Effects of phosphorus on biofilm disinfections in model drinking water distribution systems

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ABSTRACT

Drinking water biofilm development is affected by the available nutrient levels and the presence of disinfectants. Phosphorus is recognized as another important limiting nutrient besides organic carbon. In this study, drinking water biofilms were developed in annular reactors to examine the effects of phosphorus on the biofilm disinfections with free chlorine and monochloramine. Phosphorus addition was found to increase the biofilm cell number but decrease the exopolysaccharides (EPS) production. The disinfection efficacies of both free chlorine and monochloramine were increased when phosphorus was added into the reactor systems. At the same disinfection dosages, monochloramine showed greater biofilm removal efficiency than free chlorine. Monochloramine could be a better choice than free chlorine in biofilm disinfection when phosphate-based corrosion inhibitors are applied.

Key words | biofilms, drinking water, free chlorine, monochloramine, phosphorus

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INTRODUCTION

Biofilms are micro-societies where a collection of microorganisms surrounded by the exopolysaccharides (EPS) they secrete exists on the two phases interface. There is now plenty of evidence to convince us that in natural, industrial and medical habitats most bacteria are to be found colonizing surfaces in organized biofilm communities, rather than growing in suspension as individuals (Stickler 1999). The issue of biofilms has become a concern because they can be formed and be a refuge for the bacteria even in the oligo-nutrient condition of drinking water distribution systems. It has been often suggested that cells growing on the inside walls of water tanks and pipes, as biofilms, play an important role in the bacterial growth phenomenon in drinking water distribution systems (DWDS) (Characklis 1973; Donlan & Pipes 1986).

Biofilms are the source of the excess of free-floating bacteria in drinking water, some of which can cause infection and diseases in human beings. One common biofilm bacterium, *Pseudomonas aeruginosa*, is an opportunistic human pathogen which can infect damaged tissues

or people with reduced immunity with potentially fatal results if such infections occur in critical body organs such as the lungs, the urinary tract and the kidneys (Baltch & Smith 1994). Besides, biofilms can also cause corrosion in stainless steel piping systems. Biofilm control is becoming recognized as an important aspect of the operation of drinking water treatment plants and distribution systems (Gagnon *et al.* 2004). Chemical disinfectants are the most traditional and effective methods for control of biofilm microorganisms in DWDS. Traditional chlorine-based agents showed great ability to control microorganisms, and are most widely used in DWDS because they are efficient, economical and convenient (Stringer & Johnston 2001). The residual chlorine is generally effective to prevent the regrowth of the bacteria in the distribution system. However, biofilms are known to be much more disinfectant-resistant than their planktonic counterparts. Research has shown that biofilm bacteria grown on different surfaces increase resistance to different chlorine-based agents (LeChevallier *et al.* 1988).

In a survey conducted by Hu *et al.* (2005), a high biofilm growth potential in a drinking water distribution system was found even with the presence of residual chlorine. Monochloramine has become a competitive reagent in recent years because of increasing concerns about trihalo-methanes (THMs) and biofilms. Owing to its lower reactivity, the reaction potential between monochloramine and organic matters decreases, and the problem of THMs is eliminated. It has been suggested that monochloramine is better at penetrating the biofilms than free chlorine, probably because there is less reaction with the biofilm matrix, resulting in better inactivation of bacteria in the biofilms (LeChevallier *et al.* 1988). Moreover, compared with free chlorine, monochloramine is much more stable and does not dissipate from the water before it reaches the consumers. So monochloramine is often used in water distribution systems when free chlorine residuals are difficult to maintain, especially in large distribution systems where water residence time is extended (Vikesland *et al.* 2001).

Nutrients such as carbon, nitrogen and phosphorus are essential to microbial growth. Conventionally, organic carbon is thought to be the only limiting nutrient in DWDS. Recently, phosphorus has been suggested as another limiting nutrient to microbial growth in DWDS (Miettinen *et al.* 1997; Sathasivan & Ohgaki 1999). As phosphate is frequently introduced into DWDS as a corrosion inhibitor causing an increase in phosphorus concentration, the biological effects of phosphorus addition in DWDS need to be considered. Miettinen *et al.* (1997) and Sathasivan & Ohgaki (1999) suggested keeping the phosphorus level as low as possible or not introducing phosphorus to DWDS at all in order to maintain the biological stability of drinking water. Dosages from $1\,\mu g\,l^{-1}$ to $400\,\mu g\,l^{-1}$ of phosphorus have been proved to increase microbial growth in water and biofilm in DWDS (Lehtola *et al.* 2002; Chu *et al.* 2005; Hozalski *et al.* 2005). Besides the effects on microbial cell growth, there is evidence that the level of phosphorus could affect the EPS production (Hoa *et al.* 2003). Our preliminary study (Fang *et al.* 2009) has suggested that increased phosphorus level can affect not only the biofilm bacterial cell growth, but also the biofilm EPS production. The production of EPS is essential for the biofilm formation as EPS serve as the main 'cement' for the cells and cell products and EPS bind water, trap nutrients

and protect cells (Sutherland 2001). EPS production has been proposed as an antimicrobial resistance strategy of biofilm bacteria (Mah & O'Toole 2001). The protective effects of EPS against different stress conditions have also been demonstrated (Hatch & Schilier 1998; Chen *et al.* 2004). If EPS production could be affected by the addition of phosphorus, the biofilm matrix stability and disinfection resistance could also be affected. In this paper, we present the results of laboratory experiments that showed the effects of phosphorus on drinking water biofilm disinfection with free chlorine and monochloramine.

MATERIALS AND METHODS

Experimental system

Annular reactor systems (BioSurface Technologies Corporation, USA) were used to allow the development of biofilms on the surface. The annular reactor consists of two concentric cylinders: a stationary outer cylinder made of glass, and a rotating inner cylinder made of polycarbonate. The external surface of the internal cylinder was equipped with 20 removable polycarbonate slides for biofilm sampling. The reactor has a diameter of 30.5 cm and a height of 45 cm. The total liquid volume in each reactor is 1,000 ml and the hydraulic detention time was set to 3 h by controlling the influent feed rate. The rotational speed was set to 133 rpm throughout the experiment to achieve the simulating shear force under a velocity of $0.3\,m\,s^{-1}$ in 10 cm pipes. Two annular reactors were run in parallel in all the experiments for the purpose of comparison and one batch experiment of 39 days was run for each dosage of disinfectant test. Prior to the experiments, the reactor systems were washed with 70% alcohol followed by distilled water rinse and autoclaved at 121°C for 15 minutes.

Feed water

Tap water was stored for 24 h in a 60 l tank to allow the chlorine residue to decay to less than the detection limit ($0.01\,mg\,l^{-1}$ Cl_2). The dechlorinated tap water was pumped into the reactors with peristaltic pumps (Cole-Parmer Instrument Company model no. 7553-85, USA) at a flow rate of $5.5\,ml\,min^{-1}$. The characteristics of the feed water are listed in Table 1.

Table 1 | Characteristics of feed water to annular reactors ($N = 20$)

Water type parameters	Feed water (mean ± SD)
Heterotrophic plate count (HPC) (cfu ml^{-1})	45 ± 33
pH	7.75 ± 0.06
Temperature (°C)	28.5 ± 1.4
Free chlorine (mg l^{-1})	<0.01
Assimilable organic carbon (AOC) (µg l^{-1})	141 ± 12
NO$_3^-$-N (mg l^{-1})	0.59 ± 0.03
PO$_4^{3-}$-P (mg l^{-1})	<0.01

Nutrient addition

To achieve biofilm formation in a shorter experimental time and the phosphorus limiting condition, both reactors were enriched with carbon source (200 µg l^{-1} of C as sodium acetate). To compare with the control reactor, the treatment reactor was supplemented with 300 µg l^{-1} of P as K$_2$HPO$_4$ and KH$_2$PO$_4$ in 1:1 P molar ratio. The carbon and phosphorus solution was prepared in a 2l flask with deionized water and autoclaved at 121°C for 15 minutes. The nutrient stock solution was pumped into reactors at a flow rate of 0.1 ml min^{-1} throughout the experiment period.

Disinfectants preparation and application

In practice, the typical dosages vary from 0.2 to 2 mg l^{-1} for free chlorine and from 1 to 4 mg l^{-1} for monochloramine (USEPA 1999). In this study one low dose (0.5 mg l^{-1}) and one high dose (2 mg l^{-1}) were chosen to compare the disinfection efficacy of these two disinfectants. Free chlorine solution was prepared in 2l colorized bottles by NaOCl solution (available chlorine 5%) (Hayashi Pure Chemical Ind. Co. Ltd, Japan) and was diluted with distilled water. The two dosages of free chlorine examined were 0.5 and 2 mg l^{-1}, respectively. Free chlorine was measured by a Hach portable DR/890 colorimeter (Hach Company, Loveland, Colorado). Monochloramine solution was prepared in 1l colorized bottles by introducing 10 mg ml^{-1} NH$_4$Cl (Merck KgaA, Germany) into free chlorine solution to achieve a 1.5:1 molar ratio of NH$_4$Cl : NaOCl followed by slowly mixing for 1 h. Monochloramine was measured by a Hach DR/4000 spectrophotometer (Hach Company). The two dosages of monochloramine examined were 0.5

and 2 mg l^{-1}, respectively. The disinfections were applied to the reactors on the 21st day (the biofilm growth had reached a steady state) of the experiment and lasted for 18 days.

Enumeration of culturable cell number

Sample slides were rinsed with sterile MiliQ water. Biofilm samples were scraped from the slides into 50 ml autoclaved 0.9% sodium chloride solution by a sterilized rubber scraper followed by 1 minute ultrasonication (Cole-Parmer model 8891, USA). Sodium thiosulfate solution (30 g l^{-1}) was applied to the collected samples for dechlorination when chlorine residuals presented. Heterotrophic plate counts (HPCs) were obtained by spread plating appropriate dilutions of water samples on R2A medium (van der Linde *et al.* 1999). Duplicate plates were incubated at 30°C for 72 h before enumeration of colonies.

Measurement of EPS quantity

The total carbohydrate content (TCC) analysis or modified phenol-sulphuric acid method (Dubois *et al.* 1956) was used for measuring exopolysaccharides concentrations in the biofilm samples with glucose as the standard. In duplicate test tubes, 0.5 ml of phenol solution followed by 5 ml of sulfuric acid was added to 2 ml of the biofilm sample and vortex mixed. The bottles were allowed to stand for 10 minutes to react before placing them in a water bath at room temperature for 15 minutes. Then the absorbance at 490 nm was measured using a UV-visible recording spectrophotometer (Shimadzu, Japan) and the polysaccharide concentrations were obtained by comparing with a standard curve. The EPS quantity was expressed as the average polysaccharide concentration divided by the average cell count. Duplicate tests were performed for each sample.

Staining and microscopic examination of biofilm samples

The biofilms were observed with a LSM 5 PASCAL confocal laser scanning microscope (CLSM) system (Carl Zeiss, Germany) using probes as follows: bacteria were stained with the fluorescent nucleic acid stain SYTO 9

(SYTO 9 is available as part of the Live–Dead staining kit from Molecular Probes Inc., USA) (excitation = 488 nm). SYTO 9 was applied to the biofilm at a concentration of $20\,\mu g\,ml^{-1}$ at 23°C for 15 minutes prior to washing three times with sterilized distilled water. A lectin probe (*Triticum vulgaris*-TRITC (tetramethyl rhodamine isothiocyanate)) (excitation = 568 nm) was used to visualize exopolymer in biofilm communities. After drawing off the water from the biofilms carefully with a tissue, the staining solution with the lectin (Sigma Chemicals, USA) (0.1 mg lectin per ml) was applied with a $50\,\mu l$ pipette as a droplet to the biofilm and the sample was incubated at 23°C for 15 minutes. After staining, the sample was washed three times with sterilized distilled water. Then the sample slide was mounted to the microscope for observation.

Statistical analyses

Biofilm formation reached steady state after 12 days of experimental run in this study. All the statistical analyses were performed based on the data from the 12th day onward. *t*-tests were used to examine the effects of experimental factors (e.g. phosphorus addition and disinfections) on various biofilm parameters (e.g. cell count and EPS production). Differences were considered significant when $p < 0.05$. Statistical analyses were performed using Sigma-Plot version 10.0 (Systat Software Inc., USA).

## RESULTS

Biofilm development before disinfections

Figures 1–4 show the biofilm development in both control and phosphorus treatment reactors (from day 0 to 21). As can be seen, the biofilm growth reached steady state after 12 days of experimental run. The mean biofilm heterotrophic bacterial counts were $5.7 \times 10^4\,cfu\,cm^{-2}$ and $4.2 \times 10^5\,cfu\,cm^{-2}$ for control and phosphorus treatment conditions, respectively. Phosphorus addition significantly increased the biofilm cell number ($p < 0.05$). The mean biofilm EPS productions were $1.0 \times 10^{-4}\,\mu g\,glucose/(cfu\,cm^2)$ and $3.2 \times 10^{-6}\,\mu g\,glucose/(cfu\,cm^2)$ for control and phosphorus treatment conditions, respectively. Phosphorus addition significantly decreased the biofilm EPS production ($p < 0.05$).
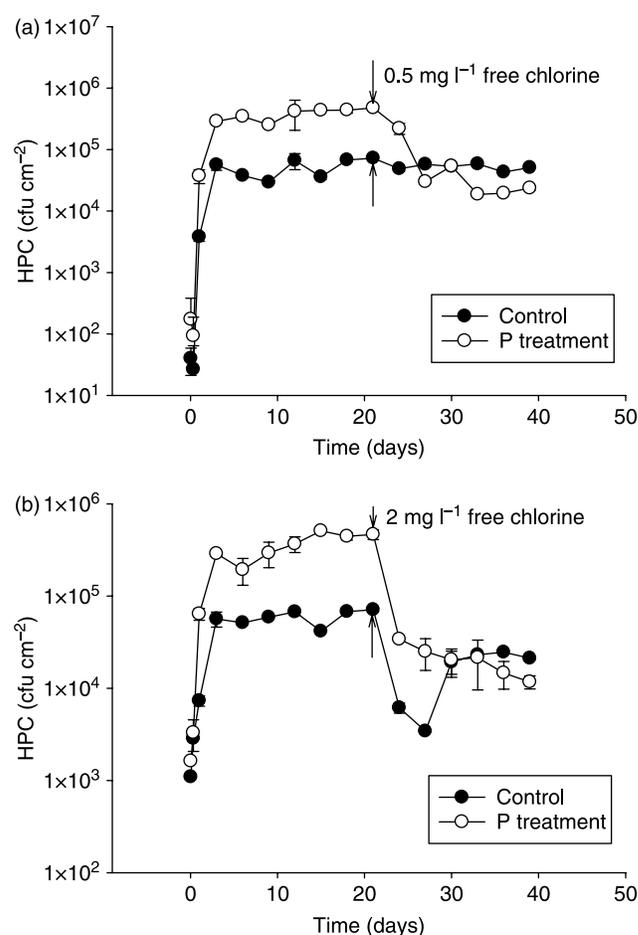
Figure 1 | Effects of phosphorus on biofilm cell number with free chlorine disinfection (a) $0.5\,mg\,l^{-1}$; (b) $2\,mg\,l^{-1}$.

Post free chlorine treatment

Figure 1 shows the biofilm cell counts after chlorine treatments (from day 24 to 39). Without phosphorus treatment (Figure 1(a)), $0.5\,mg\,l^{-1}$ free chlorine treatment caused a slight biofilm cell count decrease (mean biofilm HPC were $6.1 \times 10^4\,cfu\,cm^{-2}$ and $5.2 \times 10^4\,cfu\,cm^{-2}$ before and after chlorine treatment, respectively; no significant difference, $p > 0.2$). However, $0.5\,mg\,l^{-1}$ free chlorine treatment caused a significant decrease in biofilm cell count for the phosphorus treatment condition (mean biofilm HPC were $4.4 \times 10^5\,cfu\,cm^{-2}$ and $6.1 \times 10^4\,cfu\,cm^{-2}$ before and after chlorine treatment, respectively; significant difference, $p < 0.05$). The disinfection efficiency was improved for the control condition without phosphorus addition when chlorine dose increased to $2\,mg\,l^{-1}$ (Figure 1(b)) (mean biofilm
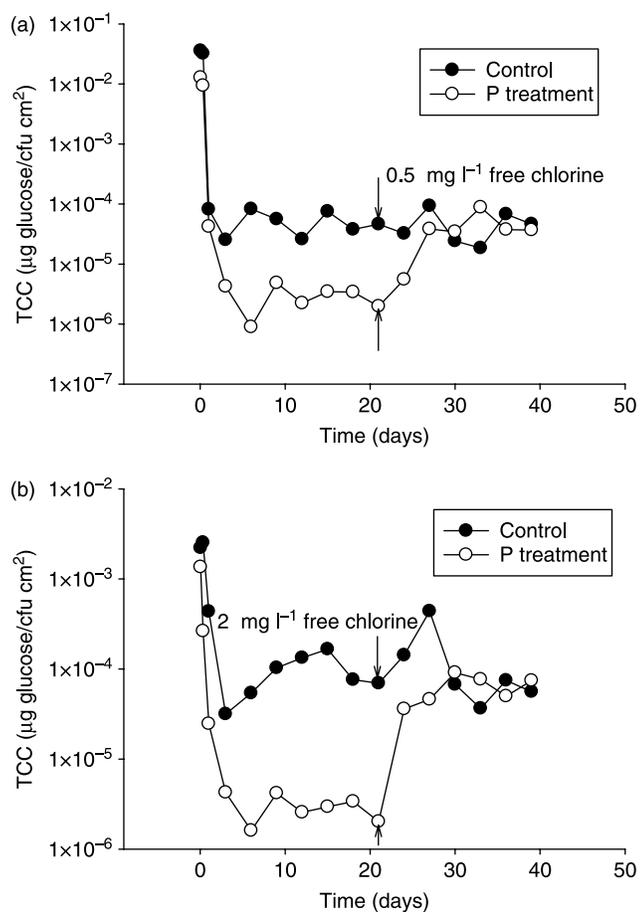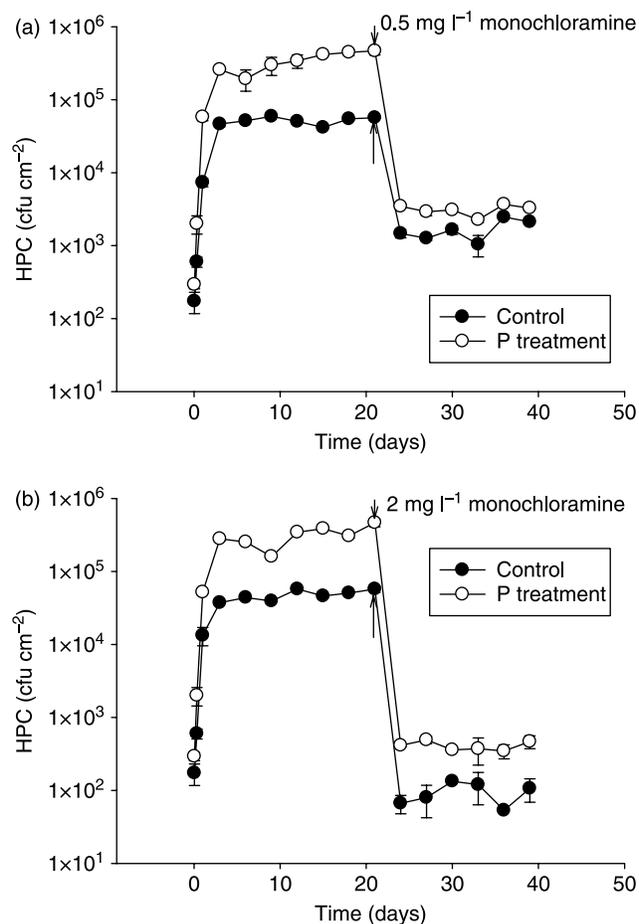
Figure 2 | Effects of phosphorus on biofilm EPS production with free chlorine disinfection (a) 0.5 mg l^{-1}; (b) 2 mg l^{-1}.

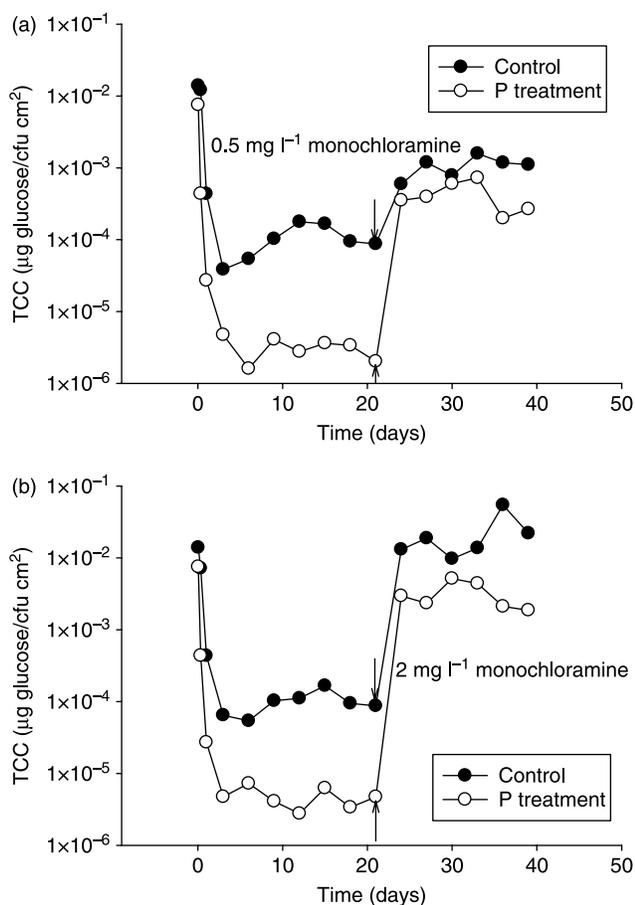HPC were 6.2×10^4 cfu cm^{-2} and 1.6×10^4 cfu cm^{-2} before and after chlorine treatment, respectively; significant difference, $p < 0.05$. The 2 mg l^{-1} free chlorine treatment also resulted in a significant decrease in biofilm cell count for the phosphorus treatment condition (mean biofilm HPC were 4.5×10^5 cfu cm^{-2} and 2.1×10^4 cfu cm^{-2} before and after chlorine treatment, respectively; significant difference, $p < 0.05$).

Figure 2 shows the biofilm EPS production after chlorine treatments. The 0.5 mg l^{-1} free chlorine treatment (Figure 2(a)) did not result in an obvious change in EPS production in control condition (mean biofilm EPS productions were 4.6×10^{-5} µg glucose/(cfu cm^2) and 4.7×10^{-5} µg glucose/(cfu cm^2) before and after chlorine treatment, respectively; no significant difference, $p > 0.9$).

However, 0.5 mg l^{-1} free chlorine treatment caused a significant increase in biofilm EPS production for the phosphorus treatment condition (mean biofilm EPS productions were 2.8×10^{-6} µg glucose/(cfu cm^2) and 4.0×10^{-5} µg glucose/(cfu cm^2) before and after chlorine treatment, respectively; significant difference, $p < 0.05$). In contrast to the effect on the biofilm cell count, 2 mg l^{-1} free chlorine treatment (Figure 2(b)) did not cause an obvious change in biofilm EPS production for the control condition (mean biofilm EPS productions were 1.1×10^{-4} µg glucose/(cfu cm^2) and 1.4×10^{-4} µg glucose/(cfu cm^2) before and after chlorine treatment, respectively; no significant difference, $p > 0.7$). For the phosphorus treatment condition, however, 2 mg l^{-1} free chlorine treatment resulted in a significant increase in biofilm EPS production

Figure 3 | Effects of phosphorus on biofilm cell number with monochloramine disinfection (a) 0.5 mg l^{-1}; (b) 2 mg l^{-1}.

Figure 4 | Effects of phosphorus on biofilm EPS production with monochloramine disinfection (a) $0.5\,\mathrm{mg\,l^{-1}}$; (b) $2\,\mathrm{mg\,l^{-1}}$.

(mean biofilm EPS productions were $2.7 \times 10^{-6}\,\mu\mathrm{g\,glucose/}$ $(\mathrm{cfu\,cm^2})$ and $6.2 \times 10^{-5}\,\mu\mathrm{g\,glucose/(cfu\,cm^2)}$ before and after chlorine treatment, respectively; significant difference, $p < 0.05$).

Post-monochloramine treatment

Figure 3 shows the biofilm cell counts after monochloramine treatments (from day 24 to 39). For the control condition (Figure 3(a)), $0.5\,\mathrm{mg\,l^{-1}}$ monochloramine treatment caused a significant biofilm cell count decrease (mean biofilm HPC were $5.1 \times 10^4\,\mathrm{cfu\,cm^{-2}}$ and $1.7 \times 10^3\,\mathrm{cfu\,cm^{-2}}$ before and after monochloramine treatment, respectively; significant difference, $p < 0.05$). However, $0.5\,\mathrm{mg\,l^{-1}}$ monochloramine treatment caused a dramatic decrease in biofilm cell count for the phosphorus treatment condition (mean biofilm HPC

were $4.2 \times 10^5\,\mathrm{cfu\,cm^{-2}}$ and $3.1 \times 10^3\,\mathrm{cfu\,cm^{-2}}$ before and after monochloramine treatment, respectively; significant difference, $p < 0.05$). The disinfection efficiencies were further improved for both control and phosphorus treatment conditions when monochloramine dose increased to $2\,\mathrm{mg\,l^{-1}}$ (Figure 3(b)). For the control condition, mean biofilm HPC were $5.3 \times 10^4\,\mathrm{cfu\,cm^{-2}}$ and $93\,\mathrm{cfu\,cm^{-2}}$ before and after monochloramine treatment, respectively (significant difference, $p < 0.05$). For the phosphorus treatment condition, mean biofilm HPC were $3.8 \times 10^5\,\mathrm{cfu\,cm^{-2}}$ and $4.1 \times 10^2\,\mathrm{cfu\,cm^{-2}}$ before and after monochloramine treatment, respectively (significant difference, $p < 0.05$).

Figure 4 shows the biofilm EPS production after monochloramine treatments. The $0.5\,\mathrm{mg\,l^{-1}}$ monochloramine treatments (Figure 4(a)) caused obvious increases in EPS production in both control and phosphorus conditions. For the control condition, mean biofilm EPS productions were $1.3 \times 10^{-4}\,\mu\mathrm{g\,glucose/(cfu\,cm^2)}$ and $1.1 \times 10^{-3}\,\mu\mathrm{g\,glucose/}$ $(\mathrm{cfu\,cm^2})$ before and after monochloramine treatment, respectively (significant difference, $p < 0.05$). For the phosphorus treatment condition, mean biofilm EPS productions were $2.9 \times 10^{-6}\,\mu\mathrm{g\,glucose/(cfu\,cm^2)}$ and $4.2 \times 10^{-4}\,\mu\mathrm{g\,glucose/(cfu\,cm^2)}$ before and after monochloramine treatment, respectively (significant difference, $p < 0.05$). Increasing the monochloramine dose from 0.5 to $2\,\mathrm{mg\,l^{-1}}$ further increased the biofilm EPS production (Figure 4(b)). For the control condition, mean biofilm EPS productions were $1.2 \times 10^{-4}\,\mu\mathrm{g\,glucose/(cfu\,cm^2)}$ and $2.2 \times 10^{-2}\,\mu\mathrm{g\,glucose/(cfu\,cm^2)}$ before and after monochloramine treatment, respectively (significant difference, $p < 0.05$). For the phosphorus treatment condition, mean biofilm EPS productions were $4.3 \times 10^{-6}\,\mu\mathrm{g\,glucose/(cfu\,cm^2)}$ and $3.1 \times 10^{-3}\,\mu\mathrm{g\,glucose/(cfu\,cm^2)}$ before and after monochloramine treatment, respectively (significant difference, $p < 0.05$).
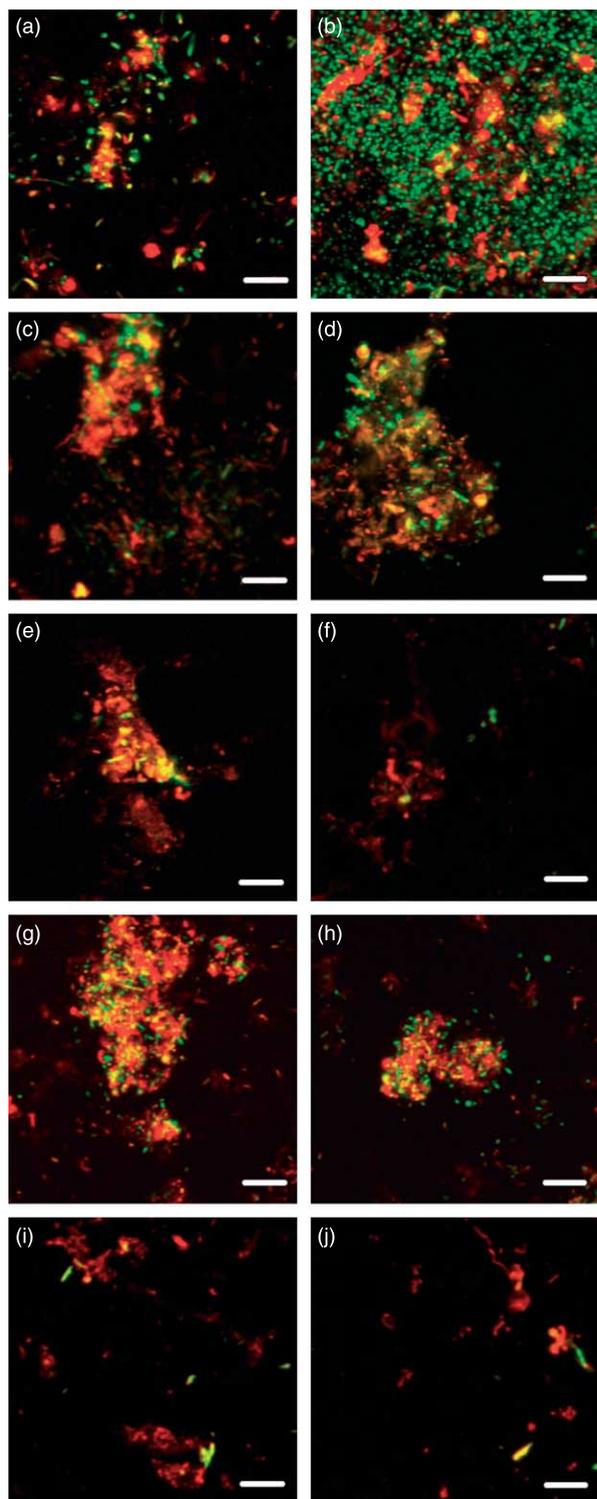
Biofilm morphology

Figure 5 shows the biofilm morphology changes from different disinfectant treatments. Figure 5(a) and (b) revealed the biofilm morphology on the 21st day before the application of disinfectants. Biofilm growth was found to be promoted by the addition of phosphorus. With the low doses of disinfectant treatments (Figure 5(c), (d), (g) and (h)), biofilm cell number reduced and the remaining cells

Figure 5 | Images from CLSM (green: cell; red: EPS). a, c, e, g and i are from control reactors; b, d, f, h and j are from phosphorus treatment reactors; a and b, 21st day biofilms before disinfection; c and d, 39th day biofilms with 0.5 mg l^{-1} free chlorine treatment; e and f, 39th day biofilms with 2 mg l^{-1} free chlorine treatment; g and h, 39th day biofilms with 0.5 mg l^{-1} monochloramine treatment; i and j, 39th day biofilms with 2 mg l^{-1} monochloramine treatment. The bar is 10 μm.

tended to congregate together and form cell colonies. When disinfectant dose increased to 2 mg l^{-1}, few cells with EPS protection remained on the surface.

DISCUSSION

The biofilm growth reached steady state in both control and phosphorus treatment reactors in 12 days, which is consistent with other biofilm studies (Camper 1996; Volk & LeChevallier 1999; Gagnon *et al.* 2004). Our results showed that phosphorus addition increased biofilm bacterial cell number, which agrees with other research work on the effects of phosphorus on biofilm growth in drinking water systems (Lehtola *et al.* 2002; Chu *et al.* 2005; Hozalski *et al.* 2005). Besides the positive effects on the biofilm cell number, we also found that the addition of phosphorus could decrease the biofilm EPS production. A similar phosphorus effect on EPS production was found in the sludge in wastewater treatment processes (Hoa *et al.* 2003). As EPS is an essential component in biofilm which maintains the structural stability of biofilm and provides protection for the entrapped cells, the decrease in EPS quantity could result in a less stable biofilm structure and the biofilm could be less resistant to disinfectants. Sutherland (2001) suggested that the amount of EPS synthesis within the biofilm depended greatly on the availability of carbon substrates (both inside and outside the cell) and on the balance between carbon and other limiting nutrients. The presence of excess available carbon substrate and limitations in other nutrients, such as nitrogen, potassium or phosphorus, promote the synthesis of EPS. So the phenomenon observed in our study may be attributed to the alteration of the nutrient condition to a more carbon-limited state by addition of phosphorus.

The internal corrosion of distribution pipes is one of the major problems faced by utilities. To prevent corrosion of

distribution pipes, phosphate-based products are normally added. Among these products, orthophosphate is a commonly used corrosion inhibitor in DWDS. McNeill & Edwards (2002) reported in their surveys of US drinking water utilities that more than half of those water suppliers surveyed reported adding phosphate inhibitors to their water. Due to the effects of phosphorus addition on biofilm development observed in this and other studies, care must be taken to maintain the drinking water in a biologically stable state when the phosphate-based corrosion control agents are applied to the DWDS. If biofilm overgrowth occurs in the distribution systems as a result of phosphorus addition, proper disinfection strategies should be applied or alternative corrosion inhibitors should be chosen.

Chlorination at low dosage ($0.5\,mg\,l^{-1}$) caused minor effects (0.07 log reduction) on the biofilm bacterial cell number in the control condition. However, better disinfection efficacy (0.58 log reduction) was achieved when chlorine dose increased to $2\,mg\,l^{-1}$. With phosphorus treatment, both 0.5 and $2\,mg\,l^{-1}$ free chlorine resulted in better disinfection efficacies (0.86 log reduction and 1.32 log reduction for 0.5 and $2\,mg\,l^{-1}$, respectively). Much better disinfection efficacies were, however, achieved with monochloramine treatments. Even $0.5\,mg\,l^{-1}$ monochloramine caused 1.48 and 2.13 log reductions for control and phosphorus treatment conditions, respectively. Log removals increased to 2.75 and 2.96 when monochloramine dose increased to $2\,mg\,l^{-1}$. Similar results were also found in other biofilm disinfection studies (Donlan *et al.* 2002; Turetgen 2004). These results confirmed that monochloramine could be a better disinfectant for biofilm compared with free chlorine (LeChevallier *et al.* 1988).

Our results also showed that the addition of phosphorus could further increase the disinfection efficacy. Except for the $2\,mg\,l^{-1}$ monochloramine treatment (a minor increase of disinfection efficacy with phosphorus treatment), disinfection efficacies with $0.5\,mg\,l^{-1}$ free chlorine, $2\,mg\,l^{-1}$ free chlorine and $0.5\,mg\,l^{-1}$ monochloramine were found to increase substantially (12.3-, 2.3- and 1.4-fold increases in disinfection efficacy for $0.5\,mg\,l^{-1}$ free chlorine, $2\,mg\,l^{-1}$ free chlorine and $0.5\,mg\,l^{-1}$ monochloramine, respectively). These results may be attributed to the reduction in EPS production resulting from the phosphorus treatment.

EPS productions were promoted when the disinfectants were applied except for chlorination in control conditions. Chlorine is known for its reaction with organic and inorganic matter and the production of biofilm EPS can therefore affect the activity of chlorine (Campanac *et al.* 2002). A thick biofilm may affect the degree of penetration of free chlorine (Turetgen 2004). In our study, biofilm EPS production was not found when free chlorine was applied to the control reactor in which more EPS was available. As free chlorine is not good at penetrating the biofilm matrix, with a relatively high EPS quantity in control conditions, the biofilm bacteria may not need to synthesize more EPS to protect themselves. However, for phosphorus treatment conditions, much less EPS are available to form the disinfectant barrier. So the biofilm bacteria may be forced to generate more EPS when free chlorine is applied as the disinfectant. When monochloramine was applied, because of its better penetration of the biofilm matrix, biofilms from both control and phosphorus treatment conditions tended to produce more EPS to form a stronger protection barrier. EPS production further implied that monochloramine could be a better biofilm disinfectant compared with free chlorine.

Along with the response in EPS production to the disinfections, biofilm structure changes may also be a strategy against disinfectant attack. After the application of the disinfectants, biofilms tended to form larger cell colonies with EPS protection in which denser biofilm communities may be able to generate a greater diffusion barrier to protect the cells from the disinfectant penetration.

CONCLUSIONS

Phosphorus addition to drinking water could increase the biofilm bacterial cell number but decrease the EPS quantity at the same time, which implies that a less biologically stable condition could be promoted by phosphorus addition. At the dosages studied, monochloramine was found to result in better biofilm disinfection than free chlorine. Monochloramine could be better than free chlorine in drinking water biofilm disinfection, when phosphorus was added as the corrosion inhibitor.

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