Characterization of changes in floc morphology, extracellular polymeric substances and heavy metals speciation of anaerobically digested biosolid under treatment with a novel chelated-Fe²⁺ catalyzed Fenton process

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HIGHLIGHTS

- Chelated-Fe²⁺ catalyzed Fenton process (CCFP) could improve sludge dewaterability.
- Highly oxidative radicals ($\cdot\text{OH}$ and $\text{HO}_2\cdot$) were responsible for sludge breakage.
- Bound water trapped in EPS was converted into free water in CCFP.
- Oxidizable and reducible fractions heavy metals were transformed into soluble fraction.

GRAPHICAL ABSTRACT

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ABSTRACT

A novel chelated-Fe²⁺ catalyzed Fenton process (CCFP) was developed to enhance dewatering performance of anaerobically digested biosolid, and changes in floc morphology, extracellular polymeric substances (EPS) and heavy metals speciation were also investigated. The results showed that addition of chelating agents caused EPS solubilization by binding multivalent cations. Like traditional Fenton, CCFP performed well in improving anaerobically digested sludge dewatering property. The highly active radicals ($\cdot\text{OH}$, $\text{O}_2\cdot^-$) produced in classical Fenton and CCFP were responsible for sludge flocs destruction and consequently degradation of biopolymers into small molecules. Furthermore, more plentiful pores and channels were presented in cake after Fenton treatment, which was conducive to water drainage under mechanical compression. Additionally, a portion of active heavy metals in the form of oxidizable and reducible states were dissolved under CCFP. Therefore, CCFP could greatly simplify the operating procedure of Fenton conditioning and improve its process adaptability for harmless treatment of biological sludge.

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1. Introduction

In recent years, sludge production was dramatically increased with the growth and development of population and urbanization. It will pose a significant threat to the ecological system without

effective treatment and disposal (Kim et al., 2002). Especially, sludge with high moisture content (usually above 99%) result in the issues of great bulk, expensive cost and difficulty of transportation. Therefore, treatment and disposal of waste sludge are also faced with many technical problems and challenges. It is indispensable to develop high-performance dewatering processes in current China to reduce the volume of sludge.

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To our best knowledge, sludge is a heterogeneous colloidal system in which small sludge particles (with the negative charge) form a stable suspension in water and is very difficult separated from the water phase. Generally speaking, it can be classified as free water (accounted for about 70%), interstitial water (about 20%), adsorbed water (about 7%) and bound water (about 3%) of the moisture in sludge system (Vaxelaire and Cézac, 2004). Additionally, the Extracellular polymeric substances (EPS), which microorganisms were embedded, account for about 60–80% of total sludge mass and significantly affect other physicochemical properties of sludge, such as surface charge, floc stability (Liu and Fang, 2003). Nielsen et al. (1997) reported that EPS can be subdivided into soluble EPS (SEPS) and bound EPS. Recent studies have shown that the bound EPS exhibits a double-layered structure including the loosely bound EPS (LB-EPS) which is diffused from tightly bound EPS (TB-EPS) that surrounds the cells (Li and Yang, 2007; Ramesh et al., 2006). Besides, it was found sludge dewaterability was only associated with LB-EPS fraction (Li and Yang, 2007; Ramesh et al., 2006). Yu et al. (2008) indicated the sludge dewatering performance was mainly influenced by LB-EPS and soluble EPS characteristics. Thus, chemical properties of soluble EPS can be used to predict the dewatering property of sludge (Sheng and Yu, 2006). Therefore, the advanced sludge conditioning processes (ASCP) have been developed to improve sludge dewaterability and reduce cake solid content by solubilizing EPS components. The established ASCP included photo-Fenton/Fenton oxidation technology (Neyens and Baeyens, 2003a), thermal treatment (Neyens and Baeyens, 2003b), acid and alkali treatment (Neyens et al., 2004), enzymatic treatment (Chen et al., 2015) and integrated processes. Many studies suggested that Fenton reagents were able to break activated sludge floc and solubilize EPS fraction, promoting the conversion from bound water to free water. In addition, the ferric hydroxo complexes produced from Fenton process could act as coagulants and skeleton builders to further enhance dewatering performance of bio-sludge (Neyens and Baeyens, 2003a). Liu et al. (2012) found that sludge conditioning with combined Fenton's reagent and skeleton builders (lime and fly ash) was an efficient mean to achieve deep dewatering. Fenton treatment resulted in the partial destruction of EPS and decrease in sludge floc size. Nevertheless, traditional Fenton reaction has a narrow action range of pH (2–3), which needs a large amount of acid and alkali, making operating procedure very complicated and costly. Additionally, anaerobically digested sludge always contained the high concentration of alkalinity in the form of HCO_3^- and would consume large amounts of acid in pH adjustment, so the traditional Fenton process was difficult to be used in chemical conditioning.

Chelating agents have several functional groups and can form multiple coordination bonds to a single metal ion (Sillanpää et al., 2011). EDTA is a hexadentate chelating ligand characterized by the higher chelating strength and a low-toxicity chemical. It belongs to the aminopolycarboxylic acids, which are able to form very stable complexes with various metals (such as Fe^{2+} , Fe^{3+}). However, EDTA complexes with heavy metals (chelates) are a potential health risk when EDTA enters into the environment. And EDTA is recalcitrant nature and poor biodegradability (Sillanpää and Oikari, 1996) and persists in the soil environment (Meers et al., 2005) causing serious environmental pollution. Ethylene diamine tetra (methylene phosphonic acid) sodium (EDTMPs) with four $\text{C}=\text{PO}(\text{OH})_2$ groups and belongs to aminopolyphosphonates. It is water-soluble and non-toxic to mammals at environmental concentration. Like EDTA, its functional groups could also form very stable and water-soluble macromolecular complexes with metal ions (such as Ca^{2+} , Mg^{2+} , Zn^{2+} , Fe^{2+}). Furthermore, bacteria have the ability to cleave the $\text{C}=\text{P}$ bond in phosphonates as a

phosphorous source for growth (Nowack, 2003). De Luca et al. (2014) found that EDTA chelated- Fe^{2+} catalyzed Fenton process could oxidize and remove a significant quantity of organic contaminants at neutral pH. Therefore, it has a high value of engineering application in integrating Fenton and chelating reagents to achieve high conditioning efficiency at neutral condition and reduce the operating cost of acid and alkali adjustment.

On the other hand, wastewater sludge is potentially a resource if reused in agriculture as fertilizer or soil amendment material (Lu et al., 2012). In China, the industrial sewage accounted for more than 35% of the capacity of total wastewater treatment plant so that the sludge is a significant environmental burden. The presence of toxic metals, or so-called “heavy metals” including Cd, Cr, Cu, Ni, Pb, Zn and As, may pose a great threat to environment as these elements are non-degradable and harmful (Smith, 2009). Hence, the land use of wastewater sludge is stringently managed in order to avoid long-term heavy metals pollution. According to Tessier et al. (1979) and Ure et al. (1993), heavy metals are classified into the following fractions: weak acid soluble fraction, which is likely to be affected by changes in water ionic composition as well as sorption-desorption processes; reducible fraction (Fe-Mn oxides or inorganically bound fraction) and oxidizable fraction (organically bound fraction) that are susceptible to changes in pH and can be degraded leading to a release of soluble metals under chemical conditions; the residual fraction of metals are quite stable which could not be taken up by organisms. Many studies have focused the mobility, bioavailability and eco-toxicity of heavy metals which were more dependent on their speciation rather than their total content (Amir et al., 2005; Zhu and Guo, 2014). Most of the studies on the chemical speciation of heavy metals have been carried out just in raw or digested sludge (Hanc et al., 2009; Kunito et al., 2001). In addition, various methods for removal and dissolution of heavy metals have been used prior to land application of sewage sludge, including chemical extraction, bioleaching process, electrokinetic process, supercritical extraction and so on (Chen et al., 2005; Solisio and Lodi, 2002).

As mentioned above, Fenton treatment is able to effectively destroy sludge flocs and solubilize EPS fractions. It is possible that heavy metals may be released at the same time, since they can form metal organic complex compounds in presence of chelates which can be separated from sludge system under mechanical dewatering. Therefore, the main objectives of this study are to: (1) compare the conditioning efficiency of chelated- Fe^{2+} catalyzed Fenton process (CCFP) and traditional Fenton process on sludge dewatering performance, and identify the active radicals responsible for sludge solubilization; (2) explore the effects of different Fenton treatment on floc morphology and microstructure of biological sludge; (3) investigate the spatial distribution and chemical composition of EPS under chemical conditioning with different Fenton system by combining conventional chemical analysis methods with three-dimensional excitation emission matrix fluorescence spectroscopy; (4) understand the effects of different Fenton conditioning on mitigation and transformation of various harmful heavy metals.

2. Materials and methods

2.1. Chemical agents

The reagents of metals analysis were guarantee reagents (GR). And the other reagents were analytical reagents (AR). In addition, methyl alcohol (CH_3OH ; Chromatographic grade; $\geq 99.99\%$) and 5, 5-Dimethyl-1-pyrroline N-oxide (DMPO, for EPR-spectroscopy; CAS: 3317-61-1) were purchased from J&K Chemical Corporation.

Table 1

Characteristics of anaerobically digested sludge.

Indicator	Moisture content (%)	pH	VSS/TSS ^a	d _{0.5} (μm) ^b	SCOD(mg.L ⁻¹) ^c	Alkalinity(mg.L ⁻¹)
Value	98.5	7.8	69.7	63.1	3340.2	4003.1

^a VSS/TSS, Volatile suspended solids; Total suspended solid.^b d_{0.5}, the average floc size.^c SCOD, Soluble chemical oxygen demand.

2.2. Source and properties of anaerobically digested sludge

The anaerobically digester sludge was obtained from the Xiaohongmen WWTP in Beijing city. This WWTP treats approximately 600,000 m³ daily and uses A²/O process. The processed sewage water conformed to the standard of the urban sewage treatment plant water pollutants discharge. The sludge characteristics were listed in Table 1.

2.3. Experimental methods

2.3.1. Effects of chelates addition on sludge properties

About 100 mL of raw sludge was placed in 250 mL beakers and kept under agitation at 200 rpm and at room temperature. Then, the following various dosages of EDTA (0.032, 0.048, 0.064, 0.096, 0.128 g/g TSS) and EDTMPS (0.067, 0.100, 0.133, 0.199, 0.265 g/g TSS) were added into each beaker. Finally, when the reaction was stopped, the suspension was centrifuged at 3000g for 10 min and the supernatant was used for TOC (total organic carbon) and fluorescence analysis later. All samples were duplicated to ensure the reproducibility of the results.

2.3.2. Sludge conditioning with different Fenton processes

A 150 mL of sludge sample was added in 250 mL Erlenmeyer flask. Then, an aliquot of EDTA or EDTMPS was spiked in the sludge sample under vigorous stirring using a magnetic stirrer. Appropriate amounts of ferrous sulfate heptahydrate salt (FeSO₄·7H₂O) and H₂O₂ (30% (w/v)) were added at varying pH values. As shown in Table 2, a set of experiments with different Fenton conditioning preparations were conducted to elucidate the mechanism of the composite conditioner to improve sludge dewaterability. At the end of the reaction, the sludge characteristics were determined.

2.4. Analytical methods

2.4.1. Determination of sludge dewaterability

The dewaterability of the sludge floc was measured with a capillary suction time (CST) instrument (Model 319, Triton, UK) equipped with an 18 mm diameter funnel and Whatman No. 17 chromatography-grade filtration paper. Centrifugal dewatering test was conducted to get a further understanding into the effects of different Fenton system on dewatering efficiency.

2.4.2. Floc morphology

Fractal dimension reflects the space availability of the complicated shapes, which is a measure of irregularity. The description is given here of the widely used light scattering method for the determination of aggregate mass fractal dimension (Wei et al., 2009). Sludge floc size and fractal dimension (D_F) were measured with Malvern Mastersizer 2000 (UK). The technique involves measurement of light intensity I as a function of the scatter vector Q .

For independently scattering aggregates, the relationship among I , Q and the fractal dimension D_F can be represented by Eq. (1):

$$I \propto Q^{D_F} \quad (1)$$

The relationship is shown in Eq. (1) indicates the determination of D_F , which can be given by the slope of log I versus log Q by fitting a straight line.

2.4.3. Metals analysis

The European Community Bureau of Reference (BCR) sequential extraction method was performed to determine the metal speciation according to previous literatures (Tessier et al., 1979; Ure et al., 1993). By centrifugation at 5000g for 10 min, the sludge was separated into the aqueous phase and solid phase. The Ca, Mg, Fe and heavy metals concentrations in the supernatant were determined after digestion of 3 mL of supernatant in a 8 mL HNO₃: HCl (1:3) mixture in a hot plate digester. Then the solid phase of sludge was extracted as the following extraction step.

Step one: Weak acid soluble fraction (f_1). The samples (0.5000 g) were introduced in a 50 mL polypropylene centrifuge tubes containing 20 mL of 0.1M CH₃COOH and then shaken for 16 h at room temperature. The solution and solid phase were separated by centrifugation at 3000g for 20 min. Subsequently, the suspension was filtered through a 0.45 μm membrane filter. The supernatant was collected and added 5 mL 5% HNO₃ into the filtrate, and then metered volume to 50 mL for metal measurement. The solid residue was flushed with 10 mL deionized water and subjected to the subsequent extractions.

Step two: Reducible fraction (f_2). 20 mL of 0.1 M NH₂OH·HCl (pH = 2 with HNO₃) was added into the solid residue of step one. The mixture was shaken for 16 h at room temperature, centrifuged and filtered. The supernatant was collected and the solid residues were for the following extraction.

Step three: Oxidizable fraction (f_3). 5 mL of 30% H₂O₂ was added into the residue of step two and digested at 25 °C (water bath) for 1 h. A second 5 mL aliquot of H₂O₂ was introduced into the mixture and digested at 85 °C (water bath) for 1 h. The mixture was then evaporated to a small volume (1–2 mL). Then, 25 mL of 1.0 M CH₃-COONH₄ (pH = 2 adjust with HNO₃) was added to the cool and moist residue, shaken, centrifuged and filtered. The supernatant was subjected to metal analysis and the solid residues were used in step four.

Step four: Residual fraction (f_4). The residue from step three was digested with HCl + HNO₃ + HF + HClO₄ (1:3:1:1) in a hot plate digester.

The determination of Ca, Mg and Fe concentrations was performed with an inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima 5300 DV, PerkinElmer, Table S1). The concentrations of heavy metals (Cu, Zn, Cd, Cr, Ni, As and Pb) in different fractions were determined by inductively coupled plasma mass spectrometry (ICP-MS; Agilent Technologies, 7700, Table S2).

2.4.4. EPS extraction procedure

An ultrasonic and heat extraction method was used to extract the LB-EPS and TB-EPS from the sludge (Niu et al., 2013). Firstly, a sludge suspension was dewatered by centrifugation in a 50 mL tube at 5000g for 10 min and the supernatant was collected as SEPS. The sludge pellet in the tube was resuspended into 20 mL of 0.05% NaCl, sonicated at 20 kHz for 2 min, shaken horizontally at 150 rpm for 10 min, sonicated again for an additional 2 min.

Table 2
Sludge conditioning with different Fenton systems.

Sludge	Sample	pH	Dosage			
			Fe ²⁺ /(g)	H ₂ O ₂ /(mL)	EDTA/(g)	EDTMPS/(g)
Raw sludge	RS	7.8	0	0	0	0
Sludge conditioned by different Fenton system	F1	3	0.1362	1	0	0
	F2	Neutral	0.1362	1	0	0
	F3	Neutral	0.1362	1	0.1433	0
	F4	Neutral	0.1362	1	0	0.4498

The extracted liquor was centrifuged at 8000g for 10 min to separate solids and supernatant. The collected supernatant was regarded as the LB-EPS. The sediment was resuspended in a 0.05% NaCl solution, sonicated for 3 min and then heated for 30 min in water-bath at the temperature of 60 °C, finally centrifuged at 12,000g for 20 min to collect TB-EPS. All of the supernatants were filtered with Polytetrafluoroethylene (PTFE) membranes of 0.45 µm and used to analyze TOC and fluorescence.

2.4.5. EPS composition analysis

2.4.5.1. Three-dimensional excitation emission matrix (3D-EEM). Fluorescence EEMs were measured on a Varian Eclipse fluorescence spectrophotometer (Hitachi F-7000, Japan) in scan mode. EEM spectra were gathered with scanning emission Em spectra from 220 to 550 nm at 5 nm increments by varying the excitation Ex wavelength from 200 to 400 nm at 5 nm increments. The spectra were recorded at a scan rate of 12000 nm/min, using excitation and emission slit bandwidths of 5 nm. As stated by Sheng and Yu (2006), the location and intensity of the fluorescence peak are not affected by the ionic strength on EEM analysis. And the fluorescence intensity of EEM could be used to measure EPS content as DOC (dissolved organic carbon) content in samples was less than 10 mg/L.

2.4.5.2. Protein, humic acid and polysaccharide measurement. The protein, humic acid and carbohydrate in extracted EPS were determined according to the procedure described by Frølund et al. (1996). The polysaccharide (PS) was measured using the anthrone method with a glucose standard (Sinopharm). Protein (PN) and humic acid (HA) were determined by the Lowry procedure using bovine serum albumin (BSA) (Sigma) and humic acid (Sigma) as standard.

2.4.6. Electron paramagnetic resonance (EPR) measurements

The active radicals were identified with a BrukerA300-10/12 (Bruker Ltd, Germany) instrument at room temperature. Solutions of different Fenton systems were mixed into 200 mmol/L of DMPO (5,5-dimethyl-1-pyrroline N-oxide) solution and transferred into 1.5 mL centrifuge tube. After mixing for about 30 s within a vortex mixer, the sample solution was then transferred into the 1.5 mL nuclear magnetic capillary tube and finally fixed in the cavity of the EPR spectrometer. They are reacted at the desired solvent systems in solutions for the detection of ·OH or HO₂[·] radicals.

2.4.7. Other indicators

Total organic carbon (TOC) determination was carried out in a TOC-VCSN TOC analyzer (Shimadzu Corporation, Kyoto, Japan). Chemical oxygen demand (COD) was determined by using potassium dichromate oxidation method. The variation in sludge floc size was determined by Malvern Mastersizer 2000 (UK). pH was measured using a pH-3C (Shanghai, China) pH meter. Other sludge parameters, including TSS (total suspended solid) and VSS (volatile suspended solids) were determined according to standard methods (APHA, 1998).

3. Results and discussion

3.1. Effects of EDTA and EDTMPS addition on sludge properties

3.1.1. CST and sludge solubilization efficiency

CST was significantly increased after addition of EDTA and EDTMPS (Fig. S1(a) and (b)). It reached the maximum of 1349.7 s and 1371.8 s at EDTA and EDTMPS dosages of 0.048 g/g TSS and 0.133 g/g TSS respectively. Meanwhile, TOC concentration reached the maximum (Fig. S1(c) and (d)), indicating that EPS was released with addition of chelates. The stability constants of Ca-EDTA and Mg-EDTA are 10.69 and 8.69, while that of Ca-EDTMPS and Mg-EDTMPS are 20.9 and 16.3 respectively. Apparently, both EDTA and EDTMPS are able to form very stable complexes with Ca²⁺ and Mg²⁺ so that the EPS bound to divalent cations were released. Thus, the release of sticky biopolymers caused deterioration of sludge filterability.

3.1.2. Effect of EDTA and EDTMPS dosage on EPS distribution and composition

EPS always contains abundant functional groups, such as carboxyl, phosphoric, amine, and hydroxyl groups, which are negatively charged at a neutral pH and are able to form organometallic complexes with multivalent cations via electrostatic attraction or other interactions and then promote bacterial aggregation (Sheng et al., 2010). Since chelates were able to bind with multivalent cations and resulted in solubilization of sludge, they can be used to extract EPS from different activated sludge systems. With the rise of EDTA dose, Ca²⁺, Mg²⁺ and Fe³⁺ content were increased first and then decreased (Fig. S2(a)). When EDTA and EDTMPS dosage was 0.064 and 0.265 g/g TSS, the supernatant concentrations of Ca²⁺, Mg²⁺ and Fe³⁺ reached the maximum (Fig. S2(a) and (b)). EDTA and EDTMPS could cause the collapse of bridge between calcium and magnesium ions and EPS, which resulted in the destruction of colloidal granule particles (Kavitha et al., 2013). The stability constants of Fe (III)-EDTA and Fe (III)-EDTMPS are respectively 25.1 and 18.3, revealing that the chelating ability of EDTMPS for Fe³⁺ were weaker.

It was observed from Fig. 1(a) that the polysaccharides content compared with the raw sludge were increased, and it reached the maximum when the dosage of EDTA was 0.096 g/g TSS, while no significant change was detected for contents of PN and HA. It was worthy to note that EDTA could interfere protein quantification by Lowry reaction, since chelating agents were able to bind with the copper ions and decrease its affinity with proteins, thereby decreasing the intensity of color development (D'Abzac et al., 2010; Smith et al., 1985). Fig. 1(b) showed that humic substances and polysaccharides in soluble EPS fraction were increased by raising EDTMPS dosage. Humic substances in sludge were always present in form of complexes with divalent cations. As mentioned above, the EDTMPS showed a higher chelating ability with Ca²⁺ and Mg²⁺ than EDTA (Fig. S2), hence the solubilization efficiency of humic substances were significantly under EDTMPS treatment.

Fig. 1. Effects of EDTA and EDTMPS addition on chemical composition of soluble EPS.

Three-dimensional fluorescence spectrum could get the intensity information of excitation and emission wavelength, is widely used for characterization of natural organic matters in various water bodies with the advantages of high sensitivity and selectivity (Zhen et al., 2012; Zhu et al., 2012). Firstly, four fluorescent peaks could be detected in SEPS fraction: Peak A ($\lambda_{ex/em} = 280/335$) - tryptophan-like protein, Peak B ($\lambda_{ex/em} = 225/340$)-aromatic proteins, Peaks C ($\lambda_{ex/em} = 330/410$)-humic substances and Peak D ($\lambda_{ex/em} = 275/425$)-fulvic acid (Fig. S3 and Table S3). It was observed that the fluorescent intensity of peaks A, B, C and D was improved from 2649, 4096, 1168 and 1185 to 3922, 5717, 1407 and 1492 when EDTA dosage was 0.096 g/g TSS. Furthermore, the intensity of all four fluorescent peaks of SEPS was increased after addition of EDTA. The fluorescent intensity of Peaks A, B, C and D intensified to 3333, 4842, 1275 and 1356 when EDTMPS dosage was 0.133 g/g TSS. These results revealed that protein-like substances and humic substances were released in bound EPS into SEPS due to complexation of EDTA and EDTMPS with Ca^{2+} , Mg^{2+} and Fe^{3+} et al.

3.2. Effects of different Fenton system on sludge properties

3.2.1. Sludge dewaterability

The acidic Fenton treatment was performed under optimal conditions according to our previous studies (Zhang et al., 2015). Molar ratios of Fe (II)-EDTA and Fe (II)-EDTMPS were 1:1 and

1:1.5, which was consistent with the report of De Luca et al. (2014). Fig. 2 clearly showed that CST was decreased from 1054.8 s to 16 s, 599.5 s, 122.4 s and 139.8 s after treatment with F1, F2, F3 and F4 respectively. Fenton treatment at pH of 7 had very limited effect on sludge dewaterability, while traditional Fenton and CCFP conditioning performed well in improving sludge dewatering performance. This observation indicated that the oxidation process played an important role in sludge conditioning. In addition, the hydrolyzed ferric ions formed in Fenton process were able to compress EPS structure and agglomerate sludge particles through charge neutralization and interfacial adsorption and act as skeleton builders, leading to decrease infiltration resistance and sludge compressibility.

3.2.2. Identification of active radicals in different Fenton systems responsible for sludge solubilization

The EPR spin-trapping technique was utilized to detect the active radicals during the reaction in different Fenton systems. DMPO (5,5-dimethyl-1-pyrroline N-oxide) was a widely used as spin trapping reagent, which could capture both carbon-centered and oxygen-centered radicals generated in chemical and biochemical systems (Cristina et al., 1997; Qian et al., 2000). As depicted in Fig. 3(a), the peaks intensity of active radical is 1:2:2:1 and the hyperfine coupling constants are $\alpha^N = 14.94G$, $\alpha^H = 14.90G$. This result indicated that DMPO-OH adducts were obviously formed in acid Fenton oxidation (sample F1) and Fenton-chelating oxidation (sample F3 and F4) in aqueous solution, though no obvious peaks of DMPO- O_2^- adducts were detected. This is likely that O_2^- is unstable in aqueous solution and rapidly easily decomposed into $\cdot OH$, and the reaction rate of DMPO with $\cdot OH$ was much faster than that of O_2^- radicals (Yin et al., 2009). Hence, it was difficult to detect the DMPO- O_2^- peaks, though it was actually formed in the reaction process (Yin et al., 2009). In order to further verify whether O_2^- is formed during different Fenton reaction, CH_3OH instead of H_2O was used to slow down the decomposition rate O_2^- . There was DMPO- or DMPO- spin adducts shown in Fig. 3(b) when different Fenton reagents were reacted in CH_3OH . Meanwhile, both peaks of DMPO- O_2^- and DMPO-OH in CCEP were weaker than F1. The radicals quenching test was performed in order to understand the reaction mechanism of Fenton oxidation. It was reported that $\cdot OH$ and O_2^- were highly active non-selective radicals and were very effective in removal of a wide variety of organic contaminants in aqueous solution. It was demonstrated that the iron species as Fe^{2+} and Fe^{3+} in solution are in the chelated form in the presence of chelating reagents, which could avoid iron precipitation and ensure catalytic

Fig. 2. Effects of different Fenton treatment on sludge dewatering performance.

Fig. 3. DMPO spin-trapping EPR spectrum and computer simulation obtained from the reaction of different Fenton systems. (a) An EPR spectrum obtained from a mixture reaction containing DMPO (200 mM), 0.1 mL of the reaction mixture under H_2O_2 ; (b) An EPR spectrum obtained from a mixture reaction containing DMPO (200 mM), 0.1 mL of the reaction mixture in the system of methyl-alcohol.

activity of irons and production of highly active radicals. Chelating agents (EDTA and EDTMPS) have played important roles in the cycle of ferrous and iron ion catalytic hydrogen peroxide to produce hydroxyl radicals (see in reaction (2) and (3)). In the cycle process, $\cdot OH$ could react with H_2O_2 and $Fe(II)-L$, and oxidize sludge structure (see in reaction (4), (5), (6) and (7)). Finally, the chain termination reaction was passed through superoxide radicals (see in reaction (8)). Obviously, the sludge that conditioned with F3 and F4 had the similar effects of acidic Fenton process.

3.2.3. Sludge floc morphological properties

The sludge floc size was significantly reduced after different Fenton treatment (Fig. S4(a)). There was no obvious difference in average floc size of sludge conditioned by Fenton-chelating agents (sample F3, F4) and acidic Fenton oxidation. The EPS components were of great importance to bioflocculation and sustained the stability of biological aggregates. The Fenton process generated large amounts of highly oxidative radicals ($\cdot OH$) through iron-catalyzed

Fig. 4. Effect of different Fenton conditioning processes on chemical composition of EPS fractions.

Fig. 5. Influence of different oxidative reaction conditions on EEM profile of different sludge EPS fractions (SEPS of raw sludge was diluted by 200 times, the other SEPS samples were diluted by 50 times; LB-EPS and TB-EPS sample was diluted by 20 times).

Fig. 6. Change of speciation distribution of heavy metals in anaerobically digested sludge with different Fenton treatment.

decomposition of H_2O_2 , which was able to effectively destroy sludge EPS and resulted in rupture of sludge flocs (Zhou et al.,

2015). It was worthy to note that CCFP could achieve the similar efficiency in comparison to acidic Fenton oxidation.

A higher fractal dimension (D_F) normally always corresponded to a more compact interior floc structure (Wang et al., 2009), and the higher D_F was always related to a more regular surface of the sludge particles. The D_F conditioned with different Fenton oxidation was decreased (Fig. S4(b)), indicating the sludge floc strength was weakened. Floc properties had important effects on the formation of sludge cake and played a decisive role in sludge filtration dewatering process.

3.2.4. Microstructure of sludge cake

Sludge filtration process could be divided into three stages: filling, cake formation and cake filtration and cake compression. Since filtration was always governed by cake-layer once formed, the structure of sludge cake had a crucial impact on sludge filtration performance (Qi et al., 2011). During filtration dewatering, the clogging of pores in the filter cake is primarily responsible for the deterioration in filtration rather than the blinding of the filtration media (Novak et al., 1988). Therefore, it is necessary to observe the structural characteristics of sludge cake after filtration dewatering. The original sludge floc obviously had a relatively smooth and closely surface (Fig. S5(a)), while after conditioned by different Fenton oxidation the surface becomes more porous. The higher degree of fragmentation on sludge floc surface can be seen (Fig. S5(b), (d) and (e)). It was noted that pore structure of sludge cake treated with Fenton oxidation was more plentiful and these pores might provide more channels for water release under pressure dewatering.

3.3. Sludge EPS changes under Fenton conditioning

Compared with the original sludge, it was found the content of SCOD (soluble chemical oxygen demand) was reduced sharply and then increasing slightly after different Fenton treatment (Fig. S6). The SCOD of sludge conditioned with EDTA and EDTMPS decreased from 3340 mg/L to 880 and 1190 mg/L respectively. Fig. 4 showed that the PN, PS and HA of the raw sludge accounted for 46.70%, 5.49% and 47.81% of the total EPS, respectively. It was reported that the high molecular weight biopolymer in SEPS had a more crucial influence on sludge filtration properties (Lyko et al., 2008). After the treatment of different Fenton oxidation, the SEPS, LB-EPS and TB-EPS concentration were reduced except sample F2 (Fenton's reagent at neutral pH). Apparently, the extractable EPS concentration reaches the minimum with the treatment of Fenton's reagent at pH of 3. In Fenton reaction, the first step was to effectively crack EPS and rupture microbial cell, and then the dissolved biopolymers were further oxidized into small molecules, such as volatile fatty acids, H_2O and CO_2 (Neyens and Baeyens, 2003a; Zhang et al.,

2015), consequently resulting in the conversion of bound water into free water. As for Fenton treatment at neutral pH, the contents of protein, polysaccharide and humic-acid were significantly increased. The oxidation process was inhibited due to hydrolysis precipitation of ferric ions (De Luca et al., 2014; Zhang et al., 2015).

It can be seen from Fig. 5 that three different Fenton processes (sample F1, F3 and F4) resulted in obvious decline of fluorescent intensity of protein-like substances (aromatic proteins I & II and tryptophan-like proteins) and humic-like substances (peak C and peak D) in SEPS and LB-EPS, while the fluorescent intensities were increased in sample F2 (Table S4). This observation indicated that sludge flocs were effectively solubilized and protein-like substances and humic-like substances were further decomposed under acidic Fenton and CCFP treatment. These results were in agreement with the analysis of EPS properties. In addition, the fluorescent intensity in TB-EPS was decreased slightly under treatment with F3 and F4.

3.4. Migration and transformation of heavy metals under chemical conditioning with different Fenton systems

The BCR results are summarized in Table 3. For all metals except As, the sum of five fractions is in good agreement with the pseudo-total concentration with satisfactory recoveries of 80–110%, which confirms the validity and reliability of the obtained results. However, the recovery of As was around 130% due to the analytic errors of the measurement instrument. Table 3 showed that the order of total metal content in anaerobically digested sludge was $Zn > Cu > Cr > Pb > As > Ni > Cd$. Moreover, the MFs (Mobility factors) for Cd, Ni, Zn, Cu, As, Cr and Pb were 61.68, 46.39, 30.35, 16.68, 14.44, 12.09 and 4.66 respectively, revealing that Cd, Ni, Zn, Cu, As and Cr exhibited high migration ability than Pb. The toxic metals with higher MFs may cause serious risks to ecosystem via constant accumulation in land use of sewage sludge (Dong et al., 2013; Zhu and Guo, 2014).

The migration and transformation of heavy metals differ greatly with each other under sludge treatment processes. As shown in Fig. 6, the Cu was primarily presented in the form of oxidizable state, which accounted for 69.56% of total content in raw sludge. The Fenton treatment at neutral pH (sample F2) did not cause obvious changes in chemical speciation of heavy metals. Conversely, the organically bound fraction of Cu reduced to 28.34%, 30.11%, and 35.42% with sample F1, F3, F4 respectively. Zn was mainly in the form of reducible and organically bound fractions in raw sludge. The inorganically bound fraction decreased to 22.61%, 7.73%, 14.77% respectively, while organically bound Zn also decreased to 5.04%, 4.92% and 4.68% with three Fenton systems

Table 3
Chemical fractionation (mg·kg⁻¹ DS*) of heavy metals in anaerobic digestion sludge.

Raw Sludge	Cu	Zn	Cd	Pb	Ni	Cr	As
Supernatant	27.42 ± 0.88	161.34 ± 8.3	0.18 ± 0.02	1.86 ± 0.18	7.37 ± 2.12	8.07 ± 1.7	2.42 ± 0.004
Weak acid soluble	37.90 ± 2.93	139.94 ± 3.27	0.48 ± 0.01	0.09 ± 0.006	9.46 ± 0.24	1.59 ± 0.05	3.56 ± 0.17
Reducible	20.29 ± 0.05	363.08 ± 7.26	0.18 ± 0.001	0.47 ± 0.013	2.4 ± 0.06	3.53 ± 0.11	3.53 ± 0.01
Oxidizable	272.46 ± 4.02	248.75 ± 4.71	0.20	22.10 ± 0.12	7.62 ± 0.25	39.99 ± 1.1	13.48 ± 0.49
Residual	33.62 ± 1.46	79.56 ± 0.70	0.03 ± 0.004	17.29 ± 0.24	9.43 ± 0.07	26.73 ± 0.70	18.43 ± 0.92
Solid phase ^a	364.27	831.34	0.90	39.95	28.91	71.84	38.99
Sum ^b	391.69	992.68	1.07	41.81	36.28	79.92	41.42
Pseudo-total ^c	489.39 ± 0.82	1160.18 ± 24.83	0.94 ± 0.04	44.61 ± 0.25	44.92 ± 0.66	80.25 ± 0.64	31.40 ± 1.08
Recovery ^d	80.0%	85.6%	113.8%	93.7%	80.8%	99.6%	131.9%
MF ^e	16.68	30.35	61.68	4.66	46.39	12.09	14.44

^a Sum of fraction in form of "Weak acid soluble, Reducible, Oxidizable, Residual".

^b Sum of all five fractions.

^c Total metal concentration in anaerobic digestate from aqua regia digestion procedure.

^d Recovery rate of heavy metals (sum/pseudo-total).

^e (Mobility factor) MF = $\frac{\text{Supernatant} + f_1}{\text{Supernatant} + f_1 + f_2 + f_3 + f_4} \times 100\%$ (Dong et al., 2013; Zhu and Guo, 2014).

* DS, dry solid.

Table 4

Estimated Operating Costs for the traditional Fenton and Chelated-Fe²⁺ Catalyzed Fenton Oxidation.

Items	Unit price (\$/kg)	Consumed (kg/ton DS ^a)	Operating costs (\$/ton DS ^a)
HCl (36%)	0.103	1121	115.46
NaOH	0.662	96.00	63.55
EDTA	1.619	63.69	103.11
EDTMPS	0.662	199.91	132.34
FeSO ₄ ·7H ₂ O	0.041	60.53	2.48
H ₂ O ₂ (30%)	0.155	502.22	77.84
Total cost of traditional Fenton		259.33	
Total cost of EDTA - Fenton treatment		183.43	
Total cost of EDTMPS - Fenton treatment		212.66	

^a DS, dry solid.

(F1, F3, F4). Zn in the form of oxidizable fraction was decreased to less than 5% after Fenton treatment.

The main existing forms of Cd and Ni were weak acid soluble, oxidizable and reducible fractions, which accounted for 44.79%, 18.95% and 16.65% of total Cd and 26.08%, 21% and 26% of total Ni respectively. These three dominant fractions of Ni and Cd were decreased under Fenton treatment and CCFP (sample F1, F3, F4). In the case of heavy metals with high toxicity (like Pb, As and Cr), they were mainly present in the form of residual and oxidizable fractions and accounted for 94.2%, 83.49%, 77.03% of total content respectively. Pb, As and Cr in the form of oxidizable fraction was reduced and removed from solid phase after Fenton treatments of F1, F3 and F4.

With regard to main element Cu and Zn, their dominant fractions were organically and reducible fractions, respectively. Other micro-elements Cd, Ni, Pb, Cr and As were mainly present as the residual fraction or oxidizable fraction. The organically and inorganically bound metals were solubilized and released in the procedure of acidic Fenton and CCFP, which did not occur at the neutral pH with Fenton process.

3.5. Practical implications

Table 4 summarized the estimated main operating costs (chemicals and sludge disposal) of different Fenton processes for sludge treatment. In traditional Fenton process, the operating cost of pH adjustment was 179.01 \$/ton DS. Total cost for traditional Fenton treatment is 259.33 \$/ton DS. The high costs and process complexities limit its engineering application. The dosages of EDTA and EDTMPS were 63.69 and 199.91 kg/ton DS, and the corresponding operating costs were 103.11 \$/ton DS and 132.34 \$/ton DS. What's more, the operating expense for EDTA and EDTMPS CCFP was 183.43 and 212.66 \$/ton DS respectively. Obviously, CCFP could significantly save the cost of Fenton treatment of waste sludge and simplify the operating procedure.

4. Conclusions

EDTA and EDTMPS chelated-Fe²⁺ catalyzed Fenton process generated ·OH and ·O₂·, which could effectively destruct flocs, solubilize EPS and disintegrate organics into small molecules. Concomitantly, CCFP performed similar effects with traditional Fenton and resulted in conversion of bound water into free water. EDTMPS is biodegradable and more environmentally friendly than EDTA in environmental application. Besides, BCR analysis showed that the dominant fraction of Cu and Zn were oxidizable and reducible fraction, while other metals were mainly present as residual or oxidizable fractions. The oxidizable and reducible fractions of heavy metals were transformed into supernatant under traditional Fenton and CCFP.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2017.06.180>.

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