Norwegian University of Life Sciences
Faculty of Veterinary Medicine
Department of Food Safety and Infection Biology

Philosophiae Doctor (PhD)
Thesis 2018:48

Use of zirconium and chitosan coagulants for physicochemical and hygienic water treatment

Ekaterina Christensen

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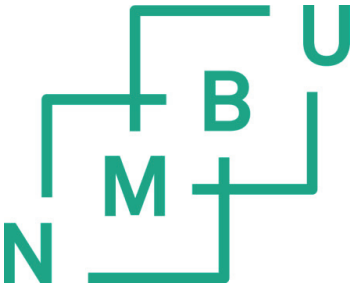

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Acknowledgement

Turning back, I realize how this project evolved from something abstract and alien to something my heart “burns” for. I already anticipate holding a hard copy of this thesis in my hands to close this chapter, and to move further to the next one. But before it happens, I would love to thank all, who were together with me during this journey.

The biggest thanks go to my supervisors. First of all, to Tor Håkonsen, a person who initiated this project. Tor, you did not hesitate to delegate this project to me, you “saw” something in me, despite my scarce knowledge on water treatment (back-then). You are extremely ambitious and have an impressive belief in things you do. Knowledge I gained about water treatment, I learnt from you. I am incredibly happy that I have encountered you on the path of my life, as a mentor, boss and friend. I am looking forward to work on more projects with you.

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me travel to a foreign country far from home. I want to dedicate this thesis to you, I would not come so far without your support.
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List of papers

I. Christensen, E., Håkonsen, T., Robertson, L. J., Myrmel, M. 2016 **Zirconium and chitosan coagulants for drinking water treatment – a pilot study**. *Aqua- Journal of Water Supply: Research and Technology*, **65**(8), 635–644. http://doi.org/10.2166/aqua.2016.162

II. Christensen, E., Nilsen, V., Håkonsen, T., Heistad, A., Gantzer, C., Robertson, L. J., Myrmel, M. 2017 **Removal of model viruses, *E.coli* and *Cryptosporidium* oocysts from surface water by zirconium and chitosan coagulants.** *Journal of Water and Health,* **15**(5) 695-705. http://doi.org/10.2166/wh.2017.055

III. Christensen, E., Myrmel, M. 2017 **Coagulant residues influence on virus enumeration as shown in a study on virus removal using aluminium, zirconium and chitosan (***in press). Journal of Water and Health*

Abbreviations

Al, aluminium
BE, beef extract
DAF dissolved air flocculation
DAPI, 4',6-diamidino-2-phenylindole
DBP, disinfection by-products
DIC, differential interference contrast microscopy
DLS, dynamic light scattering
DOC, dissolved organic carbon
EDTA, ethylenediaminetetraacetic acid
F, filter capacity
Fa, acetylation degree
Fe, iron
G, genogroup
HPC, heterotrophic plate count
HS, humic substances
IFA, immunofluorescent antibody procedure
IMS, immunomagnetic separation
Me, metal
MW, molecular weigh
NTU, nephelometric turbidity unit
NV, norovirus
NOM, natural organic matter
Non-HS, non-humic substances
PACl, polyaluminium chloride
PE, person equivalent
PFU, plaque forming unit
pI, isoelectric point
POC, particulate organic carbon
QMRA, quantitative microbial risk assessment
RT-qPCR, quantitative reverse transcription PCR
SUVA-index, UV_{254} divided by DOC
THM, trihalomethanes
TOC, total organic carbon
TSS, total suspended solids
$UV_{254,}$ UV absorbance
Vis., visible light
WHO, World Health Organisation
WTP, water treatment plant
Zr, zirconium

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Summary

Use of aluminium and ferric salts for drinking water production is almost global. Beneficial aspects of their use are extensively recognized. However, factors, such as deteriorating water quality, health concerns and rising levels of environmental pollution, pose a series of requirements for compounds intended for water treatment in the future. Alternative coagulants often possess properties that are lacking for their traditional competitors or offer a more sustainable solution. Nevertheless, their practical introduction is often difficult, as it is associated with some risks, and, therefore, scepticism. To address doubts, explicit characterization of the new chemicals, acknowledgement of practical and economical experience from the water plants using them, can be essential. Two of these alternative compounds – zirconium (Zr) and chitosan – were investigated in the research described in this thesis. The present work attempted to highlight use of Zr and chitosan coagulants for drinking water treatment in Norway and acknowledge various aspects associated with their use and stimulate further interest in these two compounds.

In **Paper 1** Zr and chitosan were assessed, together with traditionally used polyaluminium chloride (PACl), for the ability to reduce particles and natural organic matter (NOM) in water samples. The experiments were performed on river water collected at various time-points and representing different water qualities. Within the optimal dose-pH ranges, the selected coagulants provided a reduction in colour and turbidity of over 80%. Often coagulation efficiencies for PACl and Zr coagulant were found to be equally high, however, under certain conditions both PACl and chitosan were outcompeted by Zr in terms of NOM reduction. Moreover, operation of a two-media filter with Zr coagulant took at least 5-7 hours longer than for PACl, whereas the load of dry solids on the filter was similar for both coagulants. The phenomenon was attributed to higher resistance of Zr flocs against shear forces. Although significantly less sludge was formed with chitosan, this coagulant did not provide the longest filter run. Overall, chitosan performance was often sufficiently high, and also functioned over a broad pH range without affecting the pH of the treated water.

In a course of further experiments described in **Paper 2**, the ability of PACl, Zr and chitosan coagulants to reduce amounts of microorganisms in water was assessed. Reduction of virus varied between 99.7 and 99.99%, with the highest performances demonstrated by PACl and Zr. The hygienic effect against bacteria was at least 99.997% regardless of coagulant, whereas Zr and chitosan reduced *C. parvum* oocysts by at least

99.9%. The study concluded that both Zr and chitosan provided adequate removal of microorganisms from surface water.

The results also revealed that the treatment conditions defined relative to effluent turbidity and colour, were also effective for microorganism reduction, but with some limitations. Although a linear relationship between microbial reduction and residual NOM could be established, assessment of effluent turbidity did not provide all information about the coagulant hygienic efficiency. Moreover, during filter operation, virus and bacteria required slower ripening and showed earlier breakthrough, unlike the turbidity parameter that is monitored online for filter efficiency assessment.

In general, larger microorganisms were removed to a greater extent, despite the filtration theory predictions, whereas influence of the isoelectric point (pI) on microbe retention in the filter could not be detected. The present study also reported the pI of the *Salmonella* Typhimurium 28B bacteriophage for the first time.

During the experimental work for **Paper 2**, the coagulants tended to interfere with plaque assay and reverse transcriptase quantitative PCR (RT-qPCR) for bacteriophage enumeration. In order to confirm this effect, an additional study was initiated, described in **Paper 3**. The interference appeared to be most marked in the water samples with high coagulant content (typically influent). However, artificially low titres of bacteriophage were also demonstrated for the samples, in which levels of coagulants were assumed to be significantly reduced (supernatant and effluent). For plaque analysis, the interference from coagulants was minimized by treating the water sample by beef extract. Regarding virus quantitation by RT-qPCR, the influence of coagulants varied significantly according to the RNA extraction kit used.

After optimization, plaque assay and RT-qPCR were applied to evaluate Zr and chitosan performance against viral pathogens, when combined with membrane filtration. The treatment efficiency was affected by initial raw water quality. High levels of particulate and organic contaminants and, consequently coagulant doses, were shown to enhance virus removal. Regardless of water quality, chitosan treatment, combined with filtration, provided the highest virus reduction. Compared to Al and Zr, chitosan also produced water with less particles. The study indicated that virus charge characteristics influenced on virus retention on the membrane, whereas an effect of particle size or hydrophobicity

was not confirmed. MS2 bacteriophage was shown to be a suitable model for pathogenic virus aggregation and retention behaviour.

Overall, the present study addressed several issues in an attempt to extend characterization of two alternative coagulants, Zr and chitosan. This information could assist in defining a niche in water treatment for these compounds. Zr can be advantageous for treatment of colour-rich water by direct filtration, as it presumably provides a long filter run, while demonstrating substantial NOM reduction. In turn, chitosan gives no metal residues in finished water, and in discharge and sludge, must be reduced. Both compounds can be used to achieve considerable microorganism reduction, required by hygienic barrier standards.

Sammendrag

Jernsalter og aluminium brukes over hele verden og er anerkjent for sine egenskaper. Økende humusnivå i vannkilder og økende miljøforurensning, skjerper imidlertid kravene til stoffer som er beregnet for vannbehandling. Alternative koagulanter har ofte egenskaper som vi ikke finner hos tradisjonelle koagulanter og tilbyr en mer bærekraftig løsning. Dette arbeidet evaluerer mange faktorer relatert til bruk av Zr- og kitosan-koagulanter for produksjon av drikkevann, noe som gir et verdifullt innblikk i deres egenskaper.

I **Artikkel 1** ble Zr og kitosan sammenlignet med tradisjonelt brukt polyaluminiumklorid (PACl) med hensyn på reduksjon av partikler og naturlig organisk materiale (NOM) i vann. Forsøkene ble utført på elvevann av forskjellig kvalitet. Under optimale betingelser ga de utvalgte koagulantene mer enn 80% reduksjon i farge og turbiditet. Koagulasjonseffektiviteten var ofte den samme for PACl og Zr, men under betingelser som i pilot-studien, utkonkurrerte Zr både PACl og kitosan med hensyn på reduksjon av NOM. I forhold til PACl forlenget Zr driften av et to-media filter med minst 5-7 timer, mens stoffbelastningen på filteret var lik for begge koagulantene. Fenomenet kan forklares med at Zr-flokker tåler større hydrodynamisk belastning. Selv om betydelig mindre slam ble dannet med kitosan, ga ikke denne koagulanten den lengste filtergangen. Samlet sett var effekten av kitosan ofte tilstrekkelig høy og kitosan fungerte også over et bredt pH-område uten å påvirke pH i det behandlede vannet.

I løpet av forsøk beskrevet i **Artikkel 2**, ble Zr, kitosan og PACl vurdert i forbindelse med reduksjon av mikroorganismer i vann. Evnen til å redusere virus varierte mellom 99.7 og 99.99% og den høyeste effekten ble funnet for PACl og Zr. Den hygieniske effekten mot bakterier var en reduksjon på minst 99.997%, uavhengig av koagulant, mens reduksjonen av *Cryptosporidium parvum* var på mer enn 99.9% for Zr og kitosan. Studien viste at både Zr og kitosan kunne gi tilstrekkelig fjerning av mikroorganismer.

Resultatene viste at behandlingsbetingelser, basert på turbiditet og farge i filtratet, som regel også ga en effektiv reduksjon av mikroorganismer. Et lineært forhold mellom reduksjon i bakteriofag MS2 og *E.coli* og NOM ble etablert under drift av et to-media filter. På den andre siden, var ikke turbiditet en god indikator på hygienisk effekt av ulike koagulanter. Resultatene tydet også på at et filter bør modnes lenger, hvis virus og bakterier skal fjernes mest effektivt, men dette blir ikke indikert av online turbiditet. Virus

og bakterier kan også vise tidligere gjennombrudd enn partikler som overvåkes online ved hjelp av turbiditet.

Generelt ble store mikroorganismer fjernet mest effektivt mens en effekt av isoelektrisk punkt (pI) ikke kunne påvises. Studien inkluderte også en kartlegging av pI til *Salmonella* Typhimurium 28B bakteriofagen for første gang.

Under vurderingen av hygienisk effekt, viste koagulantene en tendens til å forstyrre kvantifisering av bakteriofager ved hjelp av plakktelling og RT-qPCR. Denne effekten ble ytterligere beskrevet i **Artikkel 3**. Interferensen var tydeligst i vannprøver med høyt koagulantinnhold (innløp). Kunstig lavt virus-titer ble imidlertid også påvist i prøver med redusert mengde koagulanter (supernatant og filtrat). For plakk-analyse ble interferensen minimert ved å behandle vannprøvene med biffekstrakt, mens viruskvantifisering ved RT-qPCR varierte i henhold til metode for RNA-ekstrahering.

Etter metodeoptimalisering, ble Zr- og kitosan behandling kombinert med et membranfiltreringstrinn testet for reduksjon av MS2 og patogene virus i drikkevann. Effekten syntes å være påvirket av råvannskvalitet: høyt nivå av partikulær og organisk forurensning, og følgelig høye koagulantdoser påvirket virusreduksjonen positivt. Uavhengig av vannkvalitet, ga kitosanbehandling, kombinert med filtrering, høyest virusreduksjon. Sammenliknet med Al og Zr, produserte kitosan også vann med mindre partikler. Studien viste at virusladning påvirket virusretensjon på membranen, men det var ingen effekt av partikkelstørrelse eller hydrofobicitet. MS2 virus fungerte som en passende modell for aggregering og retensjon av forskjellige patogene virus.

Denne studien bidrar til karakterisering av to alternative koagulanter, Zr og kitosan, og indikerer at disse kan ha en nisje i vannbehandling. Siden Zr kan forlenge filterdriften, kan Zr være fordelaktig for behandling av fargerikt vann ved direkte filtrering, selv om Al gir en like høy fargereduksjon. Kitosan kan anbefales for vannverk, som bruker membranfiltrering. Kitosan er også fordelaktig der bruk av metallbaserte koagulanter ikke kan anbefales på grunn av høye nivåer av metallrester i renset vann, utslipp og slam fra anlegget. Begge forbindelsene kan anvendes for å oppnå en reduksjon av mikroorganismer som er høy nok til å utgjøre en hygienisk barriere.

For reasons I have yet to understand, many people don't like chemicals...personally, I am quite comfortable with chemicals, anywhere in the universe. My favourite stars, as well as my best friends, are all made of them.

Astrophysics for people in a hurry
Neil deGrasse Tyson

1 Introduction

1.1 Water contaminants

1.1.1 Inorganic particles and organic colloids

Natural waters contain a wide variety of impurities, which include both naturally occurring substances and compounds from human activities. These constituents vary in size, density, shape, and surface properties, which define their mode of action in natural waters and during treatment processes.

Particles larger than 50 µm are usually visible as discrete objects by the unaided eye. Particles in the size range 1-1000 nm are known as *colloids*. For colloids, diffusion, i.e. Brownian motion, dominates over gravitation, preventing them from settling [1]. Very small particles (around 20 nm or less) are of a size similar to dissolved macromolecules. The line between *particulate* and *soluble matter* is rather arbitrary, but many researchers refer to compounds below 0.22-0.45 µm as soluble.

In raw waters, the inorganic particles arise mainly from natural weathering and land erosion processes and include clays, various oxides, silica and other minerals, which are relatively insoluble in aquatic environment. Their size range may vary from nano- to microscale (Figure 4). Levels of particulate matter are assessed with *total suspended solid* (TSS) parameter, which ranges typically between 2 and 200 mg/l in most water sources to 50 000 mg/l in flooding rivers [2].

Organic substances occur in water as a result of decomposition of organic materials from plants, animals and microorganisms. These substances are collectively known as natural organic matter (NOM) [3]. Natural organic matter has approximately 50% carbon by weight, and the organic carbon species found in natural water are often referred to as total organic carbon (TOC) [4]. Total organic carbon can be found in natural waters in two fractions, particulate (POC) and dissolved (DOC). The pool of POC (>0.45 µm) may include bacteria, algae, zooplankton, and organic detritus, but usually it is a small fraction (<10% for non-eutrophic waters) of TOC compared to DOC [5]. Dissolved organic carbon levels range between 0.1 and 115 mg/l, with 5.75 mg/l being reported as a global average for streams [2].

Dissolved organic carbon can be further divided into humic (HS) and non-humic (non-HS) substances [4], where the proportion of HS is often predominant [5]–[7]. Humic substances

are broadly defined as organic compounds of aromatic nature, resistant towards further biodegradation [4]. In general, the presence of natural organic matter (NOM) in drinking water is undesirable due to several reasons, described in Table 1.

The HS are usually categorized into three main fractions: humic acids, fulvic acids, and humin, based on their solubility. A model structure for aquatic HS is shown in Figure 1.

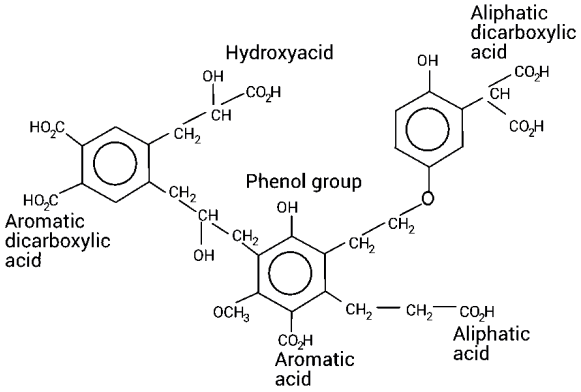

Figure 1. Model structure of aquatic HS. Adapted from Matilainen et al. [8].

All other fractions of NOM other than HS, can be referred as non-HS. Non-HS are essentially *hydrophilic* compounds, i.e. water-soluble macromolecules, whereas HS, in contrast, are mostly *hydrophobic* in nature or insoluble in water. Low charge and MW (molecular weight) of non-HS organic compounds prevent them from the removal by chemical treatment [6].

The amount, character and properties of NOM differ considerably in waters of different origins and depend on the biogeochemical cycles of the surrounding environments [8]. The properties of NOM are also affected by seasonal variations, especially for surface waters [3]. The levels of organic matter has increased in natural waters over the past few decades [9]–[11]. The main drivers for the observed increase are still up for debate [4]. There are several environmental and health concerns associated with the trend, especially with regard to its impact on drinking water [4]. Due to water quality problems and stricter regulations for drinking water treatment, there is a need for more efficient, but still economical, methods for the removal of NOM [8].

Table 1. Physicochemical water contaminants and parameters used for their assessment.

Water contaminant	Concerns	Assessment parameter	Measurement	Application in water treatment
Inorganic particles	• add cloudiness to water at concentrations of few mg/l; • shelter microorganisms from inactivation by disinfectants; • provide sorbent sites for pesticides, other synthetic organic chemicals and heavy metals	TSS	• dry weight	assess amount of suspended matter in raw water
		Turbidity	• particles ≥0.01 μm • loss of intensity of transmitted light, measured in nephelometric turbidity units (NTU),	indicates raw and finished water quality and efficiency of treatment steps; serves as an approximate indicator of pathogen removal
		Particle counts	• particles ≥2 μm passing singly through the sensing zone	convenient for clean waters (0.1-0.5 NTU) and more accurate than turbidity
NOM	• affects colour, taste and odour of water; • reacts with disinfectants and reduces their effect; • reacts with chlorine and produces disinfection by-products; • increases corrosion and growth of bacteria in a distribution system; • affects coagulant dosage	TOC/DOC	• total organic carbon	indicates raw and finished water quality and efficiency of treatment steps
		UV_{254}	• aromatic and hydrophobic humic acids (absorption of UV light)	
		Colour	• absorption at wavelength between 410 and 420, and 450 and 465 nm	

Based on the information from Pagano et al. [4]; Matilainen et al. [8]; Pernitsky [12]; Ødegaard et al. [13]; Hargesheimer et al. [14]; Bratby [15]; Gregory [1].

1.1.2 Assessment of physicochemical water quality

Various analytical measurements are used to assess water quality. Table 1 lists contaminants of concern for drinking water production together with physicochemical parameters, used for their assessment.

1.1.3 Microorganisms

Surface waters contain microorganisms, which can be natural inhabitants or faecal pathogens that enter a water source as a consequence of contamination by land runoff, bird droppings or sewage (Figure 2).

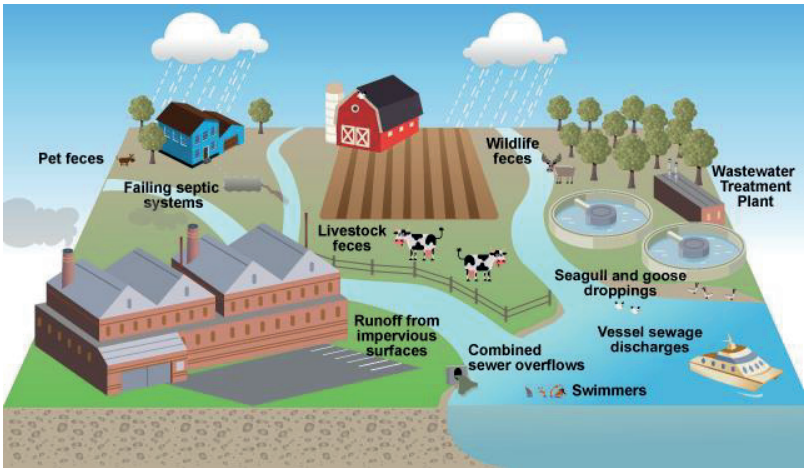

Figure 2. Sources of faecal contamination in water. Copied from http://www.microbe.com with permission.

Some waterborne pathogens are zoonotic (originate in animals and infect humans), therefore, contamination of a water source with faeces from birds, wild and farm animals is concerning [16]. However, the most common source of microbial contamination in water is human waste. Untreated or inadequately treated sewage, combined sewer overflows, and septic tanks are common sources of waterborne pathogens [17], [18], especially during extreme rainfall events [19]. The pathogens may stay infectious in water for long periods and enter water supply systems. Transmission of these infectious agents to humans occurs via consumption of insufficiently disinfected drinking water. Post-treatment contamination in a distribution system for drinking water is the other path for pathogens to reach consumers. An infected person is a potential source for further transmissions upon contact with non-infected individuals. Indirect passage is also possible in recreational waters during bathing.

Waterborne pathogens are linked to a significant disease burden worldwide. The World Health Organisation (WHO) reports that at least 1.8 billion people get drinking water from a water source that is contaminated with faeces. Faecally contaminated drinking water is estimated to cause 502 000 diarrhoeal deaths each year (www.who.int), and the situation is most critical in developing countries.

Statistical data on waterborne outbreaks in the Nordic countries in the period between 1988 to 2012 [20], [21], rated *Campylobacter,* and caliciviruses (family *Caliciviridae*), as the most frequently involved (Figure 3). In turn, the largest outbreaks, affecting most people, were caused by caliciviruses (mostly norovirus), and by *Giardia* and/or *Cryptosporidium.*

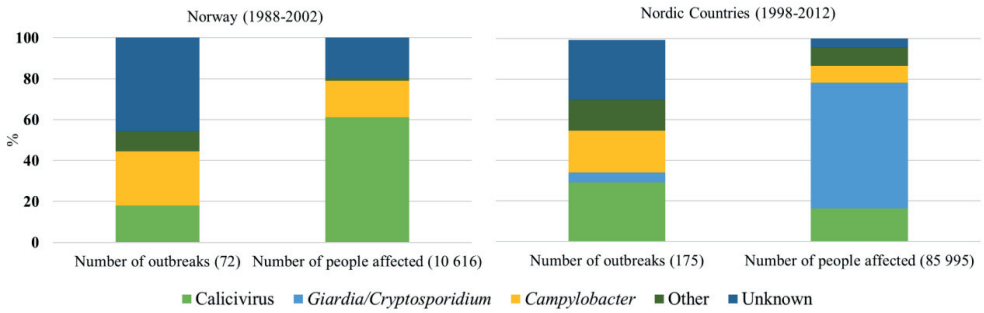

Figure 3. Pathogens implicated in waterborne outbreaks and number of people affected in Norway in the period 1988 to 2002 [21] and in the Nordic countries in the period 1998 to 2012 [20]. Total number of outbreaks and individuals are provided in parenthesis.

Waterborne pathogens that are common in Europe and their main characteristics are listed in Table 2.

Most of these pathogens cause self-limiting gastroenteritis in humans. However, long-term sequelae are not rare for some pathogens (e.g., reactive arthritis, haemolytic uremic syndrome, Guillain-Barré syndrome). Gastroenteritis can be especially dangerous to elderly, infants, pregnant women, and to persons with compromised immune systems.

Failure to provide safe drinking water may result in infectious diseases outbreaks, which may affect a large number of persons in a very short time. Therefore, the production of microbial safe drinking water is based on the use of multiple barriers in water treatment, i.e. a management strategy in which failure of one barrier does not lead to failure of the system as a whole. Water treatment with multiple *hygienic barriers* incorporates source water protection

Table 2. Common human waterborne pathogens in Europe, type of disease caused, their primary source and characteristics.

Pathogen	Disease	Primary source	Characteristics
Campylobacter jejuni	Gastroenteritis	Human and animal (poultry, cattle) faeces	Gram-negative bacterium, non-spore-forming, 4 μm in length
Norovirus (GI and GII genogroups)	Gastroenteritis		27-30 nm, naked virus, ssRNA
Rotavirus (A-C groups)	Gastroenteritis (most common in infants and young children)	Human faeces	70 nm, naked, dsRNA
Enteric adenovirus	Upper respiratory and gastrointestinal illness		70-75 nm, naked, dsDNA
Cryptosporidium* spp.** (C.parvum, C.hominis*** and other species*)*	Gastroenteritis	Human and animal (cattle, sheep, rabbits) faecal waste	oocysts are 2-5 μm in diameter, round to egg shaped dormant in the environment, but split open to release sporozoites, when ingested
Giardia *duodenalis (A and B genotypes)*		Human and animal (pets, livestock) faecal waste	exists as ovoid cysts (6-10 μm) in the environment
Toxoplasma gondii	Asymptomatic or flu like symptoms; chorioretinitis; severe encephalitis in the immunocompromised, and abortion or foetal abnormalities in primary exposures of pregnant women	Animal faeces (cats)	crescent-shaped and 2 x 6 μm in size; exists as dormant oocysts in the environment; not infectious immediately upon excretion

Based on the information from Christensen [22], Marshall et al. [23], Smith Jr. & Perdek [24], Gorchev & Ozolins [25], Jones and Dubey [26].

against contamination and appropriate treatment and disinfection processes, as well as protection during storage and distribution [25].

1.1.3.1 Campylobacter

Campylobacter, particularly *Campylobacter jejuni,* is an important cause of diarrhoea worldwide [27]. Variety animal species, including poultry, wild birds, cattle and sheep serve as potential reservoirs of *C. jejuni* [28].

Fewer than 1 000 organisms may induce infection [29]. After an incubation period of 1-4 days, the infected person develops stomach pain, diarrhoea, chills, fever and abdominal cramping [30]. Usually, the symptoms relieve after 3-7 days.

Rare, but severe complications of Guillain-Barré syndrome and reactive arthritis are reported to occur in 1 to 5% of campylobacteriosis cases [31], [32].

Survival of *C. jejuni* outside the gut of warm-blooded animals is poor, and replication does not occur readily [30]. Moreover, *C. jejuni* usually displays low resistance to free chlorine or ultraviolet radiation [33], and should be readily inactivated by adequate water treatment. Nevertheless, waterborne outbreaks with *Campylobacter* in Scandinavia are one of the most prevailing [20]. The registered outbreaks of campylobacteriosis are primarily associated with untreated drinking water served by community or private water systems [20], distribution system deficiency [34], or contamination of storage facilities [35].

1.1.3.2 Enteric viruses

Viruses are infectious agents of 20 to 350 nm size that replicate only inside a host cell. They consist of a genome (either RNA or DNA) packed inside a protective protein capsid, while enveloped viruses also have a lipid bilayer surrounding the capsid. In general, viruses are host specific (non-zoonotic), and infect only specific types of cells within a host.

Viruses that are excreted through the gastrointestinal tract are known as *enteric viruses*. Consequently, enteric viruses are present in large numbers in domestic wastewater [36]. Commonly studied groups of enteric viruses belong to the families *Picornaviridae* (polioviruses, enteroviruses, coxsakieviruses, hepatitis A virus, and echoviruses),

Adenoviridae (adenoviruses), *Caliciviridae* (noroviruses, sapoviruses), *Astroviridae* (astroviruses), and *Reoviridae* (reoviruses and rotaviruses) [37].

Although viruses, such as influenza- and coronaviruses, are not considered waterborne pathogens, as their major mode of transmission is through respiratory secretions and person-to-person contact, sewage has been suspected as the vehicle of their transmission as well (WHO, 2006). In addition water is a potential source of infection with influenza- and coronaviruses for birds and other non-human species [39], [40].

Worldwide*, noroviruses (NV)* are associated with 680 million diarrhoea cases, many of which are waterborne, and 212 000 deaths annually. Approximately 99% of the lethal cases occur in middle- and high-mortality countries. According to these estimates, norovirus is the most common cause of diarrheal cases across all ages [41]. Global economic costs of the norovirus burden are assessed by US $4.2 billion in direct health system costs and US $60.3 billion in societal costs, annually [42].

Noroviruses are non-enveloped, single-stranded RNA viruses, 27–32 nm in diameter. At least 7 norovirus genogroups (G) have been recognized from phylogenetic analyses of the capsid protein. GI, GII, and GIV are found in humans, GII viruses have been also detected in swine, GIII viruses infect cows and sheep, GV infects mice and rats, and GVI and GVII infect canine species. GI and GII viruses are responsible for the majority of disease in humans [43].

Faecal concentrations of NV reach 8-10 $\log_{10}$ viral particles per gram of stool. Viral shedding may continue until 2 weeks after symptoms resolve [44]. Norovirus is extremely contagious, and estimates show that infection occurs after ingestion of as few as 18 viral particles [45]. Like other small, non-enveloped viruses, NVs can be relatively persistent in the environment. However, commonly used doses of chlorine and UV, during water treatment, are usually effective for virus inactivation [46].

Symptoms of NV infection usually develop within 24–48 h after exposure and are characterized by acute onset of nausea, vomiting, abdominal cramps, myalgia, and non-bloody diarrhoea. Full recovery occurs within 2–3 days. In volunteers, subsequent exposure to virus 6–14 weeks later did not cause infection [47]. Recent studies have suggested that immunity to NV may last for at least 2 years [48].

A signature of NV is the genetic diversity and rapid, immune selection driven evolution [41]. Lack of an animal model and inability to cultivate NV until recently [49], has hindered understanding of pathogenesis mechanisms for this agent, and development of vaccines. In context of water research, these factors also limit broader characterisation on NV persistence in water environment, and removal by the water treatment methods.

1.1.3.3 Cryptosporidium spp.

Cryptosporidium is a one-celled apicomplexan parasite. Various *Cryptosporidium* spp. infect mammals, and nearly 20 *Cryptosporidium* species and genotypes have been identified in humans (Table 3). Livestock, particularly cattle, is one of the most important reservoirs of zoonotic *Cryptosporidium* [50].

Table 3. *Cryptosporidium* spp. commonly reported in humans and their major hosts. Adapted from Ryan et al. [50].

Species	Major host
C. muris	Rodents
C. canis	Dogs
C. hominis	Humans
C. cuniculus	Rabbits and humans
C. melegridis	Birds and humans
C. parvum	Ruminants and humans
C. ubiquitum	Ruminants, rodents, primates
C. felis	Cats

At least 64 million food- and waterborne diarrheal cases worldwide were associated with *Cryptosporidium* spp. in 2010 [27], most of them involved *C. hominis* and *C. parvum*. Waterborne outbreaks with *Cryptosporidium* spp. are often massive, as one in Milwaukee, USA, which affected over 400 000 residents [51]. Two large outbreaks with *C. hominis* took place in Sweden in 2010–2011 [52]. In both cases, inadequately treated water was a main source of the pathogen. To date, no community-wide waterborne outbreak of cryptosporidiosis has been reported in Norway.

Infected people and animals can shed up to 10 billion *C. parvum* oocysts per gram of faeces [53]; a median infectious dose is about 132 oocysts [54]. Once ingested, oocysts release sporozoites in the intestine, which go through several developmental stages [55].

Symptoms of cryptosporidiosis generally appear 2 to 10 days (average 7 days) after infection, followed by development of watery diarrhoea. Symptoms usually last about 1 to 2 weeks. Occasionally, people may experience a recurrence of symptoms after a brief

period of recovery, but symptoms can come and go for up to 30 days (https://www.cdc.gov).

The mature oocyst excreted from the body of an infected host possesses a tough trilaminar wall, resistant to harsh environment and commonly utilized disinfection techniques [56]. Chlorine dosages routinely used in water treatment to kill bacteria are generally considered as non-effective against oocysts [57]. In contrast, UV and ozone are considered as effective disinfectants for *Cryptosporidium* [58].

Development of drug treatments and vaccines against cryptosporidiosis has been unsuccessful so far.

1.1.3.4 Indicators and model organisms

Routine detection of waterborne agents in raw or treated water is often complex due to their high diversity, low concentrations, and demanding detection methods. Traditionally, physicochemical parameters and microbial indicators have been used to indicate a possible presence of faecal pathogens in water (Table 4).

Elevated levels of pollutants in the influent may be associated with pathogen presence [59]. Therefore, real-time monitoring of feed water for turbidity and TOC (alternatively, colour and UV_{254} measurements) has been widely used to assess the condition of a water source and track constant or sporadic contamination events. This easy and cheap technique allows preventive actions to be implemented at the early stages of the water production. Detection of faecal indicators, on the other hand, is more time consuming and not performed daily.

In the effluent water, physicochemical parameters and process indicators are used routinely to assess the efficacy of treatment. It has been assumed that if suspended matter (turbidity and particle count) or microbial indicators are reduced to a certain level, this assure high removal of microorganisms of a similar size, i.e. protozoa and large bacteria. Indeed, many studies have confirmed this experimentally [60]–[62]. Pathogen reduction can be further improved when the process is optimized for both reduction of turbidity and NOM [60]. Thereby, enhanced coagulation can be expected to provide increased hygienic protection that might be especially convenient for smaller colloids, such as viruses [63], [64].

Table 4. Common microbial indicators and model microorganisms in water treatment practice.

Indicators	Indicator value	Primary sources	Characteristics
Process indicators			
Heterotrophic plate count (HPC)	efficacy of treatment and disinfection (for bacteria);	naturally present in the environment	all bacteria that use organic nutrients for growth (incl. total coliforms and *Escherichia coli*)
Total coliforms	cleanliness and integrity of distribution systems and potential presence of biofilms	widely found in the environment; human and animal faeces	gram-negative, facultative anaerobic, non-spore-forming
Faecal indicators			
Escherichia coli	recent faecal contamination	human and animal faeces	gram-negative, facultative anaerobic, non-spore-forming
Intestinal enterococci	faecal contamination; more resistant to environmental conditions and disinfection than *E.coli*	part of normal intestinal flora of human and animals, but less common, than *E.coli*	gram-positive, spore-forming, strictly anaerobic
Clostridium perfringens spp. including spores	highly specific for faecal pollution; spores have been proposed as indicators of protozoa		
Model organisms			
Coliphages	faecal contamination; behaviour of enteric viruses in water and their response to treatment and disinfection	human and animal faeces; somatic coliphages might also replicate in water environment	somatic coliphages attach to receptors permanently located on the cell wall of hosts; F-RNA coliphages attach to F-fimbriae on *E.coli* host

Based on the information from Ashbolt et al. [65], Edberg et al. [66], Allen et al. [67], Gorchev & Ozolins [25].

Turbidity and particle count are extremely useful for monitoring critical phases of filter cycle dynamics (ripening period and onset of breakthrough), during which the reduction of microbes is impaired or absent [68]. In a WTP (water treatment plant), online turbidity measurements are often used to determine whether a filter needs to be backwashed or ripened a bit longer.

In turn, several full-scale experiments have revealed that drinking water nearly free from measurable particles is not necessarily free from virus [69] or protozoa [70]. Moreover, well-performing plants can still demonstrate different microorganism removal [68], even if the all the other parameters are rather the same. Finally, turbidity may not be completely accurate in predicting microorganism breakthrough [71].

Estimation of health risks through microbial indicators are also quite ambiguous [72]. Different pathogens are unlikely to behave or persist like a single indicator group [65], as has been confirmed for viruses [69], [73] and parasites [74].

Therefore, scientists generally agree that physicochemical and microbial indicators are approximate, but not quantitative, indicators of pathogen removal.

Viruses that infect bacteria are known as *bacteriophages* or simply as *phages* [65]. Bacterial viruses are abundant in sewage and can be detected by simple and inexpensive techniques [75], [76]. In addition, they have structural and morphological similarities to human enteric viruses and phages have been proposed as better indicators of faecal pollution in raw water, than indicator bacteria [77]. Phages also demonstrate low sensitivity to common disinfectants [78], [79], and are indicators of potential presence of harmful viruses in the finished water.

Tailed somatic coliphages is a commonly used parameter for water quality assessment [80]. Other extensively used bacterial viruses are F-specific RNA phages, such as MS2 and Qβ, which resemble human enteric viruses due to their naked icosahedral capsid of 21–30 nm size [81]. Use of F-specific RNA phages is also advantageous as they are not likely to replicate in the environment [82].

As for all indicators, bacteriophages are not able to replace direct measurement of enteric viruses [83], which may explain their restricted application. However, phages have been extensively used as models for reduction of enteric viruses under controlled conditions [84].

For protozoa oocysts, spores of anaerobic *Clostridium perfringens* or *Bacillus subtilis* have been proposed as suitable indicators, or model organisms, due their high resistance to adverse environmental conditions [61], [80] or chlorine [57]. However, their suitability is still controversial and discussed in the literature.

1.2 Removal of contaminants in drinking water production

The presence of impurities in natural waters is not desirable due to aesthetic reasons and human health issues. The objective of a water supply utility is to reduce the amount of these impurities below the criteria, set by regulations. Source water quality and the water quality standards define the number and operation of treatment unit processes.

Figure 4 illustrates typical contaminants, their size range and suitable water treatment methods for their removal.

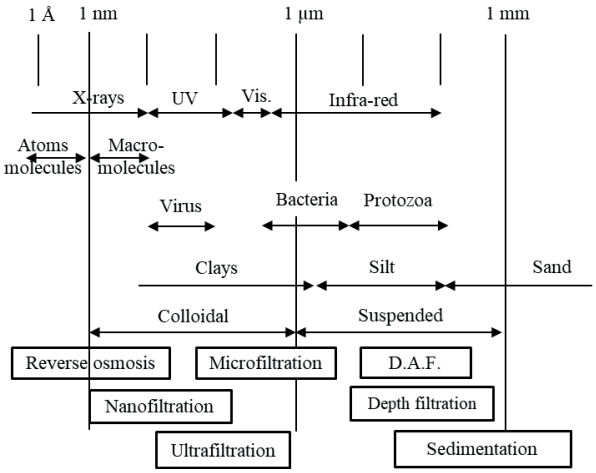

Figure 4. Diagram showing size ranges of typical aquatic particles and appropriate separation processes for their removal. Vis. - visible light; D.A.F - dissolved air flotation. Adapted from Gregory [1].

In principle, impurities are removed by sedimentation, flotation, or filtration. However, due to the small size and stabile state of the particles, direct application of these processes may not be effective or economically feasible. Therefore, initial destabilization and aggregation of impurities are often needed. Destabilization occurs by means of coagulation, which is discussed in Section 1.4. At the final steps of treatment, water is disinfected chemically (e.g. with chlorine or ozone) or by UV-inactivation.

1.3 Water supply in Norway

More than half (73%) of primary waterworks in Norway are small and serve communities with less than 1 000 PE (person equivalent). The largest plant, Oset in Oslo, provided at least 635 000 PE with drinking water in 2014.

Surface water, from mostly lakes, is the main water source suppling 61% of Norwegian WTPs and is served to almost 90% of the Norwegian population. This is the highest percentage among the Nordic countries, which use groundwater more extensively [20]. Due to minor anthropogenic pressure, water sources in Norway are generally of good quality. As a result, the majority of plants have a simple design. The number of plants using coagulation is relatively low (13%), but not in the percentage of the served population. Water production, including coagulation, flocculation, sedimentation and filtration steps, also called *conventional treatment*, is applied by the largest Norwegian plants. The majority of the WTPs using chemical treatment, exploit *contact* filtration (flocculation and sedimentation excluded), or *direct* filtration (sedimentation excluded), which are more cost-effective.

Ten percent of Norwegian WTPs do not treat (except screening, aeration or pH control) or disinfect water prior to consumption. These WTPs are primarily located in small communities and serve groundwater or surface water with low level of contaminants.

1.4 Coagulation

Coagulation is a complex chemical process that enables destabilization of the particulate and dissolved material. *Flocculation* is a physical process whereby the destabilized particles are impelled to come together, make contact, and thereby produce agglomerates (flocs). The compounds applied for coagulation in water treatment are referred to as *coagulants*. In turn, the substances applied after coagulants to enhance floc formation are defined as *flocculants* or *coagulant aids*. Traditionally, sulphate or chloride salts of the trivalent metals Al^{3+} or Fe^{3+} and synthetic organic polymers have been used for coagulation and flocculation processes, respectively [15].

The terms *coagulation* and *flocculation* are, however, not universal. In the present work, coagulation is referred to as a mechanism that impels both particle destabilization and aggregation (floc formation), induced however not by flocculants, but, as a result, of destabilized particle interaction.

1.4.1 Destabilization mechanisms

Destabilization by coagulants is accomplished through several mechanisms, which are *double-layer compression, adsorption destabilization, bridging* and *sweep floc* mechanisms.

1) Double-layer compression

Particles and colloids found in water, develop a net surface charge. Essentially, surface charge occurs when surface groups, such as -OH, $-NH_2$ or –COOH, accept or donate protons (H^+) from the solution. This charge influences the distribution of nearby ions in the media (Figure 5), and the particle gains a firmly attached layer of the counter-ions around its surface, followed by a diffuse layer of other counter-ions, held further away, but still in the vicinity of the particle. Together, these layers form an *electrical double layer* [85], [86].

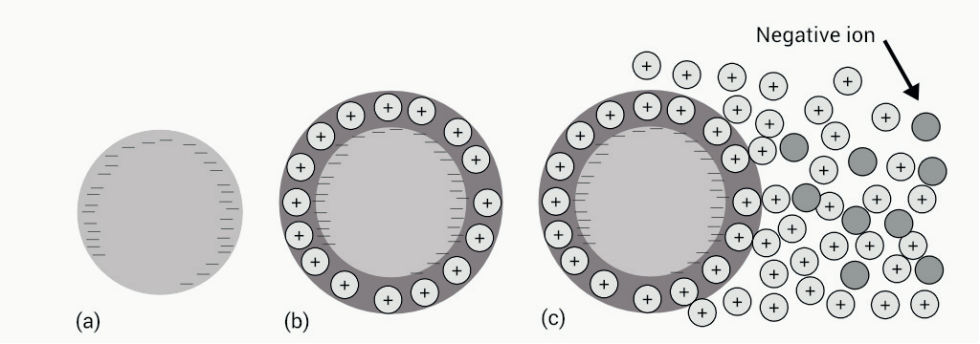

Figure 5. Colloidal particle showing: (a) negatively charged colloid surface; (b) colloid with positive counter-ions comprising fixed layer; and (c) diffuse layer. Adapted from Hendricks [87].

One essential characteristic of the double layer is the "thickness" of the diffuse region. At low ionic strength, the diffuse layer stretches far out into the solution, and, in extreme cases, such as in completely deionized water, may reach 1 000 nm [1]. In this case, when two similarly charged particles approach each other, they will repel due to overlap of their diffuse layers. Repulsion keeps the particles dispersed, or in other words, stable in the solution.

Addition of an electrolyte induces an increase in the ionic strength of the solution and effective concentration of counter-ions in the vicinity of the charged colloid. This compresses the thickness of the diffuse layer and subsequently the range of the repulsive forces, allowing particles to move into contact (Figure 6). The size of the formed

aggregates usually exceeds the colloidal range above which sedimentation is likely to take place [15], [85].

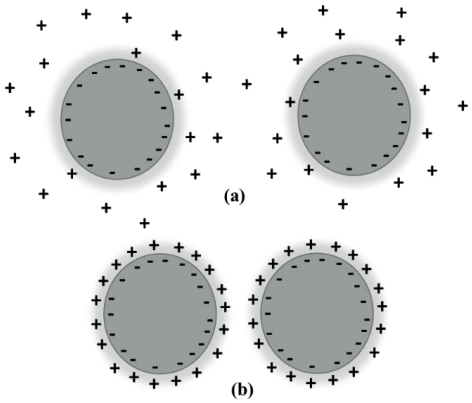

Figure 6. Schematic picture of the effect of ionic strength on the range of double-layer repulsion (a) before and (b) after electrolyte addition. Adapted from Gregory [1].

2) Adsorption-charge neutralization

Compounds used in water treatment differ from electrolytes, like NaCl. Aluminium and Fe compounds undergo rapid hydrolysis upon reaction with water. As a result, the monomeric (perhaps polymeric) hydrolysis products, such as $Fe(OH)^{2+}$, $Al(OH)_2^+$, $Al(OH)_3^o$, $Fe(OH)^{4-}$ are formed. Hydrolysed metal ions are readily adsorbed at interfaces. Adsorption of charged hydrolysis species to the particle surface of the opposite charge reduces its surface net charge, and thereby the extent of double-layer repulsion between adjacent particles [88].

In the double-layer theory, the amount of the counter-ions required for destabilization is strongly dependent on their charge. According to the Schulze-Hardy rule this dependence is proportional to the inverse power of 6 of the counter-ion charge. This rule is frequently cited to account for the fact that trivalent Al^{3+} and Fe^{3+} ions are several times more effective than Na^+ for the destabilization of negative colloids. However, this behaviour is not often found experimentally, presumably due to the fact that the availability of "free" Al^{3+} and Fe^{3+} is limited by hydrolysis reactions, as discussed here [89].

3) Sweep floc – precipitate enmeshment

During sweep floc coagulation, large amount of hydroxide precipitates, $Al(OH)_3$ and $Fe(OH)_3$, are produced by the excess of coagulant ions in the solution. The colloidal

particles are enmeshed in the precipitate as it forms, and subsequently removed from the suspension by settling [90].

4) Interparticle bridging

Interparticle bridging occurs, when a polymer spans the gap between surfaces of two or several conjunct colloids, bridging them together, and thereby promoting destabilization (Figure 7).

The bridging mechanism is common for synthetic organic polymers (flocculants). However, some authors postulate that bridging also occurs with coagulant polynuclear species [15], [91]. Moreover, bridging and patching are believed to be important destabilization mechanisms for coagulants of polymeric nature, such as chitosan.

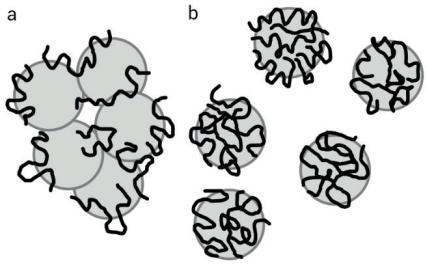

Figure 7. Schematic picture of (a) bridging flocculation and (b) restabilisation by adsorbed polymer chains. Adapted from Bolto & Gregory [2].

Another destabilization mechanism associated with coagulants of polymeric nature is *electrostatic patch* adsorption. During electrostatic patch adsorption (Figure 8), the polymer molecule is adsorbed in a relatively flat configuration on the particle surface to give a "mosaic pattern". A local excess of positive charges is introduced while the rest of the surface is free of polymer and carries the original negative charge. Regions of opposite charge are attracted between adjacent particles that end up clustering [92].

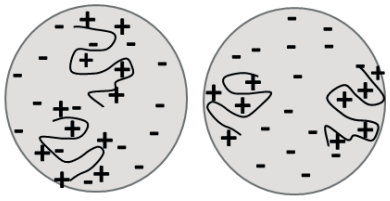

Figure 8. "Electrostatic patch" model for flocculation of negative particles by cationic polyelectrolytes. Adapted from Bolto & Gregory [2].

Often, there is no clear line between each destabilization mechanism; depending on the conditions, destabilization mechanisms may occur sequentially or overlap.

1.4.2 NOM destabilization mechanisms

NOM molecules display properties of both dissolved and colloidal matter. Edwards and Amirtharajah [93] suggested that destabilization of dissolved NOM includes a phase change i.e., complexation reactions with metal species lead to reduction of NOM molecule charge and thus solubility. NOM is thereby removed from solution by forming a solid metal humate complex, or by physical attachment of the colour molecules to the hydroxide flocs. The coagulation process modified to ensure good removal of organic matter is also known as *enhanced coagulation* [8].

1.5 Filtration

Filtration is an extensively used process in drinking water treatment for removal of particulate matter. In a general sense, *filtration* is a separation process between a fluid and its suspended matter by passage of the suspension through a porous medium [87]. The suspended matter, however, can be removed by two different ways - *depth filtration or straining*. Depth filtration is the separation process, in which the suspended particles, colloids, and particle aggregates pass through filter media, and are retained in the voids between the media granules. Normally these voids are considerably larger, than the particle size [94]. Straining describes the process of particle retention through media with pores finer than the particle size (e.g. membrane). Straining can be observed with granular filtration when the ratio of the suspended particle diameter to the grain diameter is greater than about 0.05 [95]. The next chapters present a general overview on depth filtration and rapid granular filters.

1.5.1 Filter operation

The operation of a granular filter over time follows a general pattern, determined by accumulation of the suspended matter inside the filter (Figure 9):

1) filter *ripening* - when the filter bed is clean, water of poor quality is produced due to restricted range of attraction forces between floc particles and bare filter grains [96];

2) *stable phase* - as more filter grains become covered with the flocs, there is a gradual increase in the effluent water quality;
3) *breakthrough* - at some point, the accumulation capacity of the filter is exceeded, and the effluent quality deteriorates. At this point, the filter should be cleaned by backwashing. The operating time between backwashes is called a *filter run*.

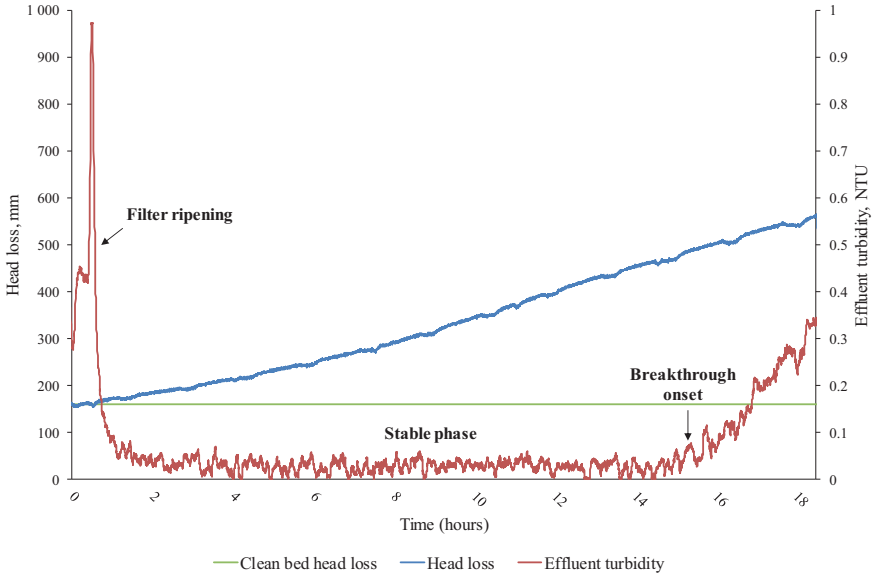

Figure 9. Effluent turbidity and head loss development over time for a rapid granular filter.

Difference in head across the filter bed is referred to as *head loss* [87]. Initial head loss in a clean filter is called *clean bed head loss*. Consequently, accumulation of suspended solids linearly increases overall head loss over time. Any deviation from linearity may indicate straining and *cake filtration*. In turn, *terminal head loss* is reached, when the water level above the filter exceeds the design value.

1.5.2 Removal mechanisms of granular media filtration

Theories describing the particle removal by depth filtration are within two approaches: *macroscopic* [97], [98] and *microscopic* [99], [100].

The macroscopic approach describes the dynamic behaviour of deep bed filters with a set of equations and model parameters that are essential for filter design. This approach does not take into consideration the complex relationship between the suspended colloids and granular media.

The microscopic approach intends to provide insights about the mechanisms of particle deposition in granular media [101]. It is postulated that retention of the suspended particles within a filter bed is usually preceded by two complementary events - particle transport in the vicinity of the filter corn and particle attachment to the corn surface. Consequently, deposited particles can be detached and dragged deeper into the filter bed, where they can reattach or be eluted with the effluent.

Depending on the size of the particles, their transport occurs by means of either *interception, settling* or Brownian diffusion forces (Figure 10). Interception occurs when a particle moving along a streamline comes into contact with the media corn. Gravitational sedimentation refers to the settling of particles onto the corn surface. Smaller particles undergo diffusion, which can result in random contact with the corn grains [102]. Diffusion is rather relevant for the particles smaller than about 1 μm, whereas larger particles are transported by interception and gravitation mechanisms. For colloids of intermediate size 1-3 μm, typically bacteria-sized particles, none the transport mechanisms is dominant, and they are believed to exhibit the lowest transport efficiency [100], [102].

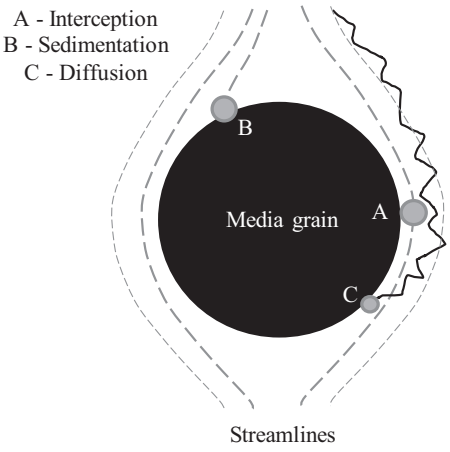

Figure 10. Transport mechanisms in water filtration. Adapted from Yao et al. [100].

The classical theory of colloidal stability predicts that adsorption of suspended particles to the filter media is primarily controlled by charge characteristics. The *attachment efficiency* between two interacting particles is balanced by the repulsive and attractive forces, and the process thereby regulated by the factors, such as the isoelectric point (*pI*,

explained in Section 2.5) of the particles, and pH and the ionic strength of the solution [103], [104]. Under favourable conditions, as in the destabilized state, the attachment rate is the highest [99]. Apart from that, factors like steric properties [105] and hydrophobicity [106], [107] may define the particle's attachment.

1.6 Common and alternative coagulants in water treatment

Salts of Al and Fe are the most commonly used coagulants in water treatment (Table 5), due to their high efficiency, good availability, low price, and detailed characterization.

Table 5. Commonly used coagulant products.

Type	Product	Coagulation conditions	
		pH	Dose[1]
Al-based	$Al_2(SO_4)_3$ $14H_2O$ (alum), $AlCl_3$	5.0-7.5	0.5 mg Al/mg C, pH 5.5 1.0 mg Al/mg C, pH 7.0
Fe-based	$Fe_2(SO_4)_3$, $FeCl_3$	4.0-6.5	1.0-1.3 mg Fe/mg C, pH 4.5 2.0 mg Fe/mg C, pH 5.8

[1] Based on information from Dennett et al [108] and Edzwald [5].

Among the major drawbacks of these coagulants are loss of the destabilization and cleaning effect at suboptimal coagulation conditions, followed by increased levels of non-reacted metal in the finished water. High metal residuals in water are associated with health hazards [109], [110], and hence regulated by health-based guidelines. Coagulation can also be impaired during periods of cold temperatures [111], Some of these problems are minimized for prepolymerised forms of coagulants [112]. These coagulants contain defined prehydrolysed species, which are less influenced by the pH and temperature of the treated water.

One of the most critical issues associated with operation of a coagulation plant is the amount of sludge generated. The sludge affects operation of the sedimentation basins, thickeners and filters, and requires handling and utilization.

The amount of sludge produced by each coagulant can be assessed, using an empirical equation for the hydrolysis reactions [85]:

$$Al^{3+} + 6H_2O = Al(OH)_3 \cdot 3H_2O\ (am) + 3H^+$$
$$Fe^{3+} + 3H_2O = Fe(OH)_3 \cdot 3H_2O\ (am) + 3H^+$$

According to these equations, each gram of Al or Fe was estimated to produce 4.9 and 2.9 g dry solids, respectively. Consequently, treatment of raw water with a moderate TOC content, i.e. 3 mg C/l, would require 1.5 g Al/m^3 or 3 g Fe/m^3 coagulant doses and result in formation of at least 7.5-9.0 g/m^3 of suspended solids in the influent. Hydroxide products are assumed to be the dominant components of the sludge. However, contributions from raw water DOC and TSS to the total amount of sludge should be taken into consideration as well. Therefore, the real amounts of the generated solids should be higher than theoretical estimates.

Due to high concentrations of metals, sludge generated with metal salts cannot be freely disposed to nature. Often, WTPs use high amounts of energy and chemicals for sludge handling, which affect the economy and waste management of the plant [113].

The search for alternative coagulants that are able to provide similar or higher treatment effects but can offer more sustainable and environmentally friendly water production, is highly relevant.

Various alternative coagulants, including *Moringa oleifera* seeds [114], alginates [115], corn starch [116], chitosan [117], lignin derivatives [118], compounds with tetravalent zirconium [119], and titanium [120] have been proposed for drinking water production. Two of these compounds – zirconium (Zr) and chitosan – were investigated in the research described in this thesis.

1.6.1 Zirconium

Zirconium (Zr) metal is a ubiquitous element on Earth. The first efforts to introduce Zr to water treatment field refer to US patent 4066542 [121], disclosing $ZrOCl_2 \cdot 8H_2O$ application on several types of waste and surface waters. Follow-up research has also investigated the effect on phosphorous [122], [123], chemical oxygen demand, total inorganic nitrogen [123] and ammonia [124] - parameters routinely monitored for wastewater, landfill leachate, and eutrophic waters. Recent studies in drinking water production have also explored the ability of Zr to reduce HS, turbidity [119], [125], and arsenic [126] in surface waters.

Some attractive properties of Zr are listed below:

1) low consumption dose - Zr is a tetravalent compound, with hydrolysis products carrying a high charge [127], [128] which is essential for particle destabilization. In theory, this implies a lower dose requirement for Zr coagulant to achieve similar removal effects, relative to trivalent agents. Several bench-scale studies have confirmed this experimentally. The work by Jarvis at al. [119] suggested a high DOC reduction already at 0.4 mg Zr/mg C dose, applied over the pH range of 5.0 to 7.0. In contrast, the work by Hussain at al. [125] proposed a ratio for Zr demand to raw water DOC within 0.6-1.2 mg Zr/mg C range for the pH between 4.5-5.1. This is somewhat higher, than suggested for Al in Table 5, but lower than for Fe.

2) enhanced DOC/TOC reduction – the literature describes Zr's advanced ability to reduce DOC in NOM rich waters. Up to 20-25% less DOC has been detected after Zr treatment, as compared to alum [129] or ferric compounds [119]. The phenomenon is likely explained by a high affinity between NOM species and Zr's charged hydrolysis products [119], [125], [130]. Moreover, Zr is claimed to be especially efficient for the removal of low to medium MW range organic compounds (<2 000 Da) that are resistant to coagulation [125]. As discussed earlier, residual DOC is undesirable in the finished water due to the risks of DBP formation and the distribution system integrity.

3) decreased sludge volume - reduced coagulant consumption gives a decreased solid load on the filter, so that extended filter runs and less backwash water could be expected. Considering the reactions of Zr in the coagulation process, the amount of sludge produced by this compound could correspond to 2.5 g dry solids for each gram Zr dosed, which is lower than for Al or Fe (Section 1.6). No studies, however, have verified the sludge production rate or filter run length for Zr coagulant.

4) stronger flocs and resistance to shear forces - Zr forms large and strong flocs [119], [125] that provide large adsorption surfaces and presumably large resistance to breakage under further liquid-solid separation processes, such as filtration, assuring longer operation.

Extensive investigation on Zr toxicity, genotoxicity, and carcinogenicity to both humans and animals has been carried out over the years. Overall, Zr compounds exhibit low toxicity, low or no bioaccumulation, and no carcinogenicity [Toxicology data network by US National Library of Medicine https://toxnet.nlm.nih.gov/ and the European Chemical Agency (https://echa.europa.eu)].

Zirconium is mined extensively for use in other manufacturing fields, and its availability is unlikely to be of concern.

There are, however, a limited number of scaled up experiments and real case studies on Zr, which are essential for its broader characterization. Moreover, Zr-based products are still under development or not in the list of officially approved products for water treatment in many countries. As a consequence, there are very few commercial Zr-based products designed for water treatment relative to conventional coagulants. Among the few found is MELsorb® (Mel Chemicals Inc, USA) treatment systems for arsenic-polluted water. Finally, the interest in Zr coagulants can be confined by its higher cost (Section 3.5).

The use of Zr for chemical treatment at several Norwegian WTPs is presented in Appendix B.

1.6.2 Chitosan

Chitin polymer is a basic component of the exoskeleton of shrimps, crabs, and other crustaceans. Numerous polymer products can be prepared from chitin, including chitosan. Chitosan material has gained considerable interest over recent decades, due to some unique characteristics and its natural origin. Today, chitosan can be purchased from many suppliers all over the world, with products intended for numerous applications, including water and wastewater treatment.

Chitosan is an unbranched cationic polymer of two randomly sequenced units, an uncharged acetylated unit (N-acetyl-D-glucosamine), normally found in chitin, and a potentially charged deacetylated unit (D-glucosamine), derived after transformation of chitin to chitosan (Figure 11). Structural characteristics of chitosan include *acetylation degree* (Fa) and MW. The acetylation degree of chitosan can be expressed as a ratio between acetylated and deacetylated fractions of chitosan. It affects the density and distribution of the glucosamine units along the polymer chain, stiffness (increases with increasing Fa), and hydrophobic characteristics of the compound [131]. The MW of chitosan defines the length of the polymer chain and thereby the viscosity of chitosan in the dissolved state.

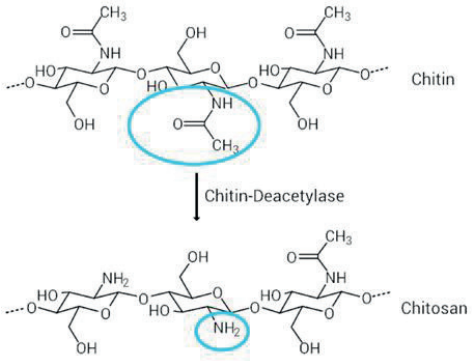

Figure 11. Structure of chitin and chitosan.

The other important parameter for chitosan is solubility, which varies with pH. At acidic conditions, the glucosamine units are protonated, and the chitosan molecule becomes soluble. Here chitosan gains the highest affinity for anionic impurities [132], and exhibits the highest destabilization efficiency as a coagulant. At neutral pH, the amino groups lose their positive charge and chitosan becomes insoluble [133].

Bench-scale tests have proven that chitosan and chitosan-derivatives are capable of removing heavy metals, phenols, dyes, pharmaceuticals and drug metabolites from variety of water effluents [134]–[136]. Chitosan has been assessed for reduction of NOM [117], [131], [137], suspended matter [138], [139], and microorganisms [140]–[142] in drinking water using both laboratory and pilot-scale setups. In many cases, this coagulant has been proven as effective as traditional Al and Fe. Substantial research on chitosan has been accomplished by The Norwegian Institute for Water Research. These data provided some unique operational experience of using chitosan for water treatment and the main findings are presented in Table C1. A TOC reduction of 30-40% was attainable with chitosan doses between 0.4 and 0.8 mg chitosan/mg C (0.05-0.15 mg chitosan/mg colour). Optimal pH varied between 4.0 and 7.0, but the coagulant demands were reduced at lower pH. Chitosan possesses several intrinsic characteristics that make it very attractive for drinking water production:

1) origin – due its organic nature, chitosan is widely regarded being a nontoxic, biologically compatible polymer [143]. It is approved for dietary applications in Japan, Italy, Finland, Norway, and several other countries.

2) no metal residue - one of the most substantial arguments for use of chitosan is lack of residual metal in the finished water. If a WTP experiences suboptimal operation, the produced water will be less harmful when produced with chitosan compared with conventional coagulants. Taking into consideration that an accidental overdosing of Al coagulant in UK in 1988 resulted in symptoms of loss of concentration and short term memory among 20 000 residents [144], one can assume that the consequences if such event could be much less detrimental with chitosan.

Chitosan application could be especially convenient in small treatment facilities, as they most frequently experience problems with high metal residue due to little buffering capacity (small dilution factor).

Similarly, sludge produced with chitosan is free of metals. Chitosan sludge is rich in organic matter and exerts less harm to the environment under deposition. Furthermore, it has a nutritional value for plants, if used as a conditioner [145], [146].

3) reduced amount of sludge - chitosan drives destabilization without the formation of additional solids in the form of a metal hydroxide precipitate. For chitosan, no by-products are formed, and 1 g of added chitosan would roughly generate 1 g of dry solids in the final sludge. Consequently, at least 2-3 times less sludge can be expected from chitosan compared to metal coagulants (Section 1.6). Chitosan also extends filter runs and 19-24 h filter operation under high water production (5-9 m/h) has been reported [145], [146].

4) chitosan also exhibits bactericidal [147], [148], antifungal [149], and antiviral activities [141], [150]. In a study by Parkpian et al. [151] prolonged contact between chitosan and sewage sludge could potentially result in 4-fold reduction of live *E.coli*. Hence, the concentration of infectious waterborne pathogens can potentially be reduced in a chitosan-rich sludge, making recycling of backwash water and disposal of the processed sludge safer than with other coagulants.

Despite many advantages, there are some factors restricting broader implementation of chitosan in the water sector. The ability of chitosan to remove NOM is considerably lower than that of metal coagulants. The phenomenon is likely to be explained by lower affinity between amino groups and NOM molecules relative to metal hydroxides, due to lower charge of the former. Attempts to enhance the efficiency of chitosan in combination with other compounds have been successful [131].

1.7 Aims of the thesis

Zirconium and chitosan coagulants have recently been introduced to the water industry, and there are some missing data for both compounds. The overall goal was to provide broader characterization of Zr and chitosan coagulants for water treatment, or more specifically to study:

1) performance in terms of dose and pH demands for efficient turbidity and colour reduction;
2) turbidity and colour reduction, compared to a conventionally used Al coagulant;
3) the amount of sludge generated;
4) the filter run lengths for a rapid two-media filter;
5) the hygienic properties against three main groups of microorganisms (virus, bacteria, and protozoa);

In pursuit of the last goal, the following topics were further investigated:

6) effluent turbidity and colour as indicators of microorganism reduction;
7) the removal patterns for microorganisms in terms of size and surface charge;
8) virus reduction as a function of raw water quality, type of coagulant and coagulant dosing;
9) the potential reversibility of the infectivity of viruses in coagulant aggregates after coagulation and flocculation;
10) the suitability of MS2 as model organism for virus reduction by coagulation-filtration.

2 Materials and Methods

2.1 Coagulants

A ready to use polyaluminium chloride (PACl) product, PAX-18, from Kemira Chemicals (Norway), was used. Zirconium (IV) oxychloride octahydrate powder (Aquator^{TM}) was obtained from Teta Vannrensing Ltd (Norway). Chitosan. KitoFlokk^{TM} (low molecular weight [MW, 100 kDa] and Fa close to 0.2) was obtained from Teta Vannrensing Ltd, Norway.

2.2 Bench-scale experiments

The jar test procedure requires neither huge amounts of water for testing, nor complex equipment and components, making it an excellent tool for simple research on water treatment chemicals (Figure 12). The tests are useful for initial screening of an unknown water source, estimation of coagulant demands in a WTP design, and correction of coagulant requirements for plant performance.

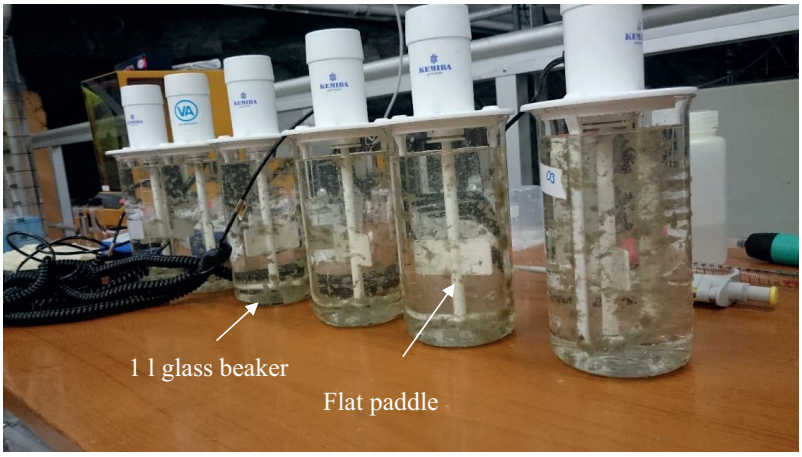

Figure 12. The jar test apparatus Flocculator 2000 (Kemira AS, Finland).

Jar tests are often criticized as they fail to represent a full-scale coagulation process precisely. The test is considered to be more appropriate for simulation of conventional treatment, for which relatively higher coagulant dosing and formation of big and settable flocs are often advantageous. In contrast, direct filtration requires less coagulant to produce smaller and compact flocs. Conditions determined by jar tests and applied during contact filtration may therefore lead to filter clogging, as happened in the present study during the initial stages of the pilot experiments. Lower doses are also hard to measure

accurately. Membrane filtration used after coagulant dosing does not provide a good representation of depth filtration, as the pore size of the glass-fibre membrane is smaller, compared to granular media, and the retaining mechanisms are different. The jar test and glass-fibre filtration procedure does not provide any information about the strength and deposition of the flocs and offers only the estimation of a filter cycle length and head loss development (by means of empirical equations), which are essential for the depth filtration.

These limitations explain why pilot-scale and full-scale research are more preferable than jar tests.

2.3 Pilot-scale experiments

An overview of the pilot plant used in the present research is outlined in Figure 13.

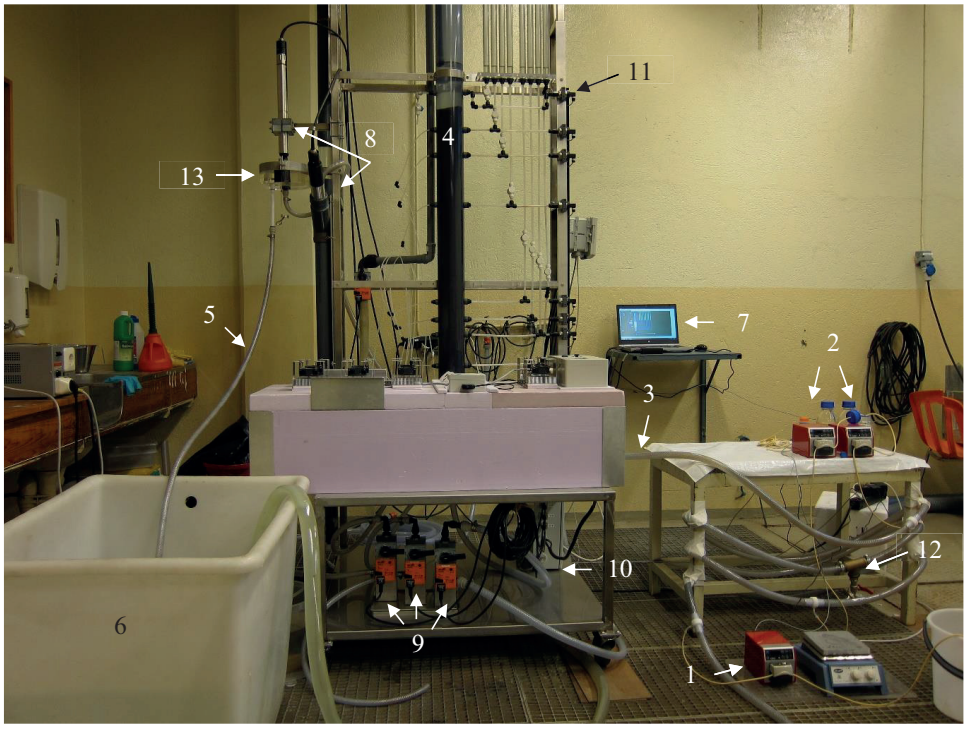

Figure 13. The main components of the pilot filter: 1 – dosing pump for seeding of microbe suspension; 2 - dosing pumps for HCl and coagulant; 3 – inlet hose from the indoor tank; 4 – two-media downstream filter; 5 – outlet hose; 6 – basin for collection and disinfection of the outlet water; 7 – operating PC; 8 – pH- and turbidimeter; 9 – valves for outlet, drain and backwash water; 10 – hose for backwash water; 11 – pressure transmitters; 12 – manual sampling point for inlet water; 13 – level control and manual sampling point for outlet water.

The pilot filter was employed in order to verify the jar test results, study model viruses, bacteria and oocysts removal, and to obtain additional operational data for the tested coagulants, such as dynamic change of the outlet turbidity over time, filter cycle length and head loss across the filter bed. Design and lag times for the pilot column resembled a classical direct filtration process. To obtain highly realistic conditions, like a full-scale plant, the experimental work focused extensively on initial testing and investigation of optimal operational conditions. Realistic conditions were also achieved through use of raw water from Glomma, which is a water source for at least three Norwegian WTPs.

The pilot column was a complex system, where component malfunction could potentially delay the work progress. The experiments could not be performed without large amounts of raw water that had to be transported and stored for some time. The long storage eventually affected the water quality, as registered in the last experiment in **Paper 1**.

As it was critical to use water with comparable quality throughout the experiments, the number of runs was restricted. As a result, just one pH-dose combination was selected for each coagulant; this considerably affected the quality of the data and restricted general comparison between the coagulants.

Finally, each run required considerable and lengthy preparation in order to avoid process disruption and ensure the run had a high chance of being successful.

2.4 Assessment of water NOM

NOM levels can be assessed by different approaches. TOC provides the most accurate estimate of the amount of NOM in a water source; a humic fraction of NOM can be assessed indirectly as light absorbency in the visible (true colour) and UV wavelength (UV_{254}) range. Consequently, a linear relationship between TOC and colour/UV_{254} parameters is often expected [152].

Historically and practically, colour analysis has been more widely used in Norway for process efficiency assessment, than TOC or DBP, e.g. trihalomethanes (THMs). This is reflected in the Norwegian Drinking Water Regulation [153], which generally requires analysis of TOC less frequently than colour. Use of the colour parameter in Norway may be partly explained by the longer processing time necessary for TOC measurements. Due to that, and other practical and economic reasons, the colour parameter was employed extensively during the present study.

2.5 Assessment of hygienic water quality

2.5.1 Plaque forming assay for bacteriophage enumeration

Infectious somatic (*Salmonella* Typhimurium 28B) and F-specific (MS2) bacteriophages were enumerated by the PFU-assay (Figure 14), following the standard 10705-1 and 10705-2 ISO procedures [75], [76].

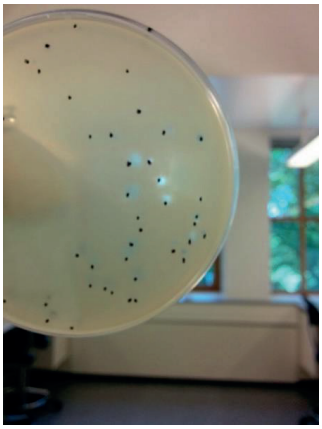

Figure 14. A petri dish with the counted plaques.

Compared with the traditional virus cell culturing techniques, the PFU assay is rapid, easy and cheap. Extensive preparations are not needed, and many samples can be processed in a short period of time, which can be especially convenient for investigation of virus removal over time.

In theory, each plaque represents a single virus. In practice, the PFU counts can be biased by virus aggregation, which has considerable potential to provide erroneously low number of infective viruses in a solution [154].

In the present study, an alkaline beef extract (BE) solution was implemented to minimize phage aggregation, caused by coagulants. Beef extract is commonly applied during concentration of viruses from water in order to elute adsorbed viruses from the filter surface [155]. The procedure included stirring of the sample in BE (13%, pH 9.5-10.0) solution for 5 h at +4°C.

2.5.2 Polymerase chain reaction (PCR) for virus detection and enumeration

The qPCR technology has been used for a variety of applications, including virus detection and quantitation of viral genomes. When RNA is the target, RNA is initially

transcribed to cDNA (copy DNA), by reverse transcriptase (RT). Variations of the PCR also include end-point and quantitative PCR (qPCR). The template quantity can be estimated from a standard curve as absolute or relative target copy number, depending on whether the absolute quantities of the standards are known or not.

Compared to cell culture for detection of viruses, qPCR is relatively easy to perform, requires less time and costs, is very sensitive and has high specificity for a certain microorganism or group of microorganisms, depending on primer/probe choice. It cannot, however, be used to determine the infectious state of an organism; it can only determine the presence or absence of pathogen-specific DNA or RNA genomes [156].

The complexity of the RT-qPCR poses also some methodological challenges. Suboptimal reaction conditions may arise for a number of reasons, including inappropriately designed primers and probes, unsuitable time or temperature conditions, variable polymerase quality, and incorrect Mg^{2+} concentration [157]. Analysis of qPCR-data can also be relatively complicated, and may require special techniques [158], and data normalization.

Another concern is RT-qPCR being sensitive to inhibitors in the sample. Alternatively, inhibitors can affect the extraction of nucleic acids. The major consequences are decreased sensitivity or false-negative results [159].

For water samples, potentially inhibitory substances include humic acids, polysaccharides and proteins [160]–[162]. Salts of sodium, potassium or calcium are also reported to affect RT-qPCR negatively [163], [164], however, it is unclear whether coagulant compounds should be classified as RT-qPCR inhibitors. In order to reveal any impact of coagulants on the RNA extraction and RT-qPCR steps, two commercial RNA extraction protocols were tested in the present work.

2.5.3 *Cryptosporidium parvum* concentration and detection

Concentration and enumeration procedures for *C.parvum* oocysts used at the Parasitology Laboratory at the University of Life Sciences are based on ISO 15553 [165] and US EPA 1623 [166] methods. The general procedure is outlined in Figure 15.

In short, a water sample was filtered through a cellulose acetate membrane (2.0 μm pore size) and adsorbed oocysts were detached with an elution buffer and pelleted. The oocysts were separated from the aquatic debris using immunomagnetic separation (IMS) and stained by IFA (immunofluorescent antibody). The stained sample was examined using

fluorescence microscopy and differential interference contrast (DIC) microscopy. Objects with correct size (3-5 μm), shape (egg-shaped), and fluorescence characteristics were counted as *Cryptosporidium* oocysts.

Oocyst losses arise, due to a high number of steps in the present method. Recovery also depends greatly on the quality of the processed water and experience of the handler. A recovery test is conducted on pure water with a known amount of spiked oocysts. If it is essential that the recovery efficiency for a particular water sample is estimated, then oocysts pre-labelled with a different fluorochrome can be used in spiking tests, but this is an additional expense on an already expensive method.

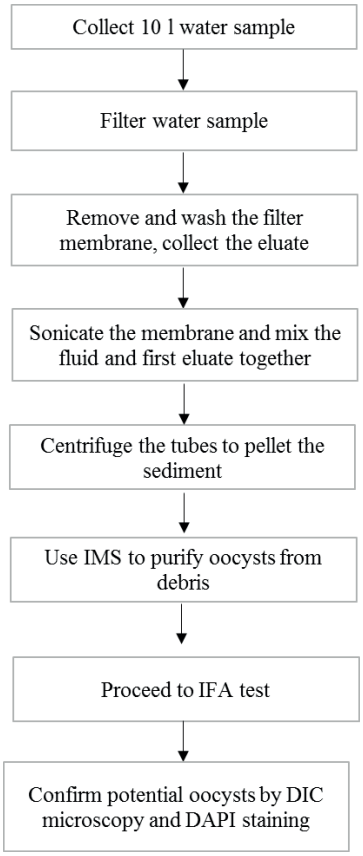

Figure 15. Flow diagram of the method used to concentrate and detect *C.parvum* oocyst in water samples. IMS - immunomagnetic separation procedure; IFA - immunofluorescent antibody procedure; DIC- differential interference contrast; DAPI - 4',6-diamidino-2-phenylindole staining.

2.5.4 Detection and quantification of *E.coli*

Standard NS-EN ISO 9308-2 [167] and its international equivalent ISO 9308-2:2012 [168] are recommended by the Norwegian Drinking Water Regulations for routine detection of total coliform bacteria and *E.coli* in water samples.

The method is based on the Colilert-18 with Quanti-Tray/2000 system (IDEXX Laboratories, USA) for cultivation of target bacteria in a liquid medium and calculation of the most probable number of organisms. Colilert-18/Quanti-Tray system was used in the present study for quantification of *E.coli.*

Colilert-18 relies upon expression of β-galactosidase from coliform bacteria, which metabolizes o-nitrophenyl-β-D-galactopyranoside and gives a colour change in positive samples after incubation at 37ºC for 18-22 hours.

For *E.coli,* the β-glucuronidase metabolizes 4-methylumbelliferyl-β-D-glucuronide hydrate which provides fluorescence to be detected with a fluorescence lamp, as shown in Figure 16.

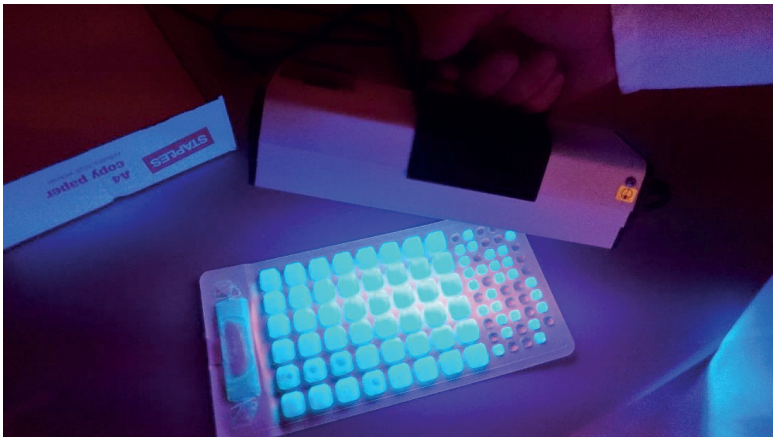

Figure 16. Detection of *E.coli* in water samples using Colilert-18/Quanti-Tray system.

The method is ideal for laboratory analysis, as it is easy, time saving and can be applied to all types of water, including those rich in suspended matter and heterotrophic bacteria.

However, some *E.coli* strains, which do not express β-glucuronidase, including pathogenic enterohemorrhagic strains of *E.coli,* cannot be detected by the Colilert technique.

2.6 Detection of isoelectric point (pI)

Charge is one of the fundamental parameters of colloidal behaviour. The net charge of a colloid is controlled among other things by protonation/deprotonation processes, and consequently by pH of the ambient solution. At a particular pH, also termed the *isoelectric point* (pI), a colloid may reach an electrically neutral state. At this state, there is no or little repulsion to prevent the colloids coming together and aggregate, and they are considered unstable.

The surface charge of the colloid is related to its velocity in an electric field. The velocity, usually referred to as *electrophoretic mobility,* can be measured by the Zetasizer instrument. Consequently, the electrophoretic mobility measured at different pH values can provide information about the pI of a colloid. This principle was applied to determine the pI of *Salmonella* Typhimurium 28B bacteriophage.

The Zetasizer also performs size measurement using dynamic light scattering (DLS). The principle of DLS relates to thermal motion of the colloids in the solution. Diffusion of colloids is connected to their size: small particles diffusing faster than large particles. The instrument measures the diffusion speed, and thereby the size and size distribution of the suspended colloids.

In this manner, the Zetasizer measures several important particle characteristics, simultaneously. Furthermore, the Zetasizer is commonly used in drinking- and wastewater treatment, as it allows monitoring the particle charge as a function of the coagulant or polymer dosing and thereby estimations on the optimum dosing range, i.e. one that makes particle unstable.

The technique is very sensitive to the presence of physical (dust, glass bits) and chemical (salts) contaminants, as their charge and size will affect the overall result. Therefore, a thorough sample preparation is paramount for obtaining accurate electrophoretic mobility and size measurements. Virus stocks, heavily contaminated by debris from host cells, salts and media components, must be purified using ultrafiltration, caesium chloride gradient centrifugation and dialysis. The colloid concentration is important for the DLS technique and should be balanced relatively to the suspended matter size. For small colloids, such as virus, high titres are usually required. This is usually not an issue for bacteriophages, which can be cultivated to high titres in a few days but can be problematic for other viruses that are grown in mammalian cell cultures.

3 Results and Discussion

3.1 Dose and pH demands for zirconium and chitosan

As outlined earlier, the coagulation phenomenon is controlled by the interrelationship between coagulant dose, the concentration of impurities, and coagulation pH [91]. The output of this relationship affects the extent of coagulation, the quality of produced water, and performance of downstream processes.

One of the aims of the present work was to characterize Zr and chitosan in terms of dose and pH requirements for optimal coagulation performance. Norwegian surface waters are often rich in NOM and low in turbidity. Since a negative charge carried by NOM colloids is about 1-2 order of magnitude greater than the charge associated with particles, coagulant dosing is often controlled by NOM levels [169]. Therefore, the coagulant doses in this chapter are estimated as a function of initial colour or TOC, whereas no dose recommendations for destabilization of suspended matter were suggested.

The data from the bench-scale experiments in **Paper 1** and **Paper 3** were combined in order to assess the effective coagulation conditions for Al, Zr and chitosan coagulants. For each coagulant, the effective dosage was defined as the minimum dose that provided residual colour below 5 mg Pt/l for the metal-based coagulants and below 10 mg Pt/l for chitosan [170]. Values slightly above 10 mg Pt/l were considered acceptable in water with high initial colour treated with chitosan, as chitosan tended not to provide high removals. The effective dosages were plotted as a function of initial water colour. The corresponding pH ranges were the values measured at the effective dosages. As a result, the following empirical models were developed:

$$\text{Al [mg/l]} = 0.09 \cdot \text{Colour} - 0.15, \text{ pH } 5.6\text{-}7.0$$
$$\text{Zr [mg/l]} = 0.16 \cdot \text{Colour} + 0.20, \text{ pH } 4.0\text{-}6.0$$
$$\text{Chitosan [mg/l]} = 0.15 \cdot \text{Colour} - 0.06, \text{ pH } 4.5\text{-}7.5$$

$$\text{Al [}\mu\text{M]} = 3.3 \cdot \text{Colour} - 10.9$$
$$\text{Zr [}\mu\text{M]} = 1.8 \cdot \text{Colour} + 2.2$$
$$\text{Chitosan [}\mu\text{M]} = 0.9 \cdot \text{Colour} - 0.3$$

Larger amounts of Zr (in mass equivalents) were required compared with Al. When expressed in moles, the consumption of Zr was slightly lower than that of Al. The lowest molar consumption was observed for chitosan.

The dosage of each compound was also expressed in terms of cationic charge equivalents. The charge density for chitosan with F_a=0.2 (pH 4.0) was calculated according to the procedure given by Kvinnesland [171]. Less Zr and chitosan were required, relative to Al, to promote destabilization of 1 mg Pt/l raw water colour.

Al [µeq/mg Pt] = 8.6
Zr [µeq/mg Pt] = 7.2
Chitosan [µeq/mg Pt] = 0.5

These results demonstrate the high destabilization power of tetravalent Zr at lower molar concentrations. Regarding chitosan, the charge from amino-groups is weaker than for Al^{3+} and Zr^{4+}. Consequently, the low charge demand was slightly unforeseen. However, for chitosan, the effective dosage was selected with the criterion 10 mg Pt/l for residual colour (versus 5 mg Pt/l, as for Al or Zr), for which higher residual charge should be expected. These results may also demonstrate a contribution of other destabilization mechanisms for this coagulant, which differ from charge neutralization. More details on this issue are presented in Section 3.3.2.

Since the data were derived from jar tests, the suggested coagulation conditions might be more suitable for conventional treatment, as discussed in Section 2.2. Doses convenient for direct filtration are expected to be 30-35% lower, as was confirmed in the pilot contact filter operation. Some inaccuracy can also be expected, as the recommended doses are suggested for a relatively broad pH range, whereas one can assume dose demands vary between the lower and upper boarders of the optimal pH window. Nevertheless, the suggested equations aim to provide dose estimates, but not precise dosages, and some adjustments may be required to improve the accuracy. Dose guidelines can be used for initial assessment of coagulant demand for Zr or chitosan. Dose recommendations should also be useful for water plants operating with these coagulants.

Similar to Fe, the pH optimum for Zr inclines towards acidic pH. Low pH has been associated with greater NOM reduction, as HS achieve electroneutrality at pH close to 2 and become more amenable to destabilization [8]. Destabilization properties of chitosan are enhanced at pH above 6.0-6.5, at which chitosan becomes soluble and gains protonation of the amino groups. Nevertheless, in the present work chitosan appeared to function over a broad pH range. Surprisingly, even under slightly alkali conditions, where chitosan was presumed to lose its solubility, the efficiency was still relatively high.

However, use of this coagulant under highly basic pH (pH>8.0) conditions, i.e. in alkaline filters, should not be recommended for removal of soluble contaminants [172].

3.2 Reduction of raw water NOM with zirconium and chitosan

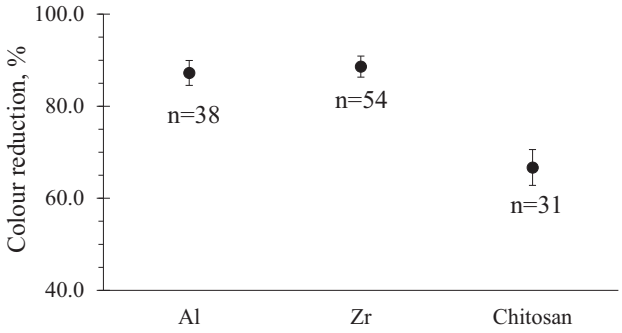

Figure 17. Colour reductions for PACl, Zr and chitosan coagulants obtained during several bench-scale experiments. The results are expressed as the mean values and 95% confidence intervals.

Colour reductions by Al, Zr and chitosan obtained during several bench-scale experiments (**Paper 1** and **Paper 3**), were surveyed and plotted in Figure 17. The doses that provided residual colour below 5 mg Pt/l for the metal-based coagulants and close to 10 mg Pt/l for chitosan [170] were included.

On average, Al, Zr and chitosan gave a colour reduction of 87, 89, and 67%, respectively. Consequently, the jar test did not confirm enhanced NOM reduction by Zr, relative to Al, as reported elsewhere [119], [125]. Failure to demonstrate advantageous NOM reductions with Zr could be attributed to use of PACl as a reference coagulant. Highly positive species like Al_{13}^{7+} have been reported for the prepolymerized Al coagulant [173], implying its high destabilization power.

Another possible explanation is the use of colour as a parameter for NOM assessment. It appears that colour fails to uncover minor differences between the coagulants when close to the detection limit, i.e. 5 mg Pt/l. Furthermore, the colour parameter essentially measures the easily removed fraction of HS. To measure the content of less compliant HS and non-HS organic matter, the SUVA-index (UV-absorption/DOC) has to be applied. In the pilot study of the present work (**Paper 1**), the SUVA parameter indicated reduced levels of low MW organic matter in the effluent produced with Zr, compared to Al and chitosan. However, as the TOC measurements were too few to confirm this effect statistically, it was concluded that Zr removes NOM similarly or slightly better than Al.

As expected, chitosan did not give the highest NOM reduction, but the performance was sufficient to provide a water quality that meets the criteria of the drinking water guidelines (Figure 17). Like other conventional coagulants, chitosan tends to remove the heavy, hydrophobic fractions of HS, while removal of hydrophilic, low MW organics is impaired [131], [137]. An effort to enhance the effectivity of chitosan with a "tiny" dose of Zr in **Paper 1** was successful to some extent, but repetition of the experiment with other pH conditions could provide relevant information. In fact, several Norwegian WTPs employ combinations of Zr and chitosan (Table B1, Appendix B). For these plants, introduction of Zr into the operation was positive and provided a considerable NOM reduction in the outlet water, in comparison to treatment with chitosan alone.

The treatment efficiencies in Figure 17 are presented to outline the quality of the finished water that can be expected for the studied coagulants. This information may help to evaluate whether each particular coagulant is able to produce water compliant with the authority's guidelines. The results here indicate that use of Zr, as well as Al, can be convenient for surface waters that are either poor or rich in colour. On the other hand, application of chitosan in waters with high initial colour can be challenging, if effluent colour below 10 mg Pt/l is preferable. This hypothesis is supported by the results in **Paper 3**, where chitosan was applied on water with colour 90 mg Pt/l. However, colours greater than 40 mg Pt/l are not recommended for contact filters [85]. Hence, the limitation is most relevant for conventional systems.

One should be aware that different fractions of NOM exhibit various susceptibilities to destabilization [13]. Both Glomma and Dragsjøen waters, used in the experiments in **Paper 1** and **Paper 3**, had a relatively high SUVA-index [131]. This implies that the organic matter in these waters is primarily composed of hydrophobic, high MW organic materials [8]; these are easier to destabilize and, subsequently, to remove. Therefore, the surveyed data in Figure 17 are valid only for waters with high MW organic materials, whereas treatment efficiency for waters rich in hydrophilic NOM should be expected to be somewhat lower.

3.2.1 Comparison of the results with existing models for predicting coagulant dose

Several empirical models predicting the dosage requirements for Al, Zr and chitosan have been developed based on initial colour or DOC levels [5], [13]. Some of these are summarized in Table 6 together with the expected NOM removal for the recommended

effective coagulant dosage. The recommendations were also compared with the results presented in the present work (**Paper 1** and **Paper 3**).

Table 6. Removal efficiencies for NOM with respect to type of coagulant, coagulant dosage (per unit of colour or unit of carbon) and pH range.

Reduction in		Effective coagulant dosage	pH	Scale	Reference
Colour	DOC/TOC				
		Aluminium			
>90%		0.05-0.07 mg/mg colour	5.7-6.7	P	[13]
	20-70% DOC	0.5 mg/mg C	5.5	F	[5]
		1.5 mg/mg C	7.0		
	37 % TOC	0.3 mg/mg C	NR	F	[146]
85-90%		0.09 mg/mg colour	5.6-7.0	B	This work
	20% TOC	0.5 mg/mg C	5.8	P	
		Zirconium			
	80-96% DOC	0.4-1.2 mg/mg C	5.0-7.0	B	[119]
	70% DOC	0.6-1.2 mg/mg C	4.5-5.1	B	[125]
86-91%		0.16 mg/mg colour	4.0-6.0	B	This work
	50% TOC	0.8 mg/mg C	4.2	P	
		Chitosan			
>60%		0.07-0.11 mg/mg colour	5.0-6.0	P	[13]
72%	32% TOC	0.08 mg/mg colour or 0.5 mg/mg C	4.0	P	[174]
67%	14% TOC	0.06 mg/mg colour or 0.4 mg/mg C	NR	F	[146]
63-71%		0.08-0.21 mg/mg colour	4.5-7.5	B	This work
	7% TOC	0.3 mg/mg C	4.2	P	

NR-not reported; B-bench-scale; P-pilot-scale; F-full-scale

The data in Table 6 indicate a good agreement with the results in this thesis. Both Al and chitosan removed slightly less TOC during the pilot tests than reported by others. A modest decrease could occur, as the treatment conditions were optimized for colour and turbidity, but not specifically for TOC.

Finally, the results in **Paper 1** and **Paper 3** were used to develop models for prediction of the remaining colour after enhanced coagulation with Al, Zr and chitosan coagulants, as a function of raw water (RW) colour, coagulant dosage, and pH:

$$\text{Colour(final)} = 0.28\ \text{RW Colour} - 3.05\text{Al} + 2.15\text{pH} - 5.91$$

$$\text{Colour(final)} = 0.39\ \text{RW Colour} - 1.11\text{Zr} + 3.71\text{pH} - 15.96$$

$$\text{Colour(final)} = \text{RW Colour}^{0.65} \cdot \text{Chit}^{-0.17}$$

3.3 Destabilization mechanisms and mode of action for zirconium and chitosan coagulants

The removal mechanisms for Zr and chitosan were not studied in the present work, although some conclusions could be drawn based on the results in **Papers 1-3**. The following chapters attempt to give a description of the studied coagulants, including their reaction products and destabilization patterns.

3.3.1 Zirconium

As for other metal coagulants, the hydrolysis reaction for Zr water is complex. When the quantity of the coagulant exceeds the solubility limit, which is almost always the case for coagulation during water treatment, a kinetic transition of free Zr^{4+} ions to soluble cationic intermediates, and then to the uncharged amorphous hydroxide precipitate $Zr(OH)_4$, occurs in the stepwise manner as the pH of the solution increases [128]. Consequently, predominant hydrolysis species, and their mean charge, will vary greatly with the Zr concentration and the pH of the medium.

$$Zr^{4+} \rightarrow Zr(OH)^{3+} \rightarrow Zr(OH)_2^{2+} \rightarrow Zr(OH)_3^{+} \rightarrow Zr(OH)_4 \rightarrow Zr(OH)_5^{-}$$

$Zr_2(OH)_6^{2+}$, $Zr_3(OH)_4^{8+}$, $Zr_4(OH)_8^{8+}$ and some other polymeric hydrolysis products have been reported for Zr as well [175], [176]. The pI-value for the amorphous Zr hydroxide is close to pH 6-7 [177]. Consequently, Zr hydrolysis products are expected to exhibit positive charge below neutral pH. Above this pH, the dominance of the uncharged $Zr(OH)_4$ and anionic Zr intermediate species could be expected. The pH values applied in **Papers 1-3** varied between 4.0 and 6.5.

As noted above, destabilization of impurities in water occurs through double layer compression, adsorption-charge neutralisation, sweep flocculation and inter particle bridging. For Zr, destabilization of colloids is apparently accomplished by double layer compression and adsorption-charge neutralisation, involving the soluble intermediates, or hydroxide precipitate [88].

Jarvis et al. [119] reported that treatment with Zr oxychloride provided a higher positive charge than Al (alum) or Fe, despite lower molar doses being applied and under similar pH conditions. A charge excess was shown to be positive for removal of impurities, such as NOM. As noted in Section 3.1, a lower molar demand for Zr, relative to PACl, was observed in the present work as well. This may indicate the presence of highly charged

polymeric species. The high charge of Zr species could improve charge neutralisation and subsequent removal of impurities. The significance of charge was also outlined in Section 3.1, where the demand of Zr was shown to be stoichiometrically related to raw water colour, and consequently, the sum of the initial negative charge in the system. Furthermore, in **Paper 1**, the Zr demand was lowered in an acidic pH environment, in which a higher positive charge from H^+ ions is expected.

A streaming current detector can be used to characterize surface charge of colloids in solution and to study the role of electrostatic effects for their destabilization. In **Paper 1**, streaming current measurements indicated that a combined effect of positive charge from pH and soluble Zr intermediates provided successful destabilization of impurities prior to their complete charge neutralization. As in the present work, Jarvis et al. [119] found that the point of charge neutralization did not coincide with the highest removal effect, and the destabilization began while the media was still negatively charged. Complete neutralization is not required for the adsorption destabilization mechanism [15].

3.3.2 Chitosan

Chitosan is not like any conventional metal coagulants. It has polyelectrolyte properties and does not form highly charged hydrolysis products. Adsorption by chitosan is accomplished when the charged polymeric chains attach to the particle surface. Charge neutralization occurs upon reaction of amino groups on the chitosan chain with negatively charged impurities in the water.

The charge density, derived from the amino groups, is influenced by the pH of the medium. Increasing the pH from 4.5 to 7.0 leads to a reduction in the charge density from 98 to 17% [178]. The charge potential is also positively influenced by the degree of deacetylation of the chitosan [131]. Therefore, the majority of the commercial chitosan products for water treatment have a high degree of deacetylation.

In the present work, the demand for chitosan was proportional to initial NOM, and subsequently, the level of negative charge in the water, indicating that charge neutralization was an important mechanism for the coagulation of HS. However, the streaming current measurements in **Paper 1** demonstrated that a point of electroneutrality for colloids did not occur using the minimal effective dose, but with an excess of chitosan. Further, the calculations in Section 3.1 show that destabilization of raw water colour with chitosan was induced with less available charge, compared with the other coagulants.

Complementary mechanisms, such as bridging or charge-patch stabilization, are common for polyelectrolytes [2] and were assumed to assist in destabilization, since these mechanisms are not necessarily accompanied by charge neutralization [15]. Furthermore, chitosan provided the highest virus reduction in **Paper 3**, probably due to polymeric chains that protrude from solution and adsorb to the filter material and impurities. Therefore, destabilization with chitosan is presumed to occur by charge neutralization and complexation with soluble matter, accompanied by bridging and electrostatic patch mechanisms. These suggestions are in the accordance with previously published conclusions [117], [134].

3.4 Filter run and sludge production with common and novel coagulants

Efforts were made to characterize Zr and chitosan in terms of sludge production and filter cycle length, which are essential for an efficient filter operation. As inferred from the results in **Paper 1**, PACl and Zr produced around 8.0-8.5 mg/l of dry solids in the influent. The amount of TSS generated by chitosan was 25% of the amount produced with metal salts, i.e. 2.1 mg/l.

In **Paper 1**, Zr provided the longest filter run: 5-7 h longer than for Al and chitosan. The phenomenon has been discussed in terms of flocs that are resistant to shear force [119]. For chitosan, at least 17 h of filter operation could be expected, if used on water with a moderate NOM content. In the present work, the filter operation with chitosan was disrupted by filter clogging. A similar phenomenon has not previously been described and could merely be an artefact of the setup used. On the other hand, the accumulation of chitosan sludge only in the top layers of the filter bed was also observed by Saltnes & Eikebrokk [179]. In the light of the results in **Paper 1** and data outlined in **Paper 3**, where membrane filtration presumably assisted chitosan coagulant in the production of high effluent quality, it was assumed that after the reaction with water contaminants, some parts of the chitosan polymer could remain active and readily adsorbed into the filter media, membrane or other surfaces. It can be speculated that the problem could be solved by increasing the reaction time and improving the mixing conditions. A strong adhesion of chitosan to solid surfaces was demonstrated indirectly in the study by Bergamasco et al. [180], in which the water that was coagulated with chitosan led to a higher membrane fouling during the ultrafiltration step for surface water treatment, compared with alum. However, in another study [181], membrane fouling using chitosan was prevented by pH

adjustment. Whether the use of chitosan is convenient for membrane filter operation, along with media filtration, should be verified in new studies.

3.4.1 Prediction of filter run

The information related to the frequency of backwashing is essential in order to meet the process objectives for a WTP. It is directly connected to the operational parameters, such as the net water production, the volume of reservoirs for backwash water, and the capacity of a unit for processed water treatment. Therefore, it is common practice, to run pilot-scale tests with intake water from a given water source to predict filter cycle lengths. In a situation, where pilot testing is not possible, empirical models can be useful tools, although less accurate.

Attempts to predict the filter run length are not numerous in the literature [182], [183]. Various factors may affect the filter cycle length, e.g. filtration velocity, filter media type and configuration, intake water quality, use of coagulant aid and others [184]. Therefore, different experimental set-ups make a direct comparison between the studies difficult. Available models are often based on empirical data, meaning that they are more specific for the conditions used during the data production. Furthermore, contact filtration has been extensively studied for Al coagulant (especially alum), whereas considerably less or no data is available for chitosan or Zr.

Nevertheless, an attempt was made to assess the consistency between the experimental data from **Paper 1** and previously published models. Since the amount of generated solids in the influent impacts the filter run more than other factors, the concentration of suspended solids after coagulation and flocculation (SS) was assessed with the equation suggested by Eikebrokk et al. [185]:

$$TSS = TSS_o + K \cdot D \quad (1)$$

where TSS_o is the concentration of suspended matter in the influent prior to coagulant dosing; D is dosage of coagulant; and K is the precipitation constant.

The prediction of coagulant dosing (D) as a function of NOM in the intake water can be assessed based on the guidelines in Table 6. The precipitation constants for alum and chitosan were defined experimentally in the work by Eikebrokk & Saltnes [186], and correspond to 4.2 and 1.1 respectively. This constant can also be calculated on the basis of the hydrolysis reactions, as in the present work (Section 1.6). Then, Al, Zr and chitosan

were estimated to produce 4.9, 2.5, and 1 g of dry solids, respectively, per 1 litre of influent.

The TSS concentration in coagulated water was calculated using equation (1) and the parameters from the pilot study. Although TSS_o was not measured, it was assumed not to exceed 2 mg/l.

$$TSS_{Al} = 2 + 4.2 \cdot 1.5 = 8.3\ mg/l$$

$$TSS_{Zr} = 2 + 2.5 \cdot 2.4 = 8.3\ mg/l$$

$$TSS_{Chit} = 2 + 1.1 \cdot 1 = 3.1\ mg/l$$

This exercise demonstrated that there was a good consistency between the modelled and experimental data.

In order to compare the filter performance in this and similar studies, the concept of *filter capacity* (F) was introduced, i.e. the amount of deposit that can accumulate inside the filter bed prior to breakthrough, expressed in kg TSS per m^3 of filter bed. For a given filter, the F value is constant and depends only on the porosity of the filter material. However, it is not rare that different filter capacities are reported for the same filter, operated with different coagulants [186]. The phenomenon is likely to be explained in terms of floc properties that are specific for each coagulant. Therefore, in this exercise, the F parameter was employed to characterize the retention properties of flocs, specific for Al, Zr, and chitosan.

The included studies had used the coagulants Al or chitosan without any coagulant aid and the filter media was identical or similar to the media employed in **Paper 1** (anthracite-sand and Filtralite-sand). For Zr, the filter run for a two-media contact filter had not yet been studied by others. If the amount of TSS in the influent had not been reported, it was estimated using equation (1). Raw water, containing both NOM and particles, was included, since the presence of NOM is apparently associated with an early breakthrough, compared to raw water that contains only particles [187]. The calculated filter capacities are presented in Table 7.

Table 7. The filter capacities of two-media contact filters, operated with Al, Zr and chitosan coagulants, expressed as the 25th to 75th percentile values.

Filter capacity, kg TSS per m^3 filter	Reference
Aluminium	
0.6-0.9	[186], [188], [189], this work
Zirconium	
0.9	This work
Chitosan	
0.3-0.6	[145], [186], this work

Several issues are noteworthy. Although the number of studies was low, there was generally a good consistency between the experimental data in the different filtration studies. More runs with Zr should be performed to determine the F value for this coagulant more accurately. The chitosan F values were abnormally low, indicating that this coagulant gave a breakthrough at an amount of accumulated sludge that corresponded to only half or one third of that accumulated with Al. However, taking into consideration that chitosan gives 25% of the sludge amount produced by Al, longer filter runs with this alternative coagulant can still be anticipated.

Finally, the filter cycle length for a contact filtration system can be estimated using the following equation:

$$t = \frac{F \cdot L}{v \cdot SS} \tag{2}$$

where t is the time of filtration until breakthrough (h), F is the filter capacity of a two-media contact filter (kg TSS/m^3 filter bed), coagulant-specific as outlined in Table 7, L is the length of the filter bed (m) (typically in the range 1.0-1.3 m), v_f is the filtration rate (m/h) and TSS is the concentration of suspended solids in the coagulated water (kg TSS/l), which can be calculated using equation (1).

3.5 Economic and environmental evaluations of zirconium and chitosan use

The use of waste as a resource, reduction of resource inputs and depletion of environmental pollution have been recognized as important elements of circular economy and sustainable production. These elements should also be applied to drinking water treatment.

This chapter attempts to estimate the annual consumption costs for use of conventional (PACl and Fe) versus alternative (Zr and chitosan) coagulants in terms of resource

demand (chemicals, clean water and energy) and handling of waste-products (sludge) during water treatment. Moreover, the use of each coagulant was evaluated in terms of environmental impact, such as the CO_2 emission connected to transportation of sludge to landfills, and the amount of disposed metal into the environment.

In this example, a theoretical middle-sized Norwegian WTP with capacity of 1 140 m^3/h is equipped with six two-media contact filters, each of 32 m^2 filtration area and a height of 1.3 m. The filtration rate corresponds to 189 m^3/h. The raw water quality, coagulant demands, filter cycle lengths and sludge concentrations were set as in the pilot filter experiment in **Paper 1**. The Fe coagulant dose (3.3 mg/l) was estimated with empirical formulas, outlined in Table 5. The theoretical filter cycle for Fe (11 h) was calculated, using equations (1) and (2), and the data provided by Eikebrokk & Saltnes [186], i.e. K=2.54, F=0.5-0.8 kg TSS/m^3.

The backwashing includes quick airing, followed by 13 min washing at 950 m^3/h. Each backwashing is assumed to be followed by a 30 min ripening period before the filter is taken into production again. The dewatering includes gravity thickening, followed by polymer dosing and centrifugation.

The analysis of operational and economic impacts is provided in Figure 18 and Table D1 (Appendix D).

Considerable costs are associated with coagulant purchase and transport. Relatively high prices for Zr and chitosan affect the overall economy negatively. However, fewer transports of Zr or chitosan are needed during a year than for PACl or Fe. Zirconium and chitosan are also able to provide a more economical filter wash. Due to less backwashing, the loss of clean water can be lowered by 32% and 12% after changing from PACl to Zr or chitosan, respectively. Moreover, reduced loss of clean water leads to a reduction in reject flows which, in some cases, can negatively affect the quality of intake water due to flow of coagulants and polymer residues and microorganisms [190].

Processed water, associated with depth filtration, is a mixture of backwash and filter-to-waste water flows. This water is characterized by low solid concentrations (Table D1). Different techniques can be applied for handling processed water. It can be delivered to a public wastewater plant without treatment. However, due to high charges for wastewater, e.g. 20 NOK/m^3, this results in considerable expenses. Fewer filter washings, as expected for Zr and chitosan, can make this solution less expensive. Direct disposal of processed

water might be preferable for small WTPs, as this alternative offers a simple plant operation, and more compact process. Moreover, some Norwegian WTPs are allowed to discharge processed water or supernatant water from thickeners back to the recipient. This is an economical, but environmentally unfriendly, alternative, especially if metal coagulants are used and thickening is poor. Discharge of processed water to the nearest watershed can be considered if chitosan is used, as it is biodegradable.

Another possibility involves treatment of processed water *in situ* and haul of dewatered sludge to landfills. The expenses include costs for dewatering, transportation and an intake fee, charged by the waste processing company. The estimate in Table D1 includes costs for *in situ* treatment. According to these estimates, the demand for polymer and energy, attributed to processed water handling, can be cut down for Zr and chitosan. For chitosan, sludge volumes can be reduced by more than 70%, compared to conventional PACl or Fe. Consequently, the transport of dewatered sludge to a disposal site can be decreased equivalently. Another advantage of using chitosan is that the sludge generated can be applied as a conditioner in agriculture [145], [146], instead of being discarded. This may give almost 0.5 mil NOK in savings. In contrast, the use of Al-rich sludge in the agricultural sector is not desirable and has been associated with depletion of phosphorous in the ground as it binds to Al [191].

The calculations indicate that despite the high costs of purchase, the use of Zr and chitosan may be economically feasible (Figure 18 and Table D1). Use of Zr is followed by reduced loss of clean water due to backwashing. The biggest savings for chitosan are achieved due to reduced costs for sludge disposal and decreased loss of clean water.

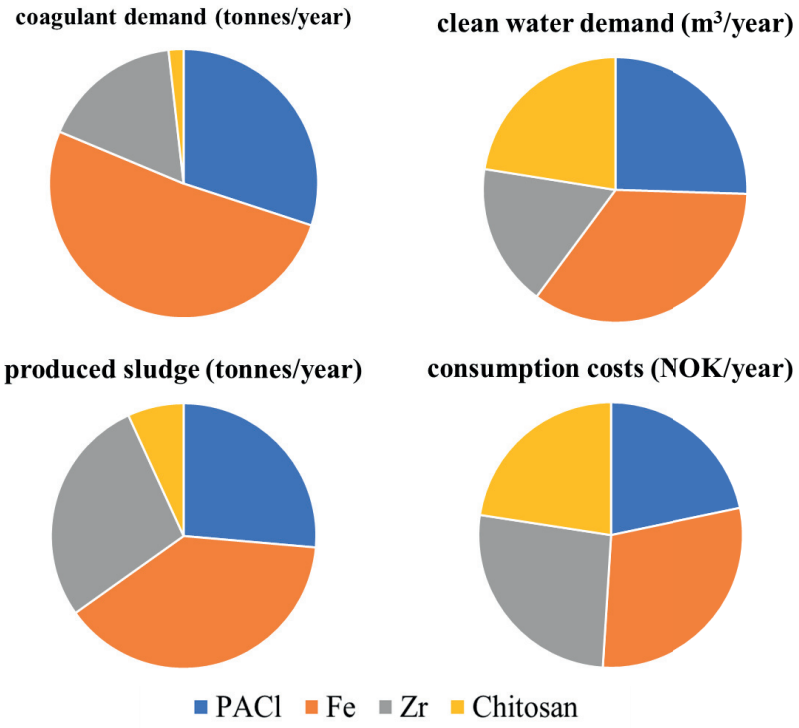

Figure 18. Aspects of water production associated with the coagulants PACl, Fe, Zr and chitosan at a theoretical middle-sized Norwegian WTP (see also Table D1, Appendix D).

The use of coagulants was assessed in terms of environmental impact (Figure 19, Table D2, Appendix D). The delivery of dewatered sludge to a disposal site is a source of CO_2 emission, and will depend on the annual number of transports, vehicle category and distance travelled. The CO_2 emission was estimated for a short distance delivery (50 km) by a truck loaded with 10 tonnes of sludge. For chitosan, fewer deliveries to a disposal site provided decrease of CO_2 by 2.9 tonnes, compared to Al. For Al and Zr, emissions were estimated to be similar and corresponded to 4 tonnes/year, which was 2.5 tonnes less than for Fe.

Generally, sludge from the plant, using conventional Al or Fe, was estimated to contribute to 15-30 tonnes of pure metal released in the nature during sludge disposal. Similar amounts are released into the environment with the use of Zr (25 tonnes/year). The effect on the eco-system is unclear and this should be addressed in future studies. In contrast, no metal pollution is generated with chitosan.

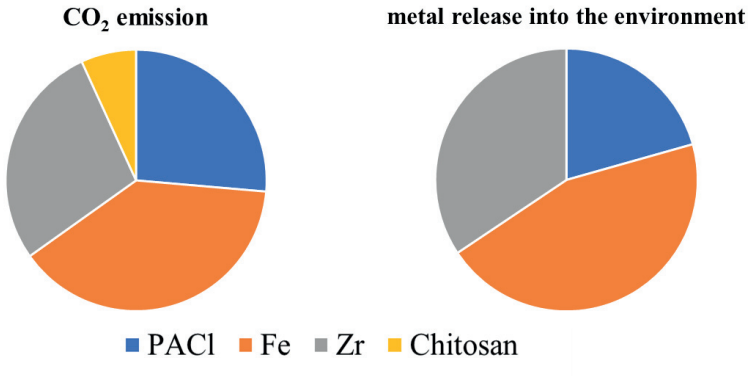

Figure 19. Aspects of environmental pollution associated with the coagulants PACl, Fe, Zr and chitosan at a theoretical middle-sized Norwegian WTP (see also Table D2, Appendix D).

3.6 Hygienic properties of zirconium and chitosan

Coagulation, combined with sedimentation and filtration, is extensively exploited for microorganism removal. Reports show that, at best, 3.4 $\log_{10}$ of enteric viruses, 3.0 $\log_{10}$ of bacteria and 5.5 $\log_{10}$ of protozoa can be removed from water with well-functioning conventional treatment (Table 8). However, there are also examples showing that almost no reduction in microorganisms occurs, despite low effluent turbidity.

Microorganism reduction obtained during the pilot filter tests with Zr and chitosan (**Paper 2**) was above the average, reported for direct filtration in Table 8. Zirconium retained 3.0-4.0 $\log_{10}$ of infectious MS2 and 28B phages, and 5.0-6.0 $\log_{10}$ of *E.coli* and *C. parvum* oocysts. Chitosan reduced bacteria in the effluent by 4.5 $\log_{10}$ and infectious phage and *C. parvum* by 2.5-3.0 $\log_{10}$. The lower hygienic performance of chitosan was partly explained by poor contact opportunities created by this coagulant. Upon reaction with water, chitosan does not produce insoluble hydroxide species, as metals do, and therefore contributes less to the increase in suspended matter. After addition of Zr to the coagulation mixture, chitosan performance was not improved and remained on the level of 2.0-2.5 $\log_{10}$ for viruses and 4.4-5.0 $\log_{10}$ for bacteria. The RT-qPCR results verified the plaque assay quantifications and ascertained a 2.2-2.6 $\log_{10}$ reduction of total MS2 for the three tested coagulants.

In **Paper 3** assessment of the coagulants with membrane filtration revealed rather different removal patterns, compared to the pilot study. Influent virus titres were reduced by 1.0-3.0 $\log_{10}$ by Al and Zr, with the highest reductions registered for colour-rich raw

water. Treatment with chitosan was more efficient, as it provided 3.0-4.0 log_{10} reduction of enteric viruses regardless of the water quality. Although bacteria and protozoa were not included, the reduction rates for these larger organisms would be expected to exceed those for viruses, independent of coagulant.

Table 8. Minimum-average-maximum log_{10}-reduction of three main groups of microorganisms by physiochemical treatment with traditional Al and Fe coagulants. Only studies that provided effluent water quality data were included. The filtration processes included a filtration rate up to 24 m/h and the turbidity in produced water was less than 0.25 NTU.

Filtration process	Log_{10}-reduction Minimum – average – maximum reduction (No of studies)		
	Virus	**Bacteria**	**Protozoa**
Conventional treatment	- *Enteric virus* 2.0 - 2.7 - 3.4 (n=2) - *Phages* 1.0 - 4.0 - 7.9 (n=3)	0.6 - 1.5 - 3.2 (n=3)	1.2 - 2.4 - 5.5 (n=4)
Coagulation/direct filtration with media-filter	- *Enteric virus* 0.2 - 1.3 - 2.5 (n=2) - *Phages* 0.1 - 2.3 - 5.1 (n=3)	1.0 - 1.5 - 2.8 (n=2)	3.0 - 3.6 - 4.2 (n=3)
References	[64], [69], [71], [84], [192], [193],	[69], [71], [189], [193], [196]	[61], [71], [74], [197], [198]

Enhanced hygienic efficiency of chitosan was presumably related to the application of higher dosages than used for the depth filter, or to the ability of chitosan to attach to a membrane filter. On the other hand, performances of Al and Zr coagulants could be affected by failure of the membrane filter to retain suspended matter effectively, as was also indicated by turbidity measurements.

In Norway, the hygienic barrier concept is applied to water treatment methods that reduce infective bacteria and viruses by a minimum of 3 log_{10} and infective parasites with 2 log_{10}. In the present work, use of Zr and chitosan in a coagulation-filtration step accomplished reduction of virus, bacteria and protozoa above 2.0-3.0 log_{10}. These results indicate that the coagulants Zr and chitosan have adequate hygienic properties for water treatment.

3.7 Turbidity and colour indicators for efficient hygienic performance

Careful control of coagulation chemistry and filter operation is essential for pathogen removal. In physicochemical treatment, effluent NOM (assessed as TOC, UV_{254} or colour) is assumed to provide insights on coagulation efficiency, while turbidity gives indication about efficiency of filtration [85].

Turbidity reduction often serves as an indicator of treatment performance for protozoan (oo)cysts [74], which are generally larger than other waterborne microbes and have size in the same range as particulate matter. The typical size range of enteric viruses is rather close to that of macromolecules, like humic compounds [199]. Similar destabilization mechanisms, i.e., complexation and adsorption, can be expected for NOM and virus [63], [64]. In contrast, neither effluent turbidity nor UV_{254} were strongly associated with removal of bacteria in the study by Xagoraraki et al. [60]. In the other study, application of a sub-optimal coagulant dose (effluent colour ca. 15-18 mg Pt/l), impaired retention of MS2 phage and *E.coli* in filter media most, compared with *C. parvum* oocysts and *Giardia* cysts [146]. Hence, destabilization and retention of particles and colloids are believed to be maximized when effluent turbidity and colour are low. Under these conditions, most microorganisms are expected to be affected to the greatest extent.

Effluent turbidity <0.2NTU and colour <10 mg Pt/l are considered to be sufficient to assure hygienic barrier requirements (reduction in infective microorganisms) during coagulation and filtering treatment [170]. For metal coagulants, the lower threshold is set to <5 mg Pt/l to reduce risks of high metal residues. In **Paper 2**, when the criteria for residual turbidity and colour were met, each coagulant fulfilled the hygienic requirements, apart from chitosan. Chitosan demonstrated slightly deficient performance against virus, along with effluent colour and TOC, in contrast to Zr and Al, which gave the highest NOM and microbial removals. Furthermore, a linear relationship was established between virus and bacteria reduction and residual TOC, and it was concluded that conditions advantageous for NOM reduction presumably contributed to elevated microbial removal. Each coagulant ensured an effluent turbidity below 0.1 NTU, for which high reduction credits should be expected [200]. Nevertheless, the turbidity parameter did not reveal differences in hygienic performances between coagulants, nor reflect the dynamics of microorganism retention during different stages of the filter cycle. In accordance with these results, WTPs should be recommended to leave the washed filter inactive, even after passing the 0.2 NTU threshold, before bringing it into operation, whereas backwashing

should be started 1-2 h ahead of the first signs of turbidity breakthrough. The drawback of this practice, however, is a drop in net water production. Curiously, some plants are operated with fixed intervals for ripening and cycle length, regardless of turbidity measurements. One can only speculate on compromised hygienic safety due to this practice.

Rather different conclusions were drawn for turbidity and colour parameters in **Paper 3**. Treatment conditions were satisfactory with regard to colour criteria, whereas the effluent turbidity was above 0.2 NTU. Microbiological criteria could still be achieved but depended on coagulant type and initial water quality.

The amount of suspended matter was expected to be elevated after coagulant addition, especially in high NOM water. Although the concentration of solids after coagulation was not measured, they could be estimated using equation (1). One could assume that the reduction of suspended matter in influent relative to effluent was greater in high NOM compared to low NOM water. Higher retention of solids on the membrane was followed by higher reduction of virus that was attached to the solids. Consequently, the retention of solids that can be assessed with turbidity or TSS measurements after coagulant addition and in the effluent was associated with removal of viruses.

A correlation between effluent colour and microorganism reduction could no longer be established, as chitosan treatment resulted in water with the highest levels of NOM and the lowest levels of viruses. An explanation could be offered in terms of distinct chitosan performance during membrane filtration, as discussed earlier. Alternatively, the relationship could be interfered due to high turbidity values.

Thereby, only the results in **Paper 2** complied with turbidity below 0.2 NTU and colour below 10 mg Pt/l (<5 mg Pt/l for metals) and low pathogen occurrence in the effluent. In **Paper 3** the results were somewhat difficult to interpret, e.g. effective virus retention could be achieved despite high effluent turbidity and colour. However, since the described phenomena were specific for high coagulant dosages or chitosan-membrane systems, they are more likely to be exceptions from the rule. Therefore, monitoring of effluent water turbidity and colour was considered as good practice in order to determine treatment conditions for microbial safe water. However, one should be aware that pathogen removal with coagulation-filtration can be deficient in the beginning and end of the filtration cycle, despite low turbidity and colour measurements in the effluent. Therefore, coagulation-

filtration step is usually accompanied by the second hygienic barrier, e.g. chlorine or UV disinfection.

Another interesting observation in **Paper 3** related to colour measurements in raw water. The influent NOM content and, subsequently, the coagulant dose, were positively associated with virus reduction. The relationship appeared to be straightforward and could be taken into considerations during assessment of coagulation-filtration barrier safety and risk analysis (QMRA). Possible explanations for this phenomenon are discussed in the next chapter.

3.8 Factors affecting microorganism removal by physicochemical treatment

According to classical filtration theory, removal of microbial particles by granular filtration is preceded by two steps: transport and attachment to the filter medium, which, in turn, are affected by the size and charge characteristics of the microorganisms (Section 1.5.2). Particle retention in a depth filter would be deficient, when their size is in the range 1-3 μm, or when their pI is not compatible with the treatment pH. An effort was made to clarify the potential impact of size and charge properties on the microbial reduction, based on the results in **Paper 2** and **Paper 3**.

Notably, microorganisms are unlikely to enter the filtration step, while still exhibiting intrinsic size and charge properties. In aquatic environments, the microorganisms tend to attach to solids [37]. In the presence of coagulants, coagulant-microbe complexes are formed upon sorption of the coagulant hydrolysis products to the microbial outer-wall [201]. This reaction changes the destabilization state of the microorganisms, and consequently their sorption behaviour. Furthermore, as the coagulant products are usually much larger (100-10 000 fold) than typical microbes [202], the transport of microorganisms embedded in flocs is expected to be altered as well. Therefore, the filtration theory predictions are not entirely supported by the empirical data [203].

In **Paper 2**, the microorganisms could be distinguished in terms of size, while the pI parameter was rather similar among the test microorganisms. The observed removal patterns in the depth filter appeared to be size-related, i.e., virus retention tended to be poorer than for bacteria or for *Cryptosporidium* oocysts. It was assumed that entrapment of viruses in the flocs was less efficient, due to the smaller size of viruses. Consequently, some virus particles proceeded to the granular filtration step as floc-free, and their retention was impaired. As the filter operation progressed and the filter bed became more

loaded, single virus colloids were more efficiently retained. This was also reflected by the gradual improvement in virus removal during the filtration course and later breakthrough. In contrast, the size factor could assist in a higher association with flocs for bacteria or protozoan oocysts, along with the log-reductions. In fact, data from Table 8 indicate deficient reduction of bacteria and enteric virus, compared to *Cryptosporidium* (and *Giardia*) spp., at least in contact filters.

Association with flocs could also explain coagulant-dependent removal patterns. As the coagulation products for PACl, Zr and chitosan are likely to exhibit varying properties, the formation of complexes with microorganisms is expected to vary as well.

In the membrane filtration tests in **Paper 3**, retention of colloids and virus was elevated in the NOM rich water (i.e. with higher coagulant doses) for Al and Zr coagulants, compared to the water sample with less NOM. Higher coagulant doses were thought to assist in production of larger and numerous flocs, advantageous for higher contact opportunities. Consequently, raw water quality, coagulant dosing, and virus reduction were assumed to be positively correlated. In fact, Dugan et al. [61] concluded that the reduction in turbidity and *Cryptosporidium* concentrations correlated positively with the turbidity of the raw water. Xagoraraki and Harrington [204] reported improvement in the reductions of *C. parvum* oocysts in natural waters with increasing DOC.

The results in **Paper 2** and **Paper 3** underline the significance of collision phenomena during the flocculation (microbe-coagulant interaction) stage. Apparently, conditions that could increase complex formation should be created for the overall removal of colloids, including small colloids like microorganisms, by a coagulation-filtration step. Apart from application of higher coagulant doses, which are inadvisable for plant operation, such conditions can be created by using coagulation aids, sufficient mixing rate, and adequate contact time.

The influence of charge characteristics on virus retention was revealed in **Paper 3**, whereas virus size (in the 30-120 nm range) or surface morphology appeared to have no impact, or the effect was not reflected by batch membrane filtration. The study indicated that viruses with a pI above 5.0 appeared to be retained more effectively. Indeed, the selected coagulation pH conditions were mostly acidic, and thereby more advantageous for destabilization of low pI viruses. While exhibiting neutral or slightly negative charge, these viruses interact readily with positively charged coagulant flocs. In contrast, removal

of viruses, which tended to achieve electroneutrality in more neutral and alkaline environment, i.e. entero-, rota- and noroviruses, and various phages [205], was suggested to be impaired during treatment with both conventional and alternative coagulants. Some studies support this hypothesis [194], [206], while other report that the charge of the microorganisms is not consistent with their removal patterns [193]. Consequently, there is no general agreement about which phages are useful models for elimination of enteric viruses by water treatment. In **Paper 3**, the $\log_{10}$-reduction of the MS2 model virus was higher than for norovirus but was not significantly different from hepatitis A virus (HAV) or coronavirus. Hence, MS2 may adequately reflect reductions of pathogenic viruses, but only under controlled enumeration conditions. Phages with higher pI may still provide more accurate data on assessment of norovirus, and are worth testing [207].

Although charge characteristics may play a role in hygienic security, they can be difficult to control during operation of the WTP, mostly because pH conditions that would result in destabilization of all present viruses can be hard to identify.

3.9 Influence of coagulants on virus enumeration techniques

Enumeration of microorganisms in raw and treated water is not a simple task. The sensitivity of the enumeration techniques may be challenged by various chemical compounds, naturally present in water or introduced during one of the operation steps. Application of methods with reduced sensitivity might result in erroneous quantification and overestimation of microorganism reduction. Data presented in **Paper 3** show that enumeration of the MS2 bacteriophage by plaque assay and RT-qPCR was sensitive to water and coagulant type, and to the amount of coagulants present in water. In samples with high amounts of coagulants, particularly chitosan, infectious MS2 titres were erroneously low when enumerated by conventional plaque assay. Also, traces of coagulants in filtrated water blocked phage infectivity, but to a much lower extent.

The plaque titres could be reversed by treating the water samples with an alkaline BE solution, indicating that virus inactivation by coagulants was not permanent. It was shown that MS2 infectivity was temporarily lost during virus complexing with coagulants, whereas BE solvent could be used to disrupt these complexes and wash coagulants away from virus capsids, thus making the virus detectable by cultivation.

Some other solvents were tested along with BE in an initial study, e.g. glycine, urea, and ethylenediaminetetraacetic acid (EDTA), but were found to be less efficient (data not shown).

This finding raises the question of whether the use of bacteriophages, or any other cultured virus, in water research using coagulants, provides reliable results. It is also unclear whether previously published work on microorganism reduction by physicochemical water treatment provides an accurate reflection of the situation. In fact, the data in Table 8 indicate that the average $\log_{10}$-reduction of bacteriophages was higher by at least 1 $\log_{10}$ compared to enteric viruses.

The RT-qPCR results suggest that also RNA extraction might be influenced by coagulants. The MS2 genome titres provided by the QIAamp® Viral RNA extraction kit were stable. For the NucliSENS miniMAG® kit, the efficiency was reduced in samples with humic matter and in samples with both high and low coagulant concentrations. This finding is probably relevant to other microorganisms that are quantified by RT-qPCR.

An initial study with the NucliSENS kit indicated that the lysis and extraction steps were most sensitive to the coagulants, whereas the amplification (RT-qPCR) step was not inhibited (results not shown).

Several inhibition scenarios could occur. Interaction with the coagulants could prevent the virus particle from releasing the nucleic acid in lysis buffer. Subsequently, after reaction with the coagulants, the nucleic acids could lose affinity to the silica material, or the coagulants could occupy the attachment sites on the silica matrix, leaving the nucleic acids behind.

3.10 Experimental relevance

Coagulants. In the present study commercial coagulant products that are used by Norwegian WTPs have been tested.

Jar-tests. Coagulant performance is difficult to predict for water of unknown quality. The use of a jar test, which was applied in the experiments with Zr and chitosan, is common practice during initial characterization of coagulant performances. The jar test procedure is a rather simple technique; however, good routines are crucial. Among important factors are water temperature, coagulant dosing, mixing intensity and duration and selection of the floc separation technique. In turn, water sampling, storage and processing are

important to ensure that the quality of the water sample is representative of the water source of interest. Both bench and pilot experiments were preceded by repeated measurements of turbidity, pH and colour to ascertain that the water quality remained stable during storage. Since the water source quality is affected in periods of stability or circulation, testing should be repeated several times during a year in order to find optimal treatment conditions.

Pilot filter. As one disadvantage of the jar test is the failure to represent a full-scale coagulation process accurately, the next stage of the coagulant characterization employed a pilot filter. The lag time and design of the pilot column simulated a classical direct granular filtration process. To obtain highly realistic conditions, as would be expected in a full-scale plant, the experimental work focused on initial testing and investigation of optimal operational conditions. Realistic conditions were also achieved through use of raw water from Glomma, which is a water source for at least three Norwegian WTPs.

Membrane filtration. The setup with a Whatman membrane was convenient for bench scale tests, where small volumes and non-complex procedures are preferable. However, the setup similarity to full-scale water treatment was restricted. Nevertheless, the results of the present study revealed that the selected bench-scale procedure with centrifugation was capable of simulating a full-scale thickening process.

The filtration with a Whatman membrane represented a microfiltration process. The efficiency of this process was significantly affected by water and coagulant type. During microfiltration retention of impurities occurs primarily by means of size exclusion, unlike in depth filtration, for which retention of colloids is controlled by many factors. It is unclear whether similar effects of water and coagulant type could be revealed in depth filters.

In order to study virus reduction using granular filtration, large volumes of high titre virus suspensions are needed, but usually not available for enteric pathogens. Bench-scale studies using Whatman filters are therefore advantageous, as small water and virus suspension volumes can be used in order to obtain accurate measurements of the treatment efficiency. In the present study, the impact of extra carbon and proteins on the raw water quality, contributed by virus solutions, was monitored, and optimization of coagulant doses was performed on water spiked with virus.

Selected microorganisms. *E.coli* is known as a faecal indicator, it can also be used as a process indicator, along with total coliforms and HPC. Structural properties between different bacteria groups can vary, however, the size of *E.coli* is not distinct from that of e.g. *Campylobacter*, which is an important waterborne pathogen. Therefore, conclusions regarding removal of *E.coli* should be relevant for other bacteria of similar size.

The present study included *C. parvum* oocysts as this protozoa is a cause of gastrointestinal illness, and transmission by contaminated and inadequately treated water is well recognised. In Scandinavia, there have been large outbreaks of waterborne cryptosporidiosis in recent years, in some cases with tens of thousands of people infected through contaminated drinking water supplies [208].

As water treatment is usually designed for reduction of bacteria and most registered outbreaks of waterborne disease are caused by viruses, some viral enteric pathogens were included in the present work. Norovirus and HAV are naked viruses that keep their infectivity in an aquatic environment for a long period. HAV can cause mild to severe liver disease and can be transmitted through unsafe water, especially in countries with low sanitary living conditions. Norovirus are important waterborne pathogen. Bovine NV was included as it is non-pathogenic for humans and very similar to human NV in terms of structure and charge. Bovine coronavirus represented enveloped viruses, which have a surface structure quite different to naked viruses. Enveloped viruses, like influenza virus and enteric coronaviruses, have potential for transmission via drinking water.

The MS2 bacteriophage is extensively employed as a model virus in water treatment studies as it is morphologically similar to many enteric viral pathogens, is non-pathogenic and can be cultivated to high titres. MS2 was also included in the present work, and this also demonstrates its suitability as a model for investigation of aggregation and retention of enteric viruses.

4 Conclusions

Sustainable and feasible coagulation step should be effective and accompanied by extensive use of eco-friendly alternatives.

Zirconium salts have been suggested for water and wastewater treatment as an alternative to Al and Fe coagulants. This novel coagulant exhibits higher charge than the conventional coagulants, which has been proposed to improve the removal of NOM. Other advantages of this coagulant include low consumption doses, low toxicity, and no bioaccumulation.

Chitosan is a biodegradable cationic polyelectrolyte, manufactured from waste from the seafood processing industries. The most substantial arguments for using chitosan include the lack of residual metal in the finished water and sludge and the reduced amount of sludge. It is assumed to pose much less harm under accidental overdosing or if returned back into the environment.

Although some commercial Zr and chitosan products are available on the market, various aspects of their operation, including chemistry, practical operation and economy are not well documented. The present work evaluated various aspects concerned with the application of Zr and chitosan coagulants in the production of potable water and provides further information and insights on their use.

Empirical models have been proposed to predict the amount of coagulant needed for effective NOM removal. In these models, the dosages of coagulants required were associated with raw water colour. The models are intended to be used by water plants that consider use of Zr and chitosan or have already adapted these two coagulants for chemical treatment.

On average, Al, Zr and chitosan gave, respectively, 87, 89, and 67% less colour in the treated water, and in the pilot study the effluent contained, respectively, 20, 50, and 7% less TOC. In this context, Zr was proposed to remove low molecular hydrophilic NOM more efficiently, and therefore is recommended for water plants that experience problems with insufficient NOM reduction.

NOM reduction with chitosan was often modest, and its application on waters with high initial colour was not recommended. However, under certain conditions chitosan

performed sufficiently well that the quality of water provided was in accordance with drinking water guidelines. An effort to enhance the effectivity of chitosan using a “tiny” dose of Zr was successful to some extent but repeating the experiment under other pH conditions may provide relevant data. In general, chitosan required neither pH-adjustment, nor affected the pH of the water to be treated. These factors make coagulation with chitosan easier to control.

Similar amounts of dry solids were produced in the influent with PACl and Zr coagulants. Nevertheless, Zr provided the longest filter run: 5-7 h longer than for Al. The duration of the filter run in contact filtration systems was reported for the first time for Zr. Chitosan generated 4-fold less sludge than Al and Zr coagulants, and provided slightly longer filter operation, than Al. Production of less sludge can reduce the CO_2 emissions associated with sludge transport to a treatment facility or deposition.

Thus, both Zr and chitosan produced more water during a single run than Al. Fewer washings enable the consumption of clean water, polymer, and energy to be decreased by 32% for Zr and 12% for chitosan, in comparison with Al. The results implied that both coagulants have the potential of providing more economical and effective filtration steps than conventional coagulants.

Use of Zr and chitosan in coagulation filtration treatment was associated with a reduction of more than 2.0-3.0 $\log_{10}$ of virus, bacteria, and protozoan oocysts, indicating that both Zr and chitosan enable achievement of adequate hygienic properties for water treatment.

There was greater removal of microbes that entered the filtration step in a floc-bound state than for those as single colloids. These results emphasise the significance of an appropriate coagulation pre-treatment and the need for adequate contact opportunities between microbes, coagulants and raw water colloids during the flocculation stage.

The turbidity and colour parameters were tested as indicators regarding treatment performance on microorganisms. A linear relationship could be established between microbial reductions and residual TOC for the granular filtration. The same observations were not confirmed for the membrane filter system, for which effective virus retention could be achieved despite high effluent turbidity or colour. However, since the described phenomena were considered to be specific for the selected setup, effluent turbidity and colour parameters are considered as relatively robust indicators to assure hygienic safety.

It was proposed that the removal patterns for microorganisms are controlled by their intrinsic characteristics. It was assumed that the entrapment of small microorganisms, such as viruses, in the flocs can be ineffective, compared to bacteria and protozoan oocysts. The impact of size seemed to be absent in the 30-120 nm range, whereas the increase in size from virus to bacteria appeared to affect retention considerably. The study demonstrated that viruses with a pI above 5.0 appeared to be retained more effectively. As a consequence, appropriate reductions in NV quantities may be difficult to achieve by coagulation-filtration treatment alone. Thus, the application of several hygienic barriers at a water plant seems a reasonable approach. The MS2 model virus has similar size and pI characteristics as several enteric viral pathogens. In the present work, MS2 served as an adequate model for investigating virus reduction by physicochemical water treatment. However, the influence of coagulants on plaque or PCR techniques for MS2 enumeration had to be assessed first. Some of the data indicate that the virus titres measured in water samples containing coagulant or coagulant traces were erroneously. Approaches that could be used to minimize this effect were investigated and described. This finding should be of use for planning and conducting future studies using model viruses.

5 Future works and perspectives

Various aspects of Zr and chitosan use in water treatment were not addressed in the present thesis, and could be considered as subjects for future research:

1) The operation of several WTPs that uses Zr and chitosan coagulants for both direct and conventional coagulation-filtration treatment should be assessed and validated for more economic and sustainable features.

2) The effect of Zr-rich sludge on the eco-system after discharge on the landfill is unclear and should be addressed in future studies.

3) Application of chitosan in microfiltration for reduction of physicochemical or microbial pollutions. This coagulant may possess some structural properties that can contribute to advanced performance in membrane filtration.

4) The relationship between raw water quality and microorganism reduction. If impaired hygienic performance can be related to physicochemical quality of water, this knowledge can be of critical value for many WTPs. It may also help in the development of practical methods for enhanced physiochemical and hygienic treatment. In this case, the floc association concept for the microorganisms could be especially relevant.

5) The data presented suggest that RNA extraction could be negatively affected by the presence of coagulants in the water. As this effect can be valid for several types of microorganisms and may affect water research, it should be investigated more closely.

References

[1] J. Gregory, *Particles in water: properties and processes*. New York, USA: CRC Press, 2005.

[2] B. Bolto and J. Gregory, "Organic polyelectrolytes in water treatment," *Water Res.*, vol. 41, no. 11, pp. 2301–2324, 2007.

[3] M. Prévost, P. Laurent, P. Servais, and J. C. Joret, *Biodegradable organic matter in drinking water treatment and distribution*. American Water Works Association, 2005.

[4] T. Pagano, M. Bida, and J. Kenny, "Trends in levels of allochthonous dissolved organic carbon in natural water: a review of potential mechanisms under a changing climate," *Water*, vol. 6, no. 10, pp. 2862–2897, 2014.

[5] J. K. Edzwald, "Coagulation in drinking water treatment: particles, organics and coagulants," *Wat. Sci. Tech.*, vol. 27, no. 11, pp. 21–35, 1993.

[6] E. L. Sharp, P. Jarvis, S. A. Parsons, and B. Jefferson, "Impact of fractional character on the coagulation of NOM," *Colloids Surfaces A Physicochem. Eng. Asp.*, vol. 286, no. 1–3, pp. 104–111, 2006.

[7] T. Carroll, S. King, S. R. Gray, B. A. Bolto, and N. A. Booker, "The fouling of microfiltration membranes by NOM after coagulation treatment," *Water Res.*, vol. 34, no. 11, pp. 2861–2868, 2000.

[8] A. Matilainen, M. Vepsäläinen, and M. Sillanpää, "Natural organic matter removal by coagulation during drinking water treatment: a review," *Adv. Colloid Interface Sci.*, vol. 159, pp. 189–197, 2010.

[9] D. T. Monteith, C. D. Evans, P. A. Henrys, G. L. Simpson, and I. A. Malcolm, "Trends in the hydrochemistry of acid-sensitive surface waters in the UK 1988-2008," *Ecol. Indic.*, vol. 37, pp. 287–303, 2014.

[10] M. D. Sanclements, G. P. Oelsner, D. M. McKnight, J. L. Stoddard, and S. J. Nelson, "New insights into the source of decadal increases of dissolved organic matter in acid-sensitive lakes of the Northeastern United States," *Environ. Sci. Technol.*, vol. 46, no. 6, pp. 3212–3219, 2012.

[11] C. D. Evans, D. T. Monteith, and D. M. Cooper, "Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts," *Environ. Pollut.*, vol. 137, no. 1, pp. 55–71, 2005.

[12] D. J. Pernitsky, "Coagulation 101," in *Alberta Water and Wastewater Operators Association (AWWOA) Annual Seminar*, 2003.

[13] H. Ødegaard, S. Østerhus, E. Melin, and B. Eikebrokk, "NOM removal technologies–Norwegian experiences," *Drink. Water Eng. Sci.*, vol. 3, no. 1, pp. 1–9, 2010.

[14] E. E. Hargesheimer, O. Conio, and J. Popovicova, *Online monitoring for drinking water utilities*. Denver, USA: AWWA Research Foundation, 2002.

[15] J. Bratby, *Coagulation and flocculation in water and wastewater treatment*, 2nd ed. London: IWA publishing, 2006.

[16] G. Kapperud and O. Rosef, "Avian wildlife reservoir of Campylobacter fetus subsp. jejuni, Yersinia spp., and Salmonella spp. in Norway.," *Appl. Environ. Microbiol.*, vol. 45, no. 2, pp. 375–380, 1983.

[17] M. Myrmel, E. M. M. Berg, B. Grinde, and E. Rimstad, "Enteric viruses in inlet and outlet samples from sewage treatment plants," *J. Water Health*, vol. 4, no. 2, pp. 197–209, 2006.

[18] J. A. E. ten Veldhuis, F. H. L. R. Clemens, G. Sterk, and B. R. Berends, "Microbial risks associated with exposure to pathogens in contaminated urban flood water," *Water Res.*, vol. 44, no. 9, pp. 2910–2918, 2010.

[19] F. C. Curriero, J. A. Patz, J. B. Rose, and S. Lele, “The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948–1994,” *Am. J. Public Health*, vol. 91, no. 8, pp. 1194–1199, 2001.
[20] B. Guzman-Herrador *et al.*, “Waterborne outbreaks in the Nordic countries, 1998 to 2012,” *Eurosurveillance*, vol. 20, no. 24, pp. 1–10, 2015.
[21] K. Nygard, “Sykdomsutbrudd forårsaket av drikkevann i Norge,” *Med. og Vitensk.*, vol. 23, no. 123, pp. 3410–3413, 2003.
[22] M. L. Christensen, “Human viral gastroenteritis,” *Clin. Microbiol. Rev.*, vol. 2, no. 1, pp. 51–89, 1989.
[23] M. M. Marshall, D. Naumovitz, Y. Ortega, and C. R. Sterling, “Waterborne protozoan pathogens,” *Clin. Microbiol. Rev.*, vol. 10, no. 1, pp. 67–85, 1997.
[24] J. E. Smith Jr. and J. M. Perdek, “Assessment and management of watershed microbial contaminants,” *Crit. Rev. Environ. Sci. Technol.*, vol. 34, no. 2, pp. 109–139, 2004.
[25] H. G. Gorchev and G. Ozolins, “WHO guidelines for drinking-water quality,” *WHO Chron.*, vol. 38, no. 3, pp. 104–108, 2011.
[26] J. L. Jones and J. P. Dubey, “Waterborne toxoplasmosis - recent developments,” *Exp. Parasitol.*, vol. 124, no. 1, pp. 10–25, 2010.
[27] M. D. Kirk *et al.*, “Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis,” *PLoS Med.*, vol. 12, no. 12, pp. 1–21, 2015.
[28] J. A. Wagenaar, D. G. Newell, R. S. Kalupahana, and L. Mughini-Gras, “Campylobacter: animal reservoirs, human infections, and options for control,” in *Zoonoses-Infections Affecting Humans and Animals*, Springer, 2015, pp. 159–177.
[29] R. E. Black, M. M. Levine, M. Lou Clements, T. P. Hughes, and M. J. Blaser, “Experimental Campylobacter jejuni infection in humans,” *J. Infect. Dis.*, vol. 157, no. 3, pp. 472–479, 1988.
[30] S. F. Altekruse, N. J. Stern, P. I. Fields, and D. L. Swerdlow, “Campylobacter jejuni - An emerging foodborne pathogen,” *Emerg. Infect. Dis.*, vol. 5, no. 1, pp. 28–35, 1999.
[31] T. Hannu *et al.*, “Campylobacter-triggered reactive arthritis: a population-based study,” *Rheumatology*, vol. 41, pp. 312–318, 2002.
[32] B. M. Allos, “Association between Campylobacter infection and Guillain-Barré syndrome,” *J. Infect. Dis.*, vol. 176, no. Suppl 2, pp. S125–S128, 1997.
[33] M. D. Sobsey, “Inactivation of health-related microorganisms in water by disinfection processes,” *Water Sci. Technol.*, vol. 2, no. 3, pp. 179–195, 1989.
[34] I. Jakopanec *et al.*, “A large waterborne outbreak of campylobacteriosis in Norway: the need to focus on distribution system safety,” *BMC Infect. Dis.*, vol. 8, no. 128, pp. 1–11, 2008.
[35] J. Engberg, P. Gerner-Smidt, F. Scheutz, E. M. Nielsen, S. L. W. On, and K. Molbak, “Water-borne Campylobacter jejuni infection in a Danish town-a 6-week continuous source outbreak,” *Clin. Microbiol. Infect.*, vol. 4, pp. 648–656, 1998.
[36] J. Hewitt, M. Leonard, G. E. Greening, and G. D. Lewis, “Influence of wastewater treatment process and the population size on human virus profiles in wastewater,” *Water Res.*, vol. 45, no. 18, pp. 6267–6276, 2011.
[37] T.-T. Fong and E. K. Lipp, “Enteric viruses of humans and animals in aquatic environments: Health risks, detection, and potential water quality assessment tools,” *Microbiol. Mol. Biol. Rev.*, vol. 69, no. 2, pp. 357–371, 2005.
[38] World Health Organization [WHO], “Review of latest available evidence on potential transmission of avian influenza (H5N1) through water and sewage and ways to reduce the risks to human health,” World Health Organization, Geneva,

Switzerland, 2006.
[39] J. S. Guy, “Virus infections of the gastrointestinal tract of poultry,” *Poult. Sci.*, vol. 77, no. 8, pp. 1166–1175, 1998.
[40] S. L. Knobler, A. Mack, A. Mahmoud, and S. M. Lemon, “Strategies for controlling avian influenza in birds and mammals,” in *The threat of pandemic influenza: are we ready?*, Washington (DC): National Academies Press (US), 2005, pp. 222–253.
[41] B. A. Lopman, D. Steele, C. D. Kirkwood, and U. D. Parashar, “The vast and varied global burden of norovirus: prospects for prevention and control,” *PLoS Med.*, vol. 13, no. 4, pp. 1–12, 2016.
[42] S. M. Bartsch, B. A. Lopman, S. Ozawa, A. J. Hall, and B. Y. Lee, “Global economic burden of norovirus gastroenteritis,” *PLoS One*, vol. 11, no. 4, pp. 1–16, 2016.
[43] J. Vinjé, “Advances in laboratory methods for detection and typing of norovirus,” *J. Clin. Microbiol.*, vol. 53, no. 2, pp. 373–381, 2015.
[44] Y. Aoki, A. Suto, K. Mizuta, T. Ahiko, K. Osaka, and Y. Matsuzaki, “Duration of norovirus excretion and the longitudinal course of viral load in norovirus-infected elderly patients,” *J. Hosp. Infect.*, vol. 75, no. 1, pp. 42–46, 2010.
[45] P. F. Teunis *et al.*, “Norwalk virus: how infectious is it?,” *J. Med. Virol.*, vol. 80, no. 8, pp. 1468–1476, 2010.
[46] R. Nims and M. Plavsic, “Inactivation of caliciviruses,” *Pharmaceuticals*, vol. 6, no. 3, pp. 358–392, 2013.
[47] R. G. Wyatt *et al.*, “Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers,” *J. Infect. Dis.*, vol. 129, no. 6, pp. 709–714, 1974.
[48] K. Simmons, M. Gambhir, J. Leon, and B. Lopman, “Duration of immunity to norovirus gastroenteritis,” *Emerg. Infect. Dis.*, vol. 19, no. 8, pp. 1260–1267, 2013.
[49] K. Ettayebi *et al.*, “Replication of human noroviruses in stem cell-Derived human enteroids,” *Sci*, vol. 353, no. 6306, pp. 1387–1393, 2016.
[50] U. N. A. Ryan, R. Fayer, and L. Xiao, “Cryptosporidium species in humans and animals: current understanding and research needs,” *Parasitology*, vol. 141, no. 13, pp. 1667–1685, 2014.
[51] W. R. Mac Kenzie *et al.*, “A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply,” *New Engl. J. Medine*, vol. 331, no. 3, pp. 161–167, 1994.
[52] M. Rehn *et al.*, “Post-infection symptoms following two large waterborne outbreaks of Cryptosporidium hominis in Northern Sweden, 2010-2011,” *BMC Public Health*, vol. 15, no. 1, p. 529, 2015.
[53] C. M. Carey, H. Lee, and J. T. Trevors, “Biology, persistence and detection of Cryptosporidium parvum and Cryptosporidium hominis oocyst,” *Water Res.*, vol. 38, no. 4, pp. 818–62, 2004.
[54] H. L. DuPont, C. L. Chappell, C. R. Sterling, P. C. Okhuysen, J. B. Rose, and W. Jakubowski, “The infectivity of Cryptosporidium parvum in healthy volunteers,” *J. Med.*, vol. 332, no. 13, pp. 855–859, 1995.
[55] U. Ryan and N. Hijjawi, “New developments in Cryptosporidium research,” *Int. J. Parasitol.*, vol. 45, no. 6, pp. 367–373, 2015.
[56] L. J. Robertson, A. T. Campbell, and H. V Smith, “Survival of Cryptosporidium parvum oocysts under various environmental pressures,” *Appl. Environ. Microbiol.*, vol. 58, no. 11, pp. 3494–500, 1992.
[57] L. V. Venczel, M. Arrowood, M. Hurd, and M. D. Sobsey, “Inactivation of Cryptosporidium parvum oocysts and Clostridium perfringens spores by a mixed-

oxidant disinfectant and by free chlorine," *Appl. Envir. Microbiol.*, vol. 63, no. 4, pp. 1598–1601, 1997.

[58] W. Q. Betancourt and J. B. Rose, "Drinking water treatment processes for removal of Cryptosporidium and Giardia," *Vet. Parasitol.*, vol. 126, no. 1, pp. 219–234, 2004.

[59] C. M. Ferguson, B. G. Coote, N. J. Ashbolt, and I. M. Stevenson, "Relationships between indicators, pathogens and water quality in an estuarine system," *Water Res.*, vol. 30, no. 9, pp. 2045–2054, Sep. 1996.

[60] I. Xagoraraki, G. W. Harrington, P. Assavasilavasukul, and J. H. Standridge, "Removal of emerging waterborne pathogens and pathogen indicators by pilot-scale conventional treatment," *J. Am. Water Work. Assoc.*, vol. 96, no. 5, pp. 102–113, 2004.

[61] N. R. Dugan, K. R. Fox, J. H. Owens, and R. J. Miltner, "Controlling Cryptosporidium oocysts using conventional treatment," *Am. Water Work. Assoc.*, vol. 93, no. 12, pp. 64–76, 2001.

[62] M. W. LeChevallier and W. D. Norton, "Examining relationships between particle counts and Giardia, Cryptosporidium, and turbidity," *J. Am. Water Work. Assoc.*, vol. 84, no. 12, pp. 54–60, 1992.

[63] T. Meyn, T. O. Leiknes, and A. Konig, "MS2 Removal from high NOM content surface water by coagulation - ceramic microfiltration, for potable water production," *AIChE J.*, vol. 58, no. 7, pp. 2270–2281, 2012.

[64] M. Abbaszadegan, B. K. Mayer, H. Ryu, and N. Nwachuku, "Efficacy of removal of CCL viruses under enhanced coagulation conditions," *Environ. Sci. Technol.*, vol. 41, no. 3, pp. 971–7, 2007.

[65] N. Ashbolt, W. Grabow, and M. Snozzi, "Indicators of microbial water quality," in *WHO Water Quality: Guidelines, Standards and Health*, London, UK: IWA Publishing, 2001, pp. 289–316.

[66] S. C. Edberg, E. W. Rice, R. J. Karlin, and M. J. Allen, "Escherichia coli: the best biological drinking water indicator for public health protection.," *Symp. Ser. Soc. Appl. Microbiol.*, no. 29, p. 106S–116S, 2000.

[67] M. J. Allen, S. C. Edberg, and D. J. Reasoner, "Heterotrophic plate count bacteria-what is their significance in drinking water?," *Int. J. Food Microbiol.*, vol. 92, no. 3, pp. 265–274, 2004.

[68] P. M. Huck *et al.*, "Using turbidity and particle counts to monitor Cryptosporidium removals by filters," *Water Sci. Technol. Water Supply*, vol. 2, no. 3, pp. 65–72, 2002.

[69] P. Payment, M. Trudel, and R. Plante, "Elimination of viruses and indicator bacteria at each step of treatment during preparation of drinking water at seven water treatment plants," *Appl. Environ. Microbiol.*, vol. 49, no. 6, pp. 1418–28, 1985.

[70] M. W. Lechevallier, W. D. Norton, and R. G. Lee, "Giardia and Cryptosporidium spp. in filtered drining water supplies," *Appl. Environ. Microbiol.*, vol. 57, no. 9, pp. 2610–2616, 1991.

[71] G. W. Harrington, I. Xagoraraki, P. Assavasilavasukul, and J. H. Standridge, "Effect of filtration conditions on removal of emerging waterborne pathogens," *J. Am. Water Work. Assoc.*, vol. 95, no. 12, pp. 95–104, 2003.

[72] G. F. Craun, P. S. Berger, and R. L. Calderon, "Coliform bacteria and waterborne disease outbreaks," *J. Am. Water Work. Assoc.*, vol. 89, no. 3, pp. 96–104, 1997.

[73] J. F. Havelaar, A.H., van Olphen, M. Schijven, A. H. Havelaar, M. Van Olphen, and J. F. Schijven, "Removal and inactivation of viruses by drinking water treatment processes under full scale conditions," *Water Sci. Technol.*, vol. 31, no. 5, pp. 55–62, 1995.

[74] E. C. Nieminski and J. E. Ongerth, “Removal of Cryptosporidium and Giardia through conventional water treatment and direct filtration,” *J. Am. Water Work. Assoc.*, pp. 96–106, 1995.
[75] International Standards Organization, “ISO 10705-1:1995. Water quality-Detection and enumeration of bacteriophages-Part 1: Enumeration of F-specific RNA bacteriophages,” Geneva, Switzerland, 1995.
[76] International Standards Organization, “ISO 10705-2:1999. Water quality-Detection and enumeration of bacteriophages-Part 2: Enumeration of somatic coliphages,” 1999.
[77] J. J. Borrego, R. Córnax, M. A. Moriñigo, E. Martínez-Manzanares, and P. Romero, “Coliphages as an indicator of faecal pollution in water. Their survival and productive infectivity in natural aquatic environments,” *Water Res.*, vol. 24, no. 1, pp. 111–116, 1990.
[78] W. O. Grabow, V. Gauss-Müller, O. W. Prozesky, and F. Deinhardt, “Inactivation of hepatitis A virus and indicator organisms in water by free chlorine residuals,” *Appl. Environ. Microbiol.*, vol. 46, no. 3, pp. 619–624, 1983.
[79] Q. S. Meng and C. P. Gerba, “Comparative inactivation of enteric adenoviruses, poliovirus and coliphages by ultraviolet irradiation,” *Water Res.*, vol. 30, no. 11, pp. 2665–2668, Nov. 1996.
[80] P. Payment and E. Franco, “Clostridium perfringens and somatic coliphages as indicators of drinking water treatment for viruses and protozoans,” *Appl. Environ. Microbiol.*, vol. 59, no. 8, pp. 2418–2424, 1993.
[81] J. Langlet, F. Gaboriaud, J. F. L. Duval, and C. Gantzer, “Aggregation and surface properties of F-specific RNA phages: implication for membrane filtration processes,” *Water Res.*, vol. 42, no. 10–11, pp. 2769–77, 2008.
[82] W. O. K. Grabow, “Bacteriophages: update on application as models for viruses in water,” *Water SA*, vol. 27, no. 2, pp. 251–268, 2001.
[83] W. O. K. Grabow, P. Coubrough, E. M. Nupen, and B. W. Bateman, “Evaluation of coliphages as indicators of the virological quality of sewage-polluted water,” *Water SA*, vol. 10, no. 1, pp. 7–14, 1984.
[84] A. Nasser, D. Weinberg, N. Dinoor, B. Fattal, and A. Adin, “Removal of hepatitis A virus (HAV), poliovirus and MS2 coliphage by coagulation and high rate filtration,” *Water Sci. Technol.*, vol. 31, no. 5, pp. 63–68, 1995.
[85] J. K. Edzwald, *Water quality & treatment: a handbook on drinking water*. New York, USA: McGraw-Hill Professional, 2011.
[86] C. P. Gerba, “Applied and theoretical aspects of virus adsorption to surfaces,” *Adv. Appl. Microbiol.*, vol. 30, pp. 133–68, 1984.
[87] D. Hendricks, *Fundamentals of water treatment unit processes: physical, chemical, and biological*. New York, USA: CRC Press, 2010.
[88] C. R. O’Melia and W. Stumm, “Aggregation of silica dispersions by iron (III),” *J. Colloid Interface Sci.*, vol. 23, no. 3, pp. 437–447, 1967.
[89] W. Stumm and J. Morgan, “Chemical aspects of coagulation,” *J. Am. Water Work. Assoc.*, vol. 54, no. 8, pp. 971–994, 1962.
[90] K. J. Howe and M. M. Clark, *Coagulation pretreatment for membrane filtration*. American Water Works Association, 2002.
[91] Stumm, W. and C. R. O’Melia, “Stoichiometry of coagulation,” *J. Am. Water Work. Assoc.*, vol. 60, no. 3, pp. 514–539, 1968.
[92] M. Ashmore, J. Hearn, and F. Karpowicz, “Flocculation of latex particles of varying surface charge densities by chitosans,” *Langmuir*, vol. 17, no. 4, pp. 1069–1073, 2000.
[93] G. A. Edwards and A. Amirtharajah, “Removing color caused by humic acids,” *J. Am. Water Work. Assoc.*, vol. 77, no. 3, pp. 50–57, 1985.

[94] M. Elimelech, J. Gregory, and X. Jia, *Particle deposition and aggregation: measurement, modelling and simulation*. Oxford, Great Britain: Butterworth-Heinemann Ltd, 2013.
[95] N. Tufenkji, "Modeling microbial transport in porous media: Traditional approaches and recent developments," *Adv. Water Resour.*, vol. 30, no. 6–7, pp. 1455–1469, 2007.
[96] S. B. Grant, E. J. List, and M. E. Lidstrom, "Kinetic analysis of virus adsorption and inactivation in batch experiments," *Water Resour. Res.*, vol. 29, no. 7, pp. 2067–2085, 1993.
[97] K. J. Ives, "Rational design of filters," *Proc. Inst. Civ. Eng.*, vol. 16, no. 2, pp. 189–193, 1960.
[98] T. Iwasaki, J. J. Slade, and W. E. Stanley, "Some notes on sand filtration," *J. Am. Water Work. Assoc.*, vol. 29, no. 10, pp. 1591–1602, 1937.
[99] C. R. O'Melia and W. Stumm, "Theory of water filtration," *J. Am. Water Work. Assoc.*, vol. 59, no. 11, pp. 1393–1412, 1967.
[100] K.-M. Yao, M. T. Habibian, and C. R. O'Melia, "Water and waste filtration: concepts and application," *Environ. Sci. Technol.*, vol. 5, no. 11, pp. 1105–1112, 1971.
[101] C. Tien and A. C. Payatakes, "Advances in deep bed filtration," *AIChE J.*, vol. 25, no. 5, pp. 737–759, 1979.
[102] N. Tufenkji and M. Elimelech, "Correlation equation for predicting single-collector efficiency in physicochemical filtration in saturated porous media," *Environ. Sci. Technol.*, vol. 38, no. 2, pp. 529–536, 2004.
[103] C. Drozd and J. Schwartzbrod, "Hydrophobic and electrostatic cell surface properties of Cryptosporidium parvum," *Appl. Environ. Microbiol.*, vol. 62, no. 4, pp. 1227–32, 1996.
[104] J. Lukasik, T. M. Scott, D. Andryshak, and S. R. Farrah, "Influence of salts on virus adsorption to microporous filters," *Appl. Envir. Microbiol.*, vol. 66, no. 7, pp. 2914–2920, 2000.
[105] H. N. Kim, S. L. Walker, and S. A. Bradford, "Coupled factors influencing the transport and retention of Cryptosporidium parvum oocysts in saturated porous media," *Water Res.*, vol. 44, no. 4, pp. 1213–1223, 2010.
[106] S. R. Farrah, "Chemical factors influencing adsorption of bacteriophage MS2 to membrane filters," *Appl. Environ. Microbiol.*, vol. 43, no. 3, pp. 659–663, 1982.
[107] X. Dai, J. Boll, M. E. Hayes, and D. E. Aston, "Adhesion of Cryptosporidium parvum and Giardia lamblia to solid surfaces: The role of surface charge and hydrophobicity," *Colloids Surfaces B Biointerfaces*, vol. 34, no. 4, pp. 259–263, 2004.
[108] K. E. Dennett, A. Amirtharajah, T. F. Moran, and J. P. Gould, "Coagulation: its effect on organic matter," *J. Am. Water Work. Assoc.*, vol. 88, no. 4, pp. 129–142, 1996.
[109] S. C. Bondy, "Prolonged exposure to low levels of aluminum leads to changes associated with brain aging and neurodegeneration," *Toxicology*, vol. 315, pp. 1–7, 2014.
[110] V. Rondeau, D. Commenges, H. Jacqmin-Gadda, and J.-F. Dartigues, "Relation between aluminum concentrations in drinking water and Alzheimer's disease: an 8-year follow-up study," *Am. J. Epidemiol.*, vol. 152, no. 1, pp. 59–66, 2000.
[111] L.-S. Kang and J. L. Cleasby, "Temperature effects on flocculation kinetics using Fe (III) coagulant," *J. Environ. Eng.*, vol. 121, no. 12, pp. 893–901, 1995.
[112] J. Q. Jiang, "Development of coagulation theory and pre-polymerized coagulants for water treatment," *Sep. Purif. Rev.*, vol. 30, no. 1, pp. 127–141, 2001.
[113] C. R. James and C. R. O'Melia, "Considering sludge production in the selection

of coagulants," *J. Am. Water Work. Assoc.*, vol. 74, no. 3, pp. 148–151, 1982.
[114] M. Pritchard, T. Craven, T. Mkandawire, A. S. Edmondson, and J. G. O'Neill, "A comparison between Moringa oleifera and chemical coagulants in the purification of drinking water - an alternative sustainable solution for developing countries," *Phys. Chem. Earth, Parts A/B/C*, vol. 35, no. 13–14, pp. 798–805, 2010.
[115] H. A. Devrimci, A. M. Yuksel, and F. D. Sanin, "Algal alginate: A potential coagulant for drinking water treatment," *Desalination*, vol. 299, pp. 16–21, 2012.
[116] C. Y. Teh, T. Y. Wu, and J. C. Juan, "Potential use of rice starch in coagulation–flocculation process of agro-industrial wastewater: Treatment performance and flocs characterization," *Ecol. Eng.*, vol. 71, pp. 509–519, 2014.
[117] E. Guibal, M. Van Vooren, B. A. Dempsey, and J. Roussy, "A review of the use of chitosan for the removal of particulate and dissolved contaminants," *Sep. Sci. Technol.*, vol. 41, no. 11, pp. 2487–2514, 2006.
[118] A. B. McKague, "Flocculating agnets derived from kraft lignin," *J. Appl. Chem. Biotechnol.*, vol. 24, no. 10, pp. 607–615, 1974.
[119] P. Jarvis, E. Sharp, M. Pidou, R. Molinder, S. A. Parsons, and B. Jefferson, "Comparison of coagulation performance and floc properties using a novel zirconium coagulant against traditional ferric and alum coagulants," *Water Res.*, vol. 46, no. 13, pp. 4179–4187, 2012.
[120] Y.-F. Wu, W. Liu, N.-Y. Gao, and T. Tao, "A study of titanium sulfate flocculation for water treatment," *Water Res.*, vol. 45, no. 12, pp. 3704–3711, 2011.
[121] B. Ayukawa, "Method of purification of waste water by treatment with zirconium salt. Patent US4066542," 1978.
[122] H. D. Kumar and L. C. Rai, "Zirconium-induced precipitation of phosphate as a means of controlling eutrophication," *Aquat. Bot.*, vol. 4, pp. 359–366, 1978.
[123] W. D. Sanville *et al.*, "Phosphorus zirconium inactivation in a eutrophic by pond," *Water Pollut. Control Fed.*, vol. 54, no. 5, pp. 434–443, 1982.
[124] S. S. Abu Amr, S. N. F. Zakaria, and H. A. Aziz, "Performance of combined ozone and zirconium tetrachloride in stabilized landfill leachate treatment," *J Mater Cycles Waste Manag*, vol. 19, no. 4, pp. 1384–1390, 2017.
[125] S. Hussain *et al.*, "Comparison of the coagulation performance of tetravalent titanium and zirconium salts with alum," *Chem. Eng. J.*, vol. 254, pp. 635–646, 2014.
[126] D. Lakshmanan, D. A. Clifford, and S. Gautam, "Arsenic coagulation with iron, aluminum, titanium, and zirconium salts," *Awwa Res. Found.*, 2008.
[127] C. Ekberg, G. Källvenius, Y. Albinsson, and P. L. Brown, "Studies on the hydrolytic behavior of zirconium (IV)," *J. Solution Chem.*, vol. 33, no. 1, pp. 47–79, 2004.
[128] P. L. Brown, E. Curti, B. Grambow, and C. Ekberg, *Chemical thermodynamics of zirconium*. Houston, USA: Gulf Professional Publishing, 2008.
[129] B. Aftab and J. Hur, "Fast tracking the molecular weight changes of humic substances in coagulation/flocculation processes via fluorescence EEM-PARAFAC," *Chemosphere*, vol. 178, pp. 317–324, 2017.
[130] P. Jarvis, J. Banks, R. Molinder, T. Stephenson, S. A. Parsons, and B. Jefferson, "Processes for enhanced NOM removal: beyond Fe and Al coagulation," *Water Sci. Technol. Water Supply*, vol. 8, no. 6, pp. 709–716, 2008.
[131] C. Vogelsang *et al.*, "Removal of humic substances by chitosan," *Water Sci. Technol. Water Supply*, vol. 4, no. 5–6, pp. 121–129, 2004.
[132] C.-Y. Chen, C.-Y. Wu, and Y.-C. Chung, "The coagulation characteristics of humic acid by using acid-soluble chitosan, water-soluble chitosan, and chitosan coagulant mixtures," *Environ. Technol.*, vol. 36, no. 9–12, pp. 1141–1146, 2015.

[133] P. Sorlier, A. Denuzière, C. Viton, and A. Domard, “Relation between the degree of acetylation and the electrostatic properties of chitin and chitosan,” *Biomacromolecules*, vol. 2, no. 3, pp. 765–772, 2001.
[134] F. Renault, B. Sancey, P.-M. Badot, and G. Crini, “Chitosan for coagulation/flocculation processes – an eco-friendly approach,” *Eur. Polym. J.*, vol. 45, pp. 1337–1348, 2009.
[135] A. Bhatnagar and M. Sillanpää, “Applications of chitin- and chitosan-derivatives for the detoxification of water and wastewater-a short review,” *Adv. Colloid Interface Sci.*, vol. 152, no. 1–2, pp. 26–38, 2009.
[136] M. N. V. Ravi Kumar, “A review of chitin and chitosan applications,” *React. Funct. Polym.*, vol. 46, no. 1, pp. 1–27, 2000.
[137] B. Eikebrokk and T. Saltnes, “NOM removal from drinking water by chitosan coagulation and filtration through lightweight expanded clay aggregate filters,” *J. Water Supply Res. Technol. - Aqua*, vol. 51, no. 6, pp. 323–332, 2002.
[138] R. Divakaran and V. N. S. Pillai, “Flocculation of river silt using chitosan,” *Water Res.*, vol. 36, no. 9, pp. 2414–8, 2002.
[139] S. Kawamura, “Effectiveness of natural polyelectrolytes in water treatment,” *J. Am. Water Work. Assoc.*, vol. 83, no. 10, pp. 88–91, 1991.
[140] S. P. Strand, S. Vandvik, M., M. Vårum, K., and K. Østgaard, “Screening of chitosans and conditions for bacterial flocculation,” *Biomacromolecules*, vol. 2, no. 1. pp. 126–33, 2001.
[141] S. N. Chirkov, “The antiviral activity of chitosan,” *Appl. Biochem. Microbiol.*, vol. 38, no. 1, pp. 5–13, 2002.
[142] T. J. Brown and M. B. Emelko, “Chitosan and metal salt coagulant impacts on Cryptosporidium and microsphere removal by filtration,” *Water Res.*, vol. 43, no. 2, pp. 331–8, 2009.
[143] T. Kean and M. Thanou, “Biodegradation, biodistribution and toxicity of chitosan,” *Adv. Drug Deliv. Rev.*, vol. 62, no. 1, pp. 3–11, 2010.
[144] C. Exley and M. M. Esiri, “Severe cerebral congophilic angiopathy coincident with increased brain aluminium in a resident of Camelford, Cornwall, UK,” *J. Neurol. Neurosurg. Psychiatry*, vol. 77, no. 7, pp. 877–879, 2006.
[145] H. Liltved, “Fjerning av farge med kitosan som koagulant ved Haugesund vannverk,” Oslo: Norsk institutt for vannforskning (NIVA), rapport LNR 4270 (in Norwegian), 2000.
[146] T. Håkonsen, “Chitosan, a coagulant for drinking water treatment: an assessment of practical conditions,” PhD [dissertation]. Ås: University of Life Sciences, Norway, 2005.
[147] E. I. Rabea, M. E.-T. Badawy, C. V. Stevens, G. Smagghe, and W. Steurbaut, “Chitosan as antimicrobial agent: applications and mode of action,” *Am. Chem. Soc.*, vol. 4, no. 6, pp. 1457–1465, 2003.
[148] H. K. No, N. Y. Park, S. H. Lee, and S. P. Meyers, “Antibacterial activity of chitosans and chitosan oligomers with different molecular weights,” *Int. J. Food Microbiol.*, vol. 74, no. 1, pp. 65–72, 2002.
[149] A. E. Ghaouth, J. Arul, J. Grenier, and A. Asselin, “Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits,” *Phytopathology*, vol. 82, no. 4, pp. 398–402, 1992.
[150] X. Su, S. Zivanovic, and D. H. D’Souza, “Effect of chitosan on the infectivity of murine norovirus, feline calicivirus, and bacteriophage MS2,” *J. Food Prot.*, vol. 72, no. 12, pp. 2623–2628, 2009.
[151] P. Parkpian, S. T. Leong, P. Laortanakul, and P. Poonpolwatanaporn, “Environmental applicability of chitosan and zeolite for amending sewage sludge,” *J. Environ. Sci. Heal. Part A*, vol. 37, no. 10, pp. 1855–1870, 2002.

[152] E. A. Vik, D. A. Carlson, A. S. Eikum, and E. T. Gjessing, “Removing aquatic humus from Norwegian lakes,” *J. Am. Water Work. Assoc.*, vol. 77, no. 3, pp. 58–66, 1985.
[153] Norwegian Food Safety Authority, *Forskrift om vannforsyning og drikkevann (Water supply and drinking water regulations) FOR 2016-12-22 nr. 1868*. 2016.
[154] P. F. M. Teunis, W. J. Lodder, S. H. Heisterkamp, and A. M. De Roda Husman, “Mixed plaques: Statistical evidence how plaque assays may underestimate virus concentrations,” *Water Res.*, vol. 39, no. 17, pp. 4240–4250, 2005.
[155] S. R. Farrah and D. R. Preston, “Concentration of viruses from water by using cellulose filters modified by in situ precipitation of ferric and aluminum hydroxides,” *Appl. Environ. Microbiol.*, vol. 50, no. 6, pp. 1502–1504, 1985.
[156] M. Abbaszadegan, P. Stewart, and M. LeChevallier, “A strategy for detection of viruses in groundwater by PCR,” *Appl. Environ. Microbiol.*, vol. 65, no. 2, pp. 444–449, 1999.
[157] I. G. Wilson, “Inhibition and facilitation of nucleic acid amplification,” *Appl. Environ. Microbiol.*, vol. 63, no. 10, pp. 3741–3751, 1997.
[158] J. M. Ruijter *et al.*, “Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data,” *Nucleic Acids Res.*, vol. 37, no. 6, pp. 1–12, 2009.
[159] C. Schrader, A. Schielke, L. Ellerbroek, and R. Johne, “PCR inhibitors - occurrence, properties and removal,” *J. Appl. Microbiol.*, vol. 113, no. 5, pp. 1014–26, 2012.
[160] D. Sutlovic, S. Gamulin, M. Definis-Gojanovic, D. Gugic, and S. Andjelinovic, “Interaction of humic acids with human DNA: Proposed mechanisms and kinetics,” *Electrophoresis*, vol. 29, no. 7, pp. 1467–1472, 2008.
[161] M. Abbaszadegan, M. S. Huber, C. P. Gerba, and I. L. Pepper, “Detection of enteroviruses in groundwater with the polymerase chain reaction,” *Appl. Environ. Microbiol.*, vol. 59, no. 5, pp. 1318–24, 1993.
[162] M. M. Ijzerman, D. R. Dahling, and G. S. Fout, “A method to remove environmental inhibitors prior to the detection of waterborne enteric viruses by reverse transcription-polymerase chain reaction,” *J. Virol. Methods*, vol. 63, no. 1–2, pp. 145–153, 1997.
[163] W. A. Al-Soud and P. Rådström, “Capacity of nine thermostable DNA polymerases to mediate DNA amplification in the presence of PCR-inhibiting samples,” *Appl. Environ. Microbiol.*, vol. 64, no. 10, pp. 3748–3753, 1998.
[164] K. L. Opel, D. Chung, and B. R. McCord, “A study of PCR inhibition mechanisms using real time PCR,” *J. Forensic Sci.*, vol. 55, no. 1, pp. 25–33, 2010.
[165] International Standards Organization, “ISO 15553:2006. Water quality - Isolation and identification of Cryptosporidium oocysts and Giardia cysts from water,” Geneva, Switzerland, 2006.
[166] USEPA, “Method 1623: Cryptosporidium and Giardia in water by filtration/IMS/FA,” Cincinnati, USA, 2005.
[167] Norsk Standard, “NS-EN ISO 9308-2:2014. Vannundersøkelse - Påvisning og telling av Escherichia coli og koliforme bakterier - Del 2: Metode med mest sannsynlige antall (in Norwegian),” 2014.
[168] International Standards Organization, “ISO 9308-2. Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 2: Most probable number method,” 2012.
[169] J. Y. Shin, R. F. Spinette, and C. R. O’Melia, “Stoichiometry of coagulation revisited,” *Environ. Sci. Technol.*, vol. 42, no. 7, pp. 2582–2589, 2008.
[170] E. Andersen, *Vannrapport 127: Vannforsyning og helse (Water supply and*

health). Oslo, Norway: Norwegian Institute Of Public Health, 2016.
[171] T. Kvinnesland, "Coagulation and flocculation of dissolved organic substances with organic polymers," PhD [dissertation]. Trondheim: Norwegian University of Science and Technology, 2002.
[172] H. Liltved, "Bruk av kitosan fra BioEffect AS for fjerning av humus fra drikkevann," Oslo: Norsk institutt for vannforskning (NIVA), rapport LNR 4174 (in Norwegian), 2000.
[173] C. Hu, H. Liu, J. Qu, D. Wang, and J. Rut, "Coagulation behavior of aluminum salts in eutrophic water: significance of Al13 species and pH control," *Environ. Sci. Technol.*, vol. 40, no. 1, pp. 325–31, 2006.
[174] H. Liltved, T. Frydenberg, and C. Vogelsang, "Bruk av biopolymerer for fjerning av humus fra drikkevann i fem Sørlandskommuner," Oslo: Norsk institutt for vannforskning (NIVA), rapport LNR 4361 (in Norwegian), 2001.
[175] A. Veyland, L. Dupont, J.-C. Pierrard, J. Rimbault, and M. Aplincourt, "Thermodynamic stability of zirconium (IV) complexes with hydroxy ions," *Eur. J. Inorg. Chem.*, vol. 1998, no. 11, pp. 1765–1770, 1998.
[176] U. S. Aja and S. A. Wood, "The aqueous geochemistry of Zr and the solubility of some Zr-bearing minerals," *Appl. Geochemistry*, vol. 10, pp. 603–620, 1995.
[177] M. Aghazadeh, A. A. M. Barmi, and M. Hosseinifard, "Nanoparticulates Zr(OH)4 and ZrO2 prepared by low-temperature cathodic electrodeposition," *Mater. Lett.*, vol. 73, pp. 28–31, 2012.
[178] B. Bolto, D. Dixon, R. Eldridge, and S. King, "Cationic polymer and clay or metal oxide combinations for natural organic matter removal," *Water Res.*, vol. 35, no. 11, pp. 2669–2676, 2001.
[179] T. Saltnes and B. Eikebrokk, "Contact filtration of humic waters: performance of an expanded clay aggregate filter (Filtralite) compared to a dual anthracite/sand filter," *Water Sci. Technol. Water Supply*, vol. 2, no. 5–6, pp. 17–23, 2002.
[180] R. Bergamasco, L. C. Konradt-Moraes, M. F. Vieira, M. R. Fagundes-Klen, and A. M. S. Vieira, "Performance of a coagulation-ultrafiltration hybrid process for water supply treatment," *Chem. Eng. J.*, vol. 166, no. 2, pp. 483–489, 2011.
[181] W. L. Ang, A. W. Mohammad, A. Benamor, and N. Hilal, "Chitosan as natural coagulant in hybrid coagulation-nanofiltration membrane process for water treatment," *J. Environ. Chem. Eng.*, vol. 4, no. 4, pp. 4857–4862, 2016.
[182] "Veiledning for dimensjonering av vannbehandlingsanlegg," Hamar: Norsk Vann Rapport no. 212 (in Norwegian), 2015.
[183] H. Mahanna, M. Fouad, K. Radwan, and H. Elgamal, "Predicting of effluent turbidity from deep bed sand filters used in water treatment," *Int. J. Sci. Eng. Res.*, vol. 6, no. 9, pp. 621–626, 2015.
[184] A. Adin and M. Rebhun, "A model to predict concentration and head-loss profiles in filtration," *J. Am. Water Work. Assoc.*, vol. 69, no. 8, pp. 444–453, 1977.
[185] B. Eikebrokk, T. Juhna, and S. W. Østerhus, "Water treatment by enhanced coagulation - operational status and optimization issues," report Techneau, D 5.3.1., 2006.
[186] B. Eikebrokk and T. Saltnes, "Removal of natural organic matter (NOM) using different coagulants and light weight expanded clay aggregate filters," *Water Sci. Technol. Water Supply*, vol. 1, no. 2, pp. 131–140, 2001.
[187] M. Rebhun, Z. Fuhrer, and A. Adin, "Contact flocculation-filtration of humic substances," *Water Res.*, vol. 18, no. 8, pp. 963–970, 1984.
[188] B. Eikebrokk, "Coagulation-direct filtration of soft, low alkality humic waters," *Wat.Sci.Tech.*, vol. 40, no. 9, pp. 55–62, 1999.
[189] J. L. Cleasby, D. J. Hilmoe, and C. J. Dimitracopoulos, "Slow sand and direct in-

line filtration of a surface water," *J. Am. Water Work. Assoc.*, pp. 44–55, 1984.
[190] H. Arora, G. Di Giovanni, and M. LeChevallier, "Spent filter backwash water: Contaminants and treatment strategies," *J. Am. Water Work. Assoc.*, vol. 93, no. 5, pp. 100–112, 2001.
[191] B. Paulsrud, "Håndtering av vannverksslam (in Norwegian)," *VANN*, vol. 1, no. 37, pp. 32–41, 2002.
[192] N. Boudaud *et al.*, "Removal of MS2, Qβ and GA bacteriophages during drinking water treatment at pilot scale," *Water Res.*, vol. 46, no. 8, pp. 2651–2664, 2012.
[193] D. W. Hendricks *et al.*, "Filtration removals of microorganisms and particles," *J. Environ. Eng.*, vol. 131, no. 12, pp. 1–12, 2005.
[194] V. C. Rao *et al.*, "Removal of hepatitis A virus and rotavirus by drinking water treatment," *J. Am. Water Works Assoc.*, vol. 80, no. 2, pp. 59–67, 1988.
[195] M. R. Templeton, R. C. Andrews, and R. Hofmann, "Removal of particle-associated bacteriophages by dual-media filtration at different filter cycle stages and impacts on subsequent UV disinfection," *Water Res.*, vol. 41, no. 11, pp. 2393–2406, 2007.
[196] C. P. Gerba, K. R. Riley, N. Nwachuku, H. Ryu, and M. Abbaszadegan, "Removal of Encephalitozoon intestinalis, calicivirus, and coliphages by conventional drinking water treatment," *J. Environ. Sci. Heal. Part A*, vol. 38, no. 7, pp. 1259–1268, 2003.
[197] P. M. Huck, B. M. Coffey, M. B. Emelko, and D. D. Maurizio, "Effects of filter operation on Cryptosporidium removal," *J. Am. Water Work. Assoc.*, vol. 94, no. 6, p. 97, 2002.
[198] N. R. Dugan and D. J. Williams, "Removal of Cryptosporidium by in-line filtration: Effects of coagulant type, filter loading rate and temperature," *J. Water Supply Res. Technol.*, vol. 53, no. 1, pp. 1–15, 2004.
[199] R. Österberg, I. Lindovist, and K. Mortensen, "Particle size of humic acid," *Soil Sci. Soc. Am. J.*, vol. 57, no. 1, pp. 283–285, 1993.
[200] H. Ødegaard and S. W. Østerhus, "Microbial barrier analysis (MBA) - a guideline. Norwegian Water Report 202/2014," 2014.
[201] C. T. Tanneru, J. D. Rimer, and S. Chellam, "Sweep flocculation and adsorption of viruses on aluminum flocs during electrochemical treatment prior to surface water microfiltration," *Environ. Sci. Technol.*, vol. 47, pp. 4612–4618, 2013.
[202] T. Liu, Y. Lian, N. Graham, W. Yu, D. Rooney, and K. Sun, "Application of polyacrylamide flocculation with and without alum coagulation for mitigating ultrafiltration membrane fouling: role of floc structure and bacterial activity," *Chem. Eng. J.*, vol. 307, pp. 41–48, 2017.
[203] W. A. M. Hijnen and G. J. Medema, *Elimination of microorganisms by water treatment processes*. London, UK, 2010.
[204] I. Xagoraraki and G. W. Harrington, "Zeta potential, dissolved organic carbon, and removal of Cryptosporidium oocysts by coagulation and sedimentation," *J. Environ. Eng.*, vol. 130, no. 12, pp. 1424–1432, 2004.
[205] B. Michen and T. Graule, "Isoelectric points of viruses," *J. Appl. Microbiol.*, vol. 109, no. 2, pp. 388–97, 2010.
[206] M. Abbaszadegan, P. Monteiro, N. Nwachuku, A. Alum, and H. Ryu, "Removal of adenovirus, calicivirus, and bacteriophages by conventional drinking water treatment," *J. Environ. Sci. Heal. Part A*, vol. 43, no. 2, pp. 171–177, 2008.
[207] N. Shirasaki, T. Matsushita, Y. Matsui, K. Murai, and A. Aochi, "Elimination of representative contaminant candidate list viruses, coxsackievirus, echovirus, hepatitis A virus, and norovirus, from water by coagulation processes," *J. Hazard. Mater.*, vol. 326, pp. 110–119, 2017.
[208] M. Widerström *et al.*, "Large outbreak of Cryptosporidium hominis infection

transmitted through the public water supply, Sweden," *Emerg. Infect. Dis.*, vol. 20, no. 4, pp. 581–589, 2014.

[209] H. Liltved and E. Norgaard, "Humusfjerning ved bruk av den naturlige polymeren kitosan som koagulant (in Norwegian)," *VANN*, vol. 3, no. 99, pp. 489–498, 1999.

[210] H. Liltved and C. Vogelsang, "Optimalisering og dokumentasjon av kitosanfellingen ved Bjoa Vannbehandlingsanlegg," Oslo: Norsk institutt for vannforskning (NIVA), rapport LNR 4565 (in Norwegian), 2002.

[211] A. Lingsten, M. Lundkvist, D. Hellström, and P. Balmér, "VA-verkens energianvändning 2008," Stockholm: Svenskt Vatten Utveckling, report no.2011-04 (in Swedish), 2011.

Paper 1

Zirconium and chitosan coagulants for drinking water treatment – a pilot study

Ekaterina Christensen, Tor Håkonsen, Lucy J. Robertson and Mette Myrmel

ABSTRACT

Scientists continuously search for alternative coagulants that would be able to outperform traditionally used aluminium (Al) and iron (Fe). Use of a novel metal coagulant zirconium (Zr) has been associated with enhanced organic matter reduction. On the other hand, eco-friendly non-metal solutions, such as chitosan, can provide non-toxic sludge and water with no metal residue. In fact, Zr and chitosan have been utilized in full-scale operation by several water plants in Norway providing over 50,000 recipients in small and large municipalities with drinking water. However, the use of these two agents is limited in other parts of the world. In the present work, Zr and chitosan coagulants were tested together with Al for drinking water production in both pilot and laboratory trials. All coagulants provided high quality effluents. However, the metals showed higher efficiencies in terms of reduction of humic substances, with better performance of Zr than Al. On the other hand, the amount of suspended solids in sludge produced with chitosan was 25% of the amount produced with metal salts. Chitosan also functioned over a broad pH range without affecting the pH of the treated water.

Key words | chitosan, coagulation, contact filter, drinking water treatment, natural organic matter, zirconium

Ekaterina Christensen (corresponding author)
Lucy J. Robertson
Mette Myrmel
Norwegian University of Life Sciences,
Department of Food Safety and Infection Biology,
P.O. Box 8146,
Oslo Dep. 0033,
Norway
E-mail: *ekaterina.christensen@norconsult.com*

Ekaterina Christensen
Tor Håkonsen
Norconsult AS,
P.O. Box 626,
Sandvika 1303
Norway

INTRODUCTION

Hydrolyzing metal salts, based on aluminium (Al) or iron (Fe), have been used routinely for the removal of particles (turbidity) and natural organic matter (NOM) in the production of potable water since the beginning of the 20th century (Gregory & Duan 2001). Considerable practical and theoretical knowledge about these two agents has been gained, and today they are the most commonly used coagulants in water treatment. However, concern about raised levels of organic matter in surface waters (Garmo *et al.* 2014) impels research on alternative coagulants that are more effective at reducing NOM. The presence of NOM in water is undesirable due to its contribution to colour, taste, and odour problems. Additionally, NOM forms potentially harmful by-products, following disinfection with chlorine (Matilainen *et al.* 2010). Reduction in NOM is associated with charge neutralization, which occurs when negatively charged particles in water react with positively charged coagulant. This implies higher neutralising power and lower minimum effective dose of four-valent zirconium (Zr), compared with three-valent Al and Fe. Despite this recognized feature, only a few studies have investigated the use of Zr (Jarvis *et al.* 2012; Hussain *et al.* 2014; Zhang *et al.* 2014), whereas two of these studies have confirmed improved NOM reduction in source water after treatment with Zr salts. Moreover, apart from the study by Jarvis *et al.* (2012), previous research characterizes Zr only at bench-scale and various aspects that might stimulate its broader application in

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water treatment practice are still not fully covered in the literature.

At the same time, there is also a growing interest in natural or so-called 'green' solutions in the water industry, as the use of metal salts is associated with problematic sludge utilization and metal residues in drinking water. Eco-friendly alternatives could be of great interest due to their biodegradability and biocompatibility features. Chitosan is derived from marine crustaceans and may be one of the more promising materials among eco-friendly alternative coagulants (Renault *et al.* 2009). First attempts to use chitosan for treating aqueous medium were made as far back as 1970 (Johnson & Peniston 1970), and numerous studies on its application in water and wastewater treatment followed, as reviewed in Renault *et al.* (2009). However, only a few of these studies describe the use of chitosan in pilot or full-scale trials for drinking water production (Saltnes & Eikebrokk 2002) and therefore additional research on chitosan is still required.

The present work evaluates Zr and chitosan coagulants for drinking water treatment in bench- and pilot-scale conditions, and compares them to traditionally used polyaluminium chloride (PACl). The optimal coagulation doses and pH were estimated by a jar-test procedure using river water from a Norwegian drinking water source. Optimization was carried out by comparing residual colour and turbidity in the finished water. During pilot-scale testing, the coagulants were dosed prior to contact filtration, and the effluents were assessed for turbidity, NOM-indicators (such as UV254 and colour), total organic carbon (TOC) and metal residue, along with other operational parameters.

MATERIAL AND METHODS

Raw water samples

All experiments were performed using water from the Glomma River obtained from a feed pipe at Nedre Romerike Water Treatment Plant in Strømmen, Norway. Raw water for the jar-tests was collected in May, August and November in 2013 and March 2014. The water was stored in plastic containers in a dark and cold room (4 °C) for no longer than one month. The pilot experiments used water collected in May 2015. This water was stored in a 30 m^3 underground stainless steel tank, equipped with a submersible recirculating pump. During storage for seven months, water temperature ranged between 5–15 °C. Both bench and pilot experiments were preceded by repeated measurements of turbidity, pH and colour to assure that the water quality remained stable upon storage. Raw water parameters exhibited only minor changes during storage, except for the last pilot-scale experiment (Run 4), for which considerable changes in pH, colour, and TOC parameters were registered. Raw water characteristics are given in Tables 1 and 2.

Coagulants

PACl

A commercial PACl product, PAX-18, was obtained from Kemira Chemicals (Norway). The product had basicity of 42% and contained 9% (w/w) of Al.

Zirconium oxychloride

Zirconium (IV) oxychloride octahydrate powder, containing 27% (w/w) of pure Zr, was obtained from Teta Vannrensing Ltd (Norway). A working solution of 15% (w/w) was prepared in distilled water.

Chitosan

The chitosan product KitoFlokk™, Teta Vannrensing Ltd (Norway) was of low molecular weight (100 kDa) and had

Table 1 | Characteristics of the Glomma water samples

Sampling month	pH	Turbidity, NTU	Colour, mg Pt L^{-1}	SCV[a]
May 2013	6.7	98.0	26	–490
August 2013	6.9	1.9	14	–342
November 2013	7.1	7.8	28	–386
March 2014	7.0	3.2	29	NM[b]
Online data (average values for 2013)[c]	7.20	4.4	24	NM

[a]SCV, Streaming current value.
[b]NM, Not measured.
[c]Retrieved from www.nrva.no.

Table 2 | Summary of various physicochemical parameters for the raw, influent (M2, after coagulant dosing) and effluents waters (M3, after filtration), treated with Al, Zr and chitosan coagulants in the pilot study

	Coagulant			
Parameter	**Al**	**Zr**	**Chitosan**	**Chitosan + Zr**
Raw water (May 2015)				
pH	7.3	7.0	7.2	7.6
Turbidity, NTU	0.8	0.8	0.5	0.7
Colour, mg Pt L^{-1}	26	24	23	19
UV254, cm L^{-1}	0.13	0.13	0.13	0.10
TOC, mg L^{-1}	3.0	3.0	3.0	2.9
M2 – influent				
Dose, mg L^{-1}	1.5	2.5	1.0	1.1 + 0.3
Coagulation pH	5.8	4.2	4.2	6.0
Turbidity, NTU	2.3	2.4	0.8	1.4
Hydraulic head development, mmH_2O $hour^{-1}$	35	35	31	37
TSS, mg L^{-1}	8.2	8.6	2.1	NM[a]
M3 – effluent				
Turbidity, NTU	0.05	0.05	0.05	0.06
Colour, mg Pt L^{-1}	3	2	9	6
UV254, cm L^{-1}	0.039	0.025	0.090	0.060
TOC, mg L^{-1}	2.4	1.4	2.8	2.6
Residual metal, μg L^{-1}	<10	<10	NM[a]	NM[a]
Filter cycle length, h	15	22	17	15[b]

[a]NM, Not measured.
[b]Terminated due to insufficient volume of raw water to complete the run.

an acetylation degree (F_A) close to 0.2. The preparation was made by dissolving chitosan powder in 0.1 M HCl. The concentrations of the working solutions were 2% (w/v) and 0.5% (w/v) for the bench and pilot experiments, respectively.

The working solutions were stored at room temperature, the chitosan solution was stored for no longer than 2 weeks.

Water analysis

Turbidity was measured using a 2100N IS turbidimeter (Hach Company, USA). Colour was measured by a Shimadzu UV Visible Spectrophotometer UVmini-1240 (Shimadzu Corporation, Japan) and by DR3900 Hach spectrophotometer (Hach, USA), following either Standard APHA Method 2120C (λ = 455 nm) or ISO 7887:2011 (λ = 410 nm) for the bench- and pilot-scale tests, respectively. UV254 was measured by a Shimadzu UV Visible Spectrophotometer UVmini-1240 (Shimadzu Corporation, Japan). Prior to colour and UV254 measurements, the samples were filtered through a 0.45 μm syringe polypropylene membrane (VWR, USA), in order to avoid the influence of turbidity. pH was measured with SenTix® 41 pH-sensor (WTW, Germany). Total organic carbon, residual Al and Zr were analysed by commercial laboratories (ALS Laboratory Group Norway AS or Noranalyse AS), following ISO 8245:1999, ISO 17294-1 and ISO 17294-2 procedures, respectively. Total suspended solids (TSS) parameter was determined using ISO 11923:1997 in technical duplicates. Colour and turbidity parameters were analysed in technical triplicates, whereas data for TOC and residual Al and Zr were obtained from single measurements.

To assess the charge neutralization dose, a streaming current parameter (SCV) was used. Measurements of SCV were performed under gradual addition of coagulant until zero SCV was achieved. SCV was registered using an ECA2100 Charge Analyzer (Chemtrac, Inc., USA).

Jar-tests

Two experimental setups were used to estimate the optimal coagulant dose (Setup A) and pH (Setup B). Further details for each setup are given under the relevant sections below. For both setups, the container with water was shaken for 30 s to resuspend settled solids, and 500 mL was poured into glass beakers and held at room temperature for at least 1 h. Cylindrical 1 L glass beakers and a Flocculator 2000 apparatus (Kemira AS, Finland), equipped with six flat paddles, were used to conduct jar-tests. After adding coagulants, the water was stirred for 30 s at 400 rpm (413 s^{-1}) followed by 10 min at 50 rpm (18 s^{-1}). For pH adjustment, predetermined amounts of 1 M NaOH or 1 M HCl were added to the water prior to the coagulant. Floc settling was for a minimum of 2 h to ensure complete settling, as determined by initial tests. After sedimentation, 100 mL supernatant was collected 2–3 cm below the surface and analysed for turbidity, colour and pH.

Optimization of coagulant dose (Setup A)

Coagulant doses varied between 2 and 11 mg L^{-1} (as metal or chitosan concentration). One individual test was

conducted per dose and repeated three times on the May, March and November waters. In order to detect the primary dose requirements and their impact on pH, initial tests with Zr and chitosan were conducted without pH adjustment.

Coagulant dose and coagulation pH (Setup B)

Two-factor experiments were performed with the March water. pH values between 3 and 8 were tested. The coagulant doses for PaCl were within the dose range applied at the drinking water treatment plant, which provided water for the experiments, whereas doses for Zr and chitosan were selected based on the results in Setup A: (a) 1, 3 and 5 mg Al L^{-1}; (b) 5, 9 and 12 mg Zr L^{-1} and (c) 1, 2 and 4 mg L^{-1} of chitosan. Two individual tests were conducted for each dose-pH combination.

Pilot tests

The pilot-scale system combined coagulation with filtration in a dual-media contact filter column, which is schematically presented in Figure 1. The column, 10 cm in diameter and 2.5 m high, was packed with support gravel (0.1 m), followed by 0.5 m-layer of Rådasand® sand (Rådasand AB, Sweden, $d_{10} = 0.4$ mm) and 0.8 m of Filtralite®NC 0.8–1.6 mm (Weber Leca Raelingen, Norway, $d_{10} = 0.95$ mm, 500 kg m^{-3} dry bulk density). Filtralite is a coarse filter media, consisting of expanded clay aggregates (Eikebrokk & Saltnes 2001). The column design was similar to the filtration system at Nedre Romerike Water Treatment Plant.

Several days prior to the experiment, approximately 1,500 L of raw water was pumped to an indoor feed tank, equipped with a paddle mixer, in order to equilibrate water to room temperature, usually within 11–18 °C. Thereafter, the water was continuously fed to the filtration column, using a peristaltic pump P1 (620 U, Watson-Marlow, UK) at a constant filtration rate of 5.9 m h^{-1}. After each run, the filter was backwashed for 15–30 min by upflow of tap water at about 55 m h^{-1}.

Hydrochloric acid (1 M) and coagulants were continuously fed to the inlet pipe at constant flow rates by two peristaltic dosing pumps (120 U/DV, Watson-Marlow, UK). The dose of HCl depended on the coagulant used and desired process pH. Three static mixers along the inlet pipe were used to provide uniform suspension. Contact time between the coagulants and water was close to 7 min. Three ports were available for manual sampling: (a) inlet pipe (M1, prior to coagulant dosing point); (b) above the Filtralite® layer (M2, after coagulant dosing point); and (c) outlet pipe (M3).

On-line monitoring of pH (SensoLyt 700 IQ, WTW, Germany) and turbidity (VisoTurb 700 IQ, WTW, Germany) was performed on the inlet and outlet water. Prior to each run, online instruments were calibrated. The column was equipped with eight pressure transmitters, which were used to monitor a course of pressure within the filter bed. All online measurements were logged by LabView software (National Instruments, USA). On-line readings were also controlled by manual measurements. Several litres of water were collected above the filter bed (port M2) by the end of each run for TSS measurement.

As the present study was limited to testing of a single coagulant dose-pH combination, application of minimal effective doses was implemented. The effective doses were identified by a new series of jar-tests, using turbidity and colour as indicators. However, application of these doses was accompanied by cake filtration in the initial pilot tests, and additional dose optimization was performed prior to the final pilot experiments. Treatment conditions for three coagulants, including Al (Run 1), Zr (Run 2), chitosan (Run 3) and a mixture of chitosan with Zr (Run 4) were optimized to assure turbidity <0.2 NTU, colour <5 mg Pt L^{-1} and residual Al and Zr <0.15 mg L^{-1} in the outlet water, as required by the Norwegian and European drinking water regulations (European Directive 98/83) (European Commission 1998). For non-metal agents, like chitosan, colour up to 10 mg Pt L^{-1} is allowed, as there is no problem with metal residue. The same principle was used for all coagulants. However, pH below 5.7–5.8 (PACl) and 4.0–4.2 (Zr and chitosan) were not studied, since they were respectively outside the generally accepted optimal pH-range (Gregory & Duan 2001) or could potentially contribute to corrosion.

The following treatment conditions were chosen: (Run 1) 1.5 mg Al L^{-1} at pH 5.8; (Run 2) 2.4 mg Zr L^{-1} at pH 4.2; (Run 3) 1 mg L^{-1} of chitosan at pH 4.2; (Run 4) 1 mg L^{-1} of chitosan mixed with 0.3 mg Zr L^{-1} at pH 6.0. Originally, lower coagulation pH was chosen for the last run, however, a slight change in water quality during storage affected the system, and the pH was unintentionally raised to 6.0. For

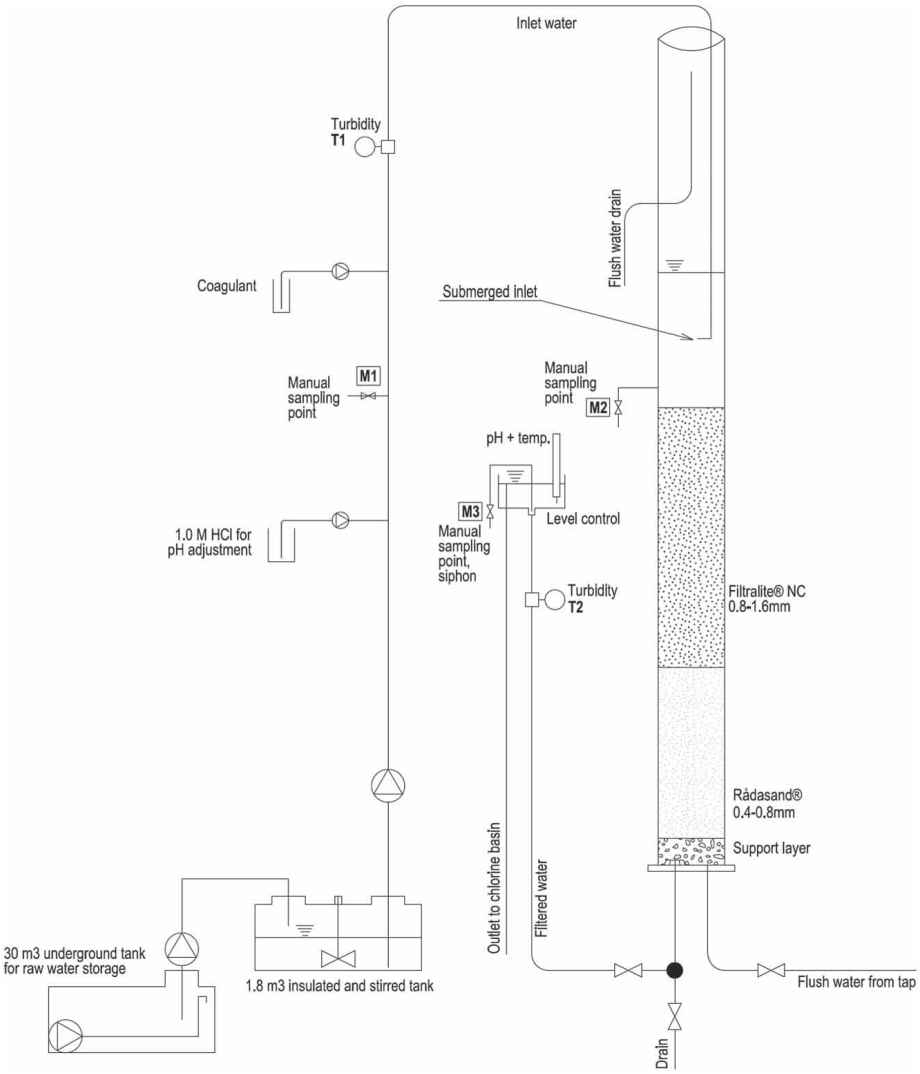

Figure 1 | A schematic of the pilot plant: M, ports for manual sampling; T, turbidimeters.

all filter runs turbidity breakthrough was reached prior to terminal head loss. An exception was Run 4, which was terminated due to insufficient volume of raw water to complete the run. Development of the head loss during an initial run with chitosan indicated solids accumulation only on the top of the filter, which is typical of cake filtration. The problem was not eliminated even after numerous efforts to adjust the treatment conditions, and was usually worse at higher chitosan dosing. Overall, conditions within 1.0–2.7 mg L^{-1} and pH 4.2–6.1 ranges were tested. Choice of chitosan dosage

was therefore a compromise between the acceptable effluent quality and prolonged filter run. For Run 4 chitosan was combined with a small amount of Zr (0.3 mg L^{-1}). The mix was screened for different coagulation/pH ratios, but not for various chitosan/Zr ratios, due to source water shortage.

RESULTS AND DISCUSSION

Raw water characteristics

Parameters of the raw water collected for the jar-tests are summarized in Table 1. Overall, the water from the Glomma river had low turbidity and medium colour. The exception was the May sample that was collected during a flood event, and which exhibited an elevated turbidity level. An increase in NOM is unusual during flooding, and therefore it was assumed that the particles that entered the water source during the flood were probably inorganic.

Jar-tests

The effectiveness and required doses of the coagulants were initially evaluated by the jar-test procedure.

The reduction in turbidity and colour as a function of coagulant dosage is shown in Figure 2. The inverted u-shaped curves depicted the system entering a state of destabilization at a minimum effective dose, followed by the restabilization phase when the coagulant was in excess or pH became suboptimal (Bratby 2006).

The high particle concentration observed in the May sample could enhance contact opportunities for aggregation and precipitation of the destabilized particles, and therefore improve reduction efficiencies. Lower coagulant doses were therefore required to induce destabilization (Shin *et al.* 2008). A related phenomenon explains the limited destabilization efficiency registered for the August sample, which had the lowest concentrations of both inorganic particles and humic substances.

For Zr, reductions above 80% were observed within 8.5–10.0 mg L^{-1} dose range for all waters. Efficiencies for chitosan were close to 60–78% for colour, and 50–95% for turbidity, when dosages between 4.0 and 6.0 mg L^{-1} were applied. The raw water pH was changed under Zr dosing, whereas chitosan had no influence on the pH of the samples. An increase in Zr dose from 8.0 to 10.0 mg L^{-1} resulted in the pH drop by 1 unit (from approximately 6.0 to 5.0). However, both coagulants could be applied without

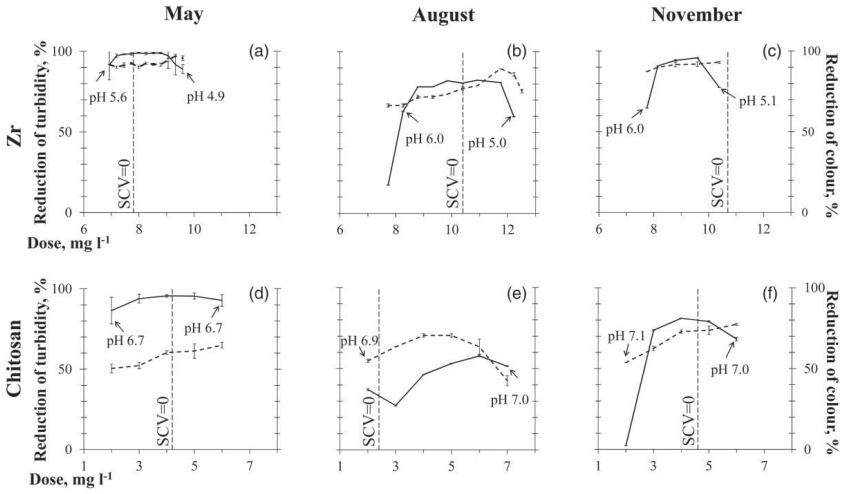

Figure 2 | Setup A: reduction (%) in turbidity (left Y-axis) and colour (right Y-axis) vs the coagulant dose for Zr (a)–(c) and chitosan (d)–(f) for water from the Glomma river, collected in May, March and November 2013. The results are expressed as the mean values and SD. Scales on X-axes vary between the coagulants.

pH-adjustment, and, as a result, consumption of the chemicals could be lowered.

Measurement of SCV enabled detection of the doses required for colloidal neutralization. Zero charge was reached between 8–11 mg Zr L^{-1} and 2–5 mg chitosan L^{-1}. These doses were consistent with high reduction efficiencies, but did not coincide exactly with the minimum effective doses. Apparently, the destabilization for these two coagulants occurs earlier than complete neutralization of colloids in the solution. A similar phenomenon was also demonstrated previously (Vogelsang *et al.* 2004; Jarvis *et al.* 2012).

The impact of pH on the destabilization efficiency is shown in Figure 3. Reductions in colour by 80–90% occurred for the metal-based coagulants at pH between 5.0–7.0 for Al and 3.5–6.3 for Zr. Efficiency between 60–80% was registered for the selected chitosan doses, applied at pH within 4.0–7.0. For all coagulants an effective minimum dose was decreasing upon protonation, implying involvement of H^+ ions in charge neutralisation mechanism (Vogelsang *et al.* 2004; Shin *et al.* 2008). Optimization of the coagulation process for the correct dose and pH was shown to be important, not only for colour reductions, but also to minimize metal residues in the treated samples (Figure 3).

Analysis of the jar-test data defined the optimal treatment conditions as 1–5 mg L^{-1} at pH 5.0–7.0 for Al, 5–12 mg L^{-1} at pH 4.5–6.3 for Zr, and 2–6 mg L^{-1} at pH 4.0–7.0 for chitosan. These data were in good agreement with previous reports. PACl dosed at 1.0–2.5 mg Al L^{-1} and pH 5.7–6.7 are recommended for contact filtration of Norwegian raw waters at colour levels of 15–50 mg Pt L^{-1} (Ødegaard *et al.* 2010). The same report prescribes 1.7–3.5 mg chitosan L^{-1} with the pH between 5 and 6. Published studies on Zr (Jarvis *et al.* 2012; Hussain *et al.* 2014; Zhang *et al.* 2014) report optimum dose and pH within ranges 5–16 mg L^{-1} and 4.5–6.0, respectively.

Pilot tests

Data from the pilot testing are summarised in Table 2. The raw and treated waters were additionally characterized for TOC and UV254 parameters. Water from a surface water source with no or low algae growth was used in the present study and it was therefore assumed that a major fraction of TOC consisted of humic matter.

The selected coagulants provided high quality effluents, although individual parameters varied for each run.

PACl is generally considered to be a robust and effective agent for water treatment (Saltnes & Eikebrokk 2002; Yan *et al.* 2007; Zhang *et al.* 2014). Indeed, the present research presented improvements of water quality by 88% for colour and 70% for UV254 for that coagulant. However, the effect of PACl on TOC was somewhat modest and did not exceed 20%. TOC provides an estimate of the amount of NOM in the water source, whereas the humic fraction of NOM is usually assessed indirectly as light absorbency in the visible (true colour) and UV wavelength range. True colour and UV254 are routinely applied surrogates for the TOC parameter in water treatment practice in Norway (Vik *et al.* 1985; Ratnaweera *et al.* 1999). However, as shown in Table 2, the correlation between colour and UV254 parameters and TOC appeared to be limited in the present study. Consequently, treatment conditions, defined via the colour surrogate, should not necessarily be interpreted as being effective for TOC removals. In such cases, coagulant assessment with respect to residual TOC could provide more reliable data. Alternatively, as the TOC data

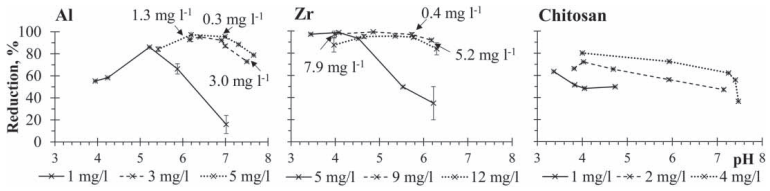

Figure 3 | Setup B: reductions (%) in colour vs coagulation pH for three different doses of Al, Zr and chitosan. The results are expressed as the mean values and SEM calculated for two independent replicates on the March water. Arrows indicate values for the residual metals measured in supernatants after sedimentation.

presented in the experiments were obtained from single measurements, poor removals that were observed for PACl and other coagulants could also be a result of measurement errors.

The effluent produced with Zr contained less organic matter than in the run with Al by 33%, as assessed by TOC measurements. In turn, results for residual colour (92% reduction) and UV254 (81% reduction) suggested a similar or slightly higher ability of Zr to reduce humic substances in water than that of Al. At these conditions, it can be speculated that Zr may be more effective against non-humic, hydrophilic NOM fractions, which, along with humic substances, contribute to water's organic carbon content. Hydrophilic NOM is undesirable in finished water and difficult to remove. Notably, the minimum effective dose of Zr was significantly lower in the pilot tests than that recorded from the jar-test results.

Chitosan is known to be less effective in NOM reduction than the metal salts due to the weaker charge derived from the amino- and hydroxyl-groups on its backbone. This is also a reason for its improved performance at acidic conditions, explained by the higher protonation of the amine groups (Guibal *et al.* 2006). Vogelsang *et al.* (2004) reported a reduction of colour by 80%, UV254 by 60% and TOC by 40% for the Glomma water after treatment with 3–4 mg L^{-1} of chitosan at pH 5.0 in jar-tests. Adequate performance of chitosan even on highly coloured water (50 mg Pt L^{-1}) was observed by Eikebrokk & Saltnes (2002). In the present work chitosan gave reliable colour reduction (by 61%), but its impact on UV254 and TOC was poor, at 31 and 7%, respectively. A somewhat moderate performance could be a result of a low chitosan dose applied under the pilot tests. Low chitosan dosage was selected to minimize an impact of cake filtration, discussed later in the text. As an explanation for low efficiency of chitosan against TOC, Eikebrokk & Saltnes (2001) suggested that high organic carbon content in the effluent, treated with chitosan, may be due to chitosan itself. Nevertheless, the quality of the effluent produced with chitosan complied with the Norwegian and European requirements for drinking water (European Directive 98/83). As demonstrated previously (Vogelsang *et al.* 2004), chitosan performance can also be strengthened by metal addition. However, in the present work a combination of chitosan and Zr was effective for colour and TOC reductions to some degree. This is probably a result of the application of a relatively high pH: acidic pH would probably be more favourable for both coagulants. Additional tests would be necessary to define the optimal pH range, effective mixing ratios between these two components and their effect on NOM.

Chitosan was noted to destabilize over a broad pH range without affecting the pH of the treated water. These properties of chitosan make its application especially convenient for small treatment facilities that might have difficulty in attaining optimal and stable coagulation steps, and sometimes lack the necessary expertise for solving specific operational problems.

Filter function

Filter cycle length describes the time the effluent turbidity is below 0.2 NTU. The filter cycle time for Zr was 5–7 hours longer than for Al or chitosan (Table 2). Both Jarvis *et al.* (2012) and Hussain *et al.* (2014) reported that flocs formed by Zr had greater strength characteristics than Al. Stronger flocs are more resistant to shear forces and may be sustained in the filter bed over a longer period of time. However, it is also possible that the different filter cycle lengths could be attributed to a pH-factor, which might affect electrostatic interactions between filter media and flocs. Longer filter runs are considered to be favourable for plant operation, as fewer filter 'starts and stops' are required and, therefore, both energy and backwash water can be saved.

Four-fold less suspended solids in the influent when using chitosan was assessed by TSS analysis. Lower sludge load in the experiment with chitosan was also reflected by the influent turbidity level and hydraulic head parameter. As hydroxides are not formed with chitosan, it is believed to produce smaller flocs and lower sludge and hydraulic loads than metal salts. As a result, chitosan is often associated with prolonged filter runs. Indeed, full-scale water plants in Norway, using chitosan in everyday practice, report doubling of the filter cycle times. However, as noted here and previously (Saltnes & Eikebrokk 2002), application of chitosan can be challenging for fine filter beds due to filter cake formation. Monitoring of head loss during the chitosan run revealed no solids accumulation at depths below 20 cm. That eventually resulted in early

turbidity breakthrough, observed already after 17 hours of operation with chitosan. In treatment practice, filter clogging is usually associated with coagulant or polymer overdose, but the chitosan dose used in the present study was too low for this. The problem was not eliminated even after numerous efforts to adjust the treatment conditions, nor after mixing with Zr. The explanation is unknown, but the phenomenon could be specific for pilot plants only.

CONCLUSIONS

In the present work the four-valent Zr and natural polymer chitosan were compared with the traditional PACl coagulant for use in drinking water treatment. The results showed that both filter operation and reduction of humic substances were improved with Zr coagulant. Therefore, it was suggested that the use of Zr could be especially convenient for the treatment of highly coloured and/or NOM-rich waters. Although chitosan gave acceptable colour reduction, it was poor for reduction of TOC in the pilot trials. Further research could potentially investigate that chitosan performance might be strengthened by use of higher doses or metal addition. Notably, operation with chitosan was accompanied by cake filtration that was difficult to explain. Another drawback with the use of chitosan is its cost, which is higher than for PACl or Zr. Nevertheless, chitosan possesses some valuable features that the metal coagulants lack: chitosan's ability to destabilize over a broad pH range without affecting the pH of the treated water, production of water with no metal residues and reduced amount of sludge under the filter operation. This makes chitosan a suitable alternative for small water treatment plants that might have difficulty in attaining optimal and stable coagulation steps or would like to reduce costs associated with sludge treatment and utilization.

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REFERENCES

Bratby, J. 2006 *Coagulation and Flocculation in Water and Wastewater Treatment*, 2nd edn. IWA Publishing, London.

Eikebrokk, B. & Saltnes, T. 2001 Removal of natural organic matter (NOM) using different coagulants and light weight expanded clay aggregate filters. *Water Sci. Technol. Water Supply* **1** (2), 131–140.

Eikebrokk, B. & Saltnes, T. 2002 NOM Removal from drinking water by chitosan coagulation and filtration through lightweight expanded clay aggregate filters. *J. Water Supply Res. Technol. Aqua* **51** (6), 323–332.

European Commission 1998 Council Directive 98/83/EC on the quality of water intended for human consumption. *Off. J. Eur. Commun. L* **330/32**, 32–54.

Garmo, Ø. A., Skjelkvåle, B. L., De Wit, H. A., Colombo, L., Curtis, C., Fölster, J. & Worsztynowicz, A. 2014 Trends in surface water chemistry in acidified areas in Europe and North America from 1990 to 2008. *Water Air Soil Pollut.* **225** (1880), 1–14. http://doi.org/10.1007/s11270-014-1880-6.

Gregory, J. & Duan, J. 2001 Hydrolyzing metal salts as coagulants. *Pure Appl. Chem.* **73** (12), 2017–2026.

Guibal, E., Van Vooren, M., Dempsey, B. A. & Roussy, J. 2006 A review of the use of chitosan for the removal of particulate and dissolved contaminants. *Separ. Sci. Technol.* **41** (11), 2487–2514.

Hussain, S., van Leeuwen, J., Chow, C. W. K., Aryal, R., Beecham, S., Duan, J. & Drikas, M. 2014 Comparison of the coagulation performance of tetravalent titanium and zirconium salts with alum. *Chem. Eng. J.* **254**, 635–646.

ISO 11923 1997 *Water Quality – Determination of Suspended Solids by Filtration through Glass-fibre Filters*. International Standards Organization, Geneva, Switzerland.

ISO 17294-1 2004 *Water Quality – Application of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) – Part 1: General Guidelines*. International Standards Organization, Geneva, Switzerland.

ISO 17294-2 2003 *Water Quality – Application of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) – Part 2: Determination of 62 Elements*. International Standards Organization, Geneva, Switzerland.

ISO 7887 2011 *Water Quality – Examination and Determination of Colour*. International Standards Organization, Geneva, Switzerland.

ISO 8245 1999 *Water Quality – Guidelines for the Determination of Total Organic Carbon (TOC) and Dissolved Organic*

Carbon (DOC). International Standards Organization, Geneva, Switzerland.

Jarvis, P., Sharp, E., Pidou, M., Molinder, R., Parsons, S. A. & Jefferson, B. 2012 Comparison of coagulation performance and floc properties using a novel zirconium coagulant against traditional ferric and alum coagulants. *Water Res.* **46** (13), 4179–4187.

Johnson, E. L. & Peniston, Q. P. 1970 Method for treating an aqueous medium with chitosan and derivatives of chitin to remove an impurity, Patent 3533940. Available from: www.google.com/patents/US3533940 (accessed 22 June 2016).

Matilainen, A., Vepsäläinen, M. & Sillanpää, M. 2010 Natural organic matter removal by coagulation during drinking water treatment: a review. *Adv. Colloid Interface Sci.* **159**, 189–197.

Ødegaard, H., Østerhus, S., Melin, E. & Eikebrokk, B. 2010 NOM removal technologies – Norwegian experiences. *Drink. Water Eng. Sci.* **3** (1), 1–9.

Ratnaweera, H., Hiller, N. & Bunse, U. 1999 Comparison of the coagulation behaviour of different Norwegian aquatic NOM. *Environ. Int.* **25** (2), 347–355.

Renault, F., Sancey, B., Badot, P.-M. & Crini, G. 2009 Chitosan for coagulation/flocculation processes – an eco-friendly approach. *Eur. Polym. J.* **45**, 1337–1348.

Saltnes, T. & Eikebrokk, B. 2002 Contact filtration of humic waters: performance of an expanded clay aggregate filter (Filtralite) compared to a dual anthracite/sand filter. *Water Sci. Technol. Water Supply* **2** (5–6), 17–23.

Shin, J. Y., Spinette, R. F. & O'Melia, C. R. 2008 Stoichiometry of coagulation revisited. *Environ. Sci. Technol.* **42** (7), 2582–2589.

Vik, E. A., Carlson, D. A., Eikum, A. S. & Gjessing, E. T. 1985 Removing aquatic humus from Norwegian lakes. *J. Am. Water Works Assoc.* **77** (3), 58–66.

Vogelsang, C., Andersen, D. O., Hey, A., Håkonsen, T., Jantsch, T. G., Müller, E. D. & Vårum, K. M. 2004 Removal of humic substances by chitosan. *Water Sci. Technol. Water Supply* **4** (5), 121–129.

Yan, M., Wang, D., Qu, J., He, W. & Chow, C. W. K. 2007 Relative importance of hydrolyzed Al(III) species (Al(a), Al(b), and Al (c)) during coagulation with polyaluminum chloride: a case study with the typical micro-polluted source waters. *J. Colloid Interface Sci.* **316**, 482–489.

Zhang, Z., Wu, C., Wu, Y. & Hu, C. 2014 Comparison of coagulation performance and floc properties of a novel zirconium-glycine complex coagulant with traditional coagulants. *Environ. Sci. Pollut. Res.* **21** (10), 6632–6639.

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Paper 2

Removal of model viruses, *E. coli* and *Cryptosporidium* oocysts from surface water by zirconium and chitosan coagulants

Ekaterina Christensen, Vegard Nilsen, Tor Håkonsen, Arve Heistad, Christophe Gantzer, Lucy J. Robertson and Mette Myrmel

ABSTRACT

The present work evaluates the effect of contact filtration, preceded by coagulation with zirconium (Zr) and chitosan coagulants, on model microorganisms and waterborne pathogens. River water intended for potable water production was spiked with MS2 and *Salmonella* Typhimurium 28B bacteriophages, *Escherichia coli*, and *Cryptosporidium parvum* oocysts prior to coagulation. The hygienic performance demonstrated by Zr comprised 3.0–4.0 $\log_{10}$ removal of viruses and 5.0–6.0 $\log_{10}$ removal of *E. coli* and *C. parvum* oocysts. Treatment with chitosan resulted in a removal of 2.5–3.0 $\log_{10}$ of viruses and parasites, and 4.5–5.0 $\log_{10}$ of bacteria. A reference coagulant, polyaluminium chloride (PACl), gave a 2.5–3.0 $\log_{10}$ removal of viruses and 4.5 $\log_{10}$ of *E. coli*. These results indicate that both Zr and chitosan enable adequate removal of microorganisms from surface water. The present study also attempts to assess removal rates of the selected microorganisms with regard to their size and surface properties. The isoelectric point of the *Salmonella* Typhimurium 28B bacteriophage is reported for the first time. The retention of the selected microorganisms in the filter bed appeared to have some correlation with their size, but the effect of the charge remained unclear.

Key words | chitosan, coagulation, contact filtration, drinking water treatment, waterborne pathogens, zirconium

Ekaterina Christensen (corresponding author)
Lucy J. Robertson
Mette Myrmel
Department of Food Safety and Infection Biology – Faculty of Veterinary Medicine,
Norwegian University of Life Sciences,
P.O. Box 8146, Dep.,
0033 Oslo, Norway
E-mail: *ekaterina.christensen@norconsult.com*

Ekaterina Christensen
Tor Håkonsen
Norconsult AS,
PO Box 626,
1303 Sandvika, Norway

Vegard Nilsen
Arve Heistad
Department of Mathematical Sciences and Technology,
Norwegian University of Life Sciences,
P.O. Box 5003,
N-1432 Aas, Norway

Christophe Gantzer
Laboratoire de Chimie, Physique et Microbiologie pour l'Environnement (LCPME),
Université de Lorraine,
UMR 7564, Faculté de Pharmacie,
Nancy F-54000, France
and
CNRS, LCPME, UMR 7564,
Nancy F-54000, France

INTRODUCTION

All source waters can potentially be contaminated with human pathogens, originating from animal and human excreta. In order to achieve an acceptable drinking water quality, hygienic barriers are implemented as a part of drinking water treatment. A hygienic barrier is a natural or artificial physical or chemical barrier for removal or inactivation of pathogens. Norwegian Drinking Water Regulations require at least two independent hygienic barriers in the water supply system (Norwegian Food Safety Authority 2001). In order to be considered as a hygienic barrier, a water treatment method should be able to reduce bacteria and virus concentrations by a minimum of 99.9% (3 $\log_{10}$) and parasites by 99% (2 $\log_{10}$) (Norwegian Food Safety Authority 2001).

The combination of coagulation, flocculation and sedimentation followed by rapid granular filtration (conventional treatment) is a commonly employed hygienic barrier. The effectiveness of conventional treatment has been evaluated by numerous studies, for which reported removals range from less than 1 $\log_{10}$ (Harrington *et al.* 2003) up to 7 $\log_{10}$ (Abbaszadegan *et al.* 2007), depending on the operational conditions and microbial

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agents to be removed. Some studies have attempted to assess the relationship between coagulant type and microbial log-removals (Rao *et al.* 1988; Brown & Emelko 2009). The work by Brown & Emelko (2009) indicated that alternative coagulants can provide similar or higher removal effects on *Cryptosporidium parvum*, as conventional aluminium (Al) and ferric (Fe). Previous studies have reported high coagulation efficiencies for zirconium (Zr) and chitosan alternative coagulants in terms of colour and turbidity reductions (Christensen *et al.* 2016); however, little is known about their effects on various waterborne microorganisms. Thus, broader characterization of these alternative agents in terms of their hygienic effects could provide information of relevance.

Removal efficiencies of microorganisms under physicochemical treatment processes are potentially influenced by their morphological, physical, and biochemical characteristics. In the present study, bacteriophages MS2 and *Salmonella* Typhimurium 28B, *Escherichia coli* and *Cryptosporidium parvum* oocysts were selected to cover the size range for waterborne pathogens (Table 2). Data on the isoelectric points (pI) of the selected species are also provided. As pI characteristics for the 28B phage were not available, the present study employed electrophoretic mobility measurements for this microorganism.

The overall aim of the present study was to evaluate the impacts of Zr and chitosan coagulants on the removal of model microorganisms and waterborne pathogens from surface water, relative to a well-characterized and effective polyaluminium chloride (PACl) agent. The present work also assesses the ability of all three coagulants to fulfil the hygienic barrier requirements.

MATERIAL AND METHODS

Raw water samples

All experiments were performed on water collected from the Glomma River, at Nedre Romerike Water Treatment Plant in Strømmen, Norway in May 2015. The water was stored in a stainless steel tank for 7 months, during which period the water temperature ranged between 5 and 15 °C. The tank was equipped with a recirculating pump, working continuously to resuspend settled particles. The water quality was assessed prior to each run (Table 1).

Table 1 | Characteristics of the Glomma water used for testing; colour measurements are expressed as means ($n = 3$); TOC values were measured once

Run	Coagulant	Run month (2015)	pH	Turbidity, NTU	Colour, mg Pt/L Average	TOC, mg/L
1	Al	May	7.3	0.8	26	3.0
2	Zr	September	7.0	0.8	24	3.0
3	Chitosan	October	7.2	0.5	23	3.0
4	Chitosan + Zr	December	7.6	0.7	19	2.9

TOC, total organic carbon.

Table 2 | Microorganism characteristics

Microorganisms	Size, nm	pI	Reference	Reason
Bacteriophage MS2	29	3.9	Langlet *et al.* (2008)	Common model for naked, enteric viral pathogens
Bacteriophage 28B	head 50 nm; tail 10 nm	–	Svenson *et al.* (1979)	Model for viruses resistant to environmental stress (Hoglund *et al.* 2002)
E. coli	2 μm long	acidic	Alves *et al.* (2010)	Model for waterborne bacteria
C. parvum	oocysts are 3–5 μm in diameter	2.5	Dumètre *et al.* (2012)	Protozoan parasite, pathogenic to various species including humans

Water analyses

Turbidity was measured using a 2100 N IS turbidimeter (Hach Company, USA). Colour was measured by DR3900 Hach spectrophotometer (Hach, USA), following ISO 7887:2011 ($\lambda = 410$ nm) procedure. Prior to colour measurements, the samples were filtered through a 0.45 μm syringe polypropylene membrane (514-0065, VWR, USA), in order to avoid the influence of turbidity. pH was measured with SenTix® 41 pH-sensor (WTW, Germany). Total organic carbon (TOC), residual Al and Zr were analysed by commercial laboratories (ALS Laboratory Group Norway AS or Noranalyse AS), following ISO 8245:1999, ISO 17294-1:2004 and ISO 17294-2:2003 procedures, respectively. Inlet and outlet water were also monitored on-line for pH (SensoLyt 700 IQ, WTW, Germany) and turbidity (VisoTurb 700 IQ, WTW, Germany).

Microorganisms

Microorganisms included in the present study, their main characteristics and the rationale for their inclusion in the study are provided in Table 2.

The bacteriophage MS2 was propagated using *Salmonella* Typhimurium WG49 (NCTC 12484) as host (ISO 10705-1:1995). Infectious MS2 was enumerated by a PFU (plaque forming unit) assay, using *Escherichia coli* Famp as bacterial host, as previously described by Debartolomeist & Cabelli (1991), with minor modifications.

Initial testing revealed that MS2 plaque counts were reduced in the presence of coagulant in both influent and effluent samples. In order to decrease this effect, treatment with an alkaline beef extract (BE) solution was included (Shirasaki *et al.* 2009). Prior to the MS2 phage enumeration, samples (1 mL) were mixed 1:10 with BE and stirred at 1,500 rpm at +4 °C for a minimum of 5 hours. Virus dilutions were also prepared with the BE solution. Thirteen per cent BE (211520, Becton-Dickinson and Company, USA) was prepared in sterile water, followed by adjustment to pH 9.5–10.0 with 5 N NaOH. The solution was stored at +4 °C and used within three days.

Propagation and enumeration of the *Salmonella* Typhimurium 28B phage was conducted according to ISO 10705-1 (1995), with some modifications. *Salmonella* Typhimurium type 5 was used as a host strain. The sample (0.5 mL) was mixed with 0.5 mL of host culture and 4 mL of a soft agar. Bottom base agar contained 8.0 g of nutrient broth (105443, Merck, Germany), 0.5 g of yeast extract (111926, Merck, Germany), and 15.0 g of agar in 1 L of distilled water. Soft agar was prepared similarly, but contained 6.5 g of agar only. Unlike MS2, enumeration of infectious 28B phage was influenced only by Zr coagulant; however, BE treatment was not used for that virus.

Escherichia coli (CCUG 17620) was cultivated in brain–heart infusion broth (237500, Becton-Dickinson and Company, USA). The overnight culture (16–18 hours at 37 °C) was centrifuged at 1,500 g for 20 min and washed twice with peptone saline diluent (CM0733, Oxoid Ltd, UK), and stored at +4 °C for no longer than 5 days. Enumeration was performed using Colilert-18 with Quanti-Tray/2000 (IDEXX Laboratories, USA) according to the manufacturer's instructions.

Cryptosporidium parvum oocysts were purchased from Moredun Scientific Limited (Scotland, UK). For detection of oocysts in the influent, samples were vortexed for 60 s, pipetted directly on a slide (100 μL) and enumerated by IFAT (immunofluorescent antibody test). Oocysts in the effluent samples (10 L) were concentrated by membrane filtration and centrifugation, followed by separation by IMS (immunomagnetic separation) prior to IFAT. Each slide was counted twice. Concentration and enumeration procedures were based on standard methods (ISO 15553 (2006) and US EPA Method 1623 (2005)). Prior to analysis, two quality control samples, prepared with 10 L tap water and oocysts EasySeed™ spike (TCS Biosciences, UK), were analysed to assess recovery efficiency, which was shown to be between 60 and 70%.

Infectious bacteriophages were enumerated in technical duplicates, whereas *E. coli* and *C. parvum* oocysts were quantified from a single measurement.

Molecular quantification of MS2

In an additional attempt to decrease the impact of the coagulants, enumeration of MS2 was also performed by quantitative reverse transcription polymerase chain reaction (RT-qPCR). Samples (140 μL) were added to lysis buffer (560 μL) immediately after sampling and stored at −80 °C. RNA was extracted with the QIAamp® Viral RNA Mini kit and QIAcube automated system (Qiagen, Germany) according to the manufacturer's instructions. Carrier-RNA (3.1 μg

per sample) was spiked after thawing and prior to RNA-extraction. RT-qPCR was performed in a Stratagene AriaMx Real-Time PCR System (Agilent Technologies, Inc., USA) using the RNA UltraSense™ One-Step Quantitative RT-PCR System kit (Invitrogen, USA). Three μL RNA was used in a total volume of 20 μL, using primers, probe and RT-qPCR conditions listed in Table 3 (Dreier *et al.* 2005). ROX was used as passive reference, and positive and negative controls were included in each run. Each sample was run in technical duplicates and the results were analysed using Agilent AriaMx 1.1 Software and Microsoft Excel.

Relative quantification was performed using a standard curve, prepared from 10-fold serial dilutions of homologous viral RNA, run in technical triplicates. The amount of viral RNA was expressed in PCR units (PCRU) per mL: 1 RT-PCR unit was defined as the amount in the highest dilution of the standard, from which MS2 RNA could be amplified. Aliquoted homologous RNA was included in all plates and used as an inter-plate calibrator (IPC) (Hellemans *et al.* 2007). Thresholds for the individual plates were adjusted manually so that Ct-values for each IPC became similar. Finally, the number of PCRU was expressed using the formula:

$$N_s = N_{IPC} \cdot (1+E)^{(Ct_{IPC}-Ct_s)} \quad (1)$$

where N_s is the amount of viral RNA in the sample; N_{IPC} is the amount of viral RNA in the IPC; Ct_{IPC} and Ct_s are threshold cycles for the IPC and sample, respectively; E is efficiency of amplification.

An initial experiment was run in order to test whether the presence of coagulant would affect the RT-qPCR-assay. Distilled water was spiked with MS2, followed by coagulants (PACl, Zr or chitosan), and compared with a control sample without coagulants. Viral RNA was extracted and enumerated as described earlier. No inhibitory effect was observed for coagulant concentrations up to 10 mg/L.

Seeding of microorganisms

The main seed suspension consisted of MS2, 28B and *E. coli* prepared in 15 L of distilled water. Additionally, 1 L of this suspension was spiked with *C. parvum*. The main suspension was continuously seeded into inlet water during the first 13–15 h of the pilot plant operation. Thereafter, the suspension with *C. parvum* was seeded for the next 2 h. Two influent and two effluent samples were collected within this period. The first effluent sample was collected after 1 h of *C. parvum* seeding, corresponding to five pore volumes. Finally, the suspension without *C. parvum* was seeded again. *C. parvum* was seeded only during Runs 2 and 3. The seed suspension was cooled on ice and stirred continuously during each run. Influent titres were 6–7 $\log_{10}$ PFU/mL for each of the two phages, 6 $\log_{10}$/100 mL for *E. coli* and ~3 $\log_{10}$ oocysts/mL for *C. parvum*.

Microorganism seed suspensions were not purified before use and could therefore elevate TOC concentrations in the raw water, and, consequently, impact the coagulation performances. However, the crude microorganism stocks were diluted at least by 2,500-fold; first, during the preparation of the seed suspensions and then after mixing with raw water. Hence, the uptake of the residual component of the culture medium was considered as minimal. The initial tests also confirmed that the surplus of TOC in raw water did not affect the outlet water quality.

Coagulants

Polyaluminium chloride

A commercial PACl product, PAX-18, was obtained from Kemira Chemicals (Norway). The product had basicity of 42% and specific gravity of 1.37 g/mL.

Table 3 | Primers/probe and RT-qPCR conditions

Primers and probe[a]	Sequence (5′-3′)	RT-qPCR conditions
MS2-TM2-F (400 nM)	TGCTCGCGGATACCCG	30 min at 55 °C, 2 min at 95 °C and 45 cycles of 15 s at 95 °C, 30 s at 58 °C
MS2-TM2-R (400 nM)	AACTTGCGTTCTCGAGCGAT	
MS2-TM2FAM (50 nM)	FAM-ACCTCGGGTTTCCGTCTTGCTCGT- BHQ1	

[a]Retrieved from Dreier *et al.* (2005) with some modifications in cycling conditions and primers/probe volumes.

Zirconium oxychloride

Zirconium (IV) oxychloride octahydrate powder was obtained from Teta Vannrensing Ltd (Norway). A working solution of 15% (w/w) was prepared in distilled water.

Chitosan

Commercial chitosan product KitoFlokk™ (low molecular weight [MW, 100 kDa] and deacetylation degree [DD] close to 0.8) was obtained from Teta Vannrensing Ltd, Norway. The concentration of the working solution was 0.5% (w/v) in 0.1 M HCl. All working solutions were stored at room temperature and the chitosan solution was prepared fresh prior to each run.

Pilot tests

A schematic and detailed experimental procedure for the filtration system has been described previously (Christensen *et al.* 2016). Briefly, the pilot-scale system combined coagulation with filtration in a dual-media contact filter column. The column, 10 cm in diameter and 2.5 m high, was packed with support gravel (0.1 m), followed by 0.5 m-layer of Rådasand® sand (Rådasand AB, Sweden, $d_{10} =$ 0.4 mm) and 0.8 m of Filtralite®NC 0.8–1.6 mm material (Weber Leca Raelingen, Norway, $d_{10} = 0.95$ mm, 500 kg/m^3 dry bulk density). Raw water was pumped at a constant filtration rate of 5.9 m/h. The microorganism suspension, HCl (1 M), and coagulants were fed sequentially to the inlet pipe at constant flow rates. The amount of HCl was set depending on the used coagulant and desired process pH.

As in water treatment practice, the coagulation conditions were optimized in terms of particulate and organic matter removals. A single combination of coagulant dose and pH was used for each selected coagulant. Optimal coagulation conditions were defined, based on residual particles (turbidity) and natural organic matter (NOM, colour) in the filtrate samples. Each coagulant was screened under stable pH conditions, which corresponded to the lower limit of the optimal pH ranges, reported in the literature: 5.7–5.8 for PACl and 4.0–4.2 for Zr and chitosan (Ødegaard *et al.* 2010; Jarvis *et al.* 2012; Christensen *et al.* 2016). A range of low doses was further tested in pilot scale to detect the minimal effective doses that provided turbidity <0.2 NTU, colour <5 mg Pt/L and residual Al and Zr <0.15 mg/L (European Commission 1998, European Directive 98/83) in the outlet water. The following treatments were selected: Run (1) 1.5 mg Al/L at pH 5.8; Run (2) 2.4 mg Zr/L at pH 4.2; Run (3) 1 mg/L of chitosan at pH 4.2; Run (4) 1 mg/L of chitosan mixed with 0.3 mg Zr/L at pH 6.0. Originally, lower coagulation pH was chosen for the last run; however, a slight change in raw water quality during storage affected the system, and the pH was unintentionally raised to 6.0.

Raw water samples were collected as grab samples from the raw water feed tank. Influent and effluent samples were collected from two ports available for manual sampling. The ports were tapped for a couple of minutes prior to sampling. Samples for virus and *E. coli* enumeration (50–150 mL) were processed within 6 h after sampling, *C. parvum* oocysts samples were concentrated and enumerated within 2 weeks after sampling. All samples were stored at 4 °C prior to processing.

Size and electrophoretic mobility measurements of 28B phage

The procedures for phage purification and electrophoretic mobility measurements were similar to those described previously by Langlet *et al.* (2008). Forty mL of phage suspension (propagated according to ISO 10705-1) were centrifuged at 4,000 g for 10 min at 4 °C. The supernatant was filtered through a 0.22 μm membrane (SLGP033RB, Millipore, Germany) and concentrated to 6 mL by ultrafiltration (100 kDa, UFC910008, Merck, Germany) at 3,500–4,000 g for 20 min at 20 °C. Caesium chloride (3.65 g, Carl Roth Gmbh, Germany) was dissolved in 5 mL of the phage suspension and centrifuged at 100,000 g for 15 h at 15 °C. The fraction with purified phage was dialyzed (100 kDa MWCO, 131420, Spectrum Laboratories, Inc., USA), against deionized water for 6 h, and against $NaNO_3$ (1 mM, pH 7.0) for 15 h. The phage was stored at 4 °C prior to measurement of electrophoretic mobility and size distribution using a Zetasizer Nano ZS (Malvern

Instruments Ltd, UK). The measurements were performed over a wide range of ionic strengths (1–100 mM $NaNO_3$) and pH conditions (3.0–6.6; adjusted with either HCl or NaOH). Prior to measurement, solutions with the selected ionic strength and pH were filtered through a 0.1 μm syringe filter (16553, Stedium Biotech GmbH, Germany) and spiked with the purified phage to a final concentration of approximately 10^{11} PFU/mL. Each measurement was performed in biological triplicates.

RESULTS

Filtration performance

The concentration of microorganisms in the influent remained stable during each run. Data on effluent water quality obtained with the selected coagulants are provided in Table 4 and Figure 1. For all three coagulants, the effluents were characterized by low residual turbidity and colour. The microorganism concentrations were reduced

Table 4 | Chemical characteristics for the effluents, and the percentage reduction compared with the influent water; colour measurements are expressed as means ($n = 3$); TOC values were measured once

Run	Coagulant	Colour, mg Pt/L average	Colour reduction, %	TOC, mg/L	TOC reduction, %	Residual metal, μg/L
1	Al	3	88	2.4	20	<10
2	Zr	2	92	1.4	53	<10
3	Chitosan	9	61	2.8	7	–
4	Chitosan + Zr	6	68	2.6	10	–

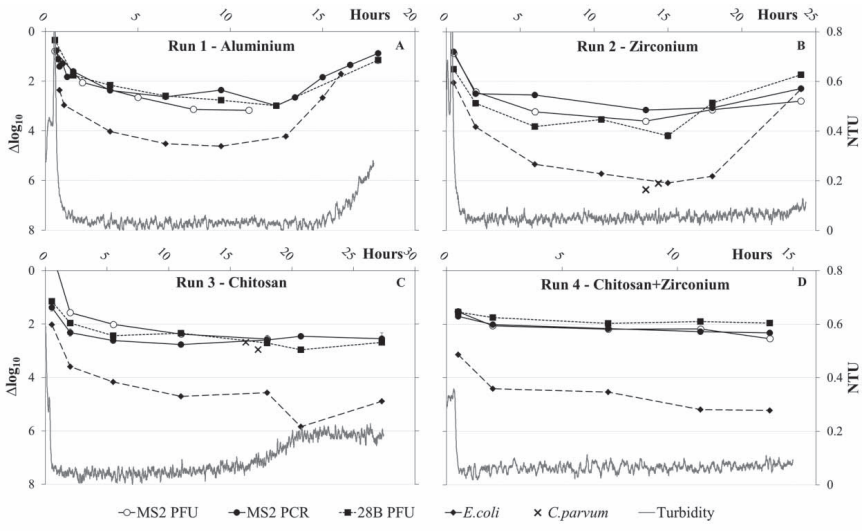

Figure 1 | Effluent turbidity and log-reductions of MS2 (PFU/mL and PCRU/mL), 28B (PFU/mL), *E. coli* (MPN/100 mL) and *C. parvum* (oocysts/10 L), as a function of the filter operation time. The conditions were: 1.5 mg Al/L at pH 5.8 (Run 1); 2.4 mg Zr/L at pH 4.2 (Run 2); 1 mg/L of chitosan at pH 4.2 (Run 3); 1 mg/L of chitosan mixed with 0.3 mg Zr/L at pH 6.0 (Run 4). *C. parvum* was sampled on two occasions (Run 2 and 3) after 13 and 15 h of operation, respectively. Log-reduction of phages is expressed as mean values and SD. *E. coli* and *C. parvum* oocysts results are derived from a single measurement.

by the coagulation-filtration treatments, although to various extents for different coagulants.

Effluent turbidity changed dynamically throughout filter operation. During ripening, the turbidity was compromised, but as the filter became loaded, the quality of the effluent improved gradually and remained stable for the next 15–22 h. Filter operation was terminated by turbidity breakthrough upon reaching maximum filter loading capacity. The dynamics of microorganism retention reflected that of the effluent turbidity to some extent. While a satisfactory turbidity (<0.2 NTU) in the effluent was achieved after 30 min of ripening, concentrations of virus and bacteria were still high. Reduction of microorganisms gradually increased as the cycle progressed. Zirconium coagulant achieved a 3–4 $\log_{10}$ removal of infectious MS2 and 28B phages and 5–6 $\log_{10}$ removal of *E. coli* and *C. parvum* (Figure 1). When PACl was used, infectious phages and bacteria were removed by 2.5–3.0 $\log_{10}$ and 4.5 $\log_{10}$, respectively. Bacteria removal by chitosan was similar to those determined for the metal salts, 4.5–5.0 $\log_{10}$, while its effect on infectious phage was slightly lower (~2.5 $\log_{10}$). Relatively poor *C. parvum* removal (~3 $\log_{10}$) was also observed during the run with chitosan. Addition of Zr to the coagulation mixture did not improve chitosan performance for the infectious phage (2.0–2.5 $\log_{10}$ removal) or bacteria (4.4–5.0 $\log_{10}$ removal).

MS2 analysis was strengthened by simultaneous enumeration of total (PCRU) and infectious (PFU) virus particles. The titres obtained for total virus were 0.1–0.2 $\log_{10}$ higher than for infectious virus; however, the removal patterns were similar (Figure 1). For all coagulants, the reduction of total MS2 was close to 2.2–2.6 $\log_{10}$.

Turbidity breakthrough had a dramatic impact on the number of microorganisms in the effluent water. For PACl and Zr coagulants, turbidity breakthrough occurred after 15 and 22 h of filter operation, respectively. However, for both coagulants, the microbiological water quality started to decline a few hours ahead of turbidity. The impairment was especially dramatic for *E. coli*. For chitosan, deterioration of the effluent turbidity started after 16–17 h of filter run; however, neither phages nor bacteria were affected and the number of microorganisms continued to decline. Due to the limited number of analyses, the effect of breakthrough on *C. parvum* was not evaluated. No signs of turbidity or microbiological breakthrough could be identified for the run with chitosan and Zr after 15 h of operation.

A positive correlation between TOC removals and log-reductions of phage ($R^2 = 0.85$) and bacteria ($R^2 = 0.70$) could be detected for Runs 1–4 (Figure 2).

Electrophoretic mobility and size distribution of 28B phage

Measurements of electrophoretic mobility and size distribution as a function of both ionic strengths and pH are presented in Figure 3. The phage exhibited a negative charge at pH close to neutrality, which was gradually weakened upon pH decrease or ionic strength increase (Figure 3(a)). The isoelectric point or point of zero charge was reached at pH 3.8. Phage particles were uniformly dispersed in the suspension within pH 4.0–7.0 (Figure 3(b)). The mean hydrodynamic diameter of each particle was close to 75–78 nm. Aggregation occurred close to pH 4.0 for 1 and 10 mM $NaNO_3$ solutions and at pH 3.4 for the 100 mM $NaNO_3$ solution.

DISCUSSION

The present study assessed the removal capacity of two non-conventional coagulants – Zr and chitosan – for physico-chemical and microbiological parameters, and compared

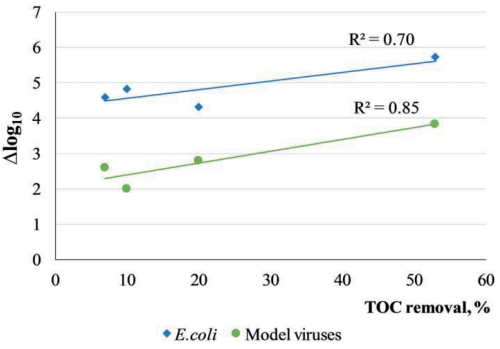

Figure 2 | Correlation between TOC removals and log-reductions of *E. coli* and bacteriophages for Runs 1–4; log-reductions during the stable filtration phases were used for calculations.

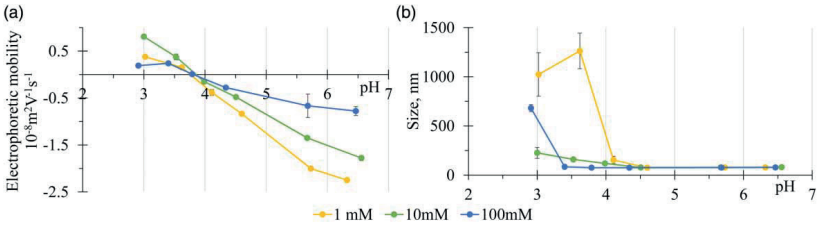

Figure 3 | Measurement of electrophoretic mobility (a) and size distribution (b) of 28B phage under varying ionic strengths ($NaNO_3$) and pH conditions; measurements are expressed as mean values ($n = 3$) and SEM (standard error of the mean).

their efficiencies with those of PACl. Log-removals for all coagulants were somewhat higher than previously published, despite the application of minimal effective doses. According to a review by Hijnen & Medema (2010), contact filtration systems with traditional Al and Fe coagulants may provide, on average, 0.9 $\log_{10}$ (0.1–1.5 $\log_{10}$ range) removal for viruses, 1.4 $\log_{10}$ (0.8–2.1 $\log_{10}$ range) for bacteria and 3.0 $\log_{10}$ (0.1–5.4 $\log_{10}$ range) for *C. parvum*. Although the low reductions could be a result of the weighting system used in this review, there is also a concern that contact filtration treatment might be considered inefficient for removal of pathogenic virus and bacteria (Hijnen & Medema 2010). In the present study, the selected coagulants fulfilled the requirements for a hygienic barrier, apart from chitosan, which gave insufficient virus reduction. Addition of Zr to the coagulation mixture did not improve chitosan performance for the removal of both MS2 and *E. coli*. Poor performance by the mixture is probably due to the relatively high coagulation pH, as acidic pH would likely be more favourable for both coagulants.

Zirconium gave 1.5 $\log_{10}$ fewer *E. coli* in the effluent than the other coagulants and retained more than 6 $\log_{10}$ of *C. parvum* oocysts. For MS2 removal, almost no difference in the efficiency of Zr and Al could be detected.

High microbial removal by Zr can be attributed to several factors. Zirconium effectiveness is usually explained in terms of its valency (Jarvis *et al.* 2012; Hussain *et al.* 2014), which presumably provides higher charge neutralization power, which is necessary for destabilization of microbial and other colloids. This also explains the higher Zr affinity to organic matter shown in the present and previous studies (Jarvis *et al.* 2012).

Chitosan is regarded as a potential substitute for the traditionally used Al and Fe coagulants, and may even be preferable due to its properties of biodegradability and non-toxicity, along with the ability to produce less sludge or water with no metal residue (Renault *et al.* 2009). Brown & Emelko (2009) assessed chitosan reduction properties for *C. parvum* oocysts in a pilot filter. Removal was shown to be dose-dependent, and the highest tested dose (3 mg chitosan/L) gave 4.2 $\log_{10}$ removal of the pathogen. Treatment with water-soluble chitosan (5 mg/L) and a small ceramic filter removed MS2 and *E. coli* by 3 and 6 $\log_{10}$, respectively (Abebe *et al.* 2016). The results achieved by chitosan for similar microorganisms in the present study were poorer. This could be due to setup differences.

It is likely that production of insoluble hydroxide-species could assist the metal-based coagulants in enhanced coagulant–microorganism interactions. Turbidity measurements revealed high levels of hydroxide species in the influent for Al and Zr (Christensen *et al.* 2016). For chitosan, which does not produce hydroxides and was dosed at low concentrations, such interactions seem to be limited. This may explain the reduced retention of virus-sized biocolloids using chitosan. Higher chitosan dosing could, potentially, enhance microorganism removal. However, high doses were avoided because of the formation of a cake layer on top of the filter (Christensen *et al.* 2016), which would affect the filtration process, and therefore the barrier properties (Logsdon *et al.* 2002). Future studies on chitosan may still be required to verify its hygienic properties. Another interesting aspect to address is inactivation of pathogens in the sludge produced with chitosan. Antimicrobial activity of chitosan towards bacteria, viruses, and fungi is well documented (Rabea *et al.* 2003; Su *et al.* 2009). A metal-free sludge with reduced levels of infective pathogens would

have fewer disposal problems and may be suitable for use in agriculture.

The treatment conditions defined on the basis of turbidity and colour were generally sufficient to meet the criteria for a hygienic barrier for the selected microorganisms. Moreover, a linear relationship could be established between microbial reductions and residual TOC. However, as the number of runs was limited, additional tests are necessary to confirm whether effluent TOC could be used to estimate coagulant efficiency for removal of microorganisms. It is noteworthy that a similarity between the mechanisms for removal of organic matter and microbes has been postulated previously for viruses (Abbaszadegan *et al.* 2007) and *C. parvum* oocysts (Xagoraraki & Harrington 2004).

The turbidity parameter is extensively used to assess the efficiency of the physicochemical treatment step for microbial removal (Xagoraraki & Harrington 2004). In the present study, turbidity was of limited reliability as a surrogate. First of all, evidence of a relationship between hygienic effects and effluent turbidity was lacking. Furthermore, the microbiological water quality was shown to be highly compromised for part of the filter cycle, but this was not reflected by online turbidity. These two observations indicate that destabilization and removal processes for inorganic particles and microorganisms differ. Alternatively, the distinction could be explained by turbidimeter failure to register fine particles, detected by microbial analysis.

The discrepancy between effluent turbidity and microbial counts was especially large at ripening and breakthrough stages, and, therefore, longer filter-to-waste periods and early cycle termination were apparently necessary to avoid water quality impairment. Filter ripening could be potentially shortened using elevated coagulant dosing. Furthermore, the cycle length is normally regulated by a fixed period of operation, which is ideally a few hours shorter than the potential turbidity breakthrough. The results indicate that this practice is advantageous in preventing microorganism leakage to the effluent.

Another interesting observation in the present study was the size-related removal pattern for the microorganisms. According to the classical colloid filtration theory, retention of microorganisms in porous media is related to their size, and appears to be more challenging for bacteria-sized particles (Yao *et al.* 1971) than for viruses and protozoa. However, introduction of a coagulation step prior to filtration changes the effective size of the microorganisms upon their attachment on flocs (Yao *et al.* 1971). In a study by Nilsen *et al.* (manuscript in preparation) microorganism removal rates within the filter column were more consistent with a particle size of 20 μm rather than with the size of a single microorganism particle. Nevertheless, the removal efficiencies, in the present study, correlated roughly with the microorganisms' size; that is, *E. coli* (0.5–1 μm) and *C. parvum* oocysts (3–5 μm) were usually retained more efficiently than viruses (29–60 nm). Although the number of replicates was insufficient for statistical analysis, it was apparent that the removal of the faecal indicator *E. coli* did not always reflect that of *C. parvum*, indicating that other factors, in addition to size, affected microorganism retention. Furthermore, breakthrough occurred earlier for bacteria than for viruses during the termination stage of filtration.

At a characteristic pH, defined as pI, the ability of microorganism colloids to aggregate or attach to filter media and flocs is enhanced (Gerba 1984). The pI of the microorganisms used in the present study were acidic (Table 2). That would imply an advantage of Zr and chitosan coagulants over Al, as they were applied at lower pH conditions. However, the data reported here support this hypothesis just partially. Furthermore, the pI value of both phages used in the present work appeared to be similar, along with their adsorptive and retention behaviour. It was, therefore, concluded that the impact of pI on microorganism removal by physicochemical processes may be important; however, additional factors are likely to be involved as well.

CONCLUSION

Three different coagulants were applied to evaluate the hygienic effects on viruses, bacteria and protozoan oocysts by the dual-media pilot contact filter. The coagulants were the conventional PACl, little characterized Zr, and eco-friendly chitosan. In general, all tested coagulants demonstrated similar or higher removal efficiencies than previously published for the traditional Al or Fe and contact filtration systems. Each coagulant fulfilled the hygienic

requirement, apart from chitosan. Nevertheless, removal of microbes provided by Zr and chitosan were adequate, and comparable to those of the reference PACl coagulant.

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REFERENCES

Abbaszadegan, M., Mayer, B. K., Ryu, H. & Nwachuku, N. 2007 Efficacy of removal of CCL viruses under enhanced coagulation conditions. *Environmental Science & Technology* **41** (3), 971–977. http://doi.org/10.1021/es061517z.

Abebe, L., Chen, X. & Sobsey, M. 2016 Chitosan coagulation to improve microbial and turbidity removal by ceramic water filtration for household drinking water treatment. *Int. J. Environ. Res. Public Health* **13** (269), 1–11. doi:10.3390/ijerph13030269.

Alves, C. S., Melo, M. N., Franquelim, H. G., Ferre, R., Planas, M., Feliu, L., Bardají, E., Kowalczyk, W., Andreu, D., Santos, N. C., Fernandes, M. X. & Castanho, M. A. R. B. 2010 *Escherichia coli* cell surface perturbation and disruption induced by antimicrobial peptides BP100 and pepR. *J. Biol. Chem.* **285** (36), 27536–27544. doi:10.1074/jbc.M110.130955.

Brown, T. J. & Emelko, M. B. 2009 Chitosan and metal salt coagulant impacts on Cryptosporidium and microsphere removal by filtration. *Water Research* **43** (2), 331–338. http://doi.org/10.1016/j.watres.2008.10.035.

Christensen, E., Håkonsen, T. & Robertson, L. J. 2016 Zirconium and chitosan coagulants for drinking water treatment: a pilot study. *Journal of Water Supply: Research and Technology-Aqua* **65** (8), 635–644. http://doi.org/10.2166/aqua.2016.162.

Debartolomeist, J. & Cabelli, V. J. 1991 Evaluation of an *Escherichia coli* host strain for enumeration of F male-specific bacteriophages. *Applied and Environmental Microbiology* **57** (5), 1301–1305.

Dreier, J., Störmer, M., Kleesiek, K. & Sto, M. 2005 Use of bacteriophage MS2 as an internal control in viral reverse transcription-PCR assays. *Journal of Clinical Microbiology* **43** (9). http://doi.org/10.1128/JCM.43.9.4551.

Dumètre, A., Aubert, D., Puech, P.-H., Hohweyer, J., Azas, N. & Villena, I. 2012 Interaction forces drive the environmental transmission of pathogenic protozoa. *Appl. Environ. Microbiol.* **78** (4), 905–912. doi:10.1128/AEM.06488-11.

European Commission 1998 Council Directive 98/83/EC on the quality of water intended for human consumption. *Off. J. Eur. Commun. L* **330/32**, 32–54.

Gerba, C. P. 1984 Applied and theoretical aspects of virus adsorption to surfaces. *Advances in Applied Microbiology* **30**, 133–168.

Harrington, G. W., Xagoraraki, I., Assavasilavasukul, P. & Standridge, J. H. 2003 Effect of filtration conditions on removal of emerging waterborne pathogens. *American Water Works Association* **95** (12), 95–104.

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F. & Vandesompele, J. 2007 Qbase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology* **8** (2), R19. http://doi.org/10.1186/gb-2007-8-2-r19.

Hijnen, W. A. M. & Medema, G. J. 2010 *Elimination of Microorganisms by Water Treatment Processes.* IWA Publishing, London, UK.

Hoglund, C., Ashbolt, N., Stenstrom, T. A. & Svensson, L. 2002 Viral persistence in source-separated human urine. *Adv. Environ. Res.* **6**, 265–275.

Hussain, S., van Leeuwen, J., Chow, C. W. K., Aryal, R., Beecham, S., Duan, J. & Drikas, M. 2014 Comparison of the coagulation performance of tetravalent titanium and zirconium salts with alum. *Chem. Eng. J.* **254**, 635–646. http://doi.org/10.1016/j.cej.2014.06.014.

ISO 10705-1 1995 *Water Quality – Detection and Enumeration of Bacteriophages-Part 1: Enumeration of F-Specific RNA Bacteriophages.* International Standards Organization, Geneva, Switzerland.

ISO 8245 1999 *Water Quality – Guidelines for the Determination of Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC).* International Standards Organization, Geneva, Switzerland.

ISO 17294-2 2003 *Water Quality – Application of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) – Part 2: Determination of 62 Elements 2003.* International Standards Organization, Geneva, Switzerland.

ISO 17294-1 2004 *Water Quality – Application of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) – Part 1: General Guidelines 2004.* International Standards Organization, Geneva, Switzerland.

ISO 15553 2006 *Water Quality – Isolation and Identification of Cryptosporidium Oocysts and Giardia Cysts From Water.* International Standards Organization, Geneva, Switzerland.

ISO 7887 2011 *Water Quality – Examination and Determination of Colour.* International Standards Organization, Geneva, Switzerland.

Jarvis, P., Sharp, E., Pidou, M., Molinder, R., Parsons, S. A. & Jefferson, B. 2012 Comparison of coagulation performance and floc properties using a novel zirconium coagulant against traditional ferric and alum coagulants. *Water Research* **46** (13), 4179–4187. http://doi.org/10.1016/j.watres.2012.04.043.

Langlet, J., Gaboriaud, F., Duval, J. F. L. & Gantzer, C. 2008 Aggregation and surface properties of F-specific RNA phages: implication for membrane filtration processes. *Water Research* **42** (10–11), 2769–2777. http://doi.org/10.1016/j.watres.2008.02.007.

Logsdon, G. S., Hess, A., Chipps, M. & Rachwa, A. 2002 *Filter Maintenance and Operations Guidance Manual.* AWWA Research Foundation and American Water Works Association, Denver, CO.

Nilsen, V., Christensen, E., Molstad, L., Myrmel, M. & Heistad, A. Dynamics of virus removal in dual-mediacontact-filtration for drinking water: experimental results and inverse modeling. Unpublished results. Manuscript in preparation.

Norwegian Food Safety Authority 2001 *Guideline to the Drinking Water Regulation FOR 2001-12-04 nr. 1372* (in Norwegian). Retrieved from: https://lovdata.no/dokument/SF/forskrift/2001-12-04-1372.

Ødegaard, H., Østerhus, S., Melin, E. & Eikebrokk, B. 2010 NOM removal technologies – Norwegian experiences. *Drink. Water Eng. Sci.* **3** (1), 1–9.

Rabea, E. I., Badawy, M. E.-T., Stevens, C. V., Smagghe, G. & Steurbaut, W. 2003 Chitosan as antimicrobial agent: applications and mode of action. *American Chemical Society* **4** (6), 1457–1465. http://doi.org/10.1021/bm034130 m.

Rao, V. C., Symons, J. M., Ling, A., Wang, P., Metcalf, T. G., Hoff, J. C. & Melnick, J. L. 1988 Removal of hepatitis A virus and rotavirus by drinking water treatment. *Journal of the American Water Works Association* **80** (2), 59–67.

Renault, F., Sancey, B., Badot, P.-M. & Crini, G. 2009 Chitosan for coagulation/flocculation processes – an eco-friendly approach. *European Polymer Journal* **45**, 1337–1348. http://doi.org/http://dx.doi.org/10.1016/j.eurpolymj.2008.12.027.

Shirasaki, N., Matsushita, T., Matsui, Y., Urasaki, T. & Ohno, K. 2009 Comparison of behaviors of two surrogates for pathogenic waterborne viruses, bacteriophages Qbeta and MS2, during the aluminum coagulation process. *Water Research* **43** (3), 605–612. http://doi.org/10.1016/j.watres.2008.11.002.

Su, X., Zivanovic, S. & D'Souza, D. H. 2009 Effect of chitosan on the infectivity of murine norovirus, feline calicivirus, and bacteriophage MS2. *Journal of Food Protection* **72** (12), 2623–2628.

Svenson, S. B., Lonngren, J., Carlin, N. & Lindberg, A. A. 1979 Salmonella bacteriophage glycanases: endorhamnosidases of Salmonella typhimurium bacteriophages. *J. Virol.* **32** (2), 583–592.

USEPA 2005 *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA.* US Environmental Protection Agency, Washington, DC, USA. Retrieved from: http://www.epa.gov.

Xagoraraki, I. & Harrington, G. W. 2004 Zeta potential, dissolved organic carbon, and removal of Cryptosporidium oocysts by coagulation and sedimentation. *Journal of Environmental Engineering* **130** (12), 1424–1432. http://doi.org/10.1061/(ASCE)0733-9372(2004)130:12(1424) CE.

Yao, K.-M., Habibian, M. T. & O'Melia, C. R. 1971 Water and waste filtration: concepts and application. *Environ. Sci. Technol.* **5** (11), 1105–1112.

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Paper 3

Coagulant residues influence on virus enumeration as shown in a study on virus removal using aluminium, zirconium and chitosan

Ekaterina Christensen[ab]*, Mette Myrmel[a]

[a] Norwegian University of Life Sciences, Department of Food Safety and Infection Biology - Faculty of Veterinary Medicine, PO Box 8146, Dep. 0033 Oslo, Norway

[b] Norconsult AS, PO Box 626, 1303 Sandvika

* Corresponding author. Phone: 47 48347908. E-mail: ekaterina.christensen@norconsult.com

Abstract

Research on microorganism reduction by physicochemical water treatment is often carried out under the assumption that the microbiological enumeration techniques are not affected by the presence of coagulants. Data presented here indicate that bacteriophage enumeration by plaque assay and RT-qPCR (reverse transcription quantitative polymerase chain reaction) can be affected by these water treatment chemicals. Treatment of water samples with an alkaline protein-rich solution prior to plaque assay and optimization of RNA extraction for RT-qPCR, were implemented to minimize the interference. The improved procedures were used in order to investigate reduction of three viral pathogens and the MS2 model virus in the presence of three coagulants. A conventional aluminium coagulant was compared to alternative agents (zirconium and chitosan) in a coagulation-filtration system. The highest virus reduction, i.e., 99.9-99.99%, was provided by chitosan, while aluminium and zirconium reduced virus by 99.9% in colour-rich water and by 90% in water with less colour, implying an effect of coagulant type and raw water quality on virus reduction. Although charge characteristics of viruses were associated with virus reduction, the results reveal that the MS2 phage is a suitable model for aggregation and retention of the selected pathogens.

Keywords: chitosan; coagulation; drinking water treatment; pathogen removal; virus quantification; zirconium

Introduction

Treatment of sewage and drinking water often involves destabilization of the particulate and dissolved matter by a coagulation step, which usually precedes separation processes, e.g., sedimentation and filtration. Drinking water plants around the world use salts of aluminium (Al) or ferric (Fe) for coagulation, however, there are some concerns regarding their use. Aluminium is a known neurotoxin, and residual Al in treated water has been linked to neurological disease at concentrations ≥0.1 mg/L (Rondeau et al., 2009). Ferric salts tend to affect pH and alkalinity of the treated water which requires increased use of chemicals for stabilization and corrosion control (Matilainen et al., 2010). Both Al and Fe have been also associated with production of high amounts of sludge (Ødegaard et al., 2010). Several reports have acknowledged that compounds, such as zirconium (Zr) and chitosan, possess properties that may enhance organic matter removal and reduce sludge production compared to Al and Fe (Christensen et al., 2016; Eikebrokk and Saltnes, 2002; Jarvis et al., 2012). However, their efficacy in reduction of waterborne pathogens is scarcely documented.

The main purposes of drinking water treatment are reduction and inactivation of pathogens. Traditionally, the effect of treatment is assessed in terms of turbidity (particle content), whereas the microbial reduction is intermittently evaluated using heterotrophic plate count (HPC). However, turbidity and HPC cannot predict the effect of treatment on other groups of microorganisms, like viruses (Hijnen and Medema, 2010).

Most of the registered waterborne outbreaks in the Nordic countries, between 1998 and 2012, were caused by viruses, mainly norovirus (NV) (Guzman-Herrador et al., 2015). Enteric viruses are excreted in high numbers by infected individuals (10^7 – 10^9 per gram) and enter the environment via waste water (Rusinol and Girones, 2017). Although 35 to 90% of viruses are removed by wastewater treatment, high levels can still enter the

recipient, e.g. a source of drinking water (Myrmel et al., 2015). As ingestion of a few virus particles, like NV (Teunis et al., 2010), can cause infection, understanding the conditions for efficient virus reduction during drinking water treatment is an important task in preventing waterborne disease.

Optimal reduction of water pollutants during physicochemical water treatment relies among others, on coagulation efficacy and factors like coagulant dose, pH and presence of other colloids (Ødegaard et al., 2010). The influence of these factors on viruses can be hard to confirm experimentally (Hendricks et al., 2005), making predictions on virus removal uncertain. Virus retention is often studied using model viruses, like bacteriophages, and not viral pathogens (Xagoraraki et al., 2004). The MS2 bacteriophages is extensively used as a model virus, due to similarities with enteric viral pathogens in size and structure (Dawson et al., 2005). Quantification of infective MS2 by plaque assay is simple, cheap and rapid, however, the enumeration can be affected by virus aggregation (Langlet et al., 2007). Virus clustering in water can be influenced by electrolytes (Floyd and Sharp, 1978), including coagulants (Shirasaki et al., 2009), whereas it is unclear whether aggregation impacts enumeration of infectious viruses in treated water, which may contain coagulant residue. This knowledge is essential in quantitative microbial risk assessment (QMRA) for water safety management.

Another commonly used technique for virus quantification is RT-qPCR (reverse transcription quantitative polymerase chain reaction), which detects the total of infective and non-infective target virus. This methodology is sensitive to a variety of inhibitors, including metals (Schrader et al., 2012), however, previous studies do not give a clear answer, whether coagulants can influence virus enumeration by RT-qPCR.

The aims of the present study were to investigate the influence of coagulants on quantitation of MS2 model virus, and to minimize any effect of these water treatment chemicals by

method optimization (part I). For quantification of infective MS2 by plaque assay, beef extract (BE) solution was tested. The choice of this basic, proteinaceous solution was explained by its ability to reduce virus adsorption to solid surfaces and other virus particles (Moore et al., 1982). Two commonly utilized RNA extraction protocols were tested for quantification of total MS2 by RT-qPCR. The two extraction methods employ different silica matrices for RNA binding (Petrich et al., 2006).

Secondly, optimized procedures were applied in a reduction study on viruses in water using Zr, chitosan and the extensively used PACl (polyaluminium chloride) coagulant (part II). Virus reduction was assessed in terms of raw and finished water turbidity and colour, type of coagulant and virus morphology. MS2 was introduced in order to assess its suitability as a model for enteric virus reduction by coagulation-filtration. The human, enteric pathogens hepatitis A virus (HAV), bovine NV (BNV) and bovine coronavirus (BCoV) were included as they represent various virus size, isoelectric points (pI) and structural properties.

Material and Methods

Raw water samples

Water was collected from Nedre Romerike Water Treatment Plant (WTP) in Strømmen (Glomma River, Norway) and Årnes WTP (lake Dragsjøen, Norway). The samples were stored in a dark room at 4°C.

The water was analysed for turbidity, true colour and pH (Table 1) after spiking with the virus suspension.

Water analyses

Turbidity was measured using a 2100AN turbidimeter (Hach Company, USA). True colour was selected as a surrogate for natural organic matter (NOM), due to its extensive use in surveillance of water treatment in Norway (Norwegian Food Safety Authority, 2016). The colour parameter was measured by a Shimadzu UV Visible Spectrophotometer UVmini-1240 (Shimadzu Corporation, Japan), following Standard APHA Method 2120C (λ=455 nm) procedure (APHA 1995). Prior to colour measurements, the samples were filtered through a 0.45 μm syringe polypropylene membrane (514-0065, VWR, USA), in order to avoid the influence of turbidity.

Coagulants

Polyaluminium chloride (PACl). A ready to use PACl product, PAX-18, was obtained from Kemira Chemicals (Norway). The product has an Al-content of 9% (17% as Al_2O_3), basicity of 42% and specific gravity of 1.37 g/mL. **Zirconium oxychloride**. Zirconium (IV) oxychloride octahydrate powder was obtained from Teta Vannrensing Ltd (Norway). A working solution of 37% (w/w) was prepared in distilled water and gave a Zr concentration close to the Al-content of 9%. **Chitosan**. KitoFlokk^{TM} (low molecular weight [MW, 100 kDa] and deacetylation degree [DD] close to 0.8) was obtained from Teta Vannrensing Ltd, Norway. The concentration of the working solution was 2% (w/v) in 0.1 M HCl. A higher chitosan concentration was avoided to prevent undissolved debris in the working solution. The chitosan solution was stored for no longer than 2 weeks. All working solutions were stored at room temperature as no change in coagulation properties had been observed under the selected storage condition.

Viruses

The size and pI characteristics of viruses included in the present study, are given in Table 2.

Propagation of MS2 bacteriophage was performed according to ISO 10705 (1995), using *Salmonella* Typhimurium WG49 (NCTC 12484) as host.

The apathogenic HAV strain pHM175 43c (kindly provided by Prof. Albert Bosch, University of Barcelona) was propagated in foetal rhesus monkey kidney cells (FRhK-4/R, ATCC® CRL-1688), as previously described (Flehmig, 1980).

The BNV (genotype III2) originated from a transmission study (Jor et al., 2010). Faecal samples were diluted 1:10 in PBS, rocked for 15 min, and centrifuged at 1 000 g for 15 min at 4 ºC. The supernatant was aliquoted, stored at -80 °C, and centrifuged at 12 000 g for 5 min at room temperature before use.

The BCoV stock was prepared in human rectal tumour cells (HRT-18G, ATCC® CRL-11663), according to the procedure described previously (Oma et al., 2016).

A spike suspension was prepared by mixing the virus stocks. The volume of individual viruses was defined by initial tests to ensure that processed samples would be virus positive. The culture medium gave a 5-10 % increase in water turbidity and colour. The spike suspension was aliquoted and stored at -80ºC.

MS2 plaque assay

Infectious MS2 was enumerated by plaque assay as previously described by Debartolomeist and Cabelli (1991). In order to study any impact of coagulants on infectious MS2 enumeration (part I), water samples were processed with (+BE) and without (-BE) beef extract (Christensen et al., 2017; Shirasaki et al., 2009).

For +BE, one mL of sample was mixed in nine mL BE (13 %) and stirred at 1 500 rpm for 5 h at +4 °C. The BE (211520, Becton-Dickinson and Company, USA) was prepared in sterile water, adjusted to pH 9.5-10.0 with 5 M NaOH, stored at +4 °C and used within three days. The quantity of MS2 is given as plaque forming units (PFU) per mL.

Extraction of RNA and RT-qPCR

In order to study any impact of coagulants on extraction of RNA and quantification of MS2 genomes by RT-qPCR (part I), two commercial RNA extraction protocols, used for processing of water samples, were compared: QIAamp® Viral RNA (Qiagen, Germany) and NucliSENS miniMAG® (Biomerieux, France), hereafter referred to as method Q, and N, respectively.

For method Q, 140 µL sample and 3.1 µg carrier RNA were added to lysis buffer (560 µL) and processed according to the manufacturer prior to RNA elution in 60 µL buffer and storage at −80°C.

For N, an identical volume of 140 µL was treated with 2 mL lysis buffer and 50 µL magnetic beads. After several washing steps, RNA was eluted from the beads in 60 µL buffer and stored at −80°C.

RT-qPCR was performed in a Stratagene AriaMx Real-Time PCR System (Agilent Technologies, Inc., USA), using the RNA UltraSense™ One-Step Quantitative RT-PCR System kit (Invitrogen, USA). Three µL RNA was used in a total volume of 20 µL, with

primers, probe and RT-qPCR conditions as listed in Table 3. ROX was used as passive reference, and positive and negative controls were included in each run. Each sample was run in technical duplicates and the results analysed with Agilent AriaMx 1.1 Software.

Relative quantification of viral RNA was performed using standard curves prepared from 10-fold serial dilutions of homologous RNA in RT-qPCR triplicates. The amount of viral RNA was expressed as RT-PCR units (RT-PCRU) per mL: one RT-PCRU was defined as the amount of target RNA in the highest dilution of the standard, which gave a positive result. Aliquoted, homologous RNA was included in all plates and used as an inter-plate calibrator (IPC). The threshold in each run was adjusted manually in order to obtain identical Ct-values for the IPC (Christensen et al., 2017).

Standard bench scale procedure

The outline of the protocol is given in Figure 1. The procedure was initially used to define suitable coagulation conditions, and later during part I and II setups. Further details are given under the relevant sections below. A water sample (400 mL) was spiked with the virus mix to achieve a final concentration of 3-6 $\log_{10}$ PFU/mL for MS2 and 4 $\log_{10}$ PCRU/mL for the other viruses. The sample was swirled prior to collection of a control (C), divided between 3 bottles (100 mL in each), and pH adjusted with 1 M NaOH or 0.3 M HCl. After adding coagulants, the bottles were immediately vortexed for 30 s (G = 262 s^{-1}), left on a rocking table for 10 min at 50 rpm (G = 5 s^{-1}), centrifuged at 112 g for 3 min, and the supernatant filtered through a Whatman membrane (glass fibre filter with 1.2 µm pore size) (Whatman GF/C, GE Healthcare, USA). Samples collected during processing were defined as under mixing (UM), supernatant (S) and filtrate (F).

Optimization of coagulant doses

As optimal conditions for treatment of water from distinct sources could differ, individual optimization was performed. Criteria for filtrate turbidity (<0.2 NTU) and colour (<10 mg Pt/L) were set, as recommended by the Norwegian Institute of Public Health (Andersen, 2016) for a coagulation-filtration step to be considered as a hygienic barrier. However, as filtration of small sample volumes with unsaturated Whatman filters was inefficient in meeting the turbidity criteria, coagulation conditions were selected in accordance with the lowest turbidity values obtained. For colour, the criteria were tightened (<5 mg Pt/L) for Al and Zr in order to avoid high metal residues, as colour and metals are often associated (Andersen, 2016). Each coagulant was titrated at an optimal pH, as reported in the literature: 5.0–7.0 for PACl, 4.5–6.3 for Zr, and 4.0–7.0 chitosan (Christensen et al., 2016; Ødegaard et al., 2010). The coagulation conditions used for part I and II and treatment results are presented in Table 4.

Part I - Optimization of virus quantification by plaque assay and RT-qPCR

Initial experiments included Glomma and diluted Dragsjøen water (Dragsjøen D) with similar concentrations of particulate and organic matter (Table 1), in order to determine whether water constituents from different water sources could influence virus quantification. After spiking with MS2, the water samples were processed using the standard bench-scale protocol, followed by collection of C, UM, S and F (Figure 1). MS2 was quantified with plaque assay (-/+BE) to explore any influence of coagulants on enumeration of viable virus. For quantification of viral genome copies, RT-qPCR was used after RNA extraction with methods Q and N.

The applicability of the most sensitive methods was finally tested using undiluted Dragsjøen water, which was included in part II.

Water samples were processed in three biological replicates with virus enumeration in duplicates. An overview of the setup is given in Figure 2.

Part II – Reduction of viruses in water; efficiency of Al, Zr and chitosan in different water types

Diluted and undiluted Dragsjøen water were spiked with MS2, HAV, BNV and BCoV, followed by the standard bench scale procedure. Samples C, S and F were collected and processed immediately. Based on the results from part I, virus was quantified using the +BE plaque assay (MS2), and RT-qPCR using RNA extracted with method Q (Figure 2). Three biological replicates, with virus enumeration in duplicates, were conducted on each water type.

Data analysis

Data analysis was performed in STATA 8.0 (Stata Corporation, USA). Differences in effluent turbidity and colour between coagulants were calculated by using one-way analysis of variance (ANOVA). A linear regression model was applied to assess the association between virus reduction ($\Delta\log_{10}$) and the explanatory variables raw water quality, type of coagulant, and virus morphology.

The required coagulant dosages were expected to be proportional to the level of NOM in Dragsjøen D and Dragsjøen samples (Ødegaard et al., 2010). Consequently, the independent variables raw water type and coagulant dosages were used interchangeably in the present work.

Results

Optimization of coagulant doses

In general, turbidity was reduced less efficiently, than colour. In water with low amounts of NOM, chitosan provided lower effluent turbidity, than Al or Zr, whereas no significant difference in turbidity reduction was found for the three coagulants in high NOM water. Chitosan was less efficient in colour reduction than Al or Zr. The criteria for colour <5 mg Pt/L (and <10 mg Pt/L for chitosan) were fulfilled in all samples with the selected coagulant doses (Table 4), except for Dragsjøen water treated with chitosan.

Part I - Optimization of virus quantification by plaque assay and RT-qPCR

In Glomma water, the number of MS2 plaques increased after treatment with BE, independently of coagulant type (Figure 3A). The efficiency of BE was specifically demonstrated for chitosan, for which a 5 $\log_{10}$ reduction in PFU was reversed with BE in UM and S samples from Dragsjøen (Figure 3B and C). An impact of chitosan was also observed in the filtered Dragsjøen sample, as the titre increased by 1.0-1.5 $\log_{10}$ after BE treatment (Figure 3D). For Al and Zr, the positive effect of BE was most significant in Glomma water.

Two RNA extraction methods (Q and N) were compared for RT-qPCR quantification of MS2 genomes in samples without (control C in Figure 4A, B) and with coagulants (Figure 4). In water samples without coagulants, the Q kit gave more than 1 $\log_{10}$ higher viral RNA titre, compared to N (Figure 4A and B). RNA dilution revealed presence of RT-qPCR inhibitors from undiluted Dragsjøen in the RNA, extracted with method N. For water samples with coagulants, virus quantification was not affected when using method Q (Figure 4A and B). For N method, the greatest interference was registered for Glomma water with a 1.0-1.5 $\log_{10,}$ reduction, depending on the coagulant. Overall, a high variability

was found for virus titres in samples processed with method N, whereas a high reproducibility was seen for the level of viral RNA extracted with method Q.

Virus titres were reduced by no more than 0.4-0.5 $\log_{10,}$ when the applicability of the +BE plaque assay and the Q extraction method, was tested on undiluted Dragsjøen UM water (worst-case scenario). The two processing methods were, therefore, used in part II of the study.

Part II - Virus reduction by coagulants

Reduction of MS2 and enteric viruses was assessed for coagulation-filtration treatment using three coagulants (Figure 5).

In the supernatants (S), 10-70% (0.1-0.5 $\log_{10}$) less virus was measured, compared with the control (C). Filtration was generally more efficient, but the results differed between the coagulants. For the metal coagulants, log-reductions were often similar and close to 90% (1 $\log_{10}$) and 99.9% (3 $\log_{10}$) for low and high NOM waters, respectively. Both metals showed a smaller reduction of BNV, compared to chitosan. Chitosan treatment provided at least 99.9-99.99% (3-4 $\log_{10}$) reduction of all viruses for low and high NOM water samples.

Regression analysis (Table 5) revealed an association between virus reduction and the independent variables, such as raw water origin, type of coagulant and virus. NOM content and, subsequently, coagulant dose, was positively associated with virus reduction after the centrifugation and filtration steps. The supernatants revealed no difference in virus reduction between the coagulants, whereas virus numbers in filtrates indicated an increased efficacy of chitosan. The reduction pattern for BNV was distinct from the other viruses. The highest virus reductions were observed for Dragsjøen water processed by Zr-centrifugation or chitosan-centrifugation-filtration. The model fit has a coefficient of determination (R^2) of 44% for supernatants and 70% for filtrates, implying that 70% of the variance in virus

reduction by coagulation-filtration, could be explained by the type of water, coagulant and virus.

Discussion

Effect of coagulants on enumeration of viable MS2 by plaque assay (aggregation effect)

The present study demonstrated that coagulants in water samples interfered reversibly with the plaque assay. The number of PFUs decreased not only in samples recently mixed with the coagulants (UM), as previously reported (Shirasaki et al., 2009), but also in supernatant and filtrates, especially when chitosan was the coagulant present.

An apparent fall in plaque number, due to coagulant presence, could be explained by several mechanisms. Apart from virus aggregation (Floyd and Sharp, 1978), coagulants could prevent virus-host interaction electrostatically (Puck et al., 1951), or physically (Tanneru et al., 2013). Phage entrapment on the flocs might also result in temporary conformational changes in the virus capsid (Taylor et al., 1980).

Treatment of the samples with BE reversed reduction in infective MS2 plaque counts almost entirely, despite high coagulant doses. The effect of BE could be caused by a synergetic effect of high pH and excess of proteins. While a solution with alkaline pH dissolves the coagulant flocs and enhances repulsion between virus-virus and virus-coagulant complexes, excess of proteins displaces viruses from the active attachment sites on undissolved flocs and naturally present suspended solids.

The impact of Al and Zr on viable MS2 quantification increased in Glomma water, compared to Dragsjøen D. For chitosan, the opposite was observed. The applied coagulation conditions varied for the two water samples. A higher amount of Zr and a lower amount of chitosan were used for Glomma compared to Dragsjøen D water. However, similar PACl doses were applied for the two waters, whereas the pH conditions varied for Al and Zr only.

Therefore, the aggregation was probably caused by a combined effect of water components, coagulant dose and pH.

The results in the present study indicate that enumeration of viable MS2 in water with coagulants and without BE treatment can result in artificially high estimates on virus reduction. This finding may provide useful insights for future studies with model viruses. For enteric virus, the BE treatment might be employed to study reversible aggregation of viruses with solids in raw water (Hejkal et al., 1981) or to improve the sensitivity of the single-hit dose-response models, which are essential in QMRA (Nilsen and Wyller, 2016).

Effect of coagulants on virus enumeration by RT-qPCR

The present study demonstrates a difference between the two extraction methods regarding purification and/or recovery of RNA from water samples. The efficacy of method Q and N was influenced differently by water quality and coagulants. While Q provided stable levels of pure viral RNA, regardless of water quality and presence of coagulants, the efficacy of N was significantly reduced by both.

The difference between the two methods might be explained by inclusion of silica in a gel membrane for method Q, while N uses silica covered magnetic beads. Furthermore, method Q uses carrier RNA to increase RNA binding to the silica (Boom et al., 1990). On the other hand other studies have demonstrated the adverse effect of high metal concentrations on the sensitivity of method Q (Chen and Chang, 2012). Therefore, it might be necessary to assess the sensitivity of a selected extraction kit prior to an experiment.

Virus removal by coagulant and filtration

In the present study, the extent of virus-floc association, and subsequent virus removal, were assessed with respect to raw water quality, coagulant type and dosage, and virus

characteristics. According to Edwards & Amirtharajah (1985), flocculation performance increases with increasing concentrations of particles and humic molecules in raw water and/or coagulant dosing. Moreover, selected coagulants may exhibit different destabilization properties and affect floc characteristics, which play a fundamental role in operation of physical separation processes (Hussain et al., 2014). Finally, efficient removal involves transport and attachment behaviour of colloids, determined by their size and charge characteristics (Yao et al., 1971).

The results of the present study revealed that the combination of flocculation and sedimentation induced 10 to 70% reduction in virus titre. At drinking water plants, sedimentation accounts for 27 to 74% decrease in virus amount (Gimbel and Clasen, 1998), implying that the selected bench scale procedure, was capable of simulating a full-scale process. Consequently, chemical pre-treatment and sedimentation did not have a substantial virus reducing effect. Without attachment to flocs, virus would remain suspended in the solution. Apparently, impaired performance could result from small size and deficient settling characteristics of the formed aggregates.

The setup with a Whatman membrane, aimed to reproduce a microfiltration process, for which straining and cake filtration are considered the predominant retention mechanisms (LeChevallier and Au, 2004). The pore size of a Whatman filter (1.2 μm) exceeds the size range for monodispersed virus particles. The filter surface is also electronegative (Blass et al., 2013), and not likely to favour adsorption of MS2, HAV and BCoV at the pH conditions used. Consequently, viruses and colloids smaller than 1.2 μm, and presumably even 0.4 μm (Hickel, 2013), were not expected to be efficiently retained on the membrane, unless their size and charge properties were modified by coagulation pre-treatment.

The filtration step showed an association between virus reduction and raw water quality. Addition of coagulants to water is followed by formation of hydrolysis species and increase

of suspended solids. In low NOM water, a relatively low dosing of the metal coagulants could restrict formation of complexes between colloids, hydroxide flocs and pathogens (Chang et al., 1958). One can also assume that under the conditions produced with Al and Zr the size of the formed flocs was below the 1.2 μm pore size. This is consistent with the deficient turbidity reduction in Dragsjøen D samples. In undiluted Dragsjøen water, increased coagulant dosages were assumed to enhance collision rates between colloids, flocs and microbes, and induce formation of numerous and larger aggregates. This could favour retention of suspended solids and associated with it virus.

On the other side, chitosan was more efficient in reducing viruses during filtration, compared to Al and Zr (Table 5). Destabilization with chitosan could possibly result in formation of larger and denser flocs, but then lower virus titres should have been seen for this coagulant after the centrifugation step. Besides, chitosan does not form hydroxides and produces less suspended matter in the influent than the metal coagulants (Christensen et al., 2016). Consequently, a cake layer formed by chitosan on the membrane surface is expected to be thinner, giving a lower retention of particles and virus. Chitosan exhibits unique destabilization mechanisms, e.g. bridging or “electrostatic patching” (Bolto and Gregory, 2007). Therefore, it could be suggested that chitosan chains protrude from the aggregated suspended matter and bind strongly to the filter material.

In the present study, regression analysis showed a slightly deficient reduction of BNV, in comparison to viruses with more acidic pI. Under the selected coagulation pH, the positive charge of BNV could prevent it from approaching positively charged coagulants. Deficient BNV reduction, however, was not observed for chitosan, which could be caused by the distinct ability of chitosan to bind the filter membrane, as discussed earlier.

The influence of particle size in the 30-120 nm range was negligible for virus retention on the membrane and the results presented imply that aggregation and retention of MS2 and enteric viruses were generally similar.

Turbidity reduction often serves as an indicator of treatment performance for (oo)cysts, which have sizes in the same range as particulate matter (Nieminski and Ongerth, 1995). The size of viruses is similar to that of humic compounds (Österberg et al., 1993), which concentrations can be assessed with colour. Thereby, treatment conditions, which are beneficial for low effluent turbidity and colour, are believed to affect most microorganisms and maximize pathogen removal during coagulation and filtration treatment.

In the present work, treatment conditions were satisfying mostly with regard to colour criteria, whereas turbidity values were above 0.2 NTU. However, efficient virus removal could still be achieved, but depended on coagulant type and initial water quality.

In Dragsjøen D water, the performance of both Al and Zr was associated with deficient turbidity and virus reduction (Table 4 and Figure 5A). In Dragsjøen water, virus removal by the same coagulants was improved, despite high effluent turbidity (Table 4 and Figure 5B). Due to higher coagulant dosing, greater increase of solid matter was expected in Dragsjøen water, compared to Dragsjøen D water. As filtrate turbidity in both Dragsjøen and Dragsjøen D waters was rather similar, it was reasonable to assume that solid retention, as well as virus removal, was higher for Dragsjøen water. Consequently, retention of solids that can be assessed with turbidity parameter was assumed to be connected to removal of viruses.

In contrast, a straightforward association between reduction in colour and virus was not observed. A high NOM residue in the filtrate, accompanied by low virus titre could be caused by adhesion of the chitosan chains to the membrane. Alternatively, the association was influenced by high turbidity, and presumably if low turbidity was achieved for Al and

Zr, these two coagulants could demonstrate similar or greater virus reduction, compared to chitosan.

Conclusions

The present study shows that enumeration of the model virus MS2, by plaque assay and RT-qPCR (RNA extraction), was sensitive to coagulant contents and other constituents in water. Treatment of water samples with BE tended to reduce the interference on enumeration by plaque assay. The sensitivity of the RT-qPCR assay relied on the RNA extraction method. The results show that the suitability of virus quantification methods should be evaluated for water studies, especially when using coagulants.

Chitosan showed a high ability to retain suspended matter on the membrane in water with low NOM content. Moreover, chitosan contributed to a higher hygienic performance in this type of water, than Al or Zr coagulants. The efficacy of the metal coagulants to reduce both suspended matter and viruses was improved, as a result of the enhanced contact opportunities between viruses and other particles in solution. Virus retention by the three coagulants could be somewhat predicted with turbidity reduction in the filtrates. In contrast, an association between effluent colour and virus reduction was not established.

In the present study, charge characteristics of viruses influenced virus reduction, however, the removal patterns for the model virus MS2 resembled the reduction of pathogenic viruses.

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References

American Public Health Association, 1995. Standard methods for the examination of water and wastewater, 19th ed. Washington, DC.

Andersen, E., 2016. Vannrapport 127: Vannforsyning og helse (Water supply and health). Norwegian Institute Of Public Health, Oslo, Norway.

Blass, J., Köhler, O., Fingerle, M., Müller, C., Ziegler, C., 2013. Properties and characteristics of wet (HF) and dry (RIE) etched borosilicate glass. Phys Status Solidi A 210, 988–993. doi:doi:10.1002/pssa.201200769

Bolto, B., Gregory, J., 2007. Organic polyelectrolytes in water treatment. Water Res. 41, 2301–2324. doi:10.1016/j.watres.2007.03.012

Boom, R., Sol, C.J., Salimans, M.M., Jansen, C.L., Wertheim-van Dillen, P.M., van der Noordaa, J., 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 28, 495–503.

Chang, S.L., Stevenson, R.E., Bryant, A.R., Woodward, R.L., Kabler, P.W., 1958. Removal of coxsackie and bacterial viruses in water by flocculation: II. Removal of coxsackie and bacterial viruses and the native bacteria in raw Ohio River water by flocculation with aluminum sulfate and ferric chloride. Am. J. Public Heal. Nations Heal. 48, 159–169.

Chen, N.-T., Chang, C.-W., 2012. Quantification of Legionella pneumophila by real-time quantitative PCR from samples with humic acid and ferric ion. Sci. Total Environ. 414, 608–613. doi:10.1016/j.scitotenv.2011.10.005

Christensen, E., Håkonsen, T., Robertson, L.J., Myrmel, M., 2016. Zirconium and chitosan coagulants for drinking water treatment – a pilot study. Aqua- J. Water Supply Res. Technol. 65, 635–644. doi:10.2166/aqua.2016.162

Christensen, E., Nilsen, V., Håkonsen, T., Heistad, A., Gantzer, C., Robertson, L.J.,

Myrmel, M., 2017. Removal of model viruses, E.coli and Cryptosporidium oocysts from surface water by zirconium and chitosan coagulants. J. Water Health 15, 695–705. doi:10.2166/wh.2017.055

Costafreda, M.I., Bosch, A., Pintó, R.M., 2006. Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. Appl. Environ. Microbiol. 72, 3846–3855.

Dawson, D.J., Paish, A., Staffell, L.M., Seymour, I.J., Appleton, H., 2005. Survival of viruses on fresh produce, using MS2 as a surrogate for norovirus. J. Appl. Microbiol. 98, 203–209. doi:10.1111/j.1365-2672.2004.02439.x

Debartolomeist, J., Cabelli, V.J., 1991. Evaluation of an Escherichia coli host strain for enumeration of F male-specific bacteriophages. Appl. Environ. Microbiol. 57, 1301–1305.

Dreier, J., Störmer, M., Kleesiek, K., Sto, M., 2005. Use of bacteriophage MS2 as an internal control in viral reverse transcription-PCR assays. J. Clin. Microbiol. 43. doi:10.1128/JCM.43.9.4551

Edwards, G.A., Amirtharajah, A., 1985. Removing color caused by humic acids. J. Am. Water Work. Assoc. 77, 50–57.

Eikebrokk, B., Saltnes, T., 2002. NOM removal from drinking water by chitosan coagulation and filtration through lightweight expanded clay aggregate filters. J. Water Supply Res. Technol. - Aqua 51, 323–332.

Flehmig, B., 1980. Hepatitis A-virus in cell culture: I. propagation of different hepatitis A-virus isolates in a fetal rhesus monkey kidney cell line (Frhk-4). Med. Microbiol. Immunol. 168, 239–248.

Floyd, R., Sharp, D.G., 1978. Viral aggregation: effects of salts on the aggregation of

poliovirus and reovirus at low pH. Appl. Envir. Microbiol. 35, 1084–1094.

Gimbel, R., Clasen, J., 1998. International report: removal of microorganisms by clarification and filtration processes. Water Supply 16, 203–208.

Guzman-Herrador, B., Carlander, A., Ethelberg, S., Freiesleben de Blasio, B., Kuusi, M., Lund, V., Löfdahl, M., MacDonald, E., Nichols, G., Schönning, C., Sudre, B., Trönnberg, L., Vold, L., Semenza, J.C., Nygård, K., 2015. Waterborne outbreaks in the Nordic countries, 1998 to 2012. Eurosurveillance 20, 1–10. doi:10.2807/1560-7917.ES2015.20.24.21160

Hejkal, T.W., Wellings, F.M., Lewis, A.L., LaRock, P.A., 1981. Distribution of viruses associated with particles in waste water. Appl Env. Microbiol 41, 628–634.

Hendricks, D.W., Asce, F., Clunie, W.F., Sturbaum, G.D., Klein, D.A., Champlin, T.L., Asce, M., Kugrens, P., Hirsch, J., Mccourt, B., Nordby, G.R., Asce, M., Sobsey, M.D., Hunt, D.J., Allen, M.J., 2005. Filtration removals of microorganisms and particles. J. Environ. Eng. 131, 1–12. doi:10.1061/(ASCE)0733-9372(2005)131:12(1621)

Hickel, W., 2013. Seston retention by Whatman GF/C glass-fiber filters. Mar. Ecol. - Prog. Ser. 16, 185–191. doi:10.3354/meps016185

Hijnen, W.A.M., Medema, G.J., 2010. Elimination of microorganisms by water treatment processes, IWA Publishing. London, UK.

Hussain, S., van Leeuwen, J., Chow, C.W.K., Aryal, R., Beecham, S., Duan, J., Drikas, M., 2014. Comparison of the coagulation performance of tetravalent titanium and zirconium salts with alum. Chem. Eng. J. 254, 635–646. doi:10.1016/j.cej.2014.06.014

International Standards Organization, 1995. ISO 10705-1:1995. Water quality-Detection and enumeration of bacteriophages-Part 1: Enumeration of F-specific RNA bacteriophages. Geneva, Switzerland.

Jarvis, P., Sharp, E., Pidou, M., Molinder, R., Parsons, S.A., Jefferson, B., 2012.

Comparison of coagulation performance and floc properties using a novel zirconium coagulant against traditional ferric and alum coagulants. Water Res. 46, 4179–4187. doi:10.1016/j.watres.2012.04.043

Jor, E., Myrmel, M., Jonassen, C.M., 2010. SYBR Green based real-time RT-PCR assay for detection and genotype prediction of bovine noroviruses and assessment of clinical significance in Norway. J. Virol. Methods 169, 1–7. doi:10.1016/j.jviromet.2010.03.028

Kapil, S., Richardson, K.L., Maag, T.R., Goyal, S.M., 1999. Characterization of bovine coronavirus isolates/from eight different states in the USA. Vet. Microbiol. 67, 221–230. doi:10.1016/S0378-1135(99)00042-5

King, B., Brian, D.A., 1982. Bovine coronavirus structural proteins. J. Virol. 42, 700–7.

Langlet, J., Gaboriaud, F., Gantzer, C., 2007. Effects of pH on plaque forming unit counts and aggregation of MS2 bacteriophage. J. Appl. Microbiol. 103, 1632–8. doi:10.1111/j.1365-2672.2007.03396.x

LeChevallier, M.W., Au, K.-K., 2004. Water treatment and pathogen control: process efficiency in achieving safe drinking-water, IWA Publishing. London, UK. doi:ISBN:1 84339 069 8

Matilainen, A., Vepsäläinen, M., Sillanpää, M., 2010. Natural organic matter removal by coagulation during drinking water treatment: a review. Adv. Colloid Interface Sci. 159, 189–197. doi:10.1016/j.cis.2010.06.007

Michen, B., Graule, T., 2010. Isoelectric points of viruses. J. Appl. Microbiol. 109, 388–97. doi:10.1111/j.1365-2672.2010.04663.x

Moore, R.S., Wait, D.A., Stokes, E.H., 1982. Improved methods for poliovirus recovery from water with electropositive adsorbent filters. Annu. Meet. Am. Soc. Microbiol. paper Q, 1–14.

Myrmel, M., Lange, H., Rimstad, E., 2015. A 1-year quantitative survey of noro-, adeno-, human boca-, and hepatitis E viruses in raw and secondarily treated sewage from two plants in Norway. Food Env. Virol 7, 213–223. doi:10.1007/s12560-015-9200-x

Nieminski, E.C., Ongerth, J.E., 1995. Removal of Cryptosporidium and Giardia through conventional water treatment and direct filtration. J. Am. Water Work. Assoc. 96–106.

Nilsen, V., Wyller, J., 2016. QMRA for drinking water: 2. The effect of pathogen clustering in single-hit dose-response models. Risk Anal. 36, 163–181.

Norwegian Food Safety Authority, 2016. Forskrift om vannforsyning og drikkevann (Water supply and drinking water regulations) FOR 2016-12-22 nr. 1868.

Oma, V.S., Tråvén, M., Alenius, S., Myrmel, M., Stokstad, M., 2016. Bovine coronavirus in naturally and experimentally exposed calves; viral shedding and the potential for transmission. Virol. J. 13, 100.

Otto, P.H., Clarke, I.N., Lambden, P.R., Salim, O., Reetz, J., Liebler-Tenorio, E.M., 2011. Infection of calves with bovine norovirus GIII. 1 strain Jena virus: an experimental model to study the pathogenesis of norovirus infection. J. Virol. 85, 12013–12021.

Petrich, A., Mahony, J., Chong, S., Broukhanski, G., Gharabaghi, F., Johnson, G., Louie, L., Luinstra, K., Willey, B., Akhaven, P., Chui, L., Jamieson, F., Louie, M., Mazzulli, T., Tellier, R., Smieja, M., Cai, W., Chernesky, M., Richardson, S.E., 2006. Multicenter comparison of nucleic acid extraction methods for detection of severe acute respiratory syndrome coronavirus RNA in stool specimens. J. Clin. Microbiol. 44, 2681–2688. doi:10.1128/JCM.02460-05

Puck, T.T., Garen, A., Cline, J., 1951. The mechanism of virus attachment to host cells. J Exp Med 93, 65–88.

Rondeau, V., Jacqmin-Gadda, H., Commenges, D., Helmer, C., Dartigues, J.F., 2009. Aluminum and silica in drinking water and the risk of Alzheimer's disease or cognitive

decline: Findings from 15-year follow-up of the PAQUID cohort. Am. J. Epidemiol. 169, 489–496. doi:10.1093/aje/kwn348

Rusinol, M., Girones, R., 2017. Summary of excreted and waterborne viruses, in: Global Water Pathogens Project (J.S Meschke, and R. Girones (Eds) Part 3 Viruses). Michigan State University, E. Lansing, MI, UNESCO.

Schrader, C., Schielke, A., Ellerbroek, L., Johne, R., 2012. PCR inhibitors - occurrence, properties and removal. J. Appl. Microbiol. 113, 1014–26. doi:10.1111/j.1365-2672.2012.05384.x

Shirasaki, N., Matsushita, T., Matsui, Y., Urasaki, T., Ohno, K., 2009. Comparison of behaviors of two surrogates for pathogenic waterborne viruses, bacteriophages Qbeta and MS2, during the aluminum coagulation process. Water Res. 43, 605–12. doi:10.1016/j.watres.2008.11.002

Tanneru, C.T., Rimer, J.D., Chellam, S., 2013. Sweep flocculation and adsorption of viruses on aluminum flocs during electrochemical treatment prior to surface water microfiltration. Environ. Sci. Technol. 47, 4612–4618. doi:dx.doi.org/10.1021/es400291e

Taylor, D.H., Bellamy, A.R., Wilson, A.T., 1980. Interaction of bacteriophage R17 and reovirus type III with the clay mineral allophane. Water Res. 14, 339–346.

Teunis, P.F., Moe, C.L., Liu, P., E Miller, S., Lindesmith, L., Baric, R.S., Pendu, J.L., Calderon, R.L., 2010. Norwalk virus: how infectious is it? J. Med. Virol. 80, 1468–1476. doi:10.1002/jmv

Xagoraraki, I., Harrington, G.W., Assavasilavasukul, P., Standridge, J.H., 2004. Removal of emerging waterborne pathogens and pathogen indicators by pilot-scale conventional treatment. J. Am. Water Work. Assoc. 96, 102–113.

Yao, K.-M., Habibian, M.T., O'Melia, C.R., 1971. Water and waste filtration: concepts and

application. Environ. Sci. Technol. 5, 1105–1112.

Ødegaard, H., Østerhus, S., Melin, E., Eikebrokk, B., 2010. NOM removal technologies–Norwegian experiences. Drink. Water Eng. Sci. 3, 1–9.

Österberg, R., Lindovist, I., Mortensen, K., 1993. Particle size of humic acid. Soil Sci. Soc. Am. J. 57, 283–285.

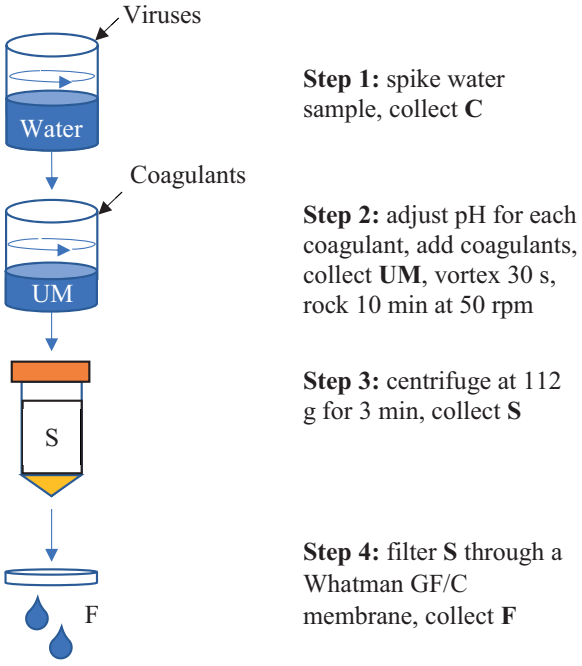


Figure 1. Bench scale procedure used for initial optimization of coagulation conditions and during part I and II setups. C – control, UM – under mixing, S – supernatant, F – filtrate.

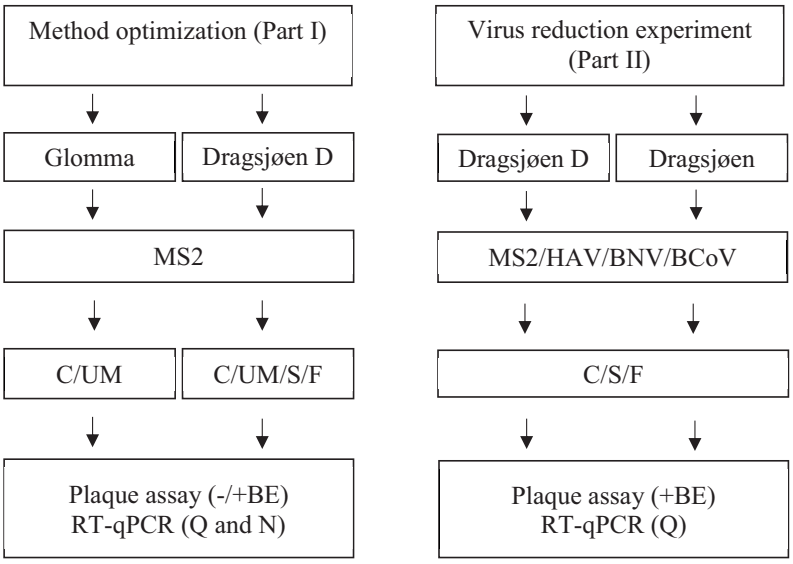


Figure 2. Outline of part I and II of the study. Dragsjøen D – diluted Dragsjøen water; C - control, UM – under mixing; S – supernatant; F – filtrate; BE – beef extract; Q – Qiagen RNA extraction; N – Nuclisens RNA extraction; MS2 – bacteriophage MS2; HAV – hepatitis A virus; BNV – bovine norovirus; BCoV – bovine coronavirus.

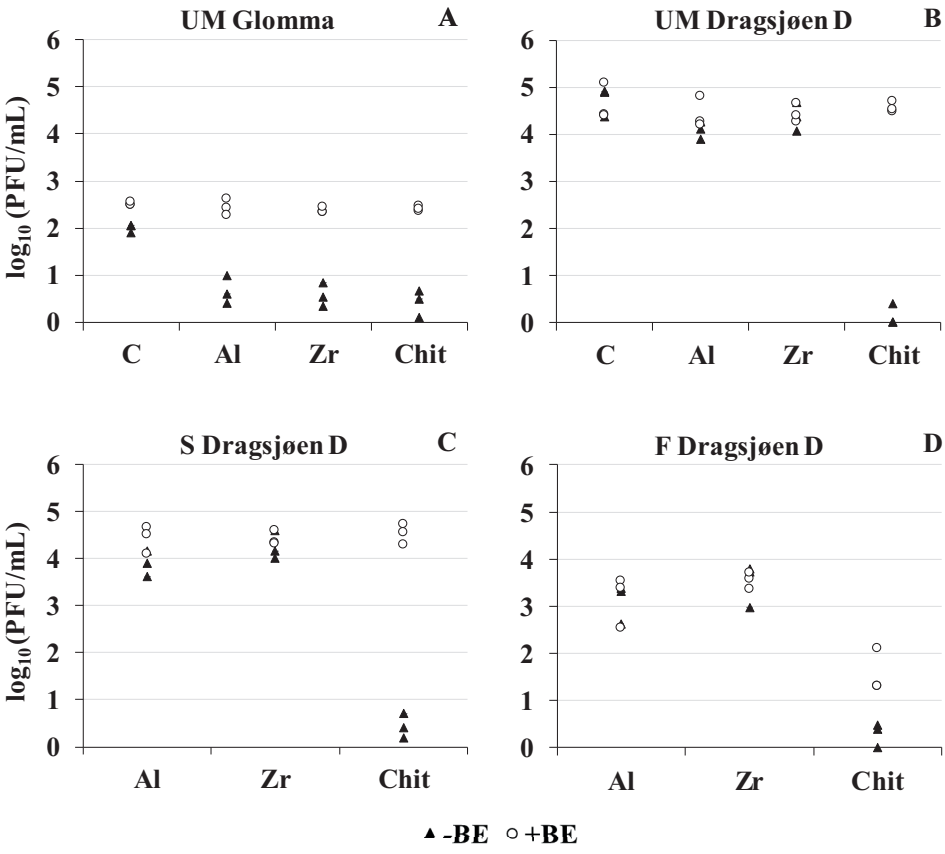


Figure 3. Influence of coagulants on MS2 enumeration by plaque assay. Viable MS2 was quantified in collected UM, S and F after treatment of Glomma (A) and Dragsjøen (B, C, D) water with Al, Zr or chitosan. Collected samples were treated with (+BE) or without (-BE) beef extract. C – control, UM – under mixing; S – supernatant; F – filtrate; Dragsjøen D – Dragsjøen diluted.

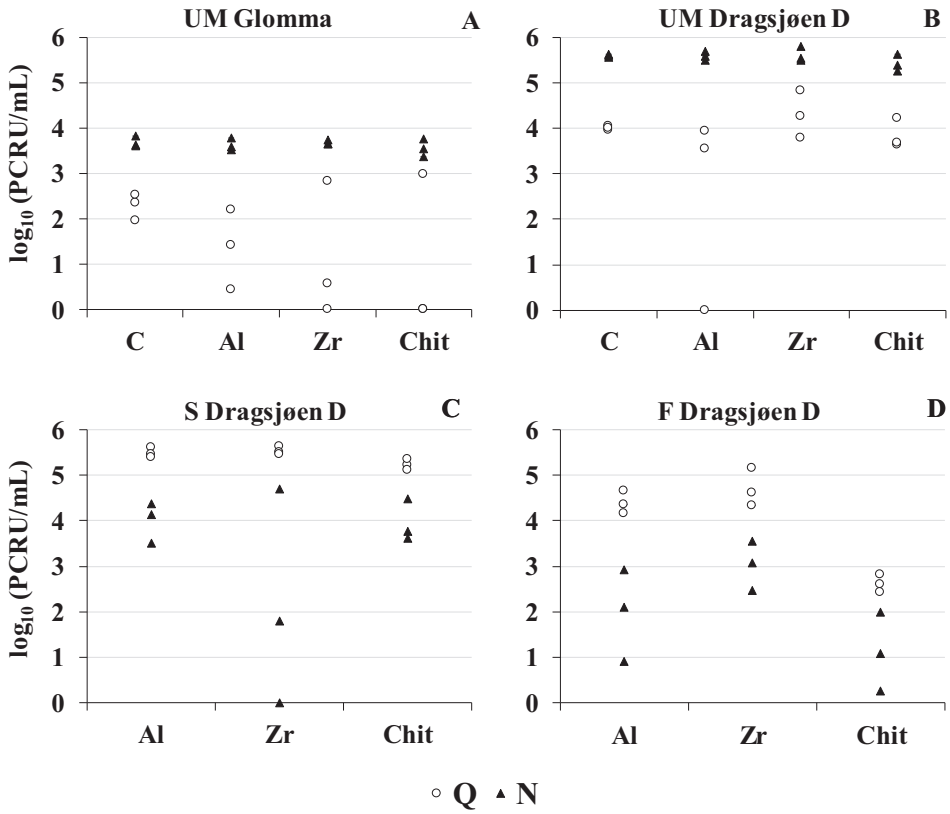


Figure 4. Influence of RNA extraction method on virus enumeration by RT-qPCR. Total MS2 was quantified in UM, S and F samples after treatment of Glomma (A) and Dragsjøen (B, C, D) water with Al, Zr or chitosan. RNA was extracted with method Q (Qiagen) and N (Nuclisense). C – control; UM – under mixing; S – supernatant; F – filtrate; Dragsjøen D – Dragsjøen diluted.

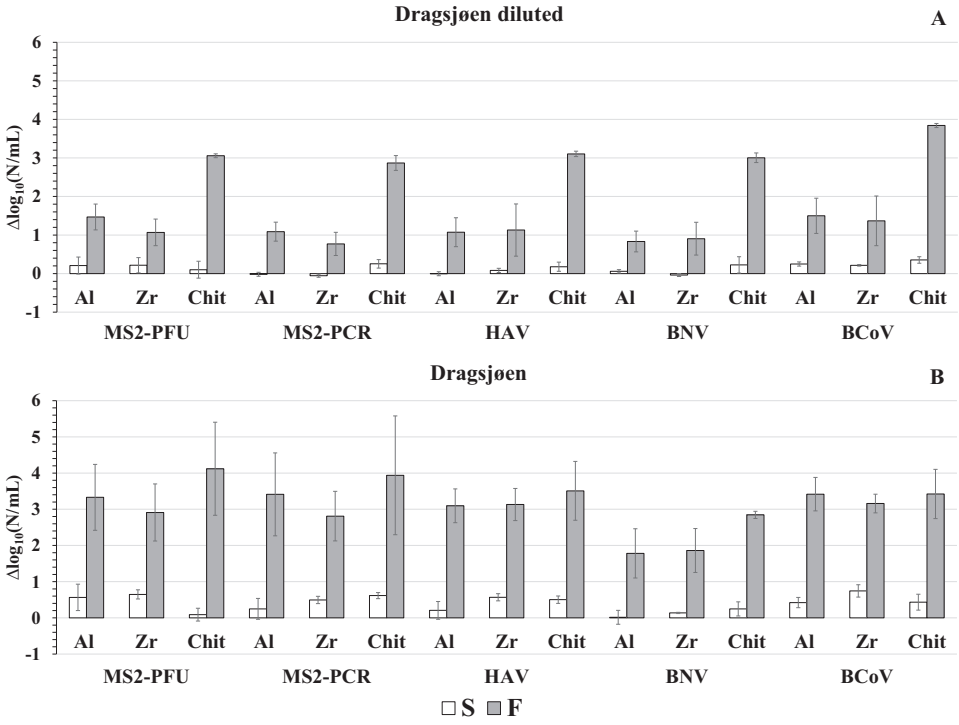


Figure 5. Log-reductions of MS2, hepatitis A virus (HAV), bovine norovirus (BNV) and bovine coronavirus (BCoV) in diluted (A) and undiluted (B) Dragsjøen water during treatment with Al, Zr and chitosan coagulants. Viable MS2 was enumerated by plaque assay including beef extract. Viral RNA was extracted from supernatant (S) and filtrate (F) with the Qiagen method and quantified by RT-qPCR. The results are expressed as mean and SD (n=3).

Table 1. Characteristics of Glomma and Dragsjøen raw water after spiking with the virus suspension. Dragsjøen diluted - Dragsjøen water diluted 1:4 by distilled water.

Water source	Raw water parameters		
	Turbidity, NTU	Colour, mg Pt/L	pH
Dragsjøen	1.1	93	6.6
Dragsjøen diluted	0.5	25	6.6
Glomma	0.8	26	7.3

Table 2. Virus characteristics; size and isoelectric point (pI).

Virus	Size, nm	pI	Reference
Bacteriophage MS2	30	3.9	Langlet et al. (2007)
Hepatitis A virus	28	2.8	Flehmig (1980); Michen & Graule (2010)
Bovine norovirus	30-35	6.0-6.3	Otto et al.(2011); Theoretical pI, calculated using the ExPASy ProtParam tool, based on the capsid protein sequence
Bovine coronavirus	80-120	4.5-4.6	Kapil et al.(1999); King & Brian (1982)

Table 3. Primers/probe and RT-qPCR conditions used for detection of viruses. MS2 – bacteriophage MS2; HAV – hepatitis A virus; BNV – bovine norovirus; BCoV – bovine coronavirus.

Virus	Primers and probes		RT-qPCR conditions	Reference or sequence
All			RT-step: 30 min at 55 °C, 2 min at 95 °C, then 40 cycles of:	
MS2	MS2-TM2-F (200 nM)	Forward	15 s at 95 °C, 30 s at 58 ºC	Dreier, Störmer, Kleesiek, & Sto (2005) [1]
	MS2-TM2-R (200 nM)	Reverse		
	MS2-TM2 (100 nM)	Probe		
HAV	HAV 68 (500 nM)	Forward	15 s at 95 °C, 1 min at 60 ºC, 8 s at 64 ºC,	Costafreda, Bosch, & Pintó (2006)[1]
	HAV 240 (900 nM)	Reverse	8 s at 68 ºC	
	HAV 150 (250 nM)	Probe		
BNV	BNVF1 (500nM)	Forward	15 s at 95 °C, 1 min at 58 ºC, 8 s at 62 ºC,	5'-GATCTTTGTGCCATCACACC
	BNVR1 (500nM)	Reverse	8 s at 66 ºC	5'-CGACTACCTTCCCACAGTGA
	BNVP1 (500 nM)	Probe		5'FAM- AACCTCATCCAAGCAAACATGGAGC-BHQ
BCoV	BCoV 1F20 (400 nM)	Forward	15 s at 95 °C, 30 s at 55 ºC, 15 s at 60 ºC	5'-TGGTGTCTATATTCATTTCTGCTG
	BCoV 1R89 (400 nM)	Reverse		5'- GGCCACTGCCTAGGATACA
	BCoV 1P48 (800 nM)	Probe		5'FAM-ACACGTCCCTGGCTGAAAGCTG-BHQ

[1] some modifications in cycling conditions and primers/probe concentrations.

Table 4. Selected conditions used for water treatment and the quality of filtrates after treatment with aluminium (Al), zirconium (Zr) and chitosan coagulants. Coagulant concentrations were optimized relative to colour reduction, at a defined pH. Dragsjøen diluted - Dragsjøen water diluted 1:4 by distilled water. ND - not done.

Water source	Selected conditions		Water parameters, filtrate		
	Coagulant	Dose, mg/L	pH	Turbidity, NTU	Colour, mg Pt/L
Dragsjøen	Al	8	5.9	0.8	5
	Zr	16	5.0	0.5	5
	Chit	15	5.0	0.5	13
Dragsjøen diluted	Al	3	5.6	0.6	5
	Zr	4	4.9	1.1	5
	Chit	4	4.9	0.3	6
Glomma	Al	3	6.6	ND	2
	Zr	9	6.1	ND	2
	Chit	2	5.1	ND	9

Table 5. Regression coefficients for the estimated virus reduction (Δlog_{10}). Standard error is in the parentheses. Dragsjøen diluted - Dragsjøen water diluted 1:4 by distilled water.

Water type	Coefficients (Std.Error)	
	Supernatant	Filtrate
Dragsjøen diluted	0.00	0.00
Dragsjøen	0.21* (0.08)	1.78** (0.24)
Coagulant type		
Al	0.00	0.00
Zr	-0.01 (0.08)	-0.15 (0.18)
Chitosan	0.12 (0.08)	2.04** (0.14)
Virus type		
MS2-PFU	0.00	0.00
MS2-PCR	-0.07 (0.07)	-0.27 (0.28)
HAV	-0.07 (0.07)	-0.16 (0.22)
BNV	-0.21** (0.07)	-0.73** (0.23)
BCoV	0.09 (0.07)	-0.08 (0.22)
Coagulant/water type		
Al-Dragsjøen	0.00	0.00
Zr-Dragsjøen	0.24* (0.11)	-0.08 (0.33)
Chitosan-Dragsjøen	-0.06 (0.12)	-1.49** (0.38)
Intercept	0.14 (0.07)	1.44 (0.19)

* and ** indicates significance at the 95%, and 99% level, respectively.

Appendix A. Norwegian water standards

Table A1. Some physicochemical water standards as accepted by Norwegian Drinking Water Regulation [153] for drinking water delivered to consumers from a water plant.

Parameter	Acceptable value by place of consumption	Notes
Colour, mg Pt/l	Acceptable for recipients	Should be <5 mg Pt/l (<10 mg Pt/l for chitosan) in the outlet water to meet hygienic barrier requirements
Trihalomethanes, µg/l	100	
Turbidity, FNU	Acceptable for recipients	Should be <0.1-0.2 FNU in the outlet water to meet hygienic barrier requirements
Residual metals, µg/l	200	150 µg/l for the big plants

Table A2. Microbiological water standards as accepted by Norwegian Drinking Water Regulation [153] for drinking water delivered to consumers from a water plant.

Organism	Acceptable value
HPC, 22 °C, 3 days	100 cfu/ml
Coliform bacteria	0 cfu (MPN)/100 ml
E.coli	0 cfu (MPN)/100 ml
Intestinal enterococci	0 cfu/100 ml
Clostridium perfringens (incl. spores)	0 cfu/100 ml

Appendix B. Use of zirconium and chitosan coagulants in Norway

Nine Norwegian WTP use Zr and/or chitosan coagulants, serving almost 50 000 PE (data for 2014, Figure B1.

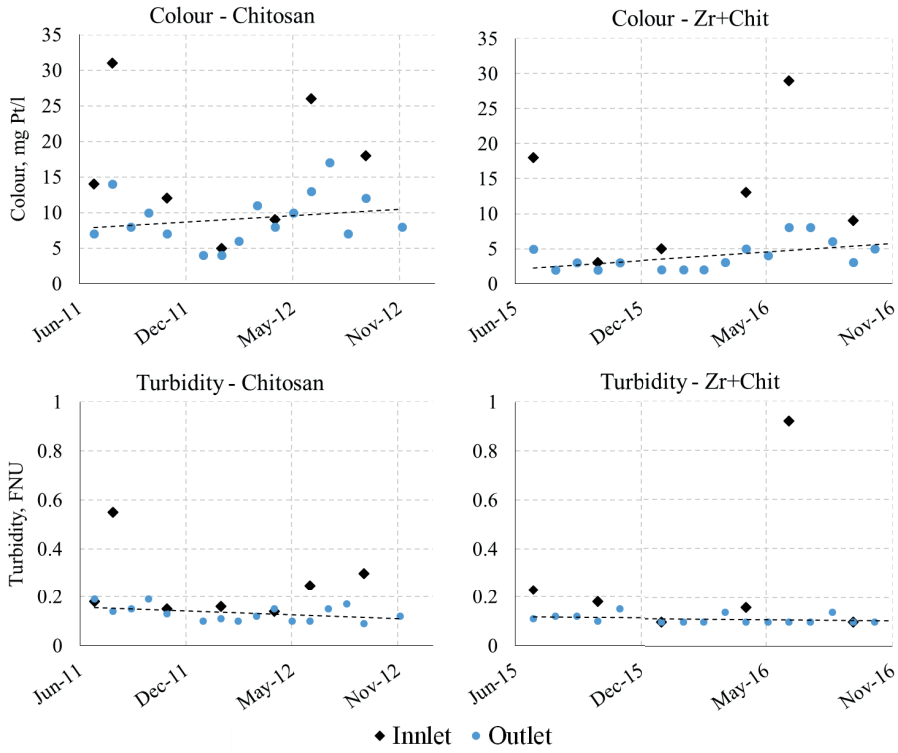
There are four WTPs that use chitosan alone (1-4), while five other plants implement a combination of Zr and chitosan (5, 6, 7 and 8), mixed in a ratio of the components of 1:2 (Zr:chitosan). Coagulant dosages per colour unit, routinely applied at these WTPs are presented in Table B1. Figures B2-B4 demonstrate quality of the inlet and outlet waters after treatment with chitosan alone or Zr-chitosan mixture at three of the outlined WTPs.

Table B1. Coagulant dosages and pH required to provide colour 5 mg Pt/l (10 mg Pt/l for chitosan used alone) in the treated water from some Norwegian water treatment plants (WTP)**.**

Raw water origin	Coagulation pH	Coagulant	Coagulant dosage per colour unit, mg/mg Pt	Data origin
Brattliflata WTP	4.0	chitosan	0.10	jar test
	6.1		0.14	
Rondablikk WTP	4.0	chitosan	0.15	
	6.8		0.25	
Haugesund WTP	4.0	chitosan	0.08	full-scale test
		Zr+chitosan (2:1)	0.08+0.04	
	7.8	Zr+chitosan	0.11+0.05	jar test
Ølen WTP	4.0	chitosan	0.08	full-scale test
		Zr+chitosan (2:1)	0.07+0.02	
Skånevik WTP	4.0	chitosan	0.12	jar test
		Zr	0.40	
		Zr+chitosan (2:1)	0.20+0.02	bench and full-scale test

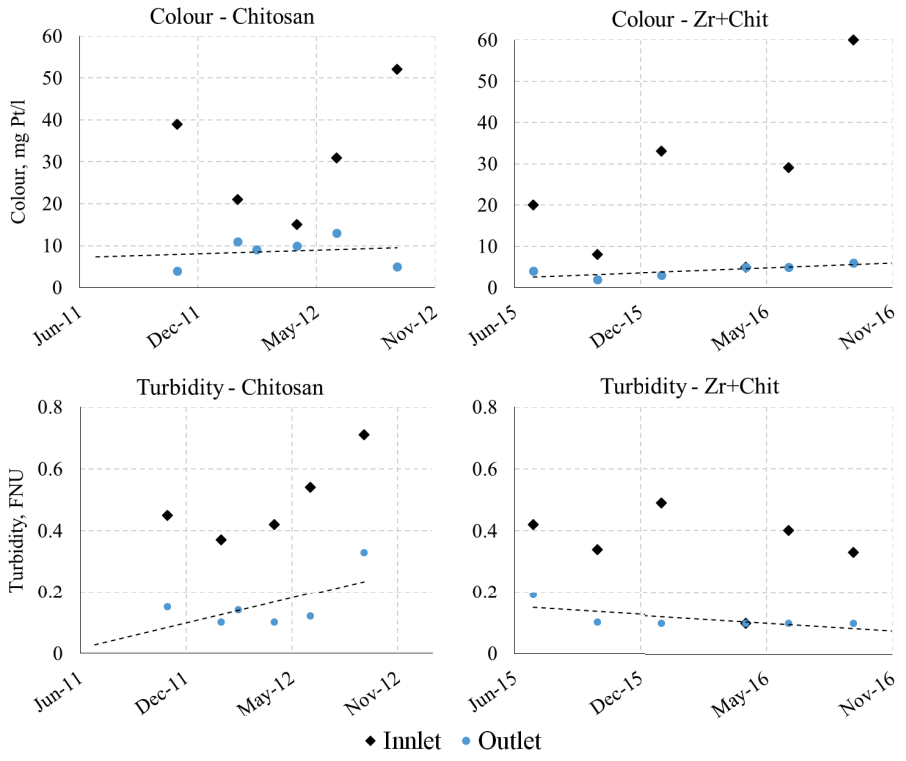
Figure B1. Location of the WTPs, employing chemical treatment with either chitosan (green), or a mixture of these two coagulants (red pins): 1) Østerbø Evangeliesenter (50 PE); 2) Brattliflata (99 PE) and Rondablikk (136 PE) WTPs; 3) Helleland WTP (395 PE); 4) Industrial and Aquatic Laboratory in Bergen; 5) Skånevik WTP (500 PE); 6) Haugesund WTP (45 000 PE); 7) Ølen WTP (2 222 PE); 8) Ølen Bjoa WTP (375 PE). Created with www.mapcustomizer.com.

Figure B2. Results of routine water analysis of raw and treated water at Ølen WTP in the periods between 01.06.2012-01.12.2013 (left column, chitosan alone) and 01.06.2015-31.12.2016 (right column, combination of Zr and chitosan)*.



* Data were adapted from www.labweb.no, and did not include samples from network mains; coagulant consumptions were approximately 1.5 g chitosan/m^3 and 1.5 g Zr/m^3+0.5 chitosan/m^3. Coagulation pH was approximately 4.0, regardless of the coagulant; treatment consists of screening, coagulation-contact filtration, UV-disinfection.

Figure B3. Results of routine water analysis of raw and treated water at Ølen Bjoa WTP in the periods between 01.06.2012-01.12.2013 (left column, chitosan alone) and 01.06.2015-31.12.2016 (right column, combination of Zr and chitosan)*.



* Data were adapted from www.labweb.no, and did not include samples from network mains; coagulant consumptions were approximately 1.5 g chitosan/m^3 and 1.5 g Zr/m^3+0.5 chitosan/m^3. Coagulation pH was approximately 4.0, regardless of the coagulant; treatment consists of screening, coagulation-contact filtration, UV-disinfection.

Figure B4. Results of routine water analysis of raw and treated water at Haugesund WTP in the periods between 01.07.2011-01.06.2013 (left column, chitosan alone) and 01.06.2015-03.02.2017 (right column, combination of Zr and chitosan)*.

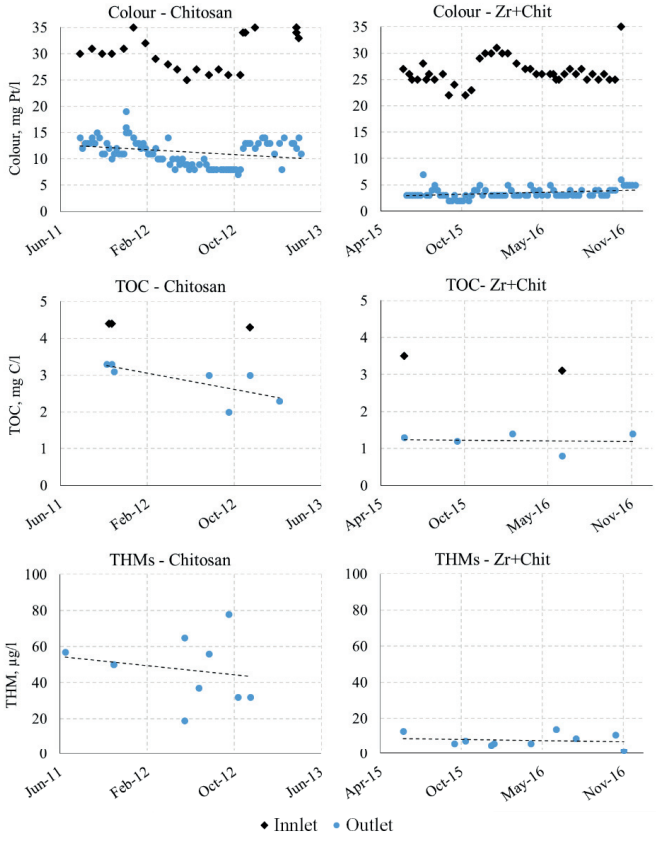
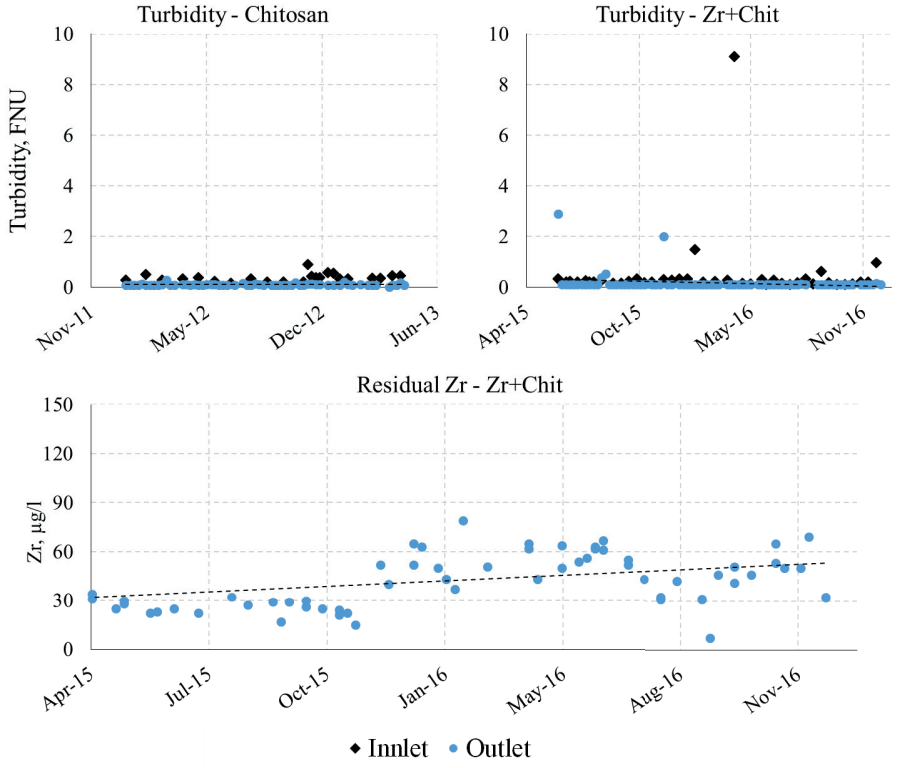


Figure B4 (cont). Results of routine water analysis of raw and treated water at Haugesund WTP in the periods between 01.01.2012-01.06.2013 (left column, chitosan alone) and 01.06.2015-03.02.2017 (right column, combination of Zr and chitosan)*.



* Data are adapted from www.mittvann.no, and do not include samples from network mains (except for TOC and THM); coagulant consumptions were approximately 2 g chitosan/m^3 and 1.9 g Zr/m^3+0.9 g chitosan/m^3. Coagulation pH was approximately 4.0-4.5, regardless of the coagulant; treatment consists of screening, coagulation-contact filtration, disinfection with chlorine (until 2014) and UV-disinfection (after 2014).

Appendix C. Pilot and full-scale research on chitosan performed in Norway

Table C1. Pilot and full-scale research on chitosan performed in Norway.

Water Source, Reference	Raw water characteristics and size of WTP	Chitosan product	Operation characteristics	Main findings
Vindkollvann, Kilsund WTP [209]	40 mg Pt/l; 7.13 mg DOC/l; 0.52 NTU	Fluka chitosan medium MW (Fluka Chemicals, USA)	4.5 mg chitosan/l, coagulation pH 6.3	80-85% reduction of colour; 35% reduction of TOC; 87-92% reduction of turbidity
			6.0 mg chitosan/l, coagulation pH 6.3; Two-media contact filter, 9.3 m/h filtration rate	2.2 $\log_{10}$ reduction of coliforms; 1.8 $\log_{10}$ reduction of HPC
Stakkestadvatnet, Haugesund WTP [145]	28 mg Pt/l; 3.3 mg TOC/l; 0.47 NTU 40 000 m^3/day	TM388 and TM578 low MW chitosan (Primex Ingredients ASA, Iceland)	1.5 mg chitosan/l, coagulation pH 4.0; Two-media contact filter, 8.5 m/h filtration rate	74% reduction of colour; 33% reduction of TOC; 57% reduction of turbidity 24 h filter cycle; Annual sludge production -250 tonnes
Rorevann, Grimstad WTP [174]	21 mg Pt/l; 3.5 mg TOC/l; 0.35 NTU 9 000 m^3/day	ChitoClear TM381 (Primex Ingredients ASA, Iceland)	1.6 mg chitosan/l, coagulation pH 4.0; Three-media contact filter, 9.2 m/h filtration rate	72% reduction of colour; 32% reduction of TOC; 86% reduction of turbidity; At least 17 h filter cycle; Annual sludge production -13 tones
Bjoa WTP [210]	18 mg Pt/l; 3.7 mg TOC/l; 1.1 NTU 360 m^3/day	Not mentioned	1.5 mg chitosan/l, coagulation pH 4.0; Two-media contact filter, 5 m/h filtration rate	72% reduction of colour; 27% reduction of TOC; 91% reduction of turbidity; No effect of low temperature on chitosan efficiency
Glomma, Nedre Romerike WTP [146]	24 mg Pt/l; 3.4 mg TOC/l; 0.23 NTU 45 000 m^3/day	Kitoflock (Teta Vannrensing Ltd, Norway)	1.5 mg chitosan/l, coagulation pH 4.0; Two-media contact filter, 4.7 m/h filtration rate	77% reduction of colour; 67% reduction of turbidity; At least 19 h filter cycle

Appendix D. Economic and environmental evaluations of zirconium and chitosan use

Table D1. Annual consumption amounts and costs for water production at a theoretical middle-sized Norwegian WTP, based on the given demands for coagulant, clean water, polymer, energy and handling of sludge during water treatment.

	Al	Fe	Zr	Chitosan
Coagulant				
Coagulant dose[1], g/m^3	1.5	3.3	2.4	1
Demand of coagulant[2], tonnes	166	284	94	10
Costs of coagulant incl. transport, mil NOK	0.4	0.5	3.2	1.8
Backwash and ripening				
Filter cycle length[1], h	15	11	22	17
Number of backwashing each day	1.6	2.2	1.1	1.4
Clean water used for backwashing and ripening[3], m^3	1 054 120	1 434 116	718 718	930 106
Costs of clean water[4], mil NOK	4.2	5.7	2.9	3.7
Polymer used for processing and backwash water[5], tonnes	16	22	11	14
Costs of polymer, incl. transport[5], mil NOK	0.5	0.7	0.3	0.4
Energy used for backwashing and dewatering[6], kWh	240 506	327 205	163 981	212 211
Costs of energy for backwashing and dewatering [7], mil NOK	0.1	0.2	0.1	0.1
Sludge				
Dry sludge produced[1], tonnes	81	119	86	21
Dewatered sludge produced[8], tonnes	404	595	429	105
Costs of sludge to be disposed, incl. transport[9], mil NOK	0.6	0.9	0.6	0.03
Total costs, mil NOK	6	8	7	6

[1] the data obtained with the pilot testing, except for Fe, for which dose and filter cycle are estimated with empirical formulas; [2] assumed PACl product, containing 9% of pure Al; $FeCl_3$ product, containing 12% of pure Fe; $ZrOCl_4$, containing 27% of pure Zr; [3] assumed backwashing time - 13 min and backwashing rate - 950 m^3/h, ripening time – 30 min at normal production; [4] costs of clean water for internal needs - 4.0 NOK/m^3; [5] assumed polymer demand - 1.5 g per m^3 of backwash water and costs 30 NOK/kg; [6] total energy consumption for water production and distribution – 0.85 kWh/m^3 [211]; backwashing and dewatering account for 10 and 20% of the total energy consumption, respectively; the energy demands are determined by multiplying electricity consumption by amounts of backwashed water and processed water, respectively, [7] electricity price – 0.5 NOK/kWh; [8] assumed total solids in dewatered sludge - 20%;[9] include costs for transportation and an intake fee for sludge (1 200 NOK/m^3).

Table D2. CO_2 emission connected to transportation of sludge to landfills, and the amount of disposed metal into the environment, attributed to use of each coagulant for water production at a theoretical middle-sized Norwegian WTP (see also Table D1).

	Al	Fe	Zr	Chitosan	Chitosan+Zr
CO_2 emission [1], tonnes	3.9	6.5	4.1	1.0	2.1
Disposed metal into the environment, tonnes	15	33	25	0	3

[1] the data obtained with the pilot testing, except for Fe, for which dose and filter cycle are estimated with empirical formulas.

20.04.2017

FORM 4.7 Errata

Correcting formal errors in the PhD thesis (cf. section15.3-2 in the PhD regulations)

The PhD candidate may after submitting the thesis apply to correct formal errors in the thesis. An application to correct formal errors must be submitted no less than four (4) weeks before the disputation. Such an application can be made only once.

Thesis title:	Use of zirconium and chitosan coagulants for physicochemical and hygienic water treatment

Page number	Paragraph	Change from	Change to
Front page		Title-normal text, size 12	Bold text, size 18
ix		BE, beef extract solution	BE, beef extract
ix		Non-HS, humic substances	Non-HS, non-humic substances
ix			Vis., visible light
5	1	The World Health Organisation	The World Health Organisation (WHO)
13	Figure 4 caption		Vis., visible light (abbreviation explanation is added)
13	Figure 4	Vis	Vis.
17	4	Another destabilization mechanism,	Another destabilization mechanism (comma is removed)
21	1	(pI, explained in Section 2.5	(*pI*, explained in Section 2.5)
22	3	environmental friendly	environmentally friendly
26	1	of	if
36	3	The effective **dosage was** plotted as a function of initial water colour. The corresponding pH ranges were the values measured at the effective **dosage**	The effective **dosages were** plotted as a function of initial water colour. The corresponding pH ranges were the values measured at the effective **dosages**
37	2	Unexpected (a cognate word "expected" is used in the next sentence, and preferably should be changed with a synonym)	unforeseen
37	2	Discussed	Presented

		(word "discussed" is also used in the next sentence, and preferably should be changed with a synonym)	
37	3	Expect (word "expect is used two times in the same sentence)	assume
44		3.3.1	3.4.1
46			Table 7 is moved to the next page
56	1	was	was not
56	4	beef extract solvent	BE solvent
70	Citation 124	S. S. Abu, A. Siti, N. Farhana, Z. Hamidi, and A. Aziz,	S. S. Abu Amr, S. N. F. Zakaria, and H. A. Aziz
71	Citation 145		«Oslo: Norsk institutt for vannforskning (NIVA)» is added
71	Citation 146		"PhD [dissertation]. Ås: University of Life Sciences" is added
73	Citation 171		"PhD [dissertation]. Trondheim: Norwegian University of Science and Technology" is added
73	Citation 172		«Oslo: Norsk institutt for vannforskning (NIVA)» is added
73	Citation 178	Aug 2001	2001
73	Citation 185		The citation has been corrected
75	Citation 210		«Oslo: Norsk institutt for vannforskning (NIVA)» is added
75	Citation 211		The citation has been corrected
6 Paper 3			APHA, 1995 - missing reference is added
21 Paper 3			Bolto, B., & Gregory, J. (2007) missing reference is added
25 Paper 3			«Norwegian Food Safety Authority. Forskrift om vannforsyning og drikkevann» missing reference is added
26 Paper 3			Rusinol, M., Girones, R., 2017. missing reference is added
76 78			Blank pages were added to hold numeration correct

90 102 104 142			
Appen-dix B		Figure B2 (cont)	Figure B3 (Figures B2 and B2(cont) are two different figures and were listed wrong Numbers of the following fig-ure were changed. Figure B3 is also listed in List of Figures now (page xii)
Appen-dix B	2	listed	Changed to "outlined", since Ølen Bjoa water treatment plant, depicted in Figure B3, is not listed in Table B1.
	Appendix C, table C1	Ton	tonnes
	Appendix C, table C1	vannverk	WTP
	Appendix D, table D1		Missing footnote is added

This form will be signed by the PhD candidate and the main supervisor and must be sent to the faculty for approval. The approved errata must be archived in the PhD candidate's doctoral archive and must be attached to the final thesis print version as the last page of the thesis.

Date and signature:

PhD candidate (Author):	Ekaterina Christensen	07.09.2018 Christensen

Main supervisor:	Mette Myrmel	7/9-18 Mette Myrmel

Errata approved by the faculty: Yes ☐ No ☐

For the faculty:	

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Norconsult

Postboks 626
1303 Sandvika, Norway
+47 67 57 10 00
http://www.norconsult.no

Forskningsrådet

P.O Box 564
N-1327 Lysaker, Norway
+47 22 03 70 00
www.forskningsradet.no

Norwegian University of Life Sciences

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

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