Characterization of changes in extracellular polymeric substances and heavy metal speciation of waste activated sludge during typical oxidation solubilization processes

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ABSTRACT

Biopolymer solubilization is considered to be the rate-limiting stage of anaerobic digestion of waste activated sludge (WAS). Oxidation processes have been proven to be effective in disrupting sludge flocs and causing solubilization of the solid biopolymers. In this study, WAS was treated by NaNO2 or H2O2 oxidation at pH of 2. The changes in extracellular polymeric substances properties and the speciation of heavy metals were investigated. The results revealed that both NaNO2 and H2O2 treatments were effective in solubilizing organics in WAS, while the conversion of biopolymers in the two treatment processes was different. Free nitrous acid destroyed the gel network structure of EPS, and organic materials were released from the solid phase to the supernatant. Indigenous peroxidase catalyzed H2O2 to produce hydroxyl radicals which caused significant solubilization of biopolymers, and the protein-like substances were further degraded into micro-molecule polypeptides or amino acids at high dosages of H2O2. During the oxidation processes, Zn, Cd and Cu, with excellent mobility, tended to migrate to the supernatant, and thus were easy to remove through the liquid–solid separation process. Ni and As showed moderate migration ability, of which the residual fraction tended to transform into reducible and soluble fractions. With poor mobility, Cr and Pb mainly existed in the forms of residual and oxidizable fractions, which were difficult to dissolve and remove from WAS. Both NaNO2 and H2O2 treatment resulted in the enhancement of sludge solubilization efficiency and heavy metal mobility in WAS, but different heavy metals showed distinct migration and transformation behaviors.

Keywords: Extracellular polymeric substances (EPS) Oxidation Biopolymers solubilization Biodegradability Heavy metals

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Introduction

Due to the rapid development of municipal wastewater treatment, wastewater sludge production has increased substantially and thus has caused enormous pressure on the ecological environment in recent decades (Kim et al., 2002; Wang et al., 2018). Waste activated sludge (WAS) is a colloidal system consisting of microorganisms (mainly bacteria) which are embedded in a biopolymeric network (Wu et al., 2017). Toxic heavy metals have been widely detected in sewage treatment systems of wastewater treatment plants (WWTPs) (Jang et al., 2001); they are easily adsorbed in sludge systems through complexation and electrical neutralization of negatively charged biopolymers (Pagnanelli et al., 2009). Therefore, as the most important by-product of the sewage treatment process, sludge contains harmful substances, such as heavy metals and organic contaminants (Liu et al., 2018). Inappropriate disposal of waste sludge may bring environmental risks, e.g., heavy metals accumulate in organisms and then enter into the human body through the food chain (Liu et al., 2018; Li et al., 2018a). Some methods have been established to remove heavy metals from waste activated sludge, such as application of ethylenediamine chelating agent (Pei et al., 2016), fungi microorganisms (Rezza et al., 2001) and biosurfactants (Reddy et al., 2010). In addition, He et al. (2017) proposed a chelated-Fe$^{3+}$ catalyzed Fenton process to treat WAS for dewaterability improvement and synchronous heavy metal removal.

Extracellular polymeric substances (EPS) are the main organic matters in sludge, consisting of polysaccharides, proteins and hemic substances, etc. (Sheng et al., 2010; Wingender et al., 1999). EPS properties have crucial effects on sludge structure, settling performance, surface charge, flocculation and dewatering properties (Mikkelsen and Keiding, 2002). Yu et al. (2008) suggested that the structure and distribution of EPS were composed of supernatant, slime, loosely bound EPS (LB-EPS), tightly bound EPS (TB-EPS) and pellet, and soluble EPS (S-EPS) fractions. EPS contain large quantities of negatively charged functional groups and have strong capabilities to adsorb heavy metals via electrostatic attraction, complexation, ion exchange, surface precipitation and other types of interaction (Pagnanelli et al., 2009; Wingender et al., 1999; Zhang et al., 2018a). Previous study has shown that heavy metals can bind to EPS with high binding capacity (Guiné et al., 2006). EPS have impacts on the speciation and toxicity of metals in biological wastewater treatment systems, and this has been shown to play an important role in metal mobilization and dispersion (Sheng et al., 2013). EPS of WAS contain various heavy metal adsorption sites, such as aromatic moieties, aliphatic groups in proteins, and hydrophobic regions in polysaccharides (Flemming et al., 1996). This also implies that EPS have a potential impact on the migration and transformation of heavy metals in biological treatment systems. Meanwhile, the binding of heavy metals can alter the structure and configuration of EPS.

Anaerobic digestion (AD) has the advantages of solid reduction, stabilization of the organic fraction and production of biogas. In the EU, more than 50% of WWTPs use anaerobic digestion for sludge treatment, and in recent years the government of China also encourages large and medium WWTPs to give preference to this process. Typically, the AD process can be divided into four stages: solubilization, hydrolysis, acidogenesis and methanogenesis. The traditional anaerobic digestion technology has the shortcomings of low gas production rate and low operating efficiency in the process of sludge treatment, and some sludge anaerobic digestion facilities are difficult to operate stably. Biopolymer solubilization and hydrolysis are considered to be the rate-limiting stages of anaerobic digestion (Zhou et al., 2015). For the efficient operation of the whole sludge anaerobic digestion system, the development of pretreatment technology is imperative. Some researchers focus on the promotion of the anaerobic digestion rate and have developed extensive forced hydrolysis processes, such as thermal, mechanical, chemical and biological methods (Lee et al., 2014; Li et al., 2016; Wang et al., 2013). Many advanced sludge digestion treatment processes have been established to destroy EPS, including enzymatic treatment (Ayol, 2005), thermal treatment (Neyens and Baeyens, 2003a), acid or alkaline treatment (Neyens et al., 2004), thermal hydrolysis pretreatment (Zhang et al., 2018b), ultrasonic treatment (Murugesan et al., 2014), microwave pretreatment (Yu et al., 2010), UV-photocatalysis (Anjum et al., 2017), and pulsed electric field pretreatment (Ki et al., 2015).

Advanced oxidation technologies, such as photo-Fenton or Fenton oxidation (Neyens and Baeyens, 2003b) and sulfate radical oxidation (Zhou et al., 2015), microwave-H$_2$O$_2$ treatment (Liu et al., 2016), electrolysis/electrocoagulation-sulfate radical oxidation (Li et al., 2016), Fe$_3$/S$_2$O$_8$– conditioning-horizontal electro-dewatering process (Li et al., 2018b) and permanganate/bisulfite conditioning-horizontal electro-dewatering (Guo et al., 2017), have been proven to be effective in improving sludge solubilization efficiency. Sun et al. (2018) found freezing with the presence of a suitable concentration of nitrite could promote sludge solubilization and dewaterability. Wei et al. (2018) reported that FNA, i.e. HNO$_2$, enhances the biodegradability of WAS, which suggests that FNA pre-treatment would enhance the destruction of volatile solids (VS) in an anaerobic sludge digester. Moreover, H$_2$O$_2$ can effectively enhance sludge anaerobic digestion under more acceptable operation conditions (Liu et al., 2016). Also, wet oxidation treatment caused the fast solubilization and release of extracellular and intracellular materials from sludge (Urrea et al., 2018). Subsequently, biopolymers underwent a degree of decomposition higher than 70% under wet oxidation treatment (Urrea et al., 2017a). In addition, the more internal biopolymers are located in cells, the more effectively they are attacked by free radicals (Urrea et al., 2017b). Meanwhile, harmful substances (such as heavy metals) may migrate and transform during this process, which brings environmental risks in the subsequent disposal of sludge. Therefore, in order to achieve the stabilization and harmlessness of sludge, the migration and transformation of harmful substances in sludge are worth investigating thoroughly.

As mentioned above, oxidation treatment may affect the composition and distribution of organic matter in sludge, which has an effect on biopolymer solubilization.
and hydrolysis. Meanwhile, the species of harmful substances (heavy metals) may be altered. In this study, the sludge solubilization efficiency of typical oxidation treatments (NaNO₂ or H₂O₂) was evaluated, and changes in EPS properties and the migration and transformation of heavy metals were investigated during the oxidation treatment processes. This study provides knowledge on the enhancement of sludge hydrolysis efficiency and heavy metals ecological risk evaluation of these sludge treatment processes.

### 1. Materials and methods

#### 1.1. Raw sludge and reagents

The sludge was sampled from the Longwangzui WWTP in Wuhan City, China. The WWTP treats approximately 300,000 m³/day wastewater, using an anaerobic/anoxic/oxic (A²O) process. The properties of the sludge were measured according to standard methods (APHA, 1998), and are as following: moisture content 94.03%, pH 7.02, VSS/TSS 0.33, Zeta potential −20.1 mV.
All the reagents for metal analysis were reagent grade (CR) and other reagents were analytical grade (AR), except methyl alcohol (CH3OH; chromatographic grade; ≥99.99%) and 5,5-Dimethyl-1-pyrroline N-oxide (DMPO, for EPR-spectroscopy; CAS: 3317-61-1), and were produced by J&K Chemical Corporation.

1.2. Batch test

Each 200 mL sludge sample aliquot was added into an Erlenmeyer flask. Then the pH of sludge was adjusted to 2 with HCl and NaOH solutions. Aliquots of NaNO2 (0, 5, 10, 20, 30, 40 and 60 mg/L) or H2O2 (0, 300, 800, 1600, 3200, 8000 and 16,000 mg/L) were spiked in the sludge samples under vigorous stirring using a magnetic stirrer. The mixtures were stirred at 200 r/min for 5 hr at ambient temperature. Samples were taken for EPS and heavy metal analysis.

1.3. Analytical methods

1.3.1. Metal analysis

The sludge was centrifuged at 5000×g for 10 min to separate the aqueous phase from the solid phase. The speciation of heavy metals in the solid phase was characterized by the European Community Bureau of Reference (BCR) sequential extraction method (Tessier et al., 1979; Ure et al., 1993). The heavy metals were classified as: soluble fraction, weak acid soluble fraction (f1), reducible fraction (f2), oxidizable fraction (f3) and residual fraction (f4). The mobility factor (MF, %) of heavy metal can be calculated using Eq. (1) (Dong et al., 2013; Zhu and Guo, 2014). The ratio of liquid to solid (L/S, %) heavy metal can be calculated using Eq. (2).

\[
MF = \frac{\text{Supernatant} + f_1}{\text{Supernatant} + f_1 + f_2 + f_3 + f_4} \times 100\%
\]  
\[
L/S = \frac{\text{Supernatant} + f_4}{\text{Supernatant} + f_1 + f_2 + f_3 + f_4} \times 100\%
\]

The concentrations of heavy metals (Cu, Zn, Cd, Cr, Ni, As and Pb) were determined by inductively coupled plasma mass spectrometry (ICP-MS; Agilent Technologies, 7700).

1.3.2. EPS characterization

1.3.2.1. EPS extraction. EPS was extracted according to the procedures described by Cao et al. (2016). An aliquot of 50 mL sludge in a 50 mL centrifugal tube was centrifuged at 2000×g for 10 min; the supernatant was collected as S-EPS. The sludge pellet in the tube was resuspended in 50 mL 0.05% NaCl, then the liquor was centrifuged at 5000×g for 10 min to separate the solids from the supernatant. The collected supernatant was LB-EPS. The residual sludge pellet in the centrifuge tube was then resuspended in a 0.05% NaCl solution, sonicated at 20 kHz for 10 min, and then centrifuged at 2000×g for 20 min to be separated as TB-EPS.

1.3.2.2. EPS analysis. The protein and carbohydrate contents of the extracted EPS were measured according to the procedure described by Frølund et al. (1996). The polysaccharide content in EPS was determined by the phenol sulfuric acid method with glucose as the standard. The protein content of EPS was determined by the Lowry procedure with bovine serum albumin (Sigma) and humic acid (Sigma) as described by Stauffer (1975).

Three-dimensional excitation emission matrix (3D-EEM) spectra were measured with an F-4500 fluorescence spectrophotometer (Hitachi, Japan) with an excitation range of 200–400 nm at 10 nm sampling intervals and an

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**Table 1 – Band assignments for fourier transform infrared spectroscopy features of extracellular polymeric substances based on previous research (Badireddy et al., 2010; Yuan et al., 2011).**

<table>
<thead>
<tr>
<th>Band region</th>
<th>Wavenumber (cm⁻¹)</th>
<th>Band assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocarbons</td>
<td>3697-3629, 3285</td>
<td>O-H stretching vibrations</td>
</tr>
<tr>
<td></td>
<td>2930</td>
<td>C-H stretching of CH₂ groups</td>
</tr>
<tr>
<td>Proteins</td>
<td>1667</td>
<td>C=O and C-N stretching (amide I)</td>
</tr>
<tr>
<td></td>
<td>1530</td>
<td>N-H and C-N stretching in –CONH– (amide II)</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>1035</td>
<td>C-OH stretching and C-O-C ring vibrations</td>
</tr>
<tr>
<td>Nucleic acids</td>
<td>930</td>
<td>O-P-O stretching vibrations</td>
</tr>
</tbody>
</table>
emission range of 280–500 nm at 10 nm sampling intervals. The spectra were recorded at a scan rate of 12,000 nm/min, using excitation and emission slit bandwidths of 10 nm. Each scan had 37 emission and 27 excitation wavelengths.

1.3.3. Electron paramagnetic resonance (EPR) measurements
The active radicals were identified with a Bruker A300-10/12 (Bruker Ltd., Germany) instrument at room temperature. Each aliquot of 50 mL EPS extracting solution was stirred with 2 mL of H2O2 solution (30%, W/W) at room temperature. Then each sample was mixed into 200 mmol/L of DMPO (5,5-dimethyl-1-pyrroline N-oxide) solution and transferred into a 1.5 mL centrifuge tube. After mixing for 30 sec with a vortex mixer, the sample solution was then transferred into a 1.5 mL capillary tube and finally fixed in the cavity of the EPR spectrometer. The samples were reacted in the desired solvent systems in solution for the detection of ·OH or HO2· radicals.

Fig. 4 – Effect of NaNO2/H2O2 dosage on sludge cake properties, FT-IR spectra of sludge cakes under NaNO2(a)/H2O2(b) treatment; XPS results for atomic percentage of conditioned sludge cakes with NaNO2(c)/H2O2(d).

Table 2 – Chemical fractionation (mg/kg DM) of heavy metals in raw sludge.

<table>
<thead>
<tr>
<th>Raw sludge</th>
<th>Cu</th>
<th>Zn</th>
<th>Cd</th>
<th>Pb</th>
<th>Ni</th>
<th>Cr</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>0.11 ± 0.0399</td>
<td>6.37 ± 0.05</td>
<td>nd</td>
<td>1.54 ± 0.08</td>
<td>2.36 ± 0.001</td>
<td>0.65 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Weak acid soluble</td>
<td>40.76 ± 0.33</td>
<td>142.35 ± 3.33</td>
<td>0.48 ± 0.01</td>
<td>0.13 ± 0.1</td>
<td>9.35 ± 0.06</td>
<td>3.90 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Reducible</td>
<td>26.22 ± 0.08</td>
<td>75.27 ± 1.67</td>
<td>0.33 ± 0.01</td>
<td>0.65 ± 0.2</td>
<td>nd</td>
<td>2.78 ± 0.03</td>
<td>1.24 ± 0.04</td>
</tr>
<tr>
<td>Oxidizable</td>
<td>162.45 ± 4.02</td>
<td>50.25 ± 1.26</td>
<td>0.32 ± 0.05</td>
<td>12.59 ± 0.13</td>
<td>3.42 ± 0.00</td>
<td>21.65 ± 0.17</td>
<td>5.24 ± 0.1</td>
</tr>
<tr>
<td>Residual</td>
<td>49.30 ± 1.92</td>
<td>108.35 ± 2.70</td>
<td>0.15 ± 0.01</td>
<td>27.09 ± 0.14</td>
<td>22.96 ± 0.38</td>
<td>72.53 ± 2.82</td>
<td>7.95 ± 0.13</td>
</tr>
<tr>
<td>Solid phaseb</td>
<td>278.73</td>
<td>376.21</td>
<td>1.28</td>
<td>40.46</td>
<td>35.73</td>
<td>97.83</td>
<td>18.33</td>
</tr>
<tr>
<td>Sumc</td>
<td>278.84</td>
<td>382.58</td>
<td>1.28</td>
<td>40.46</td>
<td>37.28</td>
<td>100.19</td>
<td>18.98</td>
</tr>
<tr>
<td>Pseudo-totald</td>
<td>340.67 ± 1.49</td>
<td>450.71 ± 5.34</td>
<td>1.47 ± 0.02</td>
<td>42.89 ± 0.53</td>
<td>42.70 ± 0.42</td>
<td>115.62 ± 3.42</td>
<td>22.80 ± 0.03</td>
</tr>
<tr>
<td>Recovery±</td>
<td>81.8%</td>
<td>84.9%</td>
<td>87%</td>
<td>94.3%</td>
<td>87.3%</td>
<td>86.7%</td>
<td>83.2%</td>
</tr>
<tr>
<td>MF</td>
<td>14.66</td>
<td>38.87</td>
<td>37.50</td>
<td>0.32</td>
<td>29.21</td>
<td>3.22</td>
<td>23.97</td>
</tr>
</tbody>
</table>

a Below the detection limit.
b Sum of all fraction in form of “Weak acid soluble, Reducible, Oxidizable, Residual”.
c Sum of all five fractions.
d Total metal concentration from aqua regia digestion procedure.
± Recovery rate of heavy metals (sum/pseudo-total).
1.3.4. Other indicators
Zeta potential was measured with a Zetasizer 3000 (Malvern Instruments, UK). Other sludge parameters, including TSS and volatile suspended solids (VSS), were determined according to standard methods (Apha, 1998). The surface functional groups of the sludge cake were analyzed using Fourier transform infrared spectroscopy (FTIR-8300, Shimadzu, Japan) with wavelength range from 500 to 4000 cm\(^{-1}\). The elemental composition of the sludge cake was analyzed using X-ray photo-electron spectroscopy (ESCALAB 250Xi, Boyue, China).

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<table>
<thead>
<tr>
<th>Percentage (%)</th>
<th>RS</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn NaNO(_2) (mg/L)</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Cu NaNO(_2) (mg/L)</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Cr NaNO(_2) (mg/L)</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Pb NaNO(_2) (mg/L)</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Ni NaNO(_2) (mg/L)</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Cd NaNO(_2) (mg/L)</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>As NaNO(_2) (mg/L)</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>

Fig. 5 – Change of speciation distribution of heavy metals in sludge with NaNO\(_2\) treatment.
Fig. 6 - Change of speciation distribution of heavy metals in sludge with H$_2$O$_2$ treatment.
1.3.5. Statistical analysis
The software OriginPro 8.5 64-bit (OriginLab Corp., Northampton, MA) was applied for statistical analysis. All assays were conducted in triplicate.

2. Results and discussion

2.1. Effect of NaNO2/H2O2 conditioning on EPS properties

2.1.1. Effect of NaNO2 dosage on EPS distribution and composition
As can be seen from Fig. 1a and b, after combined treatment of acidification and NaNO2 oxidation, the curves of polysaccharide and protein components in EPS had a similar tendency. According to the divalent cation bridging theory, the negatively charged functional groups (carboxyl, hydroxyl, phosphoric acid, etc.) within EPS can bind with divalent cations (Ca2+, Mg2+, etc.), and form a bridge between bacteria or/and flocs (Tezuka, 1969). Polysaccharide and protein components in S-EPS increased from 0.006 mg/g TSS and 0.13 mg/g TSS to 1.85 mg/g TSS and 5.65 mg/g TSS, respectively. With regard to TB-EPS, polysaccharide and protein components decreased from 0.77 mg/g TSS and 6.92 mg/g TSS to 0.35 mg/g TSS and 0.81 mg/g TSS, respectively. The organic components in LB-EPS were slightly increased under treatment. It has been reported that acidification of sludge can cause release of EPS and intracellular materials (Chen et al., 2001; Wang et al., 2017). Wang et al. (2013) suggested that pretreatment of WAS with free nitrous acid (FNA) remarkably enhanced WAS solubilization. Accordingly, it can be speculated that organic substances from TB-EPS were transformed to LB-EPS and S-EPS due to the solubilization effect of NaNO2 under acidic conditions. Wei et al. (2018) reported that volatile solids (VS) destruction was improved under FNA treatment. EPS account for 30%–42% of VS in sludge flocs (Frølund et al., 1996; Wang et al., 2014). It can be hypothesized that the FNA added under acidic conditions might lead to sludge floc lysis and breakage of the gel network structure of EPS. Thus, polysaccharides and proteins of EPS were released into the supernatant or slime fractions. Meanwhile, it has been demonstrated that FNA is effective in causing cell lysis, and thus intracellular organics may be released as well (Jiang et al., 2011). The concentration of dissolved substances was greatly increased, which improved the biodegradability of sludge.

2.1.2. Effect of H2O2 dosage on EPS distribution and composition
As depicted in Fig. 2a, after H2O2 treatment under acidic conditions, the polysaccharide component in S-EPS increased from 0.006 to 7.85 mg/g TSS, while the content of polysaccharide in TB-EPS decreased from 0.77 to 0.14 mg/g TSS. However, there was no significant change in the content of polysaccharide in LB-EPS. Fig. 2b plots protein content in S-EPS, which increased from 0.13 mg/g TSS and reached the maximum of 6.22 mg/g TSS at H2O2 dosage of 800 mg/L, then decreased to 3.26 mg/g TSS with further increase in H2O2 dosage. At the same time, the content of protein in TB-EPS decreased from 6.93 to 1.35 mg/g TSS. As to LB-EPS, protein content increased from 0.42 to 1.53 mg/g TSS and then decreased to 0.27 mg/g TSS. It was reported that EPS was dissolved and released into the sludge bulk under strong acid conditions (Lu et al., 2017; Zhang et al., 2015). Catalase is widely distributed in aerobic microorganisms and exerts a significant impact on the activity or oxidative metabolism of activated sludge (Gabbita and Hzuang, 1984). Catalase is able to catalyze the decomposition of H2O2 and produce active radicals, which can degrade the organic contaminants in the environment. In this work, EPR measurement was used to detect the radicals in the indigenous peroxidase-catalyzed H2O2 process. The results confirmed that superoxide and hydroxyl radicals were generated with the addition of H2O2, which was very likely to be associated with the catalase-catalyzed peroxidation process (Appendix A Fig. S1). Acidic conditions caused the release of EPS into the supernatant or slime by sludge floc lysis with oxidation of hydroxyl radicals. With the enhancement of dissolved substance dissolution, the biodegradability of sludge was increased, which promoted the anaerobic digestion efficiency. Furthermore, proteins were oxidized to micro-molecule polypeptides or amino acids at high dosages of H2O2. However, polysaccharides had stronger antioxidant ability and thereby were not decomposed.
2.1.3. 3D-EEM analysis

In general, 3D-EEM spectra are classified into four regions: Peak A (Ex/Em = (250–400) nm/(280–380) nm), indicating tryptophan-like protein (TPN); Peak B (Ex/Em = (200–250) nm/(280–380) nm), indicating aromatic protein; Peak C (Ex/Em = (250–400) nm/(>380) nm), indicating humic acid (HA); and Peak D (Ex/Em = (200–250) nm/(>380) nm), indicating fulvic acid (FA) (Chen et al., 2003a, 2016). To explore the changes of biopolymers, parallel factor analysis (PARAFAC) was applied to resolve the overlapped fluorescent components in EEM spectra. Two components were identified and the Ex/Em of peaks were located at (220, 275) nm/330 nm and (275, 355) nm/445 nm for C1 and C2, respectively (Appendix A Fig. S2). The C1 component indicates the existence of tryptophan proteins (TPN) and aromatic proteins (APN). The C2 component is associated with HA. With increased NaN03 concentration, the fluorescence intensity of the C1 component increased slightly while C2 enhanced significantly, suggesting that C2 was sensitive to acidic treatment (Fig. 3). In addition, the fluorescence intensity of C1 went through a maximum and then decreased, while the fluorescence intensity of C2 was weakened when the H2O2 dosage was raised, which was in agreement with the protein analysis (Fig. 3). These results revealed that both the protein-like substances and humic substances were dissolved at low H2O2 dosages; the former were then oxidized into small molecules at higher H2O2 dosages, while the latter were more stable and resistant to chemical oxidation. It was due to the oxidation of biopolymers by superoxide and hydroxyl radicals, which were produced in indigenous peroxidase catalyzing H2O2 process (Appendix A Fig. S1). EEM analysis also confirmed that the mechanism of EPS solubilization is different in the two oxidative processes: both oxidative treatments caused significant EPS dissolution, while protein-like substances were further degraded into small molecules under treatment by high dosages of H2O2.

2.2. Effect of NaNO3/H2O2 conditioning on sludge cake properties

FT-IR analysis was further carried out to clarify the identity of the functional groups of sludge during the reaction process. FT-IR associated with EPS components can be divided into four regions (Table 1): hydrocarbons, proteins, polysaccharides and nucleic acids (Yin et al., 2015). Fig. 4a and b show that there are seven characteristic peaks in raw sludge. The peaks between 3697 and 3629 and at 3285 cm−1 can be attributed to the stretching vibrations of O-H. The peak at 2930 cm−1 can be assigned to the C-H stretching of CH2 groups. This group is associated with the aliphatic chain of hydrocarbons. C=O and C-N stretching of amide I appear at 1667 cm−1 and the peak at 1530 cm−1 is ascribed to the N-H and C-N stretching in α-CO-NH– of amide II. In addition, the characteristic peak at 1035 cm−1 is due to C-OH stretching and C–O–C ring vibrations from polysaccharides. Meanwhile, the transmission spectrum at 930 cm−1 originates from the O–P–O stretching vibrations from nucleic acids. With the addition of NaN03 or H2O2, the positions of the characteristic peaks were unchanged, while the transmission intensities were weakened generally (Fig. 4a and b). The results suggested that polysaccharide, protein and humic acid of EPS were released from the solid phase to the supernatant or slime under NaN03 or H2O2 treatment, coinciding with the results obtained by EPS distribution and composition analysis. Accordingly, NaN03 under acidic conditions may cause sludge floc lysis and destroy the structure of EPS; and hydroxyl radicals, which are created by catalyzed H2O2, may oxidize the biopolymers and cause destruction of sludge flocs. Zhou et al. (2016) has found that the major functional groups in the biopolymers, including carboxyl, hydroxyl, amine and phosphoric groups, are closely related to the adsorption of heavy metals. It has also been reported that nitrogen in amino-sugars and oxygen in hydroxyl and carboxyl are the main electron donor atoms and are prone to preferentially bind with soft metal cations with strong covalent characteristics and then form inner-square complexes (Fang et al., 2013).

XPS was also performed to analyze the elemental composition of sludge samples. Fig. 4c and d show that C and O were the main elements in sludge and the metals (Ca, Mg and Fe) had low concentrations. Under oxidation treatment by NaN03 or H2O2, the binding energy of each element was unchanged, indicating that the chemical stability of each element was not affected by the oxidative processes. However, the relative content of carbon was increased, while that of oxygen was reduced in oxidative treatment (Appendix A Tables S1 and S2). Comparing the two treatment approaches, the carbon and oxygen contents in WAS were lower under H2O2 treatment, and it was likely that hydroxyl radicals generated in peroxidation performed better in oxidizing proteins into micromolecule polypeptides or amino acids, which were released into the supernatant.

2.3. Effect of NaNO3/H2O2 conditioning on the migration and transformation of heavy metals

The BCR results are summarized in Table 2. According to the sequential extraction procedures (Tessier et al., 1979; Ure et al., 1993), four fractions of metals can be defined, the acid soluble/exchangeable fraction (f1, exchangeable metal and carbonate-associated fractions), the reducible fraction (f2, fraction associated with Fe and Mn), the oxidizable fraction (f3, fraction bound to organic matters) and the residual fraction (f4). The exchangeable and reducible fractions, considered as direct toxicity fractions, have low-energy bonding and are bioavailable (Chen et al., 2015). The oxidizable fraction can be degraded and release soluble metals under oxidative conditions, recognized as the potential toxicity fraction (Yuan et al., 2011). It has also been reported that the oxidizable fraction of heavy metals is a key mediator for ecological risk (Lin et al., 2018). The residual fraction is stable and non-accessible to organisms, and regarded as the nontoxic fraction (Chen et al., 2015, 2008). For all heavy metals, the sum of the five fractions was in good agreement with the pseudo total concentration with satisfactory recoveries of 80%–110%, which confirms the validity and reliability of the obtained results.

As depicted in Table 2, the order of heavy metal contents in sludge was as follows: Zn > Cu > Cr > Pb > Ni > As > Cd, and the order of heavy metal mobility was Zn > Cd > Ni > As > Cu > Cr > Pb. Among them, Zn, Cd, Ni, As and Cu possessed high mobility, with mobility factors of 38.87, 37.50, 29.21, 23.97
and 14.66, respectively. The mobility of heavy metals is an indicator of bioavailability and eco-toxicity (Şanların et al., 2000). Once these heavy metals with high migration ability enter into an ecosystem, they will accumulate and jeopardize the ecological environment (Álvarez-Valero et al., 2009). The Cr and Pb were mainly in the forms of oxidizable and residual fractions, and their mobility factors were low at 3.22 and 0.32, indicating that they exert less impact on established ecosystems.

As depicted in Figs. 5 and 6, the migration and transformation of heavy metals differed greatly from each other under oxidative treatment processes. Figs. 5 and 6 show that Zn was mainly present in the form of weak acid soluble, residual and reducible fractions, which accounted for 37.21%, 28.32% and 19.67%, respectively. With the addition of 5 mg/L NaNO2 or 300 mg/L H2O2, the contents of acid soluble, residual and reducible fractions decreased to 24.49%, 17.3% and 14.85%, 13.2% and 0.77%, 3.6%, respectively. Various forms of Zn were released to the supernatant in the oxidative treatments.

Cu mainly existed in the oxidizable fraction, which represented 58.26% of total Cu. It has been reported that the oxidizable fraction is composed of chelated compounds of Cu and carboxyl groups in sludge organics. On addition of 5 or 60 mg/L NaNO2 and 300 mg/L H2O2, the oxidizable fraction of Cu reduced to 19.17% and 15.81%, respectively. The reducible and residual fractions of Cu were also released to the supernatant or transferred into the acid-soluble fraction. Under H2O2 treatment, residual and reducible fractions decreased and were transformed into the supernatant (49.60%) and weak acid soluble fraction (26.50%).

The Cr was mainly in the form of residual and oxidizable fractions, accounting for 72.39% and 21.61%. Under the treatment of NaNO2, the residual and oxidizable fractions of Cr decreased to 61.53% and 18.53%, respectively. Meanwhile, the supernatant, weak acid soluble and reducible fractions increased slightly. After H2O2 treatment, the residual and oxidizable fractions decreased to 72.39%, 21.61% to 66.12%, 17.45%, respectively, and weak acid soluble, reducible and supernatant fractions increased from 0.87%, 2.77%, 2.35% to 2.79%, 5.13%, 8.51%, respectively. This indicated that the forms of Cr in sludge were relatively stable and had difficulty migrating in the environment in oxidative treatment.

The dominant species of Pb in raw sludge were in the reducible and oxidizable fractions, which reduced from 66.96%, 31.11% to 53.83%, 30.73%, respectively under NaNO2 treatment. The released Pb was converted into weak acid soluble and reducible forms. With the addition of H2O2, the oxidizable fraction of Pb decreased to 21.31%, and the weak acid soluble and reducible forms of Pb increased from 0.33%, 1.60% to 2.4%, 13.67%, respectively. This result revealed that Pb was prone to migrate and transform between various speciations and was difficult to remove.

Ni was present mainly as residual (61.60%) and oxidizable (25.08%) species. The residual and weak acid soluble fractions reduced to 53.83%, 30.73%, respectively. In NaNO2 treatment, oxidizable forms also decreased slightly. Under treatment by H2O2, the residual and weak acid soluble fractions of Ni were reduced to 51.17% and 12.79%, respectively.

The main species of Cd in raw sludge were weak acid soluble (37.25%), reducible (25.72%) and oxidizable (25.31%) forms. In NaNO2 treatment, reducible and oxidizable species were reduced to 1.85% and 10.71%, respectively. Weak acid soluble and residual forms decreased slightly. The content of Cd in the supernatant reached 59.37%. After H2O2 treatment, weak acid soluble, reducible and oxidizable species reduced from 37.25% to 25.72%, 25.31% to 28.53%, 1.55%, 10.69%, respectively.

Generally, both oxidative treatments resulted in enhancement of heavy metal mobility in biosolids and acidification increased the solubility of the heavy metals. EPS contained a large proportion of high-molecular-weight fractions as well as a high proportion of hydrophobic fractions that can readily bind with heavy metal ions to form complexes firmly embedded within the sludge floc interior (Zhang et al., 2015). Oxidation by NaNO2 and H2O2 in a strong acid environment would cause breakage of sludge flocs and dissolution of heavy metals. However, different heavy metals showed distinct migration and transformation behaviors. Zn, Cd and Cu, with excellent mobility, tended to migrate to the supernatant and thus were easy to remove. Ni and As showed moderate migration ability, of which the residual fraction tended to transform into the reducible fraction and supernatant. With poor mobility, Cr and Pb were present in the form of residual and oxidizable fractions, which were not easy to release into the supernatant and thus were difficult to remove.

According to Fig. 7a and b, with the addition of NaNO2 or H2O2, the L/S value of Zn and Cd increased significantly, while the L/S value of Ni and Cu enhanced gently, and the As, Cr and Pb L/S value increased slightly. The results are generally consistent with MF, indicating that the higher MF of a heavy metal, the more easily it migrates to the supernatant.

### 3. Conclusions

In this study, WAS was treated by typical oxidation processes for enhancing biopolymer solubilization, and changes in EPS properties and speciation of heavy metals were investigated. Overall, both NaNO2 and H2O2 treatments caused sludge solubilization and increased heavy metal mobility in WAS, but different heavy metals showed distinct migration and transformation behaviors. Results revealed that both NaNO2 and H2O2 treatments effectively destroyed the sludge flocs and resulted in conversion of bound EPS into soluble EPS. FNA destroyed the gel network structure of EPS, and organic materials were released from the solid phase into the...
dosages of H2O2. What’s more, during the two oxidation processes, Zn, Cd and Cu, with excellent mobility, tended to migrate into the supernatant and thus were easy to remove using mechanical separation. Ni and As showed moderate migration ability, of which the residual fraction tended to transform into reducible and soluble fractions. Cr and Pb existed in the form of residual and oxidizable fractions, which were stable and difficult to remove.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.12.005.

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