Ferrate(VI) pre-treatment and subsequent chlorination of blue-green algae: Quantification of disinfection byproducts

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Abstract

Algal organic matter (AOM) from seasonal algal blooms may be an important precursor of disinfection byproducts (DBPs) in drinking water. This paper presents the effect of ferrate(VI) treatment on two blue-green algae, \textit{Chlorella} sp. and \textit{Pseudanabaena limnetica}, in eutrophic water. The results demonstrated that Fe(VI) removed the algal cells by causing cell death, apoptosis, and lost integrity, and decreased AOM (in terms of total organic carbon) in water via oxidation and coagulation. Chlorination of the Fe(VI) pre-oxidized algal water samples generated halogenated DBPs (including trihalomethanes, haloacetic acids, haloketones, chloral hydrate, haloacetonitriles, and trichloronitromethane), but the concentrations of DBPs were lower than those formed in the chlorinated samples without pre-treatment by Fe(VI). Higher Fe(VI) dose, longer oxidation time, and alkaline pH were beneficial in controlling DBPs. In bromide-containing algal solutions, negligible amount of bromo-DBPs were generated in the Fe(VI) pre-oxidation, and halogenated DBPs were mainly formed in the subsequent chlorination.

Keywords

Algal organic matter; Ferrate(VI); Chlorination; Disinfection byproducts

1. Introduction

Cyanobacterial bloom is ubiquitous in lakes, rivers and reservoirs, and becomes a global problem for drinking water supplies (Cao et al., 2019, Sun et al., 2012, Yao et al., 2019, Zhang et al., 2010). During the death of algal cells, undesirable organic matters, such as
algal toxins and algal organic matter (AOM), were produced into the surface water, causing odor and taste (Janssen 2019, Laszakovits and MacKay 2019, Li et al., 2012, Pivokonsky et al., 2015, Tomlinson et al., 2016). This poses a great challenge to the quality of drinking water. AOM is the product of cell detritus and cell lysis and contains carbohydrate, amino acids, and proteinaceous compounds, which are classified as extracellular organic matter (released by living cells), and intracellular organic matter (from cell lysis) (Fang et al., 2010, Li et al., 2012, Yang et al., 2011). The AOM in water has been shown to contribute to the formation of disinfection byproducts (DBPs) in chlorination and chloramination of drinking water, which include carbonaceous DBPs (C-DBPs) and nitrogenous DBPs (N-DBPs). The yields and speciation of DBPs are affected by the types of AOM and the water characteristics (e.g., pH) (Dong et al., 2019, Fang et al., 2010, Ge et al., 2018, Gonsior et al., 2019, Hua et al., 2018, Huang et al., 2009, Tao et al., 2019, Xiang et al., 2019, Xie et al., 2013, Zhang et al., 2016, Zhou et al., 2014, Zhu et al., 2015). In the last decade, N-DBPs such as haloacetonitriles (HANs) and trihalonitromethanes (THNMs) have attracted a lot of attention because N-DBPs are more toxic than the currently regulated C-DBPs (Plewa et al., 2008, Richardson et al., 2007, Yang et al., 2014).

Chlorination and chloramination of algae in bromide-containing drinking water can generate bromo-DBPs (Dong et al., 2019, Liu et al., 2018, Yang et al., 2011). Bromo-DBPs are significantly more toxic than their chloro-analogues (Dad et al., 2013, Liu and Zhang 2014, Sharma et al., 2014). Therefore, it is pivotal to understand the formation of AOM-derived DBPs (especially bromo-DBPs) during chlorination of eutrophic water. Conventional coagulation is the main treatment process for removing algae in drinking water (Xie et al., 2013). However, the removal is not efficient due to the electrostatic repulsion and steric effects (Ma et al., 2012). Previous studies have reported that the pre-treatment with oxidants such as ozone and chlorine dioxide may enhance the coagulation process to remove algae cells (Ma et al., 2019, Qi et al., 2016, Xie et al., 2013, Zhu et al., 2015). The current paper presents investigation of pre-treatment of algae cells using ferrate(VI) ($\text{Fe(VI)}$).

Fe(VI) may be applied as pre-oxidant because it has a strong reduction potential of $+2.2 \text{ V}$ in acid and $+0.7 \text{ V}$ in base (Wood, 1958). In addition, Fe(VI) is environmentally friendly and can be used as oxidant and coagulant for treating water without producing secondary pollution (Feng et al., 2019, Feng et al., 2018, Shao et al., 2018, Sun et al., 2018). Previous studies have shown that the pre-oxidation of M. aeruginosa (a freshwater cyanobacteria) by Fe(VI) decreased the generation of trihalomethanes (THMs) and haloacetic acids (HAAs) during the subsequent chlorination (Huang et al., 2009, Zhou et al., 2014). Currently, scant information is available on the effects of pH and bromide on the formation of N-DBPs and bromo-DBPs when the Fe(VI) pre-oxidation and subsequent chlorination process was applied to the blue-green algae containing water. In our study, we have performed Fe(VI) pre-treatment/chlorination process on two selected blue-green algal species, Chlorella sp. and Pseudanabaena limnetica (P. limnetica). These species are the most frequently found green algae and blue algae species in Taihu Lake, one of the largest freshwater lakes in China (Su et al., 2017, Tang et al., 2018). Occasional eutrophication of these two algae has also been reported in some shallow eutrophic lakes in India and Taiwan (Venugopalan et al., 1998).
In recent studies of drinking water treatment, flow cytometry was increasingly used for detecting cell activity and membrane integrity after oxidation processes (Moradinejad et al., 2019, Wert et al., 2013, Xie et al., 2013). Wert et al. used a flow cytometer along with chlorophyll-a measurements to investigate cyanobacterial cell damage and lysis (Wert et al., 2013). Moorhouse et al. used flow cytometry combined high performance liquid chromatography to characterize the major bloom in the River Thames (Moorhouse et al., 2018). Different from previous studies, this study newly detected the enzyme activities and cell integrity of algae during Fe(VI) oxidation, which could semi-quantify the early and late apoptosis, survival, and death of algal cells.

The main objectives of this study are: (i) to examine the removal of two blue-green algae Chlorella sp. and P. limnetica by Fe(VI) via evaluating the density of algal cells and determining the structure and characteristics of AOM, (ii) to comprehend the mechanism of removal by investigating the algal death, integrity and apoptosis, (iii) to quantify the generated DBPs (including 4 THMs, 4 HAAs, 5 haloacetonitriles (HANs), 2 haloketones (HKs), chloral hydrate (CH) and nitrotrichloromethane (TCNM)) in the water samples (containing the two tested algae respectively) treated with Fe(VI) pre-treatment followed by chlorination, and (iv) finally, to learn the effects of Fe(VI) dose, Fe(VI) oxidation time, pH, and bromide concentration on the generation of DBPs in order to minimize their formation.

2. Materials and methods

2.1. Reagents and algae cultivation

All reagents were of analytical grades or above and all solutions were prepared with deionized water, obtained from a Milli-Q system with resistivity > 18 MΩ cm. A free chlorine stock (1000 mg/L as Cl₂) solution was diluted from a commercial sodium hypochlorite (NaClO) solution (6–14%, Aladdin, Shanghai, China), and stored at 4 °C. The concentration of hypochlorite was determined before each experiment. Potassium ferrate (Fe(VI), K₂FeO₄) of high purity (> 92%) was synthesized in the laboratory by a wet oxidation method (Li et al., 2005). Stock solutions of Fe(VI) were freshly prepared at 30 min prior to their use. The concentrations of Fe(VI) solutions were determined by measuring the absorbance at 510 nm (ε₅₁₀ nm = 1150 M⁻¹ cm⁻¹). Two fluorescein used in flow cytometry analysis, propidium iodide (PI) and fluorescein isothiocyanate (FITC), were acquired from Sigma Aldrich (St. Louis, USA). The standards of four THMs (chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform (TBM)) and four HAAs (monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), bromochloroacetic acid (BCAA), and trichloroacetic acid (TCAA)) were obtained from Supelco (Supelco Park, PA, USA). The standards of five HANs (dichloroacetonitrile (DCAN), bromochloroacetonitrile (BCAN), trichloroacetonitrile (TCAN), bromoacetonitrile (BAN), and dibromoacetonitrile (DBAN)), CH, TCNM and two HKs (1,1-dichloro-2-propanone (1,1-DCP) and 1,1,1-trichloro-2-propanone (1,1,1-TCP)) of gas chromatographic grade were from Aladdin (Shanghai, China). Other reagents, including methyl tertiary butyl ether (MTBE, chromatographic grade), sulfuric acid and sodium thiosulfate, were purchased from Sinopharm Chemical Reagent (Shanghai, China).
Chlorella sp. (FACHB-25) and P. limnetica (FACHB-1277) were obtained from Wuhan Institute of Hydrobiology, Chinese Academy of Sciences. Chlorella sp. was observed as individual spherical cells and belongs to green algae, P. limnetica was observed as long filaments and corresponds to blue-green algae (Supporting Information Fig. S1). The two algae were cultivated in the BG11 culture medium (Fang et al., 2010) in an incubator at 25 °C under a 12 h light and 12 h dark regimen (MGC-250BP-2, Bluepard instruments). The growth curves are presented in Fig. S2. Chlorella sp. and P. limnetica at the stationary phase were harvested by centrifugation (Thermo Fisher, Germany) at 6000 rpm for 10 min (Daly et al., 2007).

2.2. Removal of AOM and algal cell by Fe(VI)

The removal of algal cells was quantified as the change of chlorophyll-a concentration in the solution (Gao et al., 2010). For each alga (Chlorella sp. or P. limnetica), five 200 mL culture solutions at pH 7.0 with 0.2 M phosphate buffer were prepared by harvesting the alga cells at the AOM concentration of 5 mg/L, measured as total organic carbon (TOC) by an analyzer (TOC-VCPH, Shimadzu). Fe(VI) stock solution was added to the algal solutions at the concentrations of 0, 4.0, 8.0, 12.0 and 16.0 mg/L, respectively. The volumes of added Fe(VI) stock solution was ≤2 mL, which did not significantly change the sample volume. After 10 min stirring (200 rpm), each sample was allowed to settle for 20 min. Then, 80 mL of the supernatant was taken from 5 to 40 cm below the surface, of which 20 mL was used for measuring TOC and chlorophyll-a, and the remaining 60 mL was subjected to chlorination (as introduced later in Section 2.4). To investigate the effect of pH on algal cells removal, the same experiments, presented above (which determined the decrease of chlorophyll-a concentration by Fe(VI) oxidation), were carried out at pH 6.0, 7.0, 8.0, 9.0, and 10.0.

2.3. Effect of Fe(VI) oxidation on algal cell activity and integrity, and AOM characteristics

For each alga (Chlorella sp. or P. limnetica), two 200 mL culture solutions at the initial algal density of 1.0 × 10^8 cells/L were prepared, maintained at pH 7.0 with 0.2 M phosphate buffer, and kept at a thermostat water bath of 20 °C. One of the solutions (of each alga) was spiked with 8.0 mg/L Fe(VI), and the other solution without Fe(VI) addition served as the control. After 10 min stirring (200 rpm), 20 mL of each sample (algae suspension) was quenched by ascorbic acid and subjected to flow cytometry analysis for the cell activity and integrity, and excitation emission matrix (EEM) analysis for AOM characteristics (details are given in Text S1 and Text S2).

2.4. Subsequent chlorination after Fe(VI) treatment

As aforementioned in Section 2.2, a series of 60 mL Fe(VI) oxidized samples were prepared for chlorination. First, each sample was adjusted to pH 7.0. Then, NaOCl was added to each solution at 30 mg/L as Cl₂, and the sample was adjusted back to pH 7.0. Each chlorinated sample was kept in darkness at 20 °C for 72 h. After 72 h, the chlorine residual in each sample was measured and quenched with Na₂S₂O₅ (Table S1). The quenched sample was divided into two aliquots (30 mL each) and subjected to the measurements of volatile DBPs (i.e., THMs, HANs, HKs, CH and TCNM) (Text S3) and HAAs (Text S4), respectively. The physical-chemical properties, limit of detection and recovery of each detected DBP are
listed in Tables S2 and S3. Besides the Fe(VI) dose (0–16 mg/L), various factors affecting the DBP formation, including Fe(VI) pre-oxidation time (5–15 min), reaction pH (6.0–10.0), and bromide concentration (0–0.4 mg/L) in the solution, were investigated in this study (Text S5). All the tests were conducted with the two selected algae at an initial AOM concentration of 5.0 mg/L as C, and the chlorine dose and contact time were fixed at 30 mg/L as Cl₂ and 72 h. The variables and baseline conditions are summarized in Table S4.

It was recently reported that the Fe(VI) oxidation of drinking water samples (containing natural organic matter and bromide) generated bromo-DBPs (Jiang et al., 2016). Thus, in this study, the effect of bromide concentration on DBP formation from Fe(VI) pre-oxidation of AOM was also studied. A group of 60 mL control samples (with Fe(VI) oxidation but without chlorination) were prepared to measure the bromo-DBPs formed from Fe(VI) oxidation of the two selected algae. The Fe(VI) oxidation time of the control samples was increased to 72 h. After 72 h, the Fe(VI) in each sample was undetectable.

3. Results and discussion

3.1. Fe(VI) treatment of algae and AOM

Fig. 1 shows the removal of algal cells and AOM by treatment with various doses of Fe(VI). The removal of *P. limnetica* and *Chlorella* sp. cells increased with the increase in the amount of Fe(VI). At the highest Fe(VI) dose of 16 mg/L, 58.1% and 46.2% of *P. limnetica* and *Chlorella* sp. were removed, respectively. This indicates that *P. limnetica* was easier to remove than *Chlorella* sp. Concentrations of AOM in *P. limnetica* and *Chlorella* sp. solutions (initially 5.0 mg/L as C) kept decreasing when the Fe(VI) dose increased; consistent with the removal of algal cells. At the Fe(VI) dose of 16 mg/L, the AOM concentrations in the *P. limnetica* and *Chlorella* sp. decreased to 2.6 mg/L and 3.4 mg/L, respectively. The removal of algal cells by Fe(VI) might be caused by the oxidation (which induced cell death or lysis) and coagulation (by which the algal cells settled down from the solution). During reaction, Fe(VI) was converted to iron oxide/hydroxide. Previous studies have shown that the iron oxide/hydroxide, formed from Fe(VI), caused coagulation of contaminants in water (Filip et al., 2011, Kralchevska et al., 2016, Prucek et al., 2015). To further distinguish the effects of oxidation vs coagulation, the removals of algal cells and AOM by Fe(VI) (8 mg/L, 0.14 mM), FeCl₃ (0.14 mM) and Fe(III) (from the decomposition of 0.14 mM Fe(VI)) were measured (Text S6 and Table S5). The results show that Fe(III) generated from the decomposition of Fe(VI) (which might be in different forms of Fe(III) complexes, iron oxide/hydroxide or Fe³⁺ ions) had similar coagulation effect on algal and AOM removal as Fe³⁺ (in the form of FeCl₃) did (Prucek et al., 2015). In total, Fe(VI) (the summation of oxidation and coagulation) removed 14.5% of *Chlorella* sp., in which 12.2% was removed by Fe(III) coagulation, and Fe(VI) removed 30.8% of *P. limnetica*, in which 22.1% was removed by Fe(III) coagulation. These indicate that coagulation contributed more than oxidation to the removal of the both algae.

The integrity of algal cell is an important index to assess the death or lysis of cells in the water. Figs. S3 and 2 compared the cell integrity and apoptosis of the two algae with and without Fe(VI) oxidation. The selected Fe(VI) dose was 8.0 mg/L, which caused significant removal of both algae (in terms of algal cell and AOM, see Fig. 1). In Fig. S3, annexin
V−/PI+ (Q1) cells were defined as death (no integrity, no apoptosis); annexin V+/PI+ (Q3) and annexin V+/PI− (Q2) cells were defined as early apoptosis (integrity, apoptosis) and late apoptosis (no integrity, apoptosis); and annexin V−/PI− (Q4) cells were defined as survival (integrity, no apoptosis). With Fe(VI) pre-oxidation, the survival percentage (Q4 in Fig. S3) of Chlorella sp. and P. limnetica cells decreased by 10.6% (i.e., from 99.2% to 88.6%), and 15.1% (i.e., from 96.1% to 81.0%), respectively. Additionally, in Fe(VI) oxidation, the percentage of late apoptosis significantly increased in both algae, and the cells with early apoptosis and death cells were almost the same as those in the control solutions without Fe(VI).

Fe(VI) oxidation induced change on the characteristics of AOM. The partition of excitation and emission matrix of the fluorescence spectra are given in Table S6. Fig. 3a shows that Chlorella sp. had a typical fluorophore peak in region IV (Em/Ex: 250–380/250–400) and V (Em/Ex: 380–540/250–400), and the highest peak was at Ex/Em of 280/335 nm, representing soluble microbial byproduct-like products (SMP). SMP includes the larger molecular weight protein macromolecular organic matters (280/325 nm) and small molecular weight phenol substances (275/306 nm) (Chen et al., 2017). After Fe(VI) oxidation, the intensities of fluorescence decreased (Fig. 3b). The fluorescence regional integration (FRI) method was used to calculate the region-specific EEM volumes (Chen et al., 2003). The EEM volumes in region IV decreased by 18.1% (Fig. 3e). The fluorescence EEM spectra of P. limnetica had the highest peak at Ex/Em of 280/335 nm (Region IV) and Ex/Em of 350/435 nm (Region V) (Fig. 3c), which represented SMP and humic acid-like region, respectively. After Fe(VI) oxidation, the intensities of fluorescence were decreased (Fig. 3d) and the EEM volumes in regions IV and V decreased by 9.7% and 21.4%, respectively (Fig. 3f). The change in AOM (in terms of concentration and characteristics) by Fe(VI) was expected to influence the formation of DBPs in subsequent chlorination.

### 3.2. DBPs formation in subsequent chlorination

#### 3.2.1. Effects of Fe(VI) dose and oxidation time—Fig. 4a and b show the effect of Fe(VI) dose on the formation of DBPs during chlorination of Chlorella sp. and P. limnetica. The concentrations of DBPs generally followed the descending order of HAAs > THMs > CH > HKs > HANs > TCNM. Previous studies also showed that HAAs and THMs were two dominant groups of DBPs generated during chlorination of cyanobacteria (Wert and Rosario-Ortiz 2013). Compared with Chlorella sp., P. limnetica generated higher concentrations of THMs, HAAs, HANs, CH, and TCNM, but lower levels of HKs (Fig. 4c–h). For each group of DBPs, similar trends of the variation of concentration with Fe(VI) dose were observed in the solutions of both algae. The formation of THMs, HAAs, HKs and CH decreased with Fe(VI) dose (Fig. 4c–f). When the Fe(VI) dose increased from 0 to 16 mg/L, the concentrations of THMs, HAAs, HKs and CH in the Chlorella sp. solution decreased by 11.7%, 45.8%, 9.5% and 5.5%, respectively; and the concentrations of THMs, HAAs, HKs and CH in the P. limnetica solution decreased by 12.1%, 36.8%, 17.8% and 45.1%, respectively (Fig. 4c, d, f, and e). Firstly, the decrease of DBPs mainly caused by the removal of AOM (Fig. 1) with Fe(VI) coagulation. Besides, the decrease of THMs might be caused by the high reactivity of Fe(VI) with electron-rich organic compounds such as amines and olefins, which are the main precursors to form THMs (Yang et al., 2013).
Fe(VI) could also oxidize the alcohol and amino acid groups in AOM, which caused a decrease in the formation of CH (Gan et al., 2015, Lee et al., 2009). Formation of HANs decreased when the Fe(VI) dose increased from 0 to 12 mg/L, then increased at the Fe(VI) dose of 16 mg/L (Fig. 4g). It has been reported that Fe(VI) oxidation could degrade the HANs precursor such as amino acids (Casbeer et al., 2013). However, with the increase of Fe(VI) dose to 16 mg/L, the pH value of solution increased, which would reduce the oxidation percentage of Fe(VI) and amino acid. Previous study showed the reaction between Fe(VI) and tryptophan/kynurine slowed down as the pH increased (Casbeer et al., 2013). The concentration of TCNM in the P. limnetica solution became lower when 4.0 mg/L Fe(VI) was added (compared with the solution without Fe(VI) addition) (Fig. 4h). The reaction between Fe(VI) and nitrogenous organic compounds, which may destroy the C-N bond to form inorganic nitrogen matters (Noorhasan et al., 2010). However, when the Fe(VI) dose was increased from 4.0 to 16 mg/L, the formation of TCNM remained increasing. Fe(VI) could oxidize the amine group in aromatic amines to produce nitrobenzene. The nitro products might proceed to form higher TCNM during subsequent chlorination. Sharma et al. certified the aniline moiety of sulfamethoxazole could be oxidized by Fe(VI) to a nitro product (Sharma et al., 2006). Different from P. limnetica, the Chlorella sp. generated almost the same amount of TCNM at different Fe(VI) doses (Fig. 4h). Compared with the Fe(VI) dose, the effect of Fe(VI) oxidation time on the DBPs formation was minor, i.e., the yields of the detected DBPs slightly decreased when the oxidation time increased from 5 to 15 min (see Fig. S4).

3.2.2. Effect of reaction pH—The stability and reactivity of Fe(VI) are highly pH dependent. Fe(VI) decomposes rapidly in acidic pH, but has the maximum stability at pH ~ 10.0. It has been reported that the half-life of Fe(VI) at pH 6.0 was $10^2$ s, which increases $10^5$ s at pH 10.0 (Lee et al., 2014). On the other hand, Fe(VI) exhibits a much stronger oxidation capacity in acidic solution than in alkaline condition (Sharma et al., 1998). Fig. S5 shows the removal percentages of algal cells by Fe(VI) pre-treatment at pH from 6.0 to 10.0. The maximum removal percentages of P. limnetica and Chlorella sp. were observed at pH 8.0 and 9.0, respectively. At higher pH, the removal of algal cells might be limited by the weak oxidation capacity of Fe(VI), and at lower pH, the decrease of algal removal percentages may be caused by the consumption of Fe(VI) by the undesirable fast self-decomposition in the solution.

The yield of DBPs in the P. limnetica solution gradually decreased with the increase in pH (Fig. 5b). The formed DBPs from Chlorella sp. decreased when the pH was increased from 6.0 to 9.0 (Fig. 5a). However, further increase of pH to 10.0, increase in levels of DBPs was observed. The variations of concentrations of specific DBPs with pH are illustrated in Fig. 5c–h. The concentration change of each DBP with pH in the Chlorella sp. solution was different from that observed in the P. limnetica solution. The differences in AOM of the two algae may result in this pattern (see results of EEM in Fig. 3). Several reasons might contribute to the various concentrations of generated DBPs. First, the removal of DBP precursors (AOM) by Fe(VI) depended on the combined effect of the reactivity (oxidation potential) and stability (half-life) of Fe(VI). Table S7 shows the residual Fe(VI) after 10 min pre-oxidation at various pH values. At acidic pH, Fe(VI) showed high potential to oxidize
AOM, but it decayed faster through self-decomposition, and at alkaline pH, Fe(VI) with low reactivity, could have enough time to react with AOM (Graham et al., 2004). Second, Fe(VI) was reduced to Fe$^{3+}$ during oxidation or self-decomposition. At alkaline pH, Fe$^{3+}$ was converted to Fe(OH)$_3$ particles, which could serve as a coagulant to remove AOM via precipitation. The optimal pH range for Fe(OH)$_3$ coagulation and precipitation was in the range from 8.0 to 10.0 (Yuan et al., 2002). Conversely, at low pH, coagulation by Fe$^{3+}$ was not evident. Third, chloride existed in the solution in the forms of HOCI and OCl$^{-}$ ($pK_a = 7.5$). HOCI exhibits higher oxidation potential and react faster with AOM to generate DBPs than OCl$^{-}$ (Ghernaout et al., 2014), i.e., higher pH inhibited the formation of DBPs. Fourth, the DBPs hydrolyzed at high pH, i.e., HKs, HANs and CH would undergo base-catalyzed hydrolysis (Yang et al., 2007).

3.2.3. Effect of bromide—To investigate the incorporation of bromide on DBPs, the bromine substitution factor (BSF) of THMs was calculated as the molar ratio of bromo-THMs to the total (including chloro-, chlorobromo-mixed and bromo-) THMs (Eq. (1)), and the BSF of DHANs was calculated as the molar ratio of bromo-DHANs to the total DHANs (Eq. (2)) (Hua et al., 2006).

\[
\text{BSF}_{\text{THMs}} = \frac{1}{3} \times \frac{\sum_n n^3 \times \text{CHCl}_{(3-n)} Br_n}{\sum_n n^3 \times \text{CHCl}_{(3-n)} Br_n}
\]

\[
\text{BSF}_{\text{DHANs}} = \frac{1}{2} \times \frac{\sum_n n^2 \times \text{C}_2HNCl_{(2-n)} Br_n}{\sum_n n^2 \times \text{C}_2HNCl_{(2-n)} Br_n}
\]

Fig. 6a and b presents the mole concentrations and BSF of THMs in the algae solutions at different bromide concentrations (treated by Fe(VI) pre-oxidation and subsequent chlorination). Four THMs, TCM, BDCM, DBCM and TBM, were detected. Within these THMs, TBM was detected in the Chlorella sp. and P. limnetica control samples (i.e., with Fe(VI) oxidation without chlorination). However, the concentrations were negligible compared with those detected in the algal solutions with chlorination (Fig. S6). As the bromide concentration increased, the concentration of TCM decreased and the concentrations of the three bromo-THMs (BDCM, DBCM and TBM) increased, and consequently, the BSF increased (Fig. 6a and b). When the bromide concentration increased from 0 to 0.4 mg/L, the formation of THMs increased by 17.9 and 9.3 μg/mg C in the solutions of Chlorella sp. and P. limnetica, respectively (Fig. S7). This was due to the conversion from TCM to bromo-THMs. The increase in DBP mass yield with bromide level in water was also observed in a previous study (Liu et al., 2018).

Fig. 6c and d present the mole concentrations of HANs and BSF of DHANs in the algae solutions at different bromide concentrations (treated by Fe(VI) pre-oxidation and subsequent chlorination). Five DHANs, BAN, DCAN, BCAN, DBAN, and TCAN, were detected in the solutions. DBAN was also detected in the Chlorella sp. and P. limnetica control samples (with Fe(VI) oxidation without chlorination), but the concentrations were much lower than those detected in the algal solutions with chlorination (Figs. S6 and S7).
With the increase of bromide concentration in the solutions of two algae, the formation of TCAN decreased. Conversely, the levels of bromo-HANs (BAN, BCAN and DBAN) had an ascending trend. The concentrations of DCAN were remained constant in the solution of *Chlorella* sp., but kept decreasing in the solution of *P. limnetica*. Generally, the BSF of DHANs increased with the increase in concentration of bromide.

The formation of THMs and HANs consistently show that the Fe(VI) pre-oxidation of algae did not generate chloro- and chlorobromo-mixed DBPs. However, trace levels of bromo-DBPs were noticed. A large portion of THMs and HANs were formed in the subsequent chlorination. Increasing bromide concentration in water could convert the DBPs from being less brominated (more chlorinated) to being more brominated (less chlorinated) during chlorination, which may increase toxicity of the chlorinated water samples.

4. Conclusions

- Fe(VI) treatment removed algal cells in the solution via oxidation and coagulation. Coagulation led to the decrease of algal cells and AOM concentration (i.e., by co-precipitation). The oxidation caused death, apoptosis, and lose of integrity of the algal cells, and changes in characteristics of AOM.
- The removal of algal cells and AOM by Fe(VI) increased when a higher Fe(VI) dose or a longer oxidation time was applied.
- Fe(VI) treatment decreased the concentrations of DBPs generated in subsequent chlorination. When the Fe(VI) dose or oxidation time increased, the generation of DBPs was further inhibited to lower levels. Besides, the formation of DBPs was also influenced by pH.
- With the presence of bromide in algal solutions, Fe(VI) pre-oxidation generated negligible amount of bromo-DBPs, and halogenated DBPs were mainly formed in the subsequent chlorination. Higher bromide levels in water led to the shift of DBPs from being less brominated to being more brominated.

Supplementary Material

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Acknowledgments

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Fig. 1.
Effect of Fe(VI) dose on the removal of algae cells and AOM.
Fig. 2.
Fe(VI) induced lost integrity, apoptosis, and death of the two tested algae.
Fig. 3.
Fluorescence EEMs of (a and b) the Chlorella sp. solutions without and with Fe(VI) oxidation, and (c and d) the P. limnetica solutions without and with Fe(VI) oxidation. The region-specific EEM volumes of (e) the Chlorella sp. solutions without and with Fe(VI) oxidation, and (f) the P. limnetica solutions without and with Fe(VI) oxidation.
Fig. 4.
(a and b) Effect of Fe(VI) dose used in pre-treatment on the DBPs formation during chlorination of *Chlorella* sp. and *P. limnetica*. (c–h) Further comparison of the formation of THMs, HAAs, CH, HKs, HANs and TCNM from the two algae.
Fig. 5.
(a and b) Formation of DBPs in Fe(VI) pre-treatment with subsequent chlorination of P. limnetica and Chlorella sp. at different pH. (c–h) Formation of THMs, HAAs, CH, HKs, HANs and TCNM in Fe(VI) pre-treatment and subsequent chlorination of Chlorella sp. and P. limnetica at different reaction pH.
Fig. 6.
The concentrations and BSFs of THMs and HANs in the *Chlorella sp.* and *P. limnetica* solutions with various bromide concentrations, which were treated with Fe(VI) pre-treatment and subsequent chlorination: (a) THMs in *Chlorella sp.* solutions, (b) THMs in *P. limnetica* solutions, (c) HANs in *Chlorella sp.* solutions, (d) HANs in *P. limnetica* solutions.