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1 **Effects of phosphate and hydrogen peroxide on the performance of a biological**  
2 **activated carbon filter for enhanced biofiltration**

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1 **Abstract**

2 Biofilm formation on biofilters can influence their hydraulic performance, thereby leading to head loss  
3 and an increase in energy use and costs for water utilities. The effects of a range of factors, including  
4 hydrogen peroxide and phosphate, on the performance of biological activated carbon (BAC) and biofilm  
5 formation were investigated using laboratory-scale columns. Head loss, total carbohydrates, and  
6 proteins were reduced in the nutrient-enhanced, oxidant-enhanced, and nutrient + oxidant-enhanced  
7 BAC filters. However, there were no changes in the removal of dissolved organic matter,  
8 trihalomethane formation potential, or selected trace organic contaminants. The biofilm formation on  
9 polyvinyl chloride and stainless steel coupons using the laboratory biofilm reactor system was lower  
10 when the effluent from a nutrient-enhanced column was used, which indicated that there was less  
11 biofilm formation in the distribution systems. This may have been because the effluent from the  
12 nutrient-enhanced column was more biologically stable. Therefore, enhanced biofiltration could be used  
13 not only to reduce head loss in biofilters, but also to delay biofilm formation in distribution systems.

14

15 *Keywords:* biological activated carbon; biofilm; hydrogen peroxide; phosphate

16

17 **1. Introduction**

18 Biological activated carbon (BAC) is a cost-effective drinking water treatment process that removes  
19 contaminants mainly via biodegradation, which is mediated by indigenous microorganisms. BAC  
20 processes have several advantages in water treatment, including the removal of diverse contaminants  
21 [1–3] and reduction of dissolved organic carbon (DOC), which includes precursors of disinfection  
22 byproducts. The hydraulic performance of BAC filters is integral to meeting the supply-demand  
23 requirements of water treatment works and can be substantially limited by biofilm development.  
24 Excessive biofilm accumulation within BAC filters leads to significant head loss and more frequent

1 backwashing, which influences the production of drinking water and the operation cost. Therefore, it is  
2 important to understand and control the process of biofilm formation within BAC filters.

3 According to Kirisits et al. [4], extracellular polymeric substances (EPS) production is significantly  
4 reduced in phosphorus-supplemented biofilters compared with that in phosphorus-deficient biofilters.  
5 Similarly, nutrient starvation promoted EPS production, within the context of microalgae cultivation  
6 [5]. It has been shown that EPS production is enhanced in response to phosphorus limitation and that  
7 this might serve as a protective mechanism (bacterial adhesion, adsorbing and storing nutrients) [6].  
8 Additionally, phosphorus limitation can impact biofilm physical structure and morphology, thus playing  
9 an important role in increasing head loss in BAC [7]. Low phosphorus concentrations in BAC influent  
10 can promote EPS production, which can increase the head loss in BAC filters and reduce the process  
11 efficiency. Generally, phosphate removal occurs with the addition of coagulants in coagulation-  
12 sedimentation-filtration processes, which results in phosphorus-deficient water at a concentration of  
13 0.01 mg/L [8]. However, the impact of phosphorus on BAC biofilms, particularly the EPS  
14 characteristics, has not yet been fully explored. According to a previous study, most biofilm  
15 extracellular matrixes account for over 90% of the dry mass, whereas microorganisms account for less  
16 than 10% [9]. Peroxide enhancement reduces terminal head loss by up to 60% compared with that of  
17 control biofilters without deterioration in water quality performance [10]. Moreover, the results from  
18 laboratory studies, such as batch reactors with sand and water, have revealed that hydrogen peroxide  
19 concentrations between 3 mg/L and 5 mg/L increase the DOC removal while reducing microbial activity  
20 [11]. However, Stoddart and Gagnon reported that such oxidant and nutrient enhancement strategies do  
21 not show any improvement in water quality performance with respect to total organic carbon (TOC),  
22 DOC, specific ultraviolet absorbance (SUVA), and disinfection byproduct formation potential [12].  
23 While biofiltration enhancement via nutrients and/or oxidants is effective in DOC removal, some  
24 studies showed that small changes in the magnitude of the mean difference have no practical operational  
25 importance [12]. Therefore, more investigations are necessary to determine the effects of nutrient and/or  
26 oxidant enhancement on the removal of DOC.

1 There have been no in-depth studies on the impacts of these parameters on EPS characteristics and their  
2 interactions with biofilter hydraulic performance, which includes any subsequent downstream impacts,  
3 such as on drinking water distribution systems (DWDSs). This study aimed to assess the impact of  
4 enhancing nutrients (phosphate) and/or oxidants (hydrogen peroxide) in BAC filters on the quantity and  
5 composition of EPS and filter performance. The performance of water quality improvement via BAC  
6 was determined by DOC, trihalomethane formation potential (THMFP), and trace organic contaminants  
7 (TrOCs). The biofilm formation in effluent discharged from the enhanced BAC filters was also  
8 investigated to assess the subsequent impacts on the DWDS.

## 9 **2. Materials and methods**

10 Detailed information on the batch experiments is explained in Text S1.

### 11 *2.1 Columns and biofilm reactors*

12 Four BAC columns were set up to assess the effects of phosphate and hydrogen peroxide enhancement  
13 on the performance of BAC filters and their biofilm characteristics, which included i) columns that did  
14 not have any phosphate or hydrogen peroxide added, ii) nutrient-enhanced columns using phosphate,  
15 iii) oxidant-enhanced columns using hydrogen peroxide, and iv) nutrient + oxidant-enhanced columns  
16 using phosphate and hydrogen peroxide. Each column was a glass cylinder with a diameter of 150 mm  
17 and height of 800 mm. All columns were filled with BAC collected from a full-scale drinking water  
18 treatment plant, and they were kept in a dark room (Fig. S1). The empty bed contact time of each column  
19 was 20 min (flow velocity of 1.2 m/h), and backwashes were conducted with air and water every 10 d.  
20 Feed water was collected after coagulation/flocculation-sedimentation followed by rapid sand filtration  
21 in a full-scale drinking water treatment plant (Seoul Metropolitan Waterworks, Seoul, Republic of  
22 Korea) (Table S1). Then, it was fed to all the BAC columns after ozone (O<sub>3</sub>) treatment at a concentration  
23 of 0.8 mg O<sub>3</sub>/mg DOC.

1 The nutrient-enhanced column was continuously fed with a C:P ratio of 100:10 in the influent, which  
2 was based on the results from the preliminary tests. The oxidant-enhanced columns were continuously  
3 fed with 1 mg/L of hydrogen peroxide determined based on the results of a previous study [10]. The  
4 nutrient + oxidant-enhanced column was injected with both phosphate and hydrogen peroxide at the  
5 same concentrations used for the nutrient-enhanced and oxidant-enhanced columns. Moreover, selected  
6 TrOCs, which included gemfibrozil, ibuprofen, pentoxifylline, naproxen, phenacetine, bezafibrate,  
7 diclofenac, ketoprofen, fenoprofen, caffeine, and carbamazepine, were introduced into the BAC  
8 columns at a concentration of about 1 µg/L (Table S2). Centre for Disease Control (CDC) biofilm  
9 reactors (CBR, Biosurface Technologies Bozeman, MT, USA) were fed with BAC filtered water to  
10 determine the biofilm formation potential of the post-filtered water (Table S3). The CDC reactors  
11 consisted of polyvinyl chloride (PVC) and stainless steel coupons, and the effluent from the BAC  
12 columns was used as feed water for the CDC biofilm reactors. The *p* values of all the experimental  
13 results were calculated through an analysis of variance test, which used a level of significance of 5%,  
14 and the analysis was performed using SPSS 18 (SPSS Inc., Chicago, IL, USA).

## 15 *2.2 Extraction and analysis of the extracellular polymeric substances*

16 In this study, sonication was used in order to recover biofilm. First, 1 g of each BAC sample was diluted  
17 in a 100 mL glass flask with 50 mL of deionized water. Then, samples were detached by vortexing for  
18 1 min and sonicating using an ultrasonicator (Cole-Parmer 8890, Vernon Hills, IL, USA) at 190 W for  
19 3 min [13]. EPS were extracted from the biofilm samples using the formaldehyde combined with  
20 heating protocol described by Evans [14], which separates the total carbohydrates and proteins. In brief,  
21 2 g of wet BAC, 10 mL of 0.01 M phosphate buffer solution (pH of 7), and 60 µL of 35% formaldehyde  
22 (Desung, Republic of Korea) were combined in a 50 mL sterile centrifuge tube [15]. After shaking at  
23 400 rpm for 1 h at 4 °C, the tube was heated in a water bath at 80 °C (WB-6, DAIHAN, Republic of  
24 Korea) for 10 min. Finally, the sample was centrifuged (MF300, Hanil, Republic of Korea) at 5000 g  
25 for 10 min. The carbohydrate content of the extracted EPS was determined using a phenol-sulfuric acid

1 assay, and glucose was used as the standard [16, 17]. A Bradford assay was used for the determination  
2 of proteins using Bradford reagent (Bradford Reagent 5x, SERVA Electrophoresis GmbH, Germany)  
3 [16]. Absorbance was measured using a UV-Vis spectrophotometer (DR5000, Hach, USA).

#### 4 *2.3 Dissolved organic matter characteristics and assimilable organic carbon*

5 BAC effluents were passed through a 0.45 µm filter (Whatman, USA). DOC and UV<sub>254</sub> were analyzed  
6 using a TOC analyzer (TOC-V CPN, Shimadzu, Japan) and spectrophotometer (DR5000, Hach, USA).  
7 A liquid chromatography-organic carbon detector (LC-OCD, Model 8, DOC Labor, Germany) was used  
8 to separate dissolved organic matter into high molecular weight (HMW) fractions, intermediate  
9 molecular weight (IMW) fractions, and low molecular weight (LMW) fractions [18]. A fluorescence  
10 excitation-emission matrix (EEM) was used to analyze the characteristics of fluorescent dissolved  
11 organic matter. The fluorescence emission was measured using a spectrofluorometer (RF-5301,  
12 Shimadzu, Japan) with an arc lamp as a light source. The details have been described in our previous  
13 study [19]. An assimilable organic carbon (AOC) analysis was performed using flow cytometry (FCM),  
14 as described by Eawag (Swiss Federal Institute of Aquatic Science and Technology), to evaluate the  
15 removal of AOC via BAC columns. Details of the survey method are reported elsewhere [20–22]. The  
16 AOC was used to determine the biological stability which determine the inability of drinking water to  
17 support microbial regrowth.

#### 18 *2.4 Total cell counts, heterotrophic plate counts, and adenosine triphosphate*

19 Total cell counts (TCC) in a sample were enumerated using FCM (Cube 6, Partec, Germany) following  
20 a protocol described in Park et al. [23]. Heterotrophic plate count (HPC) analysis was conducted to  
21 evaluate the growth potential of bacteria. In brief, 1 mL of the samples collected from the BAC columns  
22 was diluted and streaked on an R2A agar (Difco, USA) plate medium. To evaluate cell activity,  
23 adenosine triphosphate (ATP) measurements were calculated using BacTiter-Glo™ reagent (Promega,  
24 USA).

## 1 *2.5 Trihalomethane formation potential and trace organic contaminants*

2 BAC effluent samples were adjusted to a pH of 8 and buffered with a borate buffer solution. The effluent  
3 samples were chlorinated with sodium hypochlorite at a dosage of 3 mg Cl<sub>2</sub>/mg C, and were incubated  
4 at 20 °C for 48 h in headspace-free 300 mL amber bottles. After, the free chlorine remaining after the  
5 incubation was quenched with sodium sulfite prior to determining the trihalomethane concentrations  
6 using gas chromatography - electron capture detection (GC-ECD, Agilent 6890N, USA). The details  
7 have been described in our previous work [19]. The selected TrOCs were prepared in a stock solution  
8 of 1 g/L in high performance liquid chromatography (HPLC)-grade methanol (JT Baker, USA) and  
9 introduced at a concentration of 1 µg/L into the BAC columns. Selected TrOCs were pre-concentrated  
10 using the column switching method with a concentration column (Hypersil Gold aQ; Thermo Fisher  
11 Scientific) and an analytical column (Hypersil Gold C18). Mass spectrometry measurements were  
12 performed on a high-resolution full scan Orbitrap Exactive mass spectrometer (Thermo Fisher  
13 Scientific, Bremen, Germany) [24].

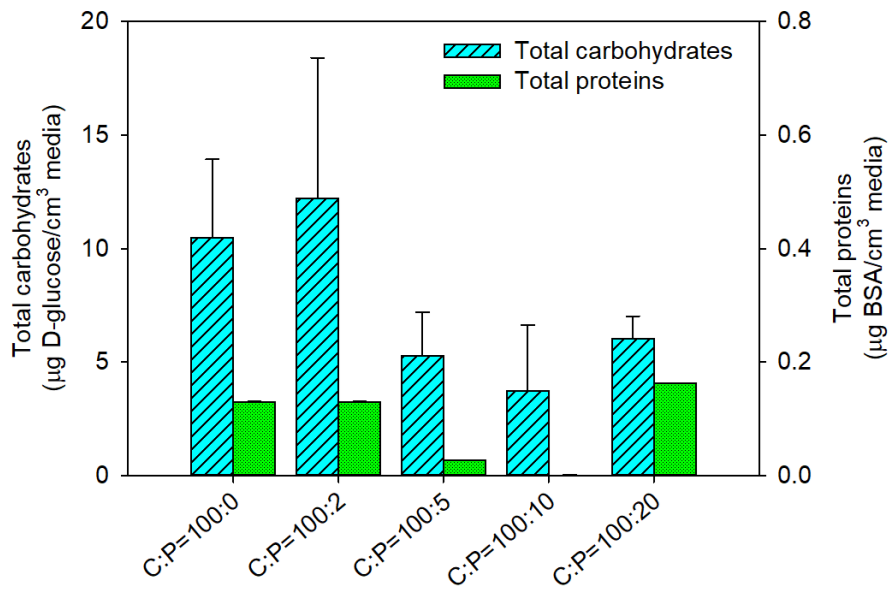
## 14 **3. Results and discussion**

### 15 *3.1 Impact of phosphate concentration on biological activated carbon biofilms*

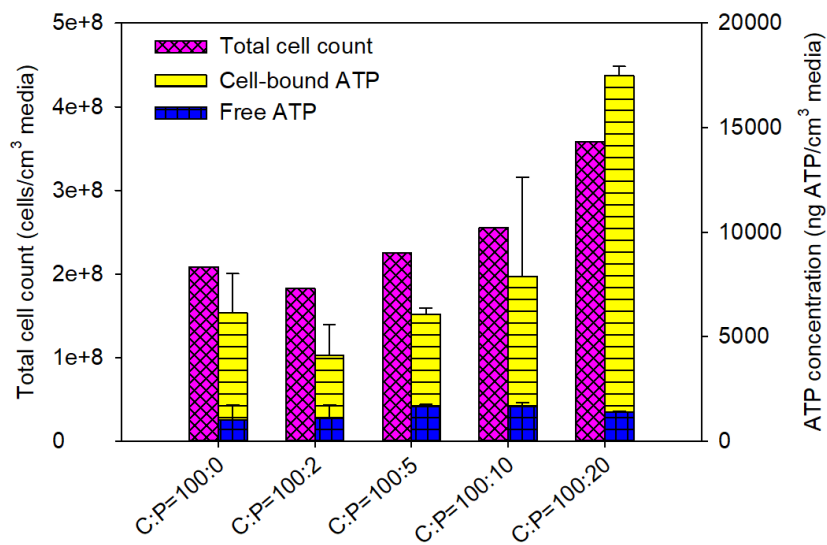
16 Comparison of the BAC biofilms developed under different C:P ratios using batch reactors showed that  
17 the highest concentration of carbohydrates and proteins, which were used as an estimate of EPS, was  
18 observed in a C:P ratio of 100:0, as shown in Fig. 1a. The EPS concentrations were 10.3, 9.0, 5.3, 3.7,  
19 and 6.0 µg/cm<sup>3</sup> BAC for C:P ratios of 100:0, 100:2, 100:5, 100:10, and 100:20, respectively (Fig. 1a).  
20 The concentration of EPS formed at the C:P ratio of 100:10 was 64% lower than that at the C:P ratio of  
21 100:0, and the TCC gradually increased as the C:P ratio decreased. The data collected indicated that  
22 EPS production was reduced with phosphate addition. However, the total EPS and protein concentration  
23 increased between the C:P ratios 100:10 and 100:20. This trend has not previously been reported and  
24 requires further exploration, which was beyond the scope of the current study. However, there may be



1 a phosphate threshold that favors changes in the EPS characteristics when a different microbial  
 2 community is selected. Alternatively, the excess phosphate caused a substantial increase in cell activity  
 3 and promoted EPS production. The total ATP in BAC increased with phosphate addition and showed a  
 4 similar trend to that of the TCC (Fig. 1b). The C:P ratio of 100:10 was selected and used in the nutrient-  
 5 enhanced columns because the lowest formation of EPS was observed in the batch study.



(a)

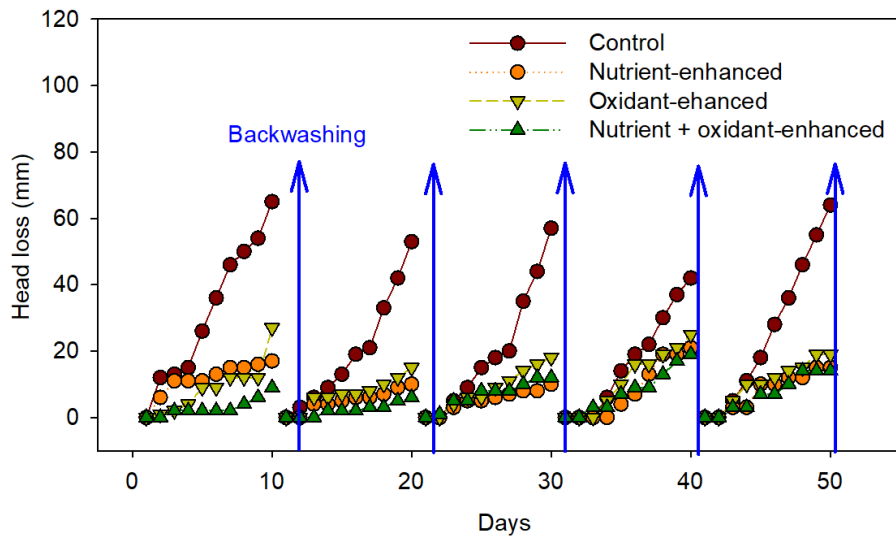


1 (b)

2 **Fig. 1.** (a) Total carbohydrates and total proteins associated with biological activated carbon (BAC) and  
3 (b) total cell counts and adenosine triphosphate (ATP) associated with BAC (n = 6).

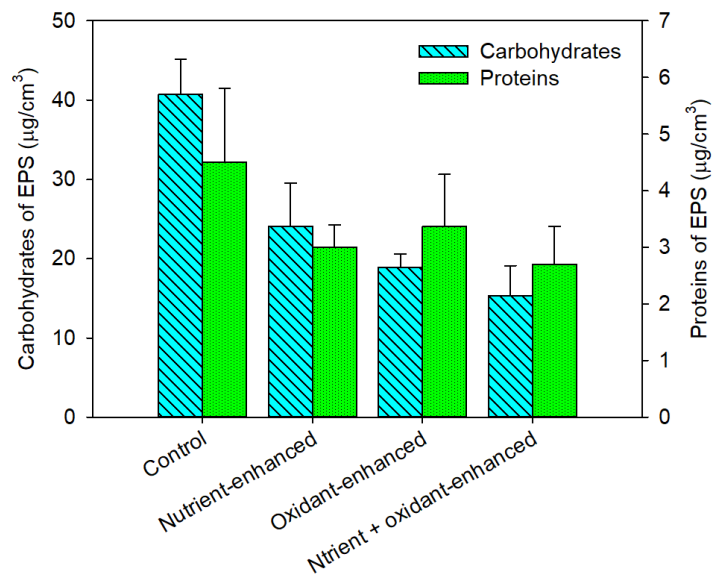
4 *3.2 Head loss and extracellular polymeric substances characteristics in enhanced biological activated*  
5 *carbon filters*

6 Fig. 2 shows the changes in head loss in the BAC columns as a function of backwashes that were  
7 conducted every 10 d ( $p < 0.05$ ) for 50 d of operation. The maximum head losses were 56, 15, 21, and  
8 12 mm for control, nutrient-enhanced, oxidant-enhanced, and nutrient + oxidant-enhanced columns,  
9 respectively. In the oxidant-enhanced columns, the head loss resulted in a 63% reduction. Nutrient-  
10 enhanced and nutrient + oxidant-enhanced columns reduced the head loss by 74% and 79%,  
11 respectively. The differences in the average head loss between the enhanced BAC columns were not  
12 significant, which suggested that any enhancement reduced head loss. Fig. 3 shows the composition  
13 and concentration of EPS associated with each of the four BAC filters. The potential formation of EPS,  
14 which was estimated as protein and carbohydrates, is known as a potential fouling agent of biological  
15 filters [10]. Therefore, the higher production of EPS in the control BAC filter (C:P ratio of 100:0) may  
16 have accelerated the head loss as a result of filter clogging. The observation of the greatest head loss in  
17 the control column has previously been attributed to greater EPS production due to a community of  
18 long-term phosphorus-deficient bacteria [25], and it is possible that this also occurred in the BAC filters  
19 of the current study.



1

2 **Fig. 2.** Head loss profiles of enhanced biofilters.



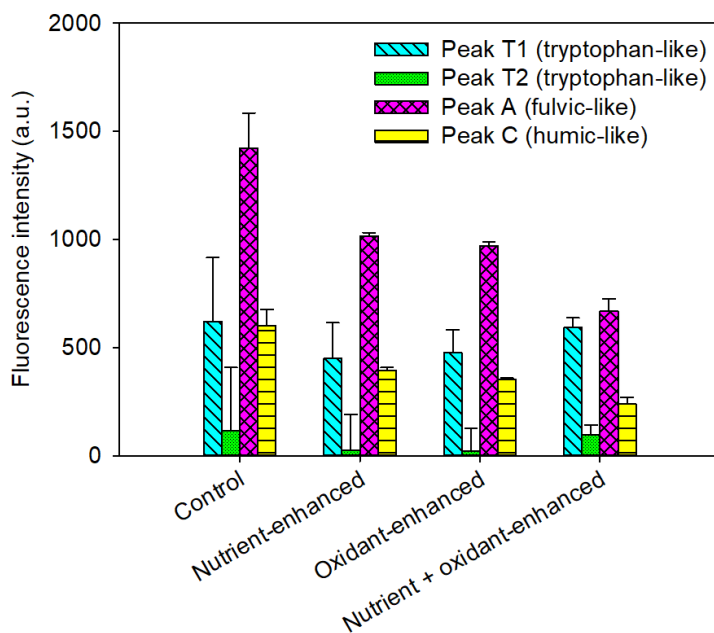
3

4 **Fig. 3.** Extracellular polymeric substances (EPS) composition in terms of total carbohydrates and total  
 5 proteins in the biological activated carbon collected from the top of the column (0–50 mm) (n = 6;  
 6  $p < 0.05$ ).

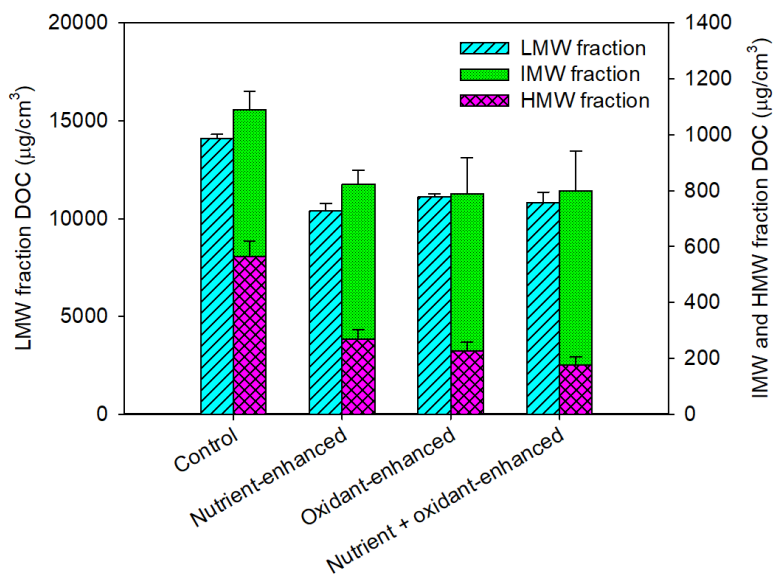
1 The concentrations of carbohydrates and proteins from biomass associated with BAC along the columns  
2 at different depths are presented in Fig. S2. The concentration of carbohydrates, which is the main  
3 component of EPS, was significantly high at the top of the control column. The EPS determined by  
4 summing the total carbohydrates and total protein concentrations was  $41 \mu\text{g}/\text{cm}^3$  BAC in the top layer  
5 (50 mm) of the control. For the nutrient-enhanced column, the EPS concentration was  $24 \mu\text{g}/\text{cm}^3$  BAC,  
6 which was 41% less than that at the top of the control. In the case of the oxidant-enhanced column, the  
7 total EPS concentration was  $19 \mu\text{g}/\text{cm}^3$  BAC, which was 54% lower than that of the control. For the  
8 nutrient + oxidant-enhanced column, the total EPS concentration was  $15 \mu\text{g}/\text{cm}^3$  BAC, which was 63%  
9 lower than that at the top of the control. The enhancement of BAC filter columns via phosphate and/or  
10 hydrogen peroxide reduced the EPS from 41% to 63%.

11 EPS are composed of protein-like substances, and fluorescence EEM spectroscopy has been extensively  
12 utilized to determine changes in protein-like substances [26]. Fluorescence EEM spectroscopy was  
13 performed to determine the fluorescent dissolved organic matter in EPS extracted from the top of each  
14 column (0–50 mm) where the highest EPS was observed (Fig. 4). The fluorescence intensities of the  
15 protein like-species T1 and T2 were not significantly different between the control and enhanced BAC  
16 columns. As shown in Fig. 1a and Fig. 3, the concentration of proteins was also not different between  
17 the control and enhanced BAC columns. A high-intensity fulvic-like substances peak was observed in  
18 the control, which indicated that more decomposed organic matter, such as fulvic acid, was associated  
19 with the control BAC. Therefore, the main fraction in the EPS associated with the BAC was humic,  
20 such as fulvic-like substances. The fluorescence EEM can only determine organic matter that has  
21 fluorescence characteristics, such as proteins, but not polysaccharides. However, an LC-OCD can detect  
22 both proteins and polysaccharides using an OCD detector. Therefore, this analysis was also applied to  
23 the EPS extracted from the top of the BAC columns (Fig. 5). The control column showed the highest  
24 concentration of the HMW fraction ( $565 \mu\text{g}/\text{cm}^3$  BAC). The HMW fractions in the nutrient-enhanced,  
25 oxidant-enhanced, and nutrient + oxidant-enhanced columns were 53%, 60%, and 69% lower than those  
26 of the control, respectively ( $p < 0.05$ ). The concentration of carbohydrates, which was in the HMW

1 fraction, in the enhanced BAC columns showed a good correlation with the HMW fraction determined  
 2 by the LC-OCD ( $R^2 = 0.98$ ).



3  
 4 **Fig. 4.** Fluorescent dissolved organic matter characteristics in extracellular polymeric substances  
 5 collected from the top of the biological activated carbon columns (0–50 mm) (n = 6).



6  
 12

1 **Fig. 5.** Extracellular polymeric substances constituents separated into high molecular weight (HMW)  
2 fractions, intermediate molecular weight (IMW) fractions, and low molecular weight (LMW) fractions  
3 (0–50 mm) (n = 6).

### 4 *3.3 Changes in dissolved organic matter characteristics in enhanced biological activated carbon filters*

5 Changes in DOC and SUVA were observed in all four BAC columns between the influent and effluent  
6 (Fig. S3a). Across all the columns, the DOC removal rates were between 13% and 17%, and the  
7 differences were not significant between any of the BAC filters ( $p < 0.05$ ). According to previous studies,  
8 the DOC removal rate of the enhanced BAC columns was between 11.6% and 12.1% [10] and showed  
9 similar removal rates. Enhanced O<sub>3</sub>/biofiltration has been reported to result in lower organic carbon by  
10 adding phosphate to the feed water [27], but there was no improvement in the removal of DOC in the  
11 filter fed with phosphate in our study. The SUVA of the column effluent increased between 9% and  
12 18% compared with that of the influent (Fig. S3a), and again there were no significant differences  
13 between the filters. The difference in DOC removal could be confirmed by the difference in  
14 biodegradability of DOC according to the feed water characteristics and oxidizing power of O<sub>3</sub>. The  
15 increase in DOC removal confirmed that HMW organic matter could be converted into LMW organic  
16 matter by O<sub>3</sub> oxidation, which increases biodegradable DOC [10, 27]. However, biofiltration without  
17 the O<sub>3</sub> process as a pretreatment did not increase the removal of DOC [12]. The O<sub>3</sub> dose used from the  
18 previous study [27] was 2.18 mg O<sub>3</sub>/mg DOC, which was relatively higher than the dose of 0.8 mg  
19 O<sub>3</sub>/mg DOC in this study. In our study, the biodegradable organic matter of the feed water was relatively  
20 low; therefore, there was no difference in the DOC removal rate with the addition of phosphate.

21 An AOC analysis was conducted to assess the biological stability in the BAC filtrates by evaluating the  
22 bacterial growth potential (Fig. S3b). It is important to investigate the fate of AOC in BAC, which is  
23 effective in the removal of biodegradable organic matter in drinking water treatment processes. The  
24 AOC concentration in the influent was 54 µg/L on average, and the removal rate was between 57% and  
25 62% in the BAC columns. The AOC is often related to LMW dissolved organic matter, and this

1 corresponded with the LC-OCD result of the preferential removal of LMW organic matter [28]. As with  
2 DOC and SUVA, no difference in the removal of AOC was observed between the control and enhanced  
3 BAC columns, and no significant improvement was observed in the enhanced columns for AOC or  
4 DOC.

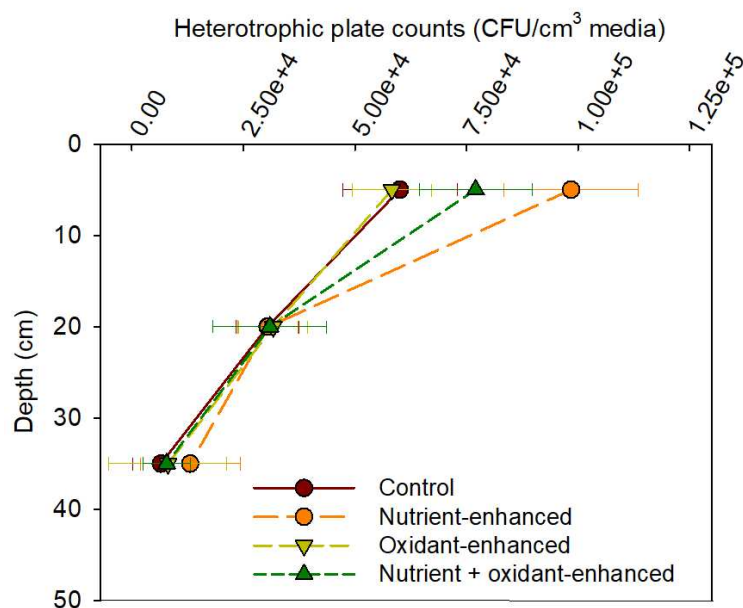
5 The LC-OCD analysis results of O<sub>3</sub>-treated water (influent) and the four column effluents are shown in  
6 Fig. S4. LMW neutrals (>71%) and acids (>98%) were effectively removed. The removal of the LMW  
7 fraction was greatest in the nutrient-enhanced column (80%), but the difference between the BAC filters  
8 was not significant. LMW organic matter, such as LMW acids and neutral compounds, were  
9 preferentially removed in relation to humic substances, which have inert organic matter characteristics.  
10 The reduction of the LMW fraction was due to biodegradation processes [20, 29], which suggested that  
11 LMW biodegradation rates were unaffected by BAC enhancements of any kind in the current study.

12 The fluorescence EEM results for the rapid sand filtrate, influent (O<sub>3</sub>-treated rapid sand filtrate), and  
13 BAC effluent were plotted according to T1, T2, A, and C peak regions (Fig. S5). The fluorescence  
14 intensities observed in the rapid sand filtrate were considerably reduced (61–90%) in selected peak  
15 regions after ozonation. This suggested that organic matter structures changed to leave fewer  
16 fluorophores. The fluorescence EEM also showed an increase in both humic-like and tryptophan-like  
17 substance regions after the BAC columns, which agreed with the increased SUVA shown in Fig. S3a.  
18 According to Lohwacharin et al. [30], there was an increase in humic-like substance regions after 6 y  
19 of BAC treatment. During biofiltration via the BAC columns, there were changes in organic matter  
20 characteristics, which indicated that the biodegradation led to more fluorophores, and these changes  
21 were similar between the four BAC filters.

### 22 *3.4 Microbial characterization*

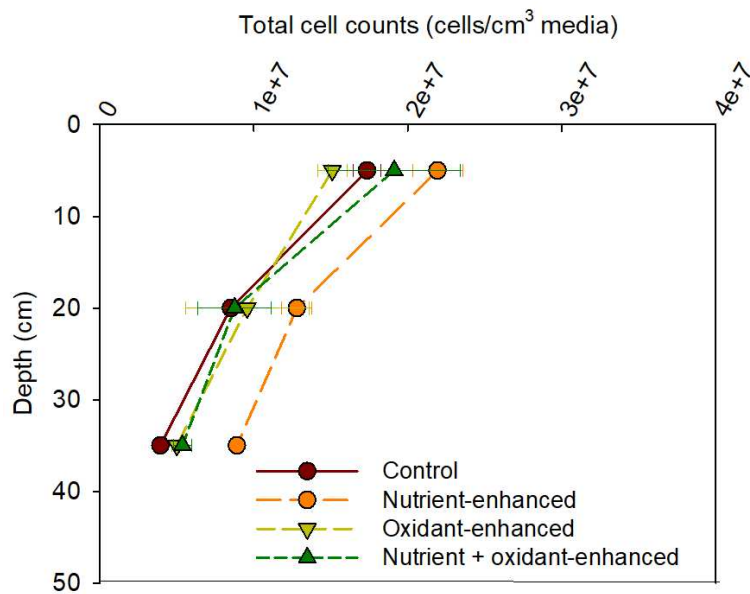
23 In order to analyze the microbial behavior in the BAC columns, HPC and TCC associated with BAC  
24 were performed (Figs. 6a and 6b). The HPC and TCC were the greatest at the top of all the columns.  
25 Ozonation of the influent likely enhanced the biodegradability in the feed water by oxidation, and thus

1 increased the AOC concentration [31–33]. Therefore, HPC and TCC associated with BAC likely  
 2 decreased with distance through the BAC filters because the amount of AOC and the dissolved oxygen  
 3 concentration would have been depleted toward the lower end of the columns. The biomass at the top  
 4 of the column was higher in the nutrient-enhanced and nutrient + oxidant-enhanced columns compared  
 5 with that of the control and oxidant-enhanced columns (Figs. 6a and 6b). At the top of the column, the  
 6 nutrient-enhanced column had higher HPC associated with BAC than those of the control column (Fig.  
 7 6a), but the EPS in the nutrient-enhanced column were lower than those in the control column. It was  
 8 confirmed that the enhancement in phosphate was effective in reducing EPS formation. The cell-bound  
 9 ATP associated with BAC along with column depth were analyzed, and are shown in Fig. S6. Similar  
 10 to HPC and TCC, the columns fed with phosphate showed relatively higher microbial activities with  
 11 respect to ATP than those of the control. The limiting nutrients in the BAC columns could lower the  
 12 microbial activity in the control.



(a)



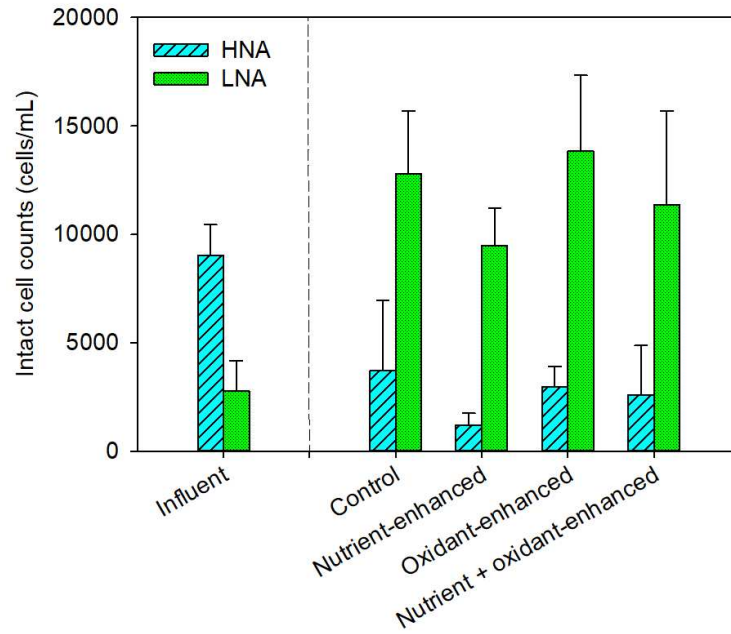


(b)

**Fig. 6.** Distribution of heterotrophic plate count bacteria (a) and total cell counts (b) at different depths (n = 3).

The average intact cell count (ICC) in the influent was  $1.2 \times 10^4$  cells/mL, and this appeared to be dominated by the high nucleic acid (HNA) group (77%; Fig. 7a.). In contrast, the ICC increased in the column effluent compared with that in the influent. The ratio of HNA group decreased because the number of cells assigned to the low nucleic acid (LNA) group was higher in the BAC effluents. HNA and cell-bound ATP showed some correlation ( $R^2 = 0.96$ ). According to Gasol and Del Giorgio, these results indicated that the HNA group could be an active group of heterotrophic bacteria [34]. In previous studies, the HPC was found to be a better indicator of microbial regrowth than TCC [35]. Herein, the average HPC of the influent water was 1368 CFU/mL, and in the BAC columns, 56%, 68%, 61%, and 66% of the HPCs was removed in the control, nutrient-enhanced, oxidant-enhanced, and nutrient + oxidant-enhanced columns, respectively. HNA and HPC in the BAC columns showed a good correlation ( $R^2 = 0.84$ ), which suggested that the cells classed as HNA were more likely to be culturable. It takes much less time to measure HNA compared with HPC, which requires incubation time; this is advantageous for evaluating biofilm formation. Owing to the presence of microbial cells (as indicated

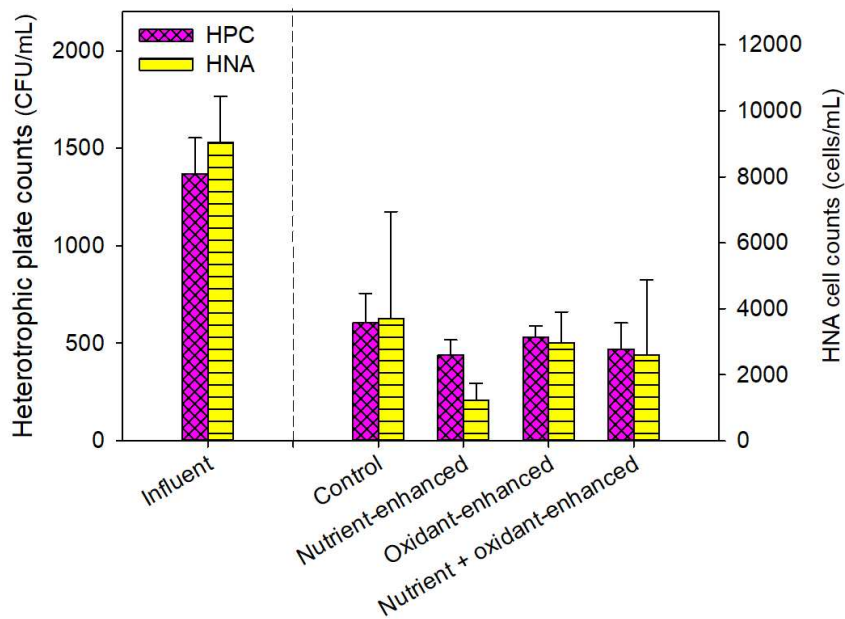
1 by ICC), specifically heterotrophic bacteria (as indicated by HPC) in effluents from the control and  
2 enhanced BAC columns, it was necessary to investigate the effects of enhanced BAC filters on the  
3 formation of biofilm in the distribution systems as a further parameter of assessing their performance.



4

5

(a)



1

2

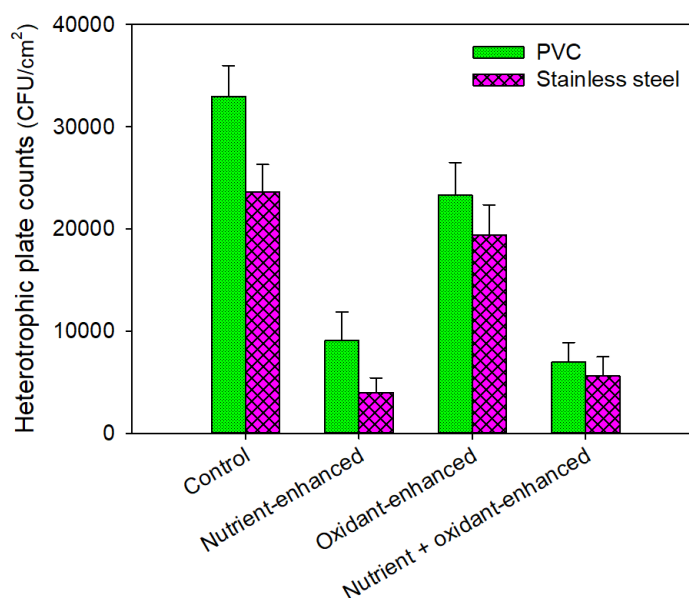
(b)

3 **Fig. 7.** (a) High nucleic acid and low nucleic acid bacteria and (b) heterotrophics plate count in the  
 4 biological activated carbon columns (n = 6).

5 *3.5 Evaluation of biofilm formation potential using Centers for Disease Control and Prevention*  
 6 *biofilm reactors*

7 CDC biofilm reactors were used to evaluate the biofilm formation potential of the effluents from BAC  
 8 columns. Subsequently, the performance data was used to determine which BAC columns would be  
 9 preferred to limit any downstream bacterial regrowth or seeding in the DWDS. The attached HPC levels  
 10 of PVC and stainless steel coupons from CDC reactors were determined (Fig. 8). The differences in  
 11 DOC and AOC in the feed water used for the CDC biofilm reactors were negligible, but the HPC was  
 12 found to be different, as shown in Fig. 8. The attached HPC levels of the coupons from the CDC reactor  
 13 fed with effluent from the control column showed  $3.3 \times 10^5 \pm 3.1 \times 10^3$  cells/cm<sup>2</sup> and  $2.4 \times 10^5 \pm 2.7 \times 10^3$   
 14 cells/cm<sup>2</sup> for PVC and stainless steel coupons, respectively. The HPC level in the nutrient-enhanced  
 15 BAC columns was 72% lower than that of the control ( $9.10 \times 10^3 \pm 2.80 \times 10^3$  cells/cm<sup>2</sup>) and 83%  
 16 ( $4.00 \times 10^3 \pm 1.44 \times 10^3$  cells/cm<sup>2</sup>) lower than that of PVC and stainless steel coupons, respectively.

1 Overall, the highest HPC level detected in a CDC biofilm reactor was related to the control column.  
2 The biofilm reactor fed with the control effluent also showed the highest HPC. Conversely, CDC  
3 reactors fed with effluents from the nutrient-enhanced column and nutrient + oxidant-enhanced column  
4 exhibited a relatively smaller biofilm formation on the coupons. Therefore, the distribution systems  
5 could also benefit from the use of nutrient-enhanced BAC, but further work to determine the community  
6 composition of the BAC effluent would be beneficial to determine the downstream impact on the  
7 DWDS.



8  
9 **Fig. 8.** Effects of nutrient-enhanced, oxidant-enhanced, and nutrient + oxidant-enhanced biological  
10 activated carbon filtration on polyvinyl chloride (PVC) and stainless steel coupons using heterotrophic  
11 plate counts (n = 3).

### 12 3.6 Trihalomethane formation potential and trace organic contaminants

13 The THMFP was analyzed to evaluate the potential for formation of disinfection byproducts in the  
14 effluent from the BAC columns (Fig. S9). The total THMFP of the influent was 103 µg/L, and the  
15 chloroform formation potential (CHCl<sub>3</sub>FP) accounted for 76% of the THMFP. Therefore, the THMFP

1 was governed by the extent of the reduction of  $\text{CHCl}_3$ ,  $\text{CHCl}_2\text{Br}$ ,  $\text{CHClBr}_2$ , and  $\text{CHBr}_3$  produced by the  
2 bromination reaction, which accounted for 14%, 1%, and 9% of the total THMFP, respectively. The  
3 total THMFP for the control, nutrient-enhanced, oxidant-enhanced, and nutrient + oxidant-enhanced  
4 columns were 70, 68, 74, and 76  $\mu\text{g/L}$ , respectively. Fig. S4 shows that more than 98% of the LMW  
5 acid fraction was effectively removed from the feed water in the BAC columns. The acidic organic  
6 matter contributed to the formation of THM [19, 36, 37]. Therefore, it was considered that the reduction  
7 of the LMW acidic fraction in the column contributed to the removal of THMFP. The  $\text{CHCl}_3$   
8 concentrations in all column effluents was between 56  $\mu\text{g/L}$  and 61  $\mu\text{g/L}$ , which was reduced by about  
9 22–29% compared with that in the feed water. The significant reduction of  $\text{CHBr}_3$  was confirmed in all  
10 the columns. Therefore, it was also confirmed that the reduced THMFP did not differ from that in the  
11 control in the enhanced BAC columns. An earlier study also reported that biofilter enhancement did not  
12 improve water quality regarding the THMFP [12].

13 The removal of selected TrOCs was not significant between the enhanced BAC columns. Table S4  
14 shows the removal performance of selected TrOCs in the BAC columns. In order to remove the effect  
15 of ozonation, selected TrOCs were introduced without ozonation into the enhanced BAC columns and  
16 more than 78% were attenuated, except for carbamazepine (18–33%). Carbamazepine, which is an  
17 antiepileptic drug, is a recalcitrant compound that is highly stable in soil and sewage treatment plant  
18 effluents. Moreover, carbamazepine was proposed as an anthropogenic marker to determine the water  
19 quality owing to its persistent characteristics in the aquatic environment [38]. Table S5 shows the  
20 removal of TrOCs via ozonation followed by BAC. Selected TrOCs further attenuated the ozonation  
21 followed by BAC (>99%;  $\text{O}_3$ +BAC). Therefore, hybrid systems combine one or more treatments in the  
22 removal of persistent compounds. However, there was no difference in the removal of selected TrOCs  
23 in the enhanced BAC columns compared with that of the control.

24

#### 25 **4. Conclusions**

1 Significant reduction with respect to head loss, namely 63–79%, was observed in the nutrient-enhanced,  
2 oxidant-enhanced, and nutrient + oxidant-enhanced BAC columns compared with that of the control  
3 column. EPS formation, which included proteins and carbohydrates, tended to be reduced in the  
4 enhanced BAC columns when compared with that of the control. Moreover, the HMW organic fractions  
5 in nutrient-enhanced, oxidant-enhanced, and nutrient + oxidant-enhanced columns were significantly  
6 reduced. The enhancement of BAC filters using phosphate was successful in promoting bacterial growth  
7 but decreased the EPS production. HNA bacteria were predominant in the influent but decreased  
8 remarkably in the effluent. CDC biofilm reactor studies showed that the microbial abundance on  
9 coupons connected to the enhanced BAC columns was relatively low compared with that of the control.  
10 Enhanced BAC filters via phosphate or hydrogen peroxide had no effect on water quality improvement  
11 with respect to the removal of DOC, THMFP, and selected TrOCs. The enhanced BAC-treated water  
12 reduced the head loss and formed less biofilm on selected coupons in this study, which indicated that  
13 there was less biofilm formation in the distribution systems.

14

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