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Variations in physical, chemical and biological properties in relation to sludge dewaterability under Fe (II) - oxone conditioning

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Abstract

The mechanism of Fe (II) – oxone conditioning to improve sludge dewaterability was investigated in this study. Five different types of sludge were tested, including raw sludge (Group 1: mixed primary and secondary sludge, waste activated sludge and anaerobic digested sludge) and pretreated sludge with prior solubilisation (Group 2: ultrasonic or thermal pretreated sludge). After Fe (II) – oxone conditioning, the concentrations of dissolved organic carbon, protein and polysaccharide of soluble extracellular polymeric substances (SB EPS) increased for Group 1, but decreased for Group 2. For all types of sludge investigated, the related organic compounds of loosely bound (LB) and tightly bound (TB) EPS decreased with Fe (II) – oxone conditioning, and increased sludge filterability showed strong and positive correlation with the

removal of low molecular weight protein and neutrals in LB EPS. Fe (II) - oxone was very effective in disintegrating cell membrane and caused potential cell lysis, as indicated by increased percentage of damaged microbial cells. From this study, the mechanism of Fe (II) - oxone conditioning was proposed and can be divided into two steps: (1) Oxidation step – sulfate radicals degraded organic compounds in LB and TB EPS in sludge and transformed bound water to free water that was trapped in TB and LB EPS; It also damaged cells membrane and may help to release intracellular water content. Sludge flocs were broken into smaller particles; (2) Coagulation step - Fe (III), generated from the oxidation step can act as a coagulant to agglomerate smaller particles into larger ones and reduce the repulsive electrostatic interactions. Combined effects from above two steps can greatly improve sludge filterability.

42 **Keywords**

43 Sludge dewaterability; Fe (II) - oxone conditioning; extracellular polymeric substances;
44 mechanism; Fe (III) coagulation; filterability and rheology.

45

46 **Abbreviations**

47	CLSM	Confocal laser-scanning microscope
48	COD	Chemical oxygen demand
49	CST	Capillary suction time
50	CST ₀	Capillary suction time before Fe (II) – oxone conditioning
51	C ₀ /C	Initial concentration divided by concentration by Fe (II) – oxone
52	conditioning	
53	D ₅₀	Medium value of particle size
54	Da	Dalton
55	DOC	Dissolved organic carbon
56	EPS	Extracellular polymeric substances
57	FeSO ₄ · 7H ₂ O	Ferrous sulfate heptahydrate
58	FeCl ₃	Iron chloride
59	<i>g</i>	gravitational acceleration

60	HB DOC	Hydrophobic dissolved organic carbon
61	HCl	Hydrochloride acid
62	HI DOC	Hydrophilic dissolved organic carbon
63	HMW	High molecular weight
64	k	Consistency index
65	$2\text{KHSO}_5 \cdot \text{KHSO}_4 \cdot \text{K}_2\text{SO}_4$	Oxone
66	LB EPS	Loosely bound extracellular polymeric substances
67	LC-OCD-OND	Organic carbon detection and organic nitrogen detection
68	LMW	Low molecular weight
69	m	Apparent viscosity correlated to the shear stress/shear rate ratio
70	mins	Minutes
71	NaCl	Sodium chloride
72	N.D.	Not detectable
73	n	Flow behavior index
74	$\cdot\text{OH}$	Hydroxyl radicals
75	$\text{OHNH}_2 \cdot \text{HCl}$	Hydroxylamine hydrochloride
76	PBS	Phosphorus saline buffer
77	POMS	Peroxymonosulfate

78	γ	Shear rate
79	rpm	Revolutions per minute
80	SB EPS	Soluble extracellular polymeric substances
81	SEC	Size-exclusion chromatography
82	$\text{SO}_4^{\cdot -}$	Sulfate radicals
83	τ	Shear stress
84	τ_y	Yield stress
85	TB EPS	Tightly bound extracellular polymeric substances
86	TS	Total solids
87	VS	Volatile solids
88	VSS	Volatile suspended solids

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1. Introduction

The treatment and disposal costs of waste sludge account for 50 – 60% of total operating expense in wastewater treatment plants (Maspolim *et al.*, 2015). Efficient sludge dewatering is of particular importance in reducing sludge volume, cutting transportation and disposal cost. Therefore, various methods and strategies have been developed to improve sludge dewatering and reduce sludge volume (Chen *et al.*, 2010, Wu *et al.*, 2015, Wu *et al.*, 2016). Recent studies have been focused on using chemical oxidation technologies (Wu *et al.*, 2014), i.e. through generating the extreme reactive species like hydroxyl radicals ($\cdot\text{OH}$) and sulfate radicals ($\text{SO}_4^{\cdot-}$) to improve sludge dewaterability. $\text{SO}_4^{\cdot-}$ based chemical oxidation process using persulfate ($\text{S}_2\text{O}_8^{2-}$) or peroxymonosulfate (POMS, HSO_5^-) as oxidants has been explored (WERF, 2008, Zhen *et al.*, 2012). Oxone ($2\text{KHSO}_5 \cdot \text{KHSO}_4 \cdot \text{K}_2\text{SO}_4$) is a stable oxidant with POMS as its active species and generates $\text{SO}_4^{\cdot-}$ after activated by Fe (II). The $\text{SO}_4^{\cdot-}$ radical owns a higher redox potential (2.5 - 3.1 V), a longer life time (30 – 40 μs) and a wide applied pH range (pH 4 – 9) than $\cdot\text{OH}$ (Kim *et al.*, 2016). Liu *et al.* (2016b) reported Fe (II) - oxone process had higher sludge dewatering efficiency by reducing over 80% capillary suction time (CST), lower oxidant dosage and wide pH applied range than Fenton process. To date, most studies mainly focused on improving Fe (II) - oxone pretreatment efficiency (Liu *et al.* 2016b) or comparing Fe (II) – oxone performance with other pretreatment methods (Kim *et al.*, 2016, Song *et al.*, 2016a), the mechanism of Fe (II) – oxone conditioning to improve sludge dewaterability and its effects on sludge physical, chemical and biological properties have not been systematically investigated. Investigation of related mechanism would help to optimize dosages and operating conditions of Fe (II) – oxone conditioning on sludge dewatering.

Sludge contains large amounts of extracellular polymeric substances (EPS), and its content, composition and stratification have been widely reported to correlate with sludge dewaterability (Zhang *et al.*, 2016). Many studies have been conducted to investigate the effects of EPS on CST during Fe (II) - oxone oxidation process, but the results were inconsistent. For example, concentrations of protein and polysaccharides in soluble EPS (SB EPS) and loosely bound (LB EPS) increased after alkali-activated or thermally-activated oxone treatments, and protein content in LB EPS were positively correlated with CST (Kim *et al.*, 2016). However, WERF (2008) reported after Fe (II) - oxone treatment, protein content in SB EPS decreased rapidly while polysaccharides content in SB EPS increased, and the correlation between EPS and CST was not established. In fact, sludge dewaterability was largely affected by the molecular weight of related compounds (Niu *et al.*, 2013). However, previous studies mainly focused on the characteristics of overall dissolved organic matter in EPS, without prior molecular weight fractionation. This may limit the understanding about the true effects of EPS composition on sludge dewaterability. Therefore, further fractionation of organic compounds in EPS based on different molecular weights during Fe (II) - oxone conditioning is necessary to better understand its mechanism.

The removal of bound water is the key step to reduce sludge water content. This is particularly important to save energy consumption for sludge drying step. Bound water is integrated with sludge through chemical bonds or capillary forces, and therefore, difficult to be removed (Song *et al.*, 2016b). Most chemical oxidation technologies focused on transforming the bound water trapped in particular fractions (i.e. LB EPS and tightly bound (TB) EPS) to free water so as to improve sludge filterability (Niu *et al.*, 2016, Zhen *et al.*, 2012). In some cases, the strong oxidation effect may further destroy microbial cells, penetrate through the microorganism

cell walls and release the water of hydration bound inside cells into free water (Zhen *et al.*, 2013). Currently, the mechanism of Fe (II) – oxone conditioning on transforming bound water from EPS fractions and cell walls remains less clear.

The rheological property of sludge is important to sludge pumping, dewatering, drying, and mixing (Xia *et al.*, 2009). Liu *et al.* (2016c) reported microwave-H₂O₂ treatment can improve sludge flowability and weaken its viscoelastic properties. Pham *et al.* (2010) indicated Fenton oxidation can increase sludge flowability. However, little is known about the effects of Fe (II) – oxone conditioning on sludge rheology and how this would affect sludge dewaterability.

The objectives of this study were to investigate the role and mechanism of Fe (II) – oxone conditioning on sludge properties and how these changes affected sludge dewaterability. The schematic diagram of this study is shown in Fig. 1. Five different types of sludge, characterized with variations in initial organic matters content, surface properties, and biological activities, were used to compare the effects derived from Fe (II) – oxone conditioning. Detailed description on the experimental procedures can be found in materials and methods.

2. Materials and method

2.1. Chemical reagents

Ferrous sulfate heptahydrate (FeSO₄ · 7H₂O) and iron chloride (FeCl₃) were purchased from Merck Pte Ltd., Singapore. Oxone was purchased from Sigma-Aldrich Pte Ltd., Singapore. Stock solutions of oxone (229.5 mM) and FeSO₄ · 7H₂O (183.6 mM) were prepared prior to experiments. Deionized water was used throughout this study from a water purified system (Mili-Q Advantages, Merck Milipore, Darmstadt, Germany). All chemicals used in this study were of analytical grade.

2.2. Sludge sources

In order to compare different responses of various types of sludge to Fe (II) - oxone conditioning, five types of sludge, which had different initial organic content (compositions and concentrations) and biological activities, were prepared for this study. The original mixed primary and secondary sludge, waste activated sludge and anaerobic digested sludge were collected from a local municipal wastewater treatment plant. Ultrasonication or thermal pretreatment was applied on raw sludge to prepare the treated sludge based on parameters shown in Xiao *et al.* (2016) and Xue *et al.* (2015). Ultrasonic pretreatment of mixed sludge was conducted with an ultrasonicator at a frequency of 20 kHz (Q700, Misonix Qsonica, Newton, CT, USA) and power input of 100 W for 2 minutes (mins). The thermal pretreated sludge was collected from a 5 L anaerobic fermenter (BIOSTAT Aplus MO, Satorius, Germany) operated at 65 °C for waste activated sludge treatment. The characteristics of five different types of sludge are listed in Table 1. The selection of original mixed sludge and waste activated sludge was to investigate the effect of Fe (II) – oxone conditioning on normal wasted sludge dewaterability and set a baseline for comparison; the selection of ultrasonic and thermal pretreated sludge was to achieve higher initial organic contents so as to investigate the efficiency of Fe (II) – oxone conditioning with the interference of high concentrations of organic compounds; while the selection of anaerobic digested sludge drawn from 30 °C and 65 °C anaerobic digesters was to investigate Fe (II) – oxone conditioning efficiency on digested sludge.

2.3. Experimental procedures

The experiments design flow can be found in Fig. 1. Briefly, EPS stratification was conducted for the five types of sludge and the contents of different EPS composition (i.e.

Dissolved organic carbon (DOC), polysaccharides and protein) were quantified. Size-exclusion chromatography (SEC) and organic carbon detection and organic nitrogen detection (LC-OCD-OND) were employed to fractionize organic compounds in EPS based on different molecular weight. The correlation (positive or negative) between the fractionized compounds and sludge filterability was established through Pearson's correlation analysis. Bound water and cell viability were tested to study the transformation of bound water in particulate fractions and cell walls by Fe (II) – oxone conditioning. Surface properties (i.e. zeta potential and particle size) and Fe (II)/Fe(III) contents of conditioned sludge were determined to investigate Fe (III) coagulation mechanism. Finally, sludge rheology was tested with three different models (Power Law, Herschel-Bulkley, and Bingham Law) to study the effects of Fe (II) – oxone conditioning on sludge flowability and viscosity.

Fe (II) – oxone conditioning step was initiated by adding a predetermined amount of oxone into reaction tubes (50 mL) that contained 40 mL sludge, after a specified dosage of Fe (II). The dosages of Fe (II) and oxone were determined from the preliminary studies at different mixing ratios, and the optimum combinations are shown in Table 1 based on results of Fig.S1 (Supplementary data). Total solids (TS) values of all sludge samples were maintained at the similar level of $15.15 \pm 0.67 \text{ g L}^{-1}$ to compare the rheology properties which is largely affected by initial TS value (Baroutian *et al.*, 2013). Oxone and Fe (II) were replaced with equivalent volume of deionized water in the control groups. The sludge samples were shaken at 150 revolutions per minute (rpm) in an incubator at room temperature (Satorius Stedim Biotech, Germany). After 20 mins incubation, sludge samples were drawn for CST, pH, bound water, Fe (III) /Fe (II) content, viability analyses and EPS extraction. The choice of 20 mins for Fe (II) - oxone conditioning time was according to the study in Liu *et al.* (2016b) to ensure complete

reaction so as for EPS extraction. Triplicates were conducted for each test and the average values with standard deviations will be reported.

To elucidate the role of Fe (III) in Fe (II) – oxone oxidation process, a separate set of experiments was conducted. Similarly, five different types of sludge were used. Four different conditions were applied in the five samples for comparison: (1) addition of Fe (II) – oxone at optimum dosages listed in Table 1, (2) Fe (II) only in form of $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ (refer to Table 1 for dosage), (3) Fe (III) only in form of FeCl_3 (equivalent molar content to Fe (II) in Table 1) and (4) raw sludge samples without addition of any chemicals. After 20 mins incubation in a shaker at 150 rpm and room temperature, sludge samples were drawn for particle size analysis. Triplicates were conducted for each test.

2.4. Analytical methods

2.4.1. TS, VS, VSS, CST and particle size

Total solids (TS), volatile solids (VS), and volatile suspended solids (VSS) of sludge were measured according to the standard methods (APHA, 2005).

Sludge dewaterability can be evaluated in two ways: residue moisture amount in sludge cake after a dewatering process (the “bound water”), and easiness of sludge filtration (the filterability) (Vesilind, 1988). Sludge filterability was evaluated by CST as a commonly used method in the literature (Kim *et al.*, 2016). CST was determined with a CST instrument (OFITE Capillary Suction Timer, OFI Testing Equipment, Houston, USA) with Whatman No.17 chromatography-grade paper. The change in sludge filterability was indicated by the reciprocal ratio of

conditioned sludge CST (CST) to its initial value (CST₀). A higher CST₀/CST value indicates the increase in filterability.

The particle size distribution was determined with a laser diffraction particle size analyser (model SALD-MS30, Shimadzu, Tokyo, Japan). pH was measured with a pH meter (Mettler-Toledo, model S220, Singapore).

2.4.2. Bound water

Bound water was determined based on a centrifugation method described in Jin *et al.* (2004). In that study, bound water was defined as the sum of interstitial water (water trapped in capillaries and voids between and inside sludge flocs), internal water (water in bacterial cells and chemically/physically bound in the sludge) and surface water (water is adhered or adsorbed onto the wet sludge). 35 mL sludge samples was centrifuged at $1057 \times$ gravitational acceleration (g) for 10 mins, and the supernatant was removed. Water content in the centrifuged biomass was determined by drying sludge sample in an oven at 105 °C overnight and was defined as bound water content.

2.4.3. Fe (III) / Fe (II) extraction and analysis

Sludge samples were spanned down at $1057 \times g$ for 10 mins, and the supernatant was removed and collected, while the remaining solids was collected as dewatered sludge cake. The concentrations of Fe (III) / Fe (II) in the supernatant and dewatered sludge cake were determined based on methods described in Yu *et al.* (2016). Briefly, Fe (III) and Fe (II) in the dewatered sludge cakes (dry mass was about 0.2 g) were dissolved in 0.5 M hydrochloride acid (HCl) solution at room temperature. Before analysis, the dissolved solution was filtered through a 0.45 μ m sterilized nylon membrane filter (Whatman, UK). By adding hydroxylamine hydrochloride

($\text{OHNH}_2 \cdot \text{HCl}$), all the Fe (III) was reduced to Fe (II) and was regarded as the total Fe (Fe (III) + Fe (II)). The concentration of total Fe in the supernatant and extraction solution was determined by the 1,10 – phenanthroline method at its maximum absorbance wavelength of 510 nm with a spectrophotometer (UV-1800, Shimadzu, Japan). While in the samples without addition of $\text{OHNH}_2 \cdot \text{HCl}$, only Fe (II) can react with 1,10 – phenanthroline, and the concentration of Fe test was Fe (II) concentration. Fe^{3+} concentration was calculated as the difference between Fe (total) and Fe (II).

2.4.4. Rheological test

Rheological behaviour of sludge was measured by a rheometer (Physica MCR101 Modular Compact Rheometer, Anton Paar, Australia), with a rotor and a cup (radius: $28.92 \pm 0.05\text{mm}$, height: 73.10 mm). 18 mL sludge was filled into the cup. A continuous ramp of shear rate increasing from $9.5 - 1000 \text{ s}^{-1}$ was applied, and then vice versa. Fifty shear rates were available for setting. Shear rates and shear stress were recorded for the typical rheograms. For making a comparison, the three most commonly used rheological models, namely Power Law (Eq.1), Herschel-Bulkley (Eq.2), and Bingham Law (Eq.3) were used to describe the rheological behavior of a non-Newtonian fluid. These models were used to fit the measured rheological data of the shear stress and the shear rate.

$$\tau = k \gamma^n \quad (\text{Eq.1})$$

$$\tau = k \gamma^n + \tau_y \quad (\text{Eq. 2})$$

$$\tau = m\gamma + \tau_y \quad (\text{Eq. 3})$$

Where,

τ = shear stress (Pa)

272 γ = shear rate (S^{-1})

273 k = consistency index ($Pa \cdot S^n$)

274 n = flow behavior index (dimensionless)

275 τ_y = yield stress (Pa)

276 m = apparent viscosity correlated to the shear stress/shear rate ratio ($Pa \cdot S$).

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278 **2.5. EPS extraction and analysis**

279 **2.5.1 EPS extraction**

280 EPS of sludge samples was extracted with the method described by Zhou *et al.* (2015).
281 Briefly, sludge sample (15 mL) was centrifuged at $4000 \times g$ at $4^\circ C$ for 15 mins, and the
282 supernatant was collected as SB EPS. The sludge pellet left was then re-suspended with 15 mL
283 of 0.05% sodium chloride (NaCl) solution. The sludge sample was sheared with a vortex mixer
284 and then the sludge suspension was incubated at $70^\circ C$ water bath for 1 min, followed by
285 centrifugation at $4000 g$ at $4^\circ C$ for 10 mins and the supernatant was collected as LB EPS. The
286 residual sludge pellet was re-suspended to its original volume by adding 0.05% NaCl solution,
287 put at $60^\circ C$ water bath for 30 mins and the sludge mixture was centrifuged at $4000 g$ at $4^\circ C$ for 15
288 mins, and the supernatant was collected as TB EPS.

289 **2.5.2. EPS analysis**

290 **2.5.2.1. Dissolved organic compounds measurement**

291 The determination of DOC, protein, and polysaccharide concentrations in SB EPS, LB EPS,
292 and TB EPS were based on the methods described by Li *et al.* (2016). Briefly, DOC was
293 determined with a total organic carbon analyser (Shimadzu, Japan). Total polysaccharide

concentration was measured with phenol-sulphuric acid method (Dubois *et al.*, 1956). Protein concentrations were measured with the modified Lowry method (Frølund *et al.*, 1995).

2.5.2.2. Size-exclusion chromatography coupled with organic carbon detection and organic nitrogen detection analysis

To quantify the major soluble organic fractions with different sizes and chemical functions in the different EPS layers, a size-exclusion chromatography, in association with organic carbon and nitrogen detection (LC-OCD-OND) was used. The flow rate of mobile phase was 1.1 mL min⁻¹ and delivered by a HPLC pump (S-100, Knauer, Berlin, Germany) to an auto-sampler (MLE, Dresden, Germany) and the chromatographic column (TSK HW 50S, 3000 theoretical plates, Toso, Japan). To analyse organic carbon, a UV detector was used (UVD 254 nm, type S-200, Knauer, Berlin, Germany). For nitrogen analyse, the same detector was used with a side stream diverted after UVD with a flow rate of 0.1 mL min⁻¹. LC-OCD-OND was employed to quantify biopolymers, high molecular weight (HMW) protein, building blocks, low molecular weight neutrals (LMW neutrals), low molecular weight acids (LMW acids) and high molecular weight polysaccharide (HMW polysaccharide) (Huber *et al.*, 2011). Concentrations of low molecular weight protein (LMW protein) and low molecular weight polysaccharide (LMW polysaccharide) were calculated by subtracting HMW protein and HMW polysaccharide from the total protein and polysaccharide concentrations tested by spectrometer method, respectively. Concentrations of total protein and polysaccharide were determined based on the modified Lowry method (Frølund *et al.*, 1995) and phenol-sulphuric acid method (Dubois *et al.*, 1956), respectively.

2.6. Viability test

The Live/Dead[®] BacLight[™] bacterial viability kit (Invitrogen Molecule Probes, L-7012) was used to determine the viable and dead cells according to methods described in Zahedi *et al.* (2016). The original sludge samples were diluted 20 times and washed with 1 × phosphorus saline buffer (PBS) for three times, and then 1 mL diluted sludge sample was mixed with 1 µL of a SYTO[®] and propidium iodide mixture solution, and incubated in dark for 15 mins at room temperature. Stained sludge samples were visualized under a confocal laser-scanning microscope (CLSM) (Zeiss LSM 880, Germany). 20 images were taken for each sample and quantification was conducted with Imaris v 8.1.2 (Bitplane AG, software available at <http://bitplane.com>). The percentage of damaged cells was calculated by dividing the area of red fluorescence (damaged cells) from the sum areas of red fluorescence (damaged cells) and green fluorescence (viable cells).

2.7. Statistical analysis

Pearson's correlation between the removal of key organic compounds in EPS and increased sludge filterability (CST_0/CST) was determined using the software SAS 8.0, developed by SAS Institute Inc. The correlations were statistically significant when the probability (p value) was less than 0.05 (Zhang *et al.*, 2015a). Rheology models were established with OriginPro 8 software.

3. Results and discussion

3.1. Sludge filterability

The CST values before (CST_0) and after (CST) Fe (II) – oxone conditioning and the relative filterability of each sludge type are shown in Fig. 2. For all types of sludge investigated, CST values decreased after conditioning (Fig. 2a). The filterability was improved by 1.91, 3.76, 9.20,

26.23 and 2.10 times for thermal pretreated sludge, waste activated sludge, mixed sludge, ultrasonic pretreated sludge and anaerobic digested sludge, respectively, at their corresponded optimal dosages (Fig. 2b). It was noted that the ultrasonic pretreated sludge showed higher improvement in filterability than the original mixed sludge. This was likely related to the initial deteriorated filterability by ultrasonic pre-treatment compared to raw mixed sludge (CST: 627.4 s vs. 96.4 s).

3.2. Responses of EPS

3.2.1. Changes in fractions of EPS

The changes of DOC, protein, and polysaccharide in different fractions of EPS (i.e. SB EPS, LB EPS, and TB EPS) were determined for different types of sludge before and after Fe (II) – oxone conditioning (Fig. 3). The total EPS was the sum of SB EPS, LB EPS and TB EPS.

Increases of DOC, protein, and polysaccharide concentrations in SB EPS were observed for waste activated sludge, mixed sludge and anaerobic digested sludge. The increases of organic compounds were likely resulted from the solubilisation of the sludge constituents and elution of organics by Fe (II) – oxone oxidation, with mostly transferred from the particulate fraction to the slime layer (SB EPS) (Niu *et al.*, 2016). However, decreases of DOC, protein, and polysaccharide concentrations in SB EPS was found for the other two pre-treated sludge types, namely, thermal pretreated sludge and ultrasonic pretreated sludge. With the prior ultrasonic and thermal pretreatment, sludge flocs were disintegrated as proven by decreased medium particle sizes (50.69 μm reduced to 7.29 μm for ultrasonic pretreatment, and 128.38 μm reduced to 90.06 μm for thermal pretreatment). Furthermore, the sludge constituents was solubilized with pre-treatment as evidenced by relatively higher initial protein, polysaccharide and DOC contents in

thermal and ultrasonic pretreated sludge compared to respective raw sludge (waste activated sludge and mixed sludge) (Table 1). Therefore, $\text{SO}_4^{\cdot-}$ could firstly react with the soluble fraction of sludge and reflected as the decrease of organic compounds in SB EPS. The mineralization might be more pronounced than the solubilisation of organic compounds under this condition (Kim *et al.*, 2016).

Decreases of DOC, protein, and polysaccharide in LB EPS, TB EPS and total EPS were noted for all sludge types. This confirmed that Fe (II) - oxone reaction can effectively degrade the EPS which are crucial for the water binding capacity. LB EPS has been reported to be closely related with sludge-water separation characteristics and contain higher bound water content than SB EPS and TB EPS (Yang and Li, 2009). TB EPS, located in the inner flocs, represents an essential structure and is important in cell adhesion and stability of sludge flocs (Liu *et al.*, 2010). The correlation between increased sludge filterability and changes in EPS fractions will be further discussed below.

3.2.2. Changes of organic composition in fractions of EPS

LC-OCD-OND was used to fractionize organic compounds in EPS based on different molecular weights. The concentrations of related compounds in SB, LB and TB EPS are shown in Fig. 4. Similar to Fig.3, the DOC (hydrophobic (HB) DOC and hydrophilic (HI) DOC) content in SB EPS increased in mixed, anaerobic digested and waste activated sludge after conditioning, however, it decreased in thermal and ultrasonic pretreated sludge. DOC (HB DOC and HI DOC) in LB EPS and TB EPS was reduced in all sludge samples. For biopolymer, HMW protein, LMW protein, LMW neutrals and LMW acids, their concentrations also decreased in SB EPS of thermal and ultrasonic pretreated sludge, while obvious increases were observed in SB EPS of

waste activated, mixed and anaerobic digested sludge. In LB and TB EPS, obvious decreases in LMW protein, building blocks, LMW neutrals and LMW acids were observed for all sludge samples.

Pearson's correlation was used to describe the relationship between increased sludge filterability (CST_0/CST) and variations in key organic compounds (initial concentration/concentration after Fe (II) – oxone conditioning, C_0/C) as shown in Table 2. The results indicated that increased sludge filterability was strongly and positively correlated with the reduction of DOC ($R = 0.8895$, $p < 0.05$), LMW protein ($R = 0.9321$, $p < 0.05$) and LMW neutrals ($R = 0.9219$, $p < 0.05$) in LB EPS. The LMW protein is defined as protein with molecular weight less than 20 kDa. Protein is known as an important compound of EPS for holding water molecules, and increase in protein content can typically worsen sludge filterability (Liu *et al.*, 2016a). LMW neutrals mainly include mono-oligosaccharides, alcohols, aldehydes and ketones, and they belong to chromatographable DOC, which represents the “truly hydrophobic” fraction that was retained due to the strong hydrophobic interactions with the resin during LC-OCD-OND analysis. High hydrophobicity is associated with a relatively poor compressibility and settleability, and therefore hinders sludge dewatering (Jin *et al.*, 2003). The results of this study further emphasized increased sludge filterability by Fe (II) – oxone conditioning was strongly associated with the degree of removal of DOC, LMW protein and LMW neutrals in LB EPS. In other words, more efforts can be focused on the removal of DOC, LMW protein and LMW neutrals in LB EPS while optimizing Fe (II) – oxone dosages and operation conditions.

3.3. Bound water and cell viability

The changes of bound water and cell viability for different types of sludge before and after Fe (II) – oxone conditioning are shown in Fig. 5. After Fe (II) - oxone conditioning, the contents of bound water in all sludge samples decreased (Fig. 5a), suggesting the possible transformation of bound water to free water (Liu *et al.*, 2016b). It was observed that after conditioning, the percentage of damaged cells increased by 10.2 % for thermal pretreated sludge, 32.2 % for waste activated sludge, 4 % for mixed sludge, 22 % for ultrasonic pretreated sludge and 6.1 % for anaerobic digested sludge, respectively (Fig. 5b).

3.4. Sludge rheology

The results of sludge rheological properties before and after Fe (II) – oxone conditioning are shown in Fig. S4. Compared to Power Law model and Bingham plastic model, Herschel-Bulkley model was found to be more efficient to describe data generated in this study (Supplementary material: Fig. S5 – S8). The statistical coefficients of the Herschel-Bulkley model for different types of sludge are listed in Table 3. The higher n value (flow behavior index) indicates the behavior of the sludge gradually tends to be a Newtonian behavior (Feng *et al.*, 2016). Lower k value (consistency) indicates sludge firmness is low or weak, and the trend of k value is typically in consistent with apparent viscosity (Liu *et al.*, 2016c).

Based on results of Herschel-Bulkley model listed in Table 3, for waste activated sludge, mixed sludge, ultrasonic pretreated sludge and anaerobic digested sludge, n values increased and k value decreased by Fe (II) – oxone conditioning. In other words, sludge flowability increased and viscosity decreased. As shown in Fig. 3, the concentrations of total organic compounds decreased, and more organic compounds, i.e. protein and polysaccharide, may be released from the inner particulate to outer layer by oxone oxidation. The deteriorated internal structure can

lead to increased flowability and decreased viscosity (Pham *et al.*, 2010). Exception was found for thermal pretreated sludge, with n value decreased and k value increased, and the results of Baudez *et al.* (2013) supported this finding that the current hydrodynamic models were not suitable for thermal pretreated sludge, as models were based on the assumption that sludge composition remained constant, however, temperature would cause irreversible composition change of sludge during thermal pretreatment. Future work would be required to monitor sludge viscosity at different temperature and the related valid model needs to be proposed for thermal pretreated sludge.

3.5. Fe (III) coagulation mechanism

Zeta potential of all sludge samples increased (Fig. S2) after conditioning, possibly due to the generation of Fe (III) and H⁺ during Fe (II) – oxone reaction based on Eqs. 4 – 7, thus causing the protonation of negatively charged functional groups in EPS (Zhang *et al.*, 2015b).

In order to further understand the role of Fe (III) generated in conditioning process. Separated coagulation experiments with five different types of sludge were carried out. As shown in Fig. 6, for all sludge samples, the medium values of particle sizes of sludge samples treated with oxone alone was smaller than raw sludge and Fe (II) – oxone conditioned sludge, indicating the oxone alone would effectively decrease sludge particle size due to destruction of

the EPS structure and breakdown of dense structure into smaller particles. The coagulation effects of Fe (III) for thermal pretreated sludge and waste activated sludge were obvious and the particle sizes were in the following order: Fe (III) > Fe (II) > Raw > Fe (II) - oxone > oxone. This trend was possibly because Fe (III) is a very good coagulant and can significantly neutralize surface charge of the particles (Katsiris and Kouzeli-Katsiri, 1987), and its effect for coagulation is better than Fe (II) (Yu *et al.*, 2016). While the coagulation effects were not obvious for mixed sludge and anaerobic digested sludge with the particle sizes in the following order: Fe (III) $\approx$ Fe (II) $\approx$ Raw > Fe (II) - oxone > oxone. More smaller particles were found in mixed and anaerobic digested sludge than thermal pretreated sludge and waste activated sludge (Fig. 6), which may require more Fe (III) dosage for coagulation (Iler, 1975). The particle size distribution for ultrasonic pretreated sludge was distinctly different from the other four types of sludge. Fe (III) and Fe (III) produced during conditioning were very effective in ultrasonic pretreated sludge.

The amount of Fe (III) in conditioned sludge samples was measured, and the results are shown in Table 4. In dewatered sludge cakes, Fe (III) concentration was obviously higher than those without conditioning. The higher Fe (III) concentration may contribute to better filterability by reducing bound water content through decreasing water-affinity surface areas (Yu *et al.*, 2016). In the supernatant, Fe (III) concentration was also higher than those without conditioning for waste activated sludge, ultrasonic pretreated sludge and mixed sludge. Fe (III) was hardly detected in the supernatant of thermal pretreated sludge and anaerobic digested sludge. This might be related with the prior anaerobic treatment for these two types of sludge. In anaerobic digestion, sulfate is reduced to sulfide, which is distributed between H₂S in the gas phase, H₂S, HS⁻, and S²⁻ in solution, and insoluble metallic sulfides (Isa *et al.*, 1986). The addition of Fe (II) may cause the partial formation of metallic precipitation FeS, thus affecting

actual Fe (II) function during Fe (II) – oxone conditioning. In other words, Fe – oxone conditioning might not be suitable for digested sludge dewatering, as indicated by relatively lower increased filterability (Fig. 2b), lower reduction of total organic compounds (Fig.3) and lower Fe (III) coagulation effect for thermal pretreated sludge and anaerobic digested sludge.

3.6. Comparison of Fe (II) – oxone conditioning for different types of sludge

For the ultrasonic and thermal pretreated sludge, the concentrations of initial organic compounds were higher than the original sludge without any pretreatment (mixed sludge and waste activated sludge) (Table 1). With Fe (II) - oxone conditioning, the ultrasonic pretreated sludge showed improved filterability than the original mixed sludge (Fig.2b), and relatively higher reduction in bound water compared to the other sludge (Fig.5a). In comparison, the anaerobic digested sludge drawn from both 30 °C digester (anaerobic digested sludge) and 65 °C digester (thermal pretreated sludge) showed lower improvements in filterability (Fig. 2b), and this may be related with the presence of sulfide, which would cause FeS precipitation and affect the actual function of Fe (II) (Isa *et al.*, 1986). The increased amount of damaged cells with Fe (II) – oxone treatment reflected the biocidal effects of Fe (II) – oxone on all types of sludge (Fig. 5b). The highest biocidal effect was found on waste activated sludge, where more live cells existed originally. Meanwhile, after conditioning, waste activated sludge also displayed a relatively higher improvement in sludge flowability and higher reduction in viscosity compared to other sludge. In fact, waste activated sludge had higher viscosity and lower flowability initially than other sludge as supported by the results of Lotito and Lotito (2014).

3.7. The mechanisms of Fe (II) – oxone conditioning

The destruction of EPS contributed to the increased sludge filterability (Fig. 2). EPS has a high affinity for bound water (Zhen *et al.*, 2012). When EPS was attacked by $\text{SO}_4^{\cdot-}$ from Fe (II) – oxone oxidation, sludge flocs were disintegrated and dissolved, in turn the bound water (i.e. interstitial and vicinal water) inside the spaces of the flocs was released (Fig. 5a). This study demonstrated that LB EPS and TB EPS layer could be effectively decomposed by Fe (II) – oxone conditioning. In particular, LMW protein and LMW neutrals present in LB EPS were significantly reduced (Table 2). The dissolution of LB EPS and TB EPS may cause the deterioration of flocs matrix (Fig. 6), release bound water (Fig. 5a) and expose microbial cells to oxidants. At this stage, the mechanism of Fe (II) – oxone conditioning can be via EPS degradation and microbial cells rupture.

Fe (III) generated in the conditioning process can act as a coagulant and agglomerate the smaller particles into larger aggregates (Fig. 6) and meanwhile reduce the repulsive electrostatic interactions (Fig. S2). The presence of Fe (III) in dewatered sludge cakes (Table 4) can further reduce bound water content by decreasing specific surface areas of sludge samples. The specific surface area was reported to contribute to the affinity of water in sludge (Yu *et al.*, 2016).

In summary, a two-step mechanism ($\text{SO}_4^{\cdot-}$ oxidation and Fe (III) coagulation) was proposed for Fe (II) – oxone sludge conditioning process as shown. In the first step, oxone oxidation resulted in degradation of organic compounds (protein and polysaccharide) in LB EPS and TB EPS (Fig. 3), disintegrated sludge flocs (Fig. 6) and release of bound water (Fig. 5). In the second step, the subsequent Fe (III) coagulation reduced the repulsive electrostatic interactions and agglomerated smaller particles into larger ones. Combined effects from above two steps can greatly improve the sludge filterability. Liu *et al.* (2016b) reported although the oxidant cost of Fe(II) – oxone was much higher than Fenton oxidation (a typical advanced oxidation method for

improving sludge dewaterability), the more excellent enhanced dewatering performance may compensate the higher operation cost. Further studies need to be conducted about detailed economic analysis of Fe (II) – oxone conditioning for sludge dewaterability so as to improve its practical implication.

4. Conclusions

This study investigated the effects of Fe (II) – oxone conditioning on sludge properties. The following conclusions can be drawn:

- (1) For the chemical properties, Fe (II) – oxone conditioning resulted in degradation of organic compounds (protein and polysaccharide) in LB EPS and TB EPS, and increased sludge filterability was strongly and positively correlated with the removal of LMW protein and LMW neutrals in LB EPS;
- (2) For the physical properties, Fe (II) – oxone conditioning increased zeta potential, altered rheology of sludge and reduced medium particle sizes, ;
- (3) For the biological properties, percentage of damaged cells increased after Fe (II) – oxone conditioning;
- (4) An overall two-step mechanism was proposed for Fe (II) – oxone conditioning, with the first step of SO_4^{2-} oxidation to disintegrate floc particles and the second step of Fe (III) coagulation to agglomerate the smaller sludge particles into larger ones, thus reducing the repulsive electrostatic interactions.

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Table 1. Characteristics of sludge samples from different sources and optimal Fe (II) – oxone dosage

Sludge type	TS (g L ⁻¹)	VS (g L ⁻¹)	Protein (ppm-C)	Polysaccharide (ppm-C)	DOC (ppm-C)	pH	Normalized CST (s L g ⁻¹ TSS)	D ₅₀ (µm)	Zeta potential (mV)	Bound water (%)	Water content (%)	Optimum dosage (mmol g ⁻¹ VS Fe (II) HS)
Commercial	15.07	11.16	720.48	213.03	1036.09	7.51	37.12	90.06	-20.05	96.70	98.68	0.81
Pre-treated	± 0.07	± 0.08	± 3.64	± 6.95	± 1.01	± 0.01	± 0.65	± 0.01	± 2.33	± 0.05	± 0.27	
Autoclaved	14.64	11.55	579.69	123.26	944.30	6.33	3.40	128.38	-13.43	95.49	98.53	0.54
Pre-treated	± 0.25	± 0.30	± 11.36	± 1.36	± 1.21	± 0.01	± 0.04	± 0.12	± 1.31	± 0.33	± 0.05	
Pre-treated	16.25	13.53	524.42	98.62	1040.50	6.04	6.75	50.69	-15.33	94.94	98.99	0.54
	± 0.13	± 0.07	± 4.41	± 0.12	± 2.23	± 0.02	± 0.33	± 0.04	± 1.03	± 0.12	± 0.01	
Ultrasonic	14.59	12.09	1413.32	239.90	1797.20	5.97	49.48	8.29	-16.67	97.12	98.41	0.54
Pre-treated	± 0.07	± 0.07	± 2.33	± 3.36	± 3.24	± 0.01	± 0.31	± 0.03	± 0.74	± 0.21	± 0.001	
Aerobic	15.19	10.98	562.50	83.17	526.80	7.55 ±	6.46	49.09	-23.40	95.72	98.44	0.54
Pre-treated	± 0.01	± 0.03	± 1.56	± 0.66	± 0.78	0.02	± 0.42	± 0.05	± 1.13	± 0.47	± 0.001

Fig.1. The schematic diagram of this study.

Fig.2. Variances of sludge filterability with Fe (II) - oxone treatment. pH changed to 7.18, 3.21, 3.59, 3.43 and 6.93 for thermal pretreated sludge, waste activated sludge, mixed sludge, ultrasonic pretreated sludge and anaerobic digested sludge, respectively after reaction at 25 °C.

Fig.3. Variances of DOC, protein, and polysaccharide by Fe (II) - oxone treatment in SB EPS, LB EPS, TB EPS and total EPS.

Fig.4. Variances of key organic compounds analysed by LC- OCD-OND in SB EPS, LB EPS, and TB EPS before (“B” denotes “before”) and after (“A” denotes “after”) Fe (II) – oxone conditioning.

Fig.5. Variations in (a) bound water and (b) cell viability before and after Fe (II) – oxone conditioning for different types of sludge samples.

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Fig.6. Particle size distribution of different raw sludge samples and conditioned sludge samples:

(a) thermal pretreated sludge; (b) waste activated sludge; (c) mixed sludge; (d) ultrasonic pretreated sludge; (e) anaerobic digested sludge.