Comparison of the effects of aluminum and iron(III) salts on ultrafiltration membrane biofouling in drinking water treatment

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ABSTRACT
Coagulation plays an important role in alleviating membrane fouling, and a noticeable problem is the development of microorganisms after long-time operation, which gradually secrete extracellular polymeric substances (EPS). To date, few studies have paid attention to the behavior of microorganisms in drinking water treatment with ultrafiltration (UF) membranes. Herein, the membrane biofouling was investigated with different aluminum and iron salts. We found that Al₂(SO₄)₃·18H₂O performed better in reducing membrane fouling due to the slower growth rate of microorganisms. In comparison to Al₂(SO₄)₃·18H₂O, more EPS were induced with Fe₂(SO₄)₃·xH₂O, both in the membrane tank and the sludge on the cake layer. We also found that bacteria were the major microorganisms, of which the concentration was much higher than those of fungi and archaea. Further analyses showed that Proteobacteria was dominant in bacterial communities, which caused severe membrane fouling by forming a biofilm, especially for Fe₂(SO₄)₃·xH₂O. Additionally, the abundances of Bacteroidetes and Verrucomicrobia were relatively higher in the presence of Al₂(SO₄)₃·18H₂O, resulting in less severe biofouling by effectively degrading the protein and polysaccharide in EPS. As a result, in terms of microorganism behaviors, Al-based salts should be given preference as coagulants during actual operations.

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Introduction

Ultrafiltration (UF) membranes have been widely used in drinking water treatment due to the improved quality of effluent produced, even with variable feed-water properties (Jermann et al., 2007). However, membrane fouling is inevitable after long-time operation, which has constrained its further utilization (De Souza and Basu, 2013). Previous studies have shown that membrane fouling leads to the reduction of membrane flux and an increase in energy consumption, resulting in increased cost during water treatment (Leiknes, 2009).

Three main fouling mechanisms are known to occur as a function of time: pore constriction, pore blocking and cake layer formation (Wang and Tarabara, 2008). To effectively alleviate membrane fouling, various kinds of technologies have been investigated, such as coagulation, adsorption, preparing new membrane materials (Dong et al., 2007; Hua et al., 2008; Gong et al., 2015), etc. However, owing to its lower cost, easier operation procedure, and higher removal
efficiency for pollutants, coagulation is still the most promising method in alleviating membrane fouling currently, and it has already been widely applied in water plants (Gao et al., 2011).

For coagulation, Al-based salts and Fe-based salts are the most commonly used coagulants. To reduce membrane fouling, a number of studies have paid much attention to improving the removal efficiency, and it has been demonstrated that the hydrolyzed flocs play an important role (Jiang and Graham, 1996; Jeong et al., 2014; Wang et al., 2017). Further research has revealed that aluminum speciation and iron speciation are also critical to the removal efficiency, especially aluminum speciation (Zhao et al., 2009; Ma et al., 2015). It has been shown that monomeric aluminum species are easily bound to polysaccharide and cellulosic molecules (Masion et al., 2000; Dong et al., 2014). The preferred species Al\(_{13}\) are easily bound to carboxylic groups under acidic conditions, while they are easily bound to phenolic moieties under alkaline conditions (Kazpard et al., 2006).

In recent years, the removal mechanism after coagulation has gradually become much clearer. However, a noticeable problem is the development of microorganisms after long-time operation, which can produce extracellular polymeric substances (EPS). These EPS, mainly composed of protein and polysaccharide, not only cause severe membrane fouling by forming a denser biofilm, but also can deteriorate the effluent quality (Komlenic, 2010). Most studies have focused on inhibiting the development of microorganisms through disinfection (Komlenic, 2010; Gao et al., 2011; Hook et al., 2012), while the composition and function of the microbial community in the cake layer are largely unknown.

Herein, to fully understand the membrane biofouling after coagulation, the membrane performance was investigated with Al\(_2\)(SO\(_4\))\(_3\)-18H\(_2\)O and Fe\(_2\)(SO\(_4\))\(_3\)-xH\(_2\)O because of the strong corrosiveness of FeCl\(_3\)-xH\(_2\)O during actual operations (Esih et al., 2005). The purpose is to provide a better understanding of the influence of coagulants on microorganisms’ behaviors, including the growth of microorganisms, the proportion of protein and polysaccharide in EPS, and the membrane fouling contributed by bacteria, fungi, archaea, etc.

### 1. Materials and methods

#### 1.1. Materials

The chemical reagents used were analytical grade except where specified. Al\(_2\)(SO\(_4\))\(_3\)-18H\(_2\)O and Fe\(_2\)(SO\(_4\))\(_3\)-xH\(_2\)O were purchased from Sinopharm Chemical Regent, Co., Ltd. (China). To simulate micro-polluted surface water, domestic sewage was mixed from Sinopharm Chemical Regent, Co., Ltd. (China). To simulate the growth of microorganisms, the proportion of the influence of coagulants on microorganisms’ behaviors, including the growth of microorganisms, the proportion of protein and polysaccharide in EPS, and the membrane fouling contributed by bacteria, fungi, archaea, etc.

#### 1.2. Experimental setup

Fig. 1 shows the schematic diagram of the experimental setup. For the coagulation section, the concentration of Al\(_2\)(SO\(_4\))\(_3\)-18H\(_2\)O or Fe\(_2\)(SO\(_4\))\(_3\)-xH\(_2\)O was 0.05 mmol/L. A rapid mixing speed was maintained at 300 r/min for 1 min, and then decreased to 100 r/min for 14 min. For the filtration section, a polyvinylidene fluoride (PVDF) hollow fiber membrane (Motianmo, China) was used, and the average pore size was 30 nm (provided by the manufacturer).

The total surface area of the submerged membrane in the membrane tank was 0.025 m\(^2\). The constant permeate flux was kept at 20 L/m\(^2\)-hr, with a cycle of filtration for 30 min followed by 1 min backwashing (40 L/m\(^2\)-hr), with aeration (100 L/hr). The hydraulic retention time (HRT) of the membrane tank was 0.5 hr. During the operation, the transmembrane pressure (TMP) was monitored each day to reflect the development of membrane fouling. No additional disinfection method was used during filtration and the sludge was discharged every three days.

#### 1.3. Characteristics of flocs

To investigate the membrane performance in detail, floc characteristics were tested with a jar test. The beaker (1.0 L) was linked with a Mastersizer 2000 laser diffraction instrument (Malvern, UK) by a silicone tube (internal diameter: 5 mm). The water was driven by a suction peristaltic pump at a flow rate of 2 L/hr (Yu et al., 2015). For the test, the rapid mixing speed was also maintained at 300 rpm for 1 min, and then decreased to 100 r/min for 14 min. D\(_{50}\) was used to represent the average diameter of flocs. The fractal dimension (D\(_f\)) was calculated with the small angle light scattering method when flocs reached their steady-state size (Wu et al., 2002).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Feed water</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.52 ± 0.11</td>
</tr>
<tr>
<td>Water temperature (°C)</td>
<td>21.8 ± 1.7</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>2.97 ± 0.08</td>
</tr>
<tr>
<td>Average particle size (nm)</td>
<td>21.7 ± 4.8</td>
</tr>
<tr>
<td>NO(_3) (mg/L)</td>
<td>0.64 ± 0.11</td>
</tr>
<tr>
<td>NO(_2) (mg/L)</td>
<td>3.62 ± 0.45</td>
</tr>
<tr>
<td>NH(_4) (mg/L)</td>
<td>1.32 ± 0.24</td>
</tr>
<tr>
<td>UV(_254) (cm(^{-2}))</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Total organic carbon (TOC, mg/L)</td>
<td>4.09 ± 0.26</td>
</tr>
</tbody>
</table>

Table 1 – Characteristics of feed water.

#### 1.4. Measurement of EPS in cake layer and membrane tank

The foulants on the membrane surface were washed by phosphate buffer saline solution (0.01 mol/L, pH 7.4) after filtration. Then, the solution was heated at 80°C for 30 min, followed by centrifuging at 20,000 r/min for another 5 min, and then the supernatant was collected for EPS analysis (Zhang et al., 1999). The concentration of protein was determined using a BCA kit (Tiangen, China), and the concentration of polysaccharide was measured by the phenol-sulfuric acid method (Saha and Brewer, 1994). Similar methods were also employed for measuring the concentration of protein and polysaccharide in the water of membrane tank.

#### 1.5. Microscopic observation of fouling layer

Five centimeters of membrane was cut from the membrane modules at the end. These membrane fibers were placed in 0.1 mol/L phosphate buffer with 3.0% glutaraldehyde at pH 7.2.
to fix the microorganisms, and were dehydrated with a gradient ethanol series (Dizge et al., 2011). The morphology of the membrane was observed by scanning electron microscopy (SEM, Hitachi SU8000, Japan).

The sludge on the membrane surface was stained by Concanavalin A conjugated to Texas red (ConA-Texas red, Molecular Probes, USA), FITC: fluorescein isothiocyanate (Sigma, USA) and DAPI: 4',6-diamidino-2-phenylindole (Sigma, USA) to measure the concentration of microorganisms, protein and polysaccharide, respectively (Schmid et al., 2003; Crampin et al., 2005). The stained sludge was tested by confocal laser scanning microscopy (CLSM) with TCS SP5 (Leica, Germany). The images were analyzed by ImageJ software (National Institutes of Health, USA) to calculate the relative concentrations of microorganisms, protein and polysaccharide with the integrated optical density (IOD) (Matkowskyj et al., 2003).

1.6. DNA extraction, real-time quantitative PCR (qPCR) analysis and high-throughput pyrosequencing

DNA from the foulants on the membrane surface was extracted by a Fast DNA SPIN Kit for Soil (MP Biomedicals, France) according to the procedure described by the manufacturer, and the DNA was stored at −20°C prior to use.

Quantification of microorganisms was performed by qPCR using the ABI 7500 Real-Time PCR System (Life Technologies, USA). The total volume for qPCR analysis was 20 μL, which contained 10 μL 2 x SYBR Premix Ex Taq II (Takara Bio Inc.), 0.4 μL 50 x ROX Reference Dye II, 1 μL template, 1 μL forward primer, 1 μL reverse primer, and 6.6 μL sterilized Milli-Q water. The bacterial 16S rDNA was amplified using a forward primer (5′-GAGGAAATAAAGCTCACAAAGGTTC-3′), and a reverse primer (5′-CAAATTCACAAAGGTAGGATT-3′) (Lwin et al., 2011). The archaea 16S rDNA was amplified using a forward primer (5′-AAGGTTTARTCCGAGTGRRTTC-3′), and a reverse primer (5′-TGACCACCTTGAGTGCTG-3′) (Agoue et al., 2008). The procedure of PCR was as follows: 1 cycle of 95°C for initial denaturation (30 sec), 40 cycles of 95°C for denaturation (5 sec), and 60°C for extension (30 sec). To absolutely quantify the copy number by qPCR, the standard curves were made with DNA extracted from pure cultures of Escherichia coli for bacteria, Saccharomyces cerevisiae for fungi, and Methanobacterium formicicum for archaea. The minimum copy number of standard DNA was 40 copies/μg, and the maximum was $4 \times 10^7$ copies/μg.

The DNA samples were measured by pyrosequencing on the V3 and V4 regions of the 16S rDNA gene using the illumina Miseq PE 300 platform (GENEWIZ, Inc.). After pyrosequencing, the raw data were initially filtered to discard short and low-quality sequences under the following principles: (1) remove forward primer and barcode in the sequences (Trimmomatic,V0.3); (2) remove the bases with trailing quality scores under 20 and sequences shorter than 400 bps (Trimmomatic,V0.3); (3) remove chimera sequences (usearch, V8.0). The clean data with similarity 97% were clustered into one operational taxonomic unit (OTU) by QIIME (V1.7). Sequences were assigned to taxonomies on the phylum level with the SILVA database (Silva 111 16S rRNA database).

1.7. Other analytical measurements

UV$_{254}$ was measured by an ultraviolet/visible spectrometer (U-3010, Hitachi, Japan) with 0.45 μm sample pre-filtering. Nitrate and nitrite were measured with an ion chromatograph.
development of TMP decreased (days 19–36). However, more EPS was released with the further growth of microorganisms as a function of time, and faster development of TMP occurred again due to the denser cake layer formed (days 37–60). A similar phenomenon was reported by a previous study in wastewater treatment (Lin et al., 2010).

2.2. Concentration of EPS in membrane tank and cake layer

To investigate the properties of biofouling, the concentration of EPS in the membrane tank was analyzed at day 60 (Fig. 3a). The results showed that a higher concentration of EPS was observed in the presence of Fe$_2$(SO$_4$)$_3$·xH$_2$O. The specific concentrations of protein and polysaccharide were 9.85 ± 0.84 and 1.77 ± 0.23 mg/L in the presence of Fe$_2$(SO$_4$)$_3$·xH$_2$O, while the specific concentrations of protein and polysaccharide were 10.39 ± 0.69 and 1.98 ± 0.08 mg/L in the presence of Al$_2$(SO$_4$)$_3$·18H$_2$O.

For the EPS in the cake layer, it was found that the concentrations of protein and polysaccharide with Al$_2$(SO$_4$)$_3$·18H$_2$O were 106.41 ± 2.84 and 17.85 ± 0.55 μg/g, respectively. The corresponding concentrations of protein and polysaccharide were also higher in the presence of Fe$_2$(SO$_4$)$_3$·xH$_2$O, at 147.56 ± 3.83 and 24.03 ± 0.58 μg/g, respectively. The concentrations of both protein and polysaccharide induced by iron salts were approximately 1.4 times those induced by aluminum salts, indicating that microorganisms were not easily nourished with Al$_2$(SO$_4$)$_3$·18H$_2$O. A possible reason was that iron was necessary to the growth of microorganisms (Alasonati and Slaveykova, 2012), while aluminum posed potential toxicity to some extent (Auger et al., 2013).

In comparison to polysaccharide, it has been reported that the degradation of protein was lower by microorganisms because of their molecular structure (Masse et al., 2006). Thus, protein was the dominant composition in EPS, in which the concentration of protein was much higher than that of polysaccharide (Fig. 3).

2.3. SEM of cake layer and characteristics of flocs

SEM images were investigated to understand the morphology of the membranes (Fig. 4a, b), which showed that the presence of EPS in the cake layer produced with Fe$_2$(SO$_4$)$_3$·xH$_2$O was more visible. Therefore, the SEM images provided information that was consistent with the physical results discussed previously, which showed that the concentrations of EPS in the membrane tank and cake layer were higher with Fe$_2$(SO$_4$)$_3$·xH$_2$O than with Al$_2$(SO$_4$)$_3$·18H$_2$O.

Owing to the larger particle sizes of flocs formed by Al$_2$(SO$_4$)$_3$·18H$_2$O and Fe$_2$(SO$_4$)$_3$·xH$_2$O (Fig. 4c), cake formation gradually became the main fouling mechanism (Yu et al., 2013). The average particle size of flocs in both cases was much larger than the average pore size of the UF membrane (30 nm). The average particle size of flocs hydrolyzed by Al$_2$(SO$_4$)$_3$·18H$_2$O was 216.8 ± 12.7 μm, while that hydrolyzed by Fe$_2$(SO$_4$)$_3$·xH$_2$O was 432.6 ± 19.8 μm. Although the particle size of Al-based flocs was smaller, it appeared that Al-based flocs were larger on the membrane surface (Fig. 4a, b). One possible reason was the larger fractal dimension of Fe-based flocs. The larger the fractal dimension, the higher the compressibility is (Yu et al., 2015). The fractal dimension of flocs hydrolized...
by Fe$_2$(SO$_4$)$_3$·xH$_2$O was 2.78 ± 0.17, while that hydrolyzed by Al$_2$(SO$_4$)$_3$·18H$_2$O was 2.61 ± 0.23 (Fig. 4d). Another potential reason for the difference was dehydration taking place before the SEM measurement, which might change the morphology of flocs. Furthermore, it seemed that the cake layer formed by Fe-based flocs was a little denser than that formed by Al-based flocs (Fig. 4a and b), resulting in more severe membrane fouling to some extent.

2.4. CLSM of cake layer

To further confirm the substances on the membrane surface, CLSM was investigated (Fig. 5). It has been reported that blue fluorescence occurs when DAPI is conjugated with DNA, and green fluorescence occurs when FITC is conjugated with protein, while red fluorescence occurs when ConA-Texas red is conjugated with polysaccharide (Wu et al., 2002).

Fig. 5 shows the corresponding fluorescence after dyeing. The more blots were observed, the higher the concentrations of microorganisms, protein and polysaccharide were. In comparison to Al$_2$(SO$_4$)$_3$·18H$_2$O, the concentrations of microorganisms, protein and polysaccharide were approximately 2.7, 2.6 and 2.1 times those in the presence of Fe$_2$(SO$_4$)$_3$·xH$_2$O, respectively. Although the ratio of protein to polysaccharide was a little larger than that described in Section 2.2, both showed the same trend. These different ratios were caused by the heterogeneous distribution of EPS in the cake layer, while the surface cake layer was measured when taking CLSM.
2.5. qPCR analysis

It is known that the microbial community in foulants on membrane surfaces is a mixture of fungi, bacteria and archaea, and the proportions of these microorganisms vary under different operating conditions (Hai et al., 2008; Hong et al., 2013). To further understand the biofouling degree contributed by microorganisms, the compositions were analyzed. As shown in Fig. 6, the dominant microorganisms were bacteria (the concentration of archaea in the cake layer was undetectable), either after coagulating with Al$_2$(SO$_4$)$_3$·18H$_2$O or Fe$_2$(SO$_4$)$_3$·xH$_2$O. For Al$_2$(SO$_4$)$_3$·18H$_2$O, the concentration of bacteria was $(1.13 \pm 0.02) \times 10^4$ copies/ng, while the concentration of fungi was only $(0.04 \pm 0.01) \times 10^4$ copies/ng. For Fe$_2$(SO$_4$)$_3$·xH$_2$O, the concentration of bacteria was $(1.61 \pm 0.02) \times 10^4$ copies/ng, while the concentration of fungi was $(0.07 \pm 0.01) \times 10^4$ copies/ng. The concentration of bacteria was 10 times higher than that of fungi. The main reason was that environmental conditions play an important role in the growth of bacteria and fungi, and bacteria grow much faster.

Fig. 5 – Confocal laser scanning microscopy (CLSM) images of the cake layer on membrane surface. (a), (c) and (e) stained with DAPI: 4',6-diamidino-2-phenylindole, FITC: fluorescein isothiocyanate and ConA-Texas red in the presence of Al$_2$(SO$_4$)$_3$·18H$_2$O; (b), (d) and (f) stained with DAPI, FITC and ConA-Texas red in the presence of Fe$_2$(SO$_4$)$_3$·xH$_2$O.
under lower water temperature conditions (21.8 ± 1.7°C) (Pietikäinen et al., 2005). In addition, owing to the utilization of iron salts (Kawaichi et al., 2013), the concentration of bacteria induced by Fe-based flocs was approximately 1.5 times higher than that induced by Al-based flocs.

2.6. Analysis of bacterial community

The classification of bacteria was further investigated due to the higher proportion of bacteria in microorganisms. As shown in Fig. 7, the main phylum was Proteobacteria, which was in accordance with the results reported in wastewater treatment (Yu et al., 2012).

It is clear that the abundance of Proteobacteria with Fe-based salts was higher than that with Al-based salts. The Proteobacteria, such as Escherichia coli, Salmonella enterica and Pseudomonas aeruginosa, often exhibited a phenomenon called quorum sensing (Swift et al., 1997; Sauer et al., 2002; Smith and Ahmer, 2003). This is a bacterial communication system related to small diffusible signaling molecules, which can activate the expression of genes that stimulate population density control (Siddiqui et al., 2015). Quorum sensing is often associated with biofilm formation, which stimulates the production of EPS (Lin et al., 2014). The larger the proportion of Proteobacteria was, the higher the concentration of EPS on the membrane surface was. Therefore, severe membrane fouling was caused in the iron-rich environment (Fig. 2).

Lower abundance of bacteria also had significant effects on biofouling. In comparison to Al₂(SO₄)₃·18H₂O, the abundance of Bacteroidetes was lower in the iron-rich environment; these bacteria have been found to have strong degrading ability toward protein and polysaccharide (Lin et al., 2014). As a result, relatively lower concentrations of EPS were induced by Al-based salts. Verrucomicrobia also make a significant contribution to polysaccharide degradation in the whole microbial community (Freitas et al., 2012). Based on the functions of Proteobacteria, Bacteroidetes and Verrucomicrobia, the development of TMP was faster in the presence of Fe₂(SO₄)₃·xH₂O.

In addition, other bacteria, such as Nitrospirae, Acidobacteria, and Planctomycetes, contributed little to membrane biofouling due to their different functions. However, it should be particularly pointed out that Firmicutes can utilize iron salts (Li et al., 2011). As a result, the abundance of Firmicutes was higher with Fe-based salts. Fig. 7 shows that the abundance of Firmicutes was 0.21% in Al-based salt systems, while it was 1.76% in Fe-based salt systems.

3. Conclusions

Although UF membranes have been widely used in drinking water plants, less attention has been paid to the growth rate of microorganisms during UF. This study was focused on biofouling in the presence of Al₂(SO₄)₃·18H₂O and Fe₂(SO₄)₃·xH₂O. The results showed that less severe biofouling was induced with Al₂(SO₄)₃·18H₂O, because of the slower growth rate of microorganisms and lower concentrations of protein and polysaccharide in the membrane tank and cake layer.

qPCR and high-throughput pyrosequencing indicated that the dominant microorganisms were bacteria, after coagulating with either Al₂(SO₄)₃·18H₂O or Fe₂(SO₄)₃·xH₂O. Proteobacteria was the main constituent, resulting in a denser biofilm, especially for Fe₂(SO₄)₃·xH₂O. Although the abundance of Bacteroidetes and Verrucomicrobia were lower, both of them had a strong degrading ability for protein and polysaccharide. It was found that the abundances of Bacteroidetes and Verrucomicrobia were lower in the presence of iron salts. Thus, severe fouling was caused with Fe-based salts.

Based on the findings, we suggest that Al-based salts would be preferentially considered over Fe-based salts as coagulants in UF systems in terms of their microorganism performance, especially with similar removal efficiency during actual operations.

Acknowledgments

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