Biofiltration optimization: phosphorus supplementation effects on disinfection byproduct formation potential

Bahman Banihashemi, Robert Delatolla, Susan Springthorpe, Erin Gorman, Andy Campbell, Onita D. Basu and Ian P. Douglas

ABSTRACT

This study investigates the effects of phosphorus supplementation on the formation potential of total trihalomethanes (TTHMfp) and five species of haloacetic acids (HAA5fp) during exposure to clearwell disinfection contact times. In addition, the study investigates the effects of phosphorus supplementation on the dissolved oxygen, organic carbon and nitrogen removal along with biofilm coverage of the filter media and biomass viability of the attached biofilm. The uptake of total phosphorus in the P enhanced filter did not correspond to the consumption of readily assimilated nitrogen or the consumption of soluble carbon. As such, the dissolved organic carbon reduction in the biologically active filters was shown to not be phosphorus nutrient limited. The clearwell TTHMfp was shown to be reduced in all filters across all measured biological filtration times in the control and P enhanced filters. The HAA5fp increased with phosphorus-supplemented operation at specific filtration cycle times as compared to non-phosphorus-supplemented operation, indicating the potential for production of HAA5 with phosphorus supplementation. Enhanced biofilm coverage of the anthracite and sand media was observed during phosphorus supplementation. In addition, increased viability of the cells embedded in the biofilm was observed in the sand media at depth during phosphorus-supplemented operation.

Key words | biofilm, biofiltration, cell viability, disinfection byproducts, phosphorus supplementation

INTRODUCTION

Biofiltration for stable drinking water production has been shown to effectively meet particle removal goals, while simultaneously removing a wide range of dissolved organic and inorganic contaminants mostly by microbial communities that metabolize these chemicals (Zhu et al. 2010). Natural organic matter (NOM) occurring in drinking water sources is a known precursor for disinfection byproduct (DBP) formation. NOM consists mainly of microorganisms and complex organic matter, as well as naturally occurring degradation products (Gopal et al. 2007). It has been shown that bacteria and fungi can degrade components of dissolved organic matter (DOM), which is considered to constitute the majority of NOM and DBP precursors (Hur et al. 2013), in addition to degrading DBPs once formed (McRae et al. 2004). Microorganisms in source water and potable water are also thought to play a role in the formation of DBPs during disinfection processes (Wang et al. 2012; Wang et al. 2015), with these microbes having the potential to be filtered through the biofiltration process. Thus, biofiltration has been shown to not only enhance the
production of biologically stable waters but also to promote the degradation of DOM and DBP precursors (Lou et al. 2009; Liao et al. 2015). Biofilter technology can therefore help to resolve the dilemma of microbial control through chlorination and the formation of harmful byproducts (Badawy et al. 2012; Richardson & Postigo 2012). However, DBP formation is a complex process that involves various chemical and biological pathways which may overlap with DBP removal mechanisms. Thus, it is often difficult to quantify the relative contribution of various mechanisms to DBP formation or destruction. As such, the disinfection byproduct formation potential (DBPfp) is commonly used to predict the potential DBPs that might form under various conditions in place of a direct measurement of the DBPs that are in the water at that specific location in a treatment process.

Due to the direct correlation of total trihalomethanes (TTHM) and five species of haloacetic acids (HAA5) with higher incidences of cancer as well as adverse reproductive effects (Porter et al. 2005), more stringent regulations have been introduced (Richardson & Postigo 2012). The United States Environmental Protection Agency (USEPA) has posed the Stage 2 Disinfectants and Disinfection Byproducts Rule (DBPR2) and has set maximum contaminant levels (MCLs) for TTHM and HAA5 at 0.080 and 0.060 mg/L, respectively (USEPA 2010). The European Union has established a maximum allowable concentration (MAC) for TTHM of 0.100 mg/L and has yet to set the limit on HAA5 (European Commission 1998). To achieve these goals, drinking water production plants typically aim to limit the amount of disinfectant used within an optimal range and/or use upstream treatment units to decrease the concentration of NOM present in the system prior to disinfection.

Krasner (2009) studied various factors that affect NOM removal and found that the presence, variety and quantity of DBPs produced by drinking water production plants with biofiltration are influenced by the disinfectant used, and the source, composition and concentration of NOM, along with the microbes present and their state of activity. Similar results were also reported in the literature, indicating that the microbial community growing on the biofilter media (McDowall et al. 2009; Zhang et al. 2011) and the amount of essential nutrients in the biofilter system (Boon et al. 2011; Lauderdale et al. 2012) can influence biodegradation of organics and ultimately the production of DBPs. In particular, the microbial activity is believed to be affected by the access to nutrients, with a deficiency of a nutrient having the potential to limit the microbially-mediated degradation of NOM. A carbon:nitrogen:phosphorus (C:N:P) ratio of 21:5:1 by weight has been reported as being required for optimal bacterial growth; however this ratio is not typically attained in a biofilter influent (Liu et al. 2001). Some studies have shown phosphorus to be the limiting nutrient (Sang et al. 2003), with Lauderdale et al. (2012) observing a 15% decrease in head-loss and an increase in dissolved organic carbon (DOC) removal from 11% to 20% when increasing the phosphorus concentration from a C:N:P stoichiometric ratio of 100:10:1 to 100:10:2. Similar results indicating positive changes in biofilter performance by nutrient enhancement have also been reported (Sang et al. 2003; Li et al. 2010; Dhawan et al. 2017). Contrarily, studies have also observed no significant improvement in biofilter performance following phosphorus addition when phosphorus was a limiting factor (Vahala et al. 1998; Melin et al. 2002; McKie et al. 2015; Stoddart & Gagnon 2015).

This work endeavors to study the effects of phosphorus supplementation on the filtrate of a biofilter system in drinking water due to current contradictory results in literature about the impact of phosphorus on both organic carbon removal and DBP production (Miettinen et al. 1997; Lehtola et al. 2002; Yu et al. 2003; Lauderdale et al. 2012). The study was performed on two parallel dual media (anthracite/sand) pilot-scale biological filters located at the Britannia drinking water production plant in Ottawa, Ontario, Canada. In particular, the work studies the effects of phosphorus supplementation on the formation potential of total trihalomethanes (TTHMfp) and five species of haloacetic acids (HAA5fp) during the clearwell contact time of the Britannia drinking water production plant. In addition, the study investigates the effects of phosphorus supplementation on organic carbon, oxygen and nitrogen removal along with the microbial response using variable pressure scanning electron microscopy (VPSEM) analysis to measure biofilm coverage of the filter media and confocal laser scanning microscopy (CLSM) in combination with viability staining to analyze the embedded bacterial biomass viability.
METHODS

Water production pilot-plant and source water description

Two 10-in diameter pilot-scale biofilters were constructed at the Britannia drinking water production plant (Ottawa, Ontario, Canada) and operated as biofiltration units composed of anthracite and sand media (Figure 1). The pilot plant biofilters were operated in parallel to the full-scale plant. The Britannia production plant is fed with the water drawn from the Ottawa River and the production train includes flocculation (with aluminum sulfate (alum), pH adjustment and activated silica), sedimentation, biofiltration via dual-media (anthracite/sand) filters and disinfection (chlorine, pH correction and ammonia addition), where

![Figure 1](image-url)
chloramine is used as a secondary disinfectant and fluoride is added. The pilot biofilters were filled with media extracted from the full-scale biofilters and were configured to operate as the full-scale biofilters at the host site. The effluent from the full-scale sedimentation process was fed to the two pilot-scale biofilters by gravity. The influent water was saturated with oxygen; therefore, no air supply was used in the filters.

The average empty bed contact time (EBCT) of the pilot filters during the study was approximately 40 minutes. This EBCT is longer than conventional operation in the USA and Canada, however the EBCT was used for the study to simulate the longer filtration cycle conditions of the Britannia production plant. The longer filtration cycles and the lower filtration rates of the Britannia production plant are representative of water conservation efforts of the population served by the drinking water plant. The two pilot-scale filters used for this study are comprised of 0.64 m of anthracite overlaying 0.38 m of sand and 0.76 m of gravel. The filters were backwashed every 72 hours with the drinking plant service water supply (pressurized plant effluent). Backwash protocols of the pilot filters simulated that of the full-scale facility, such that, each backwash event included 2 minutes of fluidization at a low washrate of 16.6 m/h followed by 3 minutes of fluidization at a high washrate of 44.6 m/h and 7 minutes of filter wash at a flow rate of 2.4 m/h.

**Source water nutrient ratio**

The source water feeding the full-scale plant and hence the pilot filters had a C:N:P ratio of 100:32:0.2 (Table 1). An approximate C:N:P ratio of 100:10:1 is recommended for heterotrophic bacterial growth in biofilters (Redfield 1958), indicating that the source water was nitrogen sufficient and potentially phosphorus limited. To achieve a target C:N:P ratio of 100:10:1, a phosphorus dose of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Feed and effluent water characteristics (average ± standard deviation) (N – 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analyte</strong></td>
<td><strong>Control filter – feed</strong></td>
</tr>
<tr>
<td></td>
<td>t – 0</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>15.5 ± 0.7</td>
</tr>
<tr>
<td>NH$_4^+$ / NH$_3$ (mg-N L$^{-1}$)</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>NO$_3^-$ (mg-N/L)</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>TKN (mg-N/L)</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>TP (mg-P/L)</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>3.5 ± 0.2</td>
</tr>
</tbody>
</table>

| **Analyte** | **P enhanced filter – feed** | **P enhanced filter – effluent** |
|         | t – 0 | t – 24 h | t – 48 h | t – 71 h | t – 0 | t – 24 h | t – 48 h | t – 71 h |
| DO (mg/L) | 15.5 ± 0.7 | 16.4 ± 0.5 | 16.1 ± 1.3 | 16.6 ± 0.3 | 15.1 ± 0.9 | 16.1 ± 0.7 | 15.8 ± 1.4 | 16.2 ± 0.2 |
| NH$_4^+$ / NH$_3$ (mg-N L$^{-1}$) | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.01 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.01 |
| NO$_3^-$ (mg-N/L) | 0.18 ± 0.00 | 0.18 ± 0.00 | 0.19 ± 0.01 | 0.19 ± 0.01 | 0.18 ± 0.02 | 0.18 ± 0.02 | 0.17 ± 0.02 | 0.18 ± 0.00 |
| TKN (mg-N/L) | 0.19 ± 0.00 | 0.17 ± 0.00 | 0.16 ± 0.00 | 0.16 ± 0.00 | 0.15 ± 0.01 | 0.15 ± 0.01 | 0.15 ± 0.01 | 0.15 ± 0.02 |
| TP (mg-P/L) | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.01 ± 0.00 | ≤0.005 | ≤0.005 | ≤0.005 |
| TOC (mg/L) | 4.4 ± 0.3 | 4.3 ± 0.3 | 4.2 ± 0.3 | 4.5 ± 0.3 | 3.4 ± 0.2 | 3.7 ± 0.3 | 3.6 ± 0.3 | 3.8 ± 0.2 |
| DOC (mg/L) | 3.5 ± 0.2 | 3.8 ± 0.2 | 3.7 ± 0.2 | 4.0 ± 0.1 | 3.3 ± 0.2 | 3.7 ± 0.2 | 3.7 ± 0.2 | 3.9 ± 0.2 |

TNK: total Kjeldahl nitrogen.
0.02 mg-P/L was added to the feed of the first pilot biofilter (P enhanced filter). Phosphorus addition was added in the form of phosphoric acid. The second biofilter was monitored during the study as the non-supplemented filter (control filter). The P enhanced filter along with the control filter were operated for 40 days prior to the start of the experimental phase to allow for the filter microbial community to re-acclimatize to the feed water of the facility and to the feed water with added phosphorus (after being harvested from the full-scale filters and introduced into the pilot-scale filters).

**Experimental plan and analytical methods**

After 40 days of acclimation, feed and effluent samples were collected from the two pilot-scale biofilters after 0 (after backwashing of the filters), 24, 48 and 71 h of filtration, over the course of two biofiltration cycles in the two pilot-scale filters. The two filters were operated between January and April and were operated off cycle to allow the backwashing of the filters to occur on different days of the week. To minimize the effects of the feed variance on the data sets, effluent samples and filter media samples were collected one EBCT after the collection of the feed samples. Water samples were analyzed for dissolved oxygen (DO), total organic carbon (TOC), DOC, ammonia (NH$_4$/NH$_3$), nitrite (NO$_2$), nitrate (NO$_3$), and total phosphorus (TP). An Orion RDO probe and meter (Thermo Fisher Scientific, Walthan (MA), USA) was used for DO measurements, with sampled water being overflowed into the sampling beaker, and once the water was quiescent, the probe was used to measure the DO with limited air entrainment due to the sampling. TOC, DOC, NH$_4$/NH$_3$, NO$_2$, NO$_3$ and TP samples were collected and stored in the refrigerator at 4 °C and analyzed within 24 hours of sampling. Preserved, headspace-free, clean vessels were used for collection of TOC and DOC samples. TOC and DOC measurements were performed according to Standard Methods for the Examination of Water and Wastewater, method 5310 B (American Public Health Association (APHA) 1989), using an Apollo 9000 TOC/TN Analyzer (Teledyne Tekmar, Mason (OH), USA). DOC samples were first filtered using a 0.45 μm filter and then measured according to the same method as TOC. NH$_4$/NH$_3$, NO$_2$ and NO$_3$ were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA 1989), methods 4500-NH$_3$ C, 4500-NO$_2$ B and 4500-NO$_3$ B, respectively. TP was analyzed using the Ontario Ministry of the Environment (MOE) method RTNP-E3367 (February 12, 2007) acid digestion in combination with an auto-analyzer (Skalar, Breda, The Netherlands) (MOE 2010).

DBP$_{tp}$ (TTHM$_{tp}$ and HAA5$_{tp}$) samples were collected in preserved, headspace-free, clean vessels. The harvested samples were stored in the refrigerator at 4 °C and analyzed within 24 hours of sampling. The TTHM$_{tp}$ and HAA5$_{tp}$ of the clearwell at the Britannia production plant were analyzed using Standard Methods for the Examination of Water and Wastewater (APHA 1989), method 6251B, with a modified contact time of 1 hour. The modified chlorine contact time of one hour is shorter than contact times of DBP$_{tp}$ measurements used to simulate the formation potential in residual taps. The clearwell contact time was used in this study when measuring the DBP$_{tp}$ to directly correlate the results of the study to the operation of the plant (Delatolla et al. 2015). Hence, prior to DBP analysis all samples were filtered with a 0.45 μm filter and dosed with 1.5 mg/L of chlorine and stored in the dark for 1 hour. USEPA Method 524.4 (USEPA 2013) was used to measure TTHM. In particular, the TTHM samples were analyzed for chloroform, bromoform, dichlorobromomethane and dibromochloromethane. To verify the accuracy of the TTHM method, replicate samples were analyzed for each sample; with replication showing a relative standard deviation of 0.027. The group of HAA5 constituents measured includes chloroacetic acid, bromoacetic acid, dichloroacetic acid, trichloroacetic acid and bromochloroacetic acid. The HAA5 concentrations in samples were determined using the USEPA Method 552.2 (Munch et al. 1995) as a method of extraction and the MOE LaSB method OPHAA-E3583 (MOE 2010) as a method of analysis. The HAA5 samples were analyzed in replicates with a relative standard deviation of 0.015.

Ten anthracite and sand media samples (five samples from the control and five samples from the P enhanced biofilter) were collected during a sample run after 24 h of
operation. The media samples were analyzed using VPSEM and CLSM in combination with viability analysis to measure the biofilm coverage of the biofilter media and cell viability of the attached bacteria.

**Microscopic methods**

Images of biofilm attached to the filter media of the control and P enhanced filters were captured using VPSEM without pre-treatment to reduce the destructive effects of traditional pre-treatment procedures associated with conventional SEM. Media was harvested in 5 replicates from each filter after 24 h of filter run time with the harvested media being analyzed at a pressure of 40 Pa using a Vega II-XMU SEM (Tescan USA Inc., Cranberry (PA), USA) variable pressure scanning microscope. For each harvested media, 20 images were captured at random locations on the media at magnifications between ×1,000 and ×2,000. Vision Assistant 7.1 (National Instruments, LabView 8.0, Austin (TX), USA) was used to calculate the percent biofilm coverage of the media.

The quantification of the viable biomass embedded in the upper 5 μm of biofilm attached to the filter media of the two pilot filters after 24 h of filtration was investigated using CLSM combined with the Invitrogen FilmTracer LIVE/DEAD Biofilm Viability Kit (Life Technologies, Burlington (ON), Canada). STYO-9 green fluorescent nucleic acid ($\lambda_{\text{excitation}} = 482$ nm; $\lambda_{\text{emission}} = 500$ nm) was used to stain all embedded cells; while dead cells were stained with propidium iodide, red-fluorescent nucleic acid, ($\lambda_{\text{excitation}} = 490$ nm; $\lambda_{\text{emission}} = 635$ nm). Particularly, propidium iodide stains only cells that have damaged cell membranes. Therefore, live cells are presumed as those with intact cell membranes. Twenty images were captured on the surface of the media for each sample set at random locations using a ×63 water immersion lens and LSM-5 Pascal confocal microscopy (Carl Zeiss Canada Ltd., Toronto (ON), Canada) equipped with an argon laser (488 nm, 514 nm) and a HeNe laser (543 nm). The percent of live and dead cells in the biofilm was quantified from acquired confocal microscopy images using Vision Assistant 7.1 (National Instruments, LabView 8.0, Austin (TX), USA).

**Statistical analyses**

Statistically significant differences in all water quality parameters along with the TTHM$_{fp}$ and HAA5$_{fp}$ measurements were tested using a student $t$-test with $p$-values of less than 0.05 signifying statistical relevance between data collected from the control and P enhanced filters across various filtration times. The student $t$-test with $p$-values of less than 0.05 was also used to signify statistical differences in percent biofilm coverage of the filter media and percent live and dead cell coverage of the embedded bacterial biomass.

**RESULTS AND DISCUSSION**

**Constituent concentration**

Table 1 shows the average and standard deviation of feed and effluent samples measured from the control and P enhanced filters after 0, 24, 48 and 71 h of filtration. Two samples were collected and measured at each filtration time across two filtration cycles, for a total of four samples being analyzed for each filtration time. The concentration of DO showed no statistical difference between the feed and the effluent samples, which supports the small change in DOC observed across the filtration cycle. Further, the lack of significant DO change across the P enhanced filter indicates that phosphorus supplementation did not demonstrate a measurable increase in aerobic, cellular respiration.

No significant difference was observed between the feed and effluent concentrations of NH$_4$/$\text{NH}_3$ in both the control and P enhanced filters (Table 1). The concentration of NO$_3$ also did not demonstrate a significant change in concentration between the feed and the effluent samples; where the concentration of NO$_3$ ranges from 0.17 to 0.19 mg/L-N in the filter feed and 0.17 to 0.18 mg/L-N in the effluent from both filters (Table 1). In all samples analyzed, the concentrations of NO$_2$ were below the detection limits of the analytical methods, 5 μg NO$_2$-N/L.

The feed concentration of TP in the control filter was low as it ranged between below 0.005 to 0.01 mg-P/L. The feed concentration of TP in the P enhanced filter was in the range of 0.02 to 0.03 mg-P/L. This change in feed...
concentration was due to small variations in the influent pump and phosphorus addition pump. The effluent TP concentrations from both biofilters were below or close to the detection limits of the analytical methods used in this study of 5 μg-P/L. The observed significant change in the TP concentrations in the P enhanced filter demonstrates P consumption in the range of 0.03 to 0.01 mg-P/L in the P enhanced filter.

The TOC concentrations of the feed in the control biofilter ranged from 4.0 to 4.4 mg/L and the effluent ranged from 3.3 to 3.8 mg/L. The TOC concentrations of the feed in the biofilter with phosphorus addition ranged from 4.2 to 4.5 mg/L, with the effluent range being 3.4 to 3.8 mg/L (Table 1). The observed differences in feed concentrations between the two pilot filters was due to the two filters being operated off cycle to allow the backwashing of the filters to occur on different days of the week. The DOC concentrations of the influent in the control and P enhanced filters ranged from 3.5 to 4.0 mg/L and 3.5 to 4.0 mg/L, respectively (Table 1). DOC concentrations of the effluent ranged from 3.2 to 3.9 mg/L and 3.3 to 3.9 mg/L in the control and P enhanced filters, respectively (Table 1). Comparing the TOC and DOC removal across a complete filtration cycle (between backwashing events) demonstrates that shorter filtration times promote the highest removal of carbonaceous constituents and hence the largest reduction in TOC and DOC. This is in agreement with a previous study conducted on the full-scale biofilters of the Britannia drinking water production plant (Delatolla et al. 2015). The overall small reductions in TOC and DOC in the pilot filters observed in this study are likely related to the high quality source water, long filtration times and short chlorination contact times of the full-scale Britannia production plant, which were also observed by previous work performed at the Britannia facility (Delatolla et al. 2015). Finally, it should be noted that the increased uptake of TP in the P enhanced filter did not correspond to an increased consumption of readily assimilable nitrogen (NH₄⁺/NH₃ or NO₃⁻) or an increased consumption of soluble carbon (DOC). As such, the DOC reduction in the biologically active filters is shown not to be phosphorus nutrient limited.

**Disinfection byproduct formation potential (DBPfp)**

Figure 2 illustrates the correlation between filter effluent DBPfp (TTHMfp and HAA5fp) measured relative to the clearwell contact time of the operating facility and the effluent TOC and DOC concentrations measured across biofiltration cycle times of 0, 24, 48 and 71 hours. Previous studies have demonstrated the potential of NOM, as measured via TOC and/or DOC, to form DBPs in raw and produced drinking water (Gopal et al. 2007; McCormick et al. 2010; Richardson & Postigo 2012).

![Figure 2](https://iwaponline.com/wqrj/article-pdf/52/4/270/241008/wqrj0520270.pdf)
although direct correlations can be hard to establish (McKie et al. 2015; Zha et al. 2016). However, in this study, a weak correlation is observed between TOC concentration and TTHMfp ($R^2 = 0.31$), with no correlation being found between TOC concentrations and HAA5fp ($R^2 < 0.1$). Similar results are observed for DOC concentrations, where a weak correlation is observed between effluent DOC concentrations and TTHMfp ($R^2 = 0.21$), and no correlation was evident with respect to HAA5fp ($R^2 < 0.1$). These findings are likely influenced by the low measured DBPfp concentrations, which is a result of the high quality source water of the facility and the short clearwell chlorination contact time of the full-scale Britannia production plant clearwell. These low measured DBPfp concentrations are in agreement with a previous study on the full-scale biofilters at the Britannia production plant (Delatolla et al. 2015).

The effect of phosphorus addition on clearwell DBPfp was investigated by subtracting the feed TTHMfp and HAA5fp from the subsequently measured effluent TTHMfp and HAA5fp (referred to as the differences in TTHMfp and HAA5fp). The differences in TTHMfp and HAA5fp are shown in Figure 3, where positive differences in DBPfp indicate lower effluent formation potentials compared to the influent and, as such, negative differences indicate higher effluent DBPfp. The small magnitude differences in DBPfp shown in Figure 3 are a result of the high quality source water of the facility and the short chlorination contact time of the full-scale Britannia production plant clearwell. Although differences measured in this study are small due to the quality of the source water and the operation of the full-scale facility, the results are operationally meaningful as they demonstrate that the DBPfp of the biofilter effluent, measured with respect to the clearwell contact time of the operating facility, was reduced relative to the clearwell DBPfp of the feed in all samples analyzed for TTHMfp (Figure 3(a)). With respect to the HAA5fp, the results are again operationally meaningful as the effluent DBPfp of the control filter was shown to decrease across all filtration times. Hence, biofiltration demonstrated an overall reduction in the DBPfp in the control filters throughout the filtration cycle. However, while HAA5fp was decreased in samples harvested at filtration times of 0 h and 48 h in the P enhanced filter, negative HAA5fp values were observed in all samples harvested after 24 hours of filtration and just prior to the backwash event ($t = 71$ h). In particular, negative HAA5fp values were measured for all samples at filtration times of 24 h and 71 h, indicating the potential for production of HAA5 in the P enhanced filter that was not observed in the control filter.

The differences in TTHMfp and HAA5fp during the filtration cycle in both the control and P enhanced filter varied across the filtration cycle and did not
demonstrate a trend with filtration duration (Figure 3). In particular, the measured differences in the TTHM<sub>fp</sub> in the control filter were statistically similar to the differences in TTHM<sub>fp</sub> in the P enhanced filter. Hence, phosphorus supplementation of the dual media filter did not demonstrate a beneficial effect with respect to TOC, DOC or TTHM<sub>fp</sub> relative to the clearwell contact time. Comparing the control filter to the P enhanced filter shows that the measured differences in clearwell HAA5<sub>fp</sub> were statistically different in samples harvested after 24 hours of operation and just prior to the subsequent backwash event (t = 71 h). The samples harvested at 24 and 71 hours filter run time show negative HAA5<sub>fp</sub> values in the P enhanced filter, indicating an increase in the potential HAA5 production from the filter.

**Biofilm coverage and biomass viability**

Anthracite and sand filtration media were collected from both the control and P enhanced filters for biofilm and biomass analyses after 24 h of filter run time (t = 24 h). VPSEM images of the anthracite and sand media, harvested from the two filters, are shown in Figure 4 with white arrows showing areas of biofilm coverage. The VPSEM images were analyzed to quantify the average and 95% confidence intervals of the percent biofilm coverage of the media (Figure 5). The anthracite media shows a statistically significant difference in the percentage of biofilm coverage compared to the sand media after 24 h of operation in the two biofilters; 60.2 ± 4.3 and 69.0 ± 2.1% of the anthracite surface was covered with biofilm in the control and P enhanced filters, respectively, as

![VPSEM images of media at ×1000 magnification after 24 h of filtration. (a) Anthracite, control filter, (b) anthracite, P enhanced filter, (c) sand, control filter, (d) sand, P enhanced filter; arrows indicate areas of biofilm coverage.](https://iwaponline.com/wqrj/article-pdf/52/4/270/241008/wqrjc0520270.pdf)
compared to 44.2 ± 5.3 and 57.8 ± 5.5% of the sand media. Further, the addition of phosphorus to the P enhanced column showed a significant change in measured percent biofilm coverage of the anthracite and sand media after 24 h of operation as compared to the control filter. Thus, phosphorus supplementation increased the microbial biomass coverage of both the anthracite and sand media between 9–13%, as either extracellular polymeric substances (EPS) or attached cells.

CLSM in combination with viability staining was used to determine the cell viability of the upper 5 μm of the biofilm. Figure 6 shows the obtained images of live cells (green) and dead cells (red) after 24 hours of operation. The CLSM images were processed to quantify the average and 95% confidence intervals of the percent biomass coverage of the biofilm (Figure 7). The percentage of live and dead cell coverage of the biofilm attached to the anthracite media was statistically higher, compared to the sand media, in the control filter. With respect to the live cell coverage of the P enhanced filter however, this trend was reversed, with the live cell coverage of the sand media being statistically higher than the live cell coverage of the anthracite media. The dead cell coverage of the anthracite and sand media of the P enhanced column did not demonstrate a statistical difference. The percentage of live and dead cell coverage of the biofilm attached to anthracite media in the control filter as compared to the P enhanced filter did not demonstrate a statistical change at a filtration time of 24 hours (Figure 7(a)). In contrast, analysis of the sand media demonstrates that the percent of live cell coverage of the biofilm showed a statistically significant increase from 0.4 ± 0.1 to 1.4 ± 0.8% at the filtration time of 24 hours when phosphorus was added to the biofilter; with the percent coverage of dead cells not demonstrating a statistically significant change (Figure 7(b)). Hence, the dosing of phosphorus to a concentration of 0.02 mg/L in order to achieve a target C:N:P ratio of 100:10:1 did not show a statistical change in the viability of the cell coverage of the anthracite media that is located at the top of the filter. However, in the sand media that was located at depth in the filter, an increase in the percentage of viable cells was observed. This increase in the viability of cells attached to the sand is indicative of media at depth in the filter bed being susceptible to phosphorus enhancement.

Although the percentage of viable cells embedded in the biofilm is statistically greater at depth in the filter bed of the P enhanced filter compared to the control filter, the TOC and DOC removal were not shown to increase with phosphorus enhancement (Table 1). Furthermore, the DBP_{17} of the P enhanced filter relative to the control filter was...
Figure 6 | Images of live (green) and dead (red) cells embedded in the biofilm at 24 hours of filtration. (a) Anthracite, control filter, (b) sand, control filter, (c) anthracite, P enhanced filter, (d) sand, P enhanced filter. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wqrj.2017.012

Figure 7 | Average and standard deviation of the percentage of cell coverage of media in the control and P enhanced filters at 24 hours of filtration. (a) Anthracite, (b) sand.
shown to remain statistically the same or decrease at various filtration times with respect to TTHM_{fp} and HAA5_{fp}; with HAA5_{fp} values indicating the potential for production of HAA5 with phosphorus supplementation (Figure 3). Hence, the enhancement of cell viability in the attached biofilm due to phosphorus supplementation might be a potential contributor to an augmented formation of DBP precursors by means of bacterially produced biomolecules or the release of biofilm EPS and attached cells that lead to the formation of DBPs during phosphorus supplementation (Wang et al. 2012; Wang et al. 2013; Delatolla et al. 2015).

CONCLUSIONS

The study investigates the effects of phosphorus addition on clearwell TTHM_{fp} and HAA5_{fp} in pilot-scale biofilters located at the Britannia drinking water production plant in Ottawa, Ontario, Canada. Both TOC and DOC were removed in the control and P enhanced filters; however, no significant differences were observed between the quantity of TOC and DOC removed in the control and the P enhanced filters. The increased uptake of phosphorus in the P enhanced filter did not correspond to an increased consumption of readily assimilable nitrogen (NH_{4}/NH_{3} or NO_{3}) or soluble carbon (DOC) and as such, the DOC reduction in the biologically active filters is shown not to be phosphorus nutrient limited. The pilot-scale filters demonstrated an overall reduction in the clearwell TTHM_{fp} in both biofilters indicating that phosphorus supplementation of the dual media filter did not demonstrate a beneficial effect with respect to TOC, DOC or TTHM_{fp} when exposed to the clearwell contact time. Most meaningfully, the clearwell HAA5_{fp} was observed to increase at specific filtration times in the P enhanced filter, which was not observed in the control filter. Hence this study identifies the potential for the production of HAA5 due to phosphorus supplementation in biofilters and particularly drinking water production plants fed with high quality source water.

The percent coverage of biofilm on the filter media was observed to increase in the anthracite and sand media during phosphorus supplementation. In addition, the viability of the embedded cells on the sand media at depth in the filters was also shown to increase due to phosphorus supplementation. The increase in EPS and attached cell biofilm coverage of the filter media and the enhanced cell viability at depth in the P enhanced filter might contribute to the augmented formation of DBP precursors, by means of bacterially produced biomolecules or the release of biofilm EPS or cells due to phosphorus supplementation and the subsequent augmented formational potential of DBPs.

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