Enhanced performance and mechanism of KMnO₄ pre-oxidation to coagulation on the removal of the DON and proteins

Cheng Liu, Siyuan He, Bin Wang, Jie Wang and Wei Chen

ABSTRACT

Proteins were the main category of the dissolved organic nitrogen (DON) in eutrophic water sources and posed a threat to the water quality safety and common operation of water plants, while the KMnO₄ pre-oxidation and coagulation were the two important ways to control the organics. Intracellular organic matter (IOM) and phycocyanin were chosen as the target DON to study the performance of KMnO₄ pre-oxidation to the enhanced removal effects for coagulation process, and molecular weight distribution, hydrophobicity, two-dimensional electrophoresis and MnO₂ adsorption experiment were used to study the mechanism. The results showed that KMnO₄ pre-oxidation enhanced the IOM and phycocyanin removal performance of coagulation significantly, although poor removal was found for KMnO₄ oxidation alone. KMnO₄ pre-oxidation altered the molecular weight, hydrophobicity and proteins categories insignificantly. However, the in-situ formed MnO₂ showed better adsorption ability for the IOM and phycocyanin. The main enhanced removal mechanism was the adsorption of MnO₂ formed from the reduction of KMnO₄ and little difference existed between the IOM and phycocyanin. In addition, the KMnO₄ pre-oxidation could enhance the turbidity removal of the coagulation due to a similar mechanism.

Key words | algae, DON, intracellular organic matter, phycocyanin, potassium permanganate, pre-oxidation

INTRODUCTION

Dissolved organic nitrogen (DON) in water is drawing increasing attention due to the detection of nitrogenous disinfection by-products (N-DBPs), which are considered to be far more carcinogenic or mutagenic than the regulated carbonaceous disinfection by-products (C-DBPs) (Pehlivanoglu-Mantas & Sedlak 2008). DON is a complex mixture that is primarily composed of amino acids, amino sugars, amides, peptides and heterocyclic-N compounds (e.g. pyrimidine, imidazole, purine and porphyrins). The typical DON concentration in surface waters varies from less than 0.1 to higher than 10 mg N/L, with a median value of approximately 0.3 mg N/L (Dotson et al. 2009).

The DON in eutrophic water sources is mainly derived from the algae cells, and its concentration is generally higher (1.0–2.0 mg N/L) than that of other waters (Westerhoff & Mash 2002). Proteins, hydrolysable amino acids, chlorophyll and amino-sugars are considered to be the main compositions of DON in eutrophic water sources (Pivokonsky et al. 2006), and the portion of proteins in intracellular organic matter (IOM) amounts to up to 29.1% in the stationary phase (Ambonguilat et al. 2006). The main varieties of the proteins in the IOM of Microcystis aeruginosa are identified through the Tandem Mass Tags method and about 185 kinds of proteins are found (Liu et al. 2015), among which the concentration of phycocyanin is the highest (0.185 mg/L) category. In addition, the proteins are looked on as the main inhibitory substances to the coagulation and could consume polyaluminum chloride
(PACl) due to the formation of chelated complexes by these inhibitory proteins and the coagulants (Takaara et al. 2007). However, poor DON removal is found by the conventional water treatment processes (coagulation–sedimentation–filtration) (Lee et al. 2005; Chu et al. 2011). Therefore, how to remove the DON effectively and safely was a critical issue on the control of N-DBPs in drinking water.

It is well known that coagulation is the key step to remove organic matter in the water treatment process and may play an important role in the removal of DON through some kinds of enhancement (Ma et al. 1997). Pre-oxidation is one of the common ways to enhance the organics’ removal and improve the water quality of the outflow, among which potassium permanganate (KMnO4) is the most popular pre-oxidation agent. According to former studies, KMnO4 pre-oxidation can enhance the coagulation effects, improve the removal of organics, algae cells, microcystins, odours, humic acids and some inorganic ions (Eary & Rai 1987; Dietrich et al. 1995; Chen & Yeh 2005; Rodríguez et al. 2007, 2008; Liu et al. 2009; Zhao et al. 2012; Fan et al. 2013). However, little is known on the direct removal performance and enhanced coagulation effects on the removal of DON by KMnO4 pre-oxidation.

Therefore, the main purpose is to investigate the removal performance of the KMnO4 pre-oxidation to the proteins and its enhanced effects to the coagulation process. The function mechanism is discussed in the meantime. IOM taken from M. aeruginosa cells and phycocyanin (the typical proteins derived from algae cells) are chosen as the target compounds in the experiment.

**MATERIAL AND METHODS**

**Materials**

Reagents were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). All the chemicals used were at least of analytical grade, except as noted without further purification.

*Microcystis aeruginosa* was selected as the source of the IOM for its widespread existence in eutrophic surface water sources. The initial *Microcystis aeruginosa* was purchased from Institute of Hydrobiology, Chinese Academy of Sciences. BG11 medium was used in batch axenic cultures method with 1 L conical flasks as container. The conical flasks were placed in an incubator and the algal cells were cultured at the temperature of 25 °C with illumination of 5,000 lx provided for 14 h every day. The live algal suspensions were harvested with culture time between 15 and 28 days and diluted by ultra-pure water to prepare the IOM samples (the concentration of DON was diluted to about 1 mg/L). The preparation method of IOM samples refers to our former study (Liu et al. 2011).

Phycocyanin was obtained from Sigma Company (USA), and the solution was prepared by dissolving phycocyanin in distilled water with a concentration of about 10 mg/L.

**Experiments**

The raw water used in the experiment was gained through dosing a certain content of IOM or phycocyanin into the pure water. In order to supply proper alkalinity, a certain volume of NaHCO3 with concentration of 0.1 mol/L was added into the pure water at the same time.

**Coagulation**

For coagulation only, the main steps were as follows: certain dosages (5–40 mg/L) of PACl and 1,000 mL of each sample were put into a circular jar and agitated with a agitator apparatus at 150 rpm for 2 min, followed by a slow mixing at 60 rpm for 6 min, and at 30 rpm for 6 min. Samples were left for 30 min; then each sample’s supernatant (100 mL) was collected by a U-shaped pipette in order to avoid the suction of precipitated solids. The samples were used to determine the turbidity, DON, protein concentration, etc.

**KMnO4 pre-oxidation**

A certain dosage of KMnO4 (the dosage used in the experiment was normally 0.5–2.0 mg/L, sometimes amplified to 5 mg/L to better explain the function mechanism) was added into the 1,000 mL solution and agitated at 150 rpm for 60 min together, sampled at a certain time, and the DON and protein concentration were determined.
Combined process of pre-oxidation and coagulation

For coagulation combined with pre-oxidation, a certain dosage of KMnO₄ was added into the 1,000 mL solution and reacted for 30 min, then the coagulation process was carried out just as in the Coagulation section.

Adsorption of MnO₂

The adsorption of DON by MnO₂ formed in-situ in de-ionized water was studied. MnO₂ was prepared through the reduction of potassium permanganate by sodium thiosulfate in 0.015 M sodium perchlorate solution (Perez-Benito et al. 1995). The concentrations of MnO₂ corresponded to the KMnO₄ concentration of 0.5, 1.0, 1.5, 2.0 and 3.0 mg/L.

The interaction between DON and solid phase MnO₂ was allowed a total contact time of 1 h, during which the solutions were constantly stirred at 150 rpm. At the end of the chosen contact time, the solution was filtered under vacuum through a 0.45 μm membrane filter.

Analysis methods

Except for the water samples to determine the turbidity, other samples were filtered using 0.45 μm filters prior to analysis. DON was determined from the difference between measured total dissolved nitrogen and sum of measured total inorganic nitrogen (DIN) species using Equation (1).

\[ \text{DON (mg/L)} = \text{TN} - (\text{NH}_3\cdot\text{N} + \text{NO}_2\cdot\text{N} + \text{NO}_3\cdot\text{N}) \]  

(1)

The Coomassie brilliant blue method was used to investigate the protein concentration.

Two-dimensional electrophoresis (2-DE) was used to determine the composition of IOM before and after oxidation, and was carried out in a commercially available electrophoresis unit (GE Ettan DALT II system, GE, USA) according to published procedures (Bollag et al. 1991).

The apparent molecular weight distributions (MWD) of the samples were determined using ultrafiltration (UF). A series of UF membranes with molecular weight cut-off of 1,000 Da, 3,000 Da, 10,000 Da, and 30,000 Da were used and then the DON level of each fraction collected was determined.

The protein surface hydrophobicity was measured with the method of fluorescence polarization. 0.4 mL of the sample was mixed with 0.6 mol/L KCl phosphate buffer (pH = 7.0), then 10 μL 8 mmol/L fluorescence probe (ANS) was added. After 10 min standing in the dark, fluorescence intensity was measured at room temperature using a fluorescence spectrophotometer (Model F-7000, Hitachi, Tokyo, Japan) in the range of 200–700 nm. The samples were excited at 374 nm, the excitation and emission slit widths were 5 nm, correction scanning speed: 1,200 nm/min.

According to the results of 2-DE, parts of the protein spots which were significantly different were selected using trypsin to conduct in-gel digestion and extract the polypeptide. The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) technique was used for analysis. 1 μL of the eluate was mixed with 1 μL of the matrix solution on the target plate. Parameter settings: mode (positive ionization mode and reflectron operation mode). The samples with the matrix were ionized by nitrogen laser pulse (λ = 337 nm), and the collision induced voltage was 20–45 V. Searching the peptide mass fingerprinting by search engine Mascot on Matrixscience website, retrieval database: NCBIInr, search condition: the error tolerant range was 100 ppm.

RESULTS AND DISCUSSION

Effect of KMnO₄ pre-oxidation on the DON removal

The raw water synthesized with IOM

Figure 1 shows the removal performance of DON by coagulation combined with the KMnO₄ pre-oxidation process at different dosages.

As seen from Figure 1, KMnO₄ pre-oxidation favoured the coagulation process to remove the DON, and the removal effects varied with the dosage of the KMnO₄ and PACl. When the dosage of PACl was 10 mg/L, the removal rate increased from 15% to 23% and 33% with the 0.5 mg/L and 1.0 mg/L of KMnO₄ pre-oxidation, respectively. As a contrast, the removal rate of DON by KMnO₄ pre-oxidation
alone was only 5.3% and 7.8%. That is to say, the removal rate of the combined process exceeded the sum of KMnO₄ pre-oxidation and coagulation, which indicated that synergistic effects exist in the combined treatment process. It can also be seen from Figure 1 that for a given removal rate, much less coagulant was needed after pre-oxidation.

The results in Figure 2 show that longer oxidation time enhanced the DON removal by the combined process, but the enhanced effects became insignificant when the time was longer than 30 min. Therefore, 30 min of oxidation time was recommended for the application in the water plant.

The raw water synthesized with phycocyanin

Phycocyanin was used to further illustrate the enhanced effect of KMnO₄ pre-oxidation to the DON removal, for it was the main category of proteins in the IOM (Liu et al. 2015). Figure 3 shows the phycocyanin removal effects of KMnO₄ pre-oxidation at different concentrations.

As shown in Figure 3(a), KMnO₄ oxidation process alone could not effectively remove phycocyanin; the removal rate was only about 10% even with higher KMnO₄ dosage and longer reaction time (2 mg/L and 1 h oxidation time). A similar trend was found for the variation of the DON concentration (Figure 3(b)). The relatively lower oxidation and reduction potential of the KMnO₄ in neutral or weakly alkaline solution may be attributed to the poor direct oxidation effects. However, just like the result of Figure 1, a poor direct oxidation effect did not equate to bad enhanced coagulation effects. Figure 4 shows the performance of the combined process of pre-oxidation and coagulation to remove phycocyanin.

Compared with the removal effect of the phycocyanin in the absence of KMnO₄, KMnO₄ pre-oxidation exhibited better removal performance irrespective of the dosage of PACL. Similarly to the removal of IOM, the removal rates of the combined process were higher than the sum of the removal rates in the treatment of the pre-oxidation and coagulation process alone, which also indicated that synergistic effects indeed existed between the two treatment processes. The KMnO₄ pre-oxidation could enhance the phycocyanin removal by coagulation. Two reasons may contribute to the enhanced effects. One was the concentration reduction of the phycocyanin through the way of direct oxidation, decreasing the coagulant demand to some extent; the other was that the main reduction product of the KMnO₄ was hydrated MnO₂, which could adsorb matters like phycocyanin. Similar studies indicated that the MnO₂ produced in-situ during permanganate pre-oxidation played an important role and suggested that MnO₂ may adsorb natural organic materials, in improving removal of organic particulates and also possibly inorganic fine particles (Yao & Millero 1996; Yadanaparthi et al. 2009).

As to the removal effects of the two parameters, Figure 4(a) and 4(b) show very different removal effects for the
proteins and DON by the same treatment process. DON could not be removed effectively even with the aid of KMnO₄ pre-oxidation; the maximum removal rate was about 28%. However, the removal rate of phycocyanin was remarkably higher than that of DON; the removal rate could reach about 80%. The reason lies in two aspects: one is the interaction between the metal ion and phycocyanin molecule and formation of complexes, which could be removed effectively during the coagulation process (Takaara et al. 2010). The other is the purity of the phycocyanin. Although the phycocyanin used in the study was extracted, large amounts of other matter containing nitrogen existed in the samples and could not be removed easily, which caused the significant removal difference between the DON and phycocyanin: that is to say, the decrease of proteins in the sample attributed to the DON removal.

**Possible enhanced DON-removing mechanism of KMnO₄ pre-oxidation**

The KMnO₄ pre-oxidation enhanced the removal performance of the coagulation to the DON, but the enhancement mechanism need to be discussed, especially for the typical
protein of IOM. Some methods were used to explain the enhanced removal mechanism.

**Effect of KMnO₄ pre-oxidation on the molecular weight of DON**

According to our former study (Liu *et al.* 2011), the coagulation process could remove part of organics with particular molecular weight effectively. Therefore, the variations of the DON MWD before and after oxidation were investigated, and the results are shown in Figure 5.

Little variation was found in the MWD of DON even with a 3 mg/L potassium permanganate dosage (Figure 5), which indicated that KMnO₄ pre-oxidation affected the molecular weight of N-containing organics indistinctively. The minor change of the MW may not contribute to the significantly enhanced effects on the DON removal.

**Effects of the pre-oxidation on the IOM hydrophobicity**

The hydrophobicity of the target compound is another main factor that affects the DON removal of coagulation; therefore, the alteration of hydrophobicity before and after the pre-oxidation need to be investigated. In view of the higher contents of algal proteins and free amino acids in IOM, exogenous fluorescence was used to investigate the change of hydrophobicity, and the results are shown in Figure 6.

As seen from Figure 6, with the increase of the oxidant concentration and oxidation time, the fluorescence intensity of algal proteins tended to increase. That is to say, the surface hydrophobicity of algal proteins increased, which indicated partial damage to the algal proteins’ structure to form new proteins and little protein was directly removed by the KMnO₄ oxidation. The increase of hydrophobicity means higher coagulant demand, and its alteration after oxidation could not explain the enhanced removal effect of the KMnO₄ pre-oxidation.

**The 2-DE of algal proteins**

2-DE combined with Image-master 2D 6.0 was used to identify the protein spots, and MALDI-TOF-MS was used to further determine the concentration of the different proteins before and after pre-oxidation. The results are shown in Figure 7 and Table 1.

Seen from Figure 7(a), there were about 230 protein spots detected in the raw water synthesized with IOM, of which the majority were located in the acidic side with MWD of 20–80 kD, while the results of Figure 7(b) show that little alteration of the protein spots occurred after the oxidation with dosage of 2 mg/L and reaction time of 1 h. About 10 protein spots were found to vary relatively much through the oxidation of KMnO₄ (Table 1), during which spots 1–8 decreased and spot 9, 10 increased. According to the total concentration of spots 1–8 and the reduction of the protein, the decrease of spots 1–8 was mainly attributed to the reduction of the total protein. However, the variation of the protein spots 1–10 could not explain...
the enhanced mechanism, but only verified the poor direct removal effect of DON by KMnO₄ pre-oxidation.

In all, the changes on the DON caused by the pre-oxidation were not the main reason for its enhanced removal performance. The reducing products of the KMnO₄ may be the crucial factor to enhance the DON removal. It is well known that the main category of the reduced KMnO₄ is MnO₂ or hydrated MnO₂, which shows better oxidation and superior adsorption ability to most of the pollutants in the raw water. The direct adsorption results of the in-situ formed MnO₂ to the phycocyanin are shown in Figure (8).

The results in Figure 8 show that the in-situ formed MnO₂ has a certain removal ability to the phycocyanin and IOM in water, and the removal was mainly contributed through the adsorption, because the MnO₂ was unable to oxidize phycocyanin and IOM due to its limited oxidation ability. Comparing the removal performance of the combined process, pre-oxidation, coagulation and adsorption, the removal rate of the combined process was similar to the sum of the other three processes, which could explain the enhanced effect of KMnO₄ pre-oxidation to coagulation. That is to say, the adsorption of in-situ formed MnO₂ was

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**Figure 7** The spectrum of 2-DE before and after oxidation (a) before pre-oxidation; (b) after pre-oxidation with dosage of 2.0 mg/L and contact time of 1 h.

| Table 1 | The alteration of 10 protein spots before and after oxidation (with KMnO₄ dosage of 2.0 mg/L and contact time of 1 h) |
|---|---|---|---|---|
| No. | Proteins category | Theoretical molecular weight (kD)/Isoelectric point | ID in NCBI | Concentration of proteins (mg/L) |
| | | | | Before oxidation | After oxidation |
| 1 | Conservative hypothetical protein | 34,073/6.27 | gi|389802325 | 0.085 | 0.067 |
| 2 | Phycocyanin α Apoenzyme | 10,095/9.66 | gi|17063593 | 0.092 | 0.075 |
| 3 | Phycocyanin β Apoenzyme | 18,324/5.12 | gi|166365186 | 0.089 | 0.07 |
| 4 | Unnamed protein product 1 | 20,447/5.60 | gi|389733951 | 0.056 | 0.043 |
| 5 | Unnamed protein product 2 | 22,046/4.75 | gi|159030269 | 0.061 | 0.048 |
| 6 | Thioredoxin peroxidase | 22,073/4.70 | gi|166366324 | 0.068 | 0.046 |
| 7 | SSU Methyltransferase Ribosome RNA G | 25,599/5.84 | gi|389883289 | 0.056 | 0.041 |
| 8 | Unnamed protein product 1 | 25,468/5.10 | gi|159026115 | 0.053 | 0.042 |
| 9 | Glutamine synthetase | 15,311/4.89 | gi|345524119 | 0.053 | 0.086 |
| 10 | NUDIX Hydrolytic enzyme | 16,099/5.07 | gi|389882725 | 0.041 | 0.068 |
the main enhanced mechanism for the DON removal, especially for the proteins' removal.

Significance of the KMnO4 pre-oxidation for the coagulation to raw water with higher content of DON

The eutrophication of the water source poses several challenges to the drinking water treatment, including the interference of algae cells with the operation of water plants, threatening the water quality (microcystins, geosmin and 2-MIB), and higher formation potential of disinfection by-products (C-DBPs, N-DBPs). According to former studies, KMnO4 shows better performance on the enhanced coagulation, improved removal of algae cells, natural organics (humics, etc.) and metabolites (Chen & Yeh 2005; Rodríguez et al. 2008; Zhao et al. 2012; Fan et al. 2013). The above results demonstrate their improved effects on the DON and proteins, but the influence of KMnO4 pre-oxidation on the coagulation performance to the raw water with plenty of proteins has not been confirmed. The corresponding experiments were done and the results are shown in Figure 9.

As seen from Figure 9, KMnO4 pre-oxidation could enhance the coagulation evidently; with dosage of 1 mg/L and reaction time of 0.5 h, the turbidity of the supernatant was obviously lower than that of coagulation alone. The reason lay in the aid of in-situ formed MnO2 and partial oxidation of proteins.

In all, KMnO4 pre-oxidation could enhance the coagulation effects and DON removal mainly through the adsorption of MnO2 formed in the oxidation process. According to the removal mechanism, some advantages on DON removal may exist for KMnO4 pre-oxidation compared with other oxidation treatments. One is the little alteration of the molecular structure of the proteins in the removal process. Proteins had relatively higher molecular and intact structure, which made them more easily removed in the following treatment than their oxidation products, especially amino acids; second is the relatively few by-products. There were few by-products found in the treatment process, and which could guarantee the safety of the treated water.

CONCLUSIONS

1. KMnO4 pre-oxidation could enhance the removal effect of coagulation to DON from the synthesized raw water with IOM and phycocyanin. However, the direct oxidation of KMnO4 had poor removal performance.
2. KMnO4 pre-oxidation altered the molecular weight, hydrophobicity and protein categories of the DON insignificantly.
3. The main enhanced mechanism of KMnO4 pre-oxidation to coagulation for DON removal mainly lies in the adsorption of in-situ formed MnO2 in the oxidation process.
4. The KMnO4 pre-oxidation could enhance the turbidity removal of the coagulation due to a similar mechanism in the meantime.
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