Simultaneous microwave extraction and synthesis of fatty acid methyl ester from the oleaginous yeast *Rhodotorula glutinis*

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Abstract

Microbial lipids have the potential to substantially reduce the use of liquid fossil fuels, though one obstacle is the energy costs associated with the extraction and subsequent conversion into a biofuel. Here we report a one-step method to produce fatty acid methyl esters (FAME) from *Rhodotorula glutinis* by combining lipid extraction in a microwave reactor with acid-catalysed transesterification. The microwave did not alter the FAME profile and over 99% of the lipid was esterified when using 25 wt% H_2SO_4 over 20 minutes at 120 °C. On using higher loadings of catalyst, similar yields were achieved over 30 seconds. Equivalent amounts of FAME were recovered in 30 seconds using this method as with a 4 hour Soxhlet extraction, run with the same solvent system. When water was present at less than a 1:1 ratio with methanol, the main product was FAME, above this the major products were FFA. Under the best conditions, the energy required for the microwave was less than

20% of the energy content of the biodiesel produced. Increasing the temperature did not change the energy return on investment (EROI) substantially; however, longer reaction times used an equivalent amount of energy to the total energy content of the biodiesel.

Keywords : Microwave, extraction, yeast, biodiesel, biofuel, catalysis,

Introduction

Due to the increasing costs, the associated environmental impact and the uncertainty of supply, renewable replacements for fossil fuels are being increasingly sought. One potential source of liquid fuels are lipids derived from oleaginous organisms grown using waste resources, such as microalgae from waste water (1), or yeasts cultivated on effluent or agricultural waste (2).

One such organism is *Rhodotorula glutinis*, which can accumulate up to 60% dry weight in lipids, and has the ability to grow on a variety of hexose and pentose sugars, as well as waste streams (3-5). Biomass production has been reported as high as 185 g L^{-1}, using a fed batch methodology, though 10-15 g L^{-1} is normal in a batch system (6, 7). The lipid profile obtained from *R. glutinis* contains palmitic acid (16:0), stearic acid (18:0) oleic acid (18:1) and linoleic acid (18:2) as the main fatty acids (8).

The lipids from these organisms can then be chemically upgraded into fuels, such as biodiesel, that can be used blended with current fossil fuels, without significant engine modification. However, there are a number of energy intensive steps in the production of these fuels that limit their adoption for large-scale production. These include the cultivation of the organisms, harvesting of the biomass, the extraction of the lipid and the subsequent

chemical conversion into a suitable biofuel (9). It is essential that lower energy techniques are investigated in each of these areas.

Often, combining production stages in the chemical industry leads to a more economic process. For example, in the production of biodiesel a large research effort has been underway to develop heterogeneous catalysts that can convert both free fatty acids (FFA) and glycerides simultaneously to avoid a multi-stage acid catalysed and then base catalysed process (10). Further work has suggested that the combination of reactive and separation stages in the manufacture of biodiesel will also provide a more economic process (11).

Lipid extraction is reportedly the most costly component of the production process, and a number of techniques that have been investigated to reduce this cost (12, 13). These include using mechanical stress to break open the cell (14, 15), cavitation through sonication (15), Soxhlet solvent extraction with organic solvents, and using CO_2 at high pressures and temperatures (16, 17). Microwave extraction also has potential to extract lipids over a short time-frame (18, 19). In this method, first reported in the 1980s, the cell walls are shattered by a combination of the high frequency microwaves and the rapid, simultaneous, localised heating throughout the sample (20).

For effective microwave heating, the solvent molecules must either have a dipole moment or a charge. For molecules with a dipole moment, the applied oscillating electric field causes the dipole to constantly realign creating heat in the form of friction. For charged molecules, the electric field moves ions continually backwards and forwards, causing collisions and therefore producing heat (21). Compared to conventional heating methods, where energy transfer is dependent on thermal conductivity and convection currents, microwave heating is considerably faster since solvents directly absorb and distribute the

energy to the surrounding solution more efficiently (22). Since the efficiency of microwave heating is therefore dependent on the polarity of the solvent, polar solvents such as water, methanol or DMSO (dimethyl sulfoxide) are necessary rather than less polar solvents such as chloroform or hexane.

Currently, microwaves are used on an industrial scale for a number of high throughput applications, including in the food industry to dry pasta and snack foods and to pasteurize food before packaging, to heat rubber to a workable elasticity, drying paint in timber production and in other chemical, textile, plastic, paper, tobacco, pharmaceutical, oil, leather and coal industries (23–25).

The industrial-scale conversion of vegetable oils into biodiesel uses potassium or sodium alkoxide catalysts with conventional heating (26). The transesterification reaction, using a sodium methoxide catalyst, has also been successfully combined with microwave heating (27). Since this method of heating is extremely efficient, shorter reaction times and lower catalyst loadings than traditional transesterification have been reported (28). However, when water or free fatty acids are present in the lipid these basic catalysts react to form soaps reducing the yield and making the extraction of the lipids problematic. In the conversion of microbial resources, the removal of all the water and polar lipids is impractical and therefore a Brønsted acid catalyst, such as H_2SO_4 must be employed. However, H_2SO_4 is roughly 4000 times slower than sodium methoxide at 65 °C (29), but significantly has been shown to convert lipids more rapidly with microwave heating (30, 31). The non-catalytic production of FAME from fatty acids using microwave heating has also been demonstrated at elevated temperatures (150-225 °C), though with low efficiency with only a maximum of 60% FAME recovered after one hour (32).

The processing time and cost could potentially be reduced significantly by combining the transesterification step with the microwave extraction, to eliminate the time-consuming catalytic second stage. With reduced extraction times and simplified method, microwaves allow fast throughputs of consistent samples due to the uniform heating and the automated settings on the microwave. The volume of solvent required per sample is also small, reducing overall solvent consumption and improving the economics of the process (23). The aim of this study was to combine the microwave extraction process of microbial lipids, from the oleaginous yeast *Rhodotorula glutinis,* and the transesterification reaction converting the lipids to FAME in one step. This technique was then compared with the Soxhlet solvent extraction.

2. Materials and methods

All chemicals and solvents were purchased from the Sigma-Aldrich chemical company apart from deuterated chloroform ($CDCl_3$), which was purchased from Fluorochem. All reactants were used as received with no additional purification.

2.1 Microbial cultivation

R. glutinis (2439) was purchased from the National Collection of Yeast Cultures, Norwich, UK and was cultured aerobically in a 1.5 litre jacketed, airlift fermentor at 30 °C as this temperature was previously found to promote high growth rates and lipid accumulation (8). The culture medium was a standard YMG media (containing by weight 10% glucose, 5% bactopeptone, 3% malt extract and 3% yeast extract) used in previous studies to promote lipid accumulation in *R. glutinis* (8). After 7 days culture, the yeast was concentrated through settling, the supernatant removed and replaced with a 2% glucose solution to

promote lipid accumulation, the yeast was held in this stage for 5 days. On completion, the yeast biomass was centrifuged for 10 minutes at 6000 rpm, the supernatant was removed and the resulting biomass washed with distilled water and freeze dried. The resulting powder (9.1 g) was stored in an air-tight container at -20 °C prior to use.

2.2 Soxhlet extraction

0.1 g of microbial biomass was added to a cellulose finger in Soxhlet glassware and the lipids were extracted over 0.5, 1, 2, 4, 12, 24 or 48 hours with a 2:1 $CHCl_3$/MeOH mixture (50 ml). This solvent mixture has been demonstrated to be suitable for lipid extraction, and is polar enough to be suitable for use with microwave heating (33). On completion the volatiles were removed under reduced pressure and the resulting lipid was added to MeOH (10 ml) and H_2SO_4 (0.1 g) and refluxed for a further 8 hours. The excess methanol was removed under reduced pressure and the lipid extracted into $CHCl_3$. The organic layer was washed with water to remove the acid catalyst and glycerol and the volatiles were removed under reduced pressure prior to analysis.

2.3 Microwave extraction

Microwave extraction was undertaken using an Anton Parr monowave 300 microwave reactor equipped with a MAS 24 autosampler capable of loading 10 ml sealable reaction vessels capable of sustaining a pressure of 30 bar. The biomass (0.1 g) was suspended in a 2:1 $CHCl_3$/MeOH mixture (6 ml) with H_2SO_4, 1wt% (0.001g), 10wt% (0.01g), 25 wt% (0.025g) or 100 wt% (0.1g) and a stir bar. In the experiments containing water, the microbial mass (0.1g) was added to the reaction vessel with the requisite amount of distilled water; this

meant that while the level of solvent increased in the reaction vessel, the same amount of biomass and lipid was present in each of the samples.

The microwave was set on an automated cycle containing 1) heating to the desired temperature and pressure (typically taking less than 1 minute) with 1000 rpm stirring, 2) the reaction (0.5-20 minutes, 1000 rpm stirring) 3) fast cooling using compressed N_2 (typically less than 2 minutes depending on temperature). The resulting lipid was extracted into chloroform and washed with water three times; the chloroform was then removed under reduced pressure prior to the analysis.

2.4 Lipid analysis

The FFA content of the resulting lipids was calculated by ^{1}H NMR, following the procedure given by Satyarthi *et al.* (34).The FAME conversion was calculated by dissolving a fraction of the sample in $CDCl_3$ and analysing by ^{1}H NMR in an adapted method given by Knothe (35). In this method the integration of the peak assignable to the glyceride backbone (δ 4.0 - 4.5 ppm) was compared that of the methyl ester (δ 3.6 ppm). FFA content was calculated by comparison of the α-CH_2 group of the carbonyl group (δ 2.29-2.32 ppm) with the glyceride backbone and methyl ester peaks. The lipid content and FAME profile were calculated by GC-MS calibrated to known standards. The GC-MS analysis was carried out using an Agilent 7890A Gas Chromatograph equipped with a capillary column (60m × 0.250mm internal diameter) coated with DB-23 ([50%-cyanpropyl]-methylpolysiloxane) stationary phase (0.25µm film thickness) and a He mobile phase (flow rate: 1.2ml/min) coupled with an Agilent 5975C inert MSD with Triple Axis Detector. A portion of the biodiesel samples (approximately 50mg) was initially dissolved in 10ml dioxane and 1µl of this solution was

loaded onto the column, pre-heated to 150°C. This temperature was held for 5 minutes and then heated to 250°C at a rate of 4°C/min and then held for 2 minutes.

2.5 Energy return on investment (EROI)

Energy content of fuels was determined using a Parr 1341 plain jacket adiabatic bomb calorimeter using a Parr 1108 oxygen combustion bomb. Approximately 0.3 g of each sample was placed in the crucible within the bomb and the bomb then filled with oxygen to a pressure of approximately 25 bar. The temperature change of the water within the stirred calorimeter was determined to an accuracy of 0.0005 °*C.* The lower heating value of the microbial lipid was found to be 39.99 MJ kg^{-1}, the lower hearing value of the resulting biodiesel (with a FAME profile given in table 1) was found to be 40.12 MJ kg^{-1}. The power used for the microwave was calculated by integrating the energy output from the microwave using the trapezium rule with a step change of 0.05. The experimental data from the study was used to calculate the EROI based on both the lipid extracted and the biodiesel produced. Calculations for an increased amount of lipid were undertaken assuming the same power output would be used irrespective of the level of lipid extracted.

3. Results and Discussion

3.1 Soxhlet extraction

Soxhlet extraction has been the main method of laboratory lipid extraction for over a century (36). While hexane is the main solvent used in industry for microbial lipid extraction, hexane is too non-polar (dielectric constant, ε, is 1.88) for efficient microwave heating (9). An alternative solvent system, first proposed by Bligh and Dyer (33), is a $CHCl_3$ and MeOH mixture (2:1 w/v), this mixture is more polar with the dielectric constants of $CHCl_3$ being

4.81, and 32.70 respectively. Due to containing one of the reactants, the suitability for the extraction and the polarity, this solvent system was then used for the extraction and subsequent transesterification. In the present work, the yeast lipid was first extracted by using Soxhlet glassware, over a range of different reaction times (fig. 1). Following extraction, samples were transesterified with an excess of methanol using conventional heating at reflux with 10 wt% H_2SO_4 as the catalyst. The total saponifiable lipid (lipid that can be converted into biodiesel), was then calculated by GC-MS. In the Soxhlet extraction around 20% of the lipid was extracted within an hour, but complete lipid recovery (32%) required 4 hours of extraction. The lipid level, as well as the FAME profile, remained constant from this point onwards demonstrating that no significant degradation of the lipid is observed over the longer reaction times.

3.2 Microwave extraction

An Anton Parr 300 monowave was used to extract the lipid from *R. glutinis* biomass. In this reactor the temperature, pressure and wattage was recorded (fig. 2). The pressure and temperature increased gradually during the first minute of the reaction as the microwave heated the mixture to the set temperature. The sample temperature was then held for the length of the reaction, during which time the pressure of the sample remained constant. Once the set time had elapsed, the system was cooled with high pressure of nitrogen causing the pressure in the system to return to its initial state. The initial period of sample heating required the largest energy input. As the set temperature was reached, the power input reduced. For all the microwave reactions an excess of methanol and chloroform (6 ml equates to approximately 50 mmols of each solvent) was used and the effect of H_2SO_4 on the conversion was established by ^1H NMR (fig. 3). Three reaction times, 0.5, 5 and 20

minutes were selected, three temperatures 80 °C, 100 °C and 120 °C and four catalyst loadings of 1 wt%, 10 wt%, 25 wt% and 100 wt% in relation to the biomass.

The lowest catalyst loading was completely ineffective in the conversion of lipids with only a maximum of 7% of the glycerides converted into biodiesel. Though this improves using 10 wt% H_2SO_4, only a maximum 60% conversion was observed at the higher temperature and longer reaction times. In contrast, using 25 wt% catalyst achieved conversions over 95% at 120 °C and 5 minutes. Similarly high conversions were achieved after 20 minutes microwave time at the lower temperatures.

On using 100 wt% catalyst, yields of over 98% were obtained at all temperatures over 5 minutes. For the conversion of lipid to FAME in the microwave reactor large catalyst loadings are needed to fully transesterify the lipids under reasonable reaction times. This is largely in accord with previous studies where full transesterification of castor oil required 10 wt% catalyst and a 30 minute reaction time (30), and full conversion of soybean oil required 40 minutes with 5 wt% H_2SO_4 catalyst (31).

To measure the FAME yield, all unreacted lipids from these reactions were esterified under reflux conditions and the resulting FAME analysed by GC-MS (fig. 4). A total of 32 dry wt.% lipid was recovered using Soxhlet after 4 hours. At 0.5 minute reaction times, irrespective of the catalyst loading or temperature, a majority of the lipid was extracted using the microwave reactor. On the addition of 1 wt% H_2SO_4 the same amount of lipid was recovered at 120 °C as the Soxhlet reaction, though the yield was slightly reduced at lower temperatures. On increasing the amount of H_2SO_4 to 10 wt% the majority of lipid was recovered at 0.5 minutes over the entire temperature range investigated. However, on running the reaction for 20 minutes, a reduction in the total lipid was observed at 120 °C. This effect was further exacerbated at a catalyst loading of 25wt% and 100 wt% suggesting

that very harsh conditions have an inverse effect on lipid yield, possibly due to degradation to alternative products. A reduction in the biodiesel yield over longer reaction times was also observed on the transesterification of waste and rapeseed oils using microwave heating (37, 38).

The FAME profile of microwave extracted and transesterified lipid was analysed using GC-MS (table 1; fig. 5). On extraction no significant change in the FAME profile was observed irrespective of the reaction conditions or the quantity of lipid extracted. This demonstrates that the FAME is not degrading significantly even at high catalyst loadings and longer reaction times.

The main mechanism of lipid degradation is oxidative (39). Polyunsaturated esters are more prone to oxidation than the monounsaturated or saturated counterparts (40). Consequently, lipid degradation through this mechanism would be expected to preferentially consume polyunsaturated esters. Since there was no change in the FAME profile, simply a reduction in the total lipid, it is unlikely that this is the cause of the observed reduction in lipid yield. There is some evidence that at high power loading, or for long reaction times, water can be produced from the glyceride oils and as a result elevated production of FFA is observed (40, 41). It is possible that when elevated levels of H_2SO_4 were used, other parts of the microbial cells were broken down to unknown by-products, that reacted with the lipid to reduce the total FAME yield.

However, under optimum conditions this effect was evidently minor since the same amount of FAME was recovered as from the Soxhlet extraction. Using the microwave, the extraction of lipids was relatively facile with 30 seconds being sufficient time to recover the majority of the lipids; the esterification of these lipids to FAME required the longer reaction times or elevated temperatures.

3.3 Effect of water on the extraction and conversion of yeast lipid

The *R. glutinis* biomass used in this study was freeze-dried prior to use. However, the process of drying microbial lipids is energy intensive and therefore the complete removal of water is not feasible for the industrial production of microbial fuels (42). While microwave reactors allow low-energy extraction and transesterification of microbial lipids, the ability to extract lipid from wet biomass would further increase the utility of the process. To examine the effect of water on the microwave extraction process, distilled water was added to the freeze-dried yeast cells to simulate wet biomass (Fig. 6a). The concentration of yeast was kept constant to allow comparability with previous experiments. The experimental conditions were 100 °C, over five minutes with 100 wt% catalyst. A similar level of lipid was recovered from each run, irrespective of the amount of water in the sample.

H_2SO_4 is very effective at converting the glyceride lipids under these conditions. When no additional water was added the level of glyceride was less than 4 % of the reaction mixture. At low water levels (below 25% of the total biomass) the FFA content remained a relatively small part of the total. However, increasing the water content to 50 wt%, which is an equivalent molar ratio to the methanol, resulted in 40% FFA. The level of FFA in the product mixture increased as the molar ratio of water to methanol increased. However, presumably due to the increased solubility of the methanol and lipid in chloroform, the FFA content was only 58% even at the highest water to methanol ratio.

The data above suggests that the production of FFA could be minimised by ensuring that the water/methanol ratio is kept below 0.5. However, on an industrial scale, elevated levels of water in the biomass would consume energy via heating. In the reactor

used in this study for example, heating a sample containing 95% water used 1.5x the energy used to extract lipid from the freeze dried sample (Fig. 6b).

3.4 Energy return on investment (EROI)

The energy used in the microwave extraction step was calculated as a percentage of the energy present in the lipid (fig. 7). The higher temperatures and longer reaction times require the largest input of energy and at 120 °C over 20 minutes, more energy is used for the extraction than is present in the extracted lipid. However, shorter reaction times resulted in a large increase in the EROI. For example, extraction at 80 °C over 30 seconds, produced lipid with 6x the amount of energy than was consumed in its extraction.

It seems a reasonable assumption that the energy input will not change substantially as a result of increased cellular lipid content. By using the data presented above, the effect of the lipid content on the EROI was also extrapolated. Unsurprisingly, higher lipid contents changed the EROI substantially, though this effect has arguably less impact on the energy invested than the time of reaction. While it seems logical to aim for an oil content of 60-70% of the cell, Ratledge *et al.* reasoned that due to the increased level of nutrients and decreased production of biomass, the optimal level of lipid production for heterotrophic organisms is roughly 40% dry weight (43).

The EROI calculation was then applied to the biodiesel produced from the microwave reactions (fig. 8). As the conversion to biodiesel was significantly reduced at 10wt% catalyst loading, a substantial amount of energy, in most cases more than is present in the resulting biodiesel, was needed to extract and convert the lipid. This demonstrates the need for effective catalysts under these conditions. At higher loadings of catalyst the biodiesel yield is increased and over a 30 second reaction time, only 20% of the energy

present in the fuel is needed to run the microwave system. Irrespective of the catalyst loading the higher temperatures, undertaken over 20 minutes, used at least 80% of the energy present in the biodiesel produced. This demonstrates that high biodiesel yields are important, though it is more efficient to use a higher temperature than an increased reaction time to achieve this. While a large amount of sulphuric acid is necessary to achieve this conversion this level of loading is consistent with the use for FFA esterification in a two stage biodiesel production process (44).

In terms of the whole system, it can be misleading to compare one set of laboratory results to other microwave systems or to a scaled industrial process though the EROI for the extraction of lipids is extremely promising. Currently, there are no industrial processes involved in the production of yeast oil biodiesel, so it is difficult to place this figure in a relevant industrial context. However, a pilot scale process to produce a cocoa butter (oil) substitute using a yeast was developed by DSIR Industrial Development in the earlier 90s (45). In this process they calculated that the feedstock was the most expensive part of the process, with only waste whey being close to economically viable (46). The authors also reasoned that reducing the water to just 5% would be economically unviable to produce cost-effective lipid, in this case wet biomass must be used. The extraction technology used in this pilot scale study was a bead mill followed by hexane extraction, though the researchers suggested that an additional polar solvent (such as methanol or chloroform) was also needed. The process economics of the extraction were most severely affected by the length of time beyond any other variable. The process data from these extensive pilot plant experiments coupled with a large scale fermentation trial in a dairy factory gave the total cost of the oil to be near £1,500 t^{-1}, taking into account plant depreciation, interest on capital investment, manufacturing overheads and todays elevated costs of production (43).

While there is little work published on yeast oils, a large body of economic and life cycle analysis data has been presented for microalgae. Algal biodiesel is estimated in most studies to cost more than the figure estimated for the yeast oil, though a large amount of the processing costs will be similar (43). Sills *et al.* recently published a comprehensive uncertainty analysis, investigating the environmental impact of algal biofuels (47). In this analysis, the researchers determined that using a solvent extraction with hexane, followed by a transesterification stage, would then account for 54% of the energy present in the biofuel. While the best EROI found in the course of this work for these stages was found to be less than 20%, it should be noted this has not been optimised on a similar scale to that presented in the literature.

While this is a significant proportion of the fuel, the authors demonstrated that the major cost in this type of processing were the drying stages, which accounted for over 180% of the energy present in the resulting biodiesel. A traditional base-catalysed biodiesel process cannot be operated with a water content over 0.5% (due to the competing saponification reaction) though acid catalysts can be used effectively with elevated levels of water. In this case, another advantage of using a microwave system is that the microwave heating accelerates the rate of the reaction, with a Brønsted acid, to allow extraction and transesterification in a reasonable timeframe.

While the EROI would change substantially for a scaled up process, it seems likely that the length of reaction and level of lipid in the yeast would be the predominant factors in determining an efficient microwave-extraction process.

4. Conclusions

Biodiesel sourced from terrestrial crops has a large ecological impact, a negative public image and can only meet a fraction of fuel demand. Yeast lipids offer a promising future feedstock for fuel production, however, the estimated cost of producing these lipids, especially from lignocellulosic resources must be reduced substantially if they are to compete with current terrestrial oil and fossil fuel alternatives. Three key areas that warrant future research in this area are:

1. To reduce the costs of the lignocellulosic feedstock and move to producing the lipids from waste resources.
2. Producing fuels from wet biomass. The cost of drying the biomass is substantial and any technology that will be used in the future must avoid this costly step.
3. A reduction in the cost and energy impact of the existing processes. For example the extraction and chemical conversion have been estimated to use up over half of the energy present in microalgal biodiesel, this figure must be reduced substantially to provide an effective process.

In this study the second and third point were addressed using a promising oleaginous yeast, *Rhodotorula glutinis*. A microwave reactor was shown to be suitable for the simultaneous extraction and transesterification of lipids, even from wet biomass. The lipids can be extracted in 30 seconds at 120 °C, as opposed to 4 hours by Soxhlet solvent extraction. However, catalyst loadings of 25 wt% H_2SO_4 and increased reaction times were necessary to complete the associated transesterification to FAME. Irrespective of the conditions, the microwave extraction did not significantly alter the FAME profile and water was tolerated up to 25 wt% without a large increase in the total FFA content. Consequently, the

simultaneous extraction and transesterification of lipids using microwave irradiation is both a powerful technique for laboratory use and has the potential to reduce energy costs in future industrialised biofuel production. While it is difficult to estimate the energy costs of a scaled up microwave process, the EROI of the optimal process for the microwave reactor used in this study is promising, provided increasing the time of reaction, which has the largest negative impact on EROI, is avoided.

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Figures

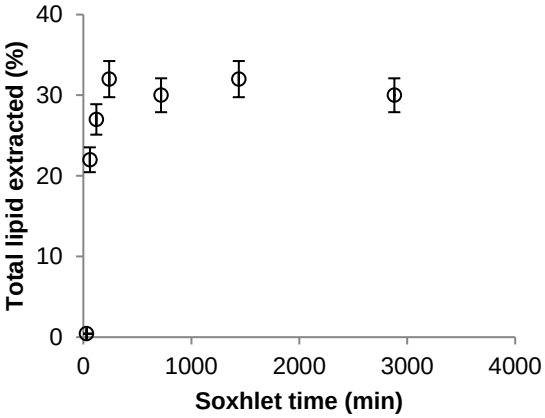


Fig. 1 Extraction of lipids from *R. glutinis* in the Soxhlet extraction set-up, using 50 ml of $CHCl_3$:MeOH, over 24 hours

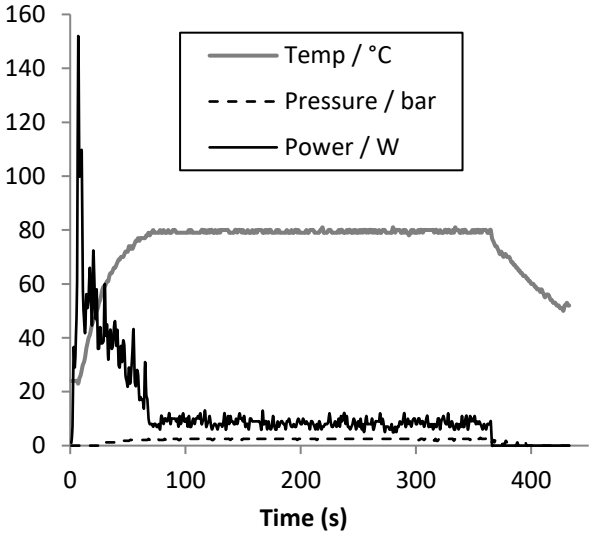


Fig. 2 Reaction conditions for Anton Parr monowave 300, showing the pressure, temperature and power of the microwave extraction with 100 wt% H_2SO_4 at 80 °C over 5 minutes.

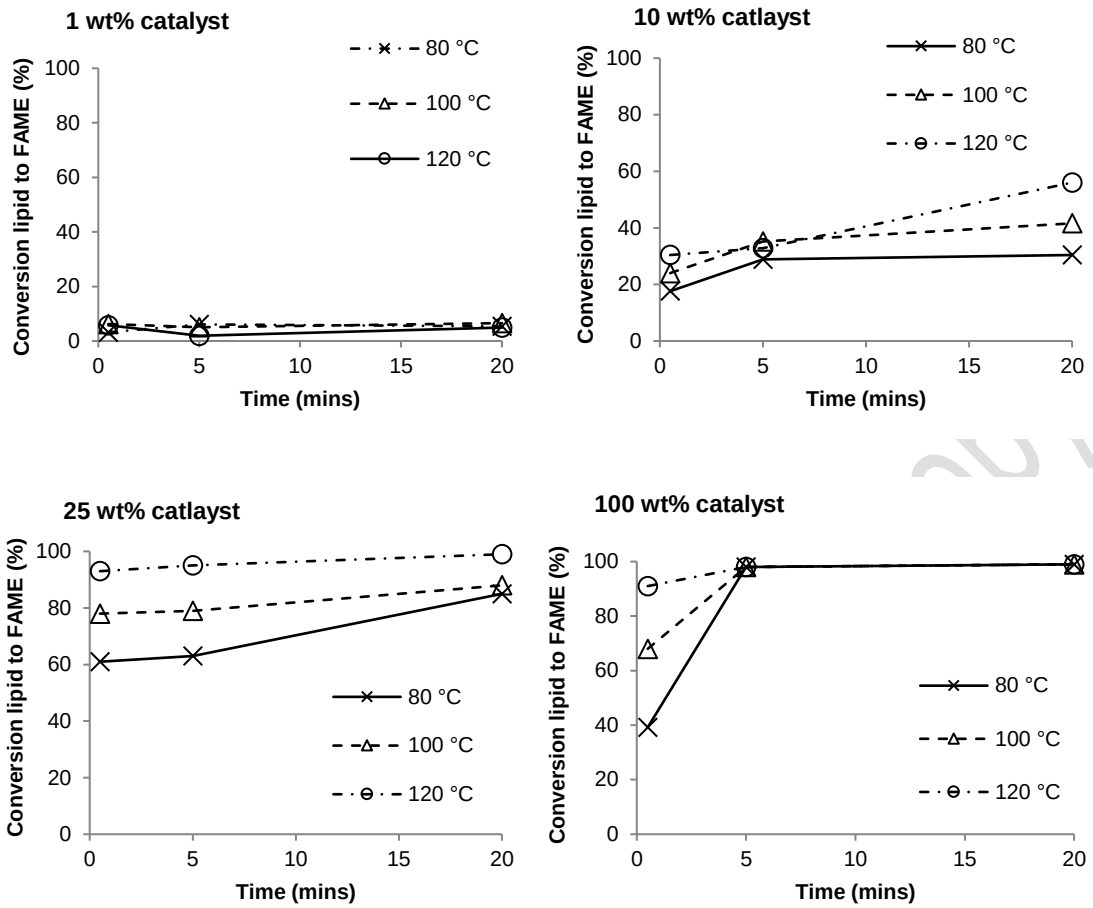


Fig. 3 Conversion of extracted lipid to FAME as a function of catalyst loading for the microwave irradiated samples, using the Anton Parr monowave 300 reactor.

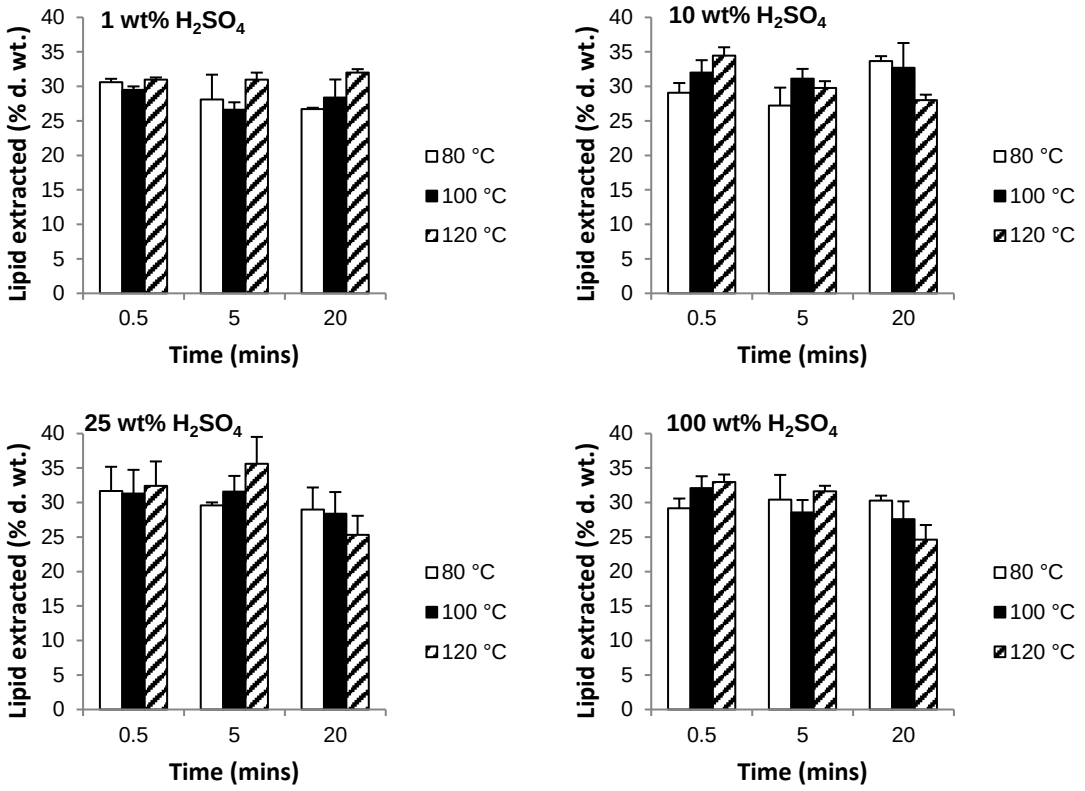


Fig. 4 The effect of the length and temperature of the microwave reaction on the total lipid extracted from *R. glutinis*, using the Anton Parr monowave 300 reactor.

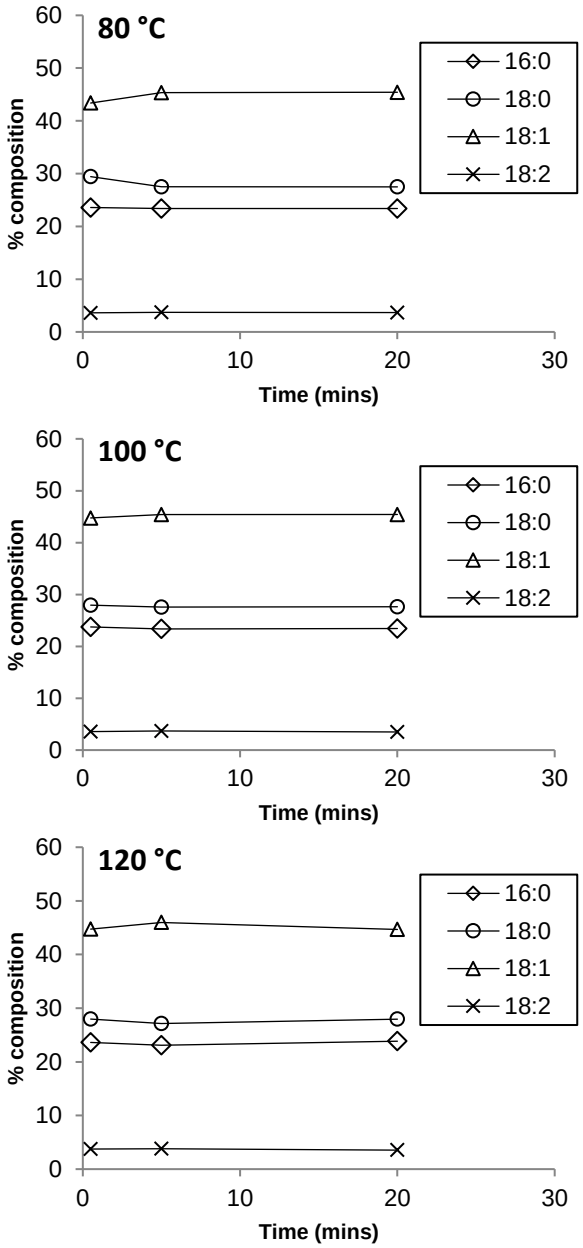


Fig. 5 Change in the major FAME components on varying the length and temperature of the microwave reaction, using the Anton Parr monowave 300 reactor, for *R. glutinis* using 100 wt% H_2SO_4

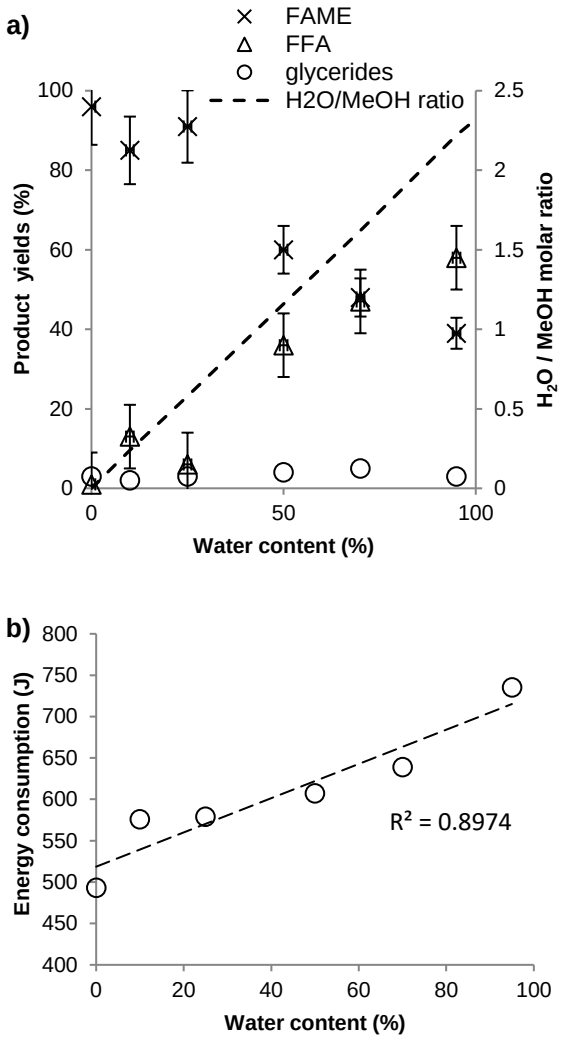
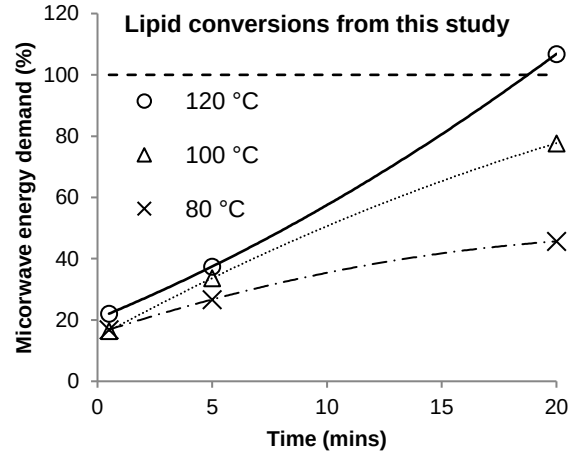
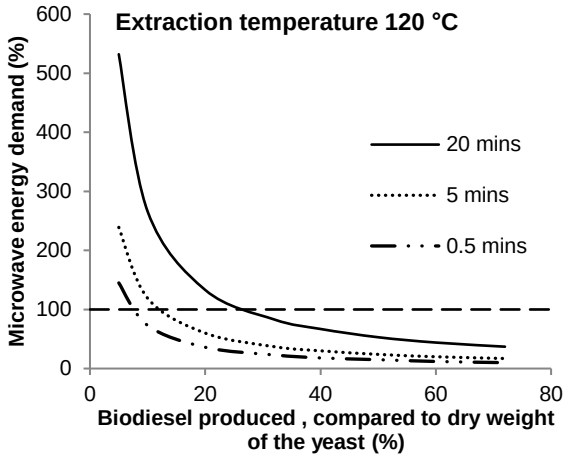


Fig. 6 a) Effect of water on one-step extraction and transesterification using 100 wt% H_2SO_4, at 100 °C over 5 minutes b) The total energy used by the Anton Parr monowave 300 microwave reactor for this step





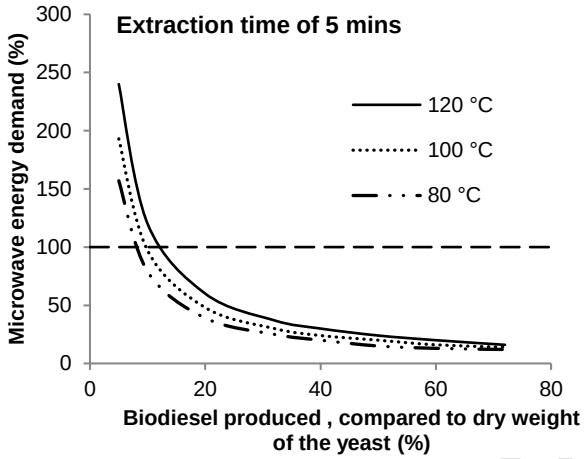
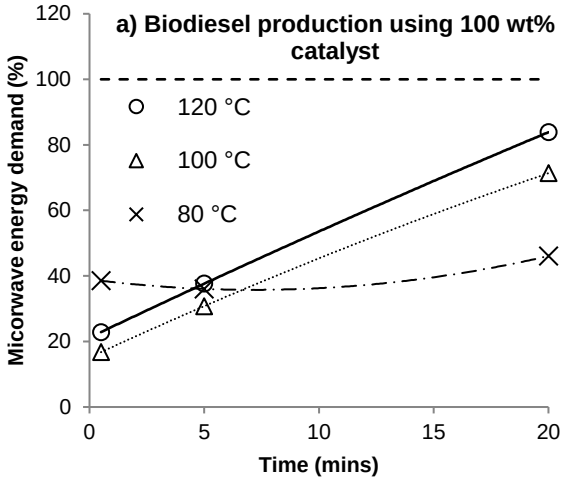
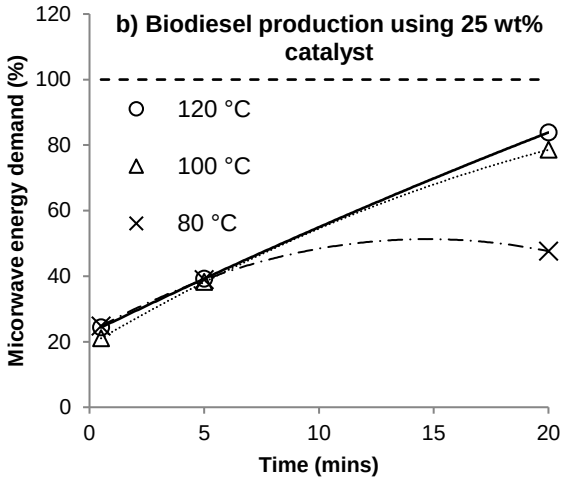


Fig. 7 Energy Return On Investment of the microwave step, using a) the lipid content found in the yeast biomass over the course of the study, b) an extrapolation based on a variable lipid content at 120 °C, c) an extrapolation based on a variable lipid content over 5 minutes.





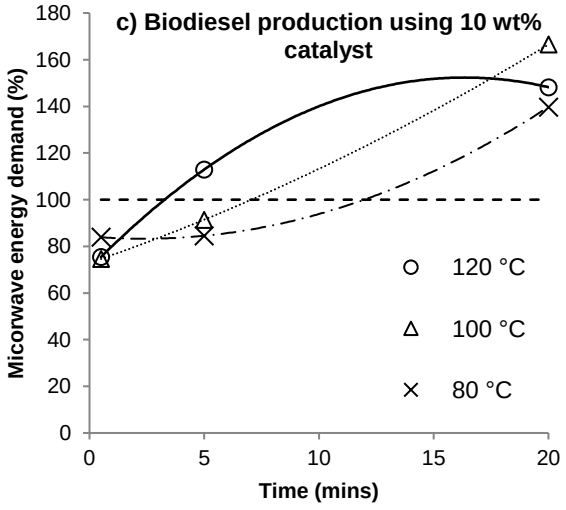


Fig. 8 Energy Return On Investment of the microwave step, for the biodiesel produced using a) 100 wt% catalyst, b) 25 wt% catalyst c) 10 wt% catalyst, using a lower heating value of the biodiesel of 40.12 MJ kg^{-1}.

FAME	***R. glutinis***
Lipid content (% dry weight)	31
14:0	1.0
16:0	23.8
16:1	1.0
18:0	29.1
18:1	41.7
18:2	3.4

Table 1. FAME profile for *R. glutinis* calculated from the microwave extraction, using an Anton Parr monowave 300 reactor, held at 80 °C over 5 minutes. The biodiesel produced was found to contain 0.7% glycerides, this level falls within the European fuel standard EN 14 214.