULSD and WCOB at full load from a stationary engine for illustration.

The pertinent information of the TD and RfD, HQ, inhalation SF and ELCR for adults and children are shown in Tables 2 and 3. The concentrations of metals used for this illustration

are adopted from Betha et al. (2011b) (shown in Table 4) and CDI is calculated using Eqns (7) – (9). The concentrations reported in Betha et al., 2011b represent those emitted from the raw engine exhaust. However, in reality the engine exhaust is diluted by ambient air once it is released to the atmosphere. The dilution factor is typically 1000 times when exhaust is released to the ambient atmosphere on road conditions (Zielinska, 2005). The mean concentrations of elements were divided by 1000 to be used in health risk calculations. The deposition efficiency E for particle with 2.5 μm is nearly 0.70. The CDI is calculated by first estimating the TD of the each element (Tables 2 and 3).

| **ULSD** | | | | | |
|---|---|---|---|---|---|
| **Metals** | **CDI (mg kg$^{-1}$ day$^{-1}$)** | **RfD (mg kg$^{-1}$ day$^{-1}$)** | **HQ** | **SF (mg kg$^{-1}$ day)** | **ELCR** |
| Non carcinogenic metals | | | | | |
| Al | 9.84 x 10$^{-6}$ | 1.43 x 10$^{-3}$ | 6.88 x 10$^{-3}$ | | |
| Cr | 1.22 x 10$^{-6}$ | 2.86 x 10$^{-5}$ | 4.26 x 10$^{-2}$ | | |
| Mn | 9.18 x 10$^{-8}$ | 1.43 x 10$^{-5}$ | 6.42 x 10$^{-3}$ | | |
| Carcinogenic metals | | | | | |
| As | 2.51 x 10$^{-8}$ | | | 15.1 | 3.79 x 10$^{-7}$ |
| Cd | 3.79 x 10$^{-9}$ | | | 6.3 | 2.39 x 10$^{-8}$ |
| Cr | 1.22 x 10$^{-6}$ | | | 4.2 | 5.12 x 10$^{-6}$ |
| Ni | 8.02 x 10$^{-8}$ | | | 84 | 6.74 x 10$^{-6}$ |
| | | | ∑=5.59 x10$^{-2}$ | | ∑ =12.3 x10$^{-6}$ |
| **WCOB** | | | | | |
| **Metals** | **CDI (mg kg$^{-1}$ day$^{-1}$)** | **RfD (mg kg$^{-1}$ day$^{-1}$)** | **HQ** | **SF (mg kg$^{-1}$ day)** | **ELCR** |
| Non carcinogenic metals | | | | | |
| Al | 6.42 x 10$^{-6}$ | 1.43 x 10$^{-3}$ | 4.49 x 10$^{-3}$ | | |
| Cr | 2.18 x 10$^{-6}$ | 2.86 x 10$^{-5}$ | 7.63 x 10$^{-2}$ | | |
| Mn | 3.85 x 10$^{-8}$ | 1.43 x 10$^{-5}$ | 2.69 x 10$^{-3}$ | | |
| Carcinogenic metals | | | | | |
| As | 9.57 x 10$^{-9}$ | | | 15.1 | 1.45 x 10$^{-7}$ |
| Cd | 2.31 x 10$^{-9}$ | | | 6.3 | 1.45 x 10$^{-8}$ |
| Cr | 2.18 x 10$^{-6}$ | | | 4.2 | 9.16 x 10$^{-6}$ |
| Ni | 8.72 x 10$^{-8}$ | | | 84 | 7.33 x 10$^{-6}$ |
| | | | ∑=8.34x 10$^{-2}$ | | ∑=16.6 x 10$^{-6}$ |

**Table 2.** Estimation of human health risk in adults due to particulate bound elements from $PM_{2.5}$ emitted from WCOB and ULSD (Betha et al., 2011b)

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| **ULSD** | | | | | |
|---|---|---|---|---|---|
| **Metals** | **CDI (mg kg$^{-1}$day$^{-1}$)** | **RfD (mg kg$^{-1}$day$^{-1}$)** | **HQ** | **SF (mg kg$^{-1}$day)** | **ELCR** |
| Non carcinogenic metals | | | | | |
| Al | 2.30 x 10$^{-5}$ | 1.43 x 10$^{-3}$ | 1.61 x 10$^{-2}$ | | |
| Cr | 2.85 x 10$^{-6}$ | 2.86 x 10$^{-5}$ | 9.95 x 10$^{-2}$ | | |
| Mn | 2.14 x 10$^{-7}$ | 1.43 x 10$^{-5}$ | 1.50 x 10$^{-2}$ | | |
| Carcinogenic metals | | | | | |
| As | 5.85 x 10$^{-8}$ | | | 15.1 | 8.84 x 10$^{-7}$ |
| Cd | 8.85 x 10$^{-9}$ | | | 6.3 | 5.58 x 10$^{-8}$ |
| Cr | 2.85 x 10$^{-6}$ | | | 4.2 | 1.20 x 10$^{-5}$ |
| Ni | 1.87 x 10$^{-7}$ | | | 84 | 1.57 x 10$^{-5}$ |
| | | | $\sum$= 1.31 x 10$^{-2}$ | | $\sum$ = 28.6 x 10$^{-6}$ |
| **WCOB** | | | | | |
| **Metals** | **CDI (mg kg$^{-1}$day$^{-1}$)** | **RfD (mg kg$^{-1}$day$^{-1}$)** | **HQ** | **SF (mg kg$^{-1}$day)** | **ELCR** |
| Non carcinogenic metals | | | | | |
| Al | 1.50 x 10$^{-5}$ | 1.43 x 10$^{-3}$ | 1.05 x 10$^{-2}$ | | |
| Cr | 5.09 x 10$^{-6}$ | 2.86 x 10$^{-5}$ | 1.78 x 10$^{-1}$ | | |
| Mn | 8.98 x 10$^{-8}$ | 1.43 x 10$^{-5}$ | 6.28 x 10$^{-3}$ | | |
| Carcinogenic metals | | | | | |
| As | 2.23 x 10$^{-8}$ | | | 15.1 | 3.37 x 10$^{-7}$ |
| Cd | 5.38 x 10$^{-9}$ | | | 6.3 | 3.39 x 10$^{-8}$ |
| Cr | 5.09 x 10$^{-6}$ | | | 4.2 | 2.14 x 10$^{-5}$ |
| Ni | 2.04 x 10$^{-7}$ | | | 84 | 1.71 x 10$^{-5}$ |
| | | | $\sum$ = 1.95 x 10$^{-1}$ | | $\sum$ = 38.8 x 10$^{-6}$ |

**Table 3.** Estimation of human health risk in children due to particulate bound elements from $PM_{2.5}$ emitted from WCOB and ULSD (Betha et al., 2011b)

As shown in Tables 2 and 3, the levels of non-carcinogenic risk (total HQ) were estimated to be 0.06 for ULSD and 0.08 for WCOB and carcinogenic risk (total ELCR) to be 12.3 x 10$^{-6}$ for ULSD and 16.6 x 10$^{-6}$ for WCOB for adults. In the case of children, non-carcinogenic and carcinogenic risks for both the fuels are higher than those in adults. Total HQ was estimated to be 0.13 for ULSD and 0.2 for B100, while total ELCR was 28.6 x 10$^{-6}$ for ULSD and 38.8 x 10$^{-6}$ for WCOB. It implies that 28 to 29 children or 12 to 13 adults in a million can get cancer after exposure to the toxic trace metals in $PM_{2.5}$ emitted from the combustion of ULSD. In the case of biodiesel, it is even higher, 38 to 39 children or 16 to 17 adults out of a million can get cancer after exposure to $PM_{2.5}$ by B100 fuel.

| Element | ULSD (mg/m$^3$) | WCOB(mg/m$^3$) |
|---|---|---|
| Al | 147.6 | 96.3 |
| Mn | 1.4 | 0.6 |
| Cr | 18.3 | 32.7 |
| Ni | 1.2 | 1.3 |
| Cd | 0.06 | 0.03 |
| As | 0.4 | 0.14 |

**Table 4.** Concentration of particulate bound elements in raw exhaust of a stationary engine

From the results it can be deduced that the non-carcinogenic risk indicated by HQ was higher for WCOB compared to ULSD for both groups of people. However, for both ULSD and WCOB, the total HQ was very low for adults compared to children and for both the groups total HQ was below acceptable levels, (Acceptable levels for total HQ =1). On the other hand carcinogenic risk indicated by ELCR was found to be much higher than the acceptable limit for both groups and for both fuels (i.e., 1 in a million) and that ELCR for WCOB was greater than ULSD. From the risk assessment results made in this study, it appears that exposure to $PM_{2.5}$ emitted from biodiesel poses higher risk when compared to $PM_{2.5}$ emitted from ULSD. However, it is to be noted that in this study the carcinogenic risk due to particulate bound elements was used as a measure to evaluate the total carcinogenic risk. A more comprehensive and extensive research needs to be done to evaluate the complete risk assessment including many other carcinogenic compounds such as PAHs and nitro-PAHs. Studies have shown that PAH emissions from biodiesel are very much lower compared to diesel (Jalava et al., 2010; Karavalakis et al., 2011; Lin et al., 2011; Turrio-Baldassarri et al., 2004). Therefore, the total carcinogenic risk of WCOB exhaust particles might be actually lower than ULSD.

In the case of PAHs the the risk assessment for PAHs that are probable and possible human carcinogens were calculated using petency equivalency factor (PEF) relative to BaP and the CDI calculated from Eq (7). Table 5 shows the PAHs with know PEFs (Collins et al., 1998).

| PAH | Group | PEF |
|---|---|---|
| Benz(a)anthracene, BaA | 2A | 0.1 |
| Benz(a)pyrene, BaP | 2A | 1 |
| Benzo(b)fluoranthene, BbF | 2B | 0.1 |
| Benzo(k)fluoranthene, BkF | 2B | 0.01 |
| Indeno(1,2,3-cd)pyrene, Ind | 2B | 0.1 |
| 2A: Probable Human Carcinogen 2B: Possible Human Carcinogen | | |

**Table 5.** Classification of PAHs by IARC and Potency equivalency factor (PEF)

Carcinogenic risk due to individual PAHs is calculated as product of CDI and PEF. The total carcinogenic risk is the summation of individual risk.

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## 5. Summary

Particle. physical and chemical properties play a key role in determining the health effects associated with PM emissions. Smaller particles can penetrate deep inside the alveolar regions of lungs. Bio-available particulate-bound compounds pose serious health problems. WCOB had lower PNC compared to that of ULSD. However, WCOB had a higher fraction of nucleation mode particles relative to that of ULSD, and therefore, a large fraction of PM emitted from WCOB can deposit in respiratory system compared to DPM. Unlike other types of biodiesel WCOB has higher metal concentrations both in the fuel as well as particulate emissions because of the nature of feedstock. Metals are leached into the oil during cooking and also from cooking utensils. Health risk inhalation of PM was calculated by assessing the CDI estimated using the concentration of particulate-bound compounds and the deposition efficiency of PM in human body, which indicates that WCOB has higher health risk compared to ULSD in terms of particulate bound elements. However, when PAHs are also taken into consideration it can either increase or decrease the relative health risk of WCOB particles depending on the PAHs emission concentrations from both the fuels.

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This book focuses on the development of biodiesel systems from the production of feedstocks and their processing technologies to the comprehensive applications of both by-products and biodiesel. It should be of interest for students, researchers, scientists and technologists.

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