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Effects of phosphate addition on the removal of disinfection by-product formation potentials by biological activated carbon filtration

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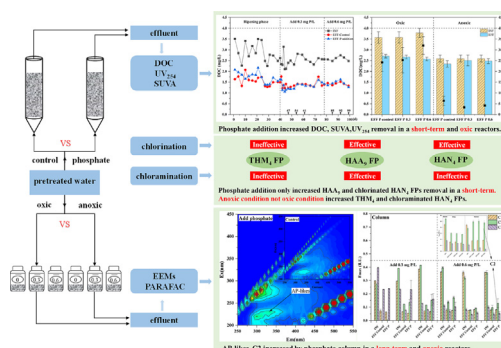
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HIGHLIGHTS

- BAC columns presented good performance for water purification and DBP control.
- DOM fluorescence substances were removed by aerobic bacteria instead of anoxic bacteria.
- P addition decreased chlori(am)inated HAA₉ and HAN₄ FPs as well as EPS on the short-term.
- Removals of SMP-like, HA-like and DBP FPs decreased on a long-term phosphate addition.
- BAC positive response to phosphate addition was attributed to aerobic bacteria not anoxic bacteria.

GRAPHICAL ABSTRACT



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ABSTRACT

In drinking water treatment plants (DWTPs), the widely used biological activated carbon filters (BACFs), as the last barrier before disinfection, can remove dissolved organic matter (DOM) known as precursors of disinfection by-products (DBPs). Whether phosphate addition can improve water purification and DBP control of BACFs is still controversial. This study investigated short-term and long-term effects of phosphate addition on controlling DBP formation potentials (FPs) by BACFs via column and batch experiments. The BAC columns presented good water purification performance: they removed around 50 % DOM, nearly all fulvic acid-like and humic acid-like as well as 5 %–70 % chlor(am)inated THM₄, HAA₉ and HAN₄ FPs (except chloraminated THM₄ FPs), which was mainly contributed by aerobic bacteria not anoxic bacteria. Phosphate addition within 7–14 days further improved removals of DOM, aromatic organics, fluorescence fractions in DOM as well as HAA₉ and HAN₄ FPs (especially TCAA FP and TCAN FP) to different extent. However, this improvement did not last longer, and removals of DOM, aromatic organics, two fluorescence fractions (soluble microbial byproduct-like and humic acid-like) and DBP FPs decreased despite long-term phosphate addition. Oxic and anoxic batch experiments showed that the positive response of water purification to short-term phosphate addition was also mainly attributed to aerobic bacteria and not to anoxic bacteria. For example, the former decreased DOM and DBP FPs, while the latter increased protein- and tryptophan-like substances as well as chloraminated THM₄ FPs. Phosphate addition resulted in EPS increase in anoxic reactors and decrease in oxic reactors. These results indicated that a high dissolved oxygen in BACFs may be helpful for water purification and DBP control.

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Overall, short-term phosphate addition into phosphorus-limited water is beneficial for BACFs to control DBPs while long-term addition has no effect. Therefore, an intermittent phosphate addition into BACFs is suggested to control DBPs in DWTPs.

1. Introduction

Dissolved organic matter (DOM) is ubiquitous in the environment, and its complexity and diversity pose challenges to drinking water treatment plants (DWTPs). DOM can react with chlorine-containing disinfectants during drinking water disinfection to form >700 regulated and unregulated disinfection by-products (DBPs) (Chu et al., 2021; Lou et al., 2014; Xiao et al., 2023), which have been linked to significant health risks such as an increased risk of birth defects, bladder cancer and miscarriages (Andersson et al., 2019; Villanueva et al., 2015). Therefore, the United States Environmental Protection Agency (USEPA) has set standards for total trihalomethanes (THM₄) and five haloacetic acids (HAA₅) at 80 and 60 µg/L respectively in drinking water (National Primary Drinking Water Regulations, 2006). EU regulated that in drinking water THM₄ and HAA₅ do not exceed 100 and 60 µg/L in the newest European Drinking Water Directive (EU, 2020). In addition, haloacetonitriles (HANs) in drinking water have also attracted much attention. The cytotoxicity and genotoxicity of HANs, a class of nitrogenous DBPs (N-DBPs), are similar to or even higher than those of THMs and HAAs, known as two classes of carbonaceous DBPs (C-DBPs) (Tan et al., 2017).

Removing DBP precursors preceding disinfection is the simplest and most effective method to reduce DBP formation (Bond et al., 2012; Sadiq and Rodriguez, 2004). Biological activated carbon filters (BACFs) have been widely used in a number of DWTPs since it is cost-effective for water purification (Onstad et al., 2008). As the last barrier before the disinfection in DWTPs, whether BACFs can effectively remove DOM, the precursors of DBPs, is of concern. An appropriate C: N: P ratio for bacterial proliferation and growth is 100: 10: 1 (Lauderdale et al., 2012). Typically, BACFs remove DOM primarily through localized microbial-mediated biodegradation. Phosphorus is an essential nutrient for microbial growth, meaning that phosphorus limitation could inhibit microbial activity (Selbes et al., 2016). In DWTPs, the coagulation process as a conventional water treatment technology can effectively remove phosphorus from source water (Juhna and Rubulis, 2004; Lauderdale et al., 2012), which results in very low phosphorus concentrations (Lehtola et al., 2002), so usually the influent of BACFs contain limited phosphorus with concentration <0.01 mg/L (Banihashemi et al., 2017). Some studies (Nishijima et al., 1997; Noh et al., 2020; Ross et al., 2019; Selbes et al., 2016) have found that adding phosphate did not improve DOC removal efficiency, while other studies showed that the removal efficiency of DOC by BACFs with phosphate addition increased by 14 % (Banihashemi et al., 2017) and 8.3 % (Lauderdale et al., 2012; Stoddart and Gagnon, 2017), respectively. Moreover, the effect of phosphate addition on DBP formation potentials (FPs) removal has been rarely studied (van der Aa et al., 2000).

Microorganisms adsorbed on the surface of BAC use dissolved oxygen (DO) to biodegrade organic matter (Korotta-Gamage and Sathasivan, 2017), and DO was consumed gradually along the depth of the filter bed in BACFs (Feng et al., 2013). Due to the change of DO, aerobic, anoxic and anaerobic microorganisms were distributed in the BACFs (Brown et al., 2001; Rui et al., 2020). Aerobic microorganisms usually fully absorb and reserve phosphate to promote the consumption of organic matter, and their demand for nutrients is C (represented by BOD): N: P = 100: 5: 1, while the phosphate uptake rate under anoxic conditions was lower than that under aerobic conditions (Artan et al., 1998; Gomez et al., 1999; Zhang et al., 2017). Therefore, the effect of phosphate addition on DBP precursors removal ability of aerobic and anoxic microorganisms might be different. There are few studies focusing on the effect of phosphate addition on aerobic and anoxic microorganisms in BACFs. To further clarify the internal mechanism of phosphate addition on DBP FPs removal by BACFs, it is

necessary to explore the physiological characteristics of aerobic and anoxic microorganisms and the response of water purification to phosphate addition.

The concentration and composition of DOM are important factors affecting the formation of DBPs (Krasner, 2009; Mian et al., 2018; Xu et al., 2022c). Many studies showed that THM and HAA FPs are closely related to humic-, fulvic-, and protein-likes in natural water environment (Jutaporn et al., 2020; Nguyen et al., 2013; Xu et al., 2021; Yang et al., 2015b). The intensities of Parallel Factor Algorithm (PARAFAC) components and the relative distributions of three-dimensional fluorescence excitation-emission matrices (EEMs) have been widely used to represent levels and chemical composition of fluorescent DOM (Coble, 1996). The effect of phosphate addition on the removal of DOM by BACFs was studied, and usually SUVA (Kitis et al., 2002) and DOC (White et al., 2003) were measured as quantitative index of DOM, and the components of DOM were rarely qualitatively analyzed. Fluorescence spectroscopy is a valuable tool for identifying and quantifying different types of DOM components. Further research is needed to better understand the relationship between different DOM components and DBP formation.

The objectives of this study were to investigate the short-term and long-term influences of phosphate addition on water purification and DBP FP removal by BACFs, and to further understand the mechanism behind it by separating aerobic and anoxic bacteria in BACFs.

2. Materials and methods

2.1. Materials

The standard products of DBPs including THMs (trichloromethane (TCM), bromodichloromethane (BDCM), chlorodibromomethane (DBCM) and tribromomethane (TBM)); HAAs (bromoacetic acid, chloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromochloroacetic acid (BCAA), bromodichloroacetic acid, dibromochloroacetic acid, dibromoacetic acid and tribromo acetic acid); HANs (trichloroacetoneitrile (TCAN), dichloroacetoneitrile (DCAN), bromochloroacetoneitrile (BCAN) and dibromoacetoneitrile (DBAN)) and chromatographic Methyl tert-butyl ether (MtBE, high performance liquid chromatography grade) were all purchased from Shanghai Balingwei Technology Co., LTD. Other chemical reagents including sodium hypochlorite, ammonium chloride, phosphate buffer salt, anhydrous sodium sulfate, hydrochloric acid, sodium hydroxide, nitric acid and sulfuric acid were purchased from Shanghai Sinopharm Chemical Reagent Co., LTD. Ultra-pure water was prepared by Millipore Milli-Q Water purification system (18 M Ω ·cm, Billerica, MA, USA). All the gases used in the experimental instruments were provided by Shanghai Chunyu Special Gas Company. The filtration membranes with 0.45 µm aperture were purchased from Shanghai Titan Technology Co., LTD. Hash residual chlorine powder pillow bag, total chlorine powder pillow bag and phosphate powder pillow bag were purchased from Shanghai Bangwoo Instrument Equipment Co, LTD. Pierce® BCA Protein Assay Kit was purchased from Shanghai Yul Biotechnology Co, LTD. All DBP standard products were of guarantee grade and other chemicals were of analytical grade.

The BAC used in this study was derived from coal and collected from a BACF which had been running for one year in a DWTP located in Jiangsu Province. The water used in this study was taken from Lake Panchi at the campus of Shanghai University every 3–5 days. To confirm the stability of the water quality, the physicochemical indexes were measured immediately every time after collection. The water quality result is shown in Table S1 in the supplemental information. The water quality met the

standard of drinking water source set in China (Su et al., 2017). In this study, Panchi Lake water was chosen as raw water, which was then coagulated and precipitated before entering the BACFs in the laboratory to simulate the drinking water treatment process. Based on the result of preliminary experiment with different dosages of polyaluminium chloride (PAC), 0.015 g PAC / L was used in the coagulation process (Fig. S1). Considering that ozonation before BACFs can oxidize the organic matter into biodegradable DOC in DWTP, 0.1 mg/L C-CH₃COONa was dosed to increase the content of biodegradable DOC in the influent of the BACFs without ozone pretreatment in this study.

2.2. Experimental design

2.2.1. Column experiments

To investigate the effect of phosphate addition on the removal of DOM and DBP FPs in BACFs, two BAC columns with and without phosphate addition (namely phosphate column and control column) were built. The columns were made of PVC, with a diameter of 20 mm and a height of 25 cm. The filling height was 20 cm, with BAC filler mentioned above. As is shown in Fig. 1, two columns are in series and they all operated in upflow mode. The filtration rate was set at 1 m/h in this study according to the actual engineering and previous studies. Usually, oxic and anoxic conditions simultaneously exist in BACFs in practice, and also in the BAC columns of this study: the influent and effluent DO was 6.64–8.09 mg/L and 0.01–0.33 mg/L respectively (Fig. S3). The pretreated raw water flowed into BAC columns through the peristaltic multi-channel pump (BT100-1 L, Shanghai Lange constant flow pump Co., Ltd.). Columns were wrapped with black plastic to ensure darkness and prevent algae growth at ambient temperature. After coagulation and sedimentation, the phosphate concentration is <0.01 mg P / L. It was found that 0.6 mg P/L addition into BAC column resulted in the lowest DBP production in drinking water distribution system (Xing et al., 2018). The maximum phosphate dosage in previous studies was 0.6 mg/L (Zhang and Andrews, 2012). Therefore, the dosage of phosphate was set as 0, 0.3 and 0.6 mg P/L in this study.

The experiment lasted for more than three months, in which the ripening phase was >1 month (day 0–40). The water was refreshed every 3 days until BAC columns reached a stable state of DOC removal. Following the ripening period was the phosphate dosing stage, which was divided into two stages. In the first phase (day 41–78), 0.3 mg P-Na₂HPO₄/L (0.3 mg P/L) was added to the phosphate column. In the second phase (day 78–99), after the effect of dosing of 0.3 mg P/L on DOC removal was

weakened, 0.6 mg P-Na₂HPO₄/L (0.6 mg P/L) was added to the phosphate column. During the experiment, the influent and effluent water samples of both BAC columns were collected every 7 days to determine DOM and DBP FPs. Each phase was sampled at least three times.

2.2.2. Batch experiments

As stated earlier, oxic and anoxic conditions simultaneously exist in BACFs usually. To investigate the mechanism of phosphate addition in the BACFs, the removal of DOM and DBP FPs after phosphate addition were studied using oxic and anoxic batch reactor experiments. The oxic and anoxic batch experimental processes are presented in Fig. 2. Twelve brown glass bottles with a volume of 500 mL were filled with 50 g (wet weight) BAC and 450 mL of pretreated water as described earlier. All batch reactors were placed in the dark at a temperature of 25 ± 1 °C. The six oxic batch reactors were open and cultured with stirring at 150 r/min to ensure enough DO in the water. The other six anoxic batch reactors were sealed using rubber stoppers and the water was stripped with nitrogen gas for 10–15 min when the water was refreshed. All batch reactors were refreshed every 5 days. Steady conditions for DOC removal in the batch reactors were reached in the ripening phase (Lekkerkerker-Teunissen et al., 2012). As is shown in Fig. S2, the removal rates of DOC were steady in the oxic and anoxic reactors on day 51 and 126, respectively. Afterwards, 0, 0.3, 0.6 mg P/L was added respectively to different batch reactors in triplicate as shown in Fig. 2. The water samples were collected at the beginning and after 24 h for analyzing DOM-related indicators and DBP FPs. Additionally, BAC samples were also collected in 24 h for analyzing extracellular polymeric substances (EPS) on BAC (Text S1).

2.3. Chlor(am)inated DBP FPs

DBP FPs tests are widely used to evaluate the formation of DBP in drinking water. DBP FPs tests were performed in 40 mL amber glass volumetric bottles under headspace-free conditions in a dark incubator at a temperature of 25.0 ± 0.5 °C (He et al., 2020; Krasner et al., 2012). NaClO stock solution with a concentration of 83 g Cl₂/L was prepared as the chlorine disinfectant, and chloramine solution was configured according to Cl₂: N = 1:1.2 (Mitch and Sedlak, 2001; Mitch and Sedlak, 2002). In DBP FPs tests, the requirements of chlorine and chloramine were calculated according to eqs. (1) and (2) (Chu et al., 2011a), and the disinfection time of chlorine and chloramine was 24 h and 72 h, respectively. At the end of the predetermined reaction time, THMs and HANs were immediately extracted by adding 2 mL MtBE to a 10 mL sample, and HAAs was extracted by adding 4 mL MtBE to a 20 mL sample. HAAs samples were treated with a water bath for 2 h by extracting the upper organic phase, then shaking the samples for 5 min using a multi-tube vortex mixer (DMT-2500, Shanghai, China) at 2300 rpm. Lastly, samples were kept in the refrigerator for <24 h before measurement.

$$\text{Chlorine (Cl}_2\text{) dosage} = 3 \times \text{DOC mg/L} + 7.6 \times \text{NH}_4^+ - \text{N mg/L} + 10 \text{ mg/L} \quad (1)$$

$$\text{NH}_2\text{Cl dosage} = 3 \times \text{DOC mg/L} \quad (2)$$

2.4. DBPs measurement

THM₄, HAA₉ and HAN₄ were decided to be the target DBPs in this study because they were frequently detected in drinking water (Zhang et al., 2020). Detailed information on DBP analytical methods are described in Table 1. They were measured by gas chromatography equipped with an electron capture detector (GC/ECD) (Clarus 680, PerkinElmer, USA) based on the United States Environmental Protection Agency method 551.1 and 552.3.

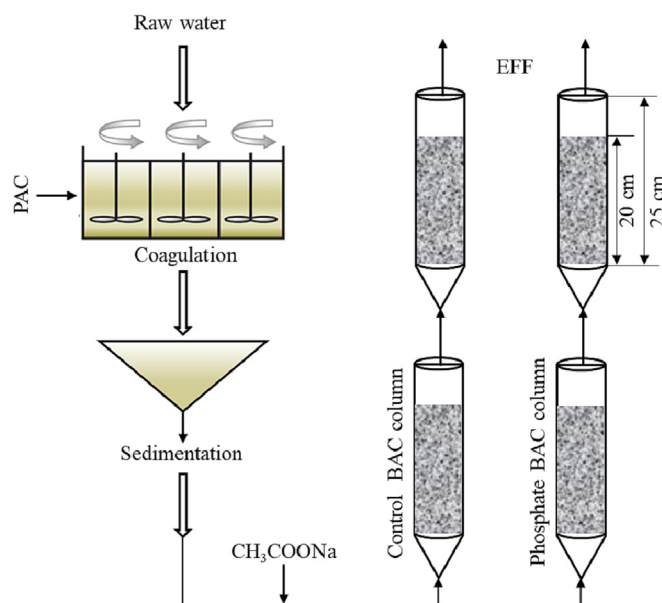


Fig. 1. Schematic overview of BAC columns.

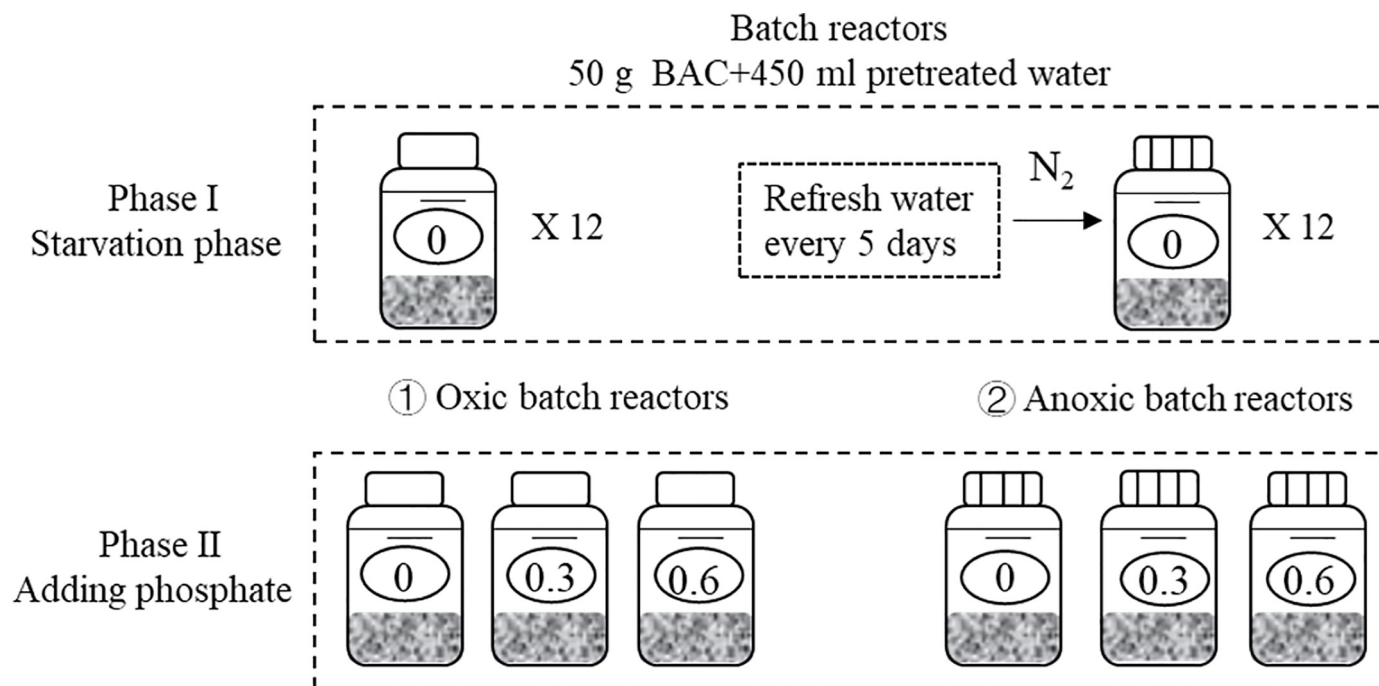


Fig. 2. Batch experiment design.

2.5. EEM, PARAFAC and other fluorescence parameters

The sources, optical properties, structures and chemical behaviors of the

fluorescence response of DOM with similar properties at each region; $I(\lambda_{ex}\lambda_{em})$ is the fluorescence intensity at each excitation/emission wavelength pair.

Table 1

Analytical methods and conditions for DBP measurement.

GC/ECD	Columns	Carrier gas	Injection volume	Vaporizing chamber	Detector	Temperature programs
Clarus 680, PerkinElmer, USA	Elite-5, 30 m × 0.25 mm ID, 0.25 μm film thickness	Nitrogen, constant flow at 3 mL per minute.	1 μL	200 °C	300 °C	THM ₄ Initial temperature at 37 °C for 3 min, then 10 °C per minute to 80 °C and hold for 2 min and finally 20 °C per minute to 220 °C and hold for 1 min. HAA ₉ Initial temperature at 40 °C for 7 min, then 2.5 °C per minute to 65 °C and 5 °C per minute to 85 °C and hold for 1 min finally 20 °C per minute to 210 °C and hold for 5 min. HAN ₄ Initial temperature at 30 °C for 10 min, then 17 °C per minute to 72 °C and hold for 1 min and finally 40 °C per minute to 200 °C and hold for 2 min.

DOM in influent and effluent samples of the BAC columns were analyzed by Fluorescence spectrophotometer (HITACHI F-7000, Japan) with xenon lamp as excitation source (Watson et al., 2018). Fluorescence spectrometer was set to excitation mode and emission mode slit width was 5 nm, excitation wavelength and emission wavelength were 200–450 nm and 210–550 nm respectively. The scanning speed was 1200 nm / min (Zhou et al., 2013). EEMs divided aquatic DOM into five distinct Regions, with aromatic protein-like substances (AP-like) (Regions I and II: Ex < 250 nm, Em < 350 nm), fulvic acid-like substances (FA-like) (Region III: Ex < 250 nm, Em > 350 nm), soluble microbial byproduct-like substances (SMP-like) (Region IV: Ex = 250–280 nm, Em < 380 nm) and humic acid-like substances (HA-like) (Region V: Ex > 280 nm, Em > 380 nm) (Yang et al., 2008). Milli-Q water was used as blank to neutralize the influence of Rayleigh and Raman scattering when the fluorescence region integration (FRI) method was utilized for the quantitative analysis of EEM spectra (Chen et al., 2003):

$$\varphi_i = \int_{ex} \int_{em} I(\lambda_{ex}\lambda_{em}) d\lambda_{ex} d\lambda_{em}$$

where φ_i is the EEM volume at region i, representing the cumulative

PARAFAC uses an iterative three-dimensional array decomposition algorithm based on the alternating least squares principle. Data analysis adopted parallel factor analysis by Matlab software and DOM Fluor toolbox. According to previous studies (Jutaporn et al., 2021; Yang et al., 2015a; Yang et al., 2015b), split-half analysis and residual analysis were used to test the validity of the PARAFAC model and to determine the optimal number of DOM components. Some of the components extracted by PARAFAC can be attributed to specific organic substances present in water samples, but they are more likely to represent groups of organic compounds with similar fluorescent properties. The fluorescence intensity was reported as the maximum fluorescence (Fmax), which is the unique value of each component in every sample that correlates with the relative amount of that fluorescing component.

2.6. DOC, UV₂₅₄ and SUVA measurements

Ultraviolet absorbance (UV₂₅₄), DOC and specific ultraviolet absorbance (SUVA) represent DOM characteristics. DOC was determined by a total organic carbon analyzer. UV absorbance at 254 nm (UV₂₅₄) was detected by a UV–visible spectrophotometer (HACH DR6000). SUVA was

determined by dividing the absorbance of each sample UV_{254} by DOC concentration and multiplying by 100. Error bars in all figures represent the standard deviation from the average of three replications ($n = 3$).

3. Results and discussion

3.1. DOC, UV_{254} and SUVA removal in BAC columns and batch reactors

Fig. 3 shows the results of DOC, UV_{254} and SUVA removal based on the column and the batch experiments with and without phosphate addition. DOC concentrations in the effluent of both BAC columns were similar and

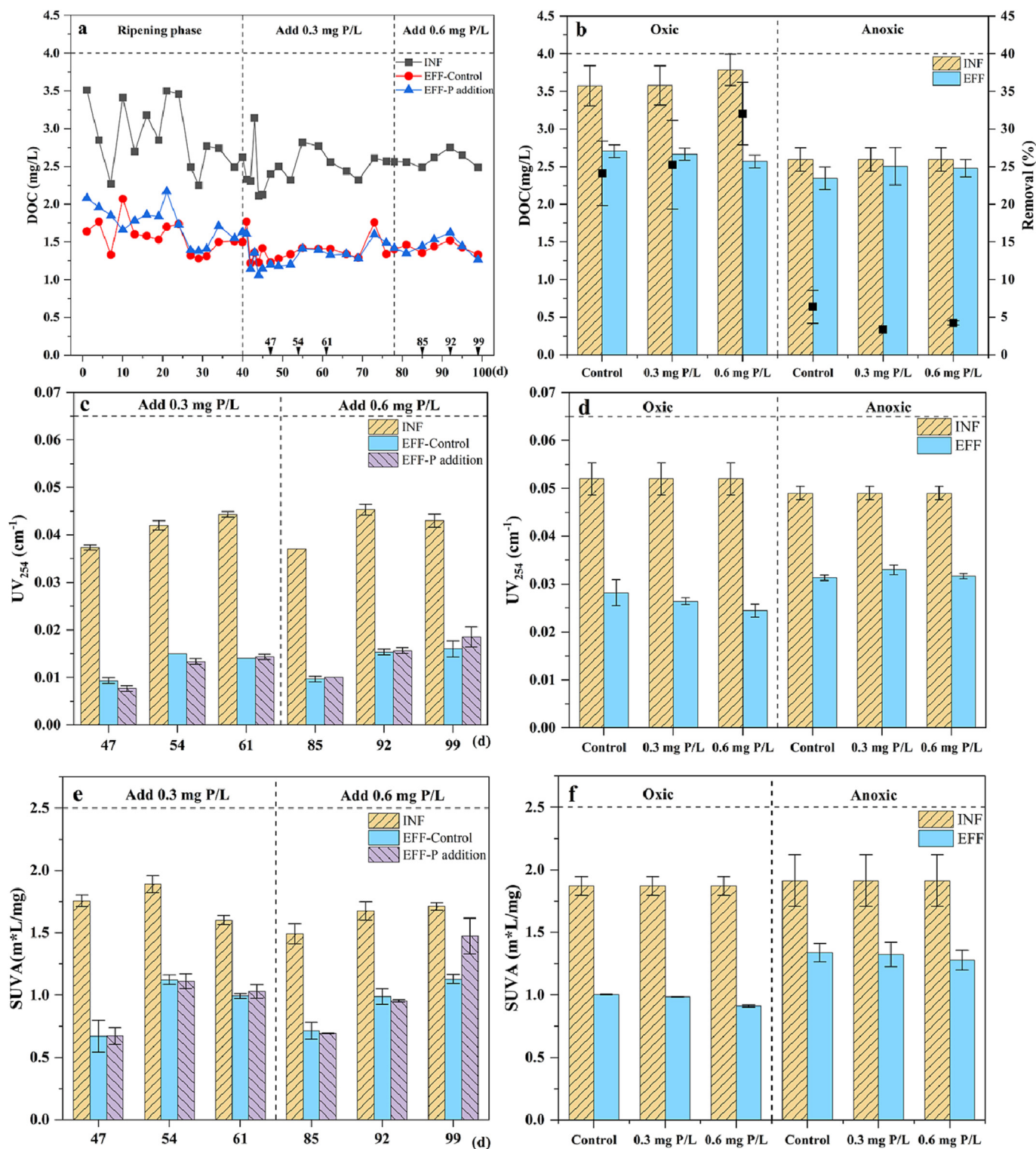


Fig. 3. DOC, UV_{254} and SUVA levels in influent and effluent of BAC columns (a, c, e) and batch reactors (b, d, f) with 0, 0.3 and 0.6 mg P/L. Day 0–40, 41–78 and 78–99 represents ripening phase, 0.3 mg P/L addition phase and 0.6 mg P/L addition phase respectively in Fig. 3a. INF and EFF of batch reactors in Fig. 3b, d, and f mean the water collected at the starting and the end of the experiment. ($n = 3$).

tended to be stable after 25 days during ripening without phosphate addition (Fig. 3a). The addition of 0.3 mg P/L decreased effluent DOC concentrations slightly in the initial 12 days of phosphate addition. The phosphate column consumed more DOC, indicating that it was in phosphorus-limited state (Sang et al., 2003). However, the continuous addition did not cause an evident difference in DOC concentration between the two columns' effluent after 12 days, indicating that long-term phosphate addition in a BACF may not be helpful to DOC removal (Nishijima et al., 1997). For 0.6 mg P/L addition, a result similar to 0.3 mg P/L addition was observed: initially the DOC removal was slightly enhanced but then the impact disappeared (Fig. 3a). The response of DOC removal to phosphate addition in the batch experiments containing oxic and anoxic reactors are shown in Fig. 3b. The removal rate of DOC in oxic reactors was significantly higher than that in anoxic reactors, which is in line with a previous study reporting that high DO was beneficial to increase microbial biological activity and DOC consumption in BACFs (Lu et al., 2020). The addition of 0.3 and 0.6 mg P/L increased DOC removal in oxic reactors, but it did not play a significant role in anoxic reactors.

The results of aromatic DOM removal from the column and batch experiments with and without phosphate addition are shown in Fig. 3c–3f. For the column experiment, the addition of 0.3 and 0.6 mg P/L both slightly lowered UV₂₅₄ and SUVA levels in the effluent at the beginning, but the effect did not last long. On day 61 and 99, UV₂₅₄ and SUVA levels even increased by $2.95 \pm 0.35\%$ and $16.34 \pm 2.44\%$ compared with the control column, respectively. The results from the batch experiments showed that phosphate addition slightly decreased the effluent UV₂₅₄ and SUVA levels under both oxic and anoxic conditions (Fig. 3d and f), indicating that the addition of phosphate was beneficial to aerobic and anoxic bacteria to decompose the aromatic compounds.

Based on both the results of columns and batch reactors above, it can be concluded that Aerobic degradation is much faster than anaerobic and phosphate addition stimulated aerobic bacteria rather than anoxic bacteria to remove more DOC in BACFs. Phosphate addition slightly increased the removal efficiency of aromatic organics on the short-term, which is contributed by both aerobic and anoxic bacteria, but decreased it on the long-term.

3.2. EEM and PARAFAC

Fluorescence EEM spectra together with FRI was performed to investigate the characteristics of DOM. The EEM spectra of influent and effluent of BAC columns and batch experiments with and without phosphate addition are given in Fig. 4. Fig. 4a–4r clearly show that the influent contained FA-likes, HA-likes, APs-likes, and SMP-likes, while all effluents were dominated by APs-like and SMP-likes, indicating BAC columns had good removal of FA-likes and HA-likes. Compared with the control group, three weeks of 0.3 mg P/L addition resulted in a higher removal of AP-likes (type II) in the first two weeks (day 47 and 54). It may be explained by the enhanced oxidation of aromatic rings in AP-likes, which is probably due to enhanced biodegradation by phosphate addition resulting in consumption of more electron donor (Wang et al., 2009). The results of FRI in Table S2 show that the removal rates of AP-likes (type II), FA-likes, SMP-likes and HA-likes increased by 12.6 %, 29.7 %, 7.6 % and 13.2 % respectively after short-time (day 40 to 47) 0.3 mg P/L addition. With time, phosphate addition could no longer increase AP-likes removal (Fig. 4i, l, o and r). Instead, it decreased removal of SMP-likes and HA-likes, indicating that long-term phosphate addition made the microorganisms got used to the phosphate level and therefore the removal percentage of fluorescence substances in DOM could not be enhanced.

Fig. 4A–4H show the EEM fractions of DOM in the influent and the effluent of oxic and anoxic batch reactors. Similar to the BAC columns, the effluent of batch reactors also mainly contained APs-likes and SMP-likes. It can be clearly observed that each fluorescence fraction was partly removed by oxic reactors, while they were hardly removed by anoxic reactors. It is noteworthy that AP-likes (type II) were formed in the anoxic reactors, as shown by the presence in the effluents. The corresponding FRI results of EEM are shown in Table S3. As is observed in Fig. 4E–4H, phosphate addition has

little effect on fluorescence fractions removal in the anoxic reactors. Compared with the control group, the removal percentages of fluorescence fractions in the oxic reactors with 0.3 and 0.6 mg P/L addition increased by 1.8 % and 4.1 %, 0.4 % and 4.3 %, 0 and 1.3 %, 0.8 % and 2.3 %, and – 0.4 % and 2.5 %, respectively. Generally, phosphate addition promoted fluorescence fractions removal in oxic reactors.

In order to further explore the change of DOM characteristic after BACFs, the EEM data above were ulteriorly analyzed by PARAFAC model. The maximum excitation / emission wavelength at a single maximum emission wavelength indicated three fluorescent components, C1, C2 and C3, present in BAC water samples (Fig. S4). The three components were identified to be fulvic-like and humic-like (C1), tryptophan-like (C2) and tyrosine-like (C3) respectively based on previous studies (Hambly et al., 2015; Xu et al., 2022a; Yamashita et al., 2010) (Table S2). Fig. 5 presents the Fmax of the three components in the influent and the effluent of BAC columns and batch reactors. Fig. 5a shows that at the beginning the Fmax removal of C1 were 77.3–82.7 % in phosphate column and 75.4–81.3 % in the control column, showing that phosphate addition slightly increased the C1 removal while decreased C2 and C3 removal on the short-term. On the long-term, phosphate addition did not impact the three main components removal by the BAC columns. The results of Fmax in oxic and anoxic batch reactors are showed in Fig. 5b. $62.9 \pm 4.3\%$ C1 was consumed in the oxic and anoxic reactors. $45.9 \pm 2.0\%$ C2 was consumed in oxic reactors while $28.1 \pm 3.7\%$ C2 was produced in the anoxic reactors, which means that aerobic bacteria instead of anoxic bacteria mainly contributed to the consumption of C2 in the BAC columns. C3 was consumed in both oxic and anoxic batch reactors, and notably its removal was much higher under anoxic conditions than under oxic conditions. In general, adding phosphate into the oxic and anoxic reactors did not significantly improve the removal of the three components. C2, C3 are tryptophan-like and tyrosine-like proteins, which are mainly related to microbial activity (Hambly et al., 2015; Yamashita et al., 2010). The results above illustrate that more DOM components were consumed by aerobic bacteria than anoxic bacteria, which is in line with previous studies that metabolism ability of aerobic bacteria to DOM is higher than that of anoxic bacteria (Xu et al., 2022a; Xu et al., 2022b).

3.3. Effect of phosphate addition on EPS characteristics of BACFs

Fig. 6 presents the effect of phosphate addition on EPS attaching on BAC particles under oxic and anoxic conditions. It shows that phosphate addition to the oxic reactors resulted in an obvious decrease in the content of EPS, mainly manifested as an evident decrease in protein (PN) content and little change in polysaccharide (PS) content. On the contrary, phosphate addition to the anoxic reactors resulted in an increase in the contents of EPS, PN and PS. When 0.3 and 0.6 mg P/L was added, PN/PS ratios in the oxic and anoxic reactors decreased significantly. It is well known that PN content has a stronger correlation than PS content with the surface properties of microbial aggregates (such as hydrophobicity and surface charge) (Liao et al., 2001). A lower PN/PS ratio in the EPS corresponded to a more negatively charged surface and lower hydrophobicity, thereby inhibiting the formation of stable microbial flocs. In this study, phosphate addition decreased the PN/PS ratio in the EPS attaching on BAC particles under oxic and anoxic conditions, indicating that phosphate addition weakened microbial cell aggregation ability and therefore EPS stability, which could result in the release of EPS into the BAC effluent. Additionally, phosphate addition resulted in EPS increase in the anoxic reactors and decrease in the oxic reactors. Therefore, it can be speculated that the addition of phosphate could increase the effluent EPS concentration of anoxic reactors. It might explain why the content of the C2 component increased in the anoxic batch reactors due to the addition of phosphate (Fig. 5b).

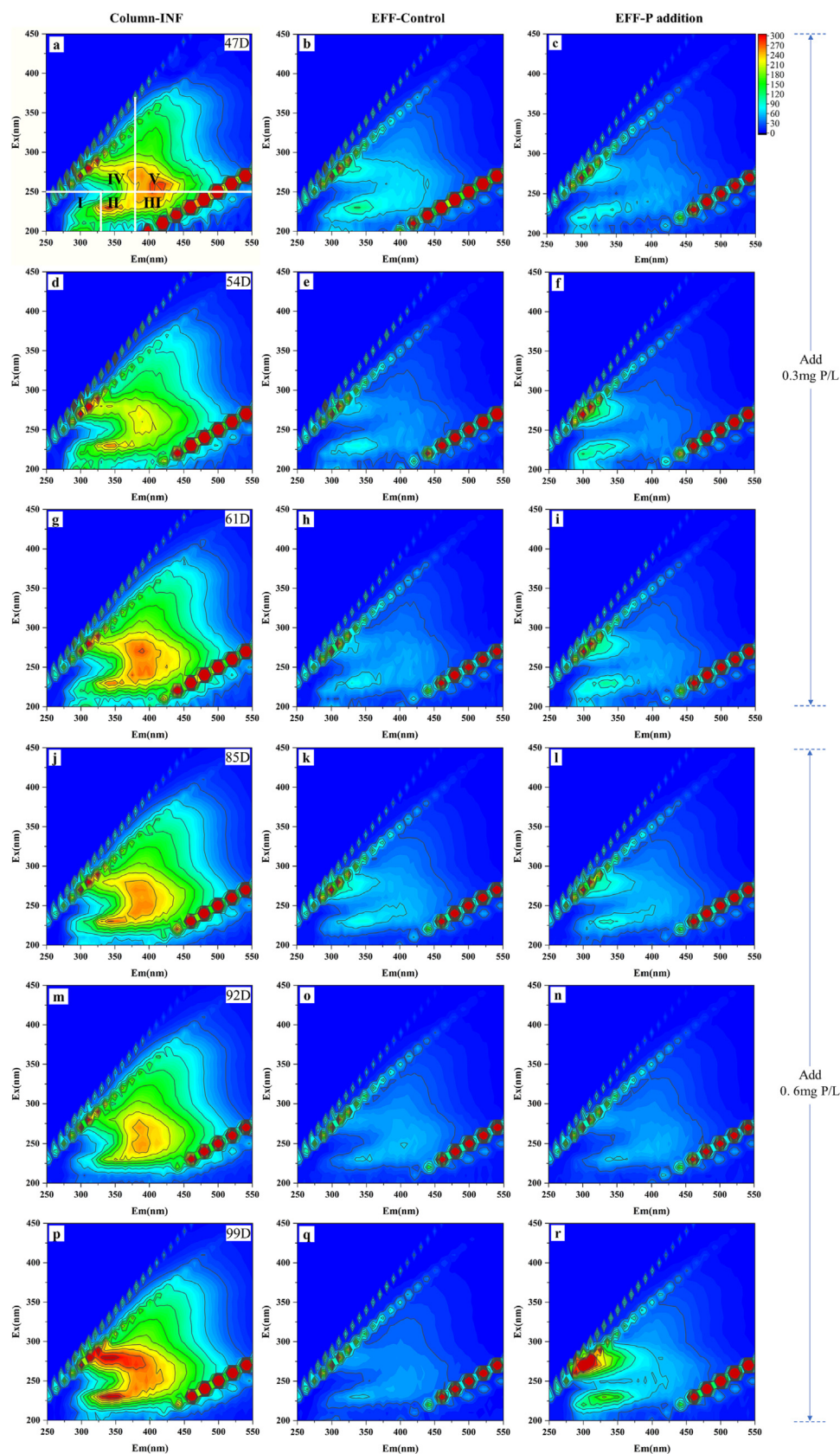


Fig. 4. Influent and effluent EEM spectra of the BAC columns and batch reactors with 0, 0.3, 0.6 mg P/L addition. Regions I-V represent aromatic protein-like substances (AP-likes) type I, aromatic protein-like substances (AP-likes) type II, fulvic acid-like substances (FA-likes), soluble microbial product-like substances (SMP-likes), and humic acid-like substances (HA-likes), respectively. 41D–61D (a-i) and 61D–99D (j-r) represent 0.3 mg P/L addition phase and 0.6 mg P/L addition phase.

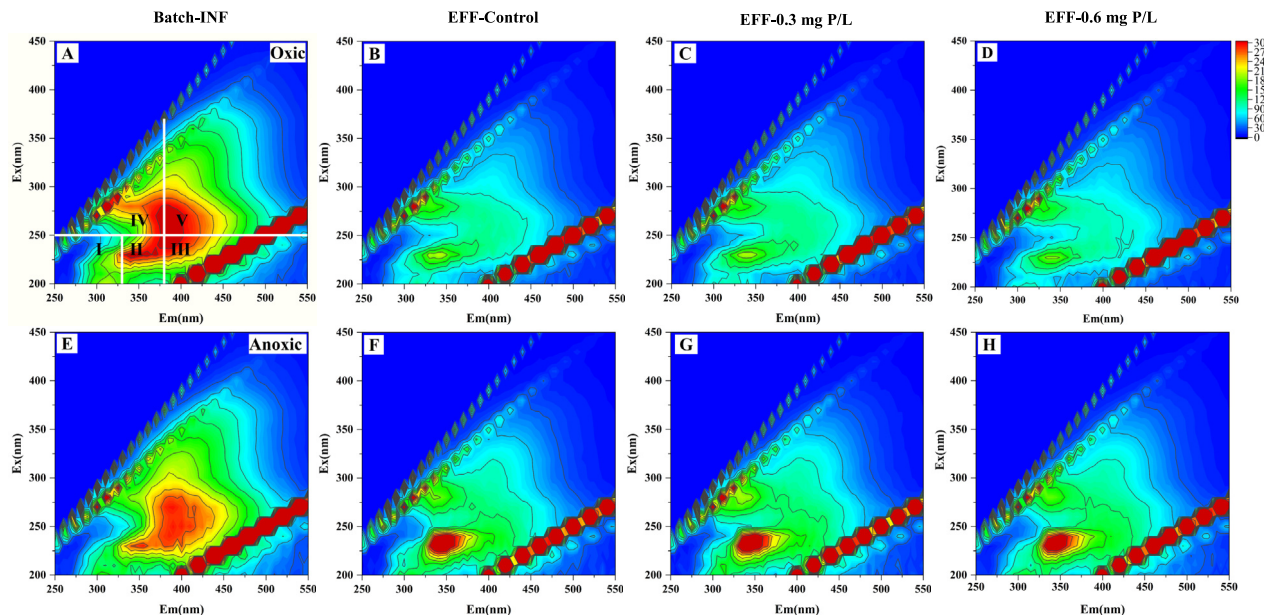


Fig. 4 (continued).

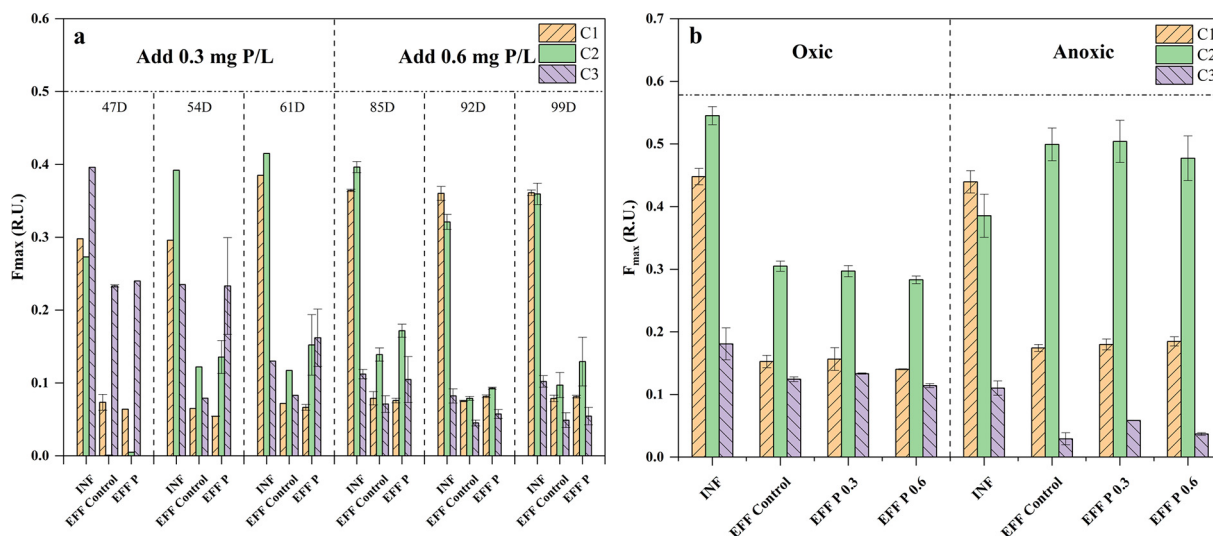


Fig. 5. The Fmax of three fluorescent components in the influent and effluent of columns (a) and batch reactors (b) with 0, 0.3 and 0.6 mg P/L dosed into BAC column and batch reactors. ($n = 3$).

3.4. Effect of phosphate on the removal of DBP FPs by BACFs

3.4.1. Removal of chlor(am)inated THM FPs by BACFs

The results of chlor(am)inated THM₄ FPs in the influent and effluent of columns and batch reactors with and without phosphate addition are presented in Fig. 7. The THM₄ FPs of BAC columns remained relatively constant during the whole experiment. The concentration of TCM FP, BDCM FP, DBCM FP and TBM FP were 194–274 $\mu\text{g/L}$, 76–101 $\mu\text{g/L}$, 20–31 $\mu\text{g/L}$ and 4–12 $\mu\text{g/L}$ in the BAC column effluent (Fig. 7a). The result was the same as a previous study in which the chloroform was the dominant THMs (Liu et al., 2011). The removal percentage of TCM FP by BAC columns was the highest among the four THMs, and TCM FP removal percentages in BAC columns with and without phosphate addition were similar. However, the removal of THM₄ FPs was lower in the phosphate column than in the control column, which is due to a reduced removal of BDCM

FP corresponding to phosphate addition. This phenomenon is consistent with previous studies (Selbes et al., 2016; Selbes et al., 2017). Fig. 7b shows the results of chlorinated THM₄ FPs in BAC batch reactors. Under both oxic and anoxic conditions, different concentrations of phosphate addition cannot significantly improve the removal of THM₄ FPs in BAC batch reactors. Different from the aerobic bacteria, the anoxic bacteria removed only TCM FP but produced DBCM, BDCM and TBM FPs to some degree.

The chloraminated THM₄ FPs of the influent and the effluent of the BAC columns with and without phosphate addition are given in Fig. 7c. Different from the chlorination, the chloraminated THM₄ FP increased greatly after BACFs. Among the THM₄ FPs, BACFs decreased TCM while it increased DBCM, BDCM and TBM FPs. The increased concentrations of BDCM and DBCM FPs in BACFs may be related to the cellular components of anoxic microorganisms in BAC and macromolecular organic compounds such as proteins, polysaccharides and HA secreted during metabolism. They may be important precursors for the formation of CHCl_2Br , CHClBr_2 and

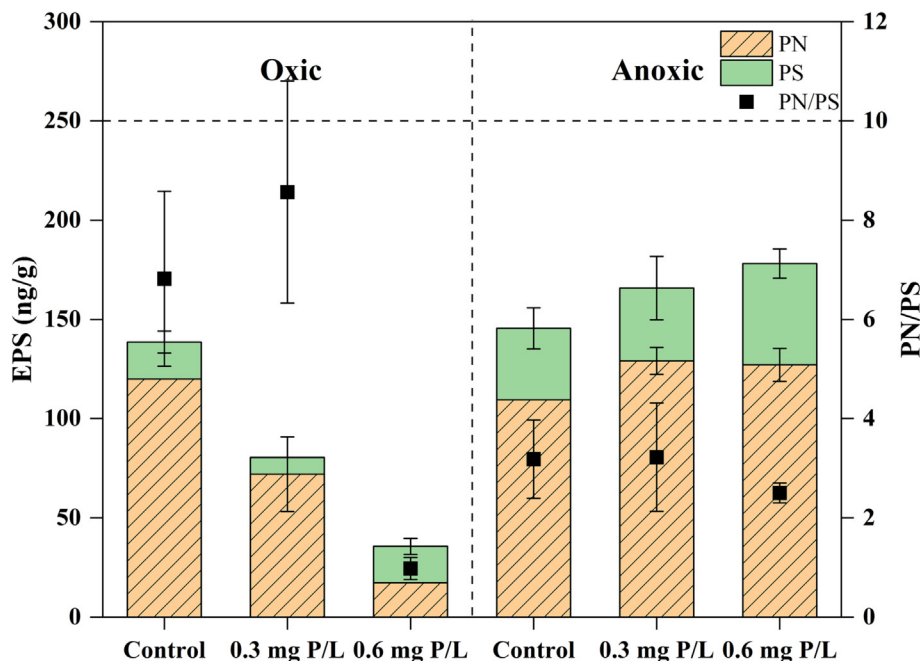


Fig. 6. The effect of phosphate on the characteristics of EPS attaching on BAC particles in oxic and anoxic batch reactors. EPS is the sum of PN and PS. ($n = 3$).

CHBr₃ during chlor(am)ination (Cowman and Singer, 1996). Additionally, phosphate addition increased THM₄ FPs of effluent, which was contributed by the increase of DBCM and BDCM FPs. Fig. 7b further shows the chloraminated THM₄ FPs of the influent and the effluent of the aerobic and anoxic BAC batch reactors. In the oxic reactors, phosphate addition increased the removal of chloraminated THM₄ FPs, which were 2.9 %, 9.3 % and 21.7 % for the control, 0.3 mg P/L and 0.6 mg P/L addition batch reactors, respectively. Nevertheless, in the anoxic reactors, phosphate addition caused a much higher chloraminated THM₄ FPs of the effluent. An explanation may be the increase of chloraminated THM₄ FPs after BACFs, due to the metabolism of anoxic bacteria instead of aerobic bacteria. In conclusion, phosphate enhancement of biofiltration cannot improve the removal THM₄ FPs.

3.4.2. Removal of chlor(am)inated HAA FPs by BACFs

The results of chlor(am)inated HAA₉ FPs in the influent and effluent of columns and batch reactors with and without phosphate addition are presented in Fig. 8. Generally, the chlor(am)ination HAA₉ FPs were removed by the BAC columns (Fig. 8a and c). Fig. 8a and b show that in chlorinated HAA₉, TCAA was predominant. The removal of DCAA, TCAA and BCAA FPs was relatively higher than the removal of other HAA FPs. It can be observed in Fig. 8a that phosphate addition slightly increased the removal of HAA₉ FPs on the short-term (day 47–61), while it reduced the removal of HAA₉ FPs on the long-term (day 85–99). Fig. 8b indicates that DCAA and TCAA FPs tend to be removed by aerobic bacteria while BCAA FP is more easily removed by anoxic bacteria.

In chloraminated HAA₉, DCAA FP was predominant (Fig. 8c and d). TCAA FP was much higher during chlorination than chloramination, and as a result the total chlorinated HAA₉ FPs was much more than the total chloraminated HAA₉ FPs. This difference might be a result of the oxidizing power of chlorine which is much higher than that of monochloramine, and TCAA FP are oxidation products while DCAA and MCAA FPs are substitution and hydrolysis products. This result is consistent with a previous study (Hong et al., 2013). Generally, no obvious difference of the removal of total HAA₉ FPs was detected between the control column and phosphate columns. However, phosphate addition increased the removal of DCAA FP. In addition, Fig. 8d shows that under both oxic and anoxic conditions, different concentrations of phosphate addition cannot apparently improve the removal of HAA₉ FPs after chloramination in BAC batch reactors. In

conclusion, phosphate addition in BACFs cannot impact the removal of chlor(am)inated HAA₉ FPs in the long run.

3.4.3. Removal of chlor(am)inated HAN FPs by BACFs

Fig. 9 shows the results of chlor(am)inated HAN₄ FPs in the BAC columns and batch reactors. The removal of chlorinated HAN₄ FPs in the BAC columns was slightly improved by phosphate addition at the beginning, day 47 and 54, whereas phosphate addition could not improve the removal of chlorinated HAN₄ FPs on the long-term (day 61–99) (Fig. 9a). In oxic batch reactors, phosphate addition improved the removal of chlorinated HAN₄ FPs from 2.9 % to around 8.5 %. In anoxic batch reactors, phosphate addition did not impact chlorinated HAN₄ FPs removal (Fig. 9b). It can be seen from Fig. 9c that among all kinds of HAN₄, the removal of chloraminated DCAN FP by BAC columns was the highest. Phosphate addition neither effectively impacted the removal of chloraminated HAN₄ FPs on both short-term (Fig. 9c and d) and long-term (Fig. 9c), nor under oxic and anoxic conditions (Fig. 9d). The oxic batch reactors mainly removed DCAN FP, while the anoxic batch reactors produced it. The chloraminated TCAN and DBAN FPs were mainly removed by the anoxic batch reactors, while BCAN and DBAN FPs were produced in the oxic batch reactors (Fig. 9d). Different from the removal of THM₄ FPs (Fig. 7) and HAA₉ FPs (Fig. 8), the removal of formation potential of N-DBPs, HAN₄, by BACFs was relatively low (Fig. 9). The possible reason is that the precursors of HANs are not only organic matter, but also have a great correlation with microbial metabolites. For example, Chu et al. (Chu et al., 2011b) reported the nitrogen-rich AP-like and SMP-like as important precursors of N-DBPs. In this study, Fig. 3e and Fig. 4 show that aromatic component and AP-like could increase after phosphate addition on the long-term, which is consistent with the results of DBPs removal by BACFs.

3.4.4. Effect of phosphate addition in different waters

The results in Fig. 7–9 demonstrate that BACFs effectively decreased all chlor(am)inated THM₄, HAA₉ and HAN₄ FPs (except chloraminated THM₄ FPs), attributed to aerobic bacteria not anoxic bacteria. Phosphate addition did not increase the removal of chlor(am)inated HAA₉ and HAN₄ FPs on the long-term. Instead, it decreased the removal of chlor(am)inated THM₄ FPs, attributed by anoxic bacteria.

Table 2 shows the removal of organic matter and DBP FPs by BAC with phosphate addition reported in previous studies. It can be found that the

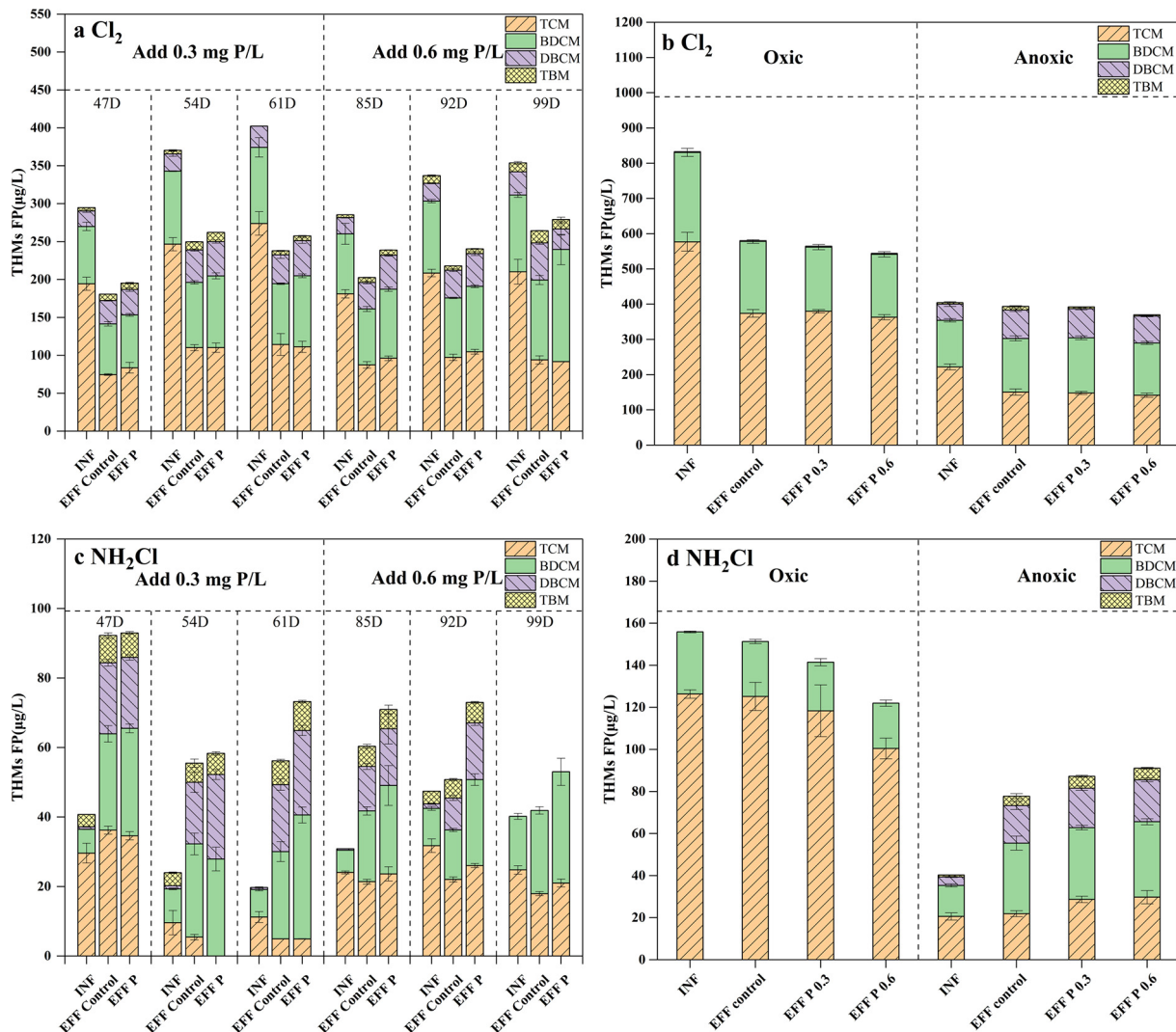


Fig. 7. The chlor(am)inated THM₄ FPs in the influent and effluent of columns (a, c) and batch reactors (b, d) with 0, 0.3 and 0.6 mg P/L addition. ($n = 3$).

effect of adding phosphate on the removal of organic matter and DBP FPs by BAC in previous studies is variable. It might be related to whether the phosphorus in the water source is limited. The appropriate mass ratio of C:N:P for bacterial growth is 100:10:1 (Selbes et al., 2016). Usually, it is considered to be phosphorus limiting when phosphorus concentration is <0.01 times of C concentration. When phosphorus in source water is not limited, phosphate addition cannot significantly affect the removal of organic matter and DBP FPs by BACFs (Selbes et al., 2016; Selbes et al., 2017; Vahala et al., 1998), otherwise adding phosphate can improve the performance of BACFs (Lauderdale et al., 2012; Ross et al., 2019; Sang et al., 2003; Stoddart and Gagnon, 2017). Additionally, adding phosphate had a significant effect on the removal of DBP FPs in BACFs for only a short time. Previous studies also showed that the effect of phosphate addition is not permanent, and diminishes over time (Lauderdale et al., 2012; Nishijima et al., 1997; Rahman et al., 2016). In this study, the phosphate in source water is limited, with concentrations <0.07 mg/L (data is not shown). The experimental results show that a short-term phosphate addition in phosphorus deficiency columns improved the removal of DOM and all the three chlorinated THM₄, HAA₉ and HAN₄ FPs, while a long-term phosphate addition hardly impact the removal of them, even negatively affected them.

Overall, when the influent of BACFs is phosphate limited, phosphate addition can improve the removal of DOM and formation potentials of various DBPs to some extent, and this improvement works on a short-term instead

of a long-term. Therefore, an intermittent phosphate addition into BACFs is suggested to control DBPs in DWTPs.

4. Conclusions

BAC columns presented good performance for water purification: 44 % DOC removal, 68.4 % UV₂₅₄ removal, and FA-likes and HA-likes complete removal, which was mainly contributed by aerobic bacteria not anoxic bacteria. All DOM fluorescence substances were partly or completely removed by aerobic bacteria but hardly removed by anoxic bacteria, and even anoxic bacteria released tryptophan-like to the effluent.

Both two dosages of phosphate decreased EPS release and improved water purification of BAC columns on the short-term: the removal of DOC, aromatic organics and DOM fluorescence fractions all increased to different extent in the first 7–14 days whereas the effect could not last longer, and even the removal of SMP-likes and HA-likes were weakened as a result of long-term phosphate addition. Anoxic bacteria presented less response to phosphate addition compared to aerobic bacteria. For example, anoxic bacteria decreased the EPS adhesion and released PN and PS into the effluent while aerobic bacteria consumed more PN.

BACFs effectively decreased all chlor(am)inated THM₄, HAA₉ and HAN₄ FPs (except chloraminated THM₄ FPs), attributed by aerobic bacteria. Phosphate addition slightly enhanced the removal of chlor(am)inated HAA₉ and HAN₄ FPs on the short-term, but not on the long-term. However, it decreased the removal of chlor(am)inated THM₄ FPs, mainly contributed

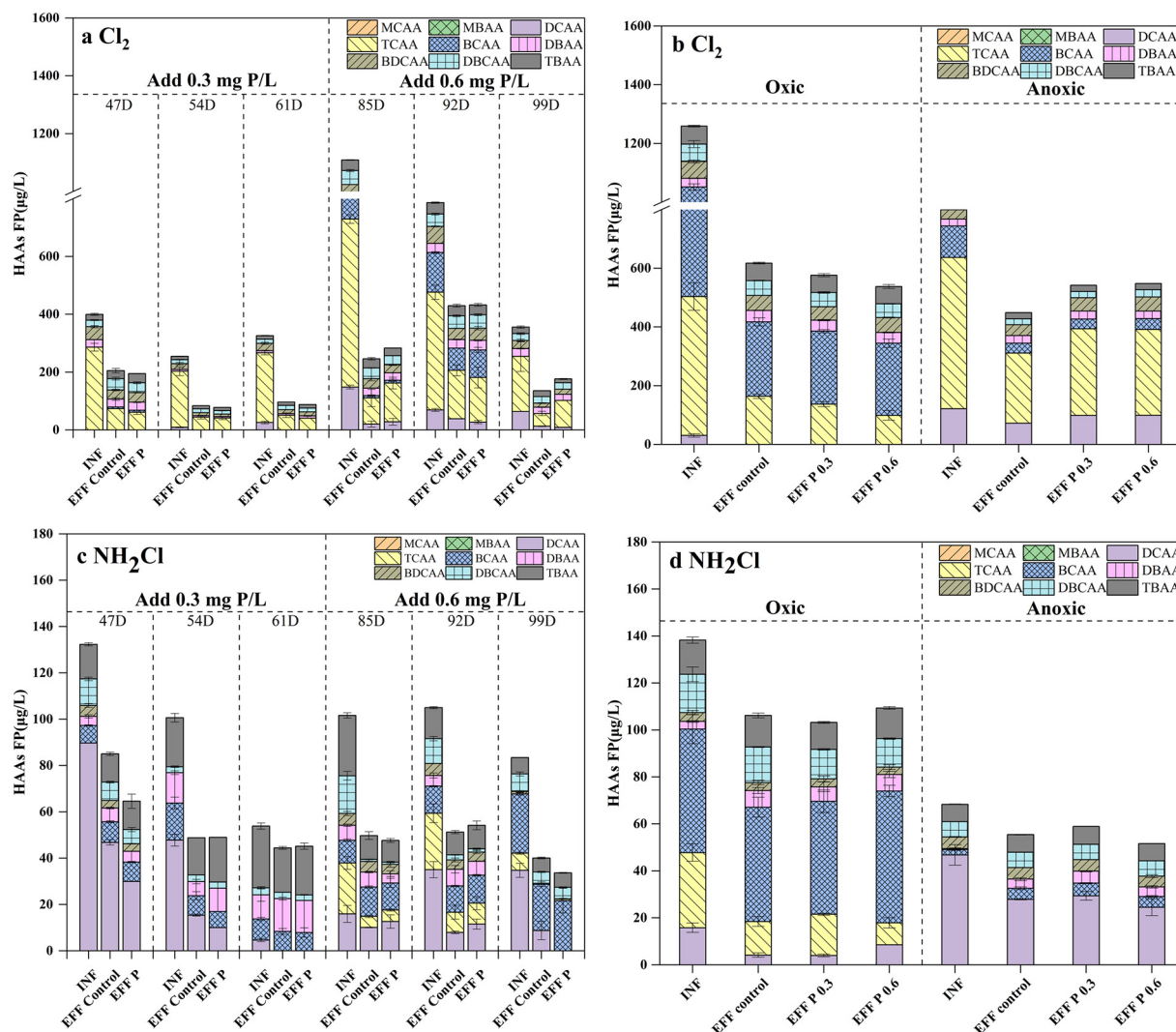


Fig. 8. The chlor(am)inated HAA₉ FPs in the influent and effluent of columns (a, c) and batch reactors (b, d) with 0, 0.3 and 0.6 mg P/L addition. ($n = 3$).

by anoxic bacteria. Based on the results of previous studies and this study, it can be concluded that phosphate addition into phosphorus-limited water can enhance the removal of DOM and formation potentials of various DBPs to some extent, but this effect works only on the short-term and not on the long-term. Therefore, an intermittent phosphate addition into BACFs is suggested to control DBPs in DWTPs.

CRediT authorship contribution statement

Feifei Wang: Conceptualization, Methodology, Writing – review & editing, Project administration. **Yulin Hu:** Investigation, Formal analysis, Validation, Writing – original draft. **Jiazhen Pan:** Investigation, Writing – review & editing. **Jie Zhou:** Investigation, Writing – review & editing. **Chiquan He:** Writing – review & editing. **J.A.M.H. Hofman:** Writing – review & editing. **Wenhai Chu:** Conceptualization, Writing – review & editing. **Jan Peter van der Hoek:** Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.163534>.

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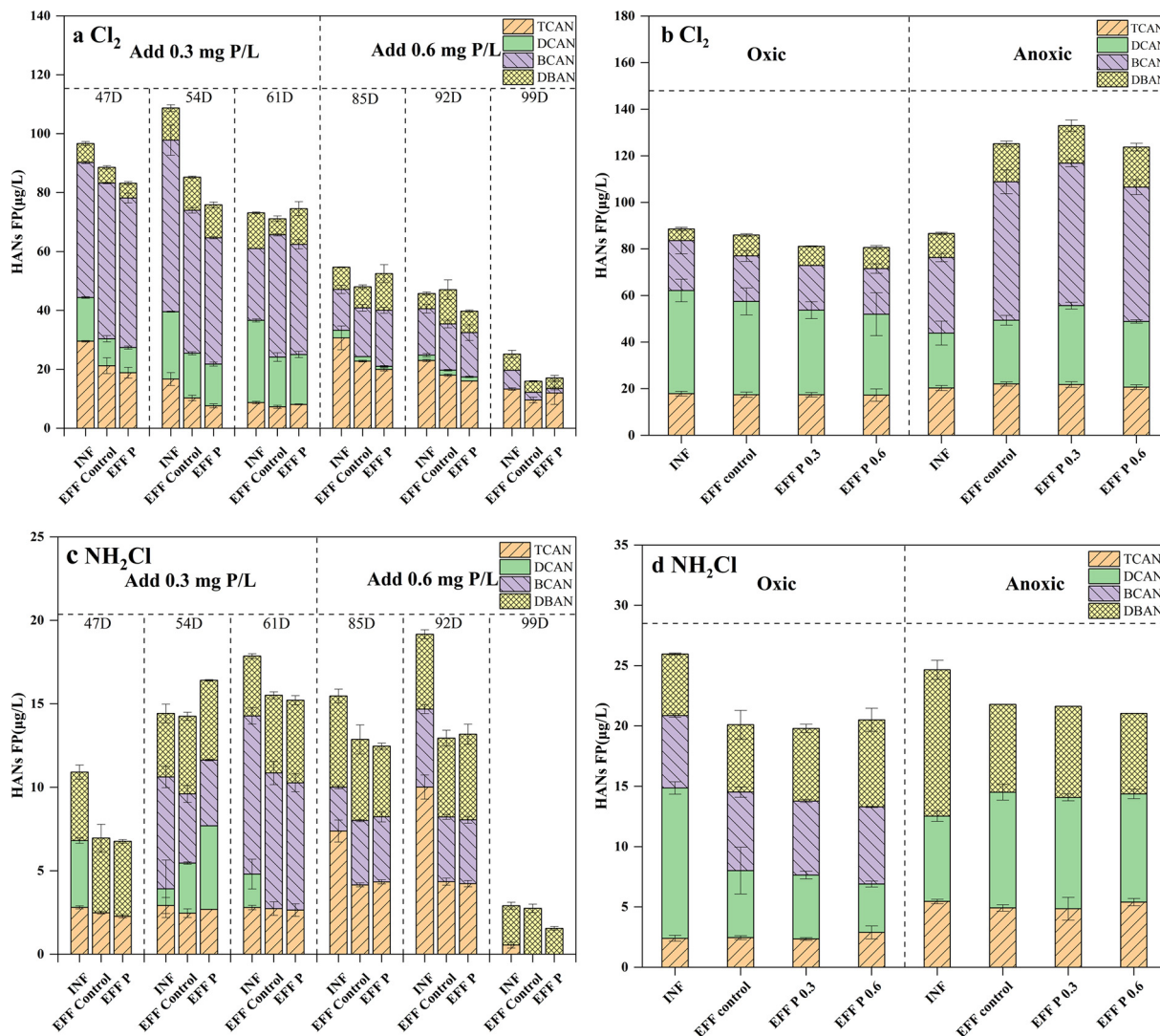


Fig. 9. The chlor(am)inated HAN₄ FPs in the influent and effluent of BAC columns (a, c) and batch reactors (b, d) with 0, 0.3 and 0.6 mg P/L addition. ($n = 3$)

Table 2

Effect of phosphate addition on removal of organic matter and DBP FPs in BACFs.

Scale	C,N,P concentration (mg/L)	C:N:P in original water	C:N:P after P addition	Effect of P addition on DOM removal	Effect of P addition on DBP FP removal	Reference
DWTP	TOC 5.7–5.8 N 0.41–0.43 P 6–8	Not P limited	100:50:30	No significant improvement	/	(Vahala et al., 1998)
DWTP	DOC 3.6 NH ₃ 0.15	100:2:~1	100:2:15	No significant improvement	No significant improvement	(Selbes et al., 2017)
DWTP	DOC 2.9 NH ₃ 0.02	100:<1:~1	100:2:6	No significant improvement	No significant improvement	(Selbes et al., 2017)
DWTP	DOC 1.3–1.6 PO ₄ ³⁻ -P 0.07–0.10	Not P limited	/	No significant improvement	No significant improvement	(Selbes et al., 2016)
DWTP	DOC 2.8–3.3 BDOC: 0.17 ± 0.09 DOC 5.5	P limited	100:/:1	Only slight improvement	Little improvement	(Stoddart and Gagnon, 2017)
DWTP	AOC 0.07 ± 0.01 PO ₄ ³⁻ -P 0.002 NH ₄ ⁺ -N 0.06	100:/:3	100:/:65	Significant improvement	/	(Ross et al., 2019)
Pilot plant	BDOC 0.4 PO ₄ ³⁻ -P 0.02	100:10:1	100:10:2	Significant improvement	/	(Lauderdale et al., 2012)
Pilot plant	NH ₄ ⁺ -N 0.96 PO ₄ ³⁻ -P 0.05	100:/:0.9	100:/:1.6	Significant improvement	/	(Sang et al., 2003)

/ means data loss.

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