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

Hygienic performance of zirconium and chitosan coagulants

Ekaterina Christensen[ab]*, Vegard Nilsen[c], Tor Håkonsen[b], Arve Heistad[c], Christophe Gantzer[de], Lucy J. Robertson[a] and 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, Norway

[c] Norwegian University of Life Sciences, Department of Mathematical Sciences and Technology, PO Box 5003, N-1432 Aas, Norway

[d] Université de Lorraine, Laboratoire de Chimie, Physique et Microbiologie pour l'Environnement (LCPME), UMR 7564, Faculté de Pharmacie, Nancy F-54000, France

[e] CNRS, LCPME, UMR 7564, Nancy F-54000, France

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

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

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 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 and 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

Water analyses

Turbidity was measured using a 2100N IS turbidimeter (Hach Company, USA). Colour was measured by DR3900 Hach spectrophotometer (Hach, USA), following ISO 7887:2011 (λ = 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, 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. 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.

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

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 and 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 5N 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 PCR (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 instruction. 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.

Table 3 | Primers/probe and RT-qPCR conditions

Primers and probe*	Sequence (5'-3')	RT-qPCR

		conditions
MS2-TM2-F (400 nM)	TGCTCGCGGATACCCG	30 min at 55 °C,
MS2-TM2-R (400 nM)	AACTTGCGTTCTCGAGCGAT	2 min at 95 °C
		and 45 cycles of
MS2-TM2FAM (50 nM)	FAM-ACCTCGGGTTTCCGTCTTGCTCGT- BHQ1	15 s at 95 °C, 30
		s at 58 ºC

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

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 (PACl)

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.

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^{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 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 matters (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 (Christensen *et al*., 2016; Jarvis *et al*., 2012; Ødegaard *et al*., 2010). 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 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 (100kDa, 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 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.

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	-

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).

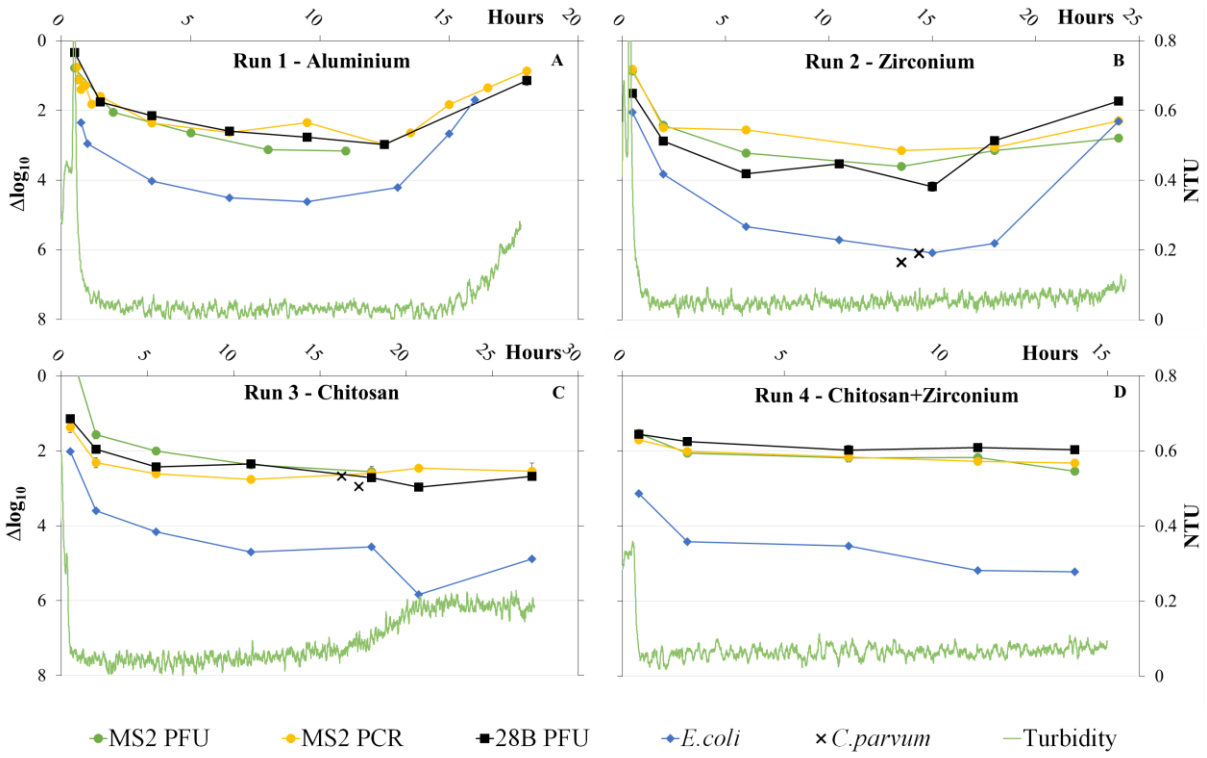

Figure 1 | Effluent turbidity and log-reductions of MS2 (PFU/mL and PCRU/mL), 28B (PFU/mL), *E. coli* (MPN/100 mL) and *C. parvum* (oocysts/10 L), as a function of the filter operation time. The conditions were: 1.5 mg Al/L at pH 5.8 (Run 1); 2.4 mg Zr/L at pH 4.2 (Run 2); 1 mg/L of chitosan at pH 4.2 (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

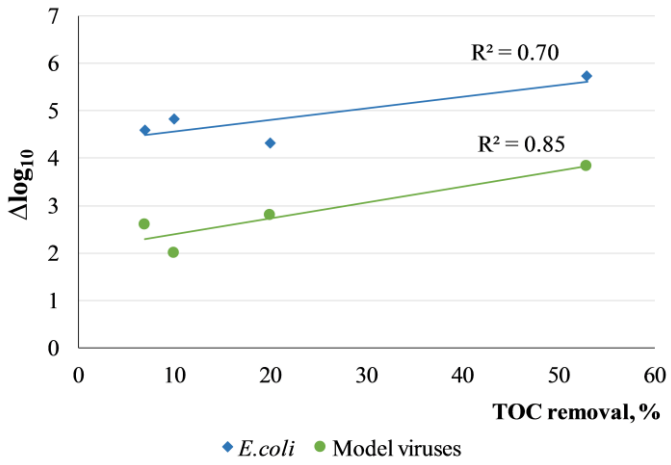

Figure 2 | Correlation between TOC removals and log-reductions of *E. coli* and bacteriophages for Runs 1–4; log-reductions during the stable filtration phases were used for calculations

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 3a). 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 3b). 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.

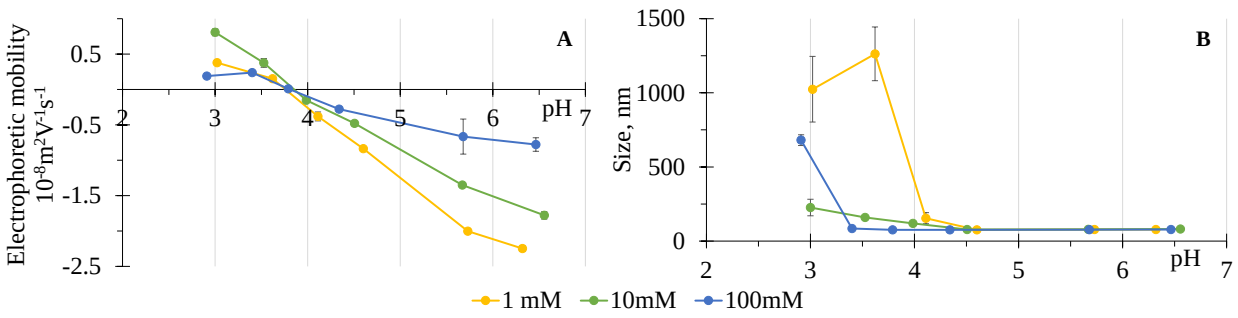

Figure 3 | Measurement of electrophoretic mobility (a) and size distribution (b) of 28B phage under varying ionic strengths ($NaNO_3$) and pH conditions; measurements are expressed as mean values (n = 3) and SEM (standard error of the mean)

DISCUSSION

The present study assessed the removal capacity of two non-conventional coagulants – Zr and chitosan – for physicochemical and microbiological parameters, and compared 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 and 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 and 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 removals 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 and 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 and Harrington, 2004).

The turbidity parameter is extensively used to assess the efficiency of the physicochemical treatment step for microbial removal (Xagoraraki and 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.

ACKNOWLEDGMENTS

The work was supported by the Research Council of Norway (grant no. 226750/O30) and Norconsult consultancy firm. The authors are grateful to the staff at Nedre Romerike Vannverk for provided water samples. The authors also thank Else Marie Aasen, Rannei Tjåland, Elisabeth Furuseth Hansen, Anne Willumsen, Torbjørn Friborg, Øyvin Østensvik, Marte Monshaugen and Olga Anna Osinska from Norwegian University of Life Sciences for the analysis assistance. The surface property measurements of phage 28B were performed using the environmental molecular biology platform from LCPME (Nancy-France) with assistance from Romain Rivet. Kemira Chemicals AS and Teta Vannrensing Ltd are acknowledged for provision of the coagulant samples.

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), 971–7. 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), 331–8. 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), 905–12. 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**, 133–68.

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. *Separation Science and Technology*, **41**(11), 2487–2514. http://doi.org/10.1080/01496390600742807

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 W*ater 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), 2769–77. 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.

Mayer, B. K., Yang, Y., Gerrity, D. W., & Abbaszadegan, M. 2015 The impact of capsid proteins on virus removal and inactivation during water treatment processes. *Microbiology Insights,* **8**(S2), 15–28. http://doi.org/10.4137/Mbi.s31441

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

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/bm034130m

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), 605–12. 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*. U.S. 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

First received 9 March 2017; accepted in revised form 2 June 2017. Available online