

Preface

This master thesis is written as a part of the Nordic Road Water (NORWAT) research and development program directed by the Norwegian Public Roads Administration (NPRA). The purpose is for the NPRA to gather knowledge, in order to plan, build and operate the road network without doing unacceptable harm to the aquatic environment. NORWAT is not only of national interest, but the program also contributes to an exchange of knowledge across borders. The NORWAT project started in January 2012.

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Tone-Lise Rustøen

Ås, 15.05.15

Summary

Water used by different machines in the road construction phase, has to be purified in accordance with limitations in the discharge permit, set by the county governor. One of the main parameters set in this permit is suspended solids. Fluctuations in particle load, and also shifts in pH, can vary from day to day during the construction process. Overdosing of inorganic precipitating agents is therefore a common problem, as small variations in inlet water can change the required dose. This can result in an excess of coagulants in outlet water, and in the case of inorganic precipitating agents, this can be harmful for the aquatic environment.

Naturally occurring organic polymers may be a better alternative to inorganic coagulants in treating water used in construction processes. Thus, the cationic polymer chitosan – extracted from crustaceans, and the anionic polymer alginate – extracted from brown seaweed, could replace the inorganic precipitating agents currently used in water treatment. This thesis researches the effectiveness of two types of both chitosan and alginate, compared to ferric chloride sulfate (PIX-318), tested on drilling fluid produced from bridge construction conducted at Knappstad, Norway. The discharge permit with regards to suspended solids at this project is a weekly average of 500 mg/L.

The performance of precipitating agents was researched in both undiluted (>6000 NTU) and diluted (4000, 3000, 2000, 1000 NTU) drilling fluid that held 21 ± 1 °C. Experiments were conducted with the use of standardized jar- test equipment (Kemira AB Flocculator 2000), and turbidity measurements with the use of a laboratory turbidity meter (Model 2100AN IS, Hath Company).

Residual turbidity <500 NTU was successfully obtained using both chitosan types in undiluted and diluted drilling fluid, whereas the same residual turbidity was not obtained using either types of alginate. The dose of PIX-318 required to achieve the same turbidity removal efficiency as chitosan, was almost 20 times lower in undiluted drilling fluid. Chitosan would be effective as a primary precipitating agent in treating water from piling under the conditions presented in this thesis. However, further research and investigation with regards to large scale use as well as the economic and environmental aspects has to be conducted before it can be recommended.

Sammendrag

Vann som brukes av ulikt maskineri i forbindelse med veibygging, må renses i henhold til begrensninger i utslippstillatelsen, gitt av fylkesmannen eller lokale myndigheter. En av de viktigste parametrene i denne tillatelsen er suspendert stoff, ofte målt ved turbiditet. Svingninger i partikkelbelastning og pH, kan variere fra dag til dag i en byggeprosess. Overdosering av uorganiske koagulanter er derfor et vanlig problem, ettersom små variasjoner i inntaksvannet kan endre den nødvendige dosen. Dette kan resultere i et overskudd av koagulant i utløpsvannet, og ved bruk av metal baserte fellingkjemikalier, kan dette være skadelig for vannmiljøet.

Naturlig forekommende organiske polymerer kan være et bedre alternativ til uorganiske fellingkjemikalier i behandling av vann som brukes i byggeprosesser. Således, kan den kationiske polymeren chitosan - ekstrahert fra skalldyr, og den anioniske polymeren alginat – ekstrahert fra brun tang, potensielt erstatte de uorganiske kjemikaliene som nå brukes i vannbehandling. Denne oppgaven undersøker renseseffekten av to typer kitosan og alginat, sammenlignet med jernklorid sulfat (PIX-318), testet på borevann produsert i forbindelse med brobygging utført i Knappstad, Norge. Utslippstillatelsen med hensyn til suspendert stoff for dette prosjektet er et ukentlig gjennomsnitt på 500 mg / L, noe som er tilnærmet lik 500 NTU.

Effekten av de ulike koagulantene ble undersøkt i både uforynnet (> 6000NTU) og fortynt (4000, 3000, 2000, 1000 NTU) borevann, som holdt 21 ± 1 ° C. Eksperimentene ble utført ved bruk av standardisert jar-test utstyr (Kemira AB Flocculator 2000), og turbiditetsmålinger ved bruk av en laboratorieturbiditetsmåler (modell 2100AN IS, Hach Company).

Slutt turbiditet <500 NTU ble oppnådd ved anvendelse av begge typer kitosan i uforynnet og fortynt borevann, dette var ikke tilfelle ved bruk av begge typer alginat. Det trengs 20 ganger lavere dose av PIX-318, sammenlignet med kitosan, i uforynnet borevann for å oppnå lik renseseffekt. Kitosan vil være effektiv som primær koagulant i behandling av vann fra brobygging under de forutsetningene som presenteres i denne avhandlingen. Ytterligere forskning med hensyn til bruk i stor skala, samt en grundig vurdering av både det økonomiske og miljørelaterte aspektet, må gjennomføres før en anbefaling kan foreligge.

List of abbreviations

CE	Coagulation Efficiency
DA	Degree of acetylation
DD	Degree of deacetylation
DW	Demineralized water
EU	European Union
HQGC	High Quality Grade Chitosan
MW	Molecular weight
NMBU	Norwegian University of Life Sciences (Norges miljø- og biovitenskapelige universitet)
NPRA	Norwegian Public Roads Administration
PAC	Poly-aluminum chloride
PIX	Ferric chloride sulfate
TGC	Technical Grade Chitosan
TR	Turbidity removal efficiency
TW	Tap water

Glossary

Clay	Inorganic particle. Size $<2\ \mu\text{m}$ (0.002 mm)
Coagulation	With respect to water treatment, the <i>process</i> of transforming a system from a stable to an unstable state
Colloid	Particle, whose size ranges from 10 nm (0.00001 mm) to $10\ \mu\text{m}$ (0.01 mm)
Destabilization	The actual occurrence of a system from stable to unstable state
Dispergation	Separation of a substance from a singular particle surface
Flocculation	Formation of lager flocs in an unstable system, the direct consequence of destabilization
Precipitating agent	A causative substance that effect the formation of suspension in solution
Sand	Inorganic particle. Size $63\ \mu\text{m} - 2\ \text{mm}$
Silt	Inorganic particle. Size $2 - 63\ \mu\text{m}$

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1 Introduction

1.1 Water from construction processes

Good infrastructure is essential in modern society. Expansion of existing road networks, maintenance, and construction of new roads are important in order to obtain a functioning and operative system that has the capacity to handle increased traffic load. The Norwegian Public Roads Administration (NPRA) has more than 500 road projects – with a total value of approximately 12 billion NOK– running at all times (NPRA 2012). However, road construction and subsequent use can adversely affect both the terrestrial and aquatic environment (Angermeier et al. 2004; Wheeler et al. 2005).

In 2007, the EU's Water Framework Directive was implemented in Norway. The main goal of the directive is to achieve good ecological and chemical status for all of Europe's surface waters and groundwater by 2027 (Fjellvør 2014). Hence, there has been an increased focus on water quality for the past decade. Particle erosion from road construction can cause siltation of water bodies which in turn might have direct and indirect negative effects on organisms (Trombulak & Frissell 2000). In order to avoid this, it is common to implement erosion control early in the construction process. The building of temporary or permanent sedimentation basins, extensive use of silt curtains, application of flocculants and pH adjustment are some measures that are adopted to avoid risk of causing unintended harm to the aquatic environment (Vikan & Meland 2012). The quality and chemical composition of water from different construction processes vary a great deal. It is well known that water from tunnel construction can have pH values >9 , due to the use of cement based grout. Whereas water from drilling processes can be around pH 7. Thus, each case has to be considered separately in order to find the optimal treatment for each construction site.

Before water from construction sites is discharged to a recipient, iron and alum based chemicals are often used in a purification process. Metal-based chemicals can accumulate in nature. There are however naturally occurring organic alternatives possible to use instead of chemicals. One is a positively charged (cationic) material, which is retrieved from the skeleton of different crustaceans, and is called chitosan. Another is a negatively charged (anionic) material, which is produced from brown seaweed, called alginate. This thesis will evaluate these organic

alternatives, with regards to turbidity removal efficiency when added to drilling fluid produced from bridge construction.

1.2 Background

The Norwegian Public Roads Administration (NPRA) is building a new stretch of road (E18) between Ørje at the Swedish border in Østfold county and Vinterbro in Akershus county, Norway (figure 1). The road will be a 70 km 4-lane motorway, to be opened in 2016. The building project is divided into nine different parts, where smaller stretches of road or allotments, are continuously completed as individual sub-projects (figure 2). Allotment number seven,



Figure 1: Geographical location of the construction site. Close-up in right corner. Modified after Google (2015)



Figure 2: The E18 project. Allotments are numbered according to construction sequence. Modified after NPRA (2015)

Knapstad- Retvet, crosses Hobøl River in Hobøl municipality, thus a bridge is a necessary part of the road construction.

Due to unstable ground conditions, the bridge needs to be piled to rock. When drilling for the installation of piles, high turbidity drilling fluids (water + clay, sand and silt) is produced. The bridge is to be founded on 90 piles. With mountain depths ranging up to 60 meters, an excessive amount of drilling fluid is produced from this project (Eriksen 2015).

The Hobøl River, which is part of Glomma river basin district (sub unit Morsa), is protected due to the habitation of river mussels and other animals found on the Norwegian red list of threatened

species. The river is characterized as having moderate ecological status, hence measures have been implemented in order to improve the water quality. To avoid pollution from the E18 project, the county authority demanded that the drilling fluid had to be treated on the construction site before being discharged into the Hobøl River. Discharge limits with regards to suspended solids (SS), was a weekly average of 500 mg/L. Thus, the drilling fluid was collected in ditches and pumped into a temporary water treatment facility established on the construction site. Ferric chloride sulfate was a coagulant used at the facility, to precipitate solids out of suspension.

1.3 Study goals

This thesis will evaluate the organic alternatives chitosan and alginate, with regards to turbidity removal efficiency and applicability as primary coagulants in water treatment, compared to ferric chloride sulfate when added to high turbidity (>6000 NTU) drilling fluid. The properties of two types of both chitosan and alginate with different characteristics will be evaluated.

First, a theoretical study will be conducted in order to try to understand which properties are of importance in a coagulation process. Second, jar-test experiments will be conducted to investigate the dose of precipitating agent required to obtain residual turbidity below 500 NTU. Both the theory and jar-test experiments will form the basis for discussing the following aspects of organic polymers, compared to chemical agents;

- Turbidity removal efficiency
- Environmental impact
- Health and safety
- Economy

2 Theory

2.1 Water treatment

There are many processes involved in water treatment, with regards to removing suspended solids. The most cost saving, but also often the most time consuming, is sedimentation (Håkonsen 2005). Because of the need for long retention time through a system, the mode of sedimentation is often combined with other processes where the aim is to speed up the sedimentation rate and reduce retention time.

Slow sedimentation rate is due to the fact that the system in question has reached a form of stable state. In order to speed up the sedimentation process, one has to reduce some of the factors involved with keeping the system stable. Hence, forcing the system into an unstable state will increase sedimentation rate (Bratby 1980).

The following subchapters will first present some of the basic concepts regarding factors causing slow sedimentation, followed by the different mechanisms involved in destabilization.

2.1.1 System stability

Most impurities suspended in water can be removed successfully by sedimentation. The challenge arises when dealing with slow settling particles and non-settable colloids. In general, the stability of a suspension depends on the number, size, density and surface properties of solid particles in suspension and the density of the medium in which the particles are suspended (Bratby 1980).

One of the fundamental properties of colloidal particles is that they have a very large specific surface area. Their capability to adsorb molecules, or ions, from the surrounding solution is an environmentally important feature, as pollutants often stick to their surface (vanLoon & Duffy 2011). The adsorption properties allow pollutants to be temporarily or permanently removed from solution. Adsorption can occur in several ways. One is due to electrostatic attraction to a charged surface.

Surface charge

In an aqueous suspension, many common environmental colloids have a negative surface charge that is relatively constant in magnitude. An example is mineral clays (i.e. SiO₂). They usually have negative electrical charges when pH is above 2, as this is their point of zero charge (pzc) or p*H*₀ (vanLoon & Duffy 2011). However, there are more variables associated with the colloid charge. Surface species, such as protonated or deprotonated functional groups, and other charged atoms in contact with the solution are affecting the charge properties of a colloid surface. These properties are often described in the terms of an electrical double layer.

The colloid charge serves to attract oppositely charged counter ions from the surrounding solution, and these form a diffuse “layer” around the particle in question. This means that there is a larger abundance of oppositely charged counter ions near the colloid surface than that in the bulk solution; where positive and negative species are balanced (normal state). Moving further away from the colloid surface - where the charge potential is at its maximum - it gradually decreases to zero, until a normal state is reached. The “thickness” of the counterion layer is defined as the distance at which the potential has decreased to 1/e (0.37) of its value at the surface (vanLoon & Duffy 2011).

Under stable conditions the repelling forces of the electrical charge are greater than the attraction forces between particles, hence aggregation does not occur (vanLoon & Duffy 2011).

Furthermore the colloids are kept in suspension by Brownian motion - the constant thermal bombardment of the colloidal particles by the relatively small molecules around them (Çoruh 2005).

2.1.2 Destabilization

In order to sediment impurities such as non-settable colloidal solids and slow- settling suspended solids, a precipitating agent is usually added to water to produce rapid-settling flocs by coagulation and/or flocculation. Hence, one manipulates the system stability. This can be done in several ways. However, it is important to keep in mind that destabilization reactions of colloids in water are quite complex, and are affected by several mechanisms and factors. Colloid properties (such as surface charge, functional groups, hydrophilic/hydrophobic etc.) as well as

factors affecting the precipitating agent (adsorption capacity, bridging capacity, charge etc.) (Bratby 1980) will influence which mechanism will dominate in a destabilization process.

Neutralization of charge

One of the main mechanism involved when using chemical agents to precipitate particles is charge neutralization. If the particles have a negative surface charge, adding positively charged aluminum or iron salts (or other cationic compounds) will neutralize particle charge, allowing aggregation, thus enhancing sedimentation (Gillberg et al. 2003). However, overdosing of counter ions can shift the equilibrium, so that there is a charge reversal on the particle surface, causing stable conditions once more.

Another way of destabilize suspended colloids is by increasing the ionic strength of the suspension. This will depress the thickness of the double layer and the potential will fall more rapidly to zero (Edzwald et al. 1974; Gregory 1978; Ødegaard et al. 2013). Thus, the repelling forces between particles will decrease, allowing the particles to settle.

Sweep coagulation

Inorganic coagulants can form solid metal residues at different concentrations and pH. When this happens suspended colloids can incidentally become trapped in/on the precipitate structure, hence settle out of suspension along with the metal residue (Bratby 1980). Thus, when discussing sweep coagulation in this thesis, it is referring to the incidental entanglement of suspended solids on a chemical precipitating agent.

Bridging

Polymers often adsorb to a particle surface either due to charge reactions, dipole-reactions, hydrogen bonding or van der Waals forces (Bratby 1980). As a polymer can have several charged sites, these are usually the first to connect to an oppositely charged particle, at least up to the point of charge neutralization (Gregory 1978). This will lead to loose strains of polymer floating freely in water, allowing incidental collisions between the polymer and other suspended particles. Thus, bridging is the mechanism in which particles are only partly covered by adsorbed polymer, so that attachments with segments from other particles can be formed (Gregory 1978). The main characteristic of the bridging mechanism is the aspect of time, as bridging occurs before the adsorption of polymer molecules to the particle surface has reached equilibrium

(Håkonsen 2005). Moreover, at excess polymer dose the particle surface can become saturated with adsorbed polymer, hence restabilization can occur.

Bridging is the mechanism by which nonionic and anionic polymers act (Ammary 1995), whereas the mode of action of cationic polymers has been, and still is to some degree, a controversial issue.

Patch theory

It is generally thought that high molecular weight cationic polymers improve the coagulation process (Ammary 1995). Due to this, researchers believed that the main mechanisms involved in flocculation were either charge neutralization alone or a combination of charge neutralization and bridging. Gregory (1973), cited by Gregory (1978), introduced the concept of electrostatic patch model which explains why the coagulation process is improved when using high molecular weight cationic polymers.

The model proposes that the improved coagulation with high molecular weight polymers are due to the uneven charge distribution of polymers on the particles surfaces, as illustrated in figure 3.



Figure 3: The "Patch theory" concept. The figure illustrates two negatively charged particles, on which cationic polymers is adsorbed unevenly (Gregory 1978)

When a high molecular weight polymer adsorb on a particle surface, there will be areas on the particle that has a high positive charge density. Gregory proposes that even though there is absorbed a sufficient amount of polymer to achieve zero net charge, there will still be patches of low negative charge density (which are the original particle surface). This uneven distribution causes increased attractive forces between the positive charged patches and negative surface

areas. It is proposed that the resulting electrostatic attraction might cause the particles to collide and form aggregates at a faster rate than previously suggested by other theories (Gregory 1978), thus enhance coagulation efficiency.

Low molecular weight cationic polymers will adsorb more evenly on the particle surface, compared to high molecular weight polymers, if assuming that the polymers have the same charge density (Ammary 1995).

2.1.3 Turbidity removal efficiency

Turbidity is a measure of light scattered by suspended particles in a liquid sample, and is often measured in Nephelometric Turbidity Unit (NTU) (Håkonsen 2005). The portion of light reflected for a given density of particles, is governed by the particle properties such as shape, color, and reflectivity. Hence, equal turbidities can be obtained from samples with highly varying characteristics. Turbidity is often used as a surrogate measure for suspended solids (Bilotta & Brazier 2008), but the correlation between the amount of suspended solids and measured turbidity varies between different suspensions. Thus, a correlation curve has to be made for each water quality.

Several studies evaluate efficiency of a precipitating agent, based on turbidity removal efficiency (%) (Divakaran & Sivasankara Pillai 2001; Divakaran & Sivasankara Pillai 2002; Zemmouri et al. 2013). Most of these studies have not defined how the turbidity removal efficiency (also called flocculation efficiency or coagulation efficiency) is calculated. Gyawali and Rajbhandari (2012) calculate the turbidity removal efficiency (TR) as shown in equation 1 .

$$TR \% = ((T_1 - T_2) / T_1) * 100 \quad (1)$$

Where T_1 is initial turbidity and T_2 is residual turbidity after treatment with precipitating agent. Thus, they indirectly define turbidity removal efficiency as the decrease in turbidity (%) after using a precipitating agent, compared to the initial turbidity (before adding the precipitating agent) of the same sample.

Research has demonstrated that the majority of cohesive solids in nature are transported in the form of larger aggregated flocs (Droppo et al. 1997; Droppo 2001; Phillips & Walling 1995).

Thus, one can expect cohesive clay particles to behave as larger aggregates in nature. This is also the reason why detention ponds work in areas where marine clays dominate the mass transport from agriculture; aggregates allow for a more rapid settling time than singular fractions of clay particles. Hence, an evaluation of whether suspended particles occur as aggregates or singular fractions can be useful as it has the potential to influence the coagulation efficiency.

2.2 Chitosan

2.2.1 General introduction

Crustaceans form a large group of arthropods, and crustacean shells, such as crab and shrimp shells, are a huge waste problem for the food industry. Every year, 750 000 tons of crustacean shells are disposed of as waste. Now, that is only within the European Union (CORDIS 2013). On a global scale, this number is much larger.

Generally, it may be beneficial to commercialize waste products (bi-products). This be profitable for the industry and society. It would also lead to a re-cycling, or re-use, of the previously unwanted material. Asia used to be a mass producer of unwanted crustacean shells. However, they have managed to turn waste into profit by extracting chitin from crustacean shells. Chitin is the raw material for commercial production of chitosan, and the difference between the two will be discussed later. Chitin is a large molecule (polymer), composed of repeating molecular units (monomers), that has unique properties. In a polymer, the number of monomers may range up to millions. Thus, it can also be described as a long chain of repeating molecular units (Mathews et al. 2013). The most common polymer on earth that we know of is cellulose. As cellulose is produced in plant cell walls, chitin is produced in shells of insects and crustaceans. Chitin is also found in e.g. algae and fungal cell walls, however it is thought to be most abundant in the exoskeleton of *arthropods* (Vårum & Smidsrød 2005). This makes chitin the second most abundant polysaccharide in the world, after cellulose (Divakaran & Sivasankara Pillai 2001; Rinaudo 2006).

Chemists describe chitin as a linear polymer of (1 → 4)-linked 2-acetoamido-2-deoxy-β-D-glucopyranose, and it was first discovered in 1811 by the French scientist Henri Braconnot (Winterowd & Sandford 1995). Chitin is biodegradable owing to the high abundance of

chitinases widely distributed in nature. Chitinases are enzymes, capable of breaking down sugar-bonds that holds the different monomers in chitin together. Chitinases are found in fungi, bacteria, plants, and also in the digestive system of many animals (Rinaudo 2006). Chitin is inert in the gastrointestinal tract of mammals, and can therefore be used in the food industry. Chitin and chitosan are also known for its nontoxicity, antibacterial- and gel forming properties, heavy metal ions chelation and affinity to proteins (Krajewska 2004). Chitosan is also found to be a potential material for arsenic adsorption on mining effluents (Westergren 2006). These characteristics open for a wide range of application areas for chitin and chitosan based material. It is especially popular in pharmaceutical and cosmetics industry, due to its unique features.

Chitosan has for the past two decades become a popular material for research regarding its possible application in water treatment processes. Chitin is a hydrophobic material, but when deacetylated to chitosan it can be dissolved in acidic solutions, and then become a polymer with positive (cationic) charge. There is however an exception, as chitin is water soluble even at neutral pH when deacetylated to about 50% (Kurita 2006). This will be explained further in subchapter 2.2.4. The charge properties of chitosan make it interesting with regard to flocculating negatively charged particles, such as clays and humic substances suspended in water.

Another interesting aspect with regard to the use of chitosan in water treatment processes is that it is known to produce less sludge than the chemical precipitating agents do. In addition, the produced sludge has the potential to work as a soil conditioner, if the treated water is not heavily contaminated to begin with (Håkonsen 2005). This is supported by Rinaudo (2006) and Arbia et al. (2013), which through their literature reviews summarized that chitosan properties related to agriculture could be; stimulation of plant growth, seed coating and frost protection, time release of fertilizers and nutrients into the soil. Adding chitin and chitosan to compost have also proven to enhance chitinase production as well as microbial diversity, which may contribute to reduce soil- borne diseases (Poulsen et al. 2008).

2.2.2 Chitin and chitosan chemistry

Chitin has a highly ordered structure, and have been found in three polymorphic forms, α -, β - and γ -chitin. Polymorphism is the ability of a solid material to exist in more than one form or crystal structure. The difference between these three forms are chain arrangement within the crystalline structure. In β -chitin the chains are parallel, in γ -chitin two chains are “up” to each chain “down”, and in the most abundant form; α -chitin, the chains are anti-parallel (Roberts 1992). Rudall (1955), cited by Roberts (1992), indicated that the three different polymeric forms are related to function rather than taxonomic grouping. This idea is interesting, as it is claimed by many (Håkonsen 2005) that it is, among other things, the difference in raw material composition that can cause different results when trying to verify experiments related to water treatment. This topic will be further enlightened in sub-chapter 2.2.5. α -chitin is found where extreme hardness is required, thus as previously mentioned it is most abundant in crustaceans, where it often is cross-linked with proteins providing strength to the outer skeleton. β - and γ -chitin are found here flexibility and toughness are required. α -chitin, however, is thought to be the most stable form, as both β - and γ -chitin may be converted to the α -structure by suitable treatments.

According to Pillai et al. (2009) there are mainly two parameters on the molecular chain that differs between chitin and chitosan. When the “R”-group on the molecular chain constitutes of an acetyl (COCH_3) group, and the degree of acetylation (DA), meaning the ratio of acetamido groups ($\text{C}_2\text{H}_4\text{NO}$) relative to amino groups ($-\text{NH}_2$) on the polymer chain, is $>50\%$ (as shown in figure 4) it is characterized as chitin. DA in chitin is typically 0.90. As deacetylation might occur during deproteinization in the extraction process, chitin may contain 5-15% amino groups.

more complex and not economical under normal circumstances. Crab shells from Europe also has a high content of CaCO_3 , preventing a cost effective conversion to chitosan (CORDIS 2013). An ongoing project called ChiBio, funded by the EU's Research and Innovation program – Horizon 2020 – , aims to improve the cost efficiency of chitin extraction, making it possible to re-cycle crustacean shells from Europe, Asia and Africa (ChiBio 2012). Regardless of these variations, crustacean shells represent the main industrial source for chitin extraction. This makes it a well distributed byproduct from the food industry (Arbia et al. 2013).

The most common extraction process of chitin from crustacean shells is by using alkaline and acid chemicals, causing deproteinization and demineralization of the crushed shells resulting in crude chitin with lightly pink color (figure 5). Chitin isolated from squid pens, are the exception, as it is completely white making discoloration unnecessary (Vázquez et al. 2013). By deacetylation of crude chitin, one can obtain chitosan with different qualities, depending upon the choice of possessing method.

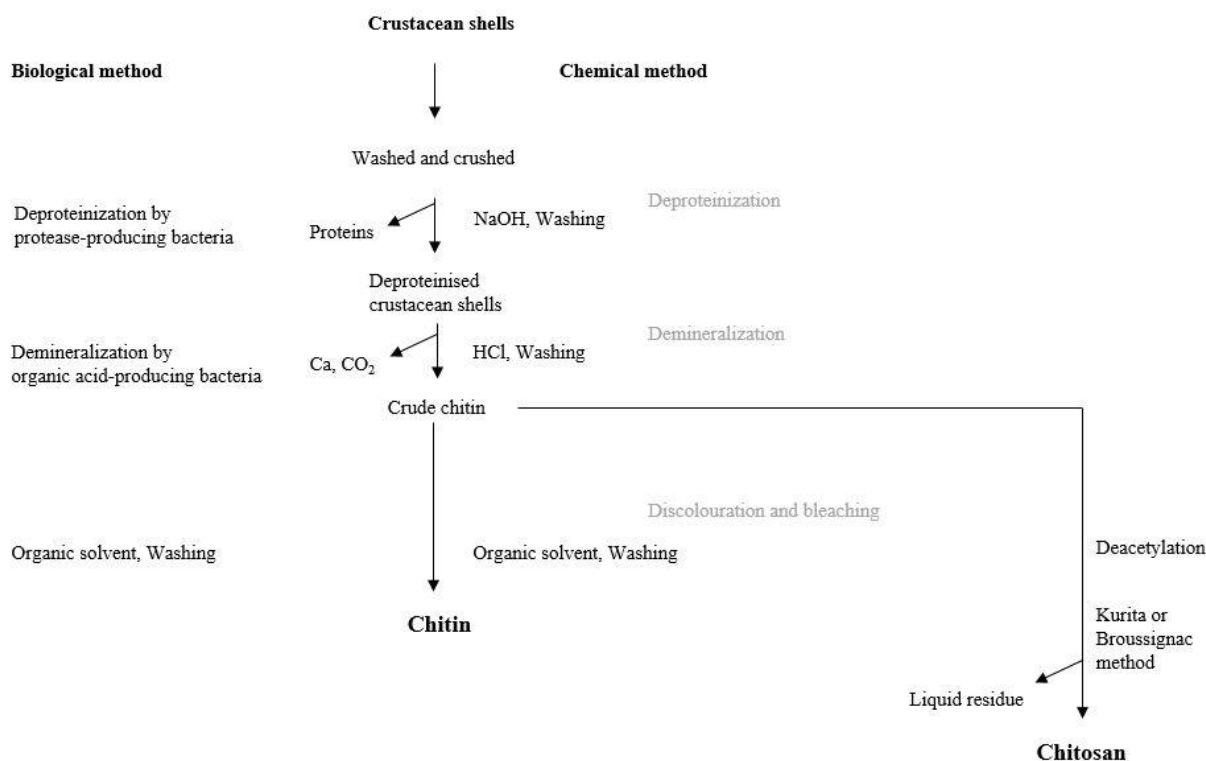


Figure 5: General procedure for biological and chemical isolation of chitin, and chemical processing of chitin to chitosan, from crustacean shells (schematically). Modified after Vårum and Smidsrød (2005), Arbia et al. (2013) and Vázquez et al. (2013).

The large variety of chitin composition, and abundance, within different sources of raw material, calls for a large variety of extraction processes. The main steps, deproteinization and demineralization, are usually carried out by the use of sodium hydroxide (NaOH) and hydrochloric acid (HCl) respectively as shown in figure 5. Deproteinization can also be conducted by use of other base agents; however, NaOH is preferred according to the literature. Roberts (1992) reported that the main varying factors related to deproteinization with NaOH is the molar concentration used during washing, temperature (ranging from room temperature to 100°C), number of repeated treatments, and total time of treatment (e.g. 30minutes and up to 72 hours). Considering demineralization, it is reported use of nitric acid (HNO₃), sulphurous acid (H₂SO₃), acetic acid (CH₃COOH) and formic acid (HCOOH); again HCl is the preferred acid. Variations in molar concentration and total treatment time differs between productions, this is also the case when the same source of raw material is used (Roberts 1992). The choice of processing conditions depends upon the purpose for which chitin is required. If chitin is subsequently to be converted to chitosan, partial deacetylation during deproteinization is not a disadvantage. Two traditionally used methods for chitosan production are described in detail by Kurita et al. (1993) and Broussignac (1968) respectively, rendered and cited by Vázquez et al. (2013).

Even though chitin extraction is considered a partial problem solved with regard to costal pollution, the traditional chemical methods of chitin extraction causes a second environmental issue – the high residual concentrations of mineral acid and alkali (Healy et al. 2003; Vázquez et al. 2013). These are usually discharged into the environment without treatment and without view to re-use (Batista et al. 2013) The process is also very energy consuming, and expensive, thus alternative biological processes are currently assessed (Arbia et al. 2013). These are however only scaled to laboratory experiments, and ongoing work are currently conducted in order to optimize for large scale production (ChiBio 2012).

2.2.4 Solubility of chitosans

When considering the use of chitosan for large scale water treatment processes, the solubility in different acidic media are of relevance. As previously mentioned, chitin can be water soluble if

deacetylated to 50% (Kurita 2006), however it is likely that its charge properties is not as strong as those required in water treatment processes.

Solubility of chitosans is a very difficult parameter to control. It is related to the degree of deacetylation, the ionic concentration of the media, pH and the nature of the acid used for protonation, as well as conditions of isolating and drying the polymer during purification (Rinaudo 2012). It is also proposed that the distribution, random or block wise, of acetyl groups along the molecular chain may influence the polymer solubility. While chitin is insoluble in most organic solvents, chitosan is readily soluble in dilute acidic solutions below pH 6.0. This is because chitosan can be considered a strong base as it possesses primary amino groups with a pKa value of 6.3 (Pillai et al. 2009). The amino groups on the polymer chain are highly electronegative, and can take up a proton and become positively charged. Whereas the N-acetylated amino groups are hydrophobic (Vårum & Smidsrød 2006).

The basic idea is that in order to dissolve chitosan, one has to protonate the free amino groups on the molecular chain, making it a cationic polymer as shown in equation 2.



This is usually done by dissolving chitosan powder in an aqueous acid. Both organic- and inorganic acids can be used, with the exception of sulfuric and phosphoric acid (Kurita 2006; Sorlier et al. 2001). HCl is used in several studies (Divakaran & Sivasankara Pillai 2001; Divakaran & Sivasankara Pillai 2002; Håkonsen 2005; Liltved & Vogelsang 2006) where the intention is to make chitosan soluble in order to use it as a precipitating agent in water treatment processes. Håkonsen (2005) reported that a chitosan concentration of 3 % when dissolved in HCl at 4 °C would be optimal with regards to practical conditions related to dissolution time. When dissolved, the recommended storage time was found to be no more than ten days.

2.2.5 Chitosan characteristics and its significance for particle aggregation

It is generally accepted that the processing conditions, as well as the biological source of chitin, strongly affect properties and characteristics of the resulting chitins and chitosans (Galed et al. 2005; Percot et al. 2003). However, when discussing this topic it's important to emphasize that Berth and Dautzenberg (2002) reported lack of significant differences between chitosans from

different sources, and that the different behavior of chitosans in solution claimed in other studies may be a result of artefacts.

As the processing conditions, such as pH, temperature and time, for demineralization and altering of the degree of acetylation becomes harsher, the molecular weight (MW) becomes lower (Percot et al. 2003). Yuan et al. (2011) also reported that harsher processing conditions had a great influence on not only molecular weight of the resulting products, but also other physiochemical properties such as ash content, protein content and crystallinity.

When considering the chitosan characteristics that might be of interest with regard to particle aggregation, high MW (thus high viscosity) could be a factor enhancing aggregation as the molecular chain would be longer thus have more binding spots for negatively charged particles, or enhance its properties as a component in bridging mechanism. Thus, longer molecules enhances the chance of physical contact between the polymer and particles suspended in water. This theory is supported by Roussy et al. (2005), who found that chitosans with high MW gave best results when tested on bentonite suspensions. Molecular weight is however the most difficult parameter to obtain precisely, and it is also a difficult parameter to control during an extraction process (Zhang & Neau 2001).

High degree of deacetylation (DD), favoring protonated amino groups on the molecular chain might also enhance aggregation as one can assume the amino-groups to be one of the main working factors in particle aggregation where particle charge plays an important role. On the other hand, as the DD increases the molecular weight becomes lower. Thus, an evaluation of what mechanisms expected to be prominent has to be considered in different water treatment cases.

2.2.6 Chitosan in water treatment processes

For the past 20 years, more environmentally friendly ways of treating drinking water, sewage and water from urban construction, has been considered, and chitosan has in periods been a popular polymer of investigation. Chitosan has the advantage over traditional chemical flocculants, such as ferric sulfate (PIX) and polyaluminium chloride (PAC), that it provides a quicker depositing velocity, higher efficiency of removing suspended solids (SS), metal ions and

chemical oxygen demand (indirect measure of organic pollutants in water), easier sludge treatment, and that there are no further pollution. However, when used as a flocculent in water treatment it will have a higher cost than that of traditional chemical flocculants (Zeng et al. 2008).

Chitosans with different characteristics have been widely tested in both laboratory scale and full-scale water treatment facilities. The following subchapters will try to summarize some of the work that has been carried out in terms of chitosan as a cationic organic polymer in water treatment processes. This study does not include experiments conducted on industrial wastewater from e.g. paper mills and dairy producers as these suspended solids have a highly different characteristic than those relevant for this scope.

Laboratory experiments

Several studies have been conducted on particle aggregation with different chitosans. Most laboratory experiments are based on mixing kaolinite or bentonite powder with an electrolyte solution (e.g. sodium chloride (NaCl)) or local tap water to desired turbidity, thus creating a synthetic suspension (Divakaran & Sivasankara Pillai 2001; Huang & Chen 1996; Roussy et al. 2005). Others have collected naturally deposited sediments from river basins, or local soils, brought it to a laboratory and mixed it with either distilled- or local tap water (Divakaran & Sivasankara Pillai 2002; Rounce et al. 2012). Experiments with crude water (Håkonsen 2005; Kure 2013; Zemmouri et al. 2013) , and excess water from tunnel construction (Liltved & Vogelsang 2006) are tested in both laboratory and full-scale experiments.

For all laboratory experiments conventional jar-tests have been used. However, both the total amount of water used in each test, and the flocculation program differs between the studies. Another difference between all tests is the type of chitosan used. In several studies, a minimum of chitosan characteristics are specified. Most have stated the raw material used for production of the given chitosans, and some have even characterized the average DD. A completed summary of what is thought to be the most influential characteristics are usually lacking. To illustrate the severe differences, a summary of some of the studies conducted on the field are presented in

table 1. Comparison between the studies are therefore difficult due to the large variability of experimental conditions, however one can evaluate trends.

Table 1: Summary of some experimental conditions from several experiments conducted with the aim to test chitosan as a flocculent of particles in water.

Suspensions	Initial turbidity/ announced characteristics	Chitosan dissolution	Type of test	pH adjustment	Uptimum dosage	Reference
Kaolinite	10-20-40-80-160 NTU	10 ml 0.1 M HCl mixed with 100 mg chitosan until dissolved, mixed with 100 ml tapwater > 1 mg chitosan/ml solution	Jar - add flocculant - 5 seconds thoroughly stirring with glass rod, 60rpm, 30min, 30min settle - collect sample from top inch, 27-31°Celsius Jar - add flocculant at 100 rpm for 3 min, 30 rpm for 20 min, 10 minutes settling time. Turbidity, settling velocity and floc diameter was measured. NaClO4 was used as ionic buffer.	0.1M HCl or 0.1M sodium carbonate solution - pH 5.6, 7, 8.9	0.75-1mg/L for all turbidities at pH 7-7.5	Dyvakaran & Sivasankara Pillai (2001)
Kaolinite, bentonite and unspecified clay	90 NTU	Chitosan dissolved in 1% HCl, and mixed with deionized water to desired concentrations (Chitosan isolated from crab shells)	Jar - add flocculant - 100rpm 2min, 30rpm 20min, 10min settle - collect sample from top inch. Measure zeta potential from coagulated flocks, light of flock volume in the sediment were measured after 30min	NaOH and HClO4 to initial pH of 3, 4, 5, 6, 7	0.8 mg/L at pH 7	Pan et al. (1999)
Kaolinite and bentonite	30-90-150-400-500-600- 1000 NTU	Acetic acid - until dissolved (heat used)	Jar - add flocculant - 100rpm 2min, 30rpm 20min, 10min settle - collect sample from top inch. Measure zeta potential from coagulated flocks, light of flock volume in the sediment were measured after 30min	NaOH and HClO4 - tested at pH 4 and 7	Ex. 1000 NTU - 12 mg/L chitosan, 500 NTU - 8 mg/L	Huang & Chen (1996)
Bentonite (from a local site)	5 gram bentonite/L	Acetic acid - 0.2M, with 0.15M ammonium acetate buffer at pH 4.5. Dissolved at room temperature overnight. (Test conducted on 10 different chitosans, with varying DD)	Jar - add flocculant at 200 rpm for 3 min, 40 rpm for 20 min. Stopped. Turbidity measurements every 5 min for 20 min, and sporadically until 60 min	NaOH and HCl - tested at pH 5 and 7	Ex. >0.10 mg/L chitosan to remove more than 95% of residual turbidity	Roussy et al. (2005)
Synthetic stormwater, based on measurements from stormwater runoff from highway construction sites	200 to 1400 NTU	Not announced	A specific dose of flocculant was added to a jar during rapid mix (1000 rpm) on a magnetic stirrer for 15 seconds. The jar was then moved onto the jar test apparatus where it was slow mixed (60 rpm) for 5 minutes. The slow mix was followed by a 5 minute settling period. After this time, the final turbidity was measured on a sample taken from the top 2.5 cm of the jar	Tested in the pH range 5-7, not defined specific pH	Ex. 1400 NTU - 3mg/L	Roussy et al. (2012)
River silt	10-20-40-80-160 NTU	10cm ³ 0.1 M HCl mixed with 100mg chitosan until dissolved, mixed with 100ml tapwater > 1mg chitosan/ml solution	Jar - add flocculant - 5 seconds thoroughly stirring with glass rod, 60rpm, 30min, 30min settle - collect sample from top inch, 27-31°Celsius	0.1M HCl or 0.1M sodium carbonate solution - pH 4 to 9	0.5mg/L for all turbidities at pH 7, efficient in pH range 5-7	Dyvakaran & Sivasankara Pillai (2002)
Water from tunnel construction	Lab- 528 NTU. Fullscale 5000 to approx. 300 NTU	Chitosan dispersed in water to a 0.5% solution, added 2M HCl to dissolve (Chitosan characteristics : 94% DD, molecular mass of 110kD)	Jar - add flocculant at 40rpm for 1 min, calm mixing for 10min, settle 3min. Fullscale- 200L 0.5% chitosan solution. Added by dosing pump to tunnel water collected in a small container	Lab- No pH adjustment- tested at pH 7.8. Fullscale- pH- variations between 6.2 and 11.3, no pH adjustment, and constant chitosan dose.	0.5mg/L regardless of other factors.	Liljed & Vogelsang (2006)
Dam water	25-50 NTU	Acetic acid (Chitosan characteristics: 85% DD)	Jar - add flocculant - 200rpm for 3min, 40rpm 20min, settle 45min. Chitosan were also tested in combination with aluminum sulfate.	HCl or NaOH - 7.8 to 8.5	0.1 to 10mg/L	Zemmouri et. Al. (2013)
Raw water	<0.33 NTU and approx. 49 mg Pt/L	Referred to unpublished work. (Chitosan characteristics >80% DD)	Jar - add flocculant - rapid stirring for 1 min, 20 min slow stirring, 20min sedimentation -> 0.45µm filtration -> water sample analyzed for color (mg Pt/L). Fullscale - raw water pH adjusted, dose added and coagulation process starts with 382G/sec	Full scale - pH 4.90 - 5.82	5mg/L gave adequate results for full scale experiments (> 15mg Pt/L)	Kure (2013)
Raw water	ca 6.2 NTU and 60 mg Pt/L	HCl (19 different chitosans with varying qualities and characteristics)	Jar- tests conducted with several combinations of stirring regime. Several fullscale experiments conducted at different waterworks.	Full scale - raw water with initial pH of 7.0 adjusted to pH 5.5 before adding chitosan	3.5mg/L for all chitosans tested	Håkmoen (2005)

Huang and Chen (1996) found that the source and properties of clay particles in raw water highly influences the chitosan coagulation efficiency. The same study also reported that using bentonite as a coagulation aid, improved the flocculation efficiency of chitosan on kaolinite particles. In addition, the pH effect on chitosan efficiency in this matter was insignificant, thus the conclusion was drawn that charge neutralization was not a major mechanism controlling the formation of flocs. Pan et al. (1999) also found that the properties of the colloid particles to be very important when comparing coagulation efficiency of chitosan and poly-aluminum chloride (PAC) on bentonite, kaolinite and a un-specified clay. When using chitosan, smaller doses were needed to achieve the same reduction in turbidity compared to PAC. The study also reported formation of larger flocs when using chitosan. Chitosan in combination with PAC has shown good results on with respect to treatment of sewage water (Zeng et al. 2008), and also treatment of raw water with aluminum sulfate as main coagulant and chitosan as coagulant aid has proven very effective (Zemmouri et al. 2013).

Kaolinite flocculation with the use of chitosan was also investigated by Divakaran and Sivasankara Pillai (2001) who concluded that the flocculation was faster at higher concentrations of kaolinite. They also drew the conclusion that the efficacy of kaolinite flocculation by chitosan is strongly dependent of pH in the test medium. The same conclusion with regard to the importance of pH and concentration of suspended solids in the test medium was drawn by Divakaran and Sivasankara Pillai (2002) when testing flocculation of river silt. Roussy et al. (2005) tested the effect of ten chitosans with different molecular weights (MW) and degrees of deacetylation (DD) on bentonite suspension at both pH 5 and 7 in demineralized water (DW) and tap water (TW). The study found that for all chitosans lower doses were required at pH 5 than at pH 7, and there was an improved performance of chitosan in tap water suspensions compared with demineralized water. This could be due to the presence of counter-ions in the TW. Turbidity removal was best for the higher MW chitosans tested at both 89.5% DD and 95% DD. The study also concluded that the overall results indicated that destabilization of bentonite was achieved by the combined mechanisms of electrostatic patch and bridging. Rounce et al. (2012) researched the effectiveness of non-ionic and anionic polyacrylamides (PAM's) as well as chitosan on flocculating different synthetic storm waters based on measurements from highway constructions. The study concluded that the non-ionic PAM's and chitosan was most effective in promoting sedimentation for all synthetic runoffs tested.

Full scale experiments

Chitosans tested in full scale experiments at drinking water works has proven quite effective at relatively high doses (3-5 mg chitosan /L) (Håkonsen 2005; Kure 2013). The tests were conducted in combination with pH-adjustment of initial pH to approximately pH 5, and filtration was a part of the experiments. Both studies concluded that one could not argue for an increase in biofilm production on the pipeline with the use of chitosan. In addition, due to the fact that chitosan is effective in a broader pH range compared with metal-based coagulants, arguments related to overdosing were presented. The Norwegian institute of water research (NIVA) has also investigated the use of chitosan at several waterworks in southern Norway (Liltved et al. 2001). Their experience is that when high amount of organic material are present in the raw water, the use of iron chloride in combination with chitosan can be an advantage. Sludge production decreased with 2/3 when using chitosan alone, compared to iron chloride. Use of chitosan has a much higher cost compared with iron chloride, however NIVA concluded that the treatment process is suitable for small and medium sized waterworks (Liltved et al. 2006).

Chitosan experiments on excess water produced from tunnel construction are conducted in both laboratory and full scale experiments (Liltved & Vogelsang 2006). The conclusions were that an average of 75% of the particle load was removed when dosing 0.5-1 mg chitosan /L tunnel water, and that chitosan could work well as a coagulant if the treatment process were optimized.

2.3 Alginate

Alginate is a negatively charged (anionic) polymer occurring in relatively large amounts in nature. Its abundance is due to its presence both as a structural component in marine brown algae (*Phaeophyceae*), where the alginate comprises up to 40% of the dry matter, and as capsular polysaccharides in soil bacteria (Draget et al. 2005). It is a polymer commercially produced from the marine brown algae, colloquially known as seaweed. Alginate in brown algae was first described in 1881, by the British chemist E.C.C. Stanford (Sabra et al. 2001). Its chemical structure however was not identified until 1965 (Sivertsen 1996).

2.3.1 Alginate production process

Alginate occurs naturally in seaweed mainly in the form of calcium, magnesium and sodium salts (FMC 2003). The extraction of alginate from algal material is schematically illustrated in figure 6. Extraction of the ground algal tissue is often done with the use of mineral acid followed by alkali treatment. First, ion exchange equilibrium is reached between the seawater and the insoluble alginate counter ions with the use of acid chemical, often during the washing process or after grinding. Next, alginic acid is brought into solution by neutralization, often with the use of sodium carbonate or sodium hydroxide. Removal of alginate material is then carried out with the use of different separation methods. Finally, alginate is precipitated with the addition of mineral acid, alcohol or calcium chloride, and incorporation of elements to achieve desired alginate characteristics are conducted (Sabra & Deckwer 1998).

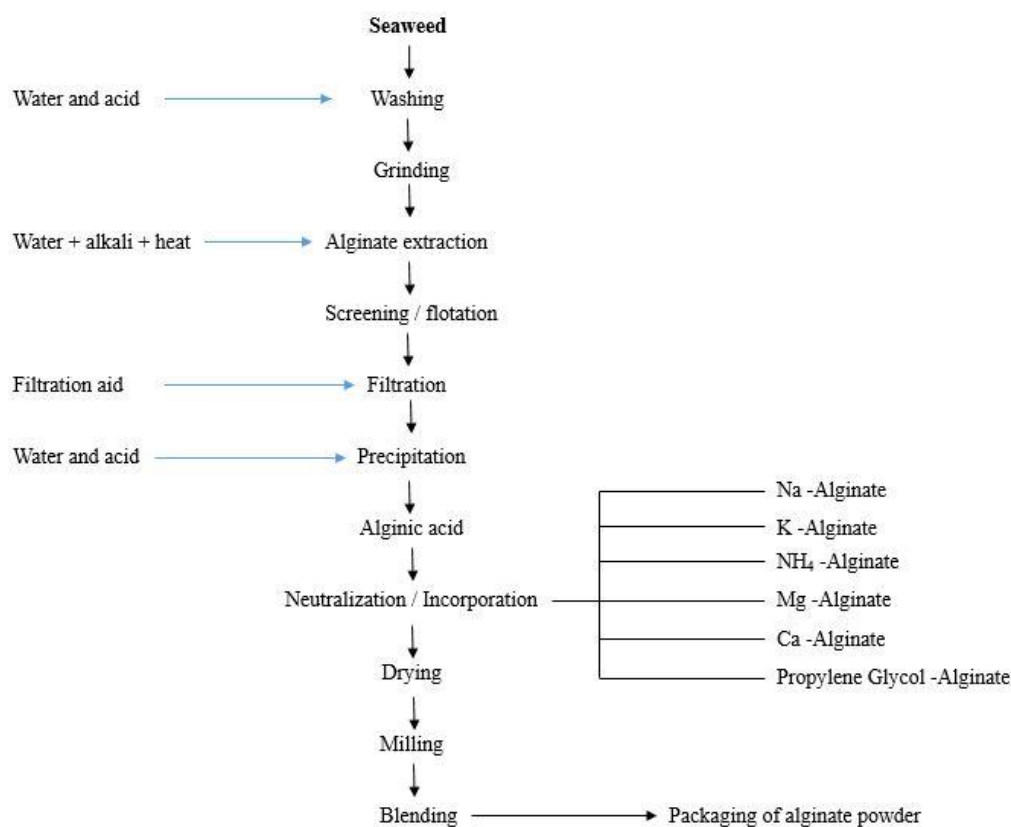


Figure 6: Flow diagram for the manufacture of different alginate powders. A simplified production process, modified after FMC (2003) and Sabra and Deckwer (1998).

There is however a great variety of production processes depending on the algal material used, and also to some extent the order of the different production steps. Sabra and Deckwer (1998)

also reported that commercial production of microbial alginate was under development. Alginates from bacteria are of special interest due to the possibilities of enabling manufacture of tailor made bacterial alginates for high value medical and biotechnological applications. However, as of 2013 farmed brown seaweed is still the only material used in commercial production of alginates (Hay et al. 2013).

2.3.2 Structure and physical properties

Alginate is a family of unbranched binary co-polymers of (1–4) linked β -D-mannuronic acid (M) and α -L-guluronic acid (G) of widely varying composition and sequential structure. The viscosity and gel-forming capacities of alginate are considered the most important characteristics in a commercial view. Block structure and chain length of the polymer largely affect these properties. Viscosity, however, is mainly a function of the molecular size as stated by Moe et al. (1995) cited by Sabra et al. (2001).

Alginate may be organized in three ways;

- (1) Hydropolymeric G-blocks (polyguluronate)
- (2) Hydropolymeric M-blocks (polymannuronate)
- (3) Heteropolymeric GM-blocks

These occur on the polymer chain in a randomly arranged G or M sequence, either as alternating MG or as short interacting G- and M-blocks, with interspersed individual M or G units (Sabra & Deckwer 1998), as shown in figure 7. The pKa-value of mannuronic and guluronic acid monomers were found to be 3.38 and 3.65, respectively (Sæther et al. 2008).

Alginates are water soluble hydrocolloids, and have the ability to make liquids viscous even in small concentrations (FMC 2003). When alginate is dissolved in water it gets the ability to form alginate gel if divalent- and/or polyvalent cations are added to the solution. Calcium ions are most often used, and these bind the G-blocks in the alginate together. The G-blocks are usually randomly placed in the alginate structure, however, when Ca^{2+} or other multivalent ions are present, the G-blocks will form a three-dimensional structure around the cations, thus forming rigid gels as shown in figure 7 (Kohn 1974; Sabra & Deckwer 1998; Sivertsen 1996). The rigid

gel structure is associated with how an eggbox (the guluronic acid) is wrapped around an egg (the cation, Ca^{2+}), thus this is known as “The egg-box model”.

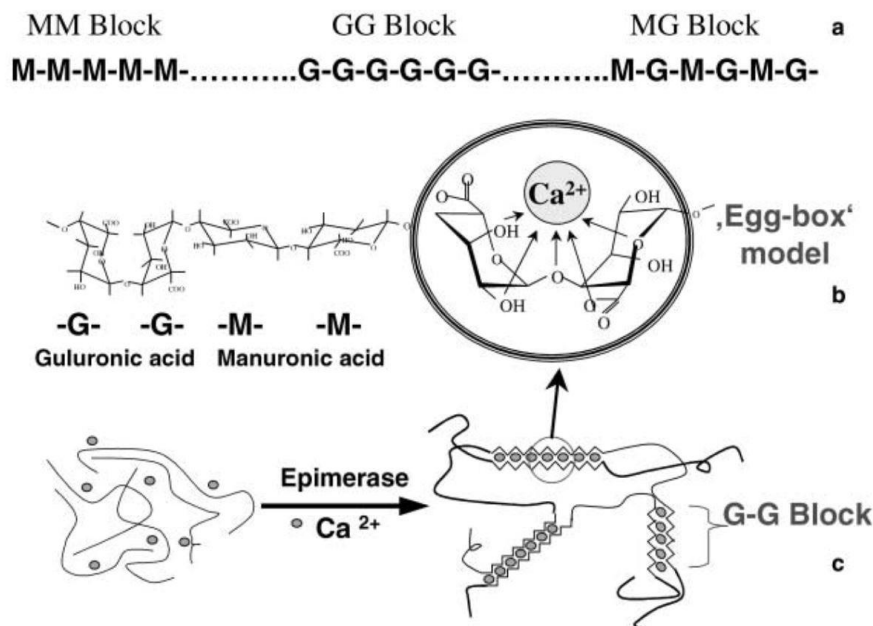
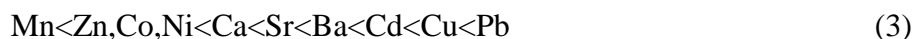


Figure 7: Alginate gel formation and egg-box model in the presence of calcium. Example; structure of *Azotobacter alginate*, (a) Block structure. (b, c) The calcium ion dependent epimerization process and the formation of the so called “egg-box model” (b), forming hard gel (Sabra et al. 2001)

Alginate has the ability to exchange and bind metallic cations. This is probably one of the most interesting properties of alginate with regard to water treatment processes. Monovalent cations in contact with dissolved alginate form soluble alginate-salts, while divalent and polyvalent cations forms a gel that precipitate cations out of solution. However, even after the gel formation has taken place the alginate can function as an ion exchanger (Sivertsen 1996). This is a result of different affinity for ions. Kohn (1974) reported that the higher the content of α -L-guluronic acid units in the alginate, the higher the selectivity in ion exchange processes. Moe et al. (1995), rendered and cited by Sabra and Deckwer (1998), indicated that the polymer affinity to ions could not only be dependent on nonspecific electrostatic binding, but some chelation caused by structural features in the G-blocks must contribute to ionic selectivity.

Martinsen (1989), cited by Sivertsen (1996), presented the alginate gel ion affinity sequence for divalent cations to increase in the order as follows:



For alkaline earth metals, Sabra and Deckwer (1998) presented that affinity of alginates increases in the following order:



The high selectivity between ions, even when they are as similar as the alkaline earth metals, support the assumption that there has to be other factors than just the mode of electrostatic binding in ion selectivity of alginate.

2.3.3 Alginate in water treatment processes

Alginate is of interest in water treatment processes due to its gelling abilities, and as previously discussed, its ability to exchange and bind cations. Several studies have been conducted on alginates' ability to remove metals from aqueous solutions (Qin et al. 2006; Sivertsen 1996; Williams et al. 1998). However, there is a limited amount of studies conducted on the field of alginate as an agent in turbidity removal. Çoruh (2005) conducted a study on alginate as a precipitating agent, in combination with calcium, as her master of science thesis in 2005. The results from this study was published by Devrimci et al. (2012). At initial turbidities of 150 NTU and 80 NTU calcium alginate proved to be a very effective coagulant causing turbidity removals generally over 98 %. The calcium concentration was then 80 mg/L for initial turbidity of 150 NTU, and most effective alginate dose of 2 mg/L was used. As for 80 NTU, calcium concentration was 120 mg/L and alginate dose of 0.4 mg/L proved most effective. Initial pH was 7.3 ± 0.1 , and 7.3 ± 0.2 after experiments were conducted, thus adding alginate did not alter pH under the given conditions.

2.4 Chitosan and alginate combined in water treatment

Oppositely charged polysaccharides in aqueous solutions interact spontaneously to form polyelectrolyte complexes (PECs) when they are mixed (Sæther et al. 2008). This opens for trials with the use of both chitosan and alginate in combination, when removing pollutants from water. Nadavala et al. (2009) reported that chitosan- calcium alginate blended biosorbent, in the

form of beads, is effective for the removal of the organic pollutants phenol and o-chlorophenol from an aqueous medium. Experiments have also been conducted with respect to removal of heavy metal ions from waste water with the use of sodium alginate and chitosan in combination (Qin et al. 2006). The study found that sodium alginate tends to form an insoluble gel structure, whilst chitosan forms a loose colloid structure, when placed in contact with heavy metal ions. When sodium alginate and chitosan are used in combination, the separation of the polymers containing the heavy metal ions from the solution is much better than when the sodium alginate and chitosan solutions are used separately. Results from the study further indicate that for monovalent metal ions, such as silver ions, it is difficult for either sodium alginate or chitosan solutions to precipitate the metal ions, however, when used in combination, there is a clear separation of the polymeric absorbent from the waste water. Hence, Qin et al. (2006) concluded that the combination of chitosan and alginate has the ability to successfully remove heavy metal ions from waste water.

2.5 Metal coagulants

Inorganic coagulants are the most common precipitating agents in water treatment processes as they are effective, cheap and easily available. Metal coagulants can mainly be divided into two categories; those based on aluminum (e.g. aluminium sulfate and aluminium chloride), and those based on iron (e.g. ferric sulfate, ferric chloride) (Bratby 1980).

The skepticism related to the extensive use of these coagulants, is unintentional discharge of large amounts of metal residues in treated water. Considering the impacts on human health, an example is that excess aluminium in drinking water is proven to contribute to early Alzheimer disease (Gupta et al. 2005). Excess iron in water from urban construction, discharged in to a river ecosystem, may lead to toxic effects along the food chain (Vuori 1995). In general, it is well known that different metal species can accumulate in nature. Acute exposure of high doses of different metals to aquatic organisms, can have a severe ecological impact.

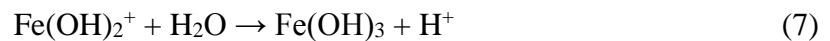
Chemical reactions

In order to understand why it is of common interest to find alternatives to the inorganic coagulants used in water treatment processes, one has to understand the basics of how they work. Inorganic coagulants are often delivered from the manufacturer as concentrated solutions of a

metal salt. When the salt is added to water, the Fe^{3+} or Al^{3+} reacts so strongly with the water that it is dissociated into hydrogen (H) and hydroxide (OH) ions, as shown in equation 5.



The newly formed iron hydroxide ion reacts with water in two stages as presented in equation 6 and 7.



All of the three reactions stated above are completed within 1 to 7 seconds (Gillberg et al. 2003). Now, it is important to emphasize that the reactions presented in equations 5 to 7 are simplified. In reality the ions also contain chemically bound water molecules. It is because of these bonded water molecules one gets an excessive sludge production with the use of inorganic chemicals – with respect to volume. Hence, extensive dewatering is needed.

Another aspect related to these reactions is that they use the alkalinity of water. An accidental release of these precipitation salts may affect e.g. the pH in the recipients, on which the magnitude is depending on general water chemistry parameters, e.g. alkalinity.

It is important to emphasize that the hydroxides produced in the reaction between the metal coagulant and water, will have a net negative or positive charge of which the outcome is highly dependent upon the pH of the medium in which the coagulant is added, and the amount of coagulant dose (Gillberg et al. 2003). The solubility of different metals is also pH dependent, as can be seen from figure 8 where iron is used as an example.

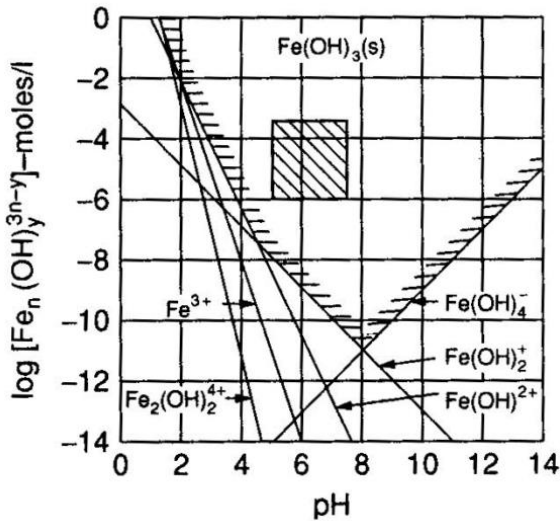


Figure 8: Solubility diagram for iron compounds at equilibrium. The shaded square rout indicated the area of which is common in conventional water treatment (Bratby 2006)

From the shaded square in figure 8, it seems that $\text{Fe}(\text{OH})_3$ (solid) is the specie that is effective in precipitating suspended colloids in conventional water treatment processes. Hence, at first glance one might suspect a bridging mechanism between the solid iron specie and the suspended colloid to be the main reason why the colloid precipitates out of solution. However, as stated by Bratby (1980), destabilization of colloids often occur much faster than the time it takes for the solution to reach equilibrium. Thus, the use of stability constants in order to identify which specie is effective during destabilization in water treatment processes may not be valid.

Due to the quick reactions related to the use of inorganic coagulants, it is especially important to ensure good rapid mixing of coagulant into the solution in question. This, so that each suspended particle will get the right amount of coagulant needed for destabilization. Also, one should not underestimate the effect of temperature on coagulation efficiency. Not only does the physical properties of water (such as viscosity and density) change at low temperatures, but there will also be a decrease in the rate of metal hydrolysis (equation 5 to 7) and rate of precipitation.

Furthermore, it is known that the optimum pH in which the coagulant in question is most effective, might change with temperature (Ammary 1995). Use of inorganic coagulants in cold waters often lead to a decrease in floc strength, thus the formation of smaller flocs is prominent.

3 Experimental work

All of the experimental work and analysis were conducted at the Norwegian University of Life Sciences, by means of different laboratory facilities available at the Department of Environmental Sciences.

3.1 Drilling fluid

Drilling fluid with high turbidity (>6000 NTU) was easily assessable from NPRA's road project (E18-Østfold). The project is divided into nine allotments (figure 2), and allotment number seven, Knapstad- Retvet is the one relevant for this study. Sample fluid was collected from this site due to its availability, as drilling was ongoing through out the period that this thesis is written.

An important note is that the sampled fluid had not previously been through a cleaning process at the onsite water treatment plant, thus it had not been reused at the construction site prior to sampling. Drilling fluid used for the coagulation experiments were collected from a steel dumpster (figure 9), that was a part of the pre-water treatment plan at the construction site. A total of approximately 140 liters of drilling fluid was collected in 25- and 5-liter polypropylene water containers (figure 10), and transported to the laboratory. All containers were stored dark in a refrigerated storage at approximately $4\text{ }^{\circ}\text{C}$ until experiments was conducted.



Figure 9: Steel dumpster of which the drilling fluid was collected. Person for scale (Photo: Lene Sørli Heier 2014).



Figure 10: 25- and 5liter polypropylene water containers filled with drilling fluid (Photo: Lene Sørli Heier 2014).

Drilling fluid characteristics

With the use of semi quantitative analysis of the mineralogy of sensitive clays, Syversen (2013) found that for eastern Norway clays is comprised of approximately 75 % illite and 25 % chlorite. This was also the case for analyzed clay samples in this project.

Particle size analysis of drilling fluid from three randomly chosen water containers were conducted as described in sub-chapter 3.5.5. The degree of sorting (S_o) for the suspended sediments in a randomly chosen container with drilling fluid, were estimated by the formula given in equation 8 (Jørgensen et al. 1997).

$$S_o = \log(Q_{75}/Q_{25}) \quad (8)$$

Where Q_{75} is the log₁₀ particle size value at 75 % volume, and Q_{25} is the log₁₀ size at 25 % volume. $S_o < 0.5$ indicates well sorted material.

Analysis of suspended dry matter and loss of ignition were conducted on water from five randomly chosen containers with two parallels form each container, and three blank samples, as described in chapter 3.5.4.

Determination of turbidity and suspended solids

The high turbidity values of the drilling fluid used in this experiment could not be measured without being diluted. Turbidity samples were collected 15 cm below liquid surface, with the use of a 5000 µl electronic pipette from Biohit, after first shaking the water container. The first turbidity measurement was conducted approximately 10 minutes after shaking, and diluted to a factor of 10 (v/v). For the next hour, turbidity measurements were conducted every five minutes with dilution factors ranging from 10 to 60. After 10 minutes, measurements were resumed and conducted every five minutes for one more hour, with the same dilution factors as for the previous hour. The next measurement was taken four hours after shaking, and another six measurements were conducted within the following 24 hours after shaking. Two subsequent measurements were conducted tree and nine days after shaking.

Preparation of sample solutions

Water containers were taken out of refrigerated storage two days in advance of each experiment, in order to ensure that the water held room temperature (approximately 21 ± 2 °C). Once out of the refrigerated storage, the containers were stored out of direct sunlight and at room temperature.

Before use of drilling fluid in any experiment, the containers were shaken with a horizontal shaker, on which shaking intensity are defined by the number of stokes per minute (expressed as mot/min). The containers were first shaken at 70 mot/min for 3 hours and then for 20 min at 200 mot/min. Containers were also shaken at 100 mot/min for approximately 30 minutes between each set of experiments.

Batches of approximately 20 liter were diluted with de-ionized water to desired turbidity, 4000 NTU, 3000 NTU, 2000 NTU and 1000 NTU respectively, from the original sample solution (>6000 NTU) when needed.

3.2 Precipitating agents

3.2.1 Chitosans

Two kind of chitosan with different physical and chemical characteristics was provided by Primex (Iceland) for the experimental work of this thesis. The stated shelf life for chitosan is 3 years, although it is a very stable and long lasting polymer (Sigridur Vigfusdottir, Sales manager, Primex). The following two subsections will first present the physical and chemical composition of the different chitosans. Instructions on how to prepare the chitosans for use in the experimental work are described in a third subsection. Both chitosans was prepared following the same procedure.

Primex ChitoClear High Quality Grade 400 (CC43020)

ChitoClear High Quality Grade 400, product code 43020, from Primex (Iceland) is a chitosan with a 95% degree of deacetylation, viscosity 563 mPa*s (1% solution in 1% acetic acid measured on a Brookfield DV-II+ viscometer, 25°C, appropriate spindle at 30 rpm), and 95% of the powder is sieved through 18 mesh (1 mm sieve opening). Raw material was fresh north

Atlantic shrimp shells, *Pandalus borealis*. Other details of interest are listed in table 2, where both the general product specification and a detailed analysis of the batch is presented. Complete product data sheet and certificate of analysis can be found in appendix A. Bought in large quantities (>1000kg) this product has an estimated cost of approximately 110 EUR per kilogram (Primex, ChitoClear – price estimate upon request).

Table 2: ChitoClear High Quality Grade 400, product code CC43020. A summary of physical and chemical properties both from the general product specification, and from the certificate of analysis.

Characteristics	General product specification	Certificate of analysis
Degree of deacetylation	≥95 %	95 %
Solubility (in 1% acetic acid)	≥99.8 %	99.9 %
Viscosity	200-600 mPa*s	563 mPa*s
Protein (as total amino acids)	≤0,1 %	≤0,1 %
Micromilled powder	95 % through 18, 50 or 100 mesh sieve	95 % through 18 mesh sieve
Raw material	<i>Pandalus borealis</i>	<i>Pandalus borealis</i>

The chitosan powder has an off-white color and constitutes of small flakes. It takes approximately 20 minutes to dissolve 0.10 g in a solution of 0.1 M acetic acid. Dissolution rate depends on acid concentration. When dissolved, ChitoClear High Quality Grade 400 is visibly more viscous than the technical grade chitosan before dilution with distilled water.

Primex ChitoClear Technical Grade (CC40500)

ChitoClear Technical Grade, product code 40500, from Primex (Iceland) is a chitosan with a 88.8% degree of deacetylation, viscosity 45 mPa*s (1% solution in 1% acetic acid measured on a Brookfield DV-II+ viscometer, 25 °C, appropriate spindle at 30 rpm). Raw material was fresh north Atlantic shrimp shells, *Pandalus borealis*. Other details of interest are listed in table 3, where both the general product specification and a detailed analysis of the batch is presented. Complete product data sheet and certificate of analysis can be found in appendix B. Bought in large quantities (>1000kg) this product has an estimated cost of approximately 25 EUR per kilogram (Primex, ChitoClear – price estimate upon request).

Table 3: ChitoClear Technical Grade, product code 40500. A summary of physical and chemical properties both from the general product specification, and from the certificate of analysis.

Characteristics	General product specification	Certificate of analysis
Degree of deacetylation	75-90 %	88.8 %
Solubility (in 1% acetic acid)	>98 %	99.6 %
Viscosity	20-200 mPa*s	45 mPa*s
Protein (as total amino acids)	N/A	N/A
Particle size	Small flakes, approx 1mm	Small flakes, approx 1mm
Raw material	<i>Pandalus borealis</i>	<i>Pandalus borealis</i>

The chitosan powder has an off-white color and constitutes of a very fine powder. It takes approximately 5 minutes to dissolve 0.10 g in a solution of 0.1 M acetic acid.

Preparation of chitosan solutions

A portion of 0.10 g of chitosan was weighed into a glass beaker (Startorius GmbH Göttingen). 10 mL of deionized water was then added to the glass beaker and mixed with the chitosan powder by means of a magnetic stirrer (IKA Big Squid) at 100 rpm. 575 μ l of 10 % 17.4 M acetic acid was added to the mixture in order to obtain an acid concentration of 0.1 M during protonation. The mixing speed was then increased to 400 rpm. After about 30 seconds of mixing, the solution was put aside to dissolve. When the chitosan was visibly completely dissolved (approximately 20minutes, depending on type of chitosan), the solution was diluted with deionized water to the 100 mL mark to obtain a solution containing 1 mg chitosan per mL of solution. When lower concentrations were needed, the solution was diluted the same way as previously described in a larger glass beaker to obtain the desired concentration. Depending on the required amount of chitosan solution, chitosan was transferred to the jar-test experiments by electronic pipettes (500-5000 μ l or 50-1000 μ l; Biohit). A new batch of chitosan solution was prepared fresh every morning before the experiments were conducted.

3.2.2 Alginates

Two kinds of alginates, with different physical and chemical characteristics, was provided by FMC BioPolymer. Both products costs approximately 30EUR per kilogram, when bought in large quantities (>1000kg) (FMC Biopolymer – price estimate upon request). The following two

subchapters will present the physical and chemical composition of the different alginates. Instructions on how to prepare the alginates for use in the experimental work are described in a third subchapter. Both alginate solutions were prepared following same procedure.

Manugel GMB sodium alginate (GMB)

Manugel GMB alginate from FMC BioPolymer (labeled Sodium alginate (E401) in EU) (appendix C), has a viscosity of 119 mPa*s, 100 % of the alginate powder is sieved through 335 micron (0,335 mm), and 99.8 % through 250 micron (0,250 mm). A comparison between some parameters of interest found in the product specification and the certificate of analysis are given in table 4. The M:G are about 40:60 in ratio, stated by Sheena M Loy – Sales Manager FMC Cooperation (exact M:G ratio are confidential to FMC).

Table 4: Manugel GMB sodium alginate. A summary of physical and chemical properties both from the general product specification, and from the certificate of analysis.

Characteristics	General product specification	Certificate of analysis
Viscosity	110-270 mPa*s	119 mPa*s
pH (1 % at 20 °C)	5.0-7.5	7.1
Particle size	>98 % through 335micron, >80 % through 250 micron	100 % through 335 micron, 99.8 % through 250 micron

The alginate powder is a white to yellowish fine powder, slowly soluble in water. Alginate powder was stored in the original package (white plastic container), at room temperature and out of direct sun light.

Manugel GHB sodium alginate (GHB)

Manugel GHB alginate from FMC BioPolymer (labeled Sodium alginate (E401) in EU) (appendix D), has a viscosity of 61 mPa*s, 100 % of the alginate powder is sieved through 335 micron (0,335 mm), and 99.8 % through 250 micron (0,250 mm). As for Manugel GMB, it is given that M:G ratio are about 40:60, stated by Sheena M Loy – Sales Manager FMC Cooperation (exact M:G ratio are confidential to FMC). Table 5 compares some interesting alginate characteristics given in the product specification and the certificate of analysis.

Table 5: Manugel GHB sodium alginate. A summary of physical and chemical properties both from the general product specification, and from the certificate of analysis.

Characteristics	General product specification	Certificate of analysis
Viscosity	50-100 mPa*s	61 mPa*s
pH (1% at 20°C)	5.0-7.5	6.9
Particle size	>98 % through 335 micron, >80 % through 250 micron	100 % through 335 micron, 99.8 % through 250 micron

The alginate powder was a white to yellowish fine powder, slowly soluble in water. However, Manugel GHB has proven slightly easier to dissolve than Manugel GMB. Alginate powder was stored in the original package (white plastic container), at room temperature and out of direct sun light.

Preparation of alginate solutions

A portion of 0,50 g of alginate powder was weighed into a 50 mL polypropylene tube (Sarstedt, Germany). The tube was then filled with distilled water to the 25 mL mark, and thoroughly shaken in order to reduce the formation of hydrophobic alginate lumps, thus reducing dissolution time. The solution was then diluted to the 50 mL mark to obtain an alginate concentration of 0,1 g/mL solution, and put aside until the alginate powder where visibly completely dissolved. If one manage to mix the alginate powder in such a manner that it does not form very large (>3 mm in diameter) lumps, nor stick to the tube wall, the alginate is visibly dissolved within approximately 15 minutes. This however is quite challenging, thus more often than not, the alginate stuck to the tube wall and it may take up to three hours for it to be completely dissolved. A new batch of alginate solution was prepared fresh each morning before experiments were conducted.

3.2.3 Ferric chloride sulfate (PIX-318)

PIX-318, an aqueous solution of ferric chloride sulfate (FeClSO_4), was obtained from Kemira Chemicals AS (appendix E). It is a solution of active trivalent iron compounds, has a dark brown color, and is slightly corrosive. Kemira PIX-318 is approved as precipitating agent in drinking water purification by the Norwegian Food Safety Authority (Mattilsynet 2014).

. Bought in large quantities (>1000 L) the price for PIX-318 is estimated to 0.8 EUR per liter (Kemira Chemicals – price estimate upon request).

PIX-318 was stored at room temperature, in a sealed glass bottle and out of direct sunlight. When lower concentrations of PIX-318 were needed, a portion was transferred to a 50 mL polypropylene tube (Sarstedt, Germany) and diluted with de-ionized water to the desired concentration.

3.3 Jar-test experiment

A series of 12 glass beakers and standardized jar-test apparatuses from Kemira chemicals (Kemira AB Flocculator 2000) were used in the experiments, as shown in figure 11. The jar-test apparatuses stirs samples with a uniform power input, allowing the same mixing conditions for each jar. A volume of 0.8 liter of drilling fluid with desired turbidity were poured into glass beakers and the equipment was assembled and turned on rapid mixing at 200 rpm.

Desired volumes of either chitosan, PIX318, or alginate was added to an in-motion sample and mixing at 200 rpm for 3 minutes was initialized. This was followed by slow stirring at 40 rpm for 15 minutes, before the samples were allowed to settle for 15 and a total of 60 minutes (figure 12). After 15 minutes settling time turbidity measurements were conducted. After one hour, another set of turbidity measurements were taken, followed by measurements of pH and conductivity. Samples for measurements were taken with great care in order not to disturb the settled flocs. Detailed instrument information is presented in chapter 3.5.



Figure 11: Setup of jar-test apparatuses during the experimental work of this thesis.

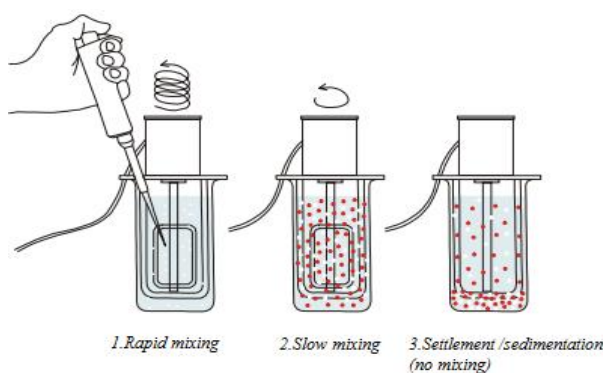


Figure 12: Schematically illustration of jar-test apparatuses. Modified after Konieczny et al. (2005).

3.4 Investigated variables

A series of variables, including turbidity, pH and ionic conductivity were determined throughout the jar-test experiments. Furthermore, initial turbidity, chitosan dose, alginate dose and required dose of PIX-318 along with settling time were tested for their effect on the process. Initial pH effect on dose efficiency were also investigated.

3.4.1 Residual turbidity as a function of dose

Turbidity is the most important variable in jar-test experiments. Initial turbidity in all kinds of water treatment processes usually varies throughout the day. In water treatment facilities on construction sites, initial turbidity depends upon activities on-site and water consumption by the machinery in construction processes.

Because initial turbidity can vary a great deal, it would be interesting to consider turbidities lower than that of the undiluted drilling fluid. Therefore, initial turbidities of undiluted drilling fluid and 4000, 3000, 2000 and 1000 NTU were investigated with respect to the performance of chitosan as a precipitation agent.

The performance of alginate as a precipitation agent were investigated using undiluted drilling fluid, and the performance of PIX-318 were investigated at 2000NTU.

30 mL samples for turbidity measure were taken approximately 3 cm below liquid surface, after both 15 minutes and one hour settling time.

Dose of precipitating agent

At the beginning of this study, PIX-318 was the coagulant used at the water treatment facilities at Knappstad construction site. Therefore, it was decided to use this as a reference when evaluating the possibilities of changing to organic polymers in the water treatment process. Preliminary analysis of PIX-318 dose at undiluted drilling fluid were conducted, however, it was chosen to proceed with investigations at turbidities 2000 NTU due to the uncertainty of how much drilling fluid that was needed for the other experiments.

After a preliminary analysis of how the different precipitating agents performed at high and low turbidities, chitosan was chosen as the polymer that would be extensively investigated. PIX-318 was investigated at turbidity 2000 NTU and at different pH for reference only. Alginate was tested at turbidity 6000 NTU as this seemed to work, based on the preliminary analysis. An overview of the different doses of each precipitating agent at altered initial turbidity and pH are shown in table 6.

Table 6: Dose of precipitating agents (mg/L) at different turbidities and pH, investigated in main experiments. * Dosed 0.65 mg/L with high quality grade chitosan, and dosed 0.125 mg/L with technical grade chitosan.

Precipitating agent	6000 NTU pH 7.5	4000 NTU pH 7.5	3000 NTU pH 7.5	2000 NTU pH 7.5	2000 NTU pH 4.5	2000 NTU pH 9.5	1000 NTU pH 7.5
Chitosan (n=3)	4, 3, 2.5, 2, 1, 0	1, 0.75, 0.5, 0.4, 0.25, 0	0.75, 0.5, 0.4, 0.25, 0.1, 0	0.75, 0.5, 0.35, 0.25, 0.65/0.125*, 0	0.25, 0.1, 0.05, 0	5, 3, 1, 0	0.3, 0.25, 0.2, 0.15, 0.1, 0
Alginate (n=3)	6.25, 4.375, 3.125, 1.875						
PIX-318 (n=3)				0.03, 0.015, 0.0225	0.015, 0.0612, 0.0075, 0.0131	0.03, 0.04, 0.16	

30 mL samples for turbidity measure were taken approximately 3 cm below the liquid surface, after both 15 minutes and one hour settling time.

3.4.2 Effect of settling time

The retention time of water through a water treatment facility can vary a great deal between different days, and activities at construction sites. Therefore the effect of residual turbidity as a function of prolonged setting time, from 15 minutes to one hour, were assessed for all experiments.

3.4.3 System pH

System pH can affect the efficiency of the precipitating agent, as different agents has their best coagulation efficiency in different intervals of pH. Therefore, a small study on the effect of initial pH on coagulation efficiency was conducted at initial turbidity 2000 NTU.

The pH of the drilling fluid was stable at 7.60 ± 0.03 (n=101) for all initial turbidities.

For turbidity 2000 NTU, chitosans and PIX-318 was tested with initial pH 4.56 ± 0.01 (n=26) and 9.58 ± 0.02 (n=25), for three different doses per precipitating agent.

pH were adjusted to 4.5 by adding 687.5 μL 10% 17.4 M acetic acid per liter diluted drilling fluid (2000 NTU), and 1250 μL 0.1 M sodiumhydroxide for pH adjustment to 9.6.

Change in system pH was measured after approximately one hour settling time.

3.4.4 Change in ionic conductivity

As the original drilling fluid was diluted with deionized water, the initial ionic conductivity is changed. Thus, measurements of ionic conductivity were conducted before and, approximately one hour, after addition of precipitating agent to the sample fluid in question.

3.5 Analytical techniques

3.5.1 Turbidity

A laboratory turbidimeter model 2100AN IS (Hath company, Loveland, CO, USA) was calibrated with standards 4000, 1000, 200 and 20 FNU from stem solution StablCal Turbidity Standard 4000 FNU (Lot 0314 from HACH) in accordance with the Instrument manual.

3.5.2 pH

pH- meter (PHM210 Standard pH meter, Copenhagen) with Ross-Sure flow combination pH electrode (Termo Scientific) was calibrated against three standard buffer solutions; 4 and 7 (CertiPUR, Germany), and 10 (LLG Labware, GmbH, Germany) respectively. The pH-meter was calibrated with appropriate buffers each morning before use, and during the day if experiments with different initial pH were tested.

3.5.3 Conductivity

Measurements of conductivity were conducted before and after the jar-tests using a CDM80 conductivity meter from Radiometer Copenhagen. Temperature was set to 20 °C and cell constant to 1 cm⁻¹, calibrated against a 0.01M KCl solution with an electrical conductivity of 1413µS/cm in accordance to Norwegian Standard NS-ISO 7888 (NSF 1993). Results are expressed in micro simens per centimeter (µS/cm).

3.5.4 Suspended solids

Suspended solids were determined according to Norwegian Standard NS-EN 872 (NSF 1996). Pre-known volumes of water were filtered through a 47 mm glass fiber filter (GF/c, Whatman). The filters were then dried at 105 °C overnight. Total suspended solids were quantified by weighing the mass of the residue retained on the filter and subtracting the weight of the empty filter. Total organic solids were quantified after burning the filters at 550 °C for 1 hour and subtracting the weight of the residue retained after burning, from the weight of the residue on the dried filter. Results are expressed as total suspended solids and organic suspended solids (mg) per liter.

3.5.5 Particle size

Particles size was determined with the use of a laser diffraction particle size analyzer (LS13 320, Beckman Coulter). Portions of 0.8 liter drilling fluid were collected in 1 L glass beakers from three different containers that had been shaken as if they were to be used in an experiment as described in chapter 3.1 – section *Preparation of sample solutions*. One container was 1/3 full, one 1/2 full and the last one full when samples were taken. This was done in order to investigate/confirm that the particle size distribution does not vary much between containers, nor between each withdrawal of original sample to dilution.

The samples were left over the weekend, allowing some settlement of the suspended solids. Remixing was then conducted with a magnetic stirring rod on a magnetic stirrer, and a 10 mL sample was transferred either directly to the particle size analyzer or via a dispergation-process. The sample was then analyzed with three replicate measurements. Polarization Intensity

Differential Scattering (PIDS) was used in the range 0.04-0.4 μm . The sample was analyzed in the range 0.04-2000 μm .

Dispergation was conducted by adding 20 ml Na-pyrophosphate to the 10 ml sample and put in an ultrasound bath for five minutes before analysis.

The particle size analysis, and fitting of the optical model, was conducted by Magdalena Rygalska, scientific assistant at NMBU.

3.5.6 Cation exchange capacity (CEC)

Cationic exchange capacity of the suspended solids in solution was determined in accordance with the ammonium acetate method, first described by Schollenberger and Simon (1945), with some practical modifications (i.e. less than 3 g solids were used in this experiment).

Procedure

A portion of 20 mL of drilling fluid was filtered through a 47 mm glass fiber filter (GF/f, Whatman) with the use of a vacuum pump. This was repeated nine times, in order to retain sufficient mass from the suspension. The residue on the filters was then scraped off with a scraper of stainless steel into a 100 mL Erlenmeyer flask with best efforts not to get any filter mass into the sample. Approximately 1.05 g of solids was obtained from the filters. A portion of 25 ml of 1 M $\text{CH}_3\text{COONH}_4$ - pH 7.00, was added to the flask. It was carefully shaken, and the suspension was left over night.

Blue ribbon filters was washed with 1 M $\text{CH}_3\text{COONH}_4$. Funnels with the washed filter paper was placed above the 250 mL volumetric flask. The solution in the 100 mL Erlenmeyer flask was again shaken and the suspension was transferred to the funnel. The flask was rinsed several times with the previously described ammonium acetate solution, which was poured into the funnel. Care was taken so that the suspension on the blue ribbon filter was kept soaked in ammonium acetate throughout a whole working day, or until a total of 250 mL ammonium acetate was filtered through. If 250 mL had not passed through the filter within the day, the filtration process was determined and ammonium acetate was filled directly into the volumetric

flask up to the 250 mL mark. The flask was carefully shaken, that is slowly turned 180° approximately 30 times.

A portion of 7 mL was then transferred to a 15 mL polypropylene tube (Sarstedt) for determination of Mg, Ca, Na, K (and Mn if required) by means of ICP-OES (PerkinElmer, Optima 5300 DV). Analyzed by Solfrid Lohne, senior engineer, NMBU.

To determine H^+ , 20 ml of the extracted solution was transferred with pipette into a plastic cup with a magnetic stirring bar. The cup was then placed on a magnetic stirrer at 350 rpm, and pH-measurements were conducted. If $pH > 7.00$, there is no need for titration. If $pH < 7.00$, titration should be conducted with 0.05 M NaOH solution to $pH 7.00 \pm 0.02$. The mass of NaOH used for titration should be recorded, and transformed into volume assuming $\rho_{NaOH} \approx 1.00 \text{ g/cm}^3$. The pH-meter was calibrated against two standard buffer solutions; 4 and 7 (CertiPUR, Germany). The ammonium acetate solution was adjusted to pH 7.00 with either ammonium acid or acetic acid.

3.5.7 Determination of trace elements and anions (ICP-MS and IC)

A portion of 50 mL of drilling fluid were collected in a 50 mL polypropylene tube (Sarstedt, Germany) for each initial turbidity before adding any precipitating agent. For all turbidities, chitosan (High Quality Grade) doses were added to achieve a residual turbidity of approximately 500 NTU, and a new 50 mL water sample was collected after 15 minutes settling time. The same were done for PIX-318 at turbidities 2000 NTU and >6000 NTU, and for alginate (GHB) at >6000 NTU. See flowcharts presented in subchapter 3.7 for more details.

Preparation of unfiltered water samples

The 50mL tubes with treated drilling fluid were thoroughly shaken, and 9.000-10.000 grams of sample water was weighed into teflon tubes and dried at 60°C for one week. 1.5 mL of HNO_3 were added to the dried samples, and decomposed by microwave technique at a maximum temperature of 260 °C for 25 minutes using Milestone UltraCLAVE®. The samples were then quantitatively transferred into a 15 mL polypropylene tubes (Sarstedt, Germany), and diluted with Milli-Q water to the 15 mL mark.

For three untreated water samples with turbidity >6000 NTU, the same procedure was conducted, however 0.3 mL of HF and 1.5 mL HNO₃ were used as solubilizing agent.

Preparation of filtered water samples

The 50 mL tubes with unfiltered samples of treated drilling fluid were put aside, and the suspended solids allowed settling for one week. A portion of 15 mL of each sample were then filtered through a 0.45 µm filter (RC Syringe filter, LLG Labware), whereas the first 5 mL were used to flush the filter, and the remaining 10 mL were transferred into a 15 mL polypropylene tube (Sarstedt, Germany), and 1 mL of HNO₃ was added to each sample.

Preparation of sludge samples

For sludge samples, high quality grade chitosan, GHB alginate and PIX-318 were added to initial turbidity >6000 NTU with a dose achieving residual turbidity of approximately 500 NTU. After 15 minutes settling time, the water was poured out of the glass beaker with great care, so that the settled sludge would stay in the jar. The same was done with a sample where no precipitating agent was added, except this was allowed to settle for one day. The sludge was then poured into five different ceramic crucibles and dried at 105 °C overnight.

The dried sludge was then crushed, and 0.200-0.300 grams of each batch were weighed into teflon tubes and 5 mL of HNO₃ were added to each sample. They were decomposed by microwave technique at a maximum temperature of 260 °C for 25 minutes using Milestone UltraCLAVE®.

Three samples from the sludge with no precipitating agent added, was prepared the same way as previously described, except that 1 mL of HF and 5 mL HNO₃ were used as extractant. The samples were diluted to 50 mL, using Milli-Q water, before analysis.

Parameters determined

Both untreated and treated drilling fluid, as well as residual sludge was analyzed for the following anions and elements:

Drilling fluid – unfiltered and filtered:

Sulfate, chloride, Fe, Mn, Al, Ca, Cd, Cr, Cu, Ni, Pb, Zn, As, Sb and P

Sludge:

Fe, Mn, Al, Ca, Cd, Cr, Cu, Ni, Pb, Zn, As, Sb and P

Trace metals were determined simultaneously with the use of inductively coupled plasma mass spectrometry (ICP-MS) from Agilent Technologies 8800 ICP-MS Triple Quad (Harris 2010).

Anions were determined with ion chromatography (IC), an analytical method based on each ions affinity to the ion exchanger, and thus allows for estimation of ions in solution (Harris 2010).

3.6 Statistical analysis

One way ANNOVA are used to decide p-values for comparison in this thesis. The model predicts one variable (usually called the dependent or response variable) from one or more other variables (usually called independent, predictor, or explanatory variables). In order to approve statistical significant differences, one of the following has to be achieved

$p < 0.05$ = significance with 95% certainty

$p < 0.01$ = significance with 99% certainty

$p < 0.001$ = significance with 99.9% certainty

Significance is for example reported as ($p < 0.01$).

The model are fitted to the different datasets by Anne-Grethe Kolnes, first consultant at institute of environmental sciences, NMBU. Statistical Analysis System (SAS) were used.

3.7 Cleaning of glassware and jar-test equipment

Water from the municipality water work is purified and de-ionized. The process used is reversed osmosis, ion exchange filters, and UV-light (Merk Millipore). De-ionized water was used for sample dilution, cleaning of glass beakers and jar-test equipment between each set of experiments, and for preparation of precipitating agents and different reagents used.

Final cleaning were done by rinsing with deionized water, followed washing with a dilute solution of nitric acid (HNO_3).

3.8 Flowchart for the experimental work

For a better overview of this study flowcharts are conducted separately for each precipitating agent. Flowchart for chitosan is presented in figure 13, alginate and PIX-318 are presented in figure 14.

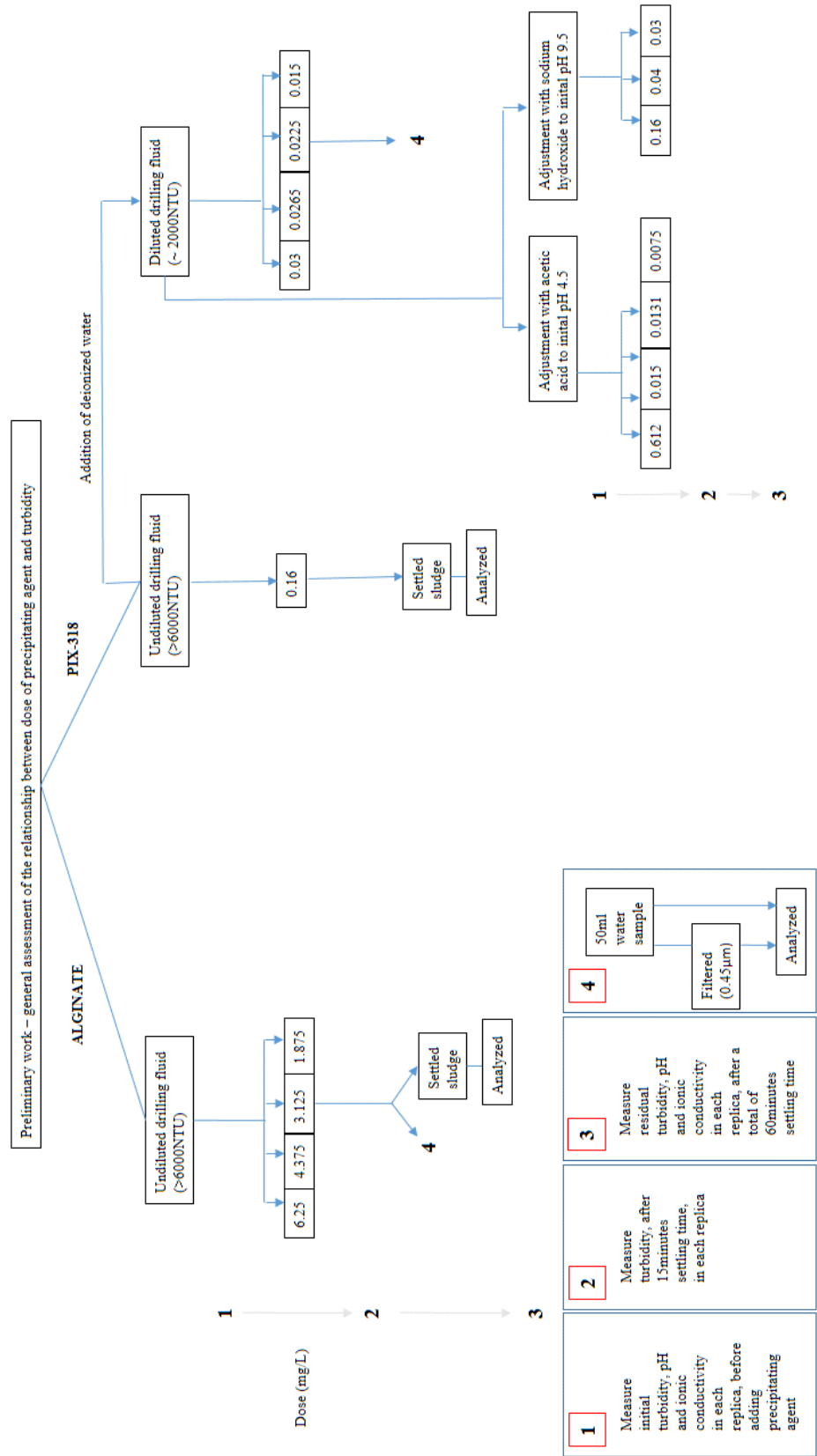


Figure 14: Flowchart for the experimental work with alginate and PIX-318, n=3.

4 Results

Table 7 presents results from samples treated with the same stirring regime through the jar tests, which have been used throughout all of the experiments, however with no added precipitating agent. These results are hereafter referred to as blank sample(s). The residual turbidities presented are used when calculating turbidity removal efficiency for all precipitating agents. However, the turbidity removal efficiency by the mode of sedimentation is calculated as described in equation 1.

Table 7: Summary of measured initial and residual turbidities in samples of drilling fluid where no precipitating agent were added (n=4). Also referred to as blank sample, or turbidity of blank sample (T_b), at pH 7.6. Turbidity removal efficiency indicates turbidity removal by the mode of sedimentation after 15 minutes and one hour settling time, calculated from initial turbidity, T_i (using mean values).

Initial turbidity referred to as	Measured initial turbidity (T_i) (NTU)	Residual turbidity (15minutes) (NTU) - T_{b15}	Turbidity removal efficiency (%) after 15 minutes	Residual turbidity (1hour) (NTU) - T_{b60}	Turbidity removal (%) after 1hour
1000 NTU	1020 ± 12	904 ± 6	11	730 ± 8	28
2000 NTU	1900 ± 137	1620 ± 27	18	1510 ± 18	23
3000 NTU	3030 ± 27	2740 ± 49	10	2610 ± 25	14
4000 NTU	4110 ± 83	3620 ± 36	12	3000 ± 61	27
>6000 NTU	>6000	>6000	0	>6000	0

The following subchapters will first present findings related to drilling fluid characteristics, followed by some general results from the preliminary experiments, and thereafter results will be divided in subchapter after type of precipitating agent.

4.1 Drilling fluid characteristics

Particle size analysis of three randomly chosen water containers showed that approximately 80 % of the suspended load were colloids (<10 μm), of which approximately 37 % were clay (<2 μm), as shown in figure 15. There was no detection of particles in the range 100- 2000 μm . The

occurrence of colloidal aggregates can be disregarded as the difference between the disperaged sample and those without were very low.

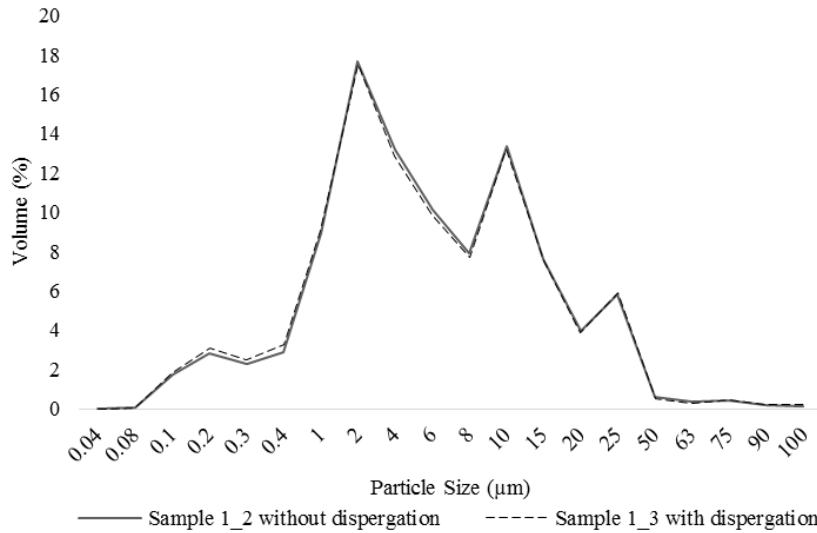


Figure 15: Particle size distribution, both with and without dispergation, of suspended solids before treatment. Average values are plotted, with $n=3$.

Furthermore the shape of the particle size distribution curve, figure 16, is typical for sorted material. The degree of sorting (S_o), equation 8, for the suspended sediments in a randomly chosen container with drilling fluid, can from figure 16 be estimated to 0.65.

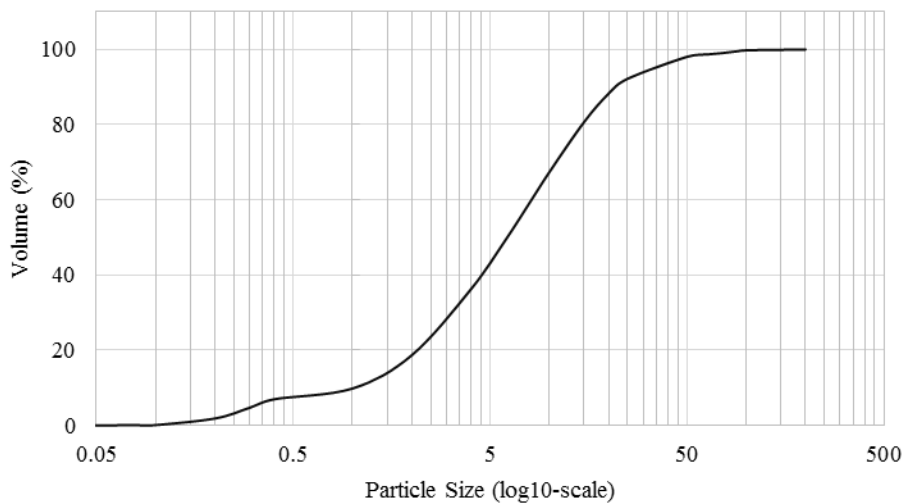


Figure 16: Particle size distribution curve of sample 1_2 without dispergation. Cumulative volume (%) of particle size (μm) presented in a \log_{10} scale

The analysis showed that total suspended dry matter was approximately 9 ± 1.7 g/L, and of this the organic matter was estimated to be 0.36 ± 0.06 g/L – which equals to a total loss of ignition of 4 ± 1 %, however when correcting for clay content the loss of ignition is 3 ± 1 %.

Furthermore, the cation exchange capacity of the colloids in suspension were found to be 39.6 cmol⁺/kg, which is common for chlorite (vanLoon & Duffy 2011).

Determination of turbidity and suspended solids

The result presented in figure 17 show decrease in turbidity 15 cm below liquid surface as a function of time. There seems to be a stable interval between 30 and 120 minutes, where the turbidity can be defined as >6000 NTU.

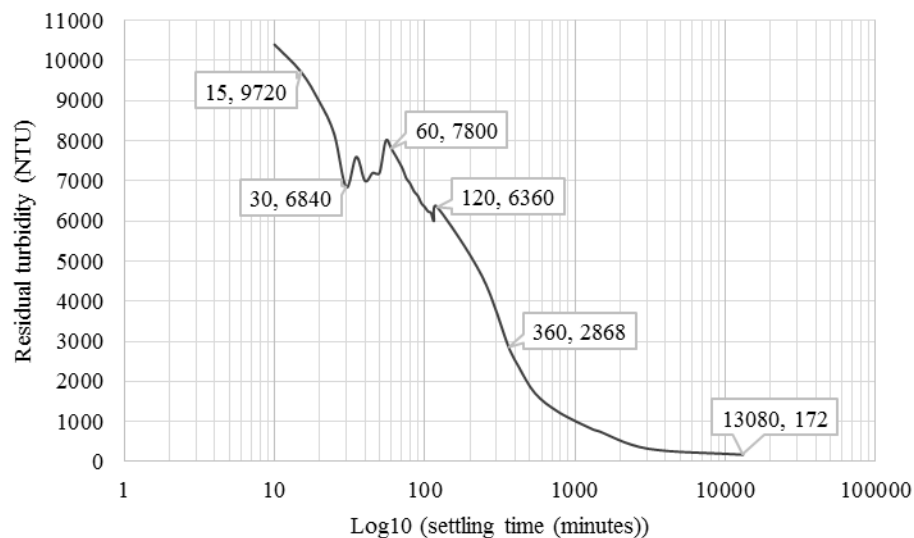


Figure 17: The decrease in turbidity (NTU) as a function of time (minutes) in a 5-liter water container with undiluted drilling fluid. Samples are collected 15cm below the liquid surface with a 5000 μ l pipette from Biotit. Data callouts presents [minutes, turbidity]. n=1

Measurements of turbidity and suspended solids (SS) are plotted in figure 18. Theoretical load of SS (y) at given turbidity (x) can be estimated by using the equation displayed on the chart. R² shows that 99% of the plotted data can be explained by the exponential equation within the turbidity range 1000 – 8000 NTU.

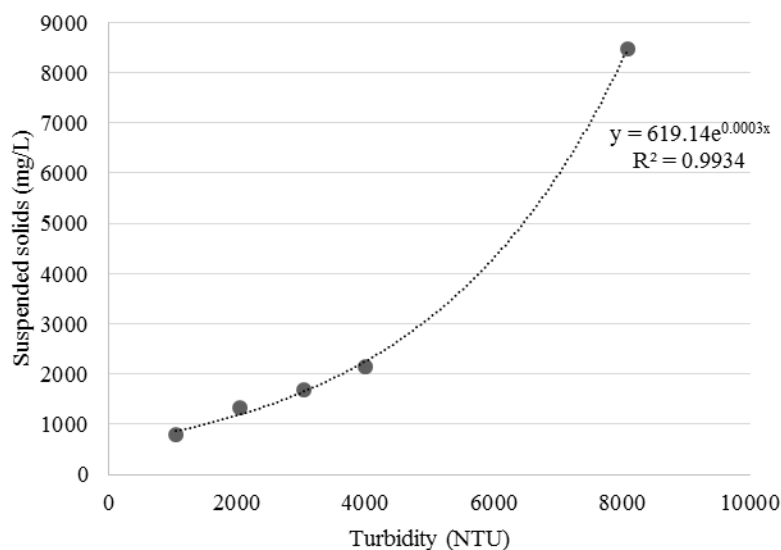


Figure 18: Correlation between turbidity (NTU) and suspended solids (SS), from diluted samples of drilling fluid. Plots present averages with $n=3$.

For turbidities below 1000 NTU, a power correlation, estimated from turbidities 1000 - 4000NTU *could* be a better fit (equation 9).

$$y=5.6305x^{0.7148} \quad (9)$$

Since there is no measurement of suspended solids in correlation with turbidities below 1000 NTU, this is only a suggestion.

Trace elements in undiluted drilling fluid

A complete overview of results from the experiment described in chapter 3.5.7 can be found in appendix F. Table 8 presents some of the elements analyzed. It seems that by adding chitosan to the suspension, the amount of phosphorus in solution is reduced.

Table 8: Characteristics of undiluted drilling fluid without treatment (>6000 NTU), and after treatment with 1.25mg HQGC/L (HQGC). Filtered sample (f), unfiltered sample (t) and untreated sludge from undiluted drilling fluid (s). $n=3$

	Mg (mg/L)	Al (mg/L)	P (μ g/L)	S (mg/L)	Ca (mg/L)	Fe (mg/L)
>6000 NTU (f)	8.20 ± 0.05	0.050 ± 0.002	16.0 ± 0.47	19 ± 0	38.60 ± 0.47	0.065 ± 0.002
HQGC (f)	8.10 ± 0.05	0.034 ± 0.004	6.70 ± 0.09	19 ± 0	38.60 ± 0.48	0.023 ± 0.003
	(g/kg)	(g/kg)	(mg/kg)	(g/kg)	(g/kg)	(g/kg)

>6000						
NTU (t)	0.36 ± 0	5.30 ± 0.12	0.025 ± 0	0.093 ± 0.002	0.85 ± 0.02	0.113 ± 0.005
HQGC (t)	0.013 ± 0	0.02 ± 0	0.223 ± 0.005	0.020 ± 0	0.05 ± 0	0.014 ± 0
>6000						
NTU (s)	17 ± 0	54.00 ± 1.90	898.00 ± 7.48	0.88 ± 0.03	8.88 ± 0.03	50.60 ± 0.8

4.2 Preliminary experiments

The first part of the experimental work was conducted in order to understand the general behavior of the different flocculants in both high (>6000 NTU) and low (2000 NTU) turbidity samples at pH 7.5. Some of these results are shown in table 4 and 5 respectively. As the preliminary work was conducted to achieve a general understanding of dose-turbidity relationship, these experiments were performed without replicates. However, as will be shown later, most of the high and low doses set during these experiments forms the basis for further experimental work.

Due to lack of published work conducted on turbidities as high as those in question has not been successful, the initial work was to some extent characterized by a lot of trying and failing. By adding an excessive amount of precipitating agent, residual turbidities <30 NTU were often obtained – resulting in an unwanted use of large amounts of drilling fluid early in the experimental phase. This was especially the case when using PIX-318 at low turbidity, which in the end had to be diluted to a factor of 20 before being added to the diluted drilling fluid at pH 7.5. Thus, the results presented in table 9 and 10 are some of the more successful results of the preliminary analysis. Furthermore, they are presented this early in the chapter in order to give the reader an idea as to what magnitude of dose is needed for the different flocculants.

Table 9: Results from the preliminary analysis of flocculent interaction in undiluted drilling fluid with turbidity >6000 NTU. The dose is given as milligrams of respective flocculent per liter of drilling fluid, and residual turbidity is measured after 15 minutes settling time (n=1).

Precipitating agent	Dose (mg/L)	Residual turbidity (NTU)
High quality grade chitosan	4	214
	1	2376
Technical grade chitosan	4	540
	1	2899
GHB alginate	6.25	810
	1.875	1001

GMB alginate	6.25	3038
	1.875	2107
PIX318	0.16	487
	0.08	6083

Table 10: Results from the preliminary analysis of flocculent interaction in diluted drilling fluid with turbidity 2000 NTU. The dose is given as milligrams of respective flocculent per liter of diluted drilling fluid, and residual turbidity is measured after 15 minutes settling time ($n=1$).

Precipitating agent	Dose (mg/L)	Residual turbidity (NTU)
High quality grade chitosan	1	37
	0.35	718
Technical grade chitosan	1	33
	0.35	426
GHB alginate	62.5	1569
	25	1806
GMB alginate	62.5	1612
	25	1751
PIX318	0.2	24.7
	0.03	360

According to the preliminary analysis presented in table 9 and 10 the high quality grade chitosan seems to perform slightly better than the technical grade at high turbidities but opposite at low turbidities. Further on it's an interesting observation that the different alginates give very different results at equal dose. These curiosities will be discussed in detail in chapter 5. As for PIX-318 overdosing was one of the main issues throughout all preliminary experiments, and it was decided not to proceed investigating the performance of this flocculent to the same extent as the chitosans.

Preliminary experiments was conducted on the different alginates at turbidities 2000 and 1000 NTU, where the results from the 2000 NTU water samples are presented in table 4.1.2. The alginates were tested with doses ranging from 0.75 mg/L to 62.5 mg/L, all with little success, as the final result was either above or equal to that of a blank sample (T_{b15}). Hence, it was decided to proceed investigating the performance of the alginate flocculants added to high turbidity water only. Addition of calcium (Ca) to enhance flocculation was considered, but after further research, the Ca doses necessary exceeded the amount in naturally occurring Ca rich water in Norway, thus this was also disregarded.

4.3 Chitosans

The following subchapters will present results from the experimental work with regard to the investigated variables described in chapter 3.4.

4.3.1 Relationship between initial turbidity and polymer dose

Different doses of chitosan were tested with the aim to achieve residual turbidity equal or less than 500 NTU, as this correspond to approximately 500 mg/L suspended solids, which is the weekly average discharge limit (for this specific project) to the Hobøl River. Residual turbidity as a function of chitosan dose after 15 minutes settling time for both high quality grade chitosan (HQGC) and technical grade chitosan (TGC) for all initial turbidities are shown in figure 19.

With initial turbidity of 1000 NTU, residual turbidity of 444 ± 5 NTU and 550 ± 19 NTU were achieved after 15 minutes settling time by dosing 0.2 mg/L of technical grade chitosan (TGC) and high quality grade chitosan (HQGC), respectively. There are no statistical significant difference between the two chitosan types, however there are a significant difference ($p < 0.001$) between the two chitosans considering each dose separately, with residual turbidity as dependent variable.

With initial turbidity of 2000 NTU acceptable residual turbidity of 420 ± 23 (TGC) and 440 ± 51 (HQG) were achieved by dosing 0.35 mg/L. Also here there is a significant difference ($p < 0.05$) between the residual turbidity result, as a function of dose and chitosan type.

Residual turbidities of 390 ± 16 (TGC) and 320 ± 11 (HQGC) were achieved by dosing 0.4 mg/L to a drilling fluid with initial turbidity of 3000 NTU. At initial turbidity of 4000 NTU, adequate residual turbidity of 580 ± 27 (TGC) and 511 ± 7 (HQGC) were obtained by a dose of 0.5 mg/L. No significant difference (n.s.) between the different chitosans for each dosing at either of the experiments were observed.

For undiluted drilling fluid (> 6000 NTU) there is a significant difference ($p > 0.001$) in residual turbidity with regard to chitosan type within the different doses. This can also easily be seen from figure 19, where satisfying residual turbidity of 450 ± 21 were retained by adding 2.5 mg/L of HQGC, and a residual turbidity of 740 ± 23 was obtained by adding the same dose of TGC to the suspension.

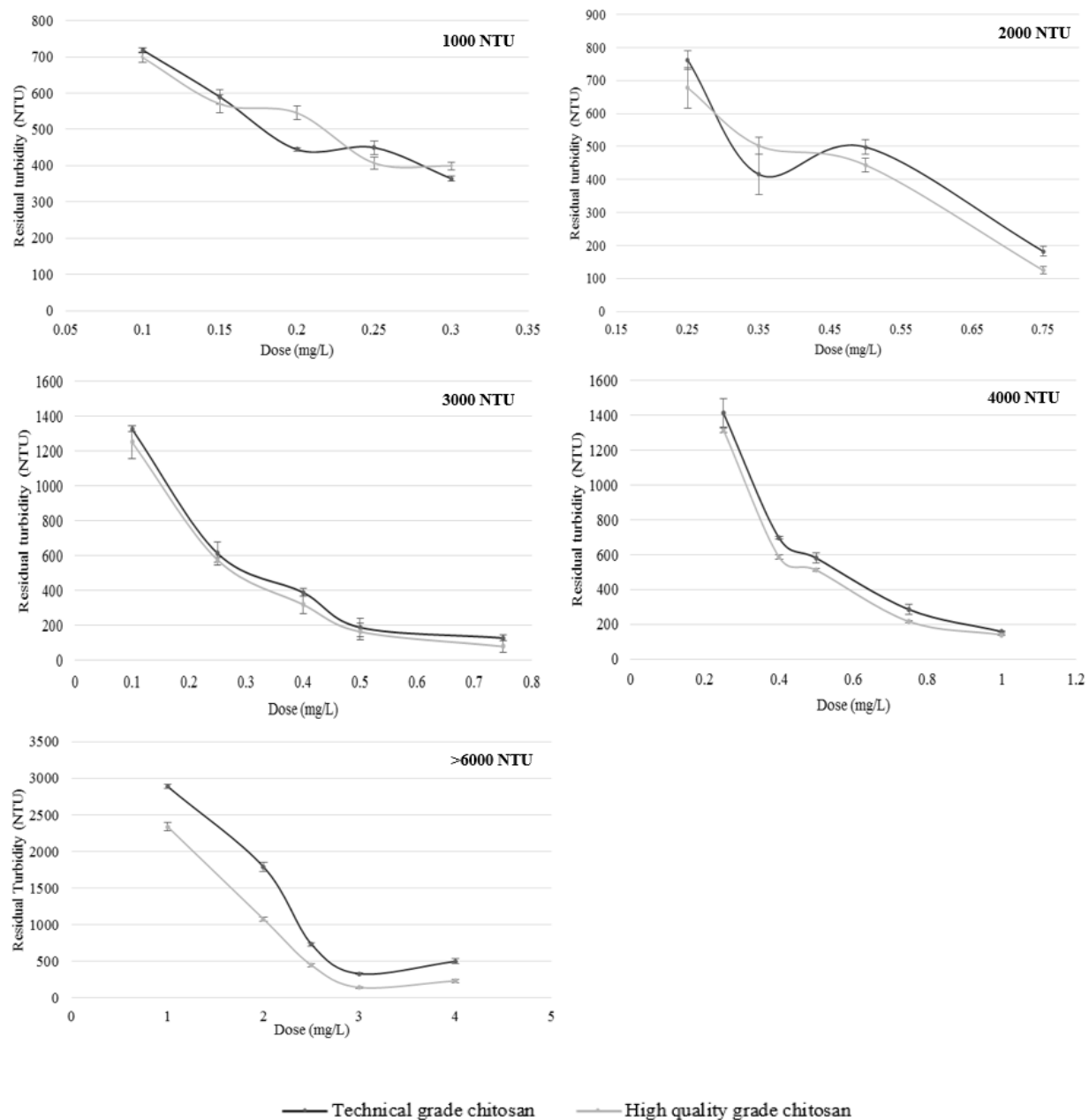


Figure 19: Residual turbidity (NTU) as a function of dose (mg/L) after 15 minutes settling time for both technical grade and high quality grade chitosan at initial turbidities 1000 NTU, 2000 NTU, 3000 NTU, 4000 NTU and >6000 NTU at pH 7.5 ($n=3$)

To summarize, there was no significant difference between the two chitosan types at any of the initial turbidities tested with the exception of undiluted drilling fluid (>6000 NTU) where there was a statistical significant difference ($p<0.001$). In addition, there was a negative correlation between dose and residual turbidity for both TGC and HQGC, i.e. increasing the dose reduces the turbidity.

4.3.2 Calculation of turbidity removal efficiency

As literature on the subject shows, turbidity removal efficiency can be calculated as described in detail in equation 1. However, this thesis proposes another approach to evaluating turbidity removal efficiency of a precipitating agent. Thus, an alternative definition is formulated; Turbidity removal efficiency can be defined as the effect (positive or negative) of a precipitating agent on particles in suspension, compared to the efficiency of sedimentation in the same time interval as the precipitating agent is allowed to work. This results in a formula presented in equation 10

$$TR \% = ((T_b - T_2) / T_b) * 100 \quad (10)$$

T_b is the turbidity of a blank sample after the same settling time as the precipitating agent is allowed to settle, and T_2 is residual turbidity after a precipitating agent is allowed to work. An illustration of the differences are presented in figure 20.

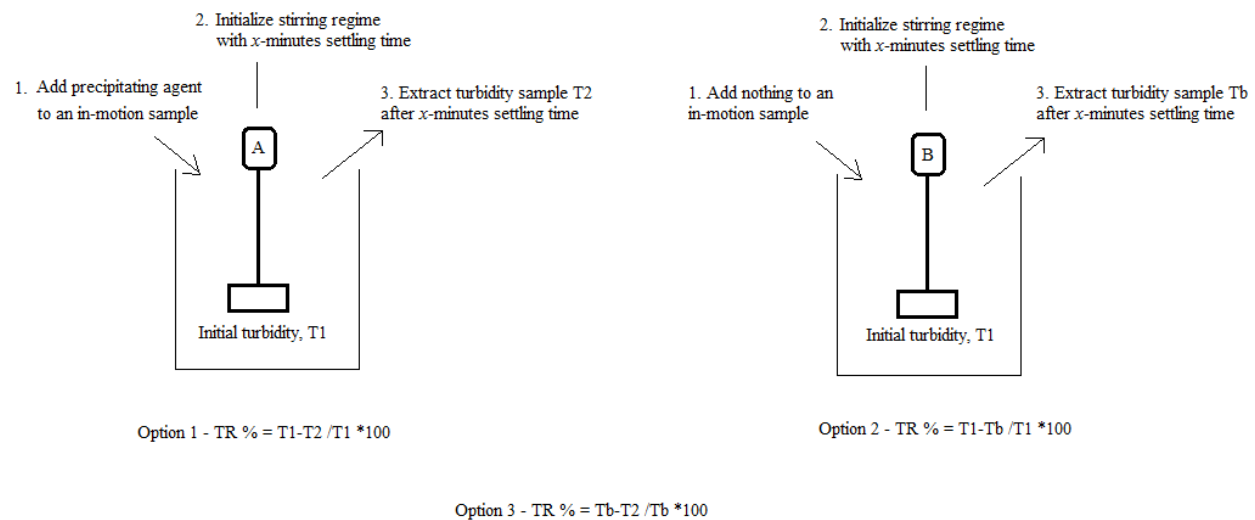


Figure 20: Illustration of different components in the turbidity removal equations, based on jar-test experiments. Jar A illustrates the one where drilling fluid is treated with a precipitating agent, and jar B illustrates the comparable blank sample where no precipitating agent is added. Option 1 is found in literature, option 2 is used when calculating turbidity removal of a blank sample, and option 3 is proposed used in this thesis when evaluating turbidity removal efficiency of a precipitating agent

In order to illustrate the possible complications that can arise from using different equations, the turbidity removal efficiency with the use of TGC at initial turbidity 1000 NTU is presented in figure 21.

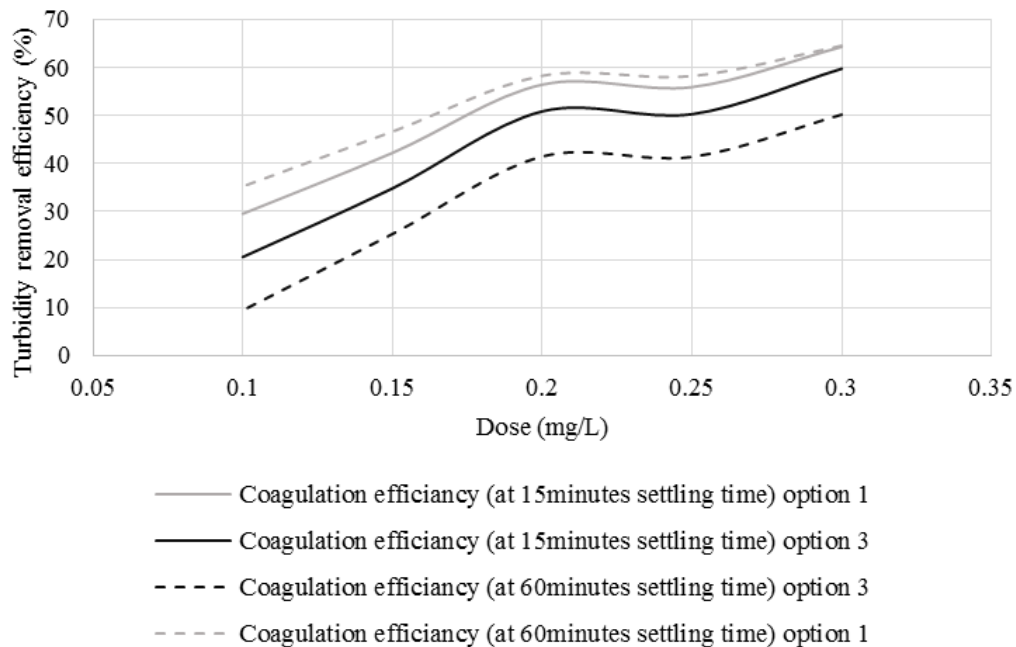


Figure 21: Estimated turbidity removal efficiency from the two different equations 1 and 10 with the use of TGC at initial turbidity 1000 NTU at 15 and 60 minutes settling time.

The difference between the two equations can increase with settling time, as shown in figure 21.

The turbidity removal efficiency after 15 minutes settling time for both chitosans, at different initial turbidities and doses, calculated using equation 10 (option 3), can be found in appendix G.

4.3.3 Effect of settling time

Residual turbidity was measured after 15, and a total of 60 minutes settling time. For initial turbidities 3000 NTU and 4000 NTU, the turbidity removal efficiency (TR) did not vary more than $\pm 2\%$ for all tested dosages with the different chitosans after allowed to settle for one hour compared to TR after 15 minutes. This is with the exception of 0.1 mg/L TGC, where an increase in coagulation efficiency by 8% was measured after one hour settling time.

Figure 22 show the general trend *expected* for this experiment. As the residual turbidity increases, the turbidity removal efficiency increases with settling time. However, figure 22 shows how the turbidity removal efficiency changes when a steady turbidity of a blank sample is applied. This means that T_b is set as 6000 NTU for both 15 and 60 minutes settling time, which in reality is wrong.

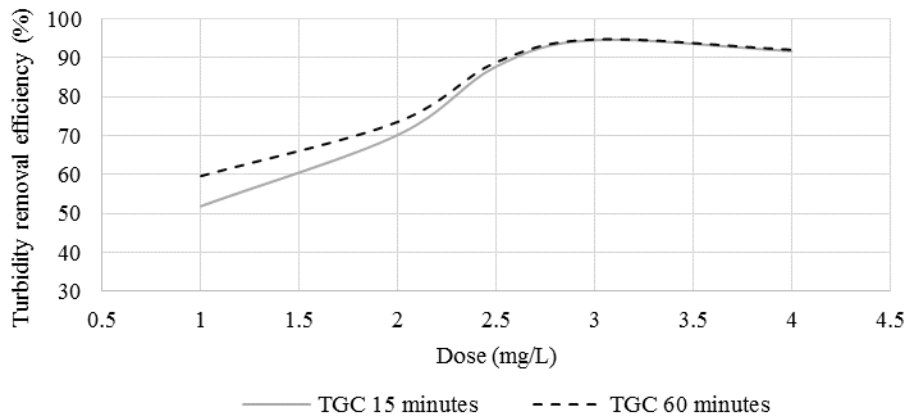


Figure 22: Change in turbidity removal efficiency (%) as a function of dose (mg/L) and time, at initial turbidity of >6000 NTU. Note that TR is calculated with no change in T_b .

For tests conducted with initial turbidities of 1000 NTU, there is a decrease in coagulation efficacy for all doses with both chitosans as settling time increases. Figure 23 show this trend for the experiment with HQGC; equal trend were observed with TGC. It is important not to be confused when evaluating the change in coagulation efficiency over time. When TR decreases, it decreases as a function of change in both residual turbidity after 1 hour measured in the experiment, and the decrease in turbidity after one hour in a blank sample. Thus, when TR decreases, the change (or Δ) between measured turbidity in an experimental sample and turbidity of a blank sample decreases. This does not necessarily mean that the residual turbidity of an experimental sample increases as TR decreases, but that the turbidity of a blank sample is more reduced in the given time interval of 45 minutes than that of the experimental sample.

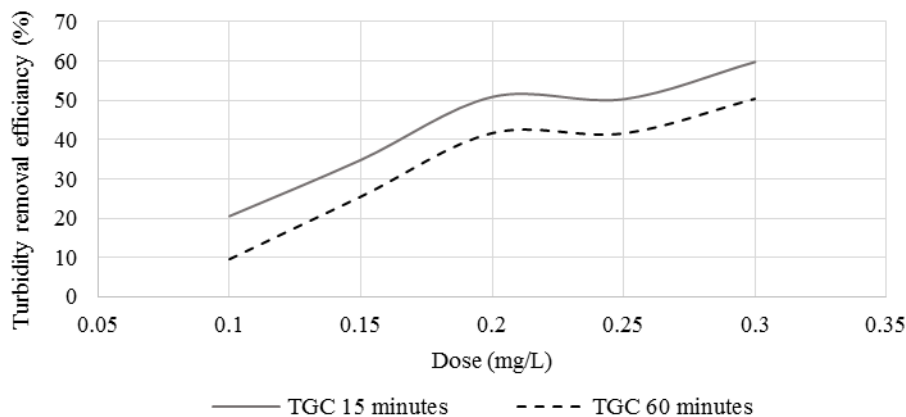


Figure 23: Change in turbidity removal efficiency (%) as a function of time, for TGC with initial turbidity of 1000NTU

For experiments conducted with initial turbidity of 2000 NTU there is a difference in turbidity removal efficiency of less than $\pm 5\%$ for all tests. Hence, both chitosans have the best *efficiency* within the first 15 minutes of settling time, compared to one hour.

However, the reduction in residual turbidity as a function of time, is another aspect. Figure 24 show that there is a very small reduction in turbidity after 60 minutes settling time with the use of HQGC compared to 15 minutes settling time in drilling fluid with initial turbidity >6000 NTU. TGC show another trend. At low doses, and with residual turbidities >1000 NTU after 15 minutes settling time, the turbidity is further reduced when allowed to settle for 60 minutes.

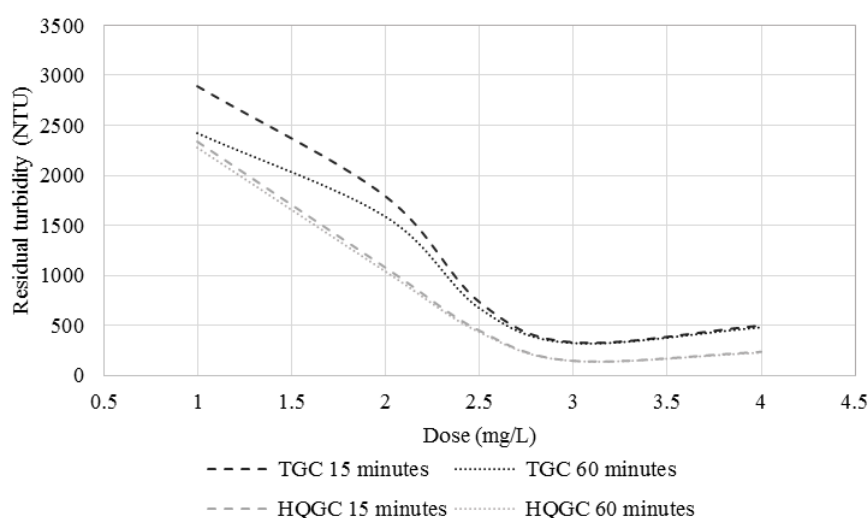


Figure 24: Residual turbidity (NTU) as a function of dose (mg/L) and settling time (15- and 60 minutes), with both high quality grade chitosan (HQGC) and technical grade chitosan (TGC) in undiluted drilling fluid (initial turbidity >6000 NTU)

As the highest residual turbidities after 15 minutes settling time for tests conducted on diluted drilling fluid were <1500 NTU, the effect of settling time is not as prominent as it is for tests conducted on undiluted drilling fluid. But the trend seems to be the same – when residual turbidity at 15 minutes settling is <1000 NTU, the change in turbidity as a function of time is almost zero.

4.3.4 Change in conductivity

Conductivity was measured before adding chitosan to the samples, and after one hour of settling time. Initial conductivity for 1000 NTU blank samples were measured to be $84 \pm 3 \mu\text{S}/\text{cm}$, however for the majority of initial conductivities measured during the experimental work, all

initial average values for electrical conductivity were found to be in the range of 49 to 58 $\mu\text{S}/\text{cm}$ at this dilution, with the highest standard deviation being 1.5.

For 2000 NTU initial conductivity were measured in the range 75 to 91 $\mu\text{S}/\text{cm}$ with the highest standard deviation being 11. Figure 25 shows that the cluster representing 2000 NTU, has the highest variation in conductivity measurements, compared to the others.

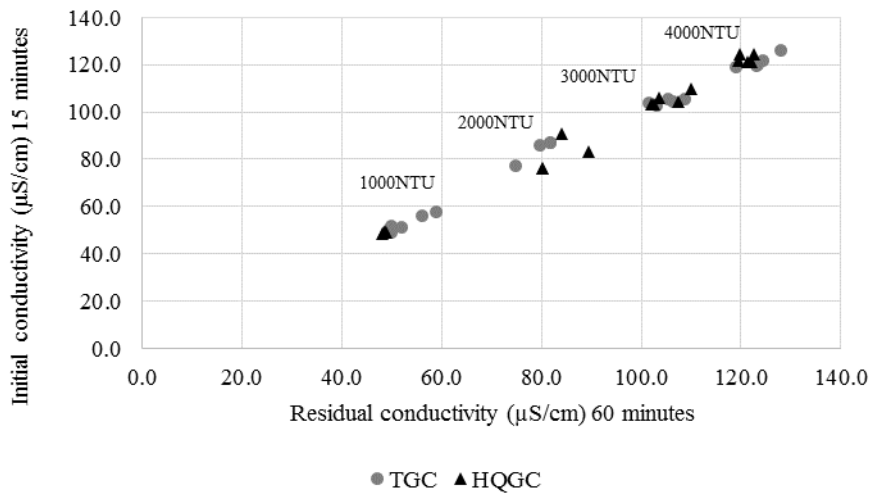


Figure 25: Initial conductivity, measured before adding chitosan, plotted against residual conductivity (after 60 minutes settling time). $n=3$

There is no apparent change in conductivity as a function of added chitosan dose at initial turbidities ranging from 1000 to 4000 NTU. For >6000 NTU, initial conductivity were measured in the range of 560-580 $\mu\text{S}/\text{cm}$ and residual in the range of 420-480 $\mu\text{S}/\text{cm}$.

4.3.5 System pH

Tests show that as the initial pH of the system increases, the chitosan dose required to achieve the same coagulation efficiency increases, as one can see from figure 26.

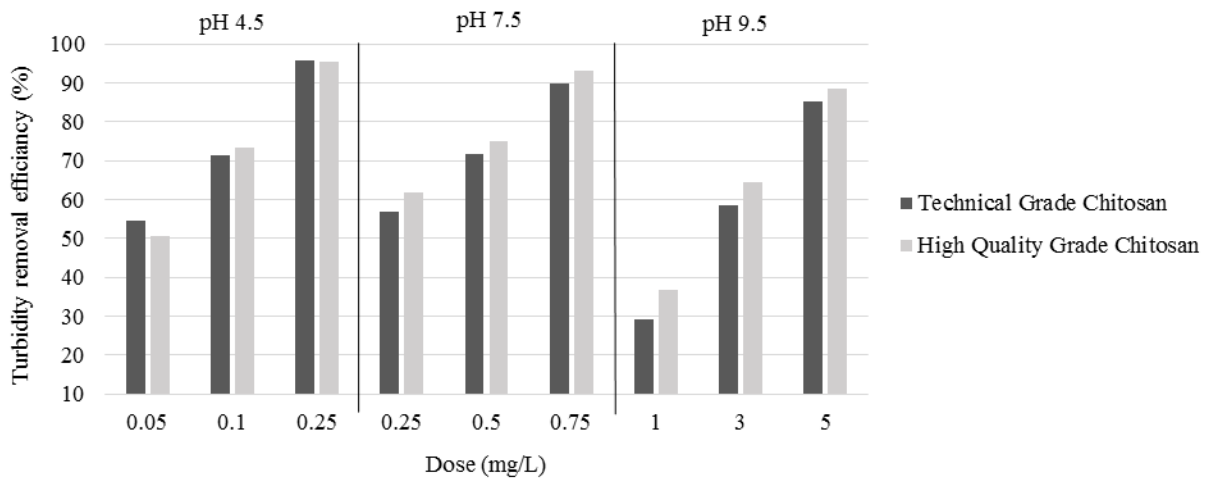


Figure 26: Turbidity removal efficiency (%) as a function of initial pH and dose (mg/L) for both technical and high quality grade chitosan at initial turbidity 2000 NTU and 15 minutes settling time ($n=3$)

4.4 Alginate

There is a significant difference ($p<0.001$) in the effect of the two alginates tested at initial turbidity >6000 NTU, as is clearly shown in figure 27. For both alginates their optimum dose was at approximately 3 mg/L, where the best residual turbidity (688 ± 4 after 15 minutes) was measured with the use of GHB alginate.

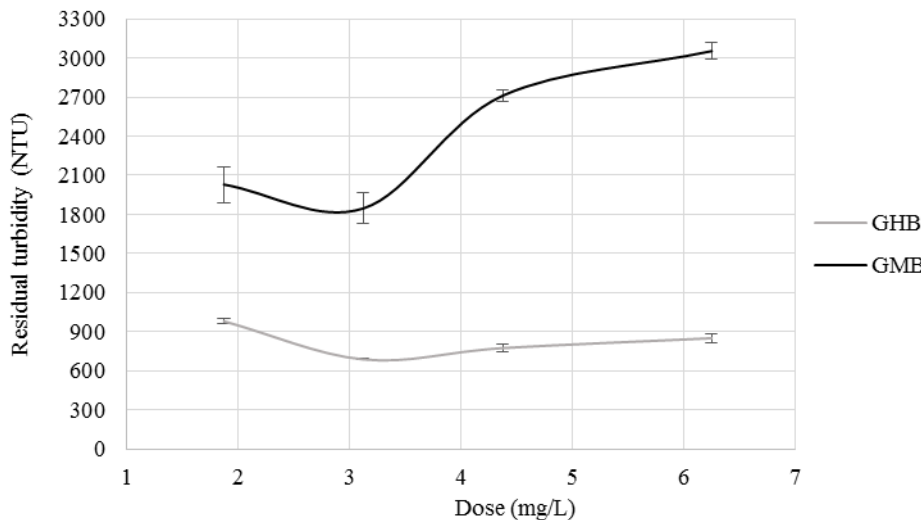


Figure 27: Residual turbidity (NTU) as a function of alginate dose (mg/L) with initial turbidity of 6000 NTU and pH 7.5, after 15 minutes settling time ($n=3$). There is a significant difference ($p<0.001$) between the two different alginates.

For both alginates, regardless of residual turbidity, alginate type and dose, the conductivity in the solution changed from approximately 570 $\mu\text{S}/\text{cm}$ to approximately 470 $\mu\text{S}/\text{cm}$.

Figure 28 illustrate the different floc formations observed with the use of the different alginates. For GHB alginate, round flocs were observed, and for GMB alginate the flocs were formed as longer strains.

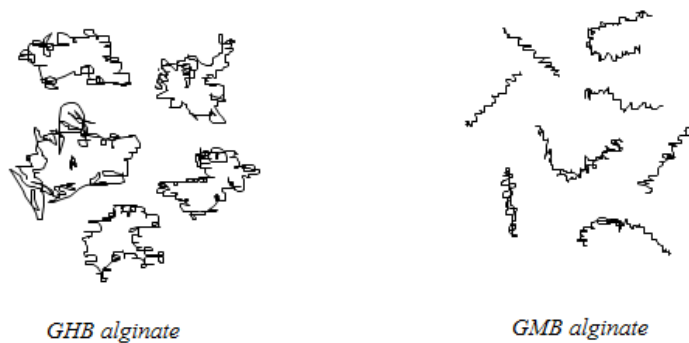


Figure 28: Illustration of different floc formation, with the use of the two alginates GMB and GHB respectively, at initial turbidity >6000 NTU (not to scale).

4.5 PIX-318

Different doses of PIX-318 were added to drilling fluid with initial turbidity of 2000 NTU and initial pH 4.5, 7.5 and 9.5 respectively. The turbidity removal efficiency (%) as a function of initial pH and dose are presented in figure 29.

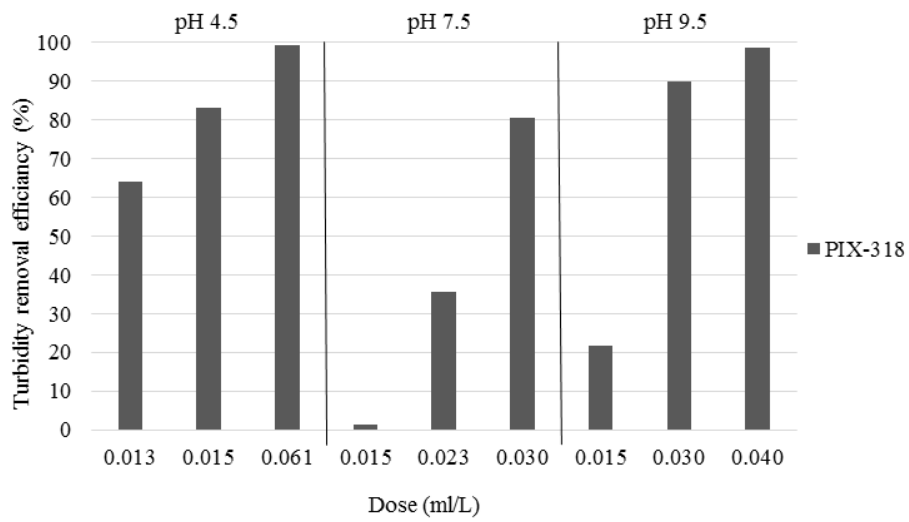


Figure 29: Turbidity removal efficiency of PIX-318 as a function of dose, with initial turbidity of 2000 NTU and initial pH 4.5, 7.5 and 9.5 respectively (n=3)

5 Discussion

5.1 Chitosan

5.1.1 Comparison between technical grade- and high quality grade chitosan

At initial turbidity >6000 NTU (undiluted drilling fluid), and average conductivity being approximately 550 $\mu\text{S}/\text{cm}$, statistical analysis showed that there was a significant difference ($p < 0.001$) between high quality grade chitosan (HQGC) and technical grade chitosan (TGC). The turbidity removal efficiency is better with the use of HQGC than for TGC (figure 19).

The main difference between HQGC and TGC was the degree of deacetylation (DD) and viscosity. DD was 95 % and viscosity 563 $\text{mPa}\cdot\text{s}$ for HQGC. For TGC the DD was 88.8 % and viscosity 45 $\text{mPa}\cdot\text{s}$. As molecular weight is a very difficult parameter to obtain precisely, as stated by Zhang and Neau (2001), using viscosity as an indirect measurement of molecular weight could be more practical. Thus, for this thesis, the assumption is made that high viscosity (e.g. 563 $\text{mPa}\cdot\text{s}$) is equal to a higher molecular weight than what is the case for low viscosity (e.g. 45 $\text{mPa}\cdot\text{s}$). The results obtained when adding chitosan to undiluted drilling fluid is in accordance with literature on the subject (Gregory 1978), as it is claimed that high molecular weight cationic polymers are better coagulants than low molecular weight polymers. This is explained by the electrostatic patch theory (introduced in chapter 2.1.2). Also, high ionic conductivity promotes coiling of the polymer chain (Gregory 1978), which enhances destabilization, as is supported by the same theory.

Furthermore, there was an abundance of suspended solids ($9 \pm 1.7 \text{ g/L}$) in the undiluted drilling fluid, which theoretically would promote bridging for both chitosan types. But if bridging were to be the only mechanism involved in destabilization, turbidity removal would be limited by the number of potential adsorption sites on the polymer – which is limited by the polymer surface area. Since both of the chitosans were dosed with the same weight/volume ratio, it is reasonable to assume that the dose of TGC had a larger total polymer surface area than that of the same dose of HQGC. This accusation is based on the previous argument with regards to viscosity and molecular weight. It follows that low molecular weight polymers, are smaller (or shorter) than high molecular weight polymers. Thus, TGC would adsorb more colloids than HQGC, in total, and theoretically remove more solids from the suspension. Assuming that the effect of floc size

does not matter within 15 minutes settling time. Figure 24 indicates that it is plausible that the floc size arising from the use of 1 mg/L TGC in undiluted drilling fluid is so small that 15 minutes settling time is not sufficient to remove all of the aggregates actually formed. However, at higher chitosan doses the effect of settling time decreases for the TGC, thus HQGC remains the most efficient polymer.

Moreover, adding chitosan to undiluted drilling fluid caused a decrease in ionic conductivity from approximately 570 $\mu\text{S}/\text{cm}$ to 450 $\mu\text{S}/\text{cm}$, for both chitosans. As the measured trend regarding change in ionic conductivity, is the same for both chitosans this can be discarded as a factor affecting the statistical significant difference between the two types.

Hence, it is likely that the difference in viscosity between the two chitosans is the *main* working polymer feature responsible for the significant difference between the chitosans when used in undiluted drilling fluid. The difference in DD could also have affected the result, however, it is likely that this could be more important at low ionic conductivities.

As the original sample was diluted with de-ionized water, two things happened. The ionic conductivity decreased, as did the number of particles per liter. When ionic conductivity decreases the polymer chain expands due to repulsion between charged segments (Gregory 1978). An expanded polymer would be beneficial if a longer distance between suspended particles in water is considered, assuming that bridging is the only mechanism responsible for flocculation. However, at low ionic conductivity, the extent of the electrical double layer from the colloid surface is larger than what it would be at higher ionic conductivities. Hence, the electrostatic attraction between the polymer and the colloid surface will decrease, thus seemingly depress the polymer properties responsible for the notable difference between the two polymers when used in undiluted drilling fluid. Thus, it is plausible that the charge density of the polymer would play a more important role at low ionic conductivity solutions.

In either case, Roussy et al. (2005) tested two similar chitosans (referred to as B1 and B4 in their article) in high ionic conductivity tap water, but with initial turbidities of 5 g bentonite/L. Their study gave similar results as this, with respect to chitosan characteristics and the difference between them as a function of turbidity removal at high ionic concentrations.

5.1.2 Influence of change in initial pH

This study found that as pH increases from 4.5 to 9.5 the chitosan dose required to achieve the same turbidity removal efficiency increases. Similar results are presented by Divakaran and Sivasankara Pillai (2002) as well as Huang and Chen (1996), where the trend was that chitosan is efficient in a broader pH range than that of metal coagulants (Gillberg et al. 2003; Håkonsen 2005). This is not in accordance with results obtained from this thesis, regarding turbidity removal efficiency as a function of pH and dose of PIX-318 (figure 29). However, as this study was quite small, and only meant as an indication as to what to expect with regards to coagulant dose, these results should be interpreted with that in mind.

Furthermore, the acids used for pH adjustment were acetic acid in this study, whereas in others HCl is often used (Divakaran & Sivasankara Pillai 2001; Roussy et al. 2005; Zemmouri et al. 2013). As HCl is a popular acid for pH adjustment in water treatment facilities, it could be more realistic to use this acid in laboratory experiments as well. However, this was not done in these experiments, as one of the main aims was to try to keep factors that can affect the result to a minimum.

5.1.3 Choice of acid for protonation

Håkonsen (2005) stated that the degree of deacetylation is an indirect measure of polymer charge, as it reflects the ratio of amino-groups relative to acetamido-groups on the polymer chain. This would be the case if one could be certain that the polymer was protonated to its full potential at all times. Several studies (Divakaran & Sivasankara Pillai 2001; Håkonsen 2005; Pan et al. 1999) have used hydrochloric acid for protonation of the amino-groups and dissolving the chitosan powder. Since HCl is primarily known as a complexing agent, it is plausible that the chloride ion would interfere with the protonated amino groups. This raises questions to whether the use of HCl can reduce the effectiveness of the respective chitosans in water treatment. In addition, Huang and Chen (1996) used heat when dissolving chitosan in acetic acid – another factor known to affect the length of the polymer chain if excessively used.

The use of different acids for pH adjustment, and chitosan protonation, is just one of several factors that contributes to uncertainty between results from different studies.

5.1.4 Efficiency of chitosan compared to other studies

This study found no clear indication of restabilization of suspended particles for either dose tested at any of the initial turbidities. This is in accordance with studies conducted by Huang and Chen (1996) who found that for kaolinite suspensions with initial turbidity of 1000 NTU there was no restabilization until exceeding a dose of 0.8 mg chitosan/L. However, Huang and Chen reported that residual turbidity after dosing 0.2 mg chitosan /L to a kaolinite suspension were 35 NTU. The same dose worked even better on bentonite suspensions. This study found that dosing 0.2 mg chitosan/L to a sample with initial turbidity of 1000 NTU gave residual turbidity of approximately 500 NTU, depending upon chitosan type. The difference in result can be caused by several factors. For one, Huang and Chen (1996) mixed bentonite and kaolinite with sodium perchlorate (NaClO_4), hence obtaining a stable ionic concentration in the medium at all turbidities tested. This study used deionized water to dilute the original sample to desired turbidity, as a consequence the ionic strength decreased in accordance with decreasing initial turbidity as presented in figure 25. The ionic strength of the suspension medium could affect the efficiency of chitosan, as was also claimed by Roussy et al. (2005) who found that chitosan worked much better when bentonite was dispersed in tap water rather than de-ionized water at either pH 5 or 7.

Furthermore, the jar-test stirring regime can affect the final result as the polymer chains can break if exposed to excessive physical stress. Also, the time span for slow mixing can influence the effect of chitosans. A short period of slow mixing can be unfavorable, as it decreases the chance for the polymer to incidentally hit suspended particles before allowed to settle. Hence the possible bridging mechanism would not be fully exploited. On the other hand, a prolonged period of slow mixing can give the polymer time to wrap around the particle, creating a stable surface, hence increasing the chance of restabilization. For this study, rapid mixing was set at 200 rpm for 3 minutes, followed by slow stirring at 40 rpm for 15 minutes. Huang and Chen (1996) used 100 rpm for 2 minutes as rapid mixing, and 30 rpm for 20 minutes as slow stirring. Thus, the differences in stirring regime could have affected the residual turbidity. However, Ammary (1995) concluded that the duration of rapid mixing had a minimal effect on the flocculation kinetics and the sedimentation process when coagulation with cationic polymers was investigated.

Moreover, several studies (Huang & Chen 1996; Pan et al. 1999; Rounce et al. 2012) stress that it is important not to underestimate the properties of the colloidal particle in suspension, as it has shown to significantly affect the result when comparing bentonite, kaolinite and clay particles.

Hence, all of the discussed factors, along with chitosan properties can affect the efficiency in water treatment processes.

5.2 Evaluation of turbidity removal efficiency

The turbidity of a blank sample after the same settling time as the precipitating agent is allowed to settle (T_b) is highly dependent upon which particles are in suspension and their settling rate. By using equation 1 (option 1), an overestimate of the actual turbidity removal efficiency of the precipitating agent in question could be concluded (figure 21). Hence, using equation 10 (option 3) gives a more modest result, even though the residual turbidity (T_2) is the same for both alternatives. Therefore it is important to know which equation is used when comparing different studies, and also when evaluating the efficiency of a precipitating agent.

It is not certain the calculation by equation 10 (option 3, figure 20) are more precise than that of equation 1 (option 1, figure 20), but this is, as mentioned, highly dependent upon the suspension in question. As an example; at low temperatures (e.g. 4 °C), the sedimentation rate of colloidal particles will be slower than what it would be at higher temperatures (e.g. 20 °C), hence the difference between the two equations will most likely be less notable at low temperatures.

Moreover, the use of equation 10 could be important when evaluating turbidity removal efficiency of a precipitating agent in a laboratory at room temperature, when the aim is to use the same agent in large scale facilities where there temperature is lower. From a practical point of view, equation 1 is probably more usable when testing precipitating agents in large scale facilities, where the retention time varies throughout the day, or from day to day, as a function of different activities at construction sites.

In either case, it is conceivable that the uncertainties related to the different measurements, at least when used in large scale facilities, can be to such an extent that the difference between the two equations is evened out.

5.3 Alginate

The main difference between GMB and GHB alginate was the viscosity. GMB alginate had a viscosity of 119 mPa*s, whereas GHB alginate had 61 mPa*s. As both of them had a M:G ratio of approximately 40:60, it is not a feature warranting consideration when evaluating the notable difference between the two alginates when used in undiluted drilling fluid (figure 27).

Ammary (1995) stated that anionic polymers could not be used in water treatment as primary coagulants. However, residual turbidity with the use of low viscosity GMB alginate came close to that of the discharge permits for Hobøl River.

As the high viscosity chitosan was the most effective when used in undiluted drilling fluid, the opposite was the case for alginate. It is possible that the idea of total polymer surface area being a limiting factor when considering particle flocculation by bridging, could be more prominent in the case of anionic polymers in high turbid fluid. This is supported by observed floc formation, illustrated in figure 28. As shown from the illustration, GHB alginate forms conglomerates rather than just long strains. This could indicate that shorter anionic polymer strains are more likely to form larger aggregates when used under the conditions set in this experiment.

Since the effectiveness of alginate seemed to reach a maximum with residual turbidity of 688 NTU, with the investigated doses in this thesis, it cannot be recommended as a primary coagulant in treating undiluted drilling fluid. However, it could be interesting to further investigate the effectiveness of alginate in combination with chitosan as this has proven successful in removal of trace metals (Qin et al. 2006) and organic pollutants (Nadavala et al. 2009).

5.4 Evaluation of practical use and economical aspects

As chitosan has proven most effective with regard to treating both diluted and undiluted drilling fluid, this will be the basis for further discussion.

Some of the most obvious challenges related to the use of chitosan as a primary coagulant is the issues of dissolving the powder, protonation, and storage. It is less likely that the use of de-ionized water to dissolve large quantities of chitosan powder will be used at a construction site. Mixing, and diluting the chitosan powder in tap water is more practical. Protonation with acetic

acid should not be a problem, however the clear disadvantage is relating to storage time. Håkonsen (2005) recommended that dissolved chitosan should not be stored for longer than a maximum of 10 days, provided cool storage. This causes challenges in an industry where the water usage, and also the use of precipitating agents, is dependent upon construction site activities.

On the other hand, it is well known that the overdosing of chemical precipitating agents is a common problem (Håkonsen 2005). This arises from the fact that a minor change in pH can shift the equilibrium of the precipitating agent, causing sweep coagulation and an excessive amount of chemical residue in the sludge. The advantage with chitosan is that it is effective in a broader pH range than that of chemical agents, thus it is easier to find an optimal dose based on turbidity inlet loggings. This could be beneficial when treating drilling fluid tested in this study, as the load of suspended solids and particle size distribution varies with drilling location and depth.

The reduction of the use of acid and base chemicals that often follows with the use of inorganic flocculants, is not only more environmentally friendly but also enhances work safety with regards to employees at the water work. Furthermore, using a precipitating agent that does not tear on the water alkalinity could potentially be better for the aquatic environment.

Another area with potential for improvement, in regard to the use of chitosan in water treatment at construction sites, is the residual sludge. For many entrepreneurs sludge dewatering, and deposition, can be a costly affair. Both Håkonsen (2005) and Zeng et al. (2008) suggested that the need for excessive dewatering is reduced when using chitosan, compared to chemical agents. Also Liltved et al. (2006) concluded that the sludge volume was reduced by 2/3 when using chitosan as the only precipitating agent in drinking water treatment, compared to using iron chloride. Hence, there is a potential for cost savings if the treatment process can be successfully optimized. Moreover, studies (Håkonsen 2005; Poulsen et al. 2008) have shown that chitosan has an unexploited potential in agriculture. Thus, using “clean sludge” as a soil enhancing medium, could potentially be beneficial for both entrepreneur and farmer.

As the product price for chitosan (and alginate) is approximately thirty times that of ferric chloride sulfate – not including the fact that the dose required with the use of PIX-318 is considerably less (approximately 160µl/L undiluted drilling fluid) than that of chitosan (3 mg/L undiluted drilling fluid) – the potential for drastic cost reductions in other areas of the water

treatment cycle has to be evaluated carefully before using chitosan as a primary coagulant. Reducing the number of employees at the water work, reducing the use of acid and base chemicals, assuming a sludge reduction of approximately 2/3, and evaluating other potential areas of application with respect to residual sludge are just some measures that has to be taken into account when considering the use of chitosan in large scale water treatment facilities.

Even though the use of chitosan has the potential to be more environmentally friendly on a local scale, it's important to keep in mind the global aspect. Kumar (2000) reported that in order to produce 1 kg of 70% deacetylated chitosan from shrimp shells, 6.3 kg of HCl and 1.8 kg of NaOH are required in addition to nitrogen, process water (0.5 tons) and cooling water (0.9 tons). Moreover, Batista et al. (2013) reported that the high residual concentrations of acid and base chemicals are often discharged into the environment without any prospects of further reusing them. Long distance transportation should also be included in a global mass budget.

The production of ferrous coagulants from ore also has its environmental challenges (Gillberg et al. 2003), however it is hard to compete with the low costs related to these products.

Thus, there are several factors to evaluate when considering the use of naturally occurring polymers in water treatment processes.

5.5 Sources of error

As the drilling fluid were filled to the 8 dL mark on the jar, there could be errors with regards to the exact amount of fluid used in the experiments. Loss of particles during pouring could also contribute to uncertainty with respect to the result. However, as the particle load in this study is high, it might not be a problem. To reduce such errors in this experiment, drilling fluid dilutions were conducted from the same water container within each dilution interval (4000, 3000 ... NTU), furthermore experiments with the different coagulants was preformed simultaneously when equal doses were to be tested. Thus, equal conditions were set for the precipitating agents tested, reducing errors related to large fluctuations in suspended solids within each dilution interval.

Another potential error is transfer of coagulant to the jar test experiment. Even though an electronic pipette was used, the difference in viscosity for the different precipitating agents tested

could cause the pipettes to fill unevenly, thus the added dose of precipitating agent could potentially not be accurate. Air bubbles could also affect the amount of dose added.

The amount of suspended solids in the drilling fluids in different containers were mapped using a 47 mm glass fiber filter - GF/c from Whatman, however it turns out that using a 47 mm glass fiber filter – GF/f from Whatman would have been more appropriate when dealing with a lot of clay particles (Thomas Rohrlack, personal communication) as it would have retained more particles in the filter – probably resulting in a higher content of organic matter than what were estimated with the use of GF/c filters.

Furthermore, there was no quality control of the turbidity, pH and conductivity measurements. However, the main contributor to measurement uncertainty would probably be the person performing the experiments. In the end, it is up to the researcher to work systematically, with precision, and using the instruments correctly.

5.6 Further work

First and foremost, it is important to emphasize that there has been a lot of work published considering use of naturally occurring polymers in water treatment, which are impossible to verify due to incomplete documentation of the experimental set-up. Due to numerous variables between different publications, comparison of results only contribute to several assumptions with regards to how effective the polymer will be when used in a similar suspension.

Developing a method that could be used as a standard, with regards to testing polymer efficiency in water treatment would be useful. However, there is a very good reason why this has not already been done. The enormous variability's with regards to properties and characteristics of both the suspension and the dispersion medium, and of course practical conditions in large scale facilities, calls for numerous flocculation programs. However, it should be possible to narrow down the number of variables by at least suggesting a standard stirring regime for the jar-tests that could be used when investigating the general effect of polymers at different suspensions in water. Hence, using existing literature to argue for a standardized stirring regime with respect to a naturally occurring cationic polymers, e.g. chitosan, could be quite useful. Especially if it were to be internationally accepted, it would benefit the ongoing “try and fail” research in this field of

study. As mixing efficiency is largely dependent upon viscosity of the suspension, modelling of optimum stirring regime at different viscosities would potentially be of use.

Furthermore, most jar tests are conducted in laboratories under warm (>20 °C) conditions. Investigating how low temperatures affect the dose necessary to achieve the same turbidity removal efficiency, as well as settling time, could be interesting for high turbidity suspensions. Conducting experiments on suspensions with steady ionic strength but different particle load, in order to evaluate the effect of ions in the dispersion medium on the polymer efficiency is another field of research with knowledge gaps. Taking it a step further, it could be interesting to map which ions, if any, that affect the coagulation efficiency.

Considering the possible variability in viscosity and degree of deacetylation between the different product batches (as shown by presenting both product specifications and certificate of analysis in chapter 3.2.1 and 3.2.2) , it is of most importance to understand what polymer characteristics that are important in water treatment.

6 Conclusion

This study concludes that chitosan would be effective as a primary precipitating agent in treating water from bridge construction under the conditions presented in this thesis. There was a notable difference between the high quality grade chitosan (HQGC) and technical grade chitosan (TGC) when used in undiluted drilling fluid, whereas the difference was not significant in diluted drilling fluids. In order to obtain a residual turbidity <500 NTU, the required dose of either chitosan type were approximately 3 mg/L when added to undiluted drilling fluid. Both chitosans had the highest turbidity removal efficiency within the first 15 minutes of settling time, compared to that of one hour at either dose. This study found no clear indication of restabilization of the suspended solids, indicating that higher chitosan doses than those tested would further reduce the residual turbidity. There were no major change in ionic conductivity before and after flocculation with the use of either chitosan type. In order to achieve the same turbidity removal efficiency, the required dose of PIX-318 were lower than that of chitosan.

GHB alginate was significantly different from that of GMB alginate when used in undiluted drilling fluid. It has not been successful to achieve residual turbidities lower than 688 ± 4 NTU with the use of GHB alginate, at the doses researched in this study. The lowest residual turbidity obtained with the use of GMB alginate were 1800 ± 119 NTU. This was acquired by dosing approximately 3 mg GMB/L undiluted drilling fluid. Adding higher doses of GMB to the suspension, gave an increase in residual turbidity – hence, restabilization was observed. Using alginate in treatment of diluted drilling fluids were unsuccessful, as the residual turbidities did not decrease more than that of a blank sample.

There is still a lot of knowledge gaps related to the use of chitosan in water treatment processes. The main issue related to the use of inorganic precipitating agents is the occurrence of overdosing, and its consequences. In order to defend the use of an expensive polymer in treating undiluted drilling fluid, further investigations related to the possibilities of generating income of residual sludge has to be investigated. In addition to an economic analysis of the different prospects, a life-cycle analysis should be conducted in order to assess environmental impacts.

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Appendix A

Certificate of analysis and product description for High Quality Grade Chitosan (Primex)

	PRODUCT DATA SHEET 		43020
	Approved by:	R. Lárusdóttir	Effective Date:
			Rev. 01 13.03.2012

PRODUCT DESCRIPTION	
Product Name	ChitoClear® HQG 400
Product Code	43020
Ingredients	Fine grade chitosan
Raw Material Source	Fresh North Atlantic Shrimp Shells, <i>Pandalus borealis</i>
Country of Origin	Iceland

PRODUCT SPECIFICATIONS	
Parameters	Test Method
DRY MATTER	CP-001
ASH	CP-002
DEGREE OF DEACETYLATION	CP-010 (Colorimetric Titration)
SOLUBILITY	CP-006 (1% acetic acid)
TURBIDITY	CP-003
VISCOSITY	CP-004 (1% solution in 1% acetic acid measured on a Brookfield DV1+ viscometer, 25°C, appropriate spindle at 30 rpm)
PROTEIN	CP-005 (as total amino acids)
MICROMICLED POWDER	CP-008
APPEARANCE	CP-007
TOTAL PLATE COUNT	NMKL no. 86, 2006
ESCHERICHIA COLI	ISO no. 7251, 2005
COLIFORM BACTERIA	ISO no. 4831, 2006
SALMONELLA PR. 25G	NMKL no. 71, 1999
MOULD AND YEAST	NMKL no. 98, 4 th ed 2005 (Compendium of methods for the microbiological examination of foods (2nd ed. 1984) 17.52)
ARSENIC	SW-846 6020 ICP/MS
CADMIUM	SW-846 6020 ICP/MS
LEAD	SW-846 6020 ICP/MS
MERCURY	SW-846 7471 CVAA

* according to customer's preferences.



Apart from our suggested standards, Primex also supplies chitosan by our customer's own specification requirements.

Certificate of analysis

ChitoClear® HQG 400
 43020
 Primex ehf
 Óskarsgata 7
 588 Stiglafjörður
 Iceland
 Tel: +354 460 6800
 Fax: +354 460 6809
 IS-A670-EFTA

Produced by:
 Primex ehf
 Óskarsgata 7
 588 Stiglafjörður
 Iceland
 Tel: +354 460 6800
 Fax: +354 460 6809
 IS-A670-EFTA

Producer name
 Primex ehf
 Generic name
 B(1→4)-D-glucosamine / N-acetyl-D-glucosamine
 Lot number
 TM4300
 Revision number
 Rev. 01
 Date of manufacture
 3.10.2014
 Access date
 3.10.2017

PARAMETER	TEST METHOD	RESULTS	Comment
Dry Matter Content	CP-001	91.7 %	
Ash	CP-002	0.5 %	
Turbidity	CP-003	13 NTU	
Viscosity	CP-004 (1% chitosan)	50 cP (mPa.s)	
Solubility	CP-006	99 %	
Deacetylation	CP-010 (Direct titration)	95 %	
Protein	As total amino acids*	<0.1 %	
Sieve Analysis	CP-008	95% through 18 mesh	
Appearance	CP-007	White powder	
Taste and odour	CP-007	No taste or smell	
Microbiological count	NMKL 86	<100 cfu/g	
Yeast and mold	NMKL 98	<10 cfu/g	
Coliform bacteria	ISO 4831	absent	
<i>E. coli</i>	ISO 7251	absent	
<i>Salmonella</i> sp.	NMKL 71	absent	
Toxic Heavy Metals			
Arsenic	SW-846 6020 ICP/MS	none detected	1.0 ppm
Cadmium	SW-846 6020 ICP/MS	none detected	0.20 ppm
Lead	SW-846 6020 ICP/MS	<1.0 ppm	0.1 ppm
Mercury	SW-846 7471 CVAA	none detected	0.01 ppm

* Analysis performed at Uppsala Biomedical Center, Sweden

- MBR – Master Batch Record
- CP – Primex Standard Test Methods
- NMKL – Nordic Committee on Food Analysis
- ISO – International Organization for Standardization
- ICP/MS – Inductively Coupled Plasma Mass Spectrometry
- CVAA – Cold Vapor Atomic Absorption

Reported By:
 Title: Laboratory Manager
 Date: 10.10.2014.

Approved By:
 Title: Quality Assurance & Technical Manager
 Date: 23.10.2014.



NOTE: List of customer specific reference files document is located in our controls

Methods of analysis available upon request

Primex ehf, Óskarsgata 7, 588 Stiglafjörður, Iceland, Phone: +354 460 6800, Fax: +354 460 6809

Appendix B

Certificate of analysis and product description for Technical Grade Chitosan (Primex)



Certificate of analysis

Product name
ChitoClear® Technical grade

Product code
40500

Source
Pandalus borealis

Generic name
B(1-4) D-glucosamine / N-acetyl-D-glucosamine

Lot number
TM4294

Revision number
Rev. 04

Date of manufacture
20.8.2014

Retest date
20.8.2017

Manufactured by:
Primex ehf
Oskarsgata 7
580 Siglufjörður
Iceland
Ph: +354 460 6900
Fax: +354 460 6909
IS-AG70-EFTA

	PRODUCT DATA SHEET		40500
	ChitoClear®		Rev. 04
Approved by:	Susanne Lieske	Effective Date:	30.10.2012

PRODUCT DESCRIPTION	
Product Name	ChitoClear® - technical grade
Product Code	40500
Ingredients	Fine grade chitosan
Raw Material Source	Fresh North Atlantic Shrimp Shells, <i>Pandalus borealis</i>
Country of Origin	Iceland

PRODUCT SPECIFICATIONS		
Parameters	Specification	Test Method
DRY MATTER	> 90%	CP-001
ASH	< 2%	CP-002
DEGREE OF DEACETYLATION	75 – 90%	CP-014 (Direct Titration)
SOLUBILITY	> 98%	CP-006 (1% acetic acid)
TURBIDITY	< 50 NTU	CP-003
VISCOSITY	20 – 200 cps	CP-004 (1% solution in 1% acetic acid measured on a Brookfield DV-II+ viscometer, 25 °C, appropriate spindle at 30 rpm)
PARTICAL SIZE	Small flake, approx 1mm	Visual
APPEARANCE	White to off white powder	CP-007
TOTAL PLATE COUNT	< 1000 cfu/g	NMML no. 86, 1999
ESCHERICHIA COLI	Absent	ISO no. 7251, 1993
COLIFORM BACTERIA	Absent	ISO no. 4831, 1991
SALMONELLA PR. 25G	Absent	NMML no. 71, 1999
MOULD AND YEAST	< 100 cfu/g	NMML no. 98, 1995 (Compendium of methods for the microbiological examination of foods (2nd ed. 1984) 17.52)
TOXIC HEAVY METALS (As, Cd, Hg and Pb)	< 5ppm total	EPA 6010B

PARAMETER	TEST METHOD	RESULTS	Comment
Dry Matter Content	CP-001	91.2 %	
Ash	CP-002	1.4 %	
Turbidity	CP-003	14 NTU	
Viscosity	CP-004 (1% chitosan)	45 cP (mPa.s)	
Solubility	CP-006	99.6 %	
Degree of Deacetylation	CP-014 (Direct titrimen)	88.8 %	
Partical size	CP-008	Small flakes, approx. 1mm	
Tap density	CP-009	0.58 g/cc	
Appearance	CP-007	White powder	
Taste and colour	CP-007	No taste or smell	
Microbiol:			<i>detection limit</i>
Aerobic plate count	NMML 86	<1000 cfu/g	10 cfu/g
Yeast and mold	NMML 98	<100 cfu/g	10 cfu/g
Coliform bacteria	ISO 4831	absent	10 cfu/g
<i>E. coli</i>	ISO 7251	absent	0.3 cfu/g
Salmonella sp.	NMML 71	absent	Neg.
Toxic Heavy Metals:			
Arsenic	SW-846 6020 ICP/MS	none detected	1.0 ppm
Cadmium	SW-846 6020 ICP/MS	none detected	0.20 ppm
Lead	SW-846 6020 ICP/MS	<1.0 ppm	0.10 ppm
Mercury	SW-846 7471 CVAA	none detected	0.01 ppm

MBR – Mister Batch Record

CP – Primex Standard Test Methods

NMML - Nordic Committee on Food Analysis

ISO – International Organization for Standardization

ICP/MS – Inductively Coupled Plasma Mass Spectrometry

CVAA – Cold Vapor Atomic Absorption



Reported By: Vela Armadottir

Title: Laboratory Manager

Date: 3. 9. 2014.

Methods of analysis available upon request

RESOURCES RESPONSIBLE

ICELANDIC INNOVATION AWARD

DS CERTIFYING

FOOD SAFETY MANAGEMENT DS/EN ISO 22000

CERTIFIED PRODUCT

TUN 6500

NOTE: Link of certified from www.certified.com document is a copy and is not controlled.

Apart from our suggested standards, Primex also supply chitosan qualities by our customers own specification requirements.

Appendix C

Certificate of analysis and product description for GMB sodium alginate (FMC Biopolymer)

FMC International
 Wallingstown, Little Island
 Co. Cork, Ireland
 Customer Service: +353-21-435-4133
 Fax: +353-21-451-7210

FMC Biopolymer
Certificate of Analysis

FMC Biopolymer
 Know how. It works.SM

Product Specifications

Manugel® GMB alginate

Product: MANUGEL GMB MGLGMB 25 Kg Bag
Lot Number: G8510201
Manufacture Date: May 06, 2014
Re-evaluation Date: May 06, 2015

Customer: FMC Biopolymer

Product Specifications

Chemical & Physical:

Viscosity 110 to 270 mPas (cP)
 pH 5.0 to 7.5
 Loss on drying maximum 15%
 Particle size minimum 98% through 335 micron
 minimum 80% through 250 micron
 minimum 38

Powder color

Heavy metals

Arsenic (As) maximum 3 ppm
 Lead (Pb) maximum 5 ppm
 Mercury (Hg) maximum 0.5 ppm
 Cadmium (Cd) maximum 0.5 ppm
 Total heavy metals (as Pb) maximum 20 ppm

Microbiology:

Total plate count maximum 5000 cfu/gram
 Mold and yeast maximum 300 cfu/gram
 Salmonella negative /25 grams
 E. coli negative /25 grams
 Coliform negative by MPN

Product Ingredients/Labeling:

US: Sodium alginate
EU: Sodium alginate (E401)

Methods of Analysis are available on request.

04122011-18131045000



Test/Standard	Specifications	Results	Method No.
LoD (IR), %	0.0 - 15.0	12.5	2081
Viscosity 1 % 20C, mPasec	110 - 270	119	2174
pH 1% 20C	5.0 - 7.5	7.1	2070
44M BS sieve Thru, %	NLT 98.0	100.0	2221
60M BS sieve Thru, %	NLT 80.0	99.8	2221
Appearance	Normal	Normal	SOM112
Powder Colour RU	NLT 38	74	SOM105B
Total Viable Count	NMT 5000	< 1000	SOM 201S
Yeast and Mould	NMT 300	< 300	SOM 203S
E. Coli in 25g	Negative by MPN	Negative by MPN	SOM204
Salmonella in 25g	Absent in 25g	Absent in 25g	0130
Arsenic by ICP (ppm)	NMT 3.00	0.40	SOM 022P
Lead by ICP (ppm)	NMT 5.00	0.50	SOM 022P
Mercury by ICP (ppm)	NMT 0.50	< 0.10	SOM 022P
Cadmium by ICP (ppm)	NMT 0.50	< 0.30	SOM 022P
Total Heavy Metals by ICP (ppm)	NMT 20	< 20	SOM 022P

PRODUCT DESCRIPTION: HIGH VISCOSITY, PURE SODIUM ALGINATE

This material complies with:
 The specifications of the current EC Directives
 The specifications of the current Food Chemicals Codex

FMC Biopolymer guarantees that at the time of shipment, the supplied lot conforms to all specifications as stated in our Sales Specification. Analytical methods are available upon request.

FMC Biopolymer (U.K.) Limited
 Ladyburn Works
 Cirvan, Ayrshire,
 Scotland KA26 9JN
 Tel: +44 (0) 1665 333000
 Fax: +44 (0) 1665 333105

Steven Hay
 Steven Hay
 Quality Manager

Appendix D

Certificate of analysis and product description for GHB sodium alginate (FMC Biopolymer)

FMC International
 Wallingstown, Little Island
 Co. Cork, Ireland
 Customer Service: +353-21-435-4133
 Fax: +353-21-451-7210

FMC Biopolymer
Certificate of Analysis

FMC Biopolymer
 Know how. It works.SM

Product Specifications

Manugel® GHB sodium alginate

Product Specifications

Chemical & Physical:

	Test Method
Viscosity	2174
pH	2070
Loss on drying	2081
Particle size	2221
Powder color	SOM 105

Heavy metals

Arsenic (As)	maximum 3 ppm
Lead (Pb)	maximum 5 ppm
Mercury (Hg)	maximum 0.5 ppm
Cadmium (Cd)	maximum 0.5 ppm

Microbiology:

Total plate count	0140
Mold and yeast	0160
Salmonella	0156
E. coli	0130
Coliform	SOM 204 EP

Product Ingredients/Labeling:

- US: Sodium alginate
- EU: Sodium alginate (E401)

Methods of Analysis are available on request.

04132011-18131058000



Customer: FMC Biopolymer

Product: MANUGEL GHB MGLGHB 25 Kg Bag
Lot Number: G8904101
Manufacture Date: November 13, 2013
Re-evaluation Date: May 14, 2015

Test/Standard	Specifications	Results	Method No.
LoD (IR), %	0.0 - 15.0	13.7	2081
pH 1% 20C	5.0 - 7.5	6.9	2070
Viscosity 1% 20C, mPasec	50 - 100	61	2174
44M BS sieve Thru, %	NLT 98.0	100.0	2221
60M BS sieve Thru, %	NLT 80.0	99.8	2221
Powder Colour RU	NLT 38	77	SOM105B
Appearance	Normal	Normal	SOM112
Total viable count, cfu/g	NNMT 5000	< 300	140
Mould and yeast, cfu/g	NNMT 300	< 300	160
E. Coli in 25g	Absent in 25g	Absent in 25g	0130
Salmonella in 25g	Absent in 25g	Absent in 25g	0156
Coliform	Negative by MPN	Negative by MPN	SOM204
Arsenic by ICP (ppm)	NNMT 3.00000	0.40000	SOM 0222P
Lead by ICP (ppm)	NNMT 5.000	0.20	SOM 0222P
Cadmium by ICP (ppm)	NNMT 0.5	< 0.3	SOM 0222P
Mercury by ICP (ppm)	NNMT 0.5	< 0.1	SOM 0222P

PRODUCT DESCRIPTION: MEDIUM VISCOSITY, PURE SODIUM ALGINATE

This material complies with:
 The specifications of the current EC Directives
 The specifications of the current Food Chemicals Codex

FMC Biopolymer guarantees that at the time of shipment, the supplied lot conforms to all specifications as stated in our Sales Specification. Analytical methods are available upon request.

FMC Biopolymer (U.K.) Limited
 Ladbarn Works
 Girvan, Ayrshire,
 Scotland KA26 9JN
 Tel: +44 (0) 1655 333000
 Fax: +44 (0) 1655 333105

Steven Hay
 Steven Hay
 Quality Manager

Appendix F

Analysis of filtered drilling fluid. Procedure described in chapter 3.5.7 and 3.8

Limit of detection, LD in (w/v) and (w/w)
 Limit of quantification, LO in (w/v) and (w/w)

Sample Name	Comment	Be µg/L	Mg mg/L	Al mg/L	P µg/L	S mg/L	Ca mg/L	V µg/L	Cr µg/L	Mn mg/L	Fe mg/L	Co µg/L	Ni µg/L	Cu µg/L	Zn µg/L	As µg/L	Mo µg/L	Cd µg/L	Sb µg/L	Tl µg/L	Pb µg/L	Bi µg/L	Th µg/L	U µg/L		
1	4000 NTU filtrert vann	<LD	2.3	0.36	14	4.5	10	1.2	0.56	5.8	0.44	0.24	0.85	2.8	2.1	0.34	1.6	<0.004	0.20	0.012	0.43	<LD	0.03	0.001	0.0004	
2	4000 NTU filtrert vann	<LD	2.3	0.36	14	4.5	10	1.2	0.56	5.8	0.44	0.24	0.85	2.8	2.1	0.34	1.6	<0.004	0.20	0.012	0.43	<LD	0.03	0.001	0.0004	
3	350 NTU filtrert vann	<LD	2.3	0.36	13	4.6	10	1.2	0.58	5.7	0.44	0.23	0.87	2.8	1.9	0.34	1.6	<0.004	0.19	0.015	0.41	<LD	0.03	0.001	0.0004	
4	350 NTU 0.25 mg HOGC filtrert vann	<LD	2.1	0.362	4.2	4.1	9.7	0.54	0.20	1.3	0.881	0.98	0.36	2.1	1.1	0.36	1.5	<0.004	0.21	0.096	<0.16	<LD	0.087	0.03	0.018	
5	375 NTU /L 4000 NTU filtrert vann	<LD	2.1	0.11	4.8	4.5	9.9	0.60	0.20	1.4	0.10	0.888	0.40	2.1	1.0	0.26	1.7	<0.004	0.20	0.016	<0.16	<LD	0.087	0.03	0.015	
6	355 NTU filtrert vann	<LD	2.2	0.096	4.3	4.4	9.9	0.54	0.13	1.2	0.079	0.077	0.39	2.1	1.3	0.26	1.6	<0.004	0.21	0.093	<0.16	<LD	0.059	0.015	0.015	
7	2000 NTU filtrert vann	<LD	1.7	0.69	17	2.9	6.7	1.7	1.0	11	0.91	0.42	1.4	2.8	3.5	0.36	1.0	0.008	0.14	0.016	0.72	<LD	0.59	0.17	0.17	
8	2000 NTU filtrert vann	<LD	1.7	0.66	17	2.9	6.8	1.7	1.1	11	0.93	0.4	1.4	2.8	3.9	0.36	1.0	0.005	0.13	0.016	0.72	<LD	0.58	0.17	0.17	
9	2000 NTU filtrert vann	<LD	1.7	0.61	16	2.9	6.7	1.5	0.97	9.9	0.81	0.36	1.3	2.7	3.6	0.36	0.99	0.008	0.14	0.016	0.66	<LD	0.54	0.16	0.16	
10	463 NTU 0.175 mg HOGC filtrert vann	<LD	1.5	0.14	6.2	2.9	6.6	0.60	0.24	2.1	0.16	0.11	0.51	1.7	1.6	0.22	1.0	<LD	0.13	0.090	<0.16	<LD	0.13	0.08	0.08	
11	470 NTU /L 2000 NTU filtrert vann	<LD	1.5	0.15	5.4	2.9	6.5	0.59	0.2	2.3	0.17	0.11	0.43	1.6	1.0	0.21	1.0	<LD	0.13	0.098	<0.16	<LD	0.13	0.076	0.076	
12	458 NTU filtrert vann	<LD	1.5	0.14	5.4	2.9	6.5	0.55	0.23	2.2	0.16	0.10	0.43	1.7	0.93	0.24	1.1	<0.004	0.13	0.096	<0.16	<LD	0.12	0.075	0.075	
13	>6000 NTU filtrert vann	<LD	8.2	0.047	16	1.9	39	1.2	0.17	1.0	0.062	0.19	1.4	6.4	1.1	0.47	5.7	0.005	0.58	0.016	<0.16	<LD	0.19	3.6	3.6	
14	>6000 NTU filtrert vann	<LD	8.2	0.053	16	1.9	38	1.2	0.16	1.2	0.066	0.18	1.3	6.5	1.3	0.49	5.7	<0.004	0.57	0.016	<0.16	<LD	0.20	3.7	3.7	
15	>6000 NTU filtrert vann	<LD	8.3	0.050	17	1.9	39	1.2	0.20	1.2	0.067	0.20	1.4	6.6	1.0	0.52	5.7	0.004	0.58	0.017	<0.16	<LD	0.20	3.7	3.7	
16	620 NTU 1.25 mg HOGC filtrert vann	<LD	8.1	0.028	6.6	1.9	39	0.8	<0.1	0.42	0.019	0.17	1.2	5.7	1.3	0.43	5.6	0.006	0.55	0.015	<LD	<LD	0.039	3.7	3.7	
17	638 NTU /L >6000 NTU filtrert vann	<LD	8.2	0.037	6.8	1.9	39	0.78	<0.1	0.45	0.025	0.17	1.2	5.8	0.88	0.43	5.7	<0.004	0.60	0.015	<LD	<LD	0.048	3.8	3.8	
18	599 NTU filtrert vann	<LD	8.2	0.037	6.8	1.9	38	0.84	<0.1	0.47	0.024	0.19	1.3	5.7	0.78	0.44	5.5	0.026	0.60	0.015	<LD	<LD	0.042	3.8	3.8	
19	521 NTU 0.028ml PIX /L filtrert vann	<LD	1.7	0.11	4.6	6.1	7.1	0.22	0.15	3.6	0.13	0.11	0.84	0.95	1.6	0.10	0.78	0.007	0.10	0.099	<0.16	<LD	0.081	0.045	0.045	
20	510 NTU 2000NTU filtrert vann	<LD	1.7	0.11	4.6	6.1	7.0	0.23	0.17	5.3	0.14	0.13	0.83	0.90	1.4	0.10	0.81	0.006	0.099	0.010	<0.16	<LD	0.085	0.035	0.035	
21	522 NTU filtrert vann	<LD	1.7	0.12	4.5	6.0	6.9	0.24	0.18	4.3	0.14	0.095	0.78	0.91	1.5	0.10	0.79	0.006	0.10	0.011	<0.16	<LD	0.085	0.031	0.031	
22	3000NTU filtrert vann	<LD	2.0	0.32	12	4.4	9.0	1.2	0.50	5.3	0.41	0.23	0.89	2.5	2.0	0.33	1.3	<0.004	0.17	0.085	0.36	<LD	0.29	0.18	0.18	
23	3000NTU filtrert vann	<LD	2.0	0.32	12	4.3	8.9	1.1	0.49	5.3	0.40	0.23	0.86	2.7	1.9	0.33	1.4	<0.004	0.17	0.079	0.36	<LD	0.28	0.17	0.17	
24	3000NTU filtrert vann	<LD	2.0	0.34	12	4.3	9.0	1.2	0.48	5.4	0.42	0.21	0.92	2.5	2.0	0.33	1.4	<0.004	0.16	0.090	0.37	<LD	0.29	0.18	0.18	
25	573 NTU 0.25mg HOGC/ 3000NTU filtrert vann	<LD	1.9	0.14	6.0	4.2	8.7	0.69	0.23	2.6	0.17	0.12	0.52	1.9	3.2	0.28	1.4	<0.004	0.19	0.056	<0.16	<LD	0.12	0.15	0.15	
26	569 NTU 3000NTU filtrert vann	<LD	1.9	0.13	6.2	4.4	8.9	0.69	0.20	2.4	0.15	0.12	0.50	1.9	1.6	0.29	1.3	<0.004	0.17	0.066	<0.16	<LD	0.11	0.15	0.15	
27	565 NTU filtrert vann	<LD	1.9	0.13	5.8	4.2	8.7	0.64	0.17	2.4	0.13	0.11	0.47	1.9	1.4	0.29	1.3	<0.004	0.17	0.055	<0.16	<LD	0.10	0.15	0.15	
28	1000 NTU filtrert vann	<LD	1.1	0.21	8.7	2.3	4.9	0.78	0.32	3.6	0.28	0.14	0.72	1.6	1.8	0.25	0.77	0.005	0.081	0.053	0.22	<LD	0.18	0.084	0.084	
29	1000 NTU filtrert vann	<LD	1.1	0.19	7.6	2.3	4.9	0.73	0.37	3.3	0.25	0.13	0.53	1.5	1.6	0.25	0.72	<0.004	0.080	0.052	0.21	<LD	0.17	0.082	0.082	
30	1000 NTU 0.15mg HOGC/L filtrert vann	<LD	1.0	0.19	7.5	2.3	4.8	0.70	0.29	3.3	0.24	0.13	0.52	1.6	1.7	0.25	0.70	<0.004	0.086	0.052	0.20	<LD	0.16	0.079	0.079	
31	305 NTU filtrert vann	<LD	1.0	0.11	4.9	2.2	4.6	0.57	0.17	3.3	0.13	0.084	0.39	1.3	3.3	0.20	0.65	<0.004	0.079	0.042	<0.16	<LD	0.084	0.061	0.061	
32	302 NTU filtrert vann	<LD	1.1	0.11	4.9	2.2	4.6	0.57	0.20	2.1	0.13	0.084	0.39	1.3	3.3	0.20	0.65	<0.004	0.081	0.042	<0.16	<LD	0.084	0.061	0.061	
33	703 NTU filtrert vann	<LD	1.1	0.12	5.2	2.3	4.6	0.48	0.21	2.7	0.14	0.086	0.36	1.3	1.7	0.21	0.64	<0.004	0.081	0.039	<0.16	<LD	0.081	0.062	0.062	
34	703 NTU 3.175 mg GHz/L filtrert vann	<LD	1.1	0.12	5.2	2.3	4.6	0.48	0.21	2.7	0.14	0.086	0.36	1.3	1.7	0.21	0.64	<0.004	0.081	0.039	<0.16	<LD	0.081	0.062	0.062	
35	684 NTU filtrert vann	<LD	1.1	0.10	5.8	2.0	3.6	0.84	<0.1	0.32	0.065	0.16	1.4	5.9	1.3	0.46	6.7	<0.004	0.059	0.014	<LD	<LD	0.019	4.8	4.8	
36	684 NTU filtrert vann	<LD	1.1	0.10	5.8	2.0	3.6	0.84	<0.1	0.28	0.065	0.16	1.4	6.0	1.0	0.44	6.6	<0.004	0.058	0.015	<LD	<LD	0.018	4.8	4.8	
37	Milli-Q Blank sample filtrert vann	<LD	7.9	0.010	6.8	2.0	3.6	0.88	<0.1	0.28	0.065	0.15	1.3	6.0	0.72	0.44	6.6	<0.004	0.058	0.015	<LD	<LD	0.018	4.8	4.8	
38	Milli-Q Blank sample filtrert vann	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
39	Milli-Q Blank sample filtrert vann	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD

Analysis of drilling fluid sludge. Procedure described in chapter 3.5.7 and 3.8.

Sample Name	Be	Mg	Al	P	S	Ca	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Mo	Cd	Sb	Ti	Pb	Bi	Th	U
7	0.208	0.005	0.08	6	0.005	0.04	0.2	0.1	0.8	0.05	0.03	0.4	1.1	0.13	0.04	0.03	0.01	0.01	0.002	0.38	0.02	0.02	0.007
8	0.250	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
9	0.205	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
10	0.204	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
11	0.204	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
12	0.205	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
13	0.211	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
14	0.230	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
15	0.300	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
16	0.257	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
17	0.254	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
18	0.254	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
19	0.273	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
20	0.268	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
21	0.283	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
22	0.270	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
23	0.263	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
24	0.264	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
25	0.250	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
26	0.250	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
27	0.233	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
28	0.248	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
29	0.271	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
30	0.277	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
31	0.251	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
32	0.233	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
33	0.242	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
34	0.239	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
35	0.250	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
36	0.250	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
37	0.275	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
38	0.260	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
39	0.256	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
40	0.250	0.016	0.061	20	0.036	0.13	0.5	0.4	2.7	0.18	0.09	0.4	1.1	0.13	0.04	0.03	0.004	0.01	0.002	0.38	0.05	0.08	0.024
1626H	14	8.1	0.15	2700	2.6	33	42	21	43	0.10	28	65	23	80	61	130	68	61	7.7	19	<LOD	1.1	1.1
1628H	14	8.1	0.14	2700	2.6	34	42	21	43	0.10	28	66	24	82	62	130	70	61	7.9	19	<LOD	1.1	1.1
1643H	14	8.0	0.14	2600	2.6	33	41	21	41	0.10	28	66	23	78	60	130	69	61	7.9	19	<LOD	1.0	1.1

Limit of detection, LD in (mV) and (w/w)
 Limit of quantification, LO in (w/v) and (w/w)
 Based on average weight, 0.25

Certificate of certified reference material, and certified values for soil reference materials

Eight Feb. 07



Analytical Methods		Composition	
Composition	Methods	Composition	Methods
Ag	AA, AAN, ES, NA	Pb	AA, ES, ICP, ID, POL, XRF
As	AA, AF, COL, POL, BS, NA, XRF	Pt	ICP, MS, NA
Au	AAN, ES	Rb	AA, PP, NA, XRF
B	COL, ES, ICP	Sb	AA, AA, HF, COL, POL, ES, NA
Ba	AA, AAN, ES, ICP, NA, MS, XRF	Sc	ES, ICP, NA, XRF
Be	AAN, COL, ES, ICP, POL	Se	AAN, AF, COL, ICP, POL
Bi	AA, AA, HF, AF, COL, POL, MS, ES, ICP	Sm	AAN, ICP, ID, MS, NA
Br	COL, ICP, NA	Sr	AAN, POL, COL, ES, MS, XRF
Ca	AA, AA, HF, ES, ICP, POL	Te	AA, AAN, ES, ICP, NA, XRF
Ce	ICP, ID, MS, NA, XRF	Ta	COL, ICP, NA
Cl	COL, ICP, SE, NA	Tb	AAN, ICP, MS, NA
Co	AA, AAN, COL, ES, ICP, NA, POL, XRF	Tc	AAN, AF, COL, POL
Cr	AA, COL, ES, ICP, NA, XRF	Ti	COL, ICP, MS, NA, POL, XRF
Cu	AA, FP, NA	Tl	COL, ICP, NA, XRF
Cy	AA, COL, ICP, POL, XRF	Tl	AAN, COL, POL, ES, MS
Dy	AAN, ICP, ID, MS, NA	Tm	AAN, ICP, MS, NA
Er	AAN, ICP, ID, MS, NA	U	COL, DNA, ID, MS, NA, POL, XRF
Bu	AAN, ICP, MS, NA	V	COL, ES, ICP, NA, POL, XRF
F	COL, SE	W	COL, NA, MS, POL, XRF
Ga	AAN, COL, POL, ES, ICP, XRF	Y	AAN, ES, ICP, MS, XRF
Gd	ICP, MS, NA	Yb	AAN, ES, ICP, ID, MS, NA
Ge	AAN, COL, POL	Zn	AA, ICP, NA, POL, XRF
Hf	COL, MS, NA, XRF	Zr	COL, ES, ICP, MS, NA, POL, XRF
Hg	AA, AA, FP, AF	SiO ₂	GR, ICP, VOL, XRF
Ho	AAN, ICP, MS, NA	Al ₂ O ₃	COL, ICP, VOL, XRF
I	COL, SE, NA, POL, VOL	TiO ₂	AA, COL, ICP, NA, VOL, XRF
Bi	AAN, ICP, MS, NA	P ₂ O ₅	ICP, VOL, XRF
La	ICP, MS, NA, XRF	MgO	AA, GR, ICP, VOL, XRF
Li	AA, FP, ICP	CaO	GR, ICP, VOL, XRF, AA
Lu	ICP, MS, NA	FeO	AA, FP, ICP, NA, XRF
Mn	AAN, ICP, NA, POL, XRF	K ₂ O	AA, FP, ICP, NA, XRF
Mo	COL, ES, NA, MS, POL, XRF	S	VOL, XRF
N	VOL	H ₂ O	GR, HL
Nb	COL, ES, ICP, MS, XRF	CO ₂	GR, VOL
Nd	ICP, ID, MS, NA, XRF	Org. C	GR, VOL
Ni	AA, AAN, COL, ES, ICP, NA, POL, XRF	LOI	GR
P	COL, ICP, XRF		

Certificate of Certified Reference Materials

NCS DC 73319 — NCS DC 73326

Soil

Note:
 AA: Flame Atomic Absorption spectrometry
 AAN: Atomic Absorption spectrometry with hydride generation
 AAN-NA: Flame Atomic Absorption spectrometry
 AF: Atomic Fluorescence spectrometry
 COL: Colorimetry
 DNA: Deuterium Neutron Activation method
 EL: Electrode method
 ES: Ion Selective Electrode method
 MS: Mass Spectrometry
 POL: Poligraphy
 PP: Plasma Pyrolysis
 GR: Gravimetry
 ICP: Inductively Coupled Plasma spectrometry
 ID: Isotope Dilution mass source mass spectrometry
 ICP-MS: Inductively Coupled Plasma Mass Spectrometry
 LP: Laser Fluorescence spectrometry
 NA: Neutron Activation analysis
 VOL: Volumetry
 XRF: X-Ray Fluorescence spectrometry

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(Beijing China)

China National Analysis Center for Iron and Steel

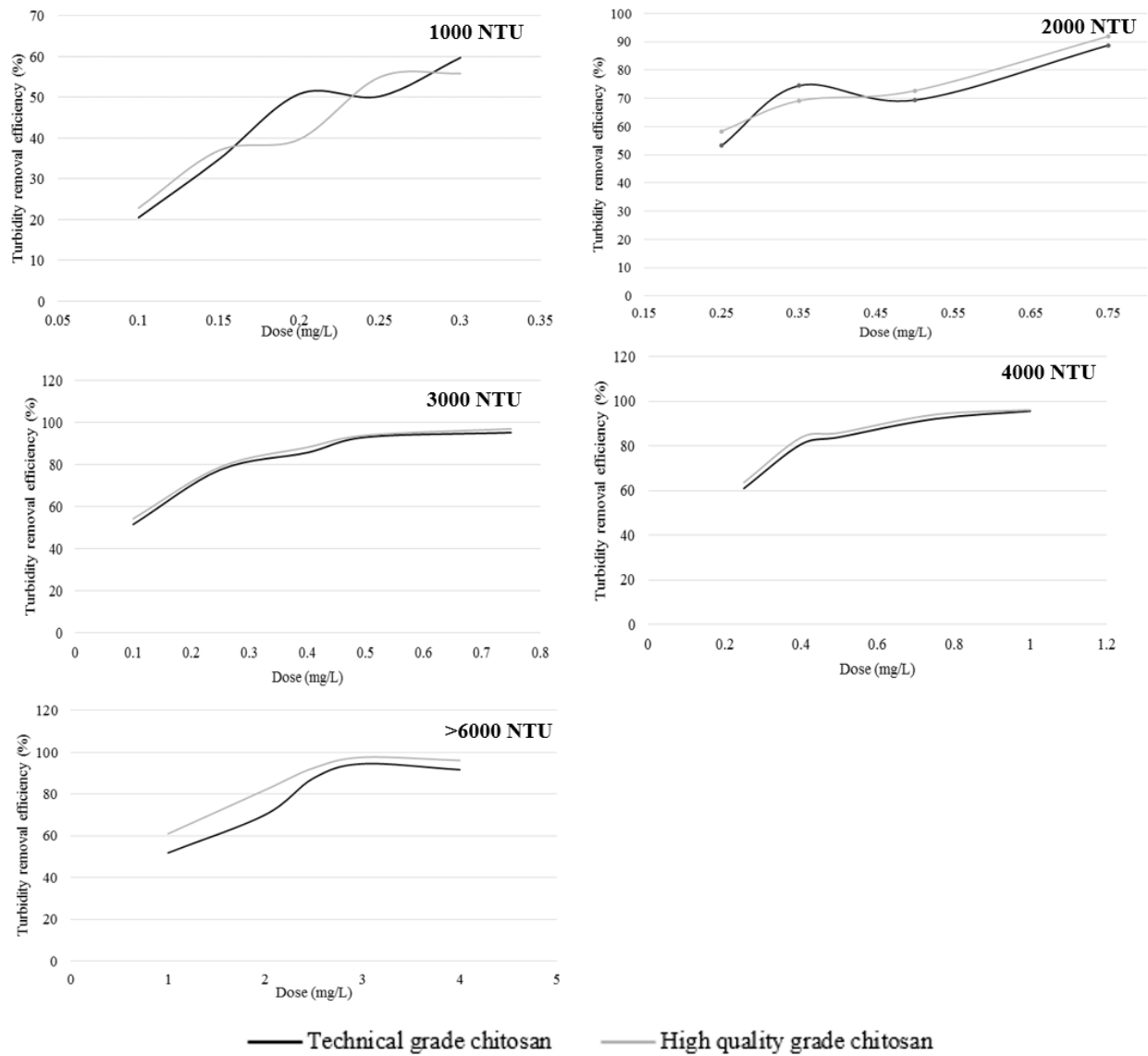
Certified values of soil reference materials

	NCS ZC 73001	NCS ZC 73002	NCS ZC 73003	NCS ZC 73004	NCS ZC 73005	NCS ZC 73006	NCS ZC 73007
Ag	0.88340(0.010)	0.89840(0.007)	0.89840(0.007)	0.89840(0.006)	0.89840(0.007)	0.89840(0.007)	0.89840(0.007)
Al	7.40(5)	7.40(5)	7.40(5)	7.40(5)	7.40(5)	7.40(5)	7.40(5)
As	3.6(3)	3.6(3)	3.6(3)	3.6(3)	3.6(3)	3.6(3)	3.6(3)
B	4.13(2)	4.13(2)	4.13(2)	4.13(2)	4.13(2)	4.13(2)	4.13(2)
Ba	2.4(0.1)	2.4(0.1)	2.4(0.1)	2.4(0.1)	2.4(0.1)	2.4(0.1)	2.4(0.1)
Be	0.27(0.02)	0.27(0.02)	0.27(0.02)	0.27(0.02)	0.27(0.02)	0.27(0.02)	0.27(0.02)
Bi	5.8(0.4)	5.8(0.4)	5.8(0.4)	5.8(0.4)	5.8(0.4)	5.8(0.4)	5.8(0.4)
Br	0.105(0.012)	0.105(0.012)	0.105(0.012)	0.105(0.012)	0.105(0.012)	0.105(0.012)	0.105(0.012)
C	70(4)	70(4)	70(4)	70(4)	70(4)	70(4)	70(4)
Ca	21.6(1.4)	21.6(1.4)	21.6(1.4)	21.6(1.4)	21.6(1.4)	21.6(1.4)	21.6(1.4)
Cl	17.9(0.5)	17.9(0.5)	17.9(0.5)	17.9(0.5)	17.9(0.5)	17.9(0.5)	17.9(0.5)
Co	6.8(0.2)	6.8(0.2)	6.8(0.2)	6.8(0.2)	6.8(0.2)	6.8(0.2)	6.8(0.2)
Cu	1.9(0.1)	1.9(0.1)	1.9(0.1)	1.9(0.1)	1.9(0.1)	1.9(0.1)	1.9(0.1)
Dy	4.7(0.3)	4.7(0.3)	4.7(0.3)	4.7(0.3)	4.7(0.3)	4.7(0.3)	4.7(0.3)
E	2.75(0.17)	2.75(0.17)	2.75(0.17)	2.75(0.17)	2.75(0.17)	2.75(0.17)	2.75(0.17)
F	452(1.6)	452(1.6)	452(1.6)	452(1.6)	452(1.6)	452(1.6)	452(1.6)
Ga	1.8(0.1)	1.8(0.1)	1.8(0.1)	1.8(0.1)	1.8(0.1)	1.8(0.1)	1.8(0.1)
Ge	1.31(0.08)	1.31(0.08)	1.31(0.08)	1.31(0.08)	1.31(0.08)	1.31(0.08)	1.31(0.08)
Hf	9.5(0.7)	9.5(0.7)	9.5(0.7)	9.5(0.7)	9.5(0.7)	9.5(0.7)	9.5(0.7)
Hg	0.013(0.0004)	0.013(0.0004)	0.013(0.0004)	0.013(0.0004)	0.013(0.0004)	0.013(0.0004)	0.013(0.0004)
Ho	0.72(0.04)	0.72(0.04)	0.72(0.04)	0.72(0.04)	0.72(0.04)	0.72(0.04)	0.72(0.04)
I	0.39(0.03)	0.39(0.03)	0.39(0.03)	0.39(0.03)	0.39(0.03)	0.39(0.03)	0.39(0.03)
Li	0.054(0.015)	0.054(0.015)	0.054(0.015)	0.054(0.015)	0.054(0.015)	0.054(0.015)	0.054(0.015)
La	35.5(1.7)	35.5(1.7)	35.5(1.7)	35.5(1.7)	35.5(1.7)	35.5(1.7)	35.5(1.7)
Lu	39.6(1.5)	39.6(1.5)	39.6(1.5)	39.6(1.5)	39.6(1.5)	39.6(1.5)	39.6(1.5)
Li	0.46(0.03)	0.46(0.03)	0.46(0.03)	0.46(0.03)	0.46(0.03)	0.46(0.03)	0.46(0.03)
Mn	681(13)	681(13)	681(13)	681(13)	681(13)	681(13)	681(13)
Mo	0.52(0.04)	0.52(0.04)	0.52(0.04)	0.52(0.04)	0.52(0.04)	0.52(0.04)	0.52(0.04)
N	1.26(0.01)	1.26(0.01)	1.26(0.01)	1.26(0.01)	1.26(0.01)	1.26(0.01)	1.26(0.01)
N%	13.8(0.6)	13.8(0.6)	13.8(0.6)	13.8(0.6)	13.8(0.6)	13.8(0.6)	13.8(0.6)
Nb	32(2)	32(2)	32(2)	32(2)	32(2)	32(2)	32(2)
Ni	2.6(0.1)	2.6(0.1)	2.6(0.1)	2.6(0.1)	2.6(0.1)	2.6(0.1)	2.6(0.1)
Na	59(2.7)	59(2.7)	59(2.7)	59(2.7)	59(2.7)	59(2.7)	59(2.7)
P	8.5(0.5)	8.5(0.5)	8.5(0.5)	8.5(0.5)	8.5(0.5)	8.5(0.5)	8.5(0.5)
Pb	1.08(0.08)	1.08(0.08)	1.08(0.08)	1.08(0.08)	1.08(0.08)	1.08(0.08)	1.08(0.08)
Re	0.77(0.24)	0.77(0.24)	0.77(0.24)	0.77(0.24)	0.77(0.24)	0.77(0.24)	0.77(0.24)
Rb	27(2.2)	27(2.2)	27(2.2)	27(2.2)	27(2.2)	27(2.2)	27(2.2)
S	0.47(0.006)	0.47(0.006)	0.47(0.006)	0.47(0.006)	0.47(0.006)	0.47(0.006)	0.47(0.006)
Se	0.88(0.05)	0.88(0.05)	0.88(0.05)	0.88(0.05)	0.88(0.05)	0.88(0.05)	0.88(0.05)
Si	10.2(0.3)	10.2(0.3)	10.2(0.3)	10.2(0.3)	10.2(0.3)	10.2(0.3)	10.2(0.3)
Sn	0.21(0.02)	0.21(0.02)	0.21(0.02)	0.21(0.02)	0.21(0.02)	0.21(0.02)	0.21(0.02)
Sr	6.0(0.2)	6.0(0.2)	6.0(0.2)	6.0(0.2)	6.0(0.2)	6.0(0.2)	6.0(0.2)
Te	1.28(0.5)	1.28(0.5)	1.28(0.5)	1.28(0.5)	1.28(0.5)	1.28(0.5)	1.28(0.5)
Ta	0.05(0.014)	0.05(0.014)	0.05(0.014)	0.05(0.014)	0.05(0.014)	0.05(0.014)	0.05(0.014)
Tb	0.64(0.05)	0.64(0.05)	0.64(0.05)	0.64(0.05)	0.64(0.05)	0.64(0.05)	0.64(0.05)
Ti	11.3(0.4)	11.3(0.4)	11.3(0.4)	11.3(0.4)	11.3(0.4)	11.3(0.4)	11.3(0.4)
Tl	0.42(0.006)	0.42(0.006)	0.42(0.006)	0.42(0.006)	0.42(0.006)	0.42(0.006)	0.42(0.006)
Tm	0.82(0.05)	0.82(0.05)	0.82(0.05)	0.82(0.05)	0.82(0.05)	0.82(0.05)	0.82(0.05)
U	2.25(0.12)	2.25(0.12)	2.25(0.12)	2.25(0.12)	2.25(0.12)	2.25(0.12)	2.25(0.12)
V	7.4(3)	7.4(3)	7.4(3)	7.4(3)	7.4(3)	7.4(3)	7.4(3)
W	1.65(0.10)	1.65(0.10)	1.65(0.10)	1.65(0.10)	1.65(0.10)	1.65(0.10)	1.65(0.10)
X	25.5(0.9)	25.5(0.9)	25.5(0.9)	25.5(0.9)	25.5(0.9)	25.5(0.9)	25.5(0.9)
Y	2.81(0.14)	2.81(0.14)	2.81(0.14)	2.81(0.14)	2.81(0.14)	2.81(0.14)	2.81(0.14)
Zn	60(5)	60(5)	60(5)	60(5)	60(5)	60(5)	60(5)
Zr	3.0(0.2)	3.0(0.2)	3.0(0.2)	3.0(0.2)	3.0(0.2)	3.0(0.2)	3.0(0.2)
%	65.5(0.012)	65.5(0.012)	65.5(0.012)	65.5(0.012)	65.5(0.012)	65.5(0.012)	65.5(0.012)
SrO ₂	13.8(0.11)	13.8(0.11)	13.8(0.11)	13.8(0.11)	13.8(0.11)	13.8(0.11)	13.8(0.11)
Al ₂ O ₃	4.17(0.03)	4.17(0.03)	4.17(0.03)	4.17(0.03)	4.17(0.03)	4.17(0.03)	4.17(0.03)
FeO	1.1(1)	1.1(1)	1.1(1)	1.1(1)	1.1(1)	1.1(1)	1.1(1)
MgO	1.20(0.03)	1.20(0.03)	1.20(0.03)	1.20(0.03)	1.20(0.03)	1.20(0.03)	1.20(0.03)
CaO	2.62(0.06)	2.62(0.06)	2.62(0.06)	2.62(0.06)	2.62(0.06)	2.62(0.06)	2.62(0.06)
Na ₂ O	1.98(0.07)	1.98(0.07)	1.98(0.07)	1.98(0.07)	1.98(0.07)	1.98(0.07)	1.98(0.07)
K ₂ O	2.65(0.05)	2.65(0.05)	2.65(0.05)	2.65(0.05)	2.65(0.05)	2.65(0.05)	2.65(0.05)
H ₂ O	0.5(0.1)	0.5(0.1)	0.5(0.1)	0.5(0.1)	0.5(0.1)	0.5(0.1)	0.5(0.1)
CO ₂	0.9(0.1)	0.9(0.1)	0.9(0.1)	0.9(0.1)	0.9(0.1)	0.9(0.1)	0.9(0.1)
SiO ₂	1.70(0.06)	1.70(0.06)	1.70(0.06)	1.70(0.06)	1.70(0.06)	1.70(0.06)	1.70(0.06)
LOI	1.25(0.11)	1.25(0.11)	1.25(0.11)	1.25(0.11)	1.25(0.11)	1.25(0.11)	1.25(0.11)
TC	1.57(0.16)	1.57(0.16)	1.57(0.16)	1.57(0.16)	1.57(0.16)	1.57(0.16)	1.57(0.16)

Note: Data behind "±" are uncertainty; Data enclosed in brackets are reference values. Sb(DA) is result with aqua regia decomposing. Sb(T) is result of total quantity method.

Appendix G

Turbidity removal efficiency (%) with the use of technical grade chitosan and high quality grade chitosan after 15 minutes settling time





Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no