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A review of selected natural coagulants in water and wastewater treatment

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Water and enviromental technology

The saddest aspect of life right now is that science gathers knowledge faster than society gathers wisdom.

Isaac Asimov (1920–1992)
Science fiction author

Acknowledgements

Ås, June, 2020

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Firstly I would like to thank my supervisors Dr. Zakhar Maletskiy and Prof. Harsha Ratnaweera for their invaluable help and guidance during my work on this thesis. Especially in the period after the COVID-19 outbreak, where they helped change my thesis from a research paper to a literature review.

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Summary

Water treatment by inorganic coagulants and synthetic polymers poses environmental challenges tied to trace metals and sludge quality/quantity. With the popularisation of chemical precipitation during the last 50 years, these challenges have become more apparent. As a way to combat the environmental challenges posed by the use of inorganic coagulants and synthetic polymers, natural coagulants have been explored for water treatment. Natural coagulants are biodegradable, non-toxic, and do not pose any of the environmental challenges that inorganic coagulants and synthetic polymers do. This literature review aims to research the treatment efficiency and coagulation mechanism of two of the most promising natural coagulants, chitosan and Moringa oleifera, and compare them to more traditional inorganic coagulants and synthetic polymers. In this context, natural coagulant refers to natural polymers used as a primary coagulant for water treatment.

Based on a review of literature on water treatment by the natural coagulants chitosan and Moringa oleifera seeds, a table for each of the natural coagulants were made where key information such as treated water type, experimental setup, achieved removal efficiency, and reported coagulation mechanisms were noted. Analysis of the results of researched literature showed that both natural coagulant excelled at removing pollutants commonly found in portable water treatment, however, few studies were found on their use in wastewater treatment. The coagulation mechanisms reported were adsorption, charge neutralization, bridging, and electrostatic patch mechanism. The results indicate that both reviewed natural coagulants can compete with inorganic coagulants for portable water treatment, but more research is needed on the treatment of wastewater before a fair comparison can be made. Further research should be directed at the implementation of chitosan in combined processes, and the use of purified protein from Moringa oleifera seeds in water treatment.

Sammendrag

Rensing av vann ved bruk av uorganiske koagulanter og syntetiske polymerer byr på utfordringer tilknyttet metallrester og slam kvalitet/mengde. Populariseringen av kjemisk rensing de siste 50 årene har ført til at disse utfordringene har blitt mer synlige. Som en måte og adressere utfordringene tilknyttet uorganiske koagulanter og syntetiske polymerer har naturlige koagulanter blitt utforsket for bruk i vannrensing. Naturlige koagulanter er nedbrytbare, giftfri og byr ikke på de samme utfordringene som inorganiske koagulanter gjør. Målet med dette litteraturstudiet er å kartlegge rensepotensialet til to lovende naturlige koagulanter, nemlig chitosan og Moringa oleifera, og sammenligne de med mer tradisjonelle inorganiske koagulanter. I denne sammenhengen er en naturlig koagulant en naturlig polymer som brukes som hovedkoagulant.

Basert på et litteratursøk på bruken av de naturlige koagulentene chitosan og Moringa oleifera for vannrensing ble en tabell for hver av de naturlige koagulantene laget, hvor nøkkelinformasjon som behandlet vanntype, eksperimentelt oppsett, oppnådd rensegrad og rapportert koagulasjonsmekanisme ble notert. Analyse av resultatene fra undersøkt litteratur, viste at begge av de undersøkte naturlige koagulantene fungerte utmerket for fjerning av forurensinger som er vanlig i drikkevann. Få studier ble funnet hvor de naturlige koagulentene ble brukt for rensing av avløpsvann. Koagulasjons mekanismer som ble rapportert var adsorpsjon, ladnigsnøytralisering, brobygging og elektrostatisk lappmekanisme. Resultatene indikerer at begge undersøkte naturlige koagulanter kan konkurrere med uorganiske koagulanter for behandling av drikkevann, men det er behov for mer forskning på behandling av avløpsvann før en rettferdig sammenligning kan gjøres. Videre forskning burde rettes på bruk av chitosan i kombinerte prosesser, og bruken av renset protein fra Moringa oleifera i vannbehandling.

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List of Acronyms

BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
DD	Degree of Deacetylation
DI	DeIonized
DO	Dissolved Oxygen
DW	Demineralized Water
GMG	Glucomoringin
MBR	Membrane BioReactor
MLVSS	Mixed Liquor Volatile Suspended Solids
MO	Moringa Oleifera
MW	Molar Weight
NOM	Natural Organic Matter
NTU	Nephelometric Turbidity Unit
PAC	PolyAliminium Chloride
RPM	Revolutions Per Minute
SDG	Sustainable Development Goal
SS	Suspended Solids
TKN	Total Kjeldhal Nitrogen
TOC	Total Organic Carbon
TOD	Total Oxygen Demand
TR	Turbidity
TW	Tap Water
UN	United Nations
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

1. Background and Objectives

1.1 Problem context

As the world's population continues to rise there is an increased amount of pressure on our water bodies from human activities and a changing climate. Modernization of the world with increasing living standards has caused the water use to increase by a factor of six over the past 100 years (UN-Water, 2020). In addition, the climate is changing and is expected to cause the sea level to rise, causing salt intrusion in groundwater aquifers currently being used as drinking water. Increasing temperatures are also expected to increase the water stress in vulnerable regions highlighted in figure 1.1.

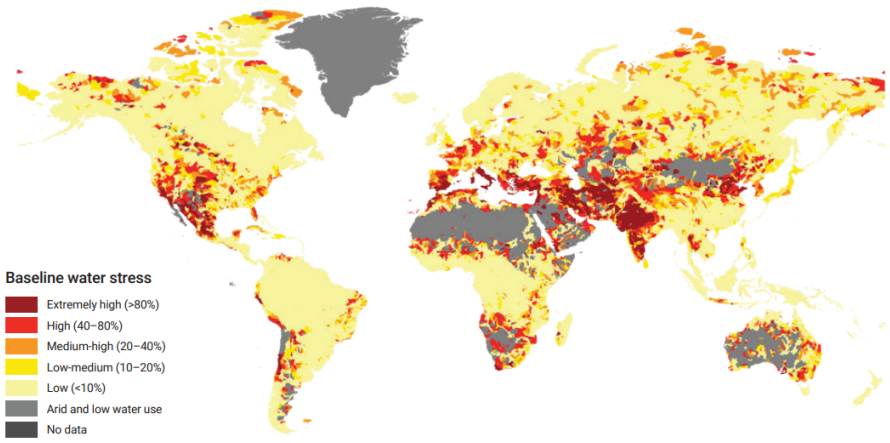


Figure 1.1: Baseline water stress measures the ratio of total water withdrawals to available renewable water supplies. (UN-Water, 2020)

Increased stress on water bodies currently being used as sources for drinking water can cause the water quality to decrease, making it necessary to treat before human consumption. As a way to combat water stress in regions with high and extremely high water stress, wastewater can be treated to drinking water quality and reused as potable water. This option is expensive, making it not viable in developing countries, and is still met with some unwarranted skepticism from the consumer.

Historically discharge of untreated wastewater has been one of the main contributors to

local pollution in the world. Untreated domestic wastewater contains organic matter, nutrients, and pathogens, while industrial wastewater can in addition to the former pollutants contain heavy metals and a variety of other substances. Uncontrolled discharge of untreated or not sufficiently treated wastewater can cause eutrophication and the spread of pathogens between humans. It is especially problematic when the recipient of the wastewater is used as a drinking water source.

The need for wastewater treatment was recognized during the mid-nineteenth century and consisted mainly of mechanical treatment focused on removing the organic matter. Since then chemical and biological treatment methods have been developed to achieve higher treatment efficiencies. Even though the technology is available not every country can afford or chooses to prioritize the cost of comprehensive wastewater treatment. A report done by WHO (2019) revealed that 22 of 79 countries who participated in the study, mainly based in Europe and America, treated 50% or less of their domestic wastewater. Most of these countries were located in northern Africa and South America. Most countries in Europe treat above 76% of domestic wastewater using mechanical, chemical, and biological treatment (WHO, 2019). United Nations (UN) sustainable development goal (SDG) 6 aims to "ensure availability and sustainable management of water and sanitation for all". Implementation of locally available and affordable natural coagulants presents a solution for developing countries that struggle to implement more traditional inorganic coagulants and thus can serve as a means to achieve SDG-6.

Norway has a long coastline with strong currents that have historically made it possible to discharge municipal wastewater with limited mechanical treatment. With a growing population, the effect of this practice became more and more apparent in areas with higher population densities and/or sensitive recipients in the form of polluted water bodies. Chemical treatment was implemented as an additional treatment step to the mechanical treatment in the early 1970s to increase the treatment efficiency of the wastewater treatment plants (WWTP). Since then the use of chemical treatment has rapidly increased and today close to 70% of the municipal wastewater is treated using chemical precipitation (Berge and Sæther, 2019).

Chemical treatment is also widely used in drinking water treatment, mainly for the removal of natural organic matter (NOM), which is prevalent in most surface waters. Degraded water quality due to the reasons mentioned earlier in the chapter often makes water treatment necessary in areas with high water stress. Inactivation of pathogens through chlorination also requires the removal of NOM to avoid disinfection byproducts, making chemical treatment in many cases necessary before human consumption.

Since the implementation of chemical treatment, inorganic coagulants have been widely used due to their effectiveness, and relative ease of use. However, recently they have

received an increased amount of attention due to a negative impact on sludge quality/quantity, and pH reduction which can cause problems for downstream processes if not controlled. As a response natural coagulants have been researched for their effectiveness in water and wastewater treatment.

1.2 Chemical treatment

After mechanical treatment, the wastewater mainly contains colloidal particles which often are quite stable. Stability, in this case, means that the particles have a high ability to remain separated and depend mainly on; the presence of a surface charge at the interface between colloidal particles and the liquid, and the hydration of surface layers of the colloid (Bratby, 2016).

The terms coagulation/flocculation and coagulant/flocculant are interpreted different based on where you are in the world. To avoid ambiguity this review adopts the following definitions proposed by Bratby (2016, p. 6) and Ratnaweera (2020).

- Coagulation is the process of destabilization of colloidal systems, leading to the agglomeration of particulate material in water and wastewater. However, coagulation in wastewater treatment is defined as the process that leads to the removal of colloids, particles, and phosphates through precipitation and agglomeration into bigger and easily settable particulate material.
- Flocculation is the process whereby destabilized particles, or various species formed as a result of destabilization, are induced to come together, make contact and thereby form large(r) agglomerates.
- Coagulant refers to that chemical or substance added to a given suspension or solution to effect destabilization.
- Flocculant (or filter) aids are those chemicals or substances added to a destabilized suspension or solution to accelerate the rate of flocculation or to strengthen flocs formed during flocculation.

A chemical treatment step normally consists of coagulation, flocculation, and particle separation. Coagulation is the addition of the coagulant which promotes destabilization of the colloidal particles in the water. How the destabilization occurs depends on the nature of the colloidal particles in the solution and the added coagulant. Generally, destabilization occurs through charge-neutralization, bridging, double-layer compression, and sweep flocculation.

Charge-neutralization involves the adsorption of a molecule (or ion) from solution onto

the surface of the added coagulant, neutralizing the charge; bridging is the formation of a "bridge" between adjacent particles promoting destabilization and is often present in coagulants with long polymeric chains.

Double-layer compression happens through the addition of positively charged coagulants which compresses the double-layer around the particles, making it possible for them to get so close that the van der Waal forces of attraction become dominant, resulting in destabilization.

Sweep flocculation is the enmeshment of particulate matter. A type of coagulant may cause destabilization by more than one mechanism dependent on the coagulant.

Flocculation is the phase where particles destabilized by coagulants are induced to form aggregates and is normally done by slow and rapid mixing in succession. Lastly, the newly formed aggregates are separated from the water phase by particle separation. Particle separation is either done by sedimentation, flotation, or mechanical filters (membrane/filters).

1.2.1 Inorganic coagulants and synthetic polymers

Inorganic coagulants

Inorganic coagulants, often called metal coagulants, are generally based on aluminum and iron. Aluminum coagulants used in water- and wastewater treatment include aluminum sulfate, aluminum chloride, aluminum chlorohydrate, sodium aluminate, polyaluminium chloride, polyaluminium sulfate chloride, polyaluminium silicate chloride, and polyaluminium chloride with organic polymers (Bratby, 2016). Iron coagulants include ferric sulfate, ferric chloride, ferrous sulfate, polyferric sulfate ferric, chloride sulfate, and ferric salts with organic polymers (Bratby, 2016). Other inorganic coagulants not based on aluminum and iron are hydrated lime and magnesium carbonate. Coagulants based on aluminum and iron are the most used coagulants in the world, mainly due to their effectiveness, availability, and relatively low cost. They are also well studied making them easier to implement and optimize which again contributes to their popularity.

The addition of inorganic coagulants to the wastewater produces a wide arrange of hydroxides with different degrees of hydrolysis. Simplified the reaction can be written as:

$$Me^{3+} + 3H_2O \leftrightarrow Me(OH)_3 + 3H^+ \tag{1.1}$$

where Me denotes a metal species, either Al or Fe. The production of H^+ ions causes the pH to drop. Distribution of Al(III) and Fe(III) species depends on the pH and is summarized in figure 1.2. Optimal pH for destabilization of a specific colloid solution

will depend on which hydrolysis species is most effective for destabilizing said solution. Pre-polymerized species of inorganic coagulants prevent rapid uncontrolled hydrolysis by slowing down the hydrolysis reaction, effectively enhancing charge interactions.

Destabilization by metal-coagulants are achieved through all of the previously mentioned mechanisms; charge-neutralization, bridging, double-layer compression, and sweep flocculation. Sweep flocculation is more dominant in metal-based coagulants due to the formation of metal-hydroxide precipitates which traps particulate matter, making it easier to remove from the water-phase. The effect of coagulant dosage on destabilization depends on the type and amount of colloid particles present in the water (figure 1.3). When electrical double-layer compression is dominant, an increase in coagulant dosage beyond the compression of the double-layer has little effect on the treatment efficiency. For solutions where adsorption is the dominant mechanism of coagulation, an increased dose beyond the point of destabilization can cause the particles to restabilize. Addition of coagulants beyond restabilization to the point of oversaturation will produce metal-hydroxide precipitates and cause sweep flocculation.

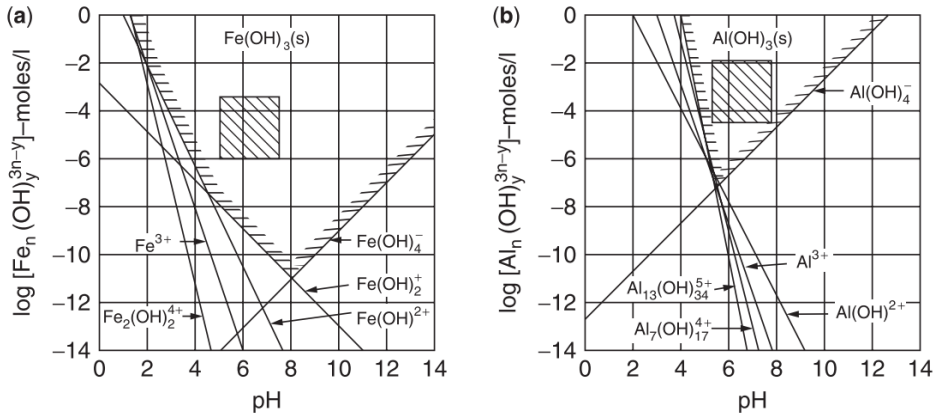


Figure 1.2: Equilibrium-solubility domains of (a) ferric hydroxide and (b) aluminium hydroxide in water. Shaded areas represent the normal working conditions of water treatment. (Stumm and O'Melia, 1968; Bratby, 2016)

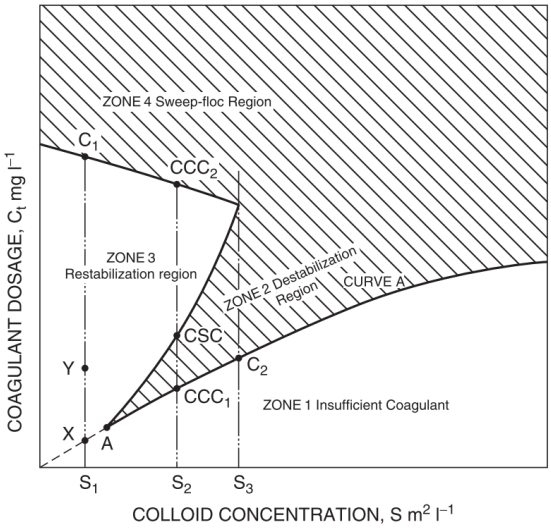


Figure 1.3: Destabilization and restabilization of colloids at a given pH value as related to colloid concentration, and coagulant dose. (Stumm and O'Melia, 1968; Bratby, 2016)

One of the main reasons metal-based coagulants are so frequently used are their ability to convert soluble phosphorus species to an insoluble form, making them possible to remove by the mechanisms mentioned above. Soluble phosphorus is present as orthophosphates, polyphosphates, pyrophosphates, and organic phosphates and makes up around 65% of the total phosphorus in domestic wastewater (Maurer and Boller, 1999). Ortophosphates and particulate phosphates are removed most rapidly by chemical precipitation, while polyphosphates and organic phosphorus are not removed as readily. Simplified the soluble orthophosphates are converted into an insoluble form through the following equation:

$$Me^{3+} + H_3PO_4 \leftrightarrow MePO_4 + 3H^+ \tag{1.2}$$

This reaction takes place alongside reaction 1.1 and also causes the pH to decrease.

As the use of inorganic coagulants has become more common, there has been an increased focus on the negative aspects of inorganic coagulants. Negative aspects with the use of inorganic coagulants are mainly connected to sludge quality and an increased amount of sludge. Besides, the use of aluminum-based coagulants can produce water with residual aluminum content. A high intake of aluminum has been linked to Alzheimer's disease (Campbell, 2002), but studies have shown that only 1-2% of the daily intake of aluminum comes from drinking water (Stauber et al., 1999). The significance of the residual aluminum in drinking water is therefore contestable, however the increased amount of sludge and decreased sludge quality poses challenges in transportation and potential reuse of the sludge.

Synthetic polymers

Synthetic polymers are polymers where properties are altered to increase the effectiveness of the polymer as a coagulant/flocculant. This is often done through altering properties such as number and type of charged units, and the molecular weight of the polymer. Functional groups attached to the backbone of the polymer may or may not carry a charge that gives an anionic character, cationic character, or ampholytic character. The charge is described by the percentage hydrolysis and charge density of the polymer. The molecular weight reflects the length of the polyelectrolyte chains. In addition to carrying charge, functional groups also serve as sites for adsorption. Popular monomers used for the synthesis of polymers are acrylamide, acrylic acid, and diallyldimethylammonium chloride (Renault et al., 2009a).

The main mechanisms of destabilization for synthetic polymers are bridging and electrostatic patch mechanism. For non-ionic and anionic polymers applied to a negatively charged dispersion, the bridging mechanism adequately accounts for the phenomena taking place (Gregory and Sheiham, 1974). For the bridging mechanism, polymers adsorb onto adjacent particles, leaving a part of the polymer extending into the solution, which then collides with other adjacent polymer-coated particles forming bridges and thus creating larger flocs. Higher molecular weight, and in turn, longer polymer chains are beneficial for destabilization through bridging. Electrostatic patch mechanism is relevant in cases where charged polymers are applied to dispersions with particles carrying surface charges of the opposite charge. Added polymer adsorbs completely to particles creating charged "patches" on the particle. Destabilization occurs when charged patches on adjacent particles align to provide strong electrostatic attraction.

Concerns with the use of synthetic polymers generally arise from unreacted monomers during use, unreacted chemicals released during the production of the polymers, and reaction by-products of the polymers in water. Common unreacted monomers are acrylamide, ethylenimine, diallydimethylammonium chloride, and trimethylolmelamine. Unpolymerized monomers suck as acrylamide are highly toxic producing neurotoxic effects (Letterman and Pero, 1990). Biosols from water treated with synthetic polymers show a long term presence of polymers, however, no harmful effect has been detected (Dentel et al., 2000).

1.2.2 Natural coagulants

Natural coagulants are coagulants which are derived from natural sources such as plants and animals and are in most cases organic polymers. Advantages with natural coagulants are that they are toxic-free, biodegradable, and often locally available. There exists a large amount of natural coagulant with over 50 being reported researched for water

treatment (Saleem and Bachmann, 2019).

To narrow the scope of the review, research on treatment efficiency will be focused on the two natural coagulants chitosan and Moringa oleifera seeds. Chitosan was chosen due to being one of the more effective and well researched natural coagulants, and to represent a non-plant based natural coagulant. Moringa oleifera was chosen based on it being a plant-based natural coagulant, and because of its ease of use, making it an option for developing countries.

Chitosan

Chitosan is the product of deacetylation of chitin (Figure 1.4), a polymer mainly found in the exoskeletons of crustaceans, arthropods and the cell wall of fungi, and is a linear copolymer of *D*-glucosamine and N-acetyl-*D*-glucosamine. The main parameters influencing the properties of chitosan is the degree of deacetylation (DD) and the molar weight (MW). DD depends on the preparation method, source of chitin, and indicates the molar fraction of deacetylated units and crystallinity. Chitin is insoluble in water, but chitosan with a degree of deacetylation (DD) over 50% can be dissolved in weakly acidic solutions (pH<5.0)(Rinaudo, 2006a).

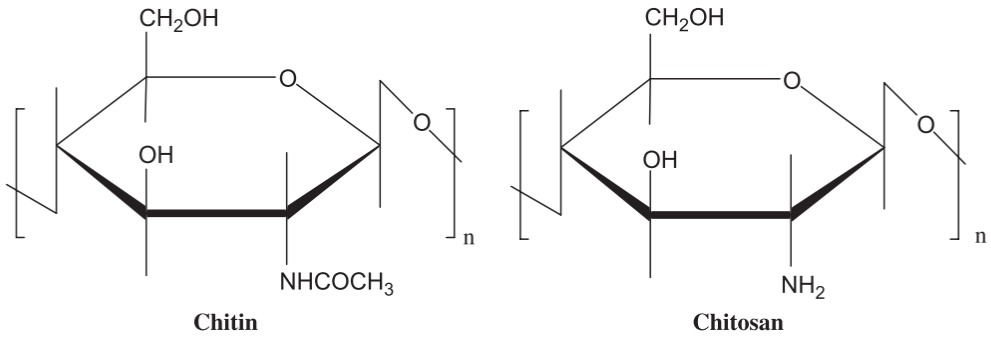


Figure 1.4: Chitin and its deacetylated form chitosan. (Crini and Badot, 2008a)

Chitin is readily available with it being the second most abundant bipolymer in the world (Renault et al., 2009a). For deacetylation to chitosan mainly chitin from crab and shrimp shells are used, due to their availability as by-products from the seafood industry. An estimate from 2014 showed that 6-8 million tonnes of crab, shrimp, and lobster shells were produced annually, and large amounts are dumped into the sea or deposited in landfills (Rødde et al., 2015). Around 30-40% of the mass of the shells consists of chitin, and in 2000 the chitosan production was estimated to be 2000 tons (Kurita, 2006). As the production of crab, shrimp, and lobsters are increasing (FAO, 2019), efficient use of their shells are becoming important to address waste disposal problems and to maximize financial return. Production of chitosan is a way to turn the

waste into a useful product. In recent decades, chitosan has been used in a range of different applications including agriculture, water & waste treatment, food & beverages, cosmetic & toiletries, and biopharmaceutics (Rinaudo, 2006a).

Several inherent characteristics make chitosan an effective coagulant/flocculant for the removal of contaminates. It can work both as a coagulant and flocculant because of its high cationic charge density and long polymer chains (Renault et al., 2009a), and it works over a broad pH range compared to inorganic coagulants. Chitosan also has antimicrobial properties and is used in the food industry for preserving food (Rabea et al., 2003). Antimicrobial mechanism of action is speculated to be interactions between positively charged chitosan molecules and negatively charged microbial cell membranes (Chung et al., 2005).

With increasing focus on the environmental effect of coagulants, chitosan has gotten increasingly more attention on account of properties such as; non-toxicity, biodegradability, biocompatibility, renewability, and antibacterial properties (Rinaudo, 2006a).

Moringa oleifera seeds

Moringa Oleifera is a multipurpose tree common across the tropics and subtropics whose seeds contain a water-soluble active agent that promotes coagulation (figure 1.5) (Ndabigengesere et al., 1995). The nature of the active agent is still not well established, but several studies have found cationic proteins to be the main active agents (Gassenschmidt et al., 1995; Ndabigengesere et al., 1995). Historically the seed extract has been used for water clarification in Malawi and Sudan (Eilert et al., 1981). The plant is fast-growing with seeds being harvestable after 10 months (Jahn et al., 1986). Harvested seeds are non-toxic and have been shown to possess antimicrobial properties. In addition to the active agent, the seeds contain up to 40% edible oil which can be extracted before using the seed as a coagulant (Rashid et al., 2008; Leone et al., 2016a).

Figure 1.5: *Moringa oleifera.* (Pritchard et al., 2010)

Preparation of the coagulant is done by extracting seeds from their pods and removing the husk covering the seed (figure 1.6). The seed is then ground into a powder using an electric blender. Research on shelled versus non-shelled MO seeds showed that shelled MO seeds were better at turbidity removal, requiring a lower dose for a higher removal rate (Ndabigengesere et al., 1995).

At this stage, the oil can be extracted from the powdered seed using ethanol but is not necessary for further use as a coagulant (Camacho et al., 2017). To extract the active agent in the MO seeds, the powdered seed is added to water (secondary process in figure 1.6). Ndabigengesere et al. (1995) recommended the standard concentration of 5% (crude powder/solvent) since higher concentrations were hard to filter. This has since been adopted as the standard and most studies on MO seeds use this concentration (Ghebremichael et al., 2005; Beltrán-Heredia et al., 2009).

As an alternative to water, salt solutions can be used for the extraction of the active agent and have shown to increase the turbidity removal in kaolin suspensions (Okuda et al., 1999). This phenomenon was explained by the presence of a higher amount of soluble protein due to the salting-in effect (E, 2014). After the addition of the crude powder to the water or salt solution, the solution is stirred for 30 min before it is filtered through a Whatman filter (0.47 μm) (Ndabigengesere et al., 1995). The crude extract (filtrate) is then used as a coagulant.

Early studies focused mainly on studying the coagulation properties of the active agent in the Moringa Oleifera (MO) seeds and comparing it with more traditional inorganic coagulants (Jahn and Dirar, 1979; Grabow et al., 1985). These studies showed good turbidity removal and after the coagulation properties of the MO seeds were established, different studies tried to identify and purify the active component.

Gassenschmidt et al. (1995) suggested that the active component was a protein with a molecular mass around 6.5 kDa and an isoelectric point above pH 10. Ndabigengesere et al. (1995) reported that the active agent was a dimeric cationic protein with a molecular weight of 13 kDa with subunits of about 6.5 kDa and an isoelectric point between 10 and 11. Okuda et al. (2001) managed to purify a non-proteic active component of 3 kDa using a salt extract solution and managed to flocculate kaolin suspension with it. Several coagulant peptides have been isolated from the MO seed, but only one of them has been sequenced (Gassenschmidt et al., 1995). This indicates that more than one coagulant protein is present and that they may differ in one or more amino acid residues.

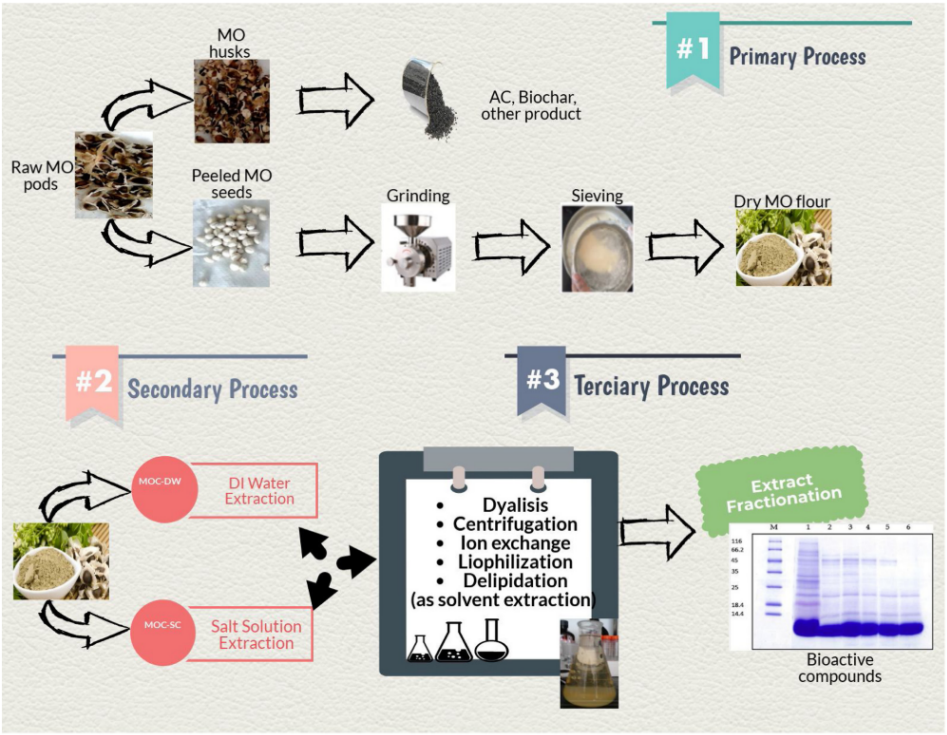


Figure 1.6: Processing techniques for MO seed. (Villaseñor-Basulto et al., 2018)

The main advantage with the use of Moringa oleifera seeds in water treatment is that it requires little to no equipment for its use at its base level, and thus can be used for water purification in areas where commercial options are not available.

Moringa oleifera seeds greatest disadvantage is its content of dissolved organic molecules and phosphates which in turn increase the chemical oxygen demand (COD) COD and phosphate levels in the treated water (Ndabigengesere and Narasiah, 1998). To overcome this challenge several studies have worked on developing ways to purify the active proteins in the crude extract (Gassenschmidt et al., 1995; Ndabigengesere et al., 1995; Okuda et al., 1999). Purification methods used are delipidation, dialysis, centrifugation, ion exchange, or lyophilization.

1.3 Existing reviews

1.3.1 Chitosan

Numerous reviews on chitosan exist, included reviews can be found in table 1.1. In total 18 reviews were included. Reviews done mainly covers the removal of specific pollutants such as heavy metals (6), microorganisms (3), and dyes (1). The 2 reviews on water treatment included the pollutants mentioned above, but also covered the removal of suspended solids and organic matter. Remaining reviews focused on the characteristics of chitosan such as production, mechanism of action, and parameters affecting coagulation.

Table 1.1: Overview of selected reviews on chitosan.

Author	Name	Scope
Bailey et al., 1999	A review of potentially low-cost sorbents for heavy metals	Heavy metal ion removal
Ravi Kumar, 2000	A review of chitin and chitosan applications	General
Rabea et al., 2003	Chitosan as antimicrobial agent: Applications and mode of action	Antimicrobial properties
Guibal, 2004	Interactions of metal ions with chitosan-based sorbents: A review	Heavy metal ion removal
Rinaudo, 2006b	Chitin and chitosan: Properties and applications	General
Gerente et al., 2007	Application of chitosan for the removal of metals from wastewaters by adsorption - Mechanisms and models review	Heavy metal ion removal
Crini and Badot, 2008b	Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: A review of recent literature	Dye removal
Bhatnagar and Sillanpää, 2009	Applications of chitin- and chitosan-derivatives for the detoxification of water and wastewater - A short review	Water treatment
Goy et al., 2009	A review of the antimicrobial activity of chitosan	Antimicrobal properties
Miretzky and Cirelli, 2009	Hg(II) removal from water by chitosan and chitosan derivatives: A review	Hg(II) removal
Kean and Thanou, 2010	Biodegradation, biodistribution and toxicity of chitosan	Toxicity
Kong et al., 2010	Antimicrobial properties of chitosan and mode of action: A state of the art review	Antimicrobal properties
Dash2011	Chitosan - A versatile semi-synthetic polymer in biomedical applications	General
Wan Ngah et al., 2011	Adsorption of dyes and heavy metal ions by chitosan composites: A review	Heavy metal ion removal
Vakili et al., 2014	Application of chitosan and its derivatives as adsorbents for dye removal from water and wastewater: A review	Dye removal
Yang et al., 2016	A review on chitosan-based flocculants and their applications in water treatment	Water treatment
Zhang et al., 2016	Removal of heavy metal ions using chitosan and modified chitosan: A review	Heavy metal ion removal
Verlee et al., 2017	Recent developments in antibacterial and antifungal chitosan and its derivatives	Antimicrobal properties

Numerous reviews on chitosan in relation to the food and drug industry exist, but they were not included due to being deemed not relevant enough. All the reviews included were assessed for eligibility (see appendix B). No relevant reviews were discarded.

1.3.2 Moringa oleifera seeds

Far less reviews on MO seeds exist, which is expected since it is less used and studied. Included reviews can be found in table 1.2. In 3 of the 8 included reviews (plant based coagulants), MO seeds are only covered subsection. The remaining reviews covers properties of MO seeds such as toxicity, and use of MO seeds in water and wastewater treatment.

Table 1.2: Overview of selected reviews on Moringa oleifera seeds.

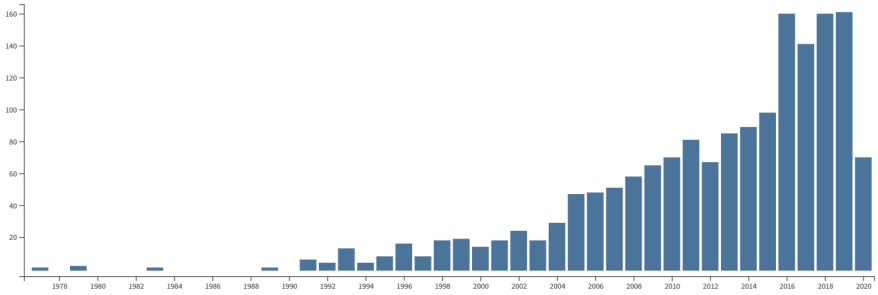
Author	Name	Scope
Yin, 2010	Emerging usage of plant-based coagulants for water and wastewater treatment	Plant-based coagulants
Mahmood et al., 2010	Moringa oleifera: A natural gift-a review	General
Bichi, 2013	A Review of the Applications of Moringa oleifera Seeds Extract in Water	Water treatment
Choy et al., 2014	Utilization of plant-based natural coagulants as future alternatives towards sustainable water clarification	Plant-based coagulants
Stohs and Hartman, 2015	Review of the Safety and Efficacy of Moringa oleifera	Safety of use
Leone et al., 2016b	Moringa oleifera seeds and oil: Characteristics and uses for human health	General
Villaseñor-Basulto et al., 2018	Wastewater treatment using Moringa oleifera Lam seeds: A review	Wastewater treatment
Saleem and Bachmann, 2019	A contemporary review on plant-based coagulants for applications in water treatment	Plant-based coagulants

Even though there exists a relative large amount of literature reviews on water and wastewater treatment by MO seed and especially chitosan, there is need for constant updates including some of the new research done. This review also goes more into depth on two different natural coagulants and indirectly compares them, which no other review found did.

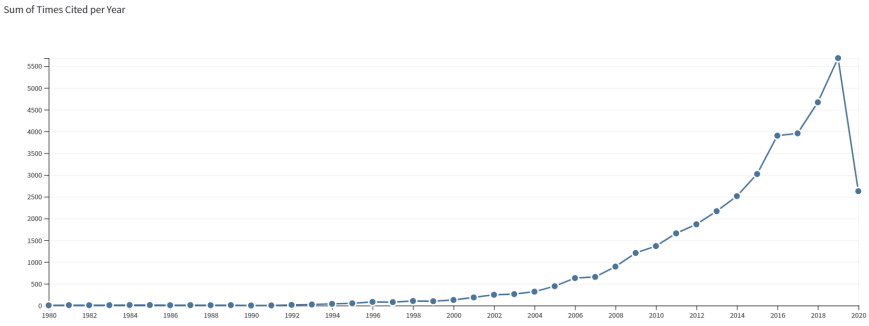
1.4 Research questions and reasoning

With chemical precipitation being popularised during the last 50 years more focus has been directed at the negative aspects of chemical precipitation. Negative aspects such

as residual aluminium, increased amount of sludge are tied to the use of inorganic coagulants, while unreacted monomers and reaction by-products are tied to the use of synthetic coagulants during chemical precipitation. Natural coagulants, which earlier was looked on as a more expensive and less effective alternative, has in the last 30 years gotten more attention for its properties such as biodegradability, non-toxicity and renewability (figure 1.7a and 1.7b).



(a) Publications



(b) Citations

Figure 1.7: Yearly publications (a) and citations (b) containing the topic "natural coagulants" in the time period 1980-2020. Data from Web of Science (*Web of Science* 2020).

Due to the share amount of potential natural coagulants, it is beneficial to classify the known natural coagulants into subgroups based on their origin and properties. This leads to the first research question.

Research question 1:

- What should be the classification of the known natural coagulants based on their origin and properties?

Classification will be based on common origins and important properties that are uncovered during the review. Because natural coagulants are not tailored with regards to charge and MW, different natural coagulants may be effective for different types of contaminants. It's therefore important to map the treatment effects and limitations of the different natural coagulants to find out where they best can be used. Comparing the treatment effects and limitations of natural coagulants to more traditional inorganic-

and synthetic coagulants/polymers make it possible to evaluate where natural coagulants can be used as an alternative to inorganic/synthetic coagulants. This leads to the second research question.

Research question 2:

- What are the treatment efficiency achieved by natural coagulants in water and wastewater treatment in comparison with inorganic/synthetic ones?

As previously mentioned, research into the treatment effect will be limited to the natural coagulants chitosan and MO seed (Moringa Oleifera seed), instead of a more shallow review of all of the natural coagulants. Treatment effects in both water and wastewater will be included. To be able to explain the treatment effects achieved by the different authors of the reviewed literature, the authors theorized mechanism of action of used natural coagulant will be noted.

Research question 3:

- What are the mechanisms of action of selected natural coagulants?

Research question 3 will be answered based on the collected explanation for achieved removal efficiency in the reviewed studies. Knowing all the mechanisms of action for the natural polymers and which one is dominant for the different water pollutants makes it possible to optimize coagulation processes.

2. Methods

2.1 Criteria for considering studies for this review

Studies included in this review were chosen based on their relevance to the research questions posed in section 1.4 and had to fit the following criteria (table 2.1).

Table 2.1: Criteria for considering studies for this review.

Treated water	Wastewater Drinking water
Coagulant type	Synthetic coagulant Natural organic coagulants
Treatment parameters	Turbidity pH Phosphates

Since synthetic coagulants are well studied, emphasis were put on studies using natural organic coagulants. To further narrow the scope of the review, studies using chitosan and moringa oleifera seeds were prioritized. Treatment parameters listed in table 2.1 are the minimum of parameters included in a study for it to be included in the literature review. These parameters were chosen due to their popularity as parameters that are easy to measure and provides sufficient information on the treatment efficiency of the coagulant, making it easier to compare treatment efficiencies.

If the study had all the criteria checked further work was put into assessing the quality of the study. Parameters used to assess the quality of the study were:

- Amount of citations
- Impact factor of journal published in
- Year published

How the parameters were applied is further described in subsection 2.3.1.

2.2 Search methods for identification of studies

To reduce reporting bias, a range of databases/search engines were used to find studies included in the review. Since studies were stored in Mendeley's library function, Mendeley's search function was primarily used to locate studies. Search engines used were Google scholar and Science direct which was mainly used to find additional studies or specific studies that were not available in Mendeley.

2.3 Data collection

2.3.1 Selection of studies

Possible eligible studies were read, keeping the criteria listed in section 2.1 in mind. The number of citations needed for a study to be eligible depended on when it was published. In general the older the study is, the more citations it needed to be included. To avoid outdated information newer studies were preferred to older studies. Older studies that are still relevant were included if no newer study covering the same topic existed. The journal where the study was published in was reviewed based on the impact factor of the journal. Only studies published in English or Norwegian were reviewed. Appendix B contains the number of citations and journal impact factor for every study included in the literature review.

2.3.2 Data collection and management

Mendeley's library function was used to store studies considered included in the review and later studies included. Mendeley was chosen because of its synergy with Overleaf, allowing the library to be synced to the document automatically. It also allows writing notes in stored pdfs. Studies not found in Mendeleys' database were added manually.

3. Results

3.1 Included studies

Table 3.1 and 3.2 on page 18 and 19, contains the reviewed studies. 33 of the 41 considered studies on chitosan were included in the review (table 3.1). 33 of the 37 considered studies on MO seeds were included in the review (table 3.2). Amount of citations and impact factor for each of the included studies can be found in appendix B. Both tables are sorted alphabetically by coagulation medium.

Table 3.1: Studies on Chitosan included in the literature review.

Coagulation medium	Chitosan	Experimental setup	Removal efficiency	Coagulation mechanism	Reference
Algae solution	DD N/A, MW N/A	Jar test	TR 93%	Charge neutralization, Bridging	Divakaran and Pillai, 2002
Anionic dye solution	DD 87%, MW $10^5 g/mol$	Jar test	Dye removal 99%	Charge neutralization, Bridging	Szyguła et al., 2009
Aquaculture wastewater	DD 90%, MW (10^5, 10^4, 10^3)	Biological filter, Jar test	Bacteria 99.9%, COD 62.8% SS 62.6%, TR 87.7%, NH_3 91.8%, PO_4 85.6-99.1%	Charge neutralization, Bridging	Chung et al., 2005
Bentonite solution	DD 95%, MW - varying	Jar test	TR 99%, TR 93%	Eletrostatic patch, Bridging, Charge neutralization	Soros et al., 2019
Bentonite solution	DD N/A, MW N/A	Jar test	TR 99%	Charge neutralization, Bridging	Hu et al., 2013a
Bentonite solution	DD N/A, MW N/A	Jar test	TR 98%	Bridging, Charge neutralization	Huang and Chen, 1996
Biodiesel wastewater	DD 93%, MW $6 * 10^5 g/mol$	Batch test	COD 88%, TSS 77%	Charge neutralization	Pitakpoolsil and Hunsom, 2013
Brewery wastewater	DD N/A, MW N/A	Jar test	COD 50%, TR95%	Bridging, Charge neutralization	Cheng et al., 2005
Clay suspension	DD 85.84%, MW N/A	Jar test	TR 61.9% - low initial turbidity TR 84.1% - medium initial turbidity TR 94.0% - high initial turbidity	Charge neutralization, Bridging	Jadhav and Mahajan, 2013
Diary wastewater	DD N/A, MW N/A	Jar test, Filtration	COD 55%, TR 97%	Bridging	Chi and Cheng, 2006
Diary wastewater	DD 88%, MW 10^4 g/mol	Jar test	COD 79%, TR 93%, TSS 73%	Bridging, Charge neutralization	Geetha Devi et al., 2012
Domestic wastewater	DD 85-98%	Jar test	COD 90.39%, SS 39%	N/A	Zeng et al., 2008
Drinking water	DD 85%, MW N/A	Jar test, Filtration	DOC 20.5%, TR 99%	N/A	Fabris et al., 2010
Fluorite solution	DD 85%, MW N/A	Batch test	Fluoride 5%	N/A	Kamble et al., 2007
Heavy metal solution	DD 85%, MW N/A	Batch test	Cu(II) 9.6%, Pb(II) 58.6%	Adsorption	Qin et al., 2006
Heavy metal solutions	DD 85-90%, MW $10^5 g/mol$	Jar test	Fe(II) 94.1%, Zn(II) 50.3% Hg(II) 97.5%, Pb(II) 91.0%	Charge neutralization	Gamage and Shahidi, 2007
Inc containing wastewater	DD 89.5%, MW $10^4 g/mol$	Jar test	COD 84%, Color 99%	Charge neutralization	Roussy et al., 2005b
Industrial wastewater	DD 75%, MW N/A	Batch test	Ag(II) 80-95%	Charge neutralization	Lasko and Hurst, 1999
Kaolin suspension	DD N/A, MW N/A	Jar test	TR 95%	N/A	Divakaran and Sivasankara Pillai, 2001
Organic suspension	DD 95%, MW N/A	Jar test	TR 90%	Charge neutralization, Bridging	Roussy et al., 2005a
Palm oil mill effluent	DD 85%, MW N/A	Jar test	SS 95%, Palm oil 99%	Charge neutralization, Bridging	Ahmad et al., 2006
Petroleum refinery wastewater	DD 90%, MW $1.7 * 10^5 g/mol$	Adsorption column	COD 65%, Oil removal 67%	Charge neutralization, (Adsorption)	Milhome et al., 2009
Pulp and paper wastewater	DD 85%, MW $1.8 * 10^5 g/mol$	Biological filter, Jar test	COD 80%, TR 85%	Charge neutralisation, Bridging, Electrostatic patch	Renault et al., 2009b
Rice mill wastewater	DD N/A, MW N/A	Batch test, Centrifugation, Filtration	COD >95%, TSS >95%	Charge neutralization	Thirugnanasambandham et al., 2013
Sea water	DD N/A, MW 10^4	Jar test	TR 97.5%	Charge neutralization, Bridging	Altaher, 2012
Surface water	DD N/A, MW N/A	Jar test, Filtration	TR 96.6%, Bacteria 87.5%	Charge neutralization, Adsorption	Mandloi et al., 2004
Surface water	DD N/A, MW N/A	Jar test	TR 77%	N/A	Rizzo et al., 2008
Surface water	DD 85%, MW N/A	Jar test	TR 16% (low initial turbidity)	Charge neutralization	Zemmouri et al., 2012
Surface water	DD 85%, MW N/A	Jar test	TR 87%	Bridging, Electrostatic patch	Zemmouri et al., 2013
Surface water	DD N/A, NW $10^5 g/mol$	Jar test	TR 50-95%, Colour 60-78%, TOC 7%	Charge neutralization	Christensen et al., 2016
Surface water	DD N/A, MW N/A	Jar test	TR 50.5% ,TSS 27.16%	Charge neutralization, Bridging	Al-Manhel et al., 2018
Textile wastewater	DD N/A, MW N/A	Batch test	COD 69.6%, Color 47.3%, SVI 331,2 mg/l	N/A	Patel and Vashi, 2013
Unhairing wastewater	DD 85%. MW N/A	Jar test	BOD 33.3%, COD 58.7%, TSS 89.0%	N/A	Sila et al., 2014

Table 3.2: Studies on Moringa oleifera seeds included in the literature review.

Coagulation medium	Extraction type (purified yes/no)	Experimental setup	Removal efficiency	Coagulation mechanism	Reference
Bacteria solution	Water (no)	Kirby-Bauer disk diffusion	Inhibited growth of Gram-positive bacteria No effect on Gram-negative bacteria	N/A	Vieira et al., 2010b
Biodiesel producer microalgae	Powder (no)	Batch test	Biomass recovery 85%	N/A	Teixeira et al., 2012
Cyanobacteria solution	Powder (no)	Coagulation, flocculation, flotation	Chlorophyll 96.5%, Colour 80.5%, TR 78.1%	Charge neutralization	Moreti et al., 2016
Dairy industry wastewater	Powder (no)	Jar test	TR 98%, Color 95%, COD 50%	Adsorption	Vieira et al., 2010a
Domestic wastewater	Water (no)	Jar test	TR 89.2%, COD increased PO_4^{3-} increased, TKN increased	N/A	Ndabigengesere and Narasiah, 1998
Domestic wastewater	Powder (no)	Batch test	TR 98.6%, BOD 11.7%, e-coli 80%	N/A	Hendrawati et al., 2016
Domestic wastewater	Water (no)	Jar test, filtration	COD 50%	N/A	Bhuptawat et al., 2007
Dye solution (tartrazine)	Powder (no)	Batch test	TR 98%, Tartrazine 95%	Adsorption	Reck et al., 2018
Dye wastewater (Congo red)	Water (no)	Jar test	Dye reduction 99.4%	Adsorption	Chethana et al., 2016
Ground water	Water (no)	Jar test	TR 92-99%, $CaCO_3$ 60-70%	N/A	Muyibi and Evison, 1995a
Ground water	Powder (no)	Batch test	TR 97.5%, BOD 18%, Cd 99% Mn 99%, Bacteria 45%	N/A	Hendrawati et al., 2016
Heavy metal solution	Powder (no)	Batch	Co 30%, Cu 82%, Pb, 95%, Cd 70%, Ag 98%	Electrostatic patch, Adsorption	Araújo et al., 2010
Kaolin suspension	Water (no)	Jar test	TR 98%	Bridging, Adsorption	Muyibi and Evison, 1995b
Kaolin suspension	Water (no)	Jar test	TR 90%	Adsorption, Charge neutralization	Ndabigengesere et al., 1995
Kaolin suspension	Water (no)	Jar test	TR 93%	N/A	Ndabigengesere and Narasiah, 1996
Kaolin suspension	Water (no)	Jar test	TR 92%, COD increased	N/A	Ndabigengesere and Subba Narasiah, 1998
Kaolin suspension	Saltwater (no)	Jar test	TR 95%	N/A	Okuda et al., 1999
Kaolin suspension	Saltwater (no)	Jar test	TR 98%, COD increased	N/A	Okuda et al., 2001
Kaolin suspension	Saltwater (yes)	Jar test	TR 98%, COD not increased	N/A	Okuda et al., 2001
Kaolin suspension	Water (no)	Jar test	TR 79% medium initial turbidity (160 NTU) TR 89% high initial turbidity (300 NTU) TR 94% very high turbidity (400 NTU)	Adsorption, Bridging	Katayon et al., 2006a
Kaolin suspension	Water (no)	Jar test	TR 90%	Charge neutralization, Bridging Electrostatic patch	Pritchard et al., 2010
Palm oil mill effluent	Water (no)	Jar test	TSS 95%, COD 52.2%	Charge neutralization	Bhatia et al., 2007
Surface water	Powder (no)	Jar test	TR 92-98%	N/A	Jahn and Dirar, 1979
Surface water	Water (no)	Jar test	TR >90%	N/A	Muyibi and Alfugara, 2003
Surface water	Powder (no)	Jar test	TR 77%	N/A	Amagloh and Benang, 2009
Surface water	Water (no)	Jar test	TR 90-99%, Bacteria 90-99%	N/A	Lea, 2014
Surface water	Water (no)	Batch test	TR 50% (low initial turbidity), Cu 90% Pb 80%, Cd 60%, Zn 50%, Cr 50%	Charge neutralization, Bridging	Subramanium et al., 2011
Surface water	Saltwater (no)	Jar test	TR 99.8%, Colour 97%	Charge neutralization	Madrona et al., 2012
Surface water	Saltwater (no)	Jar test	TR 95%	N/A	Sánchez-Martín et al., 2012
Surface water	Powder (no)	Jar test	TR 76-92.3%	N/A	E, 2014
Surface water	Powder (no)	Jar test	TR 99%	N/A	Muthuraman and Sasikala, 2014
Surface water	Water (yes)	Jar test	TR 89.71%, Colour 87.40%	Charge neutralization	Baptista et al., 2017
Surface water	Powder (no)	Jar test	TR 89% high initial turbidity TR 60% low initial turbidity	Charge neutralization	Camacho et al., 2017

3.2 Classification of natural coagulants

3.2.1 Materials used as a source of natural coagulants

A wast majority of natural coagulants explored for water treatment are polymeric and of a plant-based origin. Saleem and Bachmann, 2019 did a comprehensive review of plant-based coagulants reported used for water treatment and summarized them in a table (table 3.3). Plant parts used is for the most part seeds, fruit, leaves, and roots. In total 49 different plant species were found to have been explored for water treatment.

Other major natural coagulants which are not plants, but rather polysaccharides that are found in plants, animals, and protists are alginate, chitosan, cellulose, starch, pullulan, xanthan, and pectin (Bratby, 2016; Salehizadeh et al., 2018).

Alginate is a linear, anionic polysaccharide, and over 200 types of alginate have been identified. The extraction of alginate is mainly from seaweed with MW in the range of 32-400 kDa. The polysaccharide is widely used in biomedical, pharmaceutical, and biotechnology (Hay et al., 2009).

Cellulose is a linear polysaccharide and can be derived from different origins, such as plants, animals, and microorganisms. Plant-based cellulose is usually in a mixture with hemicellulose, lignin, pectin, and other substances while bacterial cellulose is pure (Salehizadeh et al., 2018).

Starches from various sources including potato, wheat, rice, corn, cassava, arrowroot, and yams, may be processed into polymers. Starches are highly polymerized carbohydrates and may be non-ionic, cationic, or anionic, depending on how it is processed and the substitutions. Anionic types have carboxylic substitutions, cationic types have quaternary ammonium group substitution, and non-ionic types have no substitutions (Bratby, 2016). Processes polymers are of high molecular weight, generally in the order of several million (10-1000 kDa). Research on starches in water treatment is mainly done using the starches as flocculant aid. Pullulan is a water-soluble linear bipolymer produced by fermentation of a yeast-like fungus. MW is reported in the range of 45-600 kDa. Xanthan is a branched polysaccharide that can be derived from microbial sources. Pectin is a water-soluble heterogeneous polysaccharide and is derived from the food industrial waste of fruits.

Table 3.3: List of plants explored as natural coagulants in water treatment. (Saleem and Bachmann, 2019)

Plant species	Family	Part	Coagulant type
Abelmoschus esculentus/Hibiscus esculentus (okra)	*Malvaceae*	Mucilage	Neutral*
Amorpha fruticosa (indigo bush)	*Fabaceae*	Seeds	Unknown
Aesculus hyppocastanum (Horse chestnut)	*Sapindaceae*	Seeds	Unknown
Cactus latifaria	*Cactaceae*	Mucilage	Neutral*
Cassia alata (candlebrush)	*Fabaceae*	Leaves	Unknown
Castanea sativa (European chestnut)	*Fagaceae*	Seeds	Anionic*
Ceratonia siliqua (carob; locust bean)	*Fabaceae*	Seeds	Neutral*
Cicer arietinum (chick pea)	*Fabaceae*	Grains	Unknown
Coccinia indica (ivy gourd)	*Cucurbitaceae*	Fruit	Unknown
Cuminum cyminum (cumin)	*Apiaceae*	Seeds	Unknown
Cyamopsis psoraloides/Cyamopsis tetragonoloba (guar)	*Fabaceae*	Gum	Neutral
Dolichos lablab (hyacinth bean)	*Fabaceae*	Seeds	Neutral*
Glycine max (Soybean)	*Fabaceae*	Seeds	Unknown
Hibisicus sabdariffa (red sorella)	*Malvaceae*	Seeds	Unknown
Hylocereus undatus (dragon fruit)	*Cactaceae*	Fruit foliage	Unknown
Inga edulis (ice-cream bean)	*Fabaceae – Mimosoideae*	Leaf	Unknown
Jatropha curcas (physic nut)	*Euphorbiaceae*	Seeds	Unknown
Lens culinaris (lentils; pulse)	*Fabaceae*	Seeds	Unknown
Mangifera indica (mango)	*Anacardiaceae*	Seeds	Anionic*
Manihot esculenta Crantz syn. M. utilissima (tapioca sago)	*Euphorbiaceae*	Roots	Unknown
M.oleifera (drumstick)	*Moringaceae*	Seeds	Cationic
Mucuna sloanei (mucuna bean seed)	*Fabaceae*	Seeds	Unknown
Opuntia ficus indica (nopal)	*Cactaceae*	Mucilage	Anionic & neutral*
Parkinsonia aculeate	*Fabaceae*	Seeds	Cationic
Phaseolus angularis (red beans)	*Fabaceae*	Seeds	Cationic
Phaseolus mungo/Vigna mungo	*Fabaceae*	Seeds	Unknown
Phaseolus vulgaris (common beans; kidney bean)	*Fabaceae*	Seeds	Anionic
Phoenixdactylifera (date palm)	*Arecaceae*	Seeds; pollen grains	Unknown
Plantago ovata (psyllium Indian)	*Plantginaceae*	Seed	Cationic
Pleurotus tuber regium (king tuber mushroom)	*Pleurotaceae*	Sclerotium	Unknown
Pistacia atlantica	*Anacardiaceae*	Seeds	Unknown
Pisum sativum (Pea)	*Fabaceae*	Seeds	Unknown
Prosopis juliflora (mesquite)	*Fabaceae*	Seeds	Neutral*
Prosopis laevigata (smooth mesquite)	*Fabaceae*	Seeds gum	Neutral*
Prunus armeniaca (apricot)	*Rosaceae*	Seeds	Unknown
Red maize	*Poaceae*	Grains	Unknown
Quercus robur (common oak)	*Fagaceae*	Seeds	Unknown
Quercus cerris (turkey oak)	*Fagaceae*	Seeds	Unknown
Quercus rubra (Northern red oak)	*Fagaceae*	Seeds	Unknown
Robinia pseudoacacia (black locust)	*Leguminosae*	Seeds	Unknown
Strychnos potatorumLinn (nirmali)	*Loganiaceous (Strychnaceae)*	Seeds	Neutral
Scaphium scaphigerum (Malva nut or Taiwan sweet gum)	*Sterculiaceae*	Seeds	Neutral*
Tamerindous indica (tamarind)	*Fabaceae*	Seeds	Neutral*
Trigonella foenum (fenugreek)	*Fabaceae*	Seeds	Neutral*
Vigna unguiculata	*Fabaceae*	Seeds	Cationic
Zea may (corn)	*Poaceae*	Grains	Neutral

3.2.2 Chemistry and main properties of natural coagulants

Natural coagulants are polymers with varying characteristics such as configuration, MW, and charge. Configuration of the polymer refers to the physical arrangement of monomers along its backbone and is either linear, branched, crosslinked, or in a network (figure 3.1). Most natural coagulants are either linear or branched. MW is an important parameter for polymers used in water treatment with high MW being beneficial for destabilization through the bridging mechanism. There is however a practical upper MW limit decided by the difficulty of dissolving polymers with MW over 10^7 g/mol (Kitchener, 1972). Natural coagulants can either be cationic, anionic, poly-ionic (amphoteric or ampholytes), or non-ionic (neutral) based on if their net molecule carries a charge (Salehizadeh et al., 2018). As can be seen from table 3.3 the charge of the

plant-based polymers are to a large degree still unknown.

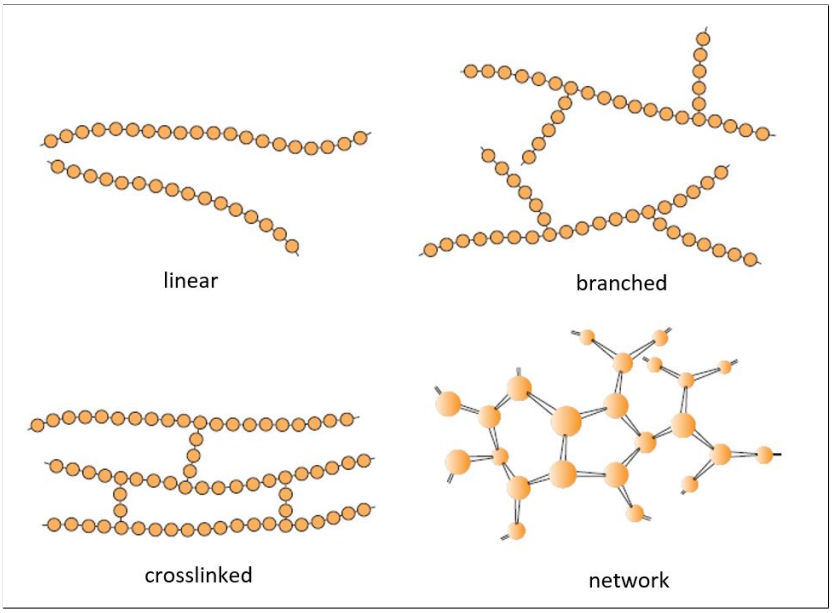


Figure 3.1: Configuration of polymers (Callister, 2007)

3.2.3 Suggested classification

The suggested classification of natural coagulants is based on their source, charge of polymer, polymer configuration (table 3.4). Origins are separated into plant-based, marine, and microbial. A wast majority of the natural coagulants found fit under the plant-based coagulant category with only alginate (marine), chitosan (marine), xanthan (microbial), and pullulan (microbial) belonging to the other categories. Coagulants are then split into 4 different categories based on their polymer configuration where most natural coagulants fit in the linear category. Polymer charge is split into the 4 different charges depending on the total charge of the polymer; cationic, anionic, poly-ionic, and non-ionic. Under this classification, chitosan would be classified as a linear cationic marine-based coagulant and MO seed as a linear cationic plant-based coagulant.

Table 3.4: Classification of natural coagulants.

Factor	Classification	Sub-class	Examples
Source	Plant-derived	**Family:**	
		Anacaridaceae	Mangifera indicia (mango)
		Arecaceae	Phoenixdactylifera (date palm)
		Apiaceae	Cuminum cyminum (cumin)
		Cactaceae	Hylocereus undatus (dragon fruit)
		Cucurvbitaceae	Coccinia indica (ivy gourd)
		Euphorbiaceae	Jatropha curcas (physic nut)
		Fabaceae	Cicer arietinum (chick pea)
		Fagaceae	Castanea sativia (European chestnut)
		Leguminosae	Robinia pseudoacacia (black locust)
		Loganiaceous	Strychnos potatorum (nirmali)
		Malvaceae	Hibiscus sabdariffa (red sorella)
		Moringaceae	Moringa Oleifera
		Plantginaceae	Plantago ovata (psyllium Indian)
		Pleurotaceae	King tuber mushroom
		Poaceae	Zea may (corn)
		Rosaceae	Prunus aremiaca (apricot)
		Sapindaceae	Horse chestnut
		Source part:	
		Fruit	Hylocereus undatus (dragon fruit)
		Grains	Zea may (corn)
		Leaves	Cassua alata (candlebrush)
		Mucilage	Cactus latifaria
		Seeds	Moringa Oleifera
	Marine	Algae	Alginate
		Crustaceans	Chitosan
	Microbial	Bacteria	Xanthan
		Fungus	Pullulan
Charge	Anionic		Tannin
	Cationic		Chitosan
			Moringa Oleifera seeds
	Poly-ionic		-
	Non-ionic		Guar gum
			Starches
Polymer configuration	Linear		Chitosan
			Moringa oleifera
	Branched		Xanthan
	Crosslinked		-
	Network		-

3.3 Treatment effect of natural coagulants

This section summarizes the findings on the treatment effect of chitosan and MO seeds on different types of treatment efficiency parameters and compares them to results from inorganic coagulants. Parameters included are only parameters that are discussed in one or more of the included studies. For each parameter, treatment efficiency is in context with the type of treated water.

3.3.1 Removal of particles

Coagulation mediums where particle removal was reported includes water types like surface water, groundwater, and synthetic surface water (bentonite and kaolinite clay solutions) which are common to encounter in drinking water treatment, and effluents from different industries that are common to encounter in wastewater treatment. All of the researched literature used small scale experimental setups like jar tests and batch tests.

Surface water and synthetic surface water (bentonite solution, kaolin solutions, and clay suspension) turbidity treatment efficiency by chitosan are reported in the range from 16% (Zemmouri et al., 2012) to 99% (Soros et al., 2019; Hu et al., 2013a).

The turbidity of water after jar test using chitosan generally lies in the range of 1-20 NTU. Low percentage turbidity removal rates in surface water tend to be associated with low initial turbidity (>50 NTU) as reported by several studies (Jadhav and Mahajan, 2013; Zemmouri et al., 2012; Christensen et al., 2016; Al-Manhel et al., 2018; Soros et al., 2019). Even though the percentage removal rates are lower for low initial turbidity water, the resulting turbidity is not necessarily lower. Fabris et al. (2010) achieved a turbidity removal rate of 99% (initial turbidity 6.7 NTU) through small scale filtration of the jar-test supernatant. Filtration was done by gravity through an 11 μm filter meant to simulate rapid sand filtration. Hu et al. (2013b) treated highly turbid (10 000 NTU) bentonite solution using chitosan. At an optimal dose of 5 g/l, residual turbidity of 50 NTU was reported which amounts in a 99% removal rate. A 99% removal sounds excellent, but residual turbidity of 50 NTU is too high for the use of sand filters.

Soros et al. (2019) did a comprehensive study on the effect of chitosan dose, molar weight (MW), and degree of deacetylation (DD) on bentonite turbidity reduction. A total of 17 chitosans were tested, 5 with similar DD (90%) and varying MW, and the remaining with similar MW (≈50000 kDa) and varying DD. The optimal removal efficiency was achieved at a dose of 3 mg/l for all types of chitosan. Doses above the optimal dose showed a significant reduction in treatment efficiency. An increase in MW had a positive effect on the removal of turbidity, while an increase in DD beyond 70% had no statistical significance on the removal of turbidity. Higher DDs did however tend to be more robust, making them less likely to overdose.

Similarly Li et al. (2013) investigated the effect of MW (1.5-232 kDa) and DD (54.6-95.3%) on the removal of turbidity of a bentonite solution with initial turbidity of 500 NTU (figure 3.2 and 3.3). Two different bentonite solutions were made from tap water (TW) and demineralized water (DW). The results for the two different solutions varied greatly due to the ionic strength in tap water. Both a high MW and high DD were

beneficial for the removal of turbidity, with MW playing a more important role than DD when treating TW. In DW both MW and DD had limited influence.

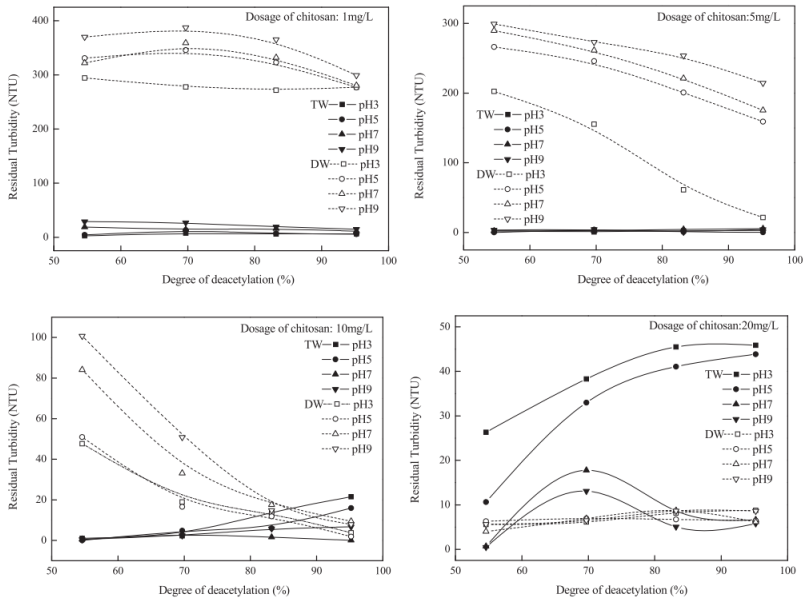


Figure 3.2: Influence of the molecular weight and chitosan dosage on the coagulation–flocculation of the bentonite suspension at different pH in demineralized water (DW) and tap water (TW). Initial turbidity of bentonite suspension 500 NTU, settling time 10 min. (Li et al., 2013)

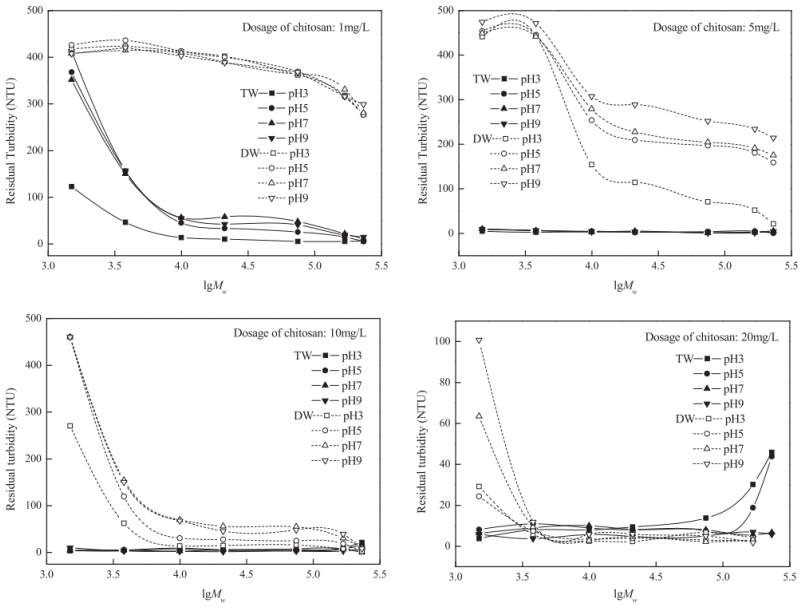


Figure 3.3: Influence of the degree of deacetylation and chitosan dosage on the coagulation–flocculation of the bentonite suspension at different pH in demineralized water (DW) and tap water (TW). Initial turbidity of bentonite suspension 500 NTU, settling time 10 min. (Li et al., 2013)

Wastewater types reported treated with chitosan where turbidity was one of the monitored parameters includes aquaculture wastewater, brewery wastewater, dairy wastewater, and pulp and paper wastewater (table 1.1).

Cheng et al. (2005) managed to achieve >95% turbidity (initial 252 NTU) removal using chitosan to treat brewery wastewater. Chi and Cheng (2006) treated wastewater from the dairy industry using chitosan in a jar test followed by filtration. Turbidity removal rates of 99% (cattle milk), and 95% (sheep milk) were achieved. Restabilization of the diary solution occurred when chitosan was dosed beyond the optimal dosage, causing an increase in turbidity

Geetha Devi et al. (2012) studied the effects of contact time, stirring speed, pH, and dosage on the treatment of dairy wastewater using low MW chitosan. The effects of dosage were similar to what Chi and Cheng (2006) found, with a decrease in the turbidity removal rate beyond the optimal dose. pH had a significant effect on the removal rates with an optimal removal rate at pH 5. Turbidity removal increased with stirring time and a slow stirring speed was favored. Optimal turbidity removal achieved was 96%.

Chung et al. (2005) treated wastewater from aquaculture using chitosan of different MW. Sampled water used in the jar test was collected after biological treatment. The optimal turbidity removal efficiency was achieved with high MW chitosan removing 87.7% (initial 26 NTU) of the turbidity.

Renault et al. (2009b) compared chitosan to polyaluminium chloride (PAC) for treating paper mill effluent. The paper mill effluent was pretreated by a biological filter. Chitosan managed the remove 85% of the turbidity compared to the 60% of PAC. The temperature was found to have no effects on the turbidity removal of chitosan.

Surface water turbidity removal rates by *Moringa oleifera* included in this review vary between 50% (Subramanium et al., 2011) and 99.8% (Madrona et al., 2012). Groundwater turbidity removal rates included in this review vary between 92-99% (Muyibi and Evison, 1995b). Most synthetic water solutions used are kaolinite solutions where treatment efficiency varies between 79% (Katayon et al., 2006b) and 98% (Okuda et al., 2001).

Camacho et al. (2017) compared the effect of direct use of MO seed powder and saline extraction on the treatment of low (5-10 NTU) and high (30-60 NTU) turbidity surface water. The necessity of oil extraction was also studied. For water with high turbidity, MO seed powder could be directly used providing the same treatment efficiency as the stock solution from saline extraction. Optimal treatment efficiency for high turbidity surface water was 85%. For the low turbidity water, saline extraction of the active proteins from MO seeds achieved a higher removal rate than the integral MO seed

powder. Optimal removal efficiency for the low initial surface water was 60%. Results showed that MO oil-extraction is not necessary when using MO seeds as a coagulant in coagulation/flocculation/sedimentation. Mechanisms for turbidity removal using saline MO extraction was explained by enmeshment in a net structure that the coagulation active component forms.

Madrona et al. (2012) compared MO seeds extracted by saline solutions to MO seeds extracted by water on the treatment of surface water turbidity (initial 450 NTU). The molar concentration of the saltwater used to extract the active proteins in the MO seeds was also varied between 0.1-1 M. Optimal removal efficiency achieved was 99.8% removal by the 1M saltwater extraction which was significantly higher than the removal efficiency achieved by the water extract (52%). The higher removal efficiency of the saltwater extract was attributed to the higher content of active proteins in the saltwater extract measured by the author (1.832 mg/l vs 4.499 mg/l). Mechanisms of coagulation reported were charge neutralization. E (2014) treated surface water with initial turbidity of 64 NTU using purified MO seed extract and saltwater MO seed extract. Coagulant dose applied varied from 30-120 mg/l and treatment efficiency increased with dose. The optimal removal efficiency was achieved by the purified MO seed extract, achieving an optimal removal efficiency of 92.3%.

Muyibi and Evison (1995b) used MO seeds to remove turbidity from a kaolinite solution. Effect of dose, initial turbidity (50-300 NTU). The optimal dose for low initial turbidity was 50 mg/l and for medium/high 100 mg/l. Removal efficiency increased with increasing initial turbidity. Turbidity increased with increasing dose beyond optimal dose which was explained by the polymer bridging theory. Large doses result in the saturation of polymer bridging sites, resulting in restabilization of the destabilized particles due to an insufficient number of particles to form more inter-particle bridges. Furthermore, due to the cationic polymeric nature of MO seeds, increasing doses lead to charge reversal with subsequent restabilization of destabilized particles. Katayon et al. (2006a) looked at the effect of storage condition and duration on its effectiveness on turbidity removal of low, medium, and high turbidity kaolinite solutions. Storage conditions were closed/open container and room temperature/refrigerator. Storage conditions did not affect the effectiveness of MO seeds. MO seeds showed a decline in coagulation efficiency after 5 months of storage. Ndabigengesere and Narasiah (1996) studied the effect of pH on turbidity removal from kaolinite solutions. pH was found not to have any significant effect on the turbidity removal using MO seeds. Pritchard et al. (2010) studied the required dose based on the initial turbidity and found a linear relationship (figure 3.4).

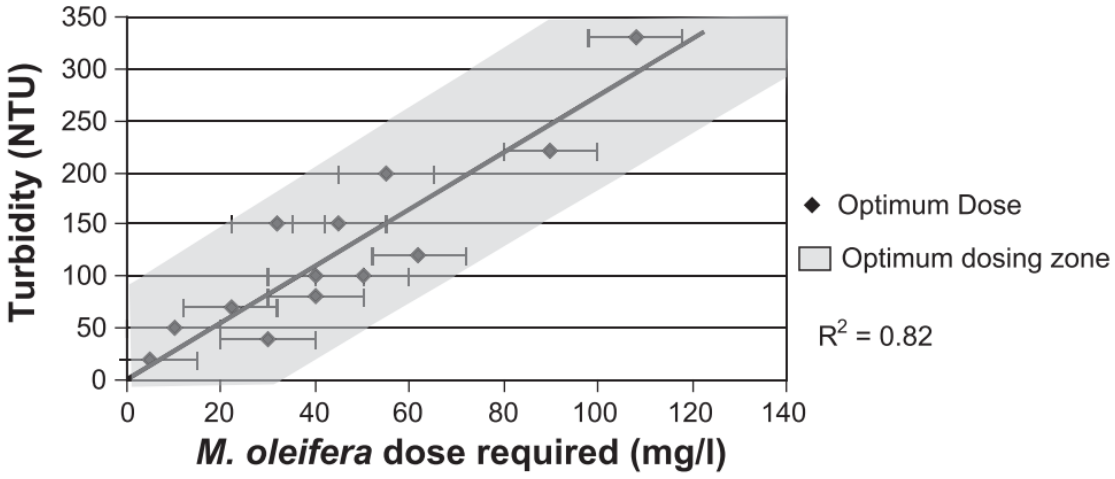


Figure 3.4: Optimum MO seed dose for turbidity reduction in water based on initial turbidity. (Pritchard et al., 2010).

Fewer studies were found on the use of MO seeds on turbidity removal in **wastewater** treatment. Ndabigengesere and Narasiah (1998) used a water extract from MO seeds and alum to treat municipal wastewater from different sources. For municipal wastewater with initial turbidity of 64.2 NTU, alum achieved a removal efficiency of 96.8%, while MO seed extract achieved an optimal removal efficiency of 95.3%. For municipal wastewater with an initial turbidity of 102 NTU, alum achieved an optimal removal efficiency of 97.5%, while MO seed extract achieved an optimal removal efficiency of 95.6%. Sludge volume from coagulation with MO seeds was 4 to 6 times less than with the use of alum. No significant change in pH was observed when using MO seeds. Hendrawati et al. (2016) treated wastewater from the textile industry using MO seed powder. The initial turbidity of the wastewater was 85.79 NTU and optimal removal efficiency achieved was 98.6%.

Baptista et al. (2017) worked on fractionating MO seed proteins and evaluating the fractions coagulation activity on the water with varying turbidity. Globulin and albumin presented the highest values of protein fractions in MO seed. For low turbidity water (50 NTU) globulin performed the best out of the protein fractions achieving 89.71% turbidity removal.

Summary and comparison with inorganic coagulants

There is ***high evidence*** in the researched literature that initial turbidity strongly influences the removal of particles by both chitosan and MO seeds. However, there is ***weak agreement*** about the overall efficiency of such treatment: removal rates of 90-99% and 16-50% were reported for high and low turbid water by chitosan, and 87-99% and 50-99% removal rates for high and low turbid water by MO seeds.

Medium evidence and ***strong agreement*** suggest that saline extraction of the MO seed is beneficial for turbidity removal at low initial turbidity due to a higher amount of active protein extracted.

High evidence in the researched literature exists on the effect of chitosan and MO dose. A ***strong agreement*** exists on the fact that the optimal dose is dependent on the type of wastewater. There is also a ***strong agreement*** that an increase in dose beyond optimal dose decrease the turbidity removal due to charge reversal and following restabilization for both chitosan and MO seeds.

Low evidence exists in the researched literature on the effect of chitosan MW on particle removal, but a ***strong agreement*** exists within the researched literature that a high MW is beneficial for turbidity removal. For MO seed a similar variation in MW is not possible and there were found no reviews where MO seeds from different regions are compared. There is ***low evidence*** for the effect of chitosan DD on turbidity removal, there is however a ***strong agreement*** that DD above 70% is beneficial for particle/turbidity removal.

Turbidity removal for high initial turbidity water by the reviewed natural coagulants are in the same range as more popular inorganic coagulants like alum (90-99%). Chitosan performs worse for the treatment of water with low initial turbidity compared to inorganic coagulants like aluminum sulfate which can remove up to 99% (Baghvand et al., 2010). Saltwater extract from MO seed can compete with aluminum sulfate in the removal of turbidity from the water with low initial turbidity (Okuda et al., 2001). Special for chitosan was that it requires a much lower dose for similar turbidity removal when compared directly to the popular inorganic coagulants like aluminum sulfate (Zemmouri et al., 2012) and aluminum chloride (Hu et al., 2013b). The natural polymers also have the advantage of working over a broader pH range and not altering the pH of the treated water.

3.3.2 Removal of organic matter

Organic matter removal is monitored by different parameters based on if it's drinking water or wastewater. For drinking organic matter removal is monitored by color removal, total organic carbon (TOC), and UV254. Removal of organic matter in wastewater is often monitored by the chemical oxygen demand (COD) removal. Organic matter causes decolorization of the water and is therefore also connected with the turbidity.

Surface water color removal by chitosan is reported in the range from 60-78% (Christensen et al., 2016) with only one of the researched studies measuring organic matter removal by another parameter than turbidity.

Christensen et al. (2016) used chitosan in a jar-test to remove the color from surface water with changing initial color value (14-29 mg Pt/l) dependent on the season. Optimal color removal efficiency achieved was 60-78% at a pH of 5, due to protonation of the amine groups. The addition of chitosan did not change the pH of the solution.

Wastewater organic matter removal by chitosan is as mentioned above mainly monitored by COD and is reported in the range of 58.7% (Sila et al., 2014) to 95% (Thirugnanasambandham et al., 2013).

Chung et al. (2005) treated aquaculture wastewater using chitosan of different MW. High MW was beneficial for the COD removal rate achieving an optimal removal rate of 62.8%. The low optimal removal rate was explained by a high hydrophilic (dissolved) COD fraction in the wastewater, which chitosan was unable to remove (figure 3.5).

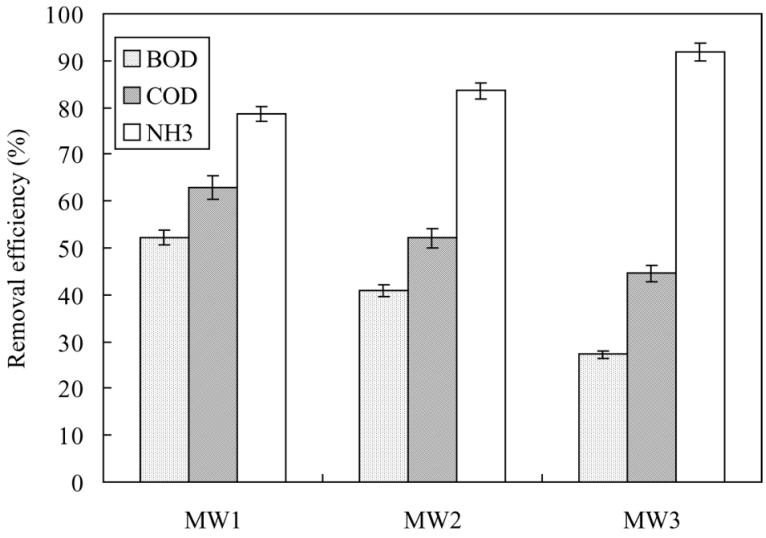


Figure 3.5: Removal efficiency of BOD, COD, and NH_3 in the secondary aquaculture effluent after coagulation by chitosan with different molecular weights. MW1, MW2, and MW3 were $3.62 * 10^5$, $4.73 * 10^4$, and $6.21 * 10^3$. (Chung et al., 2005)

Pitakpoolsil and Hunsom (2013) used chitosan flakes as an adsorbent for the removal of pollutants in biodiesel wastewater. The effect of adsorption time, initial wastewater pH, dose, and mixing rate were studied. With increasing adsorption time, the amount of adsorbed COD increased until it reached a plateau after 3 h. This increase was explained by more chances of interaction between the oil molecules and the chitosan adsorbent, and the decrease in oil-droplet size as a result of the mixing. pH affected the adsorption of COD and was most effective at a pH 4. Increasing the pH beyond 4 reduced the effectiveness of chitosan as an adsorbent of oil particles and reducing it bellow 2.5 had a similar effect. The reduced effect at strongly acidic pH was explained by the chitosan dissolving reducing the amount of adsorbent available. At pHs above 4, the reduction was explained by the deprotonation of the amine groups of chitosan,

leading to a decrease in the positive charge density of the chitosan. COD removal rates increased with increasing chitosan doses up until a dose of 3.5 g/l. Doses beyond this decreased the COD removal, which was explained by the limitation of transport of pollutant particles to the chitosan surface (figure 3.6). The optimal removal efficiency achieved was 90%.

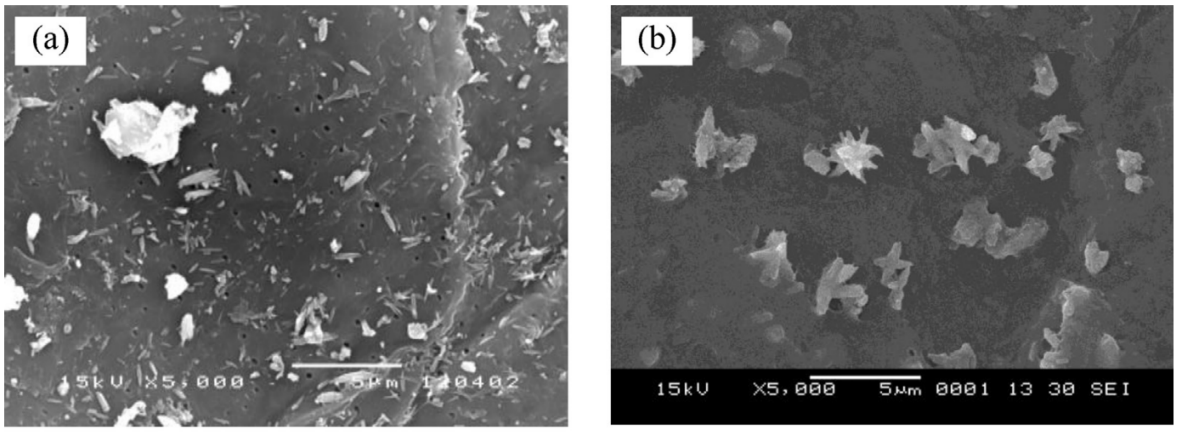


Figure 3.6: SEM images (5000∗magnification) of chitosan flakes before (a) and (b) after being used as an adsorbent (Pitakpoolsil and Hunsom, 2013).

Milhome et al. (2009) used chitosan as an adsorbent to treat wastewater from a petroleum refinery. This was done by using a column filled with chitosan. COD removal rates were showed to increase with a decreasing pH (lowest pH 2). This was explained by electrostatic interactions between ammonium groups and phenolates at low pHs. Optimal COD removal achieved was 65%.

Several studies have been done on the use of chitosan for the treatment of food industry wastewater. Cheng et al. (2005) monitored the COD removal by chitosan applied to brewery wastewater. Chitosan was applied to the wastewater after filtration and managed to remove 50% of the remaining COD. The authors reported that the remaining COD was mainly present as dissolved organic matter. In addition to monitoring turbidity Chi and Cheng (2006) also measured the COD removal rate of chitosan for treating dairy wastewater. COD removal was 55% for the sheep milk wastewater and 75% for the cattle milk wastewater. The higher removal rate for cattle milk wastewater was explained by a higher fraction of particulate COD in the cattle milk.

Renault et al. (2009c) used chitosan to remove COD from wastewater collected from a pulp and paper factory. Chitosan was applied in a jar test on wastewater pretreated by a biological step and was able to remove 80% of the COD. The temperature did not affect the COD removal by chitosan.

Geetha Devi et al. (2012) studied the effect of dose, pH, mixing time, and mixing speed on the removal of COD from dairy wastewater. An optimal dose was found to be 150

mg/l, dosing beyond the optimal dose provided no additional COD removal. For pH, the optimal removal rate was achieved at pH 5. A slow stirring speed (50 rpm) was favored for COD removal, while a stirring duration of 50 min was found to be optimal. At optimal conditions, COD reduction was 79%. Thirugnanasambandham et al. (2013) used chitosan as an adsorbent in a batch study in rice mill wastewater. The supernatant from the batch study was centrifuged and filtered before COD measurement. At optimal conditions (initial pH 4.5, agitation time 4 min, dose 600 mg/l) a COD removal rate of >95% was achieved. Patel and Vashi (2013) studied the effect of temperature on the treatment of textile wastewater using chitosan. Removal efficiency increased with increasing temperatures, reaching an optimal removal efficiency of 75.6%. The higher removal efficiency at increasing temperatures was explained by better floc settlement at high temperatures.

Surface water and kaolin solutions treated by MO seed are reported to increase the organic matter measured as COD in the treated water (Ndabigengesere and Subba Narasiah, 1998; Okuda et al., 2001).

Ndabigengesere and Subba Narasiah (1998) measured the COD of the MO seed water extract solution before using it as a coagulant. The COD value in the 5% (wt/v) MO seed solution was found to be 9630 mg/l. After treatment with the MO, water extract the COD level was higher than initial, which was in turn explained by the high COD value of the extract. Okuda et al. (2001) managed to purify the active proteins in MO seeds and used it as a coagulant for kaolin solution. The purified active proteins were compared to a saltwater extract for removal of turbidity and COD. Both performed similarly in turbidity removal, however, the purified active proteins did not increase the COD.

For MO seeds used in **wastewater** treatment, the COD does not necessarily increase. COD removal has been achieved in high initial COD waters. Vieira et al. (2010a), Baptista et al. (2017), and Bhatia et al. (2007) reported COD removal rates between 50-52.2%. COD removal rates were explained by the destabilization of suspended organic particles. The solved COD fraction was not removed. Ndabigengesere and Narasiah (1998) treated 4 different domestic wastewaters using a MO seed water extract. Initial COD values varied between 267-742. For all of the wastewater treated with the water extract, an increase in COD was observed.

Summary and comparison with inorganic coagulants

High evidence on COD removal by the natural polymers chitosan and ***low evidence*** on COD removal by MO seeds exist in the researched literature. There is a ***weak agreement*** on the achieved removal rates. COD removal rates by chitosan in jar test

vary between 50-69.6% with one outlier at 84.4% (Roussy et al., 2005b) and for MO seeds between 50-52%.

There is ***medium evidence*** and ***strong agreement*** on the need to purify the active proteins from MO seeds before use to avoid adding COD from the seeds. (Ndabigengesere and Subba Narasiah, 1998; Okuda et al., 1999; Okuda et al., 2001). There is ***high evidence*** and ***strong agreement*** for chitosan, and ***low evidence*** and ***strong agreement*** for MO seeds, on the effect of pH on COD removal. Both chitosan and MO seed achieve optimal COD removal rates at acidic pH. ***High evidence*** on chitosan and ***low evidence*** on MO seed exist on the effect of dose for COD removal in the researched literature. There is ***strong agreement*** that similar to turbidity, an increase in dose beyond a certain point causes an increase in COD. There is a ***strong agreement*** that it is caused by the restabilization of organic particles. For chitosan ***low evidence*** and ***strong agreement*** exist in the reviewed literature in the effect of MW and DD on COD removal. Optimal COD removal rates are achieved by high MW and DD above 70%.

Compared with more traditional inorganic coagulants similar results are achieved by chitosan. Neither can remove dissolved COD. MO seeds require purification before being able to compete with inorganic coagulants, but most studies with purified MO seeds are on surface water treatment.

3.3.3 Removal of nutrients

Phosphates and nitrogen compounds were the only nutrients monitored in the included reviews. They are commonly found in domestic and industrial wastewater.

Of the reviewed studies, only one monitored the removal of nutrients by chitosan, and it was in the form of PO_4^{3-} and NH_3. NH_3 is toxic to aquatic organisms and PO_4^{3-} is an important nutrient salt which causes eutrophication of water bodies. Chung et al. (2005) used chitosan of different MW to treat aquaculture wastewater pretreated by a biological filter. Optimal removal rates achieved were 91.8% for NH_3 and 99.1% for PO_4. Low MW was preferred over higher MW for the removal of both nutrients. For NH_3 removal was due to the formation of hydrogen bonds between chitosan and NH_3, and partial charge neutralization. PO_4 was removed by charge neutralization and thus low MW chitosan with a higher charge density achieved higher removal rates.

A limited amount of studies on the removal of nutrients using MO seeds were found. Use of non-purified MO seeds, either in powdered form or as an extract, has shown to increase the amount of orthophosphates and TKN (total Kjeldhal nitrogen) when used on domestic wastewater (Ndabigengesere and Narasiah, 1998). Like with COD

the increase comes from the high values of nutrients in the powdered seed and the seed extract. Ndabigengesere and Narasiah (1998) measured the amount of orthophosphates and TKN to 187 mg/l and 802 mg/l. No studies were found using purified MO seed protein for nutrient removal, but purification was mentioned by several authors as a way to avoid the addition of nutrients.

Summary and comparison with inorganic coagulants

Only a few of the reviewed studies monitored the removal of nutrients by the natural coagulants chitosan and MO seed and thus there is ***low evidence*** for their removal efficiency. The removal rate for PO_4^{3-} and NH_3 by chitosan was reported at 99.1% and 91.8%, the wastewater was however pretreated by a biological filter. Like with COD, MO seed active protein needs to be purified to avoid adding orthophosphates and TKN. ***Low evidence*** and a ***strong agreement*** were reported for the effect of MW, chitosan low MW was beneficial for the removal of both NH_3 and PO_4^{3-}. ***Low evidence*** exists in the researched literature on the effect of pH, but there is a ***strong agreement*** that nutrients are removed most efficiently at acidic pH.

It is hard to compare the selected natural coagulants to inorganic coagulants due to the low amount of research that has been done on the removal of nutrients by chitosan and MO seeds.

3.3.4 Removal of heavy metal ions

Heavy metal ions are often present in industrial wastewater and can pose an environmental hazard when discharged uncontrolled. Chitosan has shown potential as an adsorbent of heavy metal ions and several studies have been done on the subject. Studies reviewed cover the removal of the heavy metal ions Ag^{+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}.

Qin et al. (2006) treated water spiked with $CuSO_4$ using chitosan as an adsorbent. The initial concentration of Cu^{2+} was 1000 ppm. Chitosan showed a poor affinity for Cu^{2+} removal, with an optimal removal rate of 9.6%. Similarly, water was spiked with $Pb(NO_3)_2$ and treated with chitosan. The optimal removal rate of Pb^2+ was 58.6%. For both heavy metal ions, the removal efficiency increased with increasing water temperature. Chitosan-copper ion complexes are theorized to look like figure 3.7.

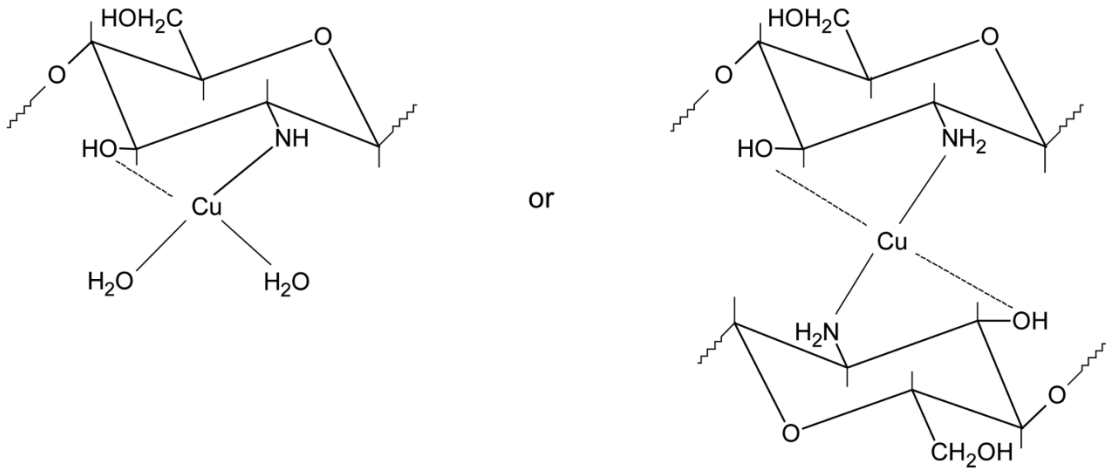


Figure 3.7: Formation of chitosan chelates with copper ions binding with one hydroxyl and two water molecules. (Gerente et al., 2007)

Gamage and Shahidi (2007) did a comprehensive study on the adsorption of different heavy metal ions by three different chitosans (type 1, 2, and 3) in an adsorption column. The different types of chitosans had slightly different DD and MW with type 1 having the highest DD. Type 1 provided the best removal efficiency for all metal ions except Mn^{2+}, explained by the author by its higher DD. pH was varied between 5-7, where pH 7 gave the best result. This was explained by the greater availability of amino groups at higher pH values. Optimal removal efficiencies achieved were: Fe^{2+} 94.1%, Mn^{2+} 23.4%, Co^{2+} 26.3%, Ni^{2+} 62.8%, Cu^{2+} 64.9%, Zn^{2+} 50.3%, As^{2+} 15.6%, Mo^{2+} 19.9%, Cd^{2+} 39.1%, Hg^{2+} 97.5%, Pb^{2+} 91.0%. Lasko and Hurst (1999) used chitosan to remove Ag^{+} from spiked water and achieved an optimal removal rate of 90%. The optimal pH for the adsorption of silver was between 4-6.

Heavy metal ions removed by MO seeds included in this review are Co, Cu, Pb, Cd, Ag, Zn, Ca. Araújo et al. (2010) used powdered MO seed to remove Cd^{2+}, Ag^{+}, Co^{2+}, Cu^{2+} and Pb^{2+}. Removal rates for each metal ion were, Cd^{2+} 70%, Ag^{+} 98%, Co^{2+} 28%, Cu^{2+} 82%, Pb^{2+} 97%. The difference in removal efficiency was due to different ionic radius size of the chemical species. For singe metal solution, ions with larger ionic radius are preferentially adsorbed. Optimal pH for the adsorption of the heavy metal ions were pH 6-7. Subramanium et al. (2011) did a similar study using powdered MO seeds for the removal of Cu^{2+}, Pb^{2+}, Cd^{2+}, Cr^{2+}, and Zn^{2+}. Optimal removal rates for the different heavy metal ions were, Cu^{2+} 90%, Pb^{2+} 80%, Cd^{2+} 60%, Zn^{2+} 50%, and Cr^{2+} 50%. Hendrawati et al. (2016) used powdered MO seed to remove the heavy metal ions Cd^{2+}, Mn^{2+}, and Cr^{2+}. With a dose of 80 mg/l, all three of the ions were removed to undetectable levels.

Summary and comparison with inorganic coagulants

Low evidence in the reviewed studies exists on the removal of heavy metal ions both by chitosan and MO seeds.

With a ***average agreement*** heavy metal ion removal rate by chitosan in reviewed studies are: Fe^{2+} 94.1%, Mn^{2+} 23.4%, Co^{2+} 26.3%, Ni^{2+} 62.8%, Cu^{2+} 9.6-64.9%, Zn^{2+} 50.3%, As^{2+} 15.6%, Mo^{2+} 19.9%, Cd^{2+} 39.1%, Hg^{2+} 97.5%, Pb^{2+} 58.6-91.0%, and Ag^{+} 90%.

Average agreement was also reported for heavy metal ion removal by Mo seeds: Cd^{2+} 60-70%, Ag^{+} 98%, Co^{2+} 28%, Cu^{2+} 82-90%, Pb^{2+} 80-97%, Cu^{2+} 90%, Zn^{2+} 50% and Cr^{2+} 50%.

For chitosan ***low evidence*** was found in the reviewed literature on the effect of DD and ***no evidence*** on the effect of MW on the removal of heavy metal ions. A high DD was beneficial in the removal of heavy metal ions. ***Medium evidence*** was found in the included studies on the effect of pH on heavy metal removal by chitosan and MO seeds. ***Average agreement*** on the optimal pH in the included studies was found. Optimal pH was similar for both chitosan and MO seeds with optimal removal being reported in the range of 4-7 pH.

For removal of inorganic heavy metal ions the natural polymers chitosan and MO seed both perform better when compared to aluminum sulfate which has reported removal rates of Cu^{2+} 46%, Pb^{2+} 37%, Zn^{2+} 36% (Bratby, 2016).

3.3.5 Removal of dyes

Dyes are a common contaminant often found in wastewater from different types of industries. Szyguła et al. (2009) used chitosan in a coagulation-flocculation experiment to remove color from a dye solution. The dye used to color the solution was Acid Blue 92 (AB92) which holds a negative charge when in solution. Formed flocs can be seen in figure 3.8. The effect of pH was evaluated and found to be optimal at acidic pHs (pH 3-7). Optimal removal efficiency achieved was 99%. Chitosan has reactive amino and hydroxyl groups that react with the negatively charged dye-surface. Coagulation mechanisms were explained by charge neutralization and bridging. Charge neutralization occurs through the hydrophobic interactions between the methyl group of the acetamide function and the -CH and $-CH_2$ groups on the glucose ring of the dye. Bridging occurs through H-bridges generated by alcohol, amine, amide, and ether functions on the chitosan chain.

Reck et al. (2018) used powdered MO seeds as an adsorbent to remove the anionic dye,

tartrazine yellow. At a dose of 91.27 mg/l, the optimal removal efficiency of 95% was achieved. Optimal pH was around 2, which was explained by protonation of the surface charge of the adsorbent at acidic pH, causing an increased interaction between the dye and the adsorbent.

Vilaseca et al. (2014) compared active protein extraction methods on the removal efficiency of dyes from water using MO seeds. The saltwater extraction method was found to be the most effective removing dyes, removing >90% of blue, crimson and navy dye solutions, and more than 85% for black dye solution. Dye removal results were almost independent of pH (5-11), being slightly better at pH 9. Increasing dose (250-1250 mg/l) provided an increase in treatment efficiency, especially for the orange dye. The temperature did not seem to be a significant factor when using MO seeds as a coagulant.

Roussy et al. (2005a) treated inc containing wastewater from cardboard box-making using two types of chitosan with varying MW. High MW was beneficial for the color removal achieving a removal rate of 99% at the optimal pH 5.

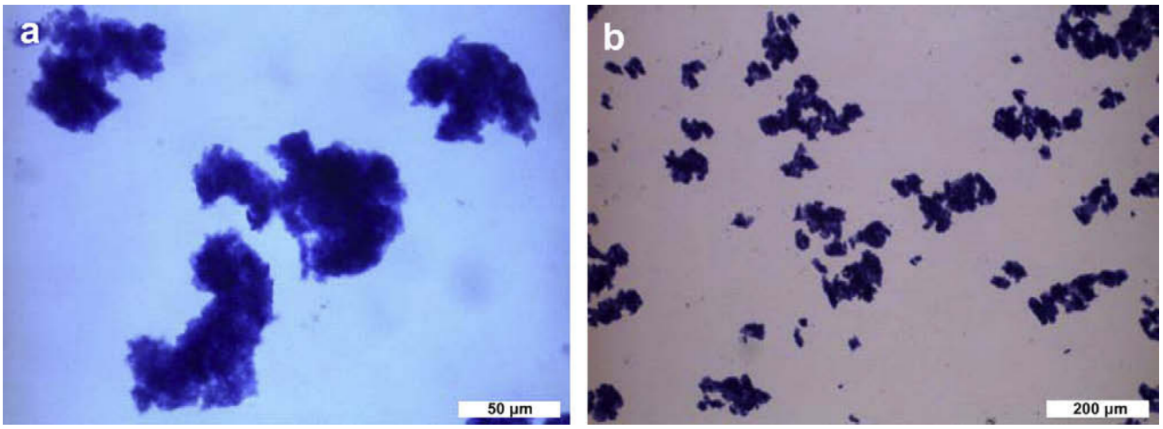


Figure 3.8: Microscope photographs of flocs formed by chitosan (a) single flocs and (b) floc aggregates (Szyguła et al., 2009).

Summary and comparison with inorganic coagulants

Low evidence on dye removal by chitosan and MO seeds exist in researched literature. Chitosan removal rates were reported at 99% for AB92 and 99% for inc containing wastewater. MO seeds removal rates for different dyes were 85-95%. ***Low evidence*** on the effect of pH was found in the reviewed literate with a ***strong agreement*** that acidic pHs were optimal for dye removal by chitosan while MO seed was less dependent on pH (5-11). Only one study on the MW effect on dye removal was found in the reviewed literature where MW was beneficial for the removal of inc containing water. Only one study was found on the optimal preparation method of MO seed for dye removal, which found that saltwater extraction performed best.

Compared with alum and PAC, both chitosan and MO seed performed similarly with respect to removal of the different dyes (Hasani Zonoozi et al., 2009; Wong et al., 2007).

3.3.6 Antimicrobial properties

There exists a large number of studies on the antimicrobial activity of chitosan, they are however mainly focused on the food industry. For water treatment there does not exist a large number of studies on the antibacterial properties of chitosan.

Chung et al. (2005) monitored the bacteria removal of chitosan as a coagulant in aquaculture wastewater treatment. An average of 99.998% removal efficiency was achieved by treating the wastewater with chitosan. Since chitosan is both a coagulant and bactericide, the author was not sure which of the properties contributed the most to the high removal rate. The mechanism of antimicrobial action of chitosan is speculated to be an interaction between the positively charged chitosan molecules and negatively charged microbial cell membranes.

Mandloi et al. (2004) used chitosan in surface water for direct filtration through a sand filter. One of the parameters monitored were bacteria and optimal removal efficiency achieved was 97.3%. Alum was used as a control for the same experiment and provided significantly less bacteria removal (78.3%).

MO seeds have antimicrobial properties that make it suitable for bacteria removal. According to Villaseñor-Basulto et al. (2018) the antimicrobial properties of MO seeds are in part due to its content of glucomoringin (GMG) which is an antimicrobial factor. Shebek et al. (2015) attributed the antimicrobial activity of MO seeds to membrane fusing. MO seeds can inhibit the growth of gram-positive bacteria, but show no effect on the inhibition of gram-negative bacteria (Vieira et al., 2010b).

Lea (2014) treated surface water using powdered MO seeds and achieved a 90-99% bacteria removal. Hendrawati et al. (2016) monitored e-coli removal efficiency when treating wastewater using powdered MO seeds. Optimal e-coli removal efficiency achieved was 80%. Moreti et al. (2016) used MO seeds in a coagulation/flocculation/dissolved air flotation process, to remove cyanobacteria. The number of cyanobacteria in the solution was monitored through the measurement of the chlorophyll level in the solution. At optimal conditions, the chlorophyll removal rate was 96.5%. The suggested coagulation mechanism was charge neutralization between algae and the coagulant.

Summary and comparison with inorganic coagulants

Medium evidence in the researched literature exists on the removal of bacteria in water treatment. ***Average agreement*** on the effectiveness of bacteria removal was

found with chitosan removing 97-99% and MO seeds removing 90-99%. No studies with chitosan looked into the effect of MW and DD on bacteria removal.

3.4 Mechanisms of action of natural coagulants

3.4.1 Interactions with particles

Particle removal in water treatment is monitored by the parameter turbidity. As presented in subsection 3.3, chitosan has proved to be potent for the removal of turbidity. Mechanism of turbidity/particle removal by chitosan is reported to be charge neutralization, bridging, electrostatic patch mechanism, and sweeping (figure 3.9).

Charge neutralization is reported to be one of the dominant destabilization mechanisms for negatively charged particles found in bentonite solutions (Soros et al., 2019), diary wastewater (Geetha Devi et al., 2012), organic suspension (Roussy et al., 2005b). Charge neutralization is especially prevalent at acidic pH due to the increased surface charge of chitosan at acidic pH.

Bridging takes place alongside charge neutralization and predominates at neutral or slightly basic conditions (Roussy et al., 2004). High chitosan MW is beneficial for bridging which is why chitosan of high MW are reported to achieve a higher turbidity removal (Roussy et al., 2004; Soros et al., 2019).

Soros et al. (2019) reported that neither charge neutralization or bridging could account for some aspects of observed coagulation of a bentonite solution at neutral pH. If bridging was the dominant mechanism an increasingly higher MW was expected to provide better results, which it did not. Low MW chitosan also performed poorly. Patch mechanism was suggested as a possible explanation for the results. Low treatment efficiency with low MW chitosan was explained by to small "patches" to cause destabilization. High MW chitosan is able to form larger patches on the particle surface, but are not able to form a greater number of patches then the medium MW chitosan. For the electrostatic patch mechanism, the number and position of patches are what determines how particles attach to each other. Sweeping, which is entrapment of particles in floccs, is theorized to take place to some degree for all polymers.

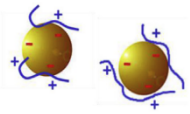
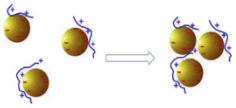
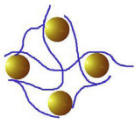
Mechanism	Description	Illustration
Simple charge neutralization	Efficient reduction of the thickness of the electric double layer and full charge neutralization.	
Charge patching	Unevenly distributed surface charges are incompletely neutralized.	
Bridging	Adsorption and connection of the primary flocs on soluble linear large-MW flocculants.	
Sweeping	Enmeshing and entrapping of small colloidal pollutants by large flocs or polymeric precipitates.	

Figure 3.9: Coagulation/flocculation mechanisms reported for chitosan. (Yang et al., 2016)

Mechanisms of turbidity/particle removal by MO seeds are reported to be adsorption, charge neutralization, and bridging (table 3.2). For the removal of turbidity in a kaolinite solution, which carries a negative charge at neutral pH, the main removal mechanisms are reported to be charge neutralization and bridging (Ndabigengesere et al., 1995). The reasoning behind charge neutralization was optimal turbidity removal when ζ-potential was around 0 mV. Bridging and charge neutralization was also reported as the main mechanisms for kaolin suspensions by Muyibi and Evison (1995b). Charge neutralization is most likely the dominant of the two, due to MO seeds low MW (6.5-14 kDa) compared to other polymers like chitosan (1-600 kDa). Compared to chitosan there is a lack of studies on the mechanisms of coagulation by MO seeds. Most studies reviewed just reference one of the few studies that looked into the coagulation mechanisms.

3.4.2 Interaction with inorganic ions

Inorganic ions reported removed by chitosan in included studies were mainly heavy metal ions and PO_4^{3-}. Reported removal mechanism for inorganic ions are adsorption (Qin et al., 2006; Gamage and Shahidi, 2007; Lasko and Hurst, 1999). Cations are adsorbed onto the free electron doublet of nitrogen on amine groups while anions are adsorbed by the protonation of amine groups in acidic solutions (Guibal, 2004). A high degree of deacetylation (DD) is beneficial due to a higher fraction of free amine groups that will be available for adsorption. Adsorption of cations is optimal around neutral pH (Gamage and Shahidi, 2007.)

Removal of heavy metal ions by MO seeds are reported to be adsorption of the metal ion onto the polymer. Adsorption takes place onto functional groups on the MO seed

active protein, mainly amino and acid groups (Araújo et al., 2010). An increase in metal adsorption with increasing pH values (5-8) can be explained competition between the proton and metal ions for the same functional groups, and a decrease in the positive surface charge. The result of this is a higher electrostatic attraction between the surface and the metal.

3.4.3 Interactions with organic molecules

Organic molecules in drinking water mainly consist of natural organic matter (NOM) which is monitored by the parameters color, ultraviolet absorption, and total organic carbon (TOC). In wastewater organic molecules present vary based on the wastewater origin. Domestic wastewater and wastewater from the food industry often have a high level of organic molecules monitored by the COD.

Organic molecules are reported removed by the same mechanisms as particles for both chitosan and MO seed due to organic molecules often being present as negatively charged particles. The minimum dose for color removal in surface water was found to be decreasing upon protonation, implying that charge neutralization is involved in color removal (Christensen et al., 2016). Neither chitosan nor MO seed was reported to remove dissolved organic molecules in the researched literature.

4. Discussion

The discussion section will be structured around the research questions posed in section 1.4 in order to properly address them.

(1) What should be the classification of the known natural coagulants based on their origin and properties?

A classification of the known natural coagulants was made based on the origin, structure, and charge of the natural coagulants. Origin was divided into the three subgroups plant-based, marine, and microbial with subgroups that further narrowed the origin, such as plant-family, plant-part, etc. The reviewed literature indicates that the vast majority of natural coagulants researched for water treatment are of a plant-based origin. The polymer structure of the natural coagulants was divided into linear, branched, crosslinked, and network. Charge of the natural coagulants was reported as anionic, cationic, poly-ionic, and non-ionic.

Suggested classification provides an easy way to categorize natural polymers/coagulants without needing to do extensive research. Origin, polymer charge, and polymer structure are all properties that do not require a large amount of research. By classifying the natural after the proposed classification it becomes apparent that the majority of natural coagulants found in the researched literature are derived from seeds of plants in the *Fabaceae* family, also known as the bean family. Polymer charge of the natural coagulants researched in reviewed literature varies between anionic, cationic, and non-ionic with no apparent pattern based on the origin of the natural coagulant. The majority of the plant-based polymers explored as natural coagulants have unknown polymer charges (table 3.3).

A limitation in the proposed classification is that it leaves the possibility of a natural coagulant not included in this review, or a newly discovered natural coagulant, not fitting under the listed classifications. An example of this could be a natural coagulant derived from land-based animals. This would however easily been implemented by adding another classification under source.

(2) What are the treatment efficiency achieved by natural coagulants in water and wastewater treatment in comparison with inorganic/synthetic ones?

The results from the litterateur review on treatment efficiency indicate that the treatment effects of selected natural coagulants are comparable with synthetic/inorganic ones for the removal of particles, inorganic ions, dyes, and bacteria. However, particle removal by natural coagulants for low initial turbid water performs worse than it's synthetic/inorganic counterparts. For the removal of organic particles and nutrients, chitosan performs at a similar level to more traditional synthetic/inorganic coagulants. Non-purified MO seeds are both reported to remove and increase the amount of COD and nutrients in the treated water. No studies were found on the use of purified MO seed for COD and nutrient removal.

Because of the large number of natural polymers that are being explored for water treatment, it is hard to make generalized statements about the treatment effects of all of them. The two natural coagulants chosen in this review represent two of the more promising natural coagulants experimented with in water treatment from their respective source classification (marine and plant-based). A large majority of the reviewed literature found was on the treatment of surface water where both chitosan and MO seed show potential. However, treatment by non-purified MO seed causes an increase in COD which can cause problems with disinfection-byproducts when disinfecting. This makes it necessary to purify the active protein in MO seed before use in the treatment of drinking water. Taken into consideration the positive sides of natural coagulants such as biodegradability, non-toxicity, and less sludge/better sludge quality natural coagulants show great promise when compared to inorganic coagulants.

The use of chitosan and MO seed in the treatment of wastewater is less researched with only a handful of studies for each coagulant being found. This may be due to researchers deeming it less suitable for wastewater, but the few studies found where chitosan was used as coagulants in wastewater it worked well enough to warrant further research. It is therefore hard to compare the natural coagulants and inorganic coagulants for wastewater treatment. Both natural coagulants showed potential as an adsorbent in the removal of heavy metal ions which can be used for pretreatment of industrial wastewater to reach levels where the industrial wastewater can be discharged to the municipal sewer line.

(3) What are the mechanisms of action of selected natural coagulants?

The results from the literature review on mechanisms of action of natural coagulants indicate that adsorption, charge neutralization, bridging, and electrostatic patch mechanism are the main mechanisms of action. To which degree each of the mechanisms

act, depends on which pollutants are present in the suspension and their concentration. For the destabilization of particles in solution by chitosan and MO seed, which are both cationic polymers, charge neutralization and bridging are reported to be the main mechanisms of action. In addition to charge neutralization and bridging, electrostatic patch mechanism were also involved in the particle destabilization of chitosan. MO seed is not theorized to destabilize through the electrostatic patch mechanisms due to low MW. Inorganic ions were reported removed by adsorption for both chitosan and MO seed. Organic molecules present as particles or bound to particles were reported, by the reviewed literature, to be removed by the same mechanisms as particles described at the beginning of this paragraph.

The reported mechanisms of action for chitosan and MO seed found in the reviewed literature are typical for cationic polymers (Bratby, 2016). Special for chitosan is the possibility of varying the MW and DD based on the origin of the chitin and the deacetylation process which in turn alter its coagulation properties.

Research into the mechanisms of action for MO seeds seems to be limited with only 44% (15/34) of the researched literature reporting mechanism of action, compared to chitosan where 79% (26/33) reported the same (table 3.1 and 3.2).

5. Conclusions

By analyzing research done on the natural coagulants chitosan and MO (Moringa Oleifera) seeds in water treatment, this review shows that natural coagulants are capable of competing with inorganic coagulants for the treatment of drinking water. Both chitosan and MO seed possess a great affinity for the removal of common pollutants in water such as particles, organic molecules, and inorganic ions with coagulation mechanisms reported to be charge-neutralization, bridging, and electrostatic patch mechanism. A drawback with the MO seed is its need to be purified before it's used as a coagulant to avoid the addition of organic matter and nutrients to the treated water. Low initial turbidity decreased the effectiveness of both natural coagulants due to less effectiveness of the destabilization mechanisms, charge neutralization, and bridging.

To little research was found on the use of natural coagulants for the treatment of wastewater where nutrient removal was monitored to form a conclusion, however, included studies indicate that chitosan is capable of phosphate removal in addition to the removal of particles, organic molecules, and inorganic ions. Non-purified MO seed did not perform well in the treatment of wastewater, and no studies were found on the use of purified MO seed for wastewater treatment.

Further research on chitosan should be directed at how it performs on a larger scale, combined processes in the production of drinking water. More research is needed on the treatment of wastewater by chitosan, focusing on the removal of nutrients. For MO seeds more research is needed on the purification of the active protein and its use in water and especially wastewater treatment.

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Appendix A. Experimental trials

As a consequence of the COVID-19 outbreak in Norway, it was deemed necessary to close down the pilot plant where I was working on my master thesis research. Due to this implementation of coagulant dosing in the pilot plant was never done. This chapter includes a summary of the work done before the lab closed down. All the time post-COVID-19 outbreak was put into the literature research and thus the content of Appendix A is not thoroughly worked through.

A.1 Background and materials

The goal of the study was to compare different types of natural coagulants with synthetic inorganic coagulants concerning treatment efficiency and optimal dose in a continuous installment. Chitosan and Moringa oleifera seeds were chosen as the natural organic coagulants in this study, and three different kinds of chitosan were bought to study the effect of MW (molar weight) and DD (degree of deacetylation). The background for doing the study was identical to the background described in chapter 1. Optimal dose was first identified in jar-tests before moving on to tests on the continuous installment (pilot plant).

A.1.1 Pilot-plant

The pilot plant intended for use in the master-thesis was a membrane bioreactor (MBR) system (figure A.1). Domestic wastewater is collected in a 1000 l holding tank to ensure a stable supply of wastewater to the system. A peristaltic pump ensures a steady supply of wastewater from the holding tank to the aeration tank which contains 150 l of mixed liquor. After biological treatment, the system is divided into three lines highlighted with different colors in figure A.1. This allows the treatment efficiency of three different coagulants to be compared simultaneously. In line 1 (blue line), an inorganic coagulant is used to serve as a base to compare the natural organic coagulants with. In line 2 (green line) natural organic coagulants are used. In line 3 (yellow line) an electro-coagulation cell is used. pH adjustment is done by peristaltic pumps.

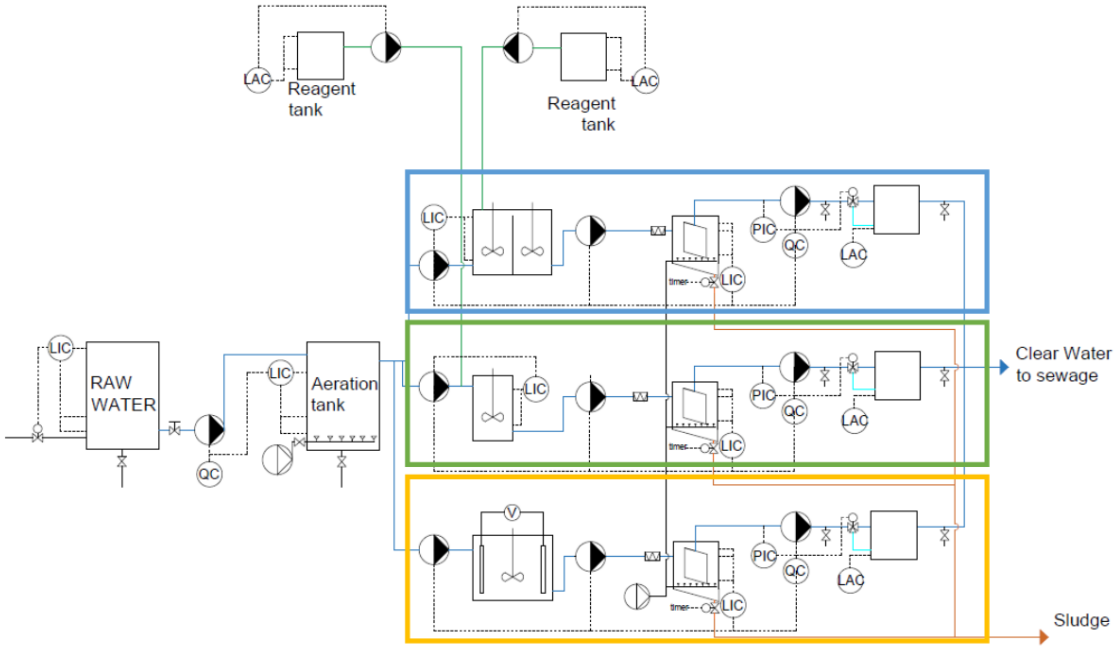


Figure A.1: Flow scheme for the pilot plant. (Shostak, 2018)

A.2 Methods

A.2.1 Preparation of coagulants

Before the coagulants could be used for the jar test they had to be prepared.

Chitosan

Three types of chitosan with varying degrees of deacetylation and molar weight were purchased from China. Information on the DD an MW was supposed to come with the chitosan, but it did not. Contacting the lab in China was not possible due to it being closed because of Covid-19. The chitosan was delivered as a white powder and needed to be dissolved in a weak acid before use as a coagulant. 0.2% (w/v) chitosan solution was made by dissolving 0.2 g of chitosan in 20 ml hydrochloric acid for an hour before filling deionized (DI) water until the total volume was 100 ml. To keep the chitosan dissolved, the pH of the stock solution was not changed before addition to the wastewater.

Moringa oleifera seed

Two different types of Moringa oleifera seeds were bought from Qingdao Honghai Bio-Tech. Both types were delivered as a powder in sealed bags. The preparation of the coagulant was done by making a 5% (w/w) solution of moringa seeds and DI water, before stirring it for 30 minutes. After stirring the solution was filtered through a 0.47 μm glass-fiber filter. The same method was followed for the saltwater extraction, but

instead of DI water, a 1 M NaCl solution was used.

A.2.2 Jar-test

Jar tests were done by filling the samples in 1 L beakers before adding the coagulant. Flocculation was done using two separate Kemira 2000 flocculators with 8 mixers connected to them. Rapid mixing was done for 40 seconds at 400 rpm, slow mixing for 10 minutes at 30 rpm, and sedimentation for 20 minutes. Coagulant was administered immediately after starting the rapid mixing and the mixers were removed after the slow mixing had ended to avoid disturbing the sedimentation. For the jar-tests with pH-correction pH was adjusted during the flocculation by the addition of 2M NaOH solution. At the end of the sedimentation, 100 ml samples were taken from the supernatant and tested for Turbidity (NTU), pH, TOD, phosphates, and ζ-potential.

A.2.3 Batch experiment

Aerobic batch experiments were performed after the method described in Experimental Methods in Wastewater (Loosdrecht et al., 2016). To make it possible to adjust the initial chemical oxygen demand (COD) level a synthetic wastewater was used for the batch experiment. A synthetic wastewater with 400 mg/l dissolved COD was made using the recipe on page 113 in Experimental Methods in Wastewater (Loosdrecht et al., 2016, p. 113). 1.5 l of mixed liquor was sampled from the aeration tank and transferred to a 4 l aerated container in the lab. The aerated mixed liquor was left to be stabilized while the pH, temperature, and dissolved oxygen (DO) was monitored. After the monitored parameters were stable a sample was taken for the calculation of MLVSS (mixed liquor volatile suspended solids) before adding 1.5 l of synthetic wastewater to the aerated mixed liquor, making the total volume 3 l. To monitor the change in dissolved COD, samples were taken every 5 min the first 30 min, every 10 min for the next 30 min and every 15 min after that until 4 hours had passed. Samples were filtered right after sampling through a 1.2 μm glass-fiber filter to stop the degradation and placed in the fridge at 4 °C. During the sampling process pH was kept stable around 7 and DO was kept above 2 mg/l. The adjustment of pH was done by adding HCl and NaOH. After all the samples were sampled and filtered TOD was measured. To make a correlation curve COD measurements were done for the samples that were taken; 5, 10, 25, 50, 90, 135, 180, 225, and 240 minutes after the start.

A.2.4 Analytical procedures

pH

pH was measured using a portable pH meter. Before daily use, the pH meter was calibrated in a buffer solution. pH value was recorded when the pH meter was stable for 1 min.

Turbidity

Measured using a standard technique on portable turbidity-meter 2100Q (Hach, USA). Sample vial belonging to the instrument was cleaned by washing it with DI-water three times between refilling the vial with a new sample. When filling a new sample to the vial, the sample was poured several times to avoid any dilution by the leftover DI water. Sample vial was stored submerged in water and handled with care to avoid damaging the vial. To verify that the instrument was working a sample containing DI water was analyzed before using the instrument.

Total suspended solids

Analysis performed according to standard procedure ISO 11923:1997. A standard glass-fiber filter with a diameter of 47 mm and a pore size of 1.2 μm (Sigma Aldrich) was first weighed on an electronic weight (Metler Toledo, Spain) with a resolution of 0.001 g. After weighing the filter a 20- or 40-ml well-mixed sample (depending on turbidity) was filtered through the glass-fiber filter before the filter was dried for at least 1 hour at 105 °C. When the filter reached a constant weight, it was taken out and left to cool down to room temperature, before weighing it again. The difference in weight of the filter before and after filtration gives the total suspended solids for the sample volume. TSS can then be calculated as:

$$TSS = \frac{m_{f2} - m_{f1}}{V_s} \left(\frac{mg}{l}\right)$$

Where m_{f1} (mg) is the mass of the filter before filtration, m_{f2} (mg) is the mass of the filter after filtration, and V_s (l) is the volume of the filtered sample.

Volatile suspended solids

25 ml of a well-mixed sample is evaporated in a weighed aluminum dish and dried to a constant weight in an oven at 105 °C. The sample is weighed again before being ignited in an oven at 550 °C for 1 hour. The loss of weight during the ignition represents the volatile suspended solids (VSS).

$$VSS = \frac{m_{d2} - m_{d1}}{V_s} \left(\frac{mg}{l}\right)$$

Where m_{d1} (mg) is the mass of the aluminum dish after drying, m_{d2} (mg) is the mass of the aluminum dish after ignition, and V_s (l) is the volume of the sample added to the dish before drying.

Total oxygen demand

For the measurement of total oxygen demand (TOD), a $QuickCOD_{lab}$ instrument (LAR, Germany) was used. The method used by the instrument is based on thermal oxidation of the sample at 1200 °C, and thereafter detection of the oxygen consumption by an O_2 detector. TOD measurements were then correlated to the chemical oxygen demand (COD) through measuring both the TOD and COD for a number of different samples, before making a correlation curve. COD was measured using the appropriate ranged TNTplus COD kits (Hach, Germany)

Phosphates

The standard chlorometric analysis proposed by USEPA Method 365.1. The analysis was done using a Systea EasyChem analyzer (Spain). Samples were first filtered to remove any particles before a minimum of 100 μl were transferred to the Systea sample vials. Reagents used by the analyzer are listed below in table A.1. To verify the validity of the measurements, samples with known concentrations were placed at intervals in the measurement series.

Table A.1: Reagents used by the instrument to measure phosphates.

Reagent	Concentration	Volume (μl)
Ammonium molybdate $(NH_4)_6Mo_7O_{24} * 4H_2O$	40 g/l	21.4
Antimony potassium tartrate $K(SbO)C_4H_4O_6$	3 g/l	7.2
Ascorbic acid $C_6H_8O_6$	18 g/l	400
Sulfuric acid H_2SO_4	5 M	71.4

Total phosphorus

Sample ($2.5ml$) was digested in an autoclave with potassium persulfate ($K_2S_2O_8$, 5%, 3 ml) and concentrated sulfuric acid (H_2SO_4, 95-97%, 75 μl) for 30 minutes until the

solution was clear and without particles. Analysis of the sample was then done using the same method as when analyzing phosphates.

A.3 Results

A.3.1 Categorisation of chitosan

As mentioned under subsection A.2.1, the MW (molar weight) and DD (degree of deacetylation) of the chitosan were unknown and the supplier was not possible to contact due to the COVID-19 outbreak. To be able to study the effect of MW and DD, a sample of each of the three types of chitosan were supposed to be sent to a lab for analysis. Unfortunately, the lab was closed before the samples were sent. MW and DD are therefor unknown which negates the ability to categorize the effect of MW and DD. To be able to distinguish the different chitosan, they were named type 1, type 2, and type 3.

A.3.2 Raw wastewater parameters

Parameters for raw wastewater used in the jar-test are presented in table A.2. Measurements for each parameter were done in triplicates.

Table A.2: Parameters of wastewater used for jar-test.

Turbidity (NTU)	pH	TOD (mg/l)	PO4 (mg/l)	Zeta potential (mV)
52 ±3.2	7.9 ±0.1	306 ±1.4	6.7 ±0.1	-19.7 ±8.1

A.3.3 Jar-test

Chitosan

The preparation of the chitosan stock solutions was done as described in A.2.1. Chitosan type 3 did not properly dissolve so concentrated HCl was added dropwise until the chitosan was dissolved. Jar-test were performed with 8 doses for each type of chitosan in the range from 0-80 mg/l. The chosen range of doses was meant to provide an overview of how the removal efficiency changed with dose and make it possible to choose a smaller range for the next set of jar tests. Only jar tests without pH correction were performed before the lab shut down. Figure A.2 shows the change in turbidity with chitosan dose for the three different types of chitosan. All of the three types show a similar development of turbidity removal with increasing dose, where the turbidity decreases before it increases again. The increase of turbidity is due to restabilization which implies

that the main mechanism of coagulation is bridging or adsorption. (Bratby, 2016, p. 86). Type 3 deviates from the two other types by reaching optimal removal rate at a lower dose and having a more rapid increase in turbidity at doses above the optimal dose. Optional removal efficiency for type 1, 2, and 3 was 77%, 80.4%, and 80% with optimal doses of 20, 20, and 15 mg/l.

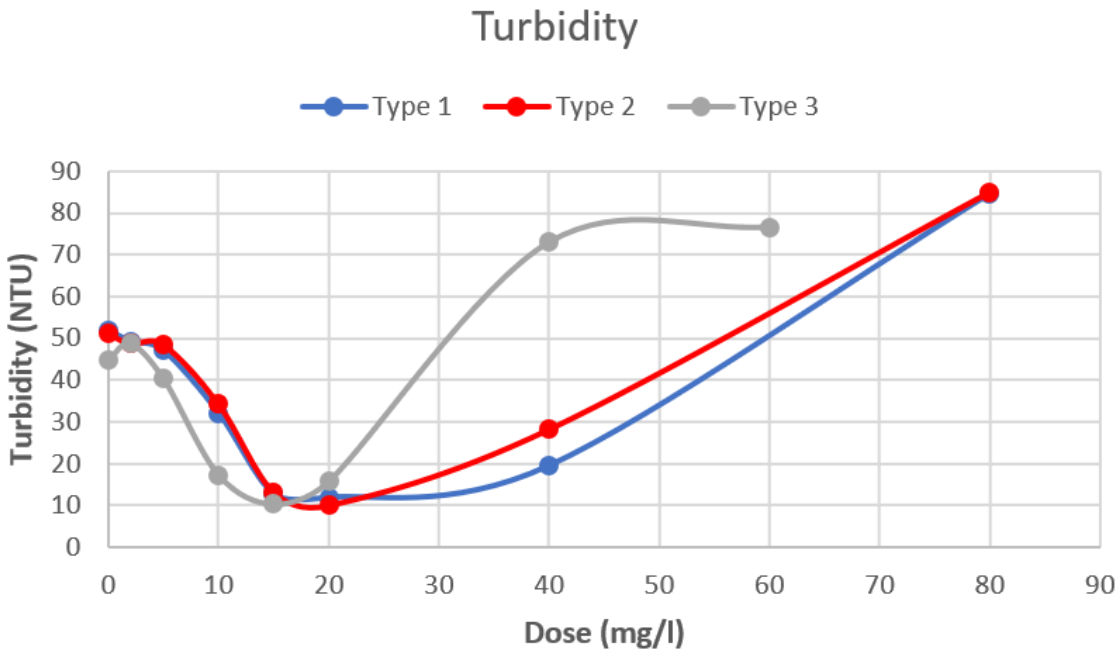


Figure A.2: Turbidity as a function of chitosan dose.

Figure A.3 shows the change in pH with an increasing chitosan dose. The decrease in pH comes from the low pH of the chitosan stock solution and for type 3 it is amplified by the additional addition of HCl added to make the chitosan dissolve.

Change in TOD with respect to chitosan dose is shown in figure A.4. Change in TOD corresponds well with the change in turbidity (figure A.2). Type 1 had a optimal dose for TOD removal of 15 mg/l achieving 33% removal, however the sample from 20 mg/l was lost. Since the turbidity was at it lowest at a dose of 20 mg/l it is likely that the TOD would have been lower as well. Type 2 had a optimal dose of 20 mg/l achieving a removal rate of 22%. For type 3 optimal dose was 15 mg/l with a removal efficiency of 32%. The low removal rates despite the high turbidity removal rates, indicates that a large portion of the TOD is present as dissolved TOD, which chitosan is not able to remove.

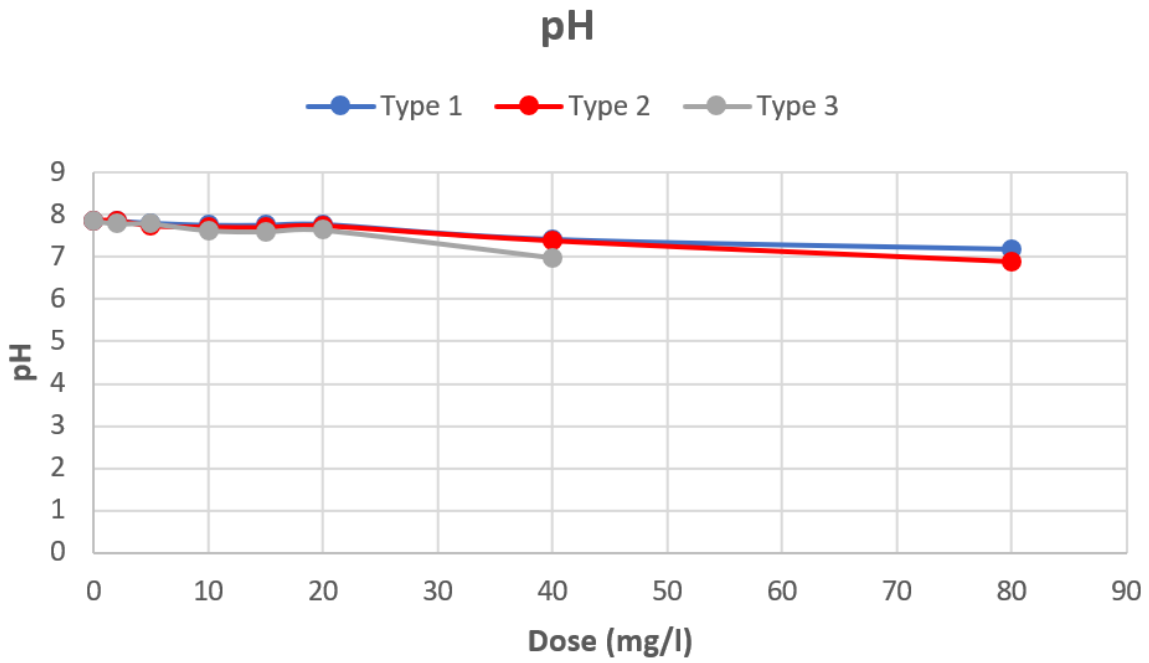


Figure A.3: pH as a function of chitosan dose

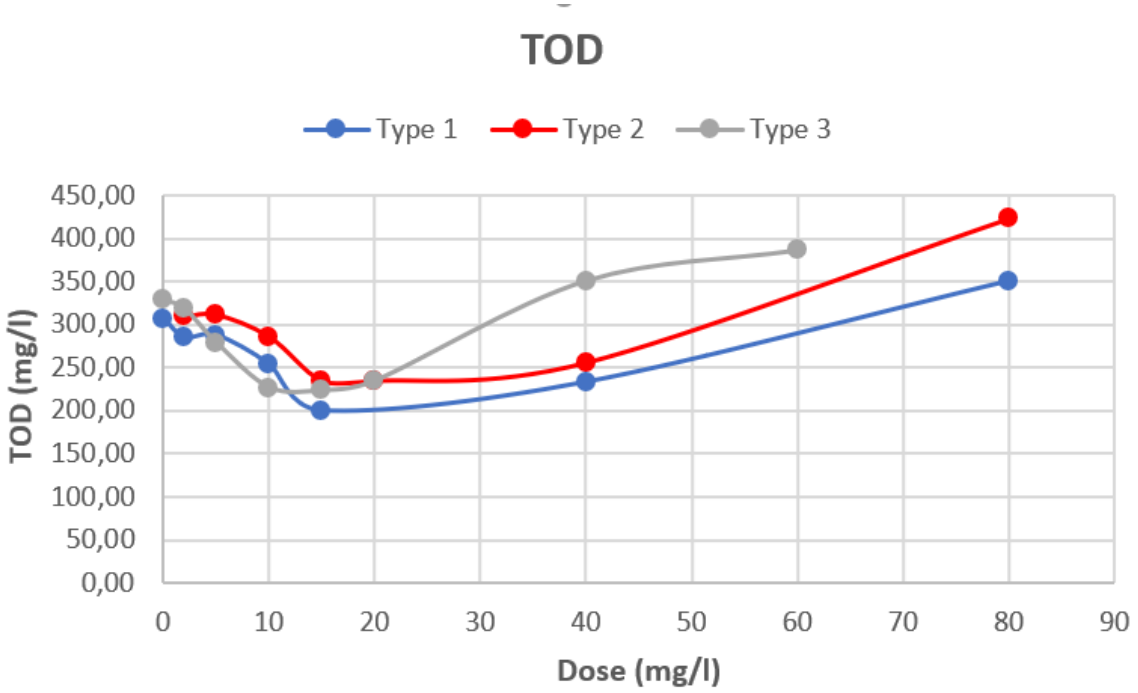


Figure A.4: Total oxygen demand (TOD) as a function of chitosan dose

Figure A.5 shows the change in phosphates with an increasing chitosan dose. As expected chitosan has no effect on the phosphorus concentration and the small variations can be explained by instrumental uncertainty.

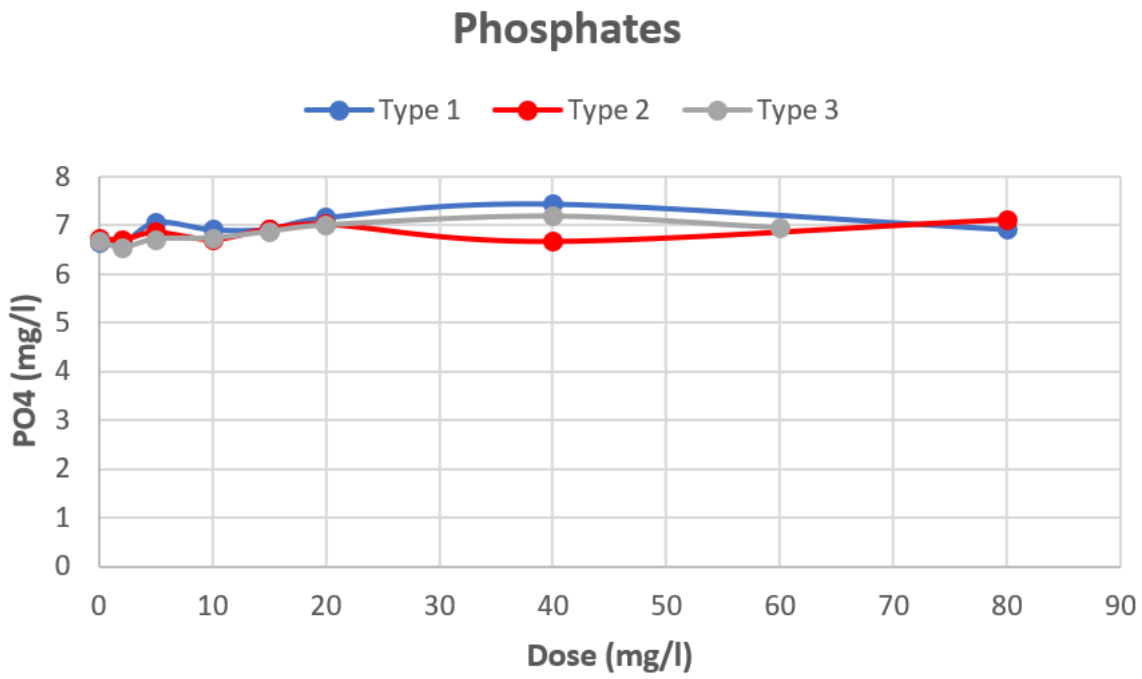


Figure A.5: Phosphates as a function of chitosan dose

Figure A.6 shows the change in ζ-potential with an increasing chitosan dose. It was expected that the ζ-potential would increase with an increasing chitosan dose, which was not the case. Instead it flattened out after reaching approximately -10 mV. Since optimal removal was around 15-20 mg/l it implies that bridging is the dominant mechanism for destabilization.

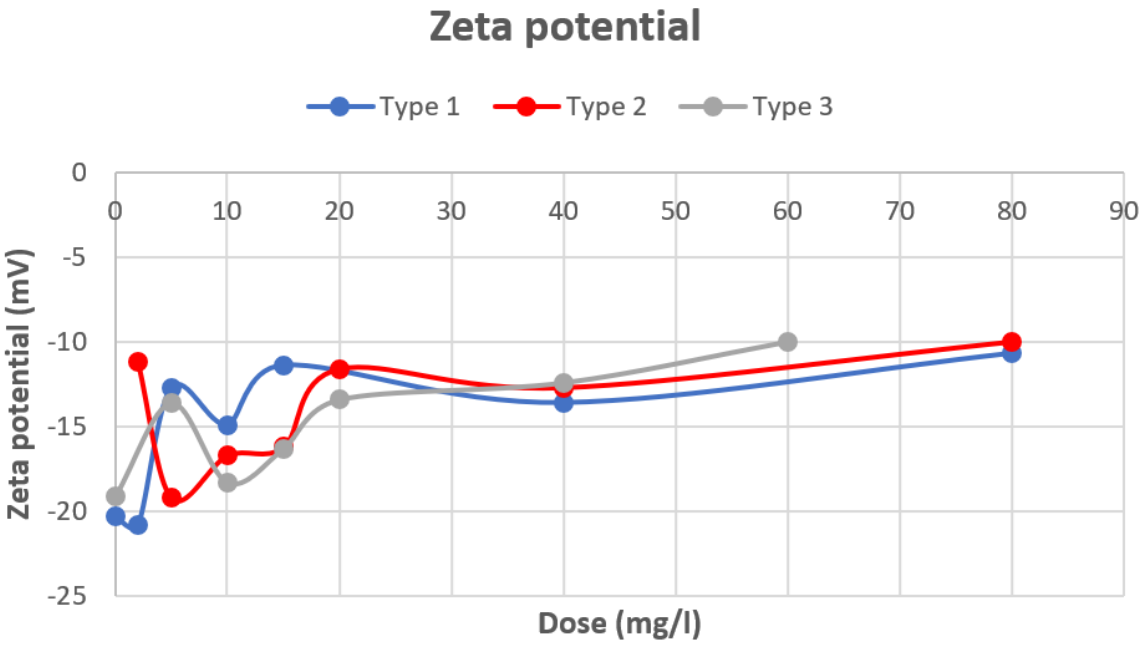


Figure A.6: ζ-potential as a function of chitosan dose

A.4 Batch experiment

Due to a malfunction with the TOD analyzer, TOD measurements were not performed for the batch experiment. Results from the COD measurements can be seen in figure A.7. In 4 hours the COD decreased from 248 to 191 mg/l which is a decrease of 57 mg/l. Since we used the same synthetic wastewater as they did in the book experimental methods in wastewater treatment (Loosdrecht et al., 2016) our results are comparable to the results from the examples in the book where they had a COD of 30-50 mg/l after 4 hours. With all the COD being available as dissolved COD, a higher removal rate was expected if the activated sludge was working optimally. It was therefore concluded that the activated sludge was not working optimally, and it was decided to restart the biological tank.

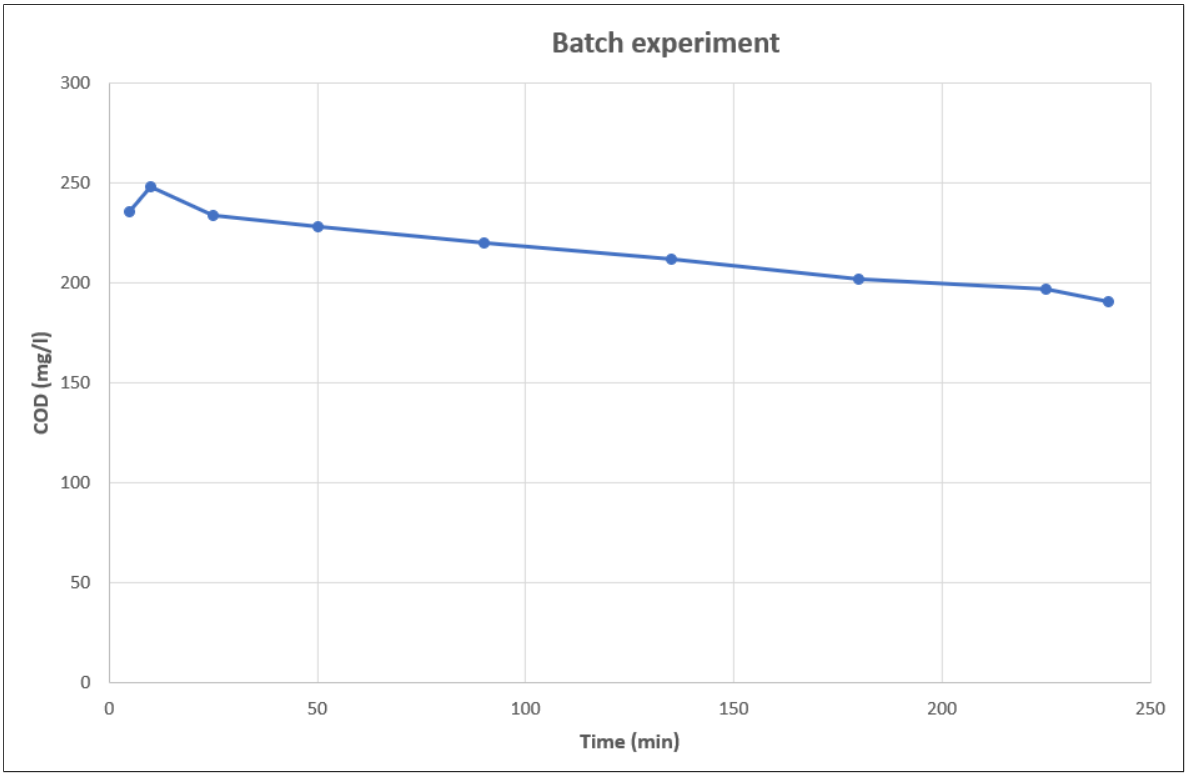


Figure A.7: Change in COD over time during the batch experiment.

A.5 Discussion and conclusion

Due to the incompleteness of the work done before COVID-19 there is to little data to draw anything from it.

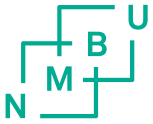
Appendix B. Reference evaluation

Table B.1: Amount of citations and journal impact factor for the studies included in the review

Reference	Amounts of citations	Journal impact factor
Ahmad et al., 2004	31	0,356
Ahmad et al., 2006	215	0,972
Al-Manhel et al., 2018	19	3,54
Altaher, 2012	34	2,27
Amagloh and Benang, 2009	63	0,41
Araújo et al., 2010	50	0,529
Bailey et al., 1999	2500	7,913
Baptista et al., 2017	46	5,107
Bhatia et al., 2007	100	2,4
Bhatnagar and Sillanpää, 2009	409	5,256
Bhuptawat et al., 2007	236	0,894
Bichi, 2013	22	5,026
Camacho et al., 2017	52	8,355
Cheng et al., 2005	42	2,765
Chethana et al., 2016	37	3,4
Chi and Cheng, 2006	66	2,896
Choy et al., 2014	75	2,61
Christensen et al., 2016	3	0,9
Chung et al., 2005	28	3,667
Crini and Badot, 2008b	1400	9,03
Dash et al., 2011	1500	27,5
Divakaran and Sivasankara Pillai, 2001	155	1,57
Divakaran and Pillai, 2002	207	2,828
E, 2014	0	0,18
Fabris et al., 2010	32	0,529
Gamage and Shahidi, 2007	119	1,475
Gerente et al., 2007	515	4,731

Goy et al., 2009	480	0,342
Guibal, 2004	1300	1,487
Hendrawati et al., 2016	22	0,149
Hu et al., 2013a	36	1,327
Huang and Chen, 1996	100	3,48
Jadhav and Mahajan, 2013	9	0,342
Jahn and Dirar, 1979	41	0,72
Kamble et al., 2007	171	7,61
Katayon et al., 2006a	115	2,52
Kean and Thanou, 2010	876	15,594
Kong et al., 2010	1300	1,61
Lasko and Hurst, 1999	97	3,367
Lea, 2014	4	1,11
Leone et al., 2016b	44	3,86
Madrona et al., 2012	27	0,419
Mahmood et al., 2010	128	0,138
Mandloi et al., 2004	16	0,65
Milhome et al., 2009	22	0,54
Miretzky and Cirelli, 2009	336	4,193
Moreti et al., 2016	9	1,918
Muthuraman and Sasikala, 2014	66	0,865
Muyibi and Evison, 1995b	125	7,913
Muyibi and Evison, 1995a	177	3,672
Muyibi and Alfugara, 2003	41	0,1
Ndabigengesere et al., 1995	406	0,72
Ndabigengesere and Narasiah, 1996	45	0,72
Ndabigengesere and Narasiah, 1998	135	1,918
Ndabigengesere and Subba Narasiah, 1998	333	0,72
Okuda et al., 1999	239	7,913
Okuda et al., 2001	245	7,913
Patel and Vashi, 2013	19	1,04
Pitakpoolsil and Hunsom, 2013	34	4,455
Pritchard et al., 2010	42	1,423
Qin et al., 2006	14	0,31
Rabea et al., 2003	1800	2,808
Ravi Kumar, 2000	3200	0,72
Reck et al., 2018	45	6,395
Renault et al., 2009b	53	1,106

Rinaudo, 2006b	4100	8,767
Rizzo et al., 2008	69	1,108
Roussy et al., 2005b	29	0,778
Roussy et al., 2005a	46	0,75
Sakkayawong et al., 2005	212	6,361
Saleem and Bachmann, 2019	5	4,978
Sánchez-Martín et al., 2012	27	1,2
Sila et al., 2014	22	2,765
Soros et al., 2019	4	1,683
Stohs and Hartman, 2015	128	3,092
Subramanium et al., 2011	17	0,379
Szyguła et al., 2009	170	0,962
Teixeira et al., 2012	60	2,601
Thirugnanasambandham et al., 2013	41	4,53
Vakili et al., 2014	453	4,77
Verlee et al., 2017	180	5,706
Vieira et al., 2010a	54	1,64
Vieira et al., 2010b	75	1,52
Villaseñor-Basulto et al., 2018	14	3,173
Wan Ngah et al., 2011	1200	4,268
Yang et al., 2016	215	7,55
Yin, 2010	202	2,883
Zemmouri et al., 2013	12	1,13
Zeng et al., 2008	130	6,044
Zhang et al., 2016	254	3,905



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