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Comparison of Permanganate Preoxidation and Preozonation on Algae Containing Water: Cell Integrity, Characteristics, and Chlorinated Disinfection Byproduct Formation

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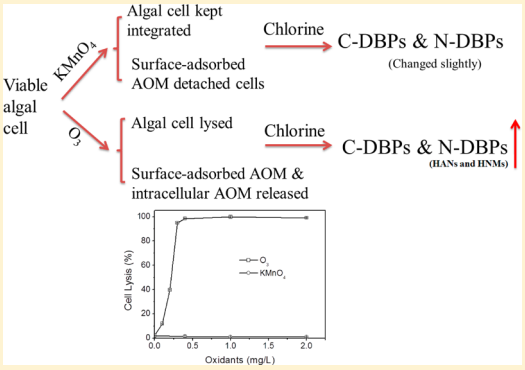
Supporting Information

ABSTRACT: Aqueous suspensions of *Microcystis aeruginosa* were preoxidized with either ozone or permanganate and then subjected to chlorination under conditions simulating drinking water purification. The impacts of the two oxidants on the algal cells and on the subsequent production of dissolved organic matter and disinfection byproducts were investigated. Preozonation dramatically increased disinfection byproduct formation during chlorination, especially the formation of haloaldehydes, haloacetonitriles, and halonitromethanes. Preoxidation with permanganate had much less effect on disinfection byproduct formation. Preozonation destroyed algal cell walls and cell membranes to release intracellular organic matter (IOM), and less than 2.0% integrated cells were left after preozonation with the dosage as low as 0.4 mg/L. Preoxidation with permanganate mainly released organic matter adsorbed on the cells' surface without causing any damage to the cells' integrity, so the increase in byproduct formation was much less. More organic nitrogen and lower molecular weight precursors were produced in a dissolved phase after preozonation than permanganate preoxidation, which contributes to the significant increase of disinfection byproducts after preozonation. The results suggest that permanganate is a better choice than ozone for controlling algae derived pollutants and disinfection byproducts.



INTRODUCTION

Algal blooms worldwide pose many challenges to drinking water production. Algal cells are stable in water due to electrostatic repulsion, surface hydrophilicity, and steric effects.[1] In addition, surface-adsorbed organic matter (S-AOM), including both the extracellular organic matter (EOM) and natural organic matter (NOM), further enhance their stability.[2,3] The stability makes algal cells difficult to remove with traditional drinking water treatment processes such as coagulation, sedimentation, and filtration. Any residual algal cells or algal organic matter (AOM) in drinking water impair its quality by releasing algal toxins, causing taste and odor problems,[4] and forming chlorinated disinfection byproducts (DBPs).[5−7] Algae are rich in organic nitrogen (org-N), which contributes to the formation of nitrogenous DBPs (N-DBPs) whose genotoxicity and carcinogenicity are 2−3 orders of magnitude higher than those of the regular DBPs such as trihalomethanes (THMs) and haloacetic acids (HAAs).[8,9]

Preoxidation is deemed effective in promoting the coagulation of algal cells and their organic matter, and it is widely used in drinking water treatment.[10] Preoxidation by ozone, chlorine, chlorine dioxide, permanganate, or ferrate has been reported to change the zeta potential and structure of algae cells and deactivate cells to promote their aggregation.[10] Preoxidation by permanganate also generates manganese dioxides *in situ*, which can adhere to the cells and promote their settling.[11] However, preoxidation by chlorine or chlorine dioxide forms undesirable DBPs such as chlorinated organics, chlorate, and chlorite.[10,12] Preoxidation by ozone, chlorine or ferrate can induce the lysis of algal cells and release intracellular organic matter (IOM) to the dissolved phase,[10,13,14] which can include algal toxins, DBP precursors, and tastes and odors.[10] Ozone and permanganate are the most effective oxidants in promoting coagulation, and they form fewer toxic byproducts, so they are widely used in water treatment. However, the impacts of preoxidation with these two oxidants, especially permanganate on the characteristics of the organic matter released from algal cells remains very unclear.

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Organic matter characteristics such as molecular size, hydrophobicity, organic nitrogen content, and aromatic content play important roles in the formation of DBPs during chlorination.[8,15−17] Preoxidation can change some of these characteristics and thus affect the formation of DBPs.[18,19] Previous studies have shown that preoxidation affects the formation of DBPs such as chloroform (TCM), HAAs, haloketones, trichloronitromethane (TCNM), N-nitrosodimethylamine, and haloacetonitriles (HANs) during chlorination.[20−23] However, the impacts of preoxidation of algal cells and AOM on DBP formation during chlorination have been much less investigated. The limited studies have mainly focused on the formation of ordinary carbonaceous DBPs (C-DBPs) such as THMs and HAAs. For example, preozonation of algae-containing water has been shown to increase the formation of THMs and HAAs significantly,[24−26] but the formation of other C-DBPs and more toxic N-DBPs such as HANs, haloacetamides (HAcAms), and halonitromethanes (HNMs)[9,46] after preoxidation of algae-containing water remains unclear.

The objectives of this study were (1) to study the impacts of ozone and permanganate preoxidation on the formation of N-DBPs and C-DBPs during the chlorination of algal suspensions and dissolved algal components and (2) to investigate the effects of the two preoxidation processes on the characteristics of algal cells and the released AOM in an attempt to explain the variation in DBPs after preoxidation. *Microcystis aeruginosa* (*M. aeruginosa*) was the algae species selected for this study, as it is the most abundant and common blue-green algae species occurring in natural water.[27]

MATERIALS AND METHODS

Test Solutions. Chemical solutions were prepared from reagent-grade chemicals and water purified using the Milli-Q biocel system (18.2 MΩ·cm). A potassium permanganate (Gaofeng, Tianjin, China) stock solution (1000 mg/L) was prepared and standardized using sodium oxalate according to the standard methods of the American Public Health Association.[28] A concentrated ozone stock solution (about 24 mg/L) was produced by continuously bubbling ozone-containing oxygen gas into a 1000 mL flask of Milli-Q water through a glass diffuser. A free chlorine stock solution (2800 mg/L as Cl_2) was prepared from a 4% sodium hypochlorite solution (NaOCl, Sigma-Aldrich, USA) and standardized periodically by the DPD ferrous titrimetric method.[27] Mixed standard kits for carrying out methods 551A and 551B of the U.S. Environmental Protection Agency and for determining chloral hydrate (CH) and nine HAAs were obtained from Supelco. Standards of dichloroacetamide (DCAcAm) and trichloroacetamide (TCAcAm) were obtained from Karlsruhe Germany and Aldrich, respectively. A dichloroacetaldehyde (DCA) standard was obtained from TCI America. Seven kinds of aldehydes of HPLC-grade were purchased from Sinopharm China, and acetone was bought from Fisher. Sixteen kinds of amino acids and four kinds of aliphatic amines were purchased from Sinopharm China and Sigma-Aldrich America, respectively.

The simulated drinking water medium was Milli-Q purified water spiked with 0.5 mM $CaCl_2$, 1 mM $NaHCO_3$, and 15 mM $NaClO_4$ and then adjusted to pH 7.0. This will hereafter be referred to as solution A. The pH was adjusted with sodium hydroxide and perchloric acid, and the pH's were monitored and maintained throughout the reactions at the predetermined pH ± 0.1.

Microcystis aeruginosa (*M. aeruginosa*) was the algae species selected for this study, as it is the most abundant and common blue-green algae species occurring in natural water.[28] Axenic cultures of *M. aeruginosa* (Collection No. HB909) were obtained from the Institute of Hydrobiology of the Chinese Academy of Sciences. The *M. aeruginosa* was cultivated for 62 days to the stationary growth phase. The details of its cultivation can be found in Fang,[5] and the growth curve is presented in Figure S1 of the Supporting Information. Cells were separated from the suspensions by centrifugation (TDL-5-A, Anke, Shanghai, China) at 4500 rpm for 12 min. The cells precipitated in the centrifuge tube were then resuspended with solution A, followed by two additional cycles of centrifugation and supernatant removal. The stock solution of algae was then prepared by suspending the resultant cells with solution A. The simulated algal suspension was prepared by adding the algae stock solution to solution A to achieve a concentration of $(1.01 \pm 0.06) \times 10^6$ cells/mL, which is equivalent to a total organic carbon (TOC) content of 5 mg/L. The dissolved organic carbon (DOC) in the suspension was determined to be 0.34 mg/L, likely due to the desorption of AOM loosely bound to the cells. The simulated algal suspension contained no EOM produced during the growth of algae by centrifugation and resuspension. This approach allowed an examination focused on the algal cell and the released IOM and excluded the effect of EOM on the preoxidation processes, because the primary purpose of the application of preoxidation on algae containing water is to enhance the removal efficiency of algal cells.[10] It should be noted that EOM in natural algae containing water can consume oxidants and react with chlorine to form DBPs.[8]

Experimental Procedures. For all the experiments, solutions without preoxidation served as the control samples. Preoxidation was carried out with 500 mL of the simulated suspension of algae in a 1000 mL glass beaker. Either potassium permanganate or ozone at the desired concentration was dosed into the solution with stirring at 150 rpm for 10 min. The sample was then divided into two portions. One was subjected to TOC and total organic nitrogen (TON) analysis, algal cell integrity, morphologies, and chlorination. The other was first filtered through a 0.45 μm glass fiber membrane (Whatman) and then subjected to DOC and dissolved organic nitrogen (DON) analysis, chlorination, and subsequent measurement of the molecular weight distribution and analyses for potassium, manganese, ammonia (NH_3 as N), aldehydes, acetone, soluble protein, amino acids, and aliphatic amines.

When preoxidation with ozone or permanganate is used in a water treatment plant, the dosage of ozone is usually no less than that of permanganate (as $KMnO_4$) in terms of mass concentration, although the molar concentration of ozone is more than three times that of permanganate when the mass concentration of permanganate is equal to that of ozone. The same mass concentration of the two oxidants is usually used in the realm of research, depending on the treatment process.[29−31] In this study, dosages of 0−2.0 mg/L (see Figure 1) of both ozone and permanganate were studied to facilitate comparison. The ozone was consumed completely after 10 min of preoxidation (data not shown), but not permanganate. Since permanganate was studied to slightly affect the formation of DBPs during chlorination of algal cells (Figure S2), no reductants were added to quench the residual oxidants after the 10 min of preoxidation before chlorination. Sodium sulfite was added to quench residual oxidants after preoxidation for the analysis of algal cells and organic matter.

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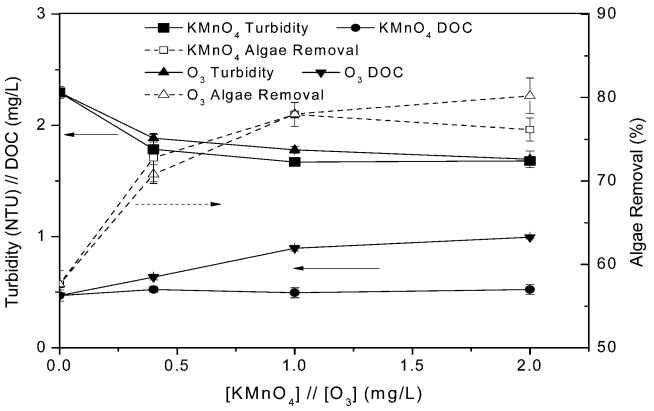

Figure 1. The effects of preoxidation with $KMnO_4$ or O_3 followed by coagulation−sedimentation on the removal of algae and the release of intracellular organic matter. Conditions: Algae cell density (1.01 ± 0.06) × 10^6 /mL, turbidity 3.94 ± 0.05 NTU, preoxidation time 10 min, $Al_2(SO_4)_3$ dosage (as Al^{3+}) 0.8 mg/L, pH 7.0, temperature 25 ± 1 °C, sedimentation time 30 min. The error bars represent the standard deviation from duplicate tests.

The preoxidation−coagulation experiments were carried out in a jar tester (TA2-2, Hengling, China) containing 500 mL of the simulated suspension of algae. Oxidants were added with mixing at 150 rpm for 5 min. Then, 0.8 mg/L aluminum sulfate (as Al^{3+}; determined by Figure S3) was added with rapid mix at 150 rpm for 1 min followed with slow mix at 53 rpm for 10 min. After settling for 30 min, 50 mL samples were extracted from 1 cm below the surface and analyzed for the concentration of algae cells, DOC, and turbidity.

The chlorination experiments were carried out with 100 mL of the preoxidized solutions at pH 7.0 (maintained with a 10 mM phosphate buffer) in amber glass bottles capped with Teflon-faced septa. The chlorinated samples were incubated at 25 ± 1 °C in the dark for 3 days and then quenched with ascorbic acid (Sinopharm, Shanghai, China) for the DBP analyses.

Analytical Methods. TOC, DOC, total nitrogen (TN), and dissolved nitrogen (DN) were measured using a TOC/TN analyzer (Multi N/C 3100, Analytik Jena, Berlin, Germany). Ammonia was measured by a colorimetric method with Nessler's reagent. [32] Nitrate and nitrite were determined using an ion chromatograph (ICS-3000, Dionex, USA). TON and DON can be estimated by subtracting ammonia, nitrate, and nitrite from TN and DN, respectively. Potassium and manganese were analyzed using an ICP-OES (Optima 5300 DV, Perkin-Elmer, Yokohama, Japan). Soluble protein was measured with the Brodford method with Bradford reagent (Sigma-Aldrich, USA) and bovine serum albumin (Solarbio, Beijing, China) using 10 cm quartz sampling cells.[33] The number of algal cells was counted in an accounting chamber using a photomicroscope (BX51, Olympus, Tokyo, Japan). The concentration of the ozone stock solution was determined by measuring its ultraviolet absorbance at 258 nm.[28] The concentrations of free chlorine were measured by DPD/FAS titration.[28] Gas chromatography (GC; model 6890, Agilent, Santa Clara, USA) was employed to measure TCM, CH, DCA, 1,1-dichloro-2-propanone (1,1-DCP), and 1,1,1-trichloro-2-propanone (1,1,1-TCP), dichloroacetonitrile (DCAN), trichloroacetonitrile (TCAN), dichloronitromethane (DCNM), TCNM, monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), and trichloroacetic acid (TCAA). GC was coupled with an electron capture detector (ECD) and an HP5 column (30 m × 0.25 mm, ID 0.32 μm) based on U.S. EPA methods 551.1 and 552.3.[34,35] For HAA9 analysis, the samples were pretreated with an extraction/derivatization procedure using methyl tert-butyl ether and acidic methanol according to U.S. EPA method 552.3.[35] DCAcAm and TCAcAm were analyzed using liquid−liquid extraction and GC/mass/mass chromatography with electron ionization (7890/7000B, Agilent, Santa Clara, USA).[36] Other conditions and the method detection limit can be found in the Supporting Information (Text S1 and Table S1). The significance of any observed differences between the mean concentrations of DBPs formed in the chlorination of the preoxidized and control samples was evaluated using Student's *t* test. [37]

The molecular weight distribution of the dissolved organic matter was determined using high performance size exclusive chromatography (HPSEC) involving a binary HPLC pump (model 1525), a multiple fluorescence detector (model 2475), and an Ultrahydrogel column (Waters Ultrahydrogel 250, 7.8 mm × 300 mm) (all from Waters, Milford, USA). The detector was set at the excitation/emission (Ex/Em) wavelength pairs of 280/340 and 275/450 nm. The other analytical details can be found in the Supporting Information (Text S2).

A flow cytometer (Accuri C6, BD Biosciences, Franklin Lakes, USA) equipped with an argon laser emitting at a fixed wavelength of 488 nm for fluorescence measurement was used to measure algal cell integrity before and after preoxidation. Fluorescent filters and detectors collected green fluorescence in channel FL1 (530 nm) and red fluorescence in channel FL3 (630 nm). SYTOX green nucleic acid stain (Invitrogen, Life Technologies, Grand Island, USA) was used to determine the cell integrity following the method of Daly.[38] SYTOX can permeate algal cells which have lost cell wall integrity and stain the nucleic acid; hence cells with higher green fluorescence (FL1) are those which have lost cell wall integrity and allowed solutes into the cell. SYTOX was spiked into samples to achieve a concentration of 0.1 μM. After a development time of 7 min, samples were injected into the flow cytometer, and the data were collected and analyzed using CFlow software version 264.15 (from BD Biosciences). Scanning electron microscopy (SEM; Phenom, FEI, Hillsboro, USA) was used to observe the morphologies of the cells before and after preoxidation, and the pretreatment details were shown in SI Text S5.

The concentrations of amino acids and aliphatic amines were analyzed with a HPLC equipped with a 2475 multiple fluorescence detector, a 1525 binary HPLC pump, and a C18 column (4.6 mm ×250 mm, i.d. × 5 μm) (all from Waters, Milford, USA) after prederivatization with fluorenylmethyl chloroformate (FMOC).[8] The 16 amino acids were histidine (His), aspartate (Asp), valine (Val), alanine (Ala), methionine (Met), glycine (Gly), trytophan (Try), phenylalanine (Phe), serine (Ser), arginine (Arg), glutamine (Glu), proline (Pro), tyrosine (Tyr), threosine (Thr), and lysine (Lys), and the four aliphatic amines were diethylamine (DEA), methylamine (MA), ethylamine (EA), and methylethylamine (MEA). The detector was set at the Ex/Em wavelength pairs of 265/310 nm. In this study, only eight kinds of amino acids of Asp, Ala, Ser, Met, Try, Arg, Phe, and Tyr and one kind of aliphatic amine of EA was detectable.

RESULTS AND DISCUSSION

Effects of Preoxidation on the Enhancement of Coagulation for Algal Removal. Figure 1 shows that

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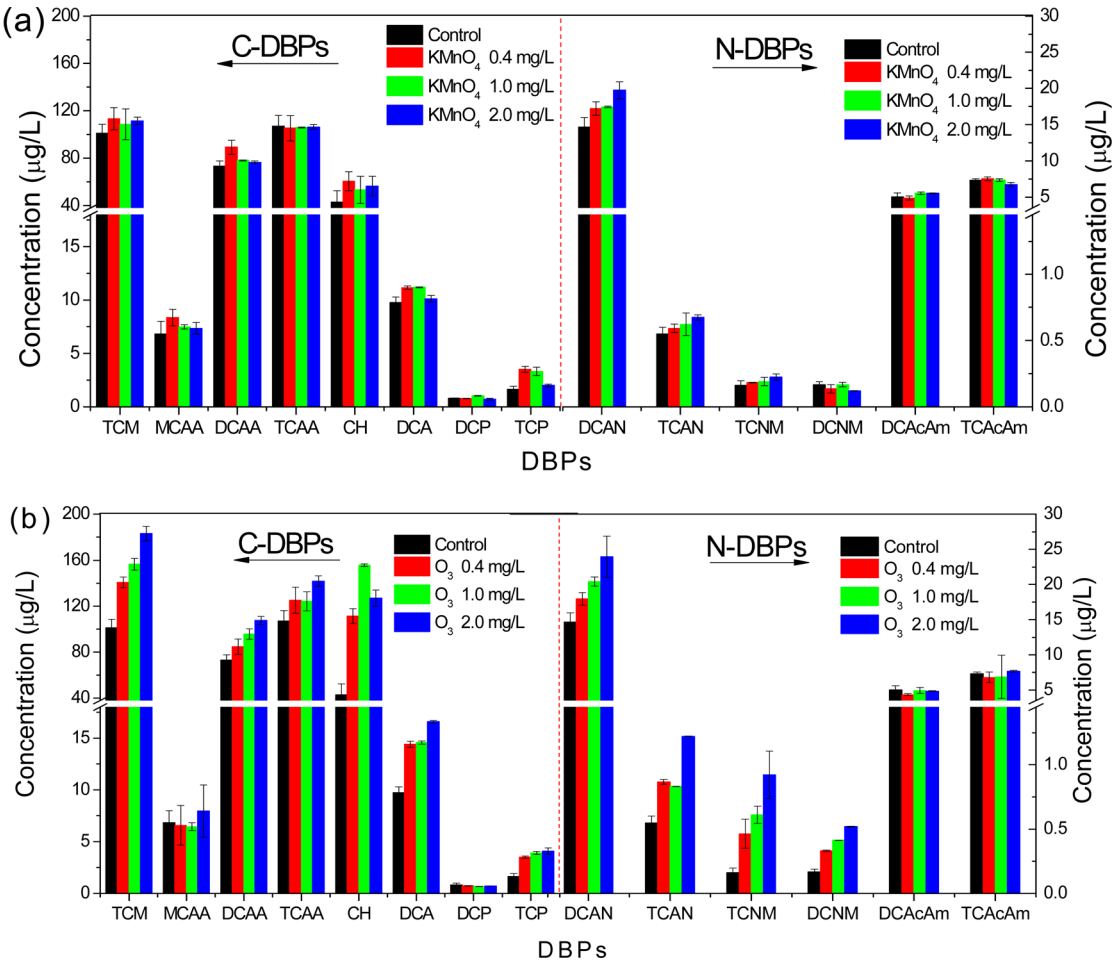

Figure 2. Carbonaceous and nitrogenous byproducts formed during the chlorination of algal suspensions preoxidized with either permanganate (a) or ozone (b). Preoxidation conditions: time 10 min, pH 7.0, and temperature 25 ± 1 °C. Chlorination conditions: chlorine dosage (as Cl_2)/TOC (mass ratio) 3:1, pH 7.0, temperature 25 ± 1 °C, and reaction time 3 days. Error bars represent the standard deviation of duplicate tests.

preoxidation with either permanganate or ozone increased the removal of algal cells after coagulation−sedimentation and lowered the residual turbidity. The removal rate of algal cells increased by more than 15% (from 56.7% to more than 72.8%) after permanganate preoxidation, with the highest removal rate (78.0%) at the 1.0 mg/L permanganate dosage. Preozonation increased the removal rate of algal cells from 56.7% to 70.8% at a 0.4 mg/L ozone dosage, to 78% at 1.0 mg/L and to 80.2% at 2.0 mg/L. DOC was unchanged after permanganate preoxidation followed by coagulation at all $KMnO_4$ dosages, but it increased from 0.42 mg/L to 1.00 mg/L with increasing ozone dosages from 0 to 2.0 mg/L. The removal of algal cells and turbidity changed slightly when the dosage of oxidant was more than 1.0 mg/L, so the dosages of 0−2.0 mg/L of both ozone and permanganate were selected in this study.

DBP Formation during the Chlorination of Algal Suspensions. Figure 2a shows the impact of permanganate preoxidation at 0−2.0 mg/L on the formation of DBPs during the chlorination of algal suspensions. Combined with the *p* values shown in Table S2 of the Supporting Information, the data show that the concentration of most DBPs changed only slightly after preoxidation with permanganate. The formation of 1,1,1-TCP exhibited an increasing and then decreasing pattern with increasing permanganate dosage. DCAN and TCNM slightly increased with increasing permanganate dosage.

Figure 2b shows the influence of preozonation at 0−2.0 mg/L on the formation of DBPs during the chlorination of algal suspensions. The concentrations of all the DBPs except for 1,1-DCP, DCAcAm, and TCAcAm rose significantly at certain ozone dosages (Table S2). The formation of most DBPs such as TCM, DCAA, TCAA, DCA, 1,1,1-TCP, HANs, and HNMs increased monotonically with increasing ozone dosage from 0 mg/L to 2.0 mg/L. The formation of CH followed an increasing and then decreasing pattern with the maximum at an ozone dosage of 1.0 mg/L, while 1,1-DCP formation monotonically decreased with increasing ozone dosage. The formation of the common DBPs (TCM, DCAA, and TCAA) increased more than 40% at an ozone dosage of 2.0 mg/L, which was similar to the results of previously reported studies.[25,26] Haloaldehydes, HANs, and HNMs increased even more sharply than the normal DBPs after preozonation. For example, CH and TCNM formation increased by 262% and 471% at ozone dosages of 1.0 mg/L and 2.0 mg/L, respectively. However, preoxidation by either ozone or permanganate had little impact on the formation of DCAcAm and TCAcAm.

DBP Formation during the Chlorination of Dissolved Algal Components. Figure 3a presents the effect of permanganate preoxidation of algal suspensions on DBP formation during the subsequent chlorination of the dissolved phase. The formation of most DBPs increased only slightly or

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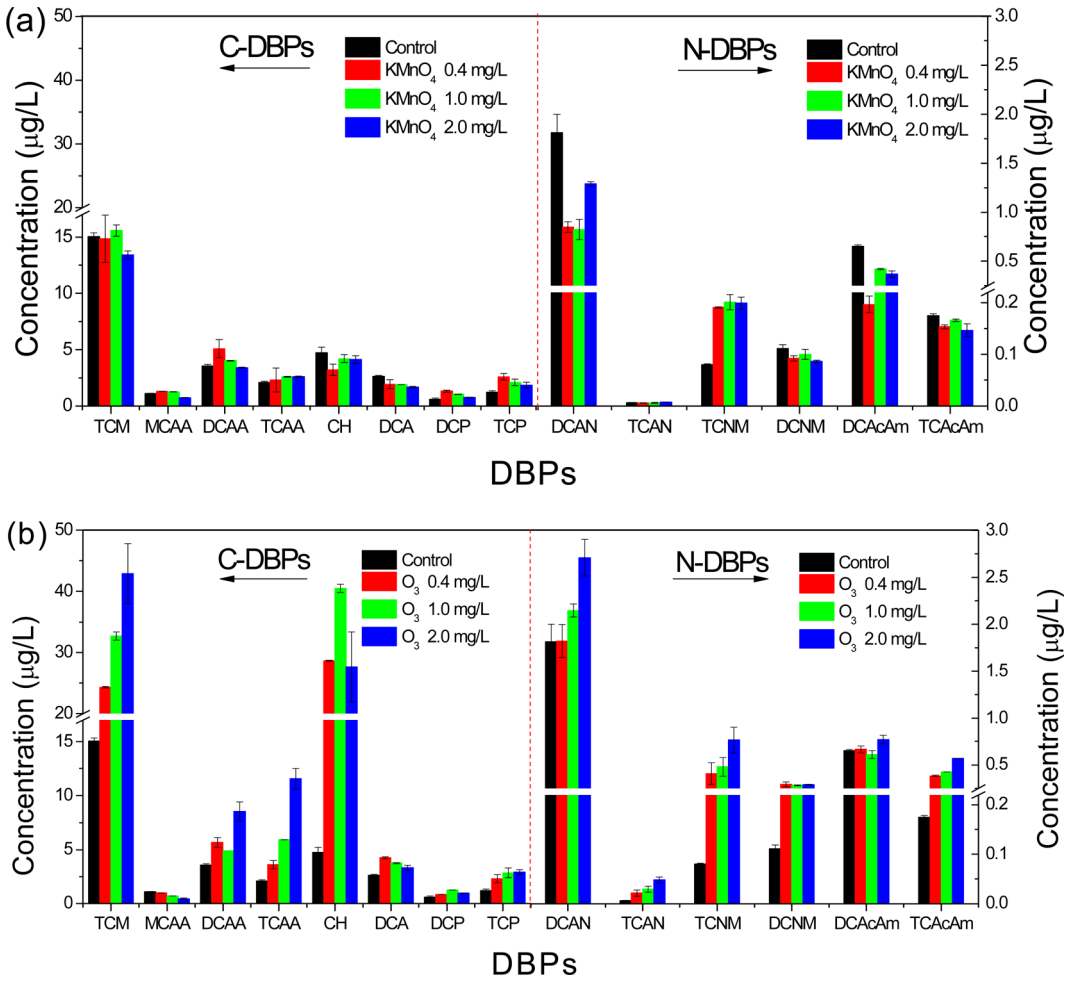

Figure 3. Carbonaceous and nitrogenous byproducts formed during the chlorination of the dissolved phase after preoxidized algal suspensions with either permanganate (a) or ozone (b). Preoxidation conditions: time 10 min, pH 7.0, and temperature 25 ± 1 °C. Chlorination conditions: chlorine dosage (as Cl_2)/DOC (mass ratio) 3:1, pH 7.0, temperature 25 ± 1 °C, and reaction time 3 days. Error bars represent the standard deviation of duplicate tests.

even decreased after permanganate preoxidation, except for TCNM, DCAA, and the haloketones (i.e., 1,1-DCP and 1,1,1-TCP) as is shown in Table S2. TCNM increased by 138% after preoxidation by permanganate at 0.4 mg/L, and it remained nearly unchanged when the permanganate dosage was further increased to 1.0 and 2.0 mg/L. DCAA, 1,1-DCP, and 1,1,1-TCP formation followed an increasing and then decreasing pattern with the maximum values appearing at a permanganate dosage of 0.4 mg/L. However, the increases of the formation of DCAA, 1,1-DCP, and 1,1,1-TCP were statistically insignificant at 2.0 mg/L of permanganate compared to the control sample (Table S2).

Figure 3b shows the effect of preozonation of algal suspensions on DBP formation during the subsequent chlorination of the dissolved phase. Combined with the figures presented in Table S2, preozonation dramatically increased the formation of most DBPs in the dissolved phase, which was similar to the DBP formation results with the algal suspensions (Figure 1b). TCM, TCAA, and N-DBPs such as DCAN, TCAN, TCNM, and TCAcAm monotonically increased with increasing ozone dosage over the range of 0.4−2.0 mg/L. At ozone dosages of 2.0 mg/L, the formation of DCAN, TCAN, DCNM, TCNM, and TCAcAm increased by 50%, 667%, 161%, 858%, and 226% respectively. The formation of CH, DCA, and 1,1-DCP followed an increasing and then decreasing pattern. CH formation increased by 752% with 1.0 mg/L of ozone, at which point it was the dominant DBP with a concentration even higher than that of TCM.

In summary, the formation of most DBPs during chlorination of algal suspensions and of the dissolved phase increased significantly after preozonation. The levels were much higher than those observed after permanganate preoxidation for equal dosages (in terms of mass concentration) of the two oxidants.

Cell Integrity and Morphology. Figure 4 shows the effect of preoxidation with permanganate and ozone on algal cell lysis using a flow cytometer to measure changes in cell permeability (R1 in Figures S4 and S5) and chlorophyll autofluorescence (R2 in Figures S4 and S5). There was no obvious change in the proportion of live cells (R2 > 97%) after 10 min of permanganate oxidation at dosages of 0.4−2.0 mg/L, indicating that it did not cause permeability or cell lysis (R1). Increasing permanganate dosage (Figure S4) and contact time (up to 2 h, data not shown) also did not change cell viability. But preozonation, by contrast, damaged algal cells severely, and there were almost no integral cells left (R2 ≤ 1%) after 10 min of ozonation at ozone dosages of 0.4−2.0 mg/L. Also, the population of lysed cells increased strongly with increasing ozone dosage when it was less than 0.4 mg/L (Figures 4 and

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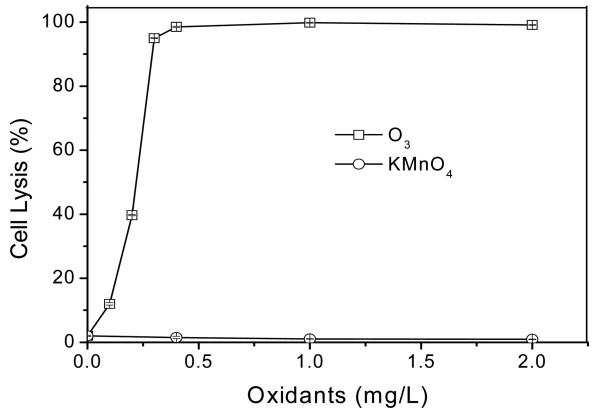

Figure 4. Algal cell lysis as a function of the ozone and permanganate dosages in preoxidation for 10 min at pH 7.0 and 25 ± 1 °C. The error bars represent the standard deviation from duplicated tests.

S5), which indicates that even when the molar concentration of ozone was less than that of permanganate, ozone still damaged algal cells more significantly than permanganate (Figure 4). As the dosage of ozone in actual water treatment is no less than the dosage of permanganate in terms of mass concentration, preozonation apparently causes much greater damage to algal cells than permanganate preoxidation.

Figure S6 shows SEM images of cells before and after preoxidation with 2.0 mg/L of permanganate or ozone. S-AOM was abundant on the raw algal cells, but it had disappeared after preoxidation with either permanganate or ozone. Preozonation, but not permanganate, caused the cells to change shape, indicating that the cell walls which maintain the shape of algal cells were damaged by preozonation, but not permanganate preoxidation.[41]

The Effect of Preoxidation on Water Parameters. Table 1 shows the variations of some water parameters after preoxidation by either permanganate or ozone at various dosages. TOC and TON of the algal suspensions decreased slightly after preozonation, while there were no apparent changes after permanganate preoxidation. This indicates that ozone mineralized some AOM in these experimental conditions and translated some organic nitrogen to inorganic nitrogen. In the dissolved phase, the concentrations of potassium, DOC, DON, soluble protein, and NH_3 increased significantly with increasing ozone dosages, from 0.37 mg/L to 1.31 mg/L, 0.39 mg/L to 1.04 mg/L, N.D. to 116.5 μg/L, 65 μg/L to 315 μg/L, and N.D. to 148 μg/L, respectively, as the ozone dosage increased from 0 mg/L to 2.0 mg/L. On the other hand, the concentrations of potassium and DOC increased only slightly with increasing permanganate dosages; soluble protein decreased to lower than the detection limit, and that of DON and NH_3 remained undetectable.

The release of potassium indicates the extent of cell membrane damage, as potassium is absorbed into the vacuole of algal cells and stored as an enzyme activator.[11,39,40] An increase in the DOC also indicates the damaging of algal cells.[11] So the changes in potassium and DOC indicate that preozonation damages the cell walls and cell membranes of algae and causes the release of IOM, while preoxidation with permanganate damages cells only slightly. A previous study found that IOM contained more proteins than extracellular organic matter.[8] The increase of soluble protein and DON after preozonation can be attributed to the release of IOM, which also indicates that preozonation damaged algal cells.

Molecular Weight Distribution of the Dissolved Organic Matter. The fluorophores centered at the Ex/Em wavelength pairs of 280/340 nm represent org-N rich substances such as proteins or protein-like materials, while the fluorophores centered at the Ex/Em wavelength pairs of 275/450 nm represent substances which are mainly rich in organic carbon (org-C) such as humic or humic-like materials.[42] The molecular weight distributions of dissolved organic matter (DOM) centered at the Ex/Em wavelength pairs of 280/340 and 275/450 nm after preoxidation are shown in Figures 5 and 6, respectively.

Figure 5 shows that permanganate preoxidation had little influence on the molecular weight distribution of the DOM rich in org-N, but preozonation caused it to change significantly. The DOM of medium or low molecular weight (retention time >17 min) increased with increasing ozone dosage, while those of high molecular weight (retention time ~8 min) decreased gradually with increasing ozone dosage from 0.4 to 2.0 mg/L. This result indicates that ozonation not only released DOM rich in org-N from the algal cells but also oxidized them into lower molecular weight organics. Permanganate, however, only slightly affected the molecular weight distribution due to slight damage to the cells.

Figure 6 shows that preoxidation with either permanganate or ozone affected the molecular weight distribution of the DOM rich in org-C significantly, which was different from the changes of org-N shown in Figure 5. The org-C content of the DOM after preoxidation with either permanganate or ozone exhibited an increasing and then decreasing pattern with increasing oxidant dosage, with the maximum occurring at 0.4 mg/L ozone and 1.0 mg/L permanganate. The molecular weight of org-C contents after permanganate preoxidation was higher than after preozonation, likely due to both permanganate and ozone being able to oxidize the released organic matter into lower molecular weight in the dissolved phase, but

Table 1. Water Characteristics before and after Preoxidation with Permanganate or Ozone[a]

		$KMnO_4$ dosage (mg/L)			O_3 dosage (mg/L)		
	control	0.4	1.0	2.0	0.4	1.0	2.0
potassium (mg/L)	0.37 ± 0.06	0.39 ± 0.09	0.46 ± 0.08	0.48 ± 0.08	0.69 ± 0.08	0.91 ± 0.08	1.31 ± 0.06
TOC (mg/L)	5.00 ± 0.12	4.97 ± 0.15	4.92 ± 0.15	4.83 ± 0.14	4.80 ± 0.17	4.62 ± 0.14	4.45 ± 0.16
DOC (mg/L)	0.39 ± 0.06	0.42 ± 0.04	0.47 ± 0.05	0.45 ± 0.03	0.84 ± 0.09	0.96 ± 0.04	1.04 ± 0.05
TON (mg/L)	0.80 ± 0.06	0.79 ± 0.07	0.76 ± 0.04	0.73 ± 0.07	0.76 ± 0.02	0.72 ± 0.09	0.66 ± 0.09
DON (μg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	116.5 ± 16.7
soluble protein (μg/L)	65 ± 15	n.d.	n.d.	n.d.	n.d.	226 ± 13	315 ± 13
NH_3 (μg/L, as N)	n.d.	n.d.	n.d.	n.d.	n.d.	55 ± 9	148 ± 11

[a]n.d. is not detectable. The error bars represent the standard deviation from duplicated tests.

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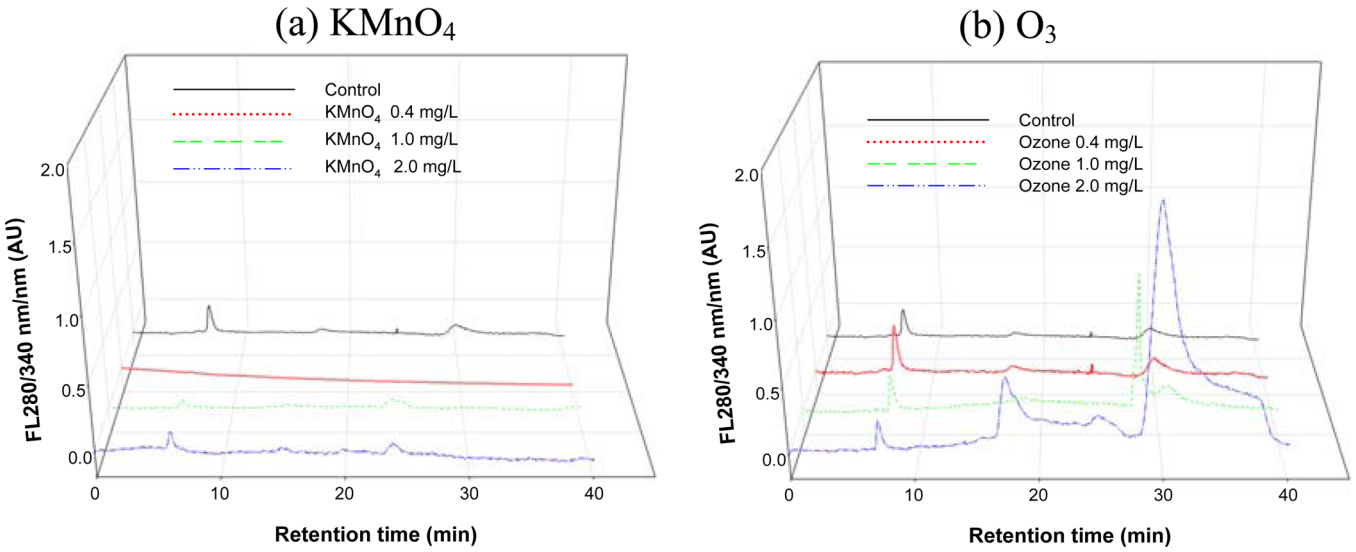

Figure 5. Chromatograms of dissolved organic matter after preoxidation of algal suspensions using permanganate (a) or ozone (b). Generated by size exclusive chromatography with a fluorescence detector set at the Ex/Em wavelength pair of 280/340 nm.

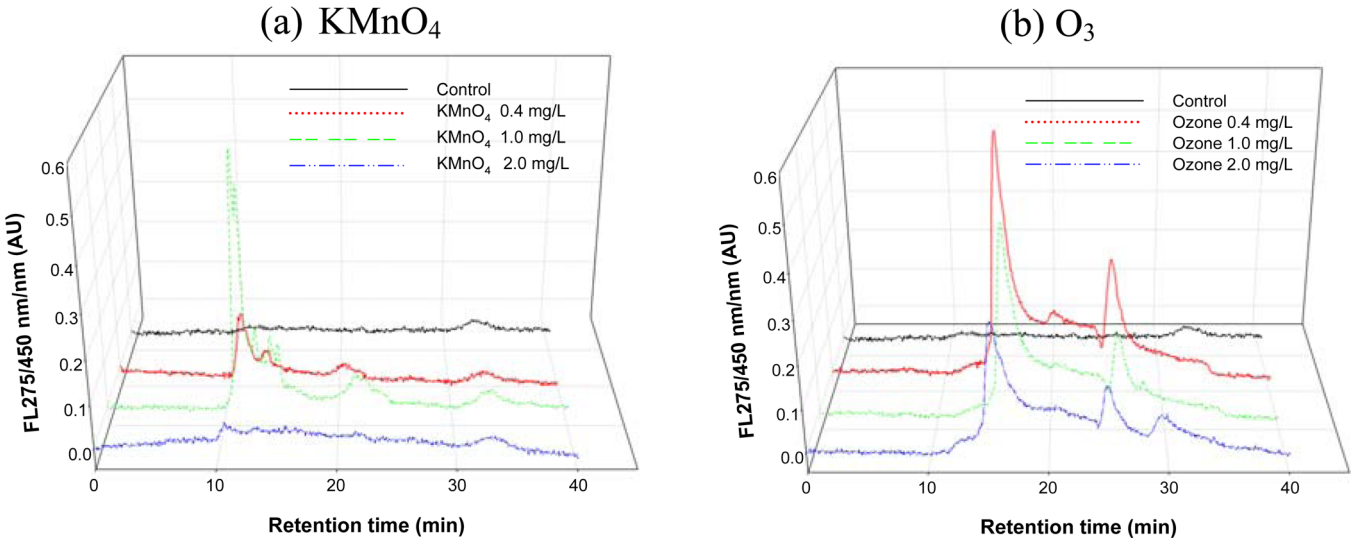

Figure 6. Chromatograms of dissolved organic matter after preoxidation of algal suspensions using permanganate (a) or ozone (b). Generated by size exclusive chromatography with a fluorescence detector set at the Ex/Em wavelength pair of 275/450 nm.

the oxidation capacity of ozone would be stronger than permanganate, and ozonation would produce lower molecular weight organics than permanganate oxidation.

Amino Acids and Aliphatic Amines in Dissolved Phase. Figure 7 shows the concentrations of specific amino acids and aliphatic amines in the dissolved phase before and after preoxidation. Preozonation of algal suspensions produced much more dissolved organic nitrogenous compounds except for Asp and EA than permanganate preoxidation. The result was in accordance with the results shown in Figure 5. EA was only detected after 1.0 and 2.0 mg/L permanganate preoxidation and 0.4 mg/L preozonation. Among the 9 kinds of organic nitrogenous compounds, Asp was comparable between preozonation and permanganate preoxidation. Asp, Ala, Ser, Met, and Arg followed a monotonous increasing pattern after preozonation over the dosage range of 0.4−2.0 mg/L. Try and Tyr followed an increasing and then decreasing pattern with increasing the dosage of ozone.

The concentration of org-N in the dissolved phase depends on their release from algal cells and their oxidation by oxidants. IOM primarily consists of org-N compounds.[8] If the IOM was released after preoxidation, the org-N would be expected to increase. So the results of algae integrity and dissolved organic matter indicate a significant release of IOM after preozonation of algal cells, but not after permanganate preoxidation. Combined with Figures 5 and 7, preozonation can also oxidize and destroy the IOM released to yield lower molecular weight organic matter and also transfer the released amino acids and aliphatic amines.

Linking the Variation of Characteristics and DBP Formation Impacted by Preoxidation. The DBPs formed in the chlorination of algal suspensions result from chlorination of both the algal cells and the dissolved components. Comparing Figures 1 and 2, it shows that the former dominates DBP formation in algal suspensions with or without preoxidation. Preozonation significantly increases DBP formation from both the cells and the dissolved phase, but DBP

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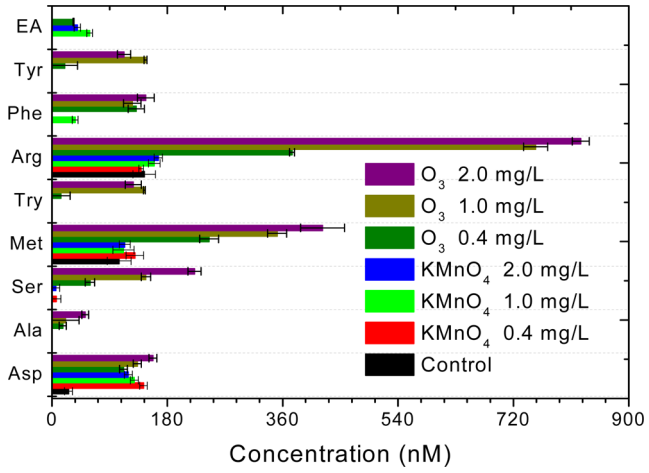

Figure 7. Concentrations of amino acids and aliphatic amines in the dissolved phase before and after preoxidation with either permanganate or ozone. Preoxidation conditions: contact time 10 min, pH 7.0, and temperature 25 ± 1 °C. The error bars represent the standard deviation from duplicated tests.

formation is affected only slightly by permanganate preoxidation. Meanwhile, the integrity and morphology of the algal cells is damaged significantly by preozonation, but not by permanganate preoxidation (Figures 4 and S6). As a result, much more organic matter is released into the dissolved phase after preozonation compared to permanganate preoxidation. But both oxidants can modify the org-N or org-C rich matter and affect subsequent DBP formation (Figures 5−7).

These results suggest that (1) oxidation with ozone or permanganate changes the characteristics of the DBP precursors of the organic matter inside and outside algal cells, but to different degrees, thus affecting DBP formation differently, and (2) preoxidation with ozone, but not permanganate, damages algal cell walls and membranes, allowing chlorine to permeate into the cells and react with IOM in the cells as well as that which has been released. The effect of preoxidation on DBP formation in the dissolved phase is largely due to the release of IOM and S-AOM and their modification. The results reported in Figures 4−7 strongly suggest that the DBP precursor in the dissolved phase after permanganate preoxidation is S-AOM detached from the algal cells, while after preozonation the DBP precursors should result largely from the release of IOM through cell lysis, in addition to the detached S-AOM.

Figures S7−S9 in the Supporting Information show the impacts of permanganate and ozone preoxidation on specific consumed chlorine and specific DBP formation (chorine consumption or DBP formed divided by DOC). In algal suspensions, the specific formation of DBPs substantially increased after preozonation but almost kept unchanged after preoxidation with permanganate. However, in the dissolved phase, the specific chlorine consumption decreased, and the specific formation of most DBPs, except for CH or TCNM, decreased or just changed insignificantly after preoxidation by either permanganate or ozone. This result indicates the modification of DBP precusors by preoxidation.

Preozonation of organic matter increased their hydrophilicity and decreased their molecular weight (Figures 5 and 6),[43] as a great number of aldehydes, ketones, and carboxylic acids formed. [44] As also shown in Figure S10, many more aldehydes were produced in the dissolved phase after preozonation than after permanganate. Hydrophilic and low molecular weight organic matter has been reported to form more TCM and HAAs during chlorination.[16,17] For example, ketonic acids, a kind of hydrophilic and low molecular organic acids, are important precursors of HAAs and TCM,[20] while aldehydes are important precursors of haloaldehyes and HANs.[45,46] Abundant org-N's with low molecular weight such as amino acids were produced after preozonation, but not after permanganate preoxidation (Figures 5 and 7), which also contributes to the increase of nitrogenous DBPs and HAAs.[46,47]

Haloacetaldehydes such as CH and DCA increased significantly after preozonation, which was similar to the findings of a prior study using natural water.[45] Haloketones such as 1,1-DCP and 1,1,1-TCP increased after either permanganate or ozone preoxidation. This result can be interpreted by the production of aldehydes and acetone during preoxidation, as shown in Figure S10. The formation of acetaldehyde is higher with ozone, while that of acetone is higher with permanganate. The formation of other aldehydes such as formaldehyde, propanal, and glyoxal was also higher with ozone. The formation of the functional groups of aldehydes and ketones should be attributable to the increase of haloaldehydes and haloketones during chlorination. However, the formation of some haloacetadehydes and haloketones decreased with further increases in the ozone or permanganate dosages. This is because aldehydes and ketones are the intermediates during oxidation, and they can be further transformed into organic acids or carbon dioxide by oxidants.[41] Besides, haloacetadehydes and haloketones are unstable, and they can go through hydrolysis reactions to form THMs and HAAs.[49]

Org-N formed after preoxidation were the most important precursors of N-DBPs. Preozonation damages algal cell and oxidized IOM to produce lots of amino acids and organic amines with low molecular weight (Figures 5 and 7), which resulted in the sharp increase of N-DBPs. In addition, preozonation of the org-N in algal suspensions can produce NH_3 (Table 1), which reacts vigorously with chlorine to form chloramines (particularly NH_2Cl and $NHCl_2$) and thus contributes to the formation of N-DBPs.[9,48]

HAN formation generally contains two pathways: one was the decarboxylation pathway, and the other was the aldehyde pathway.[46] In the decarboxylation pathway, the nitrogen was from organic nitrogen such as organic amines and amino acids. While, in the aldehyde pathway, HANs formed from the reaction between aldehydes and chloramines, which provides nitrogen.[46] For example, the reaction between formaldehyde and NH_2Cl forms cyanogen chloride, and that between aldehyde and NH_2Cl forms HANs.[46,51] In this study, amino acids and aldehydes with higher concentrations were detected after preozonation than after permanganate peroxidation (Figures 7 and S10), so preozonation increased the formation of HANs significantly (Figures 2 and 3). Also, some org-N can be translated to NH_3 by preozonation (Table 1) which can form chloramines during chlorination. So both pathways contribute to the increase of HAN formation after preozonation.

The formation of TCNM from both the suspended cells and the dissolved components during subsequent chlorination increased after preoxidation with either permanganate or ozone, and that increased more significantly after preozonation (Figures 2 and 3). This result is consistent with the findings of other studies focusing on NOM after ozonation.[22,52] There are

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abundant organic amines such as amino acids, aliphatic amines, and proteins in algal cells and AOM (Figure 7), which can be oxidized to nitro-compounds by either permanganate or ozone.[53,54] The chlorination of nitro-compounds such as nitro-alkenes and nitro-phenols favors the formation of TCNM.[22,46] In addition, hydrophilic organic matter which can form after preoxidation was a more important precursor of TCNM than hydrophobic organic matter.[52] From Figures 2 and 3, the formation of TCNM was comparable in algal suspensions and the dissolved phase, indicating that the released organic matter was the predominant precursor of TCNM. This indicates that the oxidized nitro-compounds are preferentially dissolved in the aqueous phase, rather than adsorbed on the cells.

Both preozonation and permanganate preoxidation had less impact on the formation of HAcAms compared to other N-DBPs (Figures 2 and 3), probably because preoxidation did not increase the amide functional groups, which are the important precursors of HAcAms during chlorination.[55]

■ ENGINEERING IMPLICATION

Preoxidation with either permanganate or ozone improved the removal of algal cells by coagulation−sedimentation, and with similar efficiency at identical dosages (Figure 1). Combined with Figure S3, the preoxidation process can save the coagulant dosages to achieve the same algal removal rate. The improvement was likely due to both permanganate and ozone destroying S-AOM on the cells. As mentioned in the Introduction, S-AOM plays an important role in the stability of algal cells in water, which makes algal cells difficult to remove through particle separation processes such as coagulation and flotation.[3]

Meanwhile, preozonation damaged the integrity of algal cells and resulted in the increase of DOC and DBP precursors in the dissolved phase which is hardly removed by the following coagulation process (Figure 1). However, preoxidation by permanganate kept the integrity of the algal cells and caused a slight increase of dissolved organic matter and DBP precursors in water containing algae. When algal cells were damaged, dramatic dissolved organic matter including algal toxins and DBP precursor and tastes and odors would be released outside, which can impair drinking water quality.[10,54] The release of tastes and odors and algal toxins from algal cells is expected to be much less after permanganate oxidation than after preozonation based on the cell integrity results. So, permanganate preoxidation is a good option for enhancing algal cell removal and controlling chlorinated DBPs.

■ ASSOCIATED CONTENT

Supporting Information

Operating conditions for the volatile DBP and HAA analyses and molecular weight distribution analyses, effects of permanganate on the formation of chlorinated DBPs, determination of the coagulant dosage, operation of scanning electron microscopy test, formation of aldehydes and ketones after preoxidation, additional tables and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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