Microbial reduction in wastewater treatment using Fe^{3+} and Al^{3+} coagulants and PAA disinfectant

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ABSTRACT

Wastewater is an important source of pathogenic enteric microorganisms in surface water and a major contaminating agent of drinking water. Although primary and secondary wastewater treatments reduce the numbers of microorganisms in wastewater, significant numbers of microbes can still be present in the effluent. The aim of this study was to test the feasibility of tertiary treatment for municipal wastewater treatment plants (WWTPs) using PIX ($FeCl_3$) or PAX ($AlCl_3$) coagulants and peracetic acid (PAA) the disinfectant to reduce microbial load in effluent. Our study showed that both PIX and PAX efficiently reduced microbial numbers. PAA disinfection greatly reduced the numbers of culturable indicator microorganisms (*Escherichia coli*, intestinal enterococci, F-specific RNA coliphages and somatic DNA coliphages). In addition, pathogenic microorganisms, thermotolerant *Campylobacter*, *Salmonella* and norovirus GI, were successfully reduced using the tertiary treatments. In contrast, clostridia, *Legionella*, rotavirus, norovirus GII and adenovirus showed better resistance against PAA compared to the other microorganisms. However, interpretation of polymerase chain reaction (PCR) analysis results will need further studies to clarify the infectivity of the pathogenic microbes. In conclusion, PIX and PAX flocculants followed by PAA disinfectant can be used as a tertiary treatment for municipal WWTP effluents to reduce the numbers of indicator and pathogenic microorganisms.

Key words | enteric microorganisms, PAA, PAX, PIX, wastewater treatment

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INTRODUCTION

Primary and secondary wastewater treatment typically achieves 90–99.9% reduction of enteric microbial numbers, and rapid sand contact filtration as tertiary treatment can achieve further 90–99% reductions (Koivunen *et al.* 2003; Rajala *et al.* 2003). However, these treatment processes may not always be sufficient to achieve a microbiologically safe effluent that can be discharged into natural waters or be reused. Some microorganisms, such as *Legionella*, can even thrive during the wastewater treatment process (Kusnetsov *et al.* 2010). There have also been numerous drinking water contamination incidents and waterborne outbreaks caused by wastewater intrusion into drinking water (Zacheus & Miettinen 2011). It is important to devise the efficient methods for tertiary wastewater treatment processes that fit different needs and also in consideration of the fact that wastewater composition can vary with the seasons in different climates.

The coagulation-flocculation process efficiently removes suspended solids (SS), organic matter (OM), phosphorus and also microorganisms (Ûgurlu *et al.* 2008). The performance of the coagulation–flocculation process is largely affected by the coagulant type. The most commonly used coagulants are $AlCl_3$, $Al_2(SO_4)_3$, $FeCl_3$ and $Fe_2(SO_4)_3$, because they are inexpensive and have been demonstrated to be very effective in the electro-coagulation process (Yang *et al.* 2010a). However, aluminium-based coagulants are reported

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to cause high residual aluminium concentrations in the treated water (Yang *et al.* 2010b), which raises concerns about the potential toxicity of residual aluminium. Therefore iron-based coagulants may be comparatively safer for the environment. Peracetic acid (PAA, CH_3COOOH) is an organic peroxy compound, which has strong oxidizing properties (Liberti & Notarnicola 1999). PAA treatment efficiently inactivates many pathogenic and indicator microorganisms in wastewaters (Sànchez-Ruiz *et al.* 1995; Wagner *et al.* 2002). One of the benefits of PAA disinfection is that it does not produce any significant amounts of toxic or mutagenic disinfection by-products or chemical residues, in the effluent (Booth & Lester 1995; Crebelli *et al.* 2005). PAA disinfection is already used in some wastewater treatment plants (WWTPs) and the US Environmental Protection Agency has included PAA among the five disinfectants recommended for use on combined sewer overflows (USEPA 1999).

The aim of this study is to find modern methods for improving municipal WWT processes. The work deals with tertiary treatment of municipal wastewater to improve the microbiological quality of wastewater effluents. The study hypothesis is that tertiary wastewater treatment using PIX or PAX flocculation followed by PAA disinfection would significantly reduce the large amount of enteric microbes in wastewater effluents.

MATERIALS AND METHODS

Wastewater

The WWTP of Siilinjärvi, Finland, with a hydraulic load of 3,150 m^3/day, treats domestic wastewater that is produced by 25,000 inhabitants and a commercial laundry. The WWTP uses a physical primary treatment and a secondary treatment using activated sludge followed by chemical precipitation before discharging the effluent into surface water. Both influent and effluent wastewaters were sampled and transported to the laboratory within one hour of sampling. The analyses were initiated on the same day. The wastewater quality varied according to the season, i.e. the wastewater may contain runoff water in summer but not in winter and the water temperature in summer reaches over 20 °C and in winter is less than 10 °C.

The experiment was divided into two segments: (1) pretreatments with coagulation-flocculation and (2) disinfection. In addition, dissolved air flotation (DAF) was tested at the Siilinjärvi wastewater treatment plant. The DAF unit was not functioning efficiently so only one DAF experiment is included in this study. Other experiments were carried out in the water laboratory of University of Eastern Finland, Kuopio campus. The microbiological analyses were carried out either at the University of Eastern Finland or at the Water and Health Unit, National Institute for Health and Welfare.

The indicator microorganisms (somatic coliphages, F-specific RNA coliphages, *Escherichia coli*, intestinal enterococci and the spores of sulfite-reducing clostridia (SRC) and heterotrophic bacteria) and the pathogenic microorganisms (*Salmonella*, *Legionella*, thermotolerant *Campylobacter*, norovirus GI, norovirus GII, rotavirus and adenovirus) were determined from the wastewater before and after PIX and PAX flocculation and PAA treatment. Temperature and major physico-chemical parameters were measured.

Coagulation–flocculation tests

Flocculation was done by placing 3 L of influent or effluent wastewater into a glass jar followed by the addition of PIX-111 (Kemira, Finland) so that the Fe^{3+} dose was 7.1 g Fe/m^3 and then mixing. For flocculation, the wastewater was mixed for one minute with rapid mixing, i.e. 250 rpm (250 G) (soon after addition of PIX) followed by 90 rpm (80 G) for 15 min, 50 rpm (40 G) for 15 min, and left for 45 min to settle. After stabilization and sedimentation, the upper layer (supernatant) was decanted carefully for the disinfection test. Indicator and pathogenic microorganisms were analysed from this upper layer water. This test was done in three replicates.

In the adenovirus spiking experiment, flocculation was carried out in 30 L wastewater effluents using PIX-111 at a dose of 8.2 g Fe/m^3 or PAX-18 (Kemira, Finland) at a dose of 4 g Al/m^3. In this experiment, human adenovirus 40 (ATCC VR-931) was spiked into the effluents at a concentration of 3×10^8 GC/mL. For flocculation, the wastewater was mixed for 1 min at 250 rpm (250 G) after quickly pouring in the PIX or PAX; the mixing was followed by 23 rpm (80 G) for 5 min, 18 rpm (55 G) for 10 min, 12 rpm for 10 min and 45 min for settlement.

Flotation

The pilot plant flotator used was made by Pomiltek International Ltd, Finland, and was previously used by Koivunen & Heinonen-Tanski (2008). The wastewater flow was 10.5 m^3/h and the hydraulic loading rate was 4.4 m/h. The coagulant used was PIX-111 at doses of 3–4 g/m^3. The recycle ratio was approximately 25%. In many cases, the treated water still clearly contained floc. Flotated water samples without any visible floc particles were taken for analyses.

Disinfection

The flocculated wastewater was further processed for disinfection using PAA (Kemira, Finland) within 2 hours of the sampling. In the PAA disinfection experiment, one litre of wastewater after flocculation was taken into a glass decanter. PAA (11%) was added at a dose of 5 mg PAA/L with a contact time of 5 min and mixed thoroughly using a magnetic stirrer for the entire experiment for experiment 1, i.e. for influent and effluent (results presented in Table 1). In the other experiments, PAA (11%) was dosed at 3 mg PAA/L with a contact time of 5 min. PAA was quenched by adding 1,000 μL/L sodium thiosulfate (10% $Na_2S_2O_3$) and the H_2O_2 residues were eliminated by adding 10 μL/L Sigma catalase. The test was carried out with three replicates for indicator microorganisms, but pathogenic microorganisms were determined from one pooled sample.

Similarly, in the 30 L flocculation test, 3 L of flocculated water sample was taken for disinfection by PAA (11%) using a similar method as for the 1 L disinfection test.

Microbial analysis

The number of coliphages (with hosts *E. coli* ATCC 13706 and 15597), enterococci, spores of SRC and *Salmonella* were determined by cultivation. The methods used were: the ISO/DIS 1075-2.2 (1998) for coliphages, SFS-EN-ISO 7899-2

Table 1 | Reduction of microbes in wastewater influent and effluent of Siilinjärvi WWTP flocculated by PIX-111, and followed by PAA (5 mg/L) disinfection. Log_{10} CFU/100 mL or PFU/100 mL and reduction percentage. Reduction % is calculated before log-transformation. Geometric means ± standard deviations (N = 3). LDL = less than the detection limit in all replicates, NA = not analysed. The different letters in a row indicate a statistically different result

	Without additional treatments	After flocculation	Reduction %	After PAA 5 mg/L	Reduction %
Influent					
Somatic coliphage	4.95a	4.75 ± 0.04b	36.8	3.77 ± 0.12c	89.2
F-specific RNA coliphage	4.73a	4.41 ± 0.12b	48.6	3.27 ± 0.15c	92.5
E. coli	6.93a	6.15 ± 0.04b	83.3	3.15 ± 0.12c	>99
Enterococci	5.98a	5.60 ± 0.07b	57.1	0.50c	>99
Spore of SRC	4.36a	3.86 ± 0.15a	66.4	2.97 ± 1.16a	68.8
COD mg/L	440	NA		NA	
BOD mg/L	150	NA		NA	
Total-P mg/L	7.6	NA		NA	
Effluent					
Somatic coliphage	3.85	3.61 ± 0.06	41.2	LDL	>99
F-specific RNA coliphage	2.35	1.61 ± 0.87	62.4	LDL	>99
E. coli	4.58	3.94 ± 1.12	98.5	LDL	>99
Enterococci	3.91	2.10 ± 1.22	89.5	LDL	98.2
Spore of SRC	3.19	1.46 ± 0.92	91.8	LDL	95.9
COD mg/L	48	NA		NA	
BOD mg/L	12	NA		NA	
Total-P mg/L	0.65	NA		NA	
NH_4-N mg/L	22	NA		NA	

(2000) for intestinal enterococci, SFS-EN 26461-2 (1993) for clostridial spores as direct enumeration tests and ISO 19250 (2010) for *Salmonella* as MPN (most probable number) tests using at least 3×25 ml samples for the pre-enrichment. The cultivation from selective enrichment was done to XLD and Rambach agars (both from Merck). *E. coli* was determined using the spread plate technique in ChromoCult agar (Merck) and incubated at 37 °C for 24 h and confirmed by Kovacs indole reagent.

Legionella were analysed from one portion of the wastewater sample concentrated by filtration and the second portion of the sample was diluted three-fold before processing according to ISO 11731. These portions of diluted, undiluted and concentrated samples were inoculated directly or acid-washed (pH 2.2, 4 min) or heat-treated (50 °C, 30 min) before inoculation onto GVPC medium plates (buffered charcoal yeast extract medium containing glycine, vancomycin, polymyxin B and cycloheximide, Oxoid Ltd, Cambridge, UK). These plates were incubated for 10–12 days at 36 ± 1 °C and colonies resembling *Legionella* were further confirmed by growth tests according to the standard test (ISO 11731 1998(E)). *Legionella* strains were serotyped with agglutination tests (Slidex Legionella Kit, bioMérieux Corporation, Marcy l'Etoile, France; *Legionella* Latex test, Oxoid Ltd, Cambridge, UK).

The presence of thermotolerant *Campylobacter* spp. was analysed in a semi-quantitative manner from sample volumes from 0.1 to 100 mL with a modification of the international standard method (ISO 17995 2005) and the species were identified as previously described (Pitkänen *et al.* 2008; Hokajärvi *et al.* 2013).

Norovirus genogroup I (GI) and genogroup II (GII), rotaviruses and adenoviruses were analysed by concentrating a 500 mL wastewater sample using a two-phase extraction method (WHO 2003). In the flocculation/disinfection experiment, the spiked adenoviruses were analysed directly without concentration. Viral DNA and RNA were extracted using High Pure Viral Nucleic Acid Kit and High Pure Viral RNA Kit (Roche Molecular Biochemicals Ltd, Mannheim, Germany), respectively. Viruses were detected by real time (RT-)PCR (polymerase chain reaction) assays carried out in a Rotor-Gene™ 3000 real-time rotary analyser (Qiagen). The primer and probe sequences for norovirus GI were used according to Kauppinen *et al.* (manuscript in preparation), for norovirus GII according to Loisy *et al.* (2005) (primer QNIF2d) and Kageyama *et al.* (2003) (primer COG2R and probe RING2-TP), for rotaviruses according to Jothikumar *et al.* (2009), and for adenoviruses according to Jothikumar *et al.* (2005). The real time (RT-)PCR assays for adeno- and noroviruses and the quantification using plasmid DNA standards have been described elsewhere (Kauppinen *et al.* 2012; Kauppinen *et al.* manuscript in preparation). For rotavirus, the real time RT-PCR reaction mix was the same as for noroviruses, and the real time RT-PCR amplification was performed according to Jothikumar *et al.* (2009).

The infectivity of adenoviruses was assessed by integrated cell culture PCR (ICC-PCR). For the ICC-PCR assay, 293 cells originated from human kidney (ATCC CRL-1573) were prepared and inoculated in 75 cm^2 cell culture flasks as earlier described by Mautner (1998). Briefly, the PIX and PAX flocculated and PAA disinfected wastewater samples were diluted (1:2) in Dulbecco's modified Eagle medium supplemented with 0.5% (v/v) of fetal bovine serum, l-glutamine (4 mM) and penicillin-streptomycin (100 U/mL and 100 μg/mL, respectively). Inocula were allowed to adsorb into cell monolayers for 60 min at 37 °C and 5% CO_2 with rocking every 15 min following the addition of medium. The inoculated cells were harvested after incubation for 5 days. Viruses were released by three freeze-thaw cycles and collected by centrifugation at 1,500 g for 2 min. The viruses were precipitated from the supernatant by the addition of 9% (w/v) polyethylene glycol (PEG) 6000 and 1 M NaCl and incubated with agitation overnight at 4 °C. The precipitate was recovered by centrifugation at 10,000 ***g*** for 30 min at 4 °C and suspended in PBS. The concentrated sample was used for viral DNA isolation and quantitative PCR as described above. The $\log_{10}$ growth values were determined for the inoculated samples.

Physiochemical methods

Chemical oxygen demand (COD) was measured using a HACH DR/2010 spectrophotometer according to the manufacturer's instructions. Total-P was determined from peroxy disulfate digestion (SFS 3026 1986). Biochemical oxygen demand (BOD) analysis was performed by using the Oxitop Control system (WTW, Weilheim, Germany) according to the manufacturer's instructions. NH_4-N was analysed using HACH (HACH LANGE, DR 2800) with Nessler method.

Data analysis

The microbial indicators were analysed as three replicates. In the 30 L flocculation test, adenoviruses were analysed in two replicates from a pooled sample. The data were counted with Microsoft Excel 2003 and transformed to IBM SPSS 19. Basic statistical analysis was performed before the appropriate test. Data were analysed using one-way analysis of variance (ANOVA) post-hoc and Kruskal–Wallis test. If there was a microbial number 'zero' (less than the detection limit) in some parallel assays, a value of 0.5 was used for counting geometric mean or log-value.

RESULTS

The wastewater effluent still contained high amounts of different microorganisms as can be seen in Tables 1–3. Coagulation and flocculation with PIX and the disinfection with PAA improved the quality of both wastewater influent and effluent so that significant ($P < 0.05$) microbial reductions could be achieved (Table 1). PAA disinfection was more efficient against the effluent than the influent. DAF was not operating well as shown in the microbiological results and COD, BOD, total-P and NH_4-N of effluents with (Table 2) or without DAF treatment (Table 1) were very similar. However, PAA disinfection was effective (>1 $\log_{10}$) against all the indicator microorganisms, except against spores of clostridia (Table 2). This result is similar to those presented in Table 1, where the PAA concentration was higher than in the experiment presented in Table 2. With respect to the pathogenic microorganisms, PAA also reduced the presence of *Salmonella*, *Legionella*, *Campylobacter* and norovirus GI but not rotavirus, adenovirus and norovirus GII (Table 2).

PIX and PAX flocculation clearly reduced the numbers of enterococci and clostridia in comparison to other indicator microorganisms and also PIX-PAA (PAA

Table 2 | Reduction of microbes in wastewater dissolved air flotation disinfected by 3 mg/L PAA. Microbial counts are presented as $\log_{10}$ CFU/100 mL or PFU/100 mL for indicators as geometric means ± standard deviations (N = 6). Variation between two parallel experiment dates for pathogenic microbes are indicated by a dash sign (–). LDL= less than detection limit (detection limit = 1 CFU/mL or 1 GC/mL), ND = not detected, NA = not analysed, GI = genogroup I, GII = genogroup-II, GC = genome copies. Water temperature was 21 ± 1 °C

	Dissolved air flotation treated effluent	After PAA 3 mg/L	Reduction %
Somatic coliphage	3.5 ± 0.72	1.2 ± 1.41	98.3
F-specific RNA coliphage	2.9 ± 0.39	2.1 ± 1.69	83.7
E. coli	5.4 ± 0.19	3.3 ± 0.65	99.2
Enterococci	4.1 ± 0.84	1.5 ± 1.73	99.7
Spore of SRC	3.4 ± 0.02	1.6 ± 1.67	96.5
Salmonella/25 mL	Positive	Negative	
Legionella (CFU/L) and their types	20,000 (*L. pneumophila* serogroups 1)	1,100 (*L. pneumophila* serogroups 1 and 2–15)	94.5
Thermotolerant *Campylobacter* species CFU/volume	*C. jejuni* more than 100/L – *C. jejuni* more than 1/200 mL	ND/200 mL – ND/1L	
Norovirus GI	LDL – positive/mL	LDL–LDL	
Norovirus GII	25–4 GC/mL	48–4 GC/mL	
Rotavirus	Positive/mL – ND/mL	Positive/mL – ND/mL	
Adenovirus	Positive/mL – positive/mL	Positive/mL – positive/mL	
COD mg/L	35.5 ± 7.8	NA	
BOD mg/L	8.8 ± 7.4	NA	
Total-P mg/L	0.4 ± 0.3	NA	
NH_4-N mg/L	16.05 ± 19.7	NA	

Table 3 | Microbial reduction in wastewater effluent flocculated with coagulants PIX-111 and PAX-18, and followed by 3 mg/L PAA disinfectant. *Adenoviruses were spiked to the effluent. Log_{10} CFU/100 mL and PFU/100 mL except noroviruses and adenoviruses are log_{10} genome copies/mL and *Salmonella* is determined for 25 mL. Geometric means ± standard deviations (N = 2–3). LDL = less than detection limit (detection limit = 1 CFU/mL or 1 GC/mL). Water temperature was 11 ± 1 °C

		PIX		PAX	
	Effluent	After flocculation	After PAA	After flocculation	After PAA
Heterotrophic bacteria	8.4	7.2	5.6	7.7	5.7
Somatic coliphage	3.9	3.1 ± 0.63	2.6 ± 0.04	3.1 ± 0.38	2.4 ± 0.03
F-specific RNA coliphage	3.4	1.9 ± 0.34	1.2 ± 0.31	1.1 ± 0.83	0.8 ± 0.9
E. coli	5.0	4.5 ± 0.03	2.3 ± 0.07	3.2 ± 0.71	2.1 ± 0.16
Enterococci	4.0	2.4 ± 0.25	1.5 ± 0.04	2.0	1.5 ± 0.18
Spore of SRC	3.0	LDL	LDL	LDL	LDL
Salmonella/25 mL	LDL	LDL	LDL	LDL	LDL
Legionella	4.0	2.7	2.7	1.7	1.7
Thermotolerant *Campylobacter*	LDL	LDL	LDL	LDL	LDL
Norovirus GI, GC/mL	1.8	1.0	LDL	LDL	LDL
Norovirus GII, GC/mL	1.8	1.5	LDL	LDL	LDL
Rotavirus	Positive	Positive	Positive	Positive	Positive
Adenovirus* GC/mL	8.5 ± 0.79	6.6 ± 0.63	6.4 ± 0.57	6.4 ± 0.57	6.4 ± 0.53
COD mg/L	38 ± 11.3	24.5 ± 3.5	NA	19 ± 12.7	NA
pH	6.4	6.5	NA	6.3	NA

disinfection after PIX flocculation) significantly reduced the numbers of *E. coli* compared to other indicator microorganisms; however, other indicator microorganisms were also reduced by more than 75%. PAX coagulant achieved a slightly better ($P > 0.05$) reduction of the levels of indicator microorganisms when compared to the reduction achieved by the PIX coagulant (Table 3). Although PAX flocculation achieved a better reduction in the numbers of indicator microorganisms and some of the pathogenic microorganisms compared to PIX flocculation, these reductions did not differ statistically significantly from each other ($P > 0.05$) (Figure 1 and Table 3). PIX-PAA showed a better reducing effect on *E. coli* ($P = 0.34$) and enterococci ($P = 0.05$) when compared to PAX-PAA, but this latter

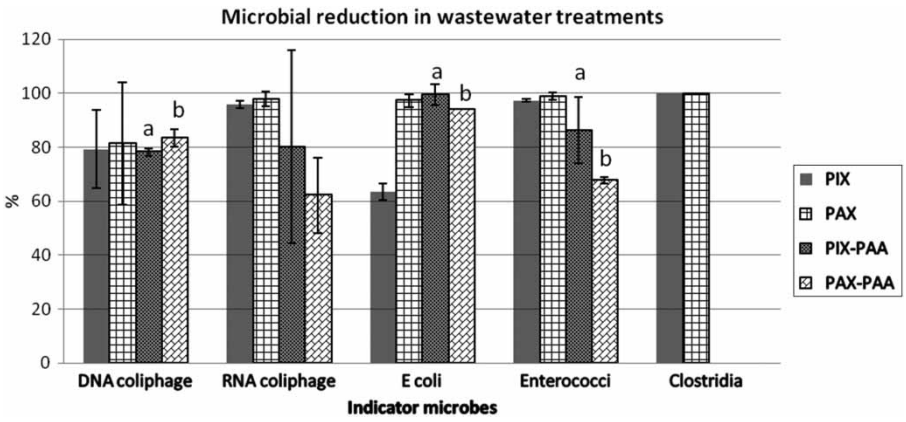

Figure 1 | Microbial reduction in wastewater effluent flocculated by PIX and PAX, and subsequent 3 mg/L PAA disinfection. The reduction of clostridia after PAX and PAA could not be determined since they were less than the detection limit (LDL) already after flocculation ($N = 3$). The letters a and b above the same microbe indicate a statistically significantly different result (Kruskal–Wallis test).

combination achieved a greater reduction of somatic coliphage ($P = 0.046$).

The low number of noroviruses in effluent hampered the precise comparison of two flocculants since these viruses were reduced to the level of less than the detection limit. However, adenovirus seemed to be resistant against PAA disinfection (Table 3) as confirmed by ICC-PCR comparing the $\log_{10}$ growth values between the flocculated and the PAA disinfected samples. The viral multiplication was in the PAX flocculated and the PAX-PAA treated samples 2.6 and 2.3 logs, respectively. Similarly, in PIX flocculated and PIX-PAA treated samples the growth was 1.6 and 1.5 logs, respectively.

DISCUSSION

The WWTP effluent can still contain rather high microbial numbers (Table 1) and some pathogenic microorganisms can also grow in wastewater effluent (Vital *et al.* 2010) increasing the health risks. There can be extensive variation in the numbers of pathogenic microorganisms and the diversity of pathogenic microorganisms as found in the effluents in this work. In addition, at least the adenoviruses remained infective even after the PAA-treatment as shown by the ICC-PCR technique and only small differences in the number of adenoviruses were observed between the flocculated and disinfected samples.

If there is a need to reduce the numbers of enteric microorganisms in WWT effluents before discharge into surface waters, then a tertiary treatment is necessary. Our results proved that flocculation was a good way to improve the wastewater quality. According to these results, both iron (PIX) and aluminium (PAX) could be used for tertiary WWT in spite of the fact that in a drinking water study aluminium flocculation achieved a better microbial reduction and better COD reduction (Fiksdal & Leiknes 2006) or a better reduction of MS2 and poliovirus (Bell *et al.* 2000) as compared to iron. In our flocculation tests, the floc was separated efficiently by both coagulants and thus the COD content was reduced. This reduction in the amount of organic matter (higher COD and SS) may have reduced the consumption of PAA in our disinfection studies (Table 1). Thus PAA could have been more efficient in destroying microorganisms present in the wastewater effluent (Lazarova *et al.* 1998). If both these coagulants can be used, PIX containing Fe^{3+} may pose a lower environmental risk compared to PAX as iron is a more essential biochemical than aluminium. We also noticed that PIX flocculation achieved a better percentage reduction in the spiking experiment (Table 3) compared to the first experiment (Table 1) possibly due to the fact that the first experiment was done with 7.1 g Fe/L, while the last experiment was conducted using 8.2 g Fe/L; also the first experiment was performed in a 3 L beaker and the last one in a 30 L tank. It is possible that microorganisms in our work easily become attached with SS during floc formation. Therefore, PIX flocculation followed by PAA disinfection seems to be very efficient for use in tertiary treatment of municipal wastewater.

After reducing the organic matter, the disinfection with PAA was successful as found by Gehr *et al.* (2003) and Dell'Erba *et al.* (2004). In these studies, the effect of PAA against non-sporing bacteria tended to be good, but sporing clostridia seemed to be more resistant against PAA and they were even more resistant in flocculated influent compared to flocculated effluent (Tables 1 and 2). Similarly, campylobacteria as non-sporing bacteria were sensitive to PAA (Table 2), confirming previous reports that PAA was effective at reducing campylobacteria on a poultry farm (Bauermeister *et al.* 2008). Furthermore, F-specific coliphage and clostridia showed more resistance against PAA when compared to other indicator microbes as also reported previously (Gehr *et al.* 2003; Dell'Erba *et al.* 2004). However, PAA did not seem to have any major effect on the concentrations of norovirus GII, rotavirus and adenovirus (Table 2), but both flocculants (before PAA disinfection) reduced the number of adenoviruses and both norovirus genotypes.

E. coli is still a relatively good indicator for pathogenic microorganisms and also F-specific coliphages indicate rather well the presence of human viruses. Thus both coagulants and PAA disinfection had similar reducing effects on most of these microbial groups. In this work with fresh WW, *E. coli* and enterococci behaved rather similarly (Tables 1–3) so that if there was a shortage of analytical capacity, then the determination of enterococci could have been omitted. After flocculation and especially after 5 mg/L or 3 mg/L PAA, the wastewater effluent would have fulfilled the guidelines and been regarded as excellent or good inland

bathing water according to the EU Bathing Water Directive (EC 2006), since it contained less than 200 cfu/100 mL intestinal enterococci and less than 500 cfu/100 mL *E. coli* (Tables 1 and 3).

The relatively high numbers of *Legionella* in municipal wastewater effluent (Tables 2 and 3) will have to be studied in the future since this bacterial group can spread into the air and cause serious illnesses, even death in humans. This bacterium has often been found in industrial wastewaters where it can grow in aeration tanks (Kusnetsov *et al.* 2010), but there is also a previous report (Huang *et al.* 2009) indicating that it may thrive in municipal wastewater aeration basins and then spread with the effluent into the environment. Nonetheless in the present work, the numbers of *Legionella* were clearly less than in industrial wastewaters (Kusnetsov *et al.* 2010). According to these results, the WWTPs should study the possibility that there can be growth of *Legionella* bacterium in their aeration tanks and evaluate whether workers should always use protecting masks or whether the aeration tanks should be covered so that *Legionella* and other pathogenic microorganisms do not spread via bioaerosols into the work environment and neighbouring areas.

In conclusion, PIX and PAX flocculation followed by PAA disinfection reduced large numbers of enteric and pathogenic microbes from wastewater effluent confirming the study hypothesis. Subsequently, these WWT processes can be suggested as good options for tertiary wastewater treatment. If better reductions of these microorganisms are needed, then it is recommended that higher doses of PAA, or a longer contact time, should be used.

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