Impact of carbon-based nutrient enhancement on biofiltration performance for drinking water treatment

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Abstract

Incorporation of a carbon-based nutrient enhancement strategy for drinking water biofiltration is an attractive option, especially for source waters which contain recalcitrant organics. This study compared biofilters that were operated in parallel and individually enhanced with amino acids (including alanine, phenylalanine, and tryptophan), inulin, and sucrose to increase biomass concentration and promote biodegradation of dissolved organic carbon (DOC) in the source water, including disinfection by-product (DBP) precursors. Biomass activity was characterized by measuring adenosine tri-phosphate (ATP), dissolved oxygen (DO) consumption, and through the use of laccase and esterase enzyme assays. Performance was evaluated in terms of headloss, turbidity, pH, DOC, UV254, and DBP formation potential (DBP FP). The introduction of carbon-based nutrients significantly increased biomass activity, where ATP values peaked at 976 ng/g of filter media, 853 ng/g, and 513 ng/g for amino acids, inulin, and sucrose-spiked biofilters, respectively, while a non-spiked control only reached 104 ng/g. DO utilization by the enhanced biofilters was significantly higher than the control, with a strong correlation between ATP and DO uptake observed for all filters ($R^2 > 0.74$). Laccase and esterase enzyme activities of enhanced biofilters were also elevated ($p > 0.05$), suggesting greater biodegradation potential. Operational parameters such as headloss development and turbidity control were not impaired by carbon supplementation strategies or an increase in biomass concentration and activity. However, the enhancement strategy did not provide improvement in terms of source water carbon removal (DOC and UV254) or DBP FP when treated filters were compared to a control.

Keywords: Biological filtration, Carbon enhancement, Biomass, Disinfection by-products, Dissolved organic carbon

Introduction

When considering biofiltration, indigenous microorganisms are allowed to colonize filter media in order to help remove biodegradable organic compounds including disinfection by-product (DBP) precursors and pharmaceuticals (McKie et al., 2015). Biofiltration performance can be impacted by various factors including media type (Emelko et al., 2006), temperature (Pharand et al., 2015), and empty bed contact time (EBCT) (Nemani et al., 2018). During the past five years, the use of nutrient enhancement to stimulate biofiltration performance has received increasing attention from both researchers and practitioners. Application of ozonation prior to biofiltration breaks down recalcitrant natural organic matter (NOM) into low molecular weight fractions thereby increasing biodegradable dissolved organic carbon (BDOC) and biodegradation (Dhawan...
Previous enhancement studies have focused on the addition of phosphorous and nitrogen, as these elements may be lacking in source waters. Theoretically, an uptake of carbon:nitrogen: phosphorous (C:N:P) molar ratio of 100:10:1 is representative of optimal microbial metabolism (LeChevalier et al., 1991; Thompson et al., 2006). Dhawan et al. (2017) reported a 15% increase in DOC removal following the addition of nitrogen and phosphorous to nutrient-limited biofilters. Others focused on the singular impact of the phosphorous (0.02 mg/L PO₄-P) and reported a 15% reduction in terminal headloss as well as higher DOC removal (0.3 mg/L) (Lauderdale et al., 2012). However augmentation with nitrogen and phosphorous addition will only improve biofilter performance if limiting conditions exist. Rahman et al. (2016) reported no improvement in biomass concentration or microbial activity when supplementing river water with 0.011 mg/L of phosphorous. Kamjune et al. (2016) observed no impact on biofilm bacterial production or DOC removal following phosphorous addition. Others observed no statistically significant difference (α = 95%) between a control and biofilters enhanced with 0.5 mg/L nitrogen and phosphorous in terms of DOC or DBP precursor removal (McKie et al., 2015).

While nitrogen/phosphorous addition and the concept of engineered biological filters with supplementation has been previously examined, few studies have focused on the benefits of carbon-based nutrients which may be essential, especially when considering waters that contain low (≤2 mg/L) or recalcitrant DOC. Amino acids (AA) represent a group of carbon-based nutrients that serve as building blocks for proteins and are essential for cellular function. Gagnon et al. (2000) investigated the impact of three types of amino acids (aspartic acid, glutamic acid, and serine) at several concentrations on biofilm growth using annular reactors with hydraulic retention times ranging from 1 to 4 hr and concluded that the amino acids could be fully biodegraded at concentrations of ≤100 mg C/L. Van der Kooij and Hijnen (1988) reported an increase in bacterial growth (measured in maximum colony count) in the presence of 21 amino acids (total 1 μg C/L). Li (2016) expanded upon this research by examining alanine, phenylalanine, tryptophan, and tyrosine using batch and bench-scale experiments. The same author observed a 4.08-log increase in ATP (Cₐ = 0 μg/ml) when adding 3 mg/L phenylalanine to a bench-scale biofilter (EBCT = 15 min), representing 10 ng/g of media. Overall, the biomass growth rate was faster for the amino acids (0.148/hr⁻¹) when compared to a control (0.058/hr⁻¹).

Biofilter performance depends on the type of microbes present and their activity level. Seeding filters with specific microbes known to degrade DOC may appear promising, however it has been shown that over time the natural flora will prevail as they are better adapted for a given water matrix (McDowall et al., 2009). As such, prebiotics addition was identified as a potential means of supplementation, whereby carbon components are added to stimulate the growth or activity of microbes to encourage higher organic carbon utilization rates. Apart from the benefits of stimulating microbial growth (Capurso, 2001), this strategy could prove to be beneficial for biofilters facing varying influent conditions. Typical prebiotics include fructose polymers such as inulin, galacto-oligosaccharide (GOS) and transgalacto-oligosaccharide (TOS), and contain 3–10 carbohydrate monomers. The use of prebiotics has been extensively studied in mammals, where others have confirmed their positive impact on stimulating gastrointestinal microbiota growth (Saad et al., 2013). A study conducted by Nakayama and Oishi (2013) on the impact of GOS on the concentration of Bifidobacterium at the proximal colon, reported a 2.5-log increase in bacterial count. Liu et al. (2015) examined the prebiotic effect of inulin supplementation on rats where the relative abundance of rhizobiales increased by 7.8-fold. Thus, if inulin can encourage the growth of rhizobiales in mammals, it stands to reason that this prebiotic may also stimulate growth and abundance in the microbiome attached to biological filters. This hypothesis is further supported by the fact that rhizobiales are one of the most abundant bacterial species in drinking water biofilters (Lautenschlager et al., 2014). Furthermore, proteobacteria in biofilters are linked to biodegradation of organic pollutants (Mahjoubi et al., 2013), which suggests that the addition of prebiotics may improve overall water quality.

The objective of this study was to increase biomass activity and improve biofiltration performance using carbon-based nutrient enhancement strategies (addition of prebiotics), including amino acids (alanine, phenylalanine, and tryptophan), inulin, and sucrose to pilot filters in the absence of a pre-oxidant. Performance was evaluated in terms of organic removal (DOC, UV₂₅₄), reduction of disinfection by-product formation potential (DBP FP, including THM₄ and HAA₅), as well as biomass activity (ATP, enzyme activity, DO uptake) and operational parameters (turbidity, pH, headloss).

1. Material and methods

1.1. Pilot-scale plant configuration and experimental design

A pilot biofiltration study was conducted at a conventional drinking water treatment plant which treats surface water from Lake Ontario, Canada. Pre-chlorination is practiced year-round for zebra mussel control; any remaining chlorine residual was quenched using sodium bisulphite (2.3 mg/L) prior to granular filtration. Four parallel filter columns made of acrylic (D = 7.62 cm) each contained 1 m of media harvested from full-scale biological activated carbon contactors (BACCs) at the same facility with an initial ATP concentration of less than 100 ng/g (Fig. 1). The media (Calgon Filtrasorb-300, effective size = 1.3–1.5 mm, uniformity coefficient = 1.4) had been in operation for over 40 months and assumed to be exhausted in terms of adsorption capacity. DOC in pilot influent varied from 2.2 to 2.5 mg/L during the study; UV₂₅₄, DO, pH, turbidity, and temperature ranged between 0.0200–0.0292 cm⁻¹, 10.1–11.6 mg/L, 5.8–7.1, 0.3–12.7 NTU, and 8.4–18.7°C, respectively. Nutrients, including 1.5 mg/L of amino acid (AA) mixture (0.5 mg/L of alanine, phenylalanine, and tryptophan), 1.5 mg/L inulin (IN), and 1.5 mg/L of sucrose (S), were added in-line directly upstream of the pilot influent. Van der Kooij and Hijnen (1988) reported bacterial growth increased in the presence of 21 amino acids (total 1 μg C/L). Gagnon et al. (2000) investigated the impact of three amino acids (aspartic acid, glutamic acid, and serine) on biofilm growth using annular reactors and concluded that amino acids can be fully biodegraded if the concentrations are below 100 mg C/L. Li (2016) expanded upon their research and investigated the impact of individual amino acids (alanine,
phenylalanine, tryptophan, and tyrosine) dosed at 3 mg/L using batch and bench-scale experiments. A lower dose of 1.5 mg/L (72 μM C/L) was selected due to the DBP FP of the amino acids and the inulin cost. As a point of interest, the DOC contribution of the nutrients was determined for each mixture at bench-scale over several incremental concentrations (1, 2, 3, 4, and 5 mg/L of each supplement were analyzed), where amino acids contributed 0.36 ± 0.08 mg/L of DOC, while the inulin and sucrose each contributed 0.466 ± 0.05 mg/L of DOC. Although empirically, the amino acids were higher, based on the DOC values these contributed similar amounts of DOC. EBCT (8 min, filtration velocity = 0.5 L/min) mimicked the operation of full-scale biologically active carbon contactors (BACCs). All pilot filters were operated for 96 hr and backwashed twice per week with their own unchlorinated effluent. Water quality parameters, including DOC, UV254, DO, pH, turbidity, and DBP FP (THM FP and HAA FP) were monitored on individual filter effluent and their corresponding influent following nutrient addition. Media samples were collected from the top 5 cm of pilot filters for ATP and enzyme (laccase and esterase) activity measurement. Water and media samples were collected twice per month immediately prior to backwash.

1.2. Analytical methods

Media samples were collected from the top 5 cm of the media, prior to backwash procedure, for biochemical characterization by way of ATP measurements and enzyme activity assays. Total ATP was determined for 1 g of media using a Luminultra analysis kit (DSA-100C, Fredericton, New Brunswick, Canada) according to instructions provided by the manufacturer.

For the enzyme assays, 2 g of media sample was dissolved in trisaminomethane buffer, sonicated 15 min and analyzed immediately. Esterase and laccase enzyme activities were analyzed with their corresponding substrate analogues, 4-methylumbelliferone acetate (4-MUB acetate) and L-3,4-dihydroxyphenlaniline (L-DOPA), respectively, as described by Lautenschlager et al. (2014), Sharma et al. (2018), and Sinsabaugh et al. (2003). For laccase activity, 200 μL of media extract was incubated with 25 μL of 20 mmol/L L-DOPA substrate (pH = 8) in a 96-well plate and assayed in quadruplicate. Absorbance was measured at 460 nm every 15 min for a minimum of 36 hr with a Sunrise™ absorbance plate reader (Tecan Group Ltd., Männedorf, Switzerland) equipped with XFlouro software V 4.5.1 for data processing. 200 μmol/L substrate solutions for 4-methylumbelliferone (4-MUB) and 4-MUB acetate in dimethyl sulfoxide (DMSO) were prepared. Calibration standards ranging from 0 to 100 μmol/L were produced by diluting the 200 μmol/L 4-MUB substrate solution. Measurements were performed with a Cary Eclipse Luminescence Spectrometer G9800A (Agilent Technologies Canada Inc., Mulgrave, Victoria, Australia) at an excitation wavelength of 370 nm and an emission wavelength of 445 nm. 1 mL of media extract was incubated with 1 mL of 4-MUB acetate and assayed in triplicate. The fluorescence of each sample was measured over time (0, 2, 5, 10, 20, 30, 45, 60, 90, and 120 min) to determine the enzyme kinetics.

DO and temperature were measured by Hach HQ30d multimeter with Hach LDO probe as per EPA-NERL 360.1 (NEPIS, 1979). pH was determined by Hach HQ11d with PHC101 electrode as described by Standard Method 4500-H+B (APHA,
Turbidity was monitored using a Hach 2100N turbidimeter as per Standard Method 2130 B (APHA, 2012). DOC was analyzed based on Standard Method 5310 D (APHA, 2012) using an O-I Corporation Model 1010 TOC Analyzer with a Model 1051 Vial Multi-Sampler (College Station, Texas, USA). UV<sub>254</sub> was measured using a CE 3055 Single Beam Cecil UV/Visible Spectrophotometer (Cambridge, England) with 1 cm quartz cells (Hewlett Packard, Mississauga, Ontario, Canada) as per EPA-NERL 415.3 (NEPIS, 1979).

DBP formation potential (THM FP, HAA FP) was examined by first chlorinating the samples in duplicate to ensure a residual of 1.0 ± 0.5 mg/L after 24 ± 2 hr at 20°C, as described by Standard Method 4500-CI G (APHA, 2012). THM and HAA samples were subjected to liquid–liquid extraction followed by gas chromatography based on Standard Method 6263 B and Standard Method 6232 B (APHA, 2012), respectively. Both were analyzed with a Hewlett Packard 5890 Series Plus gas chromatograph (Mississauga, Ontario, Canada) equipped with an electron capture detector and a DB 5 625 capillary column (Agilent Technologies Canada Inc., Mississauga, Ontario, Canada).

2. Results and discussion

2.1. Biological characterization

2.1.1. ATP

Following nutrient supplementation, biomass activity levels (as measured by ATP) demonstrated a marked increase after 14 days of operation (Fig. 2). Inulin-supplemented biofilter followed a log increase in ATP concentration between day 14 and 28, whereas amino acids and sucrose induced a prolonged activity response from day 14 to day 35. The highest ATP levels were observed when adding amino acids (976 ng/g), followed by inulin (853 ng/g) and sucrose (513 ng/g), which were all statistically significantly higher than control (100 ng/g, Table 1). The results suggest that the microbial community was carbon limited and the supplementation strategies were sufficient to encourage biofilm formation on the media surface.

2.1.2. Dissolved oxygen

All biofilters receiving nutrient addition consumed significantly higher DO than the control (Fig. 3, Table 1). A strong correlation was observed between ATP concentrations and DO uptake for all biofilters ($R^2 = 0.7414–0.9191$). As such, the performance of a given filter may be monitored in real-time by tracking DO consumption in lieu of ATP media analysis.

2.1.3. Enzyme activity

Microbial communities can utilize the easily biodegradable NOM present in water, including sugars and amino acids (Lautenschlager et al., 2014). This metabolic process involves several key enzymes; as such monitoring enzyme activity can be useful in providing biochemical characterization of the attached microbiome. Laccase enzyme catalyzes the degradation of long chain organics and acts on aromatic compounds commonly observed in humic substances.
Esterase activity is indicative of heterotrophic carbon fixation for microbial growth (Hoggs, 2013; Nybroe et al., 1992). All enhanced biofilters demonstrated higher laccase activities when compared to the control (Fig. 4a) suggesting that nutrient addition increased either metabolic activity or the concentration of microbes that express laccase. Esterase activities (Fig. 4b) were also higher when compared to the control (amino acids: $2.65 \pm 1.58 \mu\text{mol/(min·g)}$; inulin: $2.72 \pm 1.57 \mu\text{mol/(min·g)}$; sucrose: $1.87 \pm 0.63 \mu\text{mol/(min·g)}$; control: $1.04 \pm 0.19 \mu\text{mol/(min·g)}$). However, the increase in enzyme activity was not statistically significant ($\alpha = 95\%$, Table 1). Esterase activity did not correlate well with either ATP or % DO uptake (Appendix A Table S-1). However, laccase activity was shown to have a strong correlation with ATP for both the amino acids and inulin supplemented filters ($R^2 = 0.89$ and 0.74, respectively). DO utilization rate also correlated well with laccase activity for the inulin-enhanced biofilter ($R^2 = 0.71$), suggesting that

Table 1 – Statistical comparison of p-values for carbon enhanced filters versus the control using paired t-tests, where bold indicates a statistical difference at 95% significance level ($p \leq 0.05$), * denote a significant increase between the carbon-enhanced filters compared to the control filter; $\emptyset$ = number of samples.

<table>
<thead>
<tr>
<th>BAC + AA</th>
<th>BAC + IN</th>
<th>BAC + S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity (9)</td>
<td>$0.31^*$</td>
<td>0.24</td>
</tr>
<tr>
<td>Headloss (5)</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>pH (9)</td>
<td>0.04$^*$</td>
<td>0.02</td>
</tr>
<tr>
<td>ATP (9)</td>
<td>0.01$^*$</td>
<td>0.02</td>
</tr>
<tr>
<td>Dissolved oxygen (9)</td>
<td>$0.009^*$</td>
<td>0.024</td>
</tr>
<tr>
<td>Laccase activity (4)</td>
<td>0.06$^*$</td>
<td>0.23</td>
</tr>
<tr>
<td>Esterase activity (4)</td>
<td>0.12$^*$</td>
<td>0.12</td>
</tr>
<tr>
<td>DOC (6)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>UV$_{254}$ (6)</td>
<td>0.015</td>
<td>0.93</td>
</tr>
<tr>
<td>THM FP (5)</td>
<td>0.32</td>
<td>0.01$^*$</td>
</tr>
<tr>
<td>HAA FP (4)</td>
<td>0.003</td>
<td>0.32</td>
</tr>
</tbody>
</table>

BAC: biological activated carbon; AA: amino acids; IN: inulin; S: sucrose; ATP: adenosine triphosphate; DOC: dissolved organic carbon; THM: trihalomethanes; HAA: haloacetic acids; FP: formation potential.

(Shraddha et al., 2011).
the addition of prebiotics stimulated the growth or activity of aerobic microbes that express laccase.

2.2. Operational parameters

2.2.1. Turbidity, headloss, and pH

Influent turbidity fluctuated between 0.3 and 2 NTU with the exception of one value (12.7 NTU) associated with a storm event (>35 mm precipitation in 24 hr). Filters receiving supplements consistently had lower turbidity values when compared to the control, although no statistical difference was observed. All pilot filters had similar headloss accumulation rates of 6–13 cm over 7 days. The effluents of biofilters receiving nutrients had slightly elevated pH values, which was found to be statistically different (average pH: amino acids = 6.3, inulin = 6.4, sucrose = 6.4, control = 6.2, Table 1), while the influent was 6.2. In theory, anaerobic metabolism could lower the pH by way of lactic acid production (Mazzio et al., 2010), therefore these small increase in effluent pH may reflect elevated aerobic metabolism of the supplied carbon by the microbiome (Tran and Unden, 1998). This observation suggests that simple pH measurements may serve as an indicator of microbial metabolism in biofilters when monitored routinely, especially during potential treatment upsets.

2.3. Organics and DBP formation potential removal

2.3.1. DOC and UV$_{254}$

DOC and UV$_{254}$ were monitored in the influent and effluent to assess organics removal. Influent analyses (pre- and post-spike) show that 1.5 mg/L of AA contributed 0.6 mg/L DOC and 0.009/cm UV$_{254}$ (Fig. 5a, b). Inulin and sucrose both contributed 0.4 mg/L of DOC and had no impact on UV$_{254}$. Effluent DOC and UV$_{254}$ values suggest that the biofilters were simply removing the added nutrients, and unable to utilize the source water DOC due to its recalcitrant nature in the absence of ozone (Nemani et al., 2018). Statistical analyses indicated that biofilters receiving amino acids had a significantly higher DOC and UV$_{254}$ (Table 1) in the effluent, which could be evidence of carbon nutrient breakthrough. Inulin effluent DOC removal was also significantly higher than the control. Neither DOC removal nor UV$_{254}$ removal demonstrated a positive correlation with the biological characterization parameters (ATP, DO uptake, laccase and esterase activity) (Appendix A Table S-2), which indicates that the enhanced biofilters may be utilizing primarily the supplemented nutrients and unable to metabolize the recalcitrant source water DOC.

2.3.2. THM FP and HAA FP

Disinfection by-product formation potential (DBP FP) was also evaluated to (1) assess the contribution of DBP precursors associated with nutrient addition, and (2) to determine precursor removal. Amino acids in the influent contributed 59 μg/L THM FP and 45 μg/L HAA FP, while the contribution of inulin and sucrose was below the detection limit (THM: 4 μg/L, HAA: 2 μg/L). Amino acids serve as DBP precursors, likely due to their aromatic structure (phenylalanine and tryptophan) (Hong et al., 2009). The aromatic nature of these nutrients was associated with a significant increase observed in UV$_{254}$ for the biofilter influent. This biofilter utilized almost all the amino acids, as the effluent contained an average of 38 μg/L of THM FP and 31 μg/L of HAA FP, compared with the control (THM FP: 4 μg/L, HAA FP: 25 μg/L, Fig. 6). The net DBP FP reduction for the amino acid enhanced filter was 62% and 59% for THM FP and HAA FP, respectively. This filter also had the highest ATP measurements. These results are supported by the work of Li (2016) and demonstrate that tryptophan–alanine–phenylalanine addition can increase biomass concentrations the biodegradation of organics that serve as DBP precursors.

Neither inulin nor sucrose served as DBP precursors. The effluent from the inulin-enhanced biofilter had a significantly lower concentration than the control and outperformed the other
nutrient-supplemented filters in terms of THM FP (Table 1), suggesting that inulin may have stimulated the metabolism of the microbes resulting in lower THM FP. No correlation was observed between DBP FP removal and biomass characterization parameters (ATP, DO consumption, and enzyme activity) (Appendix A Table S-3) since the majority of biofilters only removed the nutrients that were introduced. A microbial community analysis on the 16S rRNA could be used in future studies to quantify the impact on microbiome abundance and composition.

3. Conclusions

Nutrient enhancement may serve as a viable option to maintain stable biomass concentration in the event of upstream ozone upsets. However, the addition of amino acids, inulin, and sucrose had no positive impact on the overall organic carbon removal through the filter in the absence of ozone; there was also no negative impact on operational parameters including headloss and turbidity, leaving room to further investigate other carbon-based strategies or dosing regimens. Nutrient type should be carefully selected to avoid potential DBP precursor breakthrough. Biofilters are aerobic; their metabolic activity was reflected by a strong correlation between dissolved oxygen consumption and ATP levels. Inulin addition represented the preferred nutrient strategy when compared to amino acids or sucrose, as this nutrient had lower effluent THM FP compared to the control. Future studies will be conducted with upstream ozonation to determine the impact of carbon supplementation in the presence of more easily biodegradable organics. In addition, studies could be conducted to determine the impact of carbon-based nutrients on the microbial composition in the biofilm.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.03.002.

REFERENCES


