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Evaluating filterability of different types of sludge by statistical analysis: the role of key organic compounds in extracellular polymeric substances

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Abstract

An investigation was conducted for 20 different types of sludge in order to identify the key organic compounds in extracellular polymeric substances (EPS) that are important in assessing variations of sludge filterability. The different types of sludge varied in initial total solids (TS) content, organic composition and pre-treatment methods. For instance, some of the sludges were pre-treated by acid, ultrasonic, thermal, alkaline, or advanced oxidation technique. The Pearson's correlation results showed significant correlations between sludge filterability and zeta potential, pH, dissolved organic carbon, protein and polysaccharide in soluble EPS (SB EPS), loosely bound EPS (LB EPS) and tightly bound EPS (TB EPS). The principal component analysis (PCA) method was used to further explore correlations between variables and similarities among EPS fractions of different types of sludge. Two principal components were extracted: principal

component 1 accounted for 59.24% of total EPS variations, while principal component 2 accounted for 25.46% of total EPS variations. Dissolved organic carbon, protein and polysaccharide in LB EPS showed higher eigenvector projection values than the corresponding compounds in SB EPS and TB EPS in principal component 1. Further characterization of fractionized key organic compounds in LB EPS was conducted with size-exclusion chromatography-organic carbon detection-organic nitrogen detection (LC-OCD-OND). A numerical multiple linear regression model was established to describe relationship between organic compounds in LB EPS and sludge filterability.

Keywords

Sludge filterability; Loosely bound extracellular polymeric substances; Principal component analysis; Pearson's correlation analysis; Fractionized organic compounds.

Abbreviations

ANOVA	Analysis of variance
BPL	Biopolymer
CST	Capillary suction time
$d_{0.50}$	Median particle size
DL	Dissolved organic carbon in loosely bound extracellular polymeric substance
DOC	Dissolved organic carbon

42	DS	Dissolved organic carbon in soluble extracellular polymeric
43		substance
44	DT	Dissolved organic carbon in tightly bound extracellular
45		polymeric substance
46	EPS	Extracellular polymeric substance
47	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	Ferrous sulfate heptahydrate
48	$2\text{KHSO}_5 \cdot \text{KHSO}_4 \cdot \text{K}_2\text{SO}_4$	oxone
49	HB DOC	Hydrophobic dissolved oxygen carbon
50	HMW	High molecular weight
51	HIDOC	Hydrophilic dissolved oxygen carbon
52	LB	Loosely bound
53	LC-OCD-OND	Size-exclusion chromatography-organic carbon detection-
54		organic nitrogen detection
55	LMW	Low molecular weight
56	Min	minutes
57	MW	Molecular weight
58	p	Probability
59	PCA	Principal component analysis

60	PC1	First component
61	PC2	Second component
62	PL	Polysaccharide
63	PLL	Polysaccharide in loosely bound extracellular polymeric
64		substance
65	PLS	Polysaccharide in soluble extracellular polymeric substance
66	PLT	Polysaccharide in tightly bound extracellular polymeric
67		substance
68	PN	Protein
69	PNS	Protein in soluble extracellular polymeric substance
70	PNL	Protein in loosely bound extracellular polymeric substance
71	PNT	Protein in tightly bound extracellular polymeric substance
72	rpm	revolutions per minute
73	R	Pearson's correlation coefficient
74	SAS	Statistical analysis system
75	SB	Soluble
76	SCST	Specific capillary suction time
77	SRT	Sludge retention time

78	TB	Tightly bound
79	TS	Total solids
80	TSS	Total suspended solids
81	US	Ultrasonication
82	VS	Volatile solids
83	VSS	Volatile suspended solids

84 1. Introduction

85 Sludge is a by-product of biological wastewater treatment process, and the cost for sludge
86 disposal and treatment accounts for 40-50 % of total operation expense in wastewater treatment
87 plants (Devlin *et al.*, 2011, Xiao *et al.*, 2016). To reduce costs of transportation and handling,
88 sludge dewatering is necessary to reduce sludge volume by water separation prior to sludge
89 disposal (Xiao *et al.*, 2016). To improve sludge filterability, sludge conditioning aiming to
90 transform bound water to free water was usually adopted before dewatering step. Currently,
91 many conditioning methods have been studied, such as acid (Huang *et al.*, 2010), thermal (Ruiz-
92 Hernando *et al.*, 2015), alkaline (Shao *et al.*, 2012), ultrasonication (Shao *et al.*, 2010), advanced
93 oxidation (Liu *et al.*, 2016a) and combined treatment methods (Liu *et al.*, 2016b).

94 EPS is an important constituent of sludge flocs and it is strongly correlated with sludge
95 formation, properties, dewaterability, rheology and compression characteristics. Previous studies
96 commonly correlated sludge properties changes with EPS content changes that were caused by
97 different conditioning methods. For example, Zhou *et al.* (2015a) reported decreased EPS
98 contents (both loosely bound (LB) EPS and tightly bound (TB) EPS) by Fenton treatment can

increase sludge filterability. Liu *et al.* (2016c) showed changes of concentrations and compositions of EPS may contribute to the variation of rheology of digested sludge, and the solubilisation of bound EPS may result in the reduction of yield stress and apparent viscosity. Raynaud *et al.* (2012) concluded EPS content can affect the compression in sludge dewatering step, and the relative hydrophobicity of sludge can be reduced with more EPS destructed and polymeric structure broken-down, therefore positively impacting the compression stage. Some studies investigated the role of EPS in sludge filterability through EPS stratification. However, the results are inconsistent. It has been reported sludge filterability was correlated with SB EPS (Yu *et al.*, 2008), LB EPS (Li and Yang, 2007), TB EPS (Zhen *et al.*, 2013) or there is no correlation at all (Chao and Keinath, 1979). The contradictory results may be related with the unique single substrate and pretreatment method used. Therefore, the exact role of EPS in sludge filterability still needs to be investigated and a set of general parameter should be identified regardless of substrates and pretreatment methods applied.

Multivariate statistical analysis methods, such as Partial Least Squares and Principal Component Analysis (PCA) have been used to identify important components or factors (principal components) that explain most of the variances from a quite large database of quantitative observations (Avella *et al.*, 2011). Statistical methods of data analysis have been used for microbial community analysis in biological sludge treatment systems. For example, Liao *et al.* (2001) used two main principal components to describe the similarity of microbial community while studying the influence of sludge retention time (SRT) on EPS and physicochemical properties of sludge. Their results showed a higher degree of variability in the microbial communities appeared at SRTs of 4 and 6 d (Liao *et al.*, 2001). In case of EPS component and the filterability of anaerobic digestate from food waste, Lü *et al.* (2015) used

Partial Least Squares modelling analysis to investigate anaerobic digestate filterability and EPS characteristics (protein, polysaccharide, protein+polysaccharide, polysaccharide/protein). Their results showed protein and polysaccharide in the extracted EPS had significant positive effects on specific resistance to filtration (SRF), capillary suction time (CST) and bound water content of the anaerobic digestate. Sludge filterability showed significant correlation with characteristics of EPS component (protein, polysaccharide, dissolved organic carbon (DOC)) in different EPS fractions (SB EPS, LB EPS and TB EPS) (Liu and Fang, 2003, Zhang *et al.*, 2015). However, the key (or most important) organic compounds in different EPS fractions have not been elucidated well. Moreover, Liu *et al.* (2016a) fractionized dissolved organic matter based into different molecular weight for organic compounds in EPS with size-exclusion chromatography (SEC) and investigated its correlation to sludge dewaterability, but the related type of fractionized compounds were not identified. This would limit the understanding about the role of EPS in sludge filterability, as both the molecular weight and the type of related compounds can largely affect sludge filterability (Niu *et al.*, 2013). Therefore, it is important to better understand the key organic compounds affecting sludge filterability.

The objectives of this study were to investigate the role of EPS in sludge filterability based on 20 different sludge types. The schematic diagram of this study is shown in Fig.1. Pearson's correlation was used to investigate correlations between sludge filterability and sludge properties. PCA was used with the goal to explore the relationship between EPS fraction and filterability of different types of sludge. To go one step further, size-exclusion chromatography-organic carbon detection-organic nitrogen detection (LC-OCD-OND) was used to investigate the distribution of fractionized key organic compounds in the extracted principal EPS fraction component, and a

numerical multiple linear regression model was used to describe their relationship with sludge filterability.

2. Materials and methods

2.1. Sludge type and source

The 20 different types of sludge with different total solids (TS) concentrations and were pre-treated by different methods. The pre-treatment methods applied in this study included ultrasonic, thermal, acid, alkaline, and advanced oxidation (i.e. Fe (II)-oxone conditioning), and the hybrid treatment processes, such as combined ultrasonic and acid, ultrasonic and alkaline, thermal and Fe (II)-oxone conditioning, and ultrasonic and Fe (II)-oxone conditioning.

The original secondary sludge, anaerobic digested sludge, and mixed primary and secondary sludge were collected from a local Water Reclamation Plant (Singapore), with respective initial TS values of $14.64 \pm 0.25 \text{ g L}^{-1}$, $15.19 \pm 0.01 \text{ g L}^{-1}$ and $40.21 \pm 0.70 \text{ g L}^{-1}$. Sludge was diluted with deionized water (Millipore, Merck, Darmstadt, Germany) to achieve different final TS values. The effects of water dilution on EPS release were considered as negligible herein. The sludge samples were stored at 4°C for a short period (4 days) before experiment.

2.2. Sludge treatment processes

Ultrasonic pretreatment was conducted with an ultrasonicator at a frequency of 20 kHz (Q700, Misonix Qsonica, Newton, CT, USA). Mixed sludge samples with different TS values were pretreated at 0.4 and 1 W mL^{-1} by adjusting the input power when applied to a fixed sludge volume for 2 mins and this duration was the optimum sonication time based on preliminary study. The specific energy inputs were $4.20 \text{ KJ g}^{-1} \text{ TS}$ (No.4), $7.25 \text{ KJ g}^{-1} \text{ TS}$ (No.5), and $6.04 \text{ KJ g}^{-1} \text{ TS}$

(No.6) for different mixed sludge. During the ultrasonication, temperature of sludge samples in the beaker was $29 \pm 1^\circ\text{C}$, and the tip of the sonication probe was centrally placed 1 cm into the sludge sample held in a beaker.

For acid / alkaline pretreatments, the mixed primary and secondary sludge with a TS value of $12.09 \pm 0.27 \text{ g L}^{-1}$ was used. The pHs of raw sludge sample were adjusted to pH 2 and 10 with HCl (5 mol L^{-1}) and/or NaOH (5 mol L^{-1}), respectively. For combined acid / alkaline-ultrasonic pretreatment, the raw mixed primary and secondary sludge was first pre-treated at 0.4 W mL^{-1} , and then final pHs were adjusted to 2 or 10, respectively.

For thermal treatment, the secondary sludge ($\text{TS} = 15.05 \pm 0.07 \text{ g L}^{-1}$) was fed daily into a fermenter with working volume of 3 L (BIOSTAT Aplus MO, Satorius, Germany) operated in semi-continuous (0.5 L of the fermented sludge was withdrawn and replaced with 0.5 L feed sludge daily, resulting in a solid retention time of 6 days) and completely mixing mode at 65°C . The effluent was collected as the thermal treated secondary sludge.

For the advanced oxidation treatment, Fe (II)-oxone conditioning was used. Fe (II) was dosed in form of ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). HSO_5^- was dosed in form of oxone ($2\text{KHSO}_5 \cdot \text{KHSO}_4 \cdot \text{K}_2\text{SO}_4$). The mixed primary and secondary sludge, secondary sludge, and anaerobic digested sludge with different TS values were chosen as the sludge samples to be oxidized. All the experiments were carried in a 50 mL centrifuge tube (Greiner Bio-one, Germany), with the sludge volume of 40 mL at its inherent pH. The reaction was initiated by adding predetermined amounts of HSO_5^- and Fe (II). The optimum condition, 0.9 mM g^{-1} volatile suspended solids (VSS) HSO_5^- and 0.54 mM g^{-1} VSS Fe (II) for mixed sludge, 0.9 mM g^{-1} VSS HSO_5^- and 0.54 mM g^{-1} VSS Fe (II) for anaerobic digested sludge, and 0.9 mM g^{-1} VSS HSO_5^-

and 0.81 mM g⁻¹ VSS Fe (II) for secondary sludge were adopted to improve sludge filterability based on preliminary studies.

For combined ultrasonic and Fe (II) - oxone oxidation pretreatment, mixed sludge samples with different TS values were used as well. The sludge was firstly ultrasonicated at 1 W mL⁻¹ for 2 mins, and then pretreated with both optimum dosages of 0.9 mM g⁻¹ VSS HSO₅⁻ and 0.54 mM g⁻¹ VSS Fe (II). For combined thermal and Fe (II)-oxone oxidation pretreatment, the effluent collected from the 65° C fermentor was treated with optimum dosage of 0.675 mM g⁻¹ VSS HSO₅⁻ and 0.81 mM g⁻¹ VSS Fe (II).

All above treated/collected sludge samples were incubated at 150 revolutions per minute (rpm) in an incubator for 30 minutes (min) at room temperature (Sartorius Stedim Biotech, Germany) to ensure homogeneity. After 30 min incubation, sludge samples were drawn for CST, pH, total suspended solids (TSS), volatile solids (VS), VSS analyses and EPS extraction. Triplicates were conducted for each test. The physicochemical properties of all types of sludge used in this study were listed in Table 1.

2.3. Analytical methods

2.3.1. Sludge characteristics

TS, volatile suspended solids (VS), TSS and VSS were measured according to standard methods (APHA, 2005). pH was measured with a pH meter (Mettler-Toledo, model S220). Zeta potential was measured with Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK). Sludge dewaterability were typically evaluated in terms of residue moisture amount in dewatered sludge cake (the “bound water”) and easiness of sludge filtration (the filterability) (Vesilind, 1988). CST was a good index for sludge filterability (Chen *et al.*, 1996), and it has positive correlations with the particulate nature of sludge factors, i.e. solid content, total suspended solid, polysaccharide and protein contents (Shi *et al.*, 2016). In this study, sludge filterability was evaluated by CST and tested with a CST instrument (OFITE Capillary Suction Timer, OFI Testing Equipment, Houston, USA). The CST values were normalized against TS concentration, and expressed in unit of seconds liter per gram TS (s L g^{-1} TS). Particle size was determined with a laser diffraction particle size analyser (model SALD-MS30, Shimadzu, Tokyo, Japan).

2.3.2. EPS extraction and analysis

EPS in sludge samples was stratified into three fractions (i.e. SB EPS, LB EPS and TB EPS) by the EPS extraction protocol described in the literature (Zhang *et al.*, 2016, Zhou *et al.*, 2015b). The extracted solution for respective SB EPS, LB EPS and TB EPS were filtered through a 0.45 μm sterilized nylon membrane filter, and DOC of organic components in SB EPS, LB EPS and TB EPS was monitored using a total organic carbon analyser (Shimadzu, Japan). Total polysaccharide (PL) of organic components was determined based on phenol-sulphuric acid

method (Dubois *et al.*, 1956). Total protein (PN) concentration of organic components was determined with the modified Lowry method (Frølund *et al.*, 1995). 1 g of polysaccharide and 1 g of protein equal to 0.444 and 0.497 g of equivalent carbon, respectively (Li *et al.*, 2016).

2.3.3. 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 LB EPS (after being filtered through a 0.45 μm nylon filter), a size-exclusion chromatography, in association with organic carbon and nitrogen detection (LC-OCD-OND) was used. Description and analysis of LC-OCD-OND operation condition can be found in Li *et al.* (2016).

2.4. Statistical analysis

Three statistical methods were applied to analyse the correlation between CST and sludge properties:

- (1) Pearson's correlation by the software Statistical Analysis System (SAS) 8.0, developed by SAS Institute Inc., was used to investigate positive or negative or absent correlation between sludge filterability and sludge properties. The Pearson's correlation coefficient (R) of 1 or -1 indicates a perfect correlation between two variables; R value of 0 denotes no relationship between two variables; R value of 0.7 to 0.9 (positive or negative) denotes strong correlations; R value of 0.4 to 0.69 (positive or negative) indicates moderate correlations; R value of 0.1 to 0.3 (positive or negative) denotes weak correlations;

(2) To remove the outlying data from the sludge properties related to sludge filterability, PCA analyses were conducted. Biplot was used to evaluate correlation of measured parameters. In the biplot, parameters close to each other are in normal correlation; relate to others by a 180° rotation are in inverse correlation; relate to others by a 90° rotation are independent; parameters close to the centre are independent of all the others in the circle;

(3) Based on the above analyzed principal component, the relationship between sludge filterability and fractionized organic compounds by LC-OCD-OND was established with a multiple linear regression method. The model reliability was checked based on the following satisfaction objectives: (1) The regression coefficient R^2 should be more than 85%, which means it can explain more than 85% of the variation, and indicated adequate satisfaction between experimental and modelled results (Baek and Cadwallader, 1995); (2) The residuals, namely the difference between the actual and predicted CST values, should be a normal probability distribution; (3) The Probability (p) values of all estimated coefficients should be less than 0.05.

3. Results and discussion

3.1. Physicochemical properties of sludge

As shown in Fig. 2, sludge samples with acid pretreatment (No.9) had a specific CST (SCST) value of 1.55 s L g⁻¹ TS and combined ultrasonic-acid pretreatment (No.11) has a CST value of 1.54 s L g⁻¹ TS. The filterability with these two treatments were much better than the raw sludge (No.1: CST value of 18.46 s L g⁻¹ TS), the sludge with alkaline pretreatment (No. 10: CST value of 36.31 s L g⁻¹ TS), and combined ultrasonic-alkaline pretreatment (No. 12: CST value of 53.66 s L g⁻¹ TS). Comparably, alkaline pretreatment would deteriorate sludge filterability (No. 10 vs. No.1) and result in the release of inner compounds, i.e. protein and polysaccharide, from sludge disintegration (Fig.2b), possibly due to alkali induced solubilisation of membrane proteins and saponification of membrane lipids (Mendonca *et al.*, 1994). Shao *et al.* (2012) also reported single alkaline pretreatment would deteriorate sludge filterability. Ultrasonic pretreatment would also deteriorate sludge filterability as indicated by higher CST values (No.4 vs. No.1; No. 5 vs. No.2), possibly due to increased adsorption effect of disintegrated fine particles for free water after ultrasonication (Wang *et al.*, 2006). Increased zeta potential value was noticed for acidified sludge samples (No. 9) than raw sludge (No.1), while alkaline (No. 10) and ultrasonic-alkaline pretreatments (No.12) would decrease zeta potential. Increased zeta potential can cause protonation of negatively charged particles, reduce electrostatic repulsion and then increase sludge filterability (Liu *et al.*, 2016a).

However, with thermal treatment at 65 °C, sludge filterability deteriorated with CST value of 31.72 s L g⁻¹ TS (No. 16) compared to the non-treated secondary sludge with CST value of 2.96 s L g⁻¹ TS (No. 15). This may be also related to the release of intracellular compounds from

sludge flocs as indicated by higher DOC, PN and PL contents (Fig. 2 b, 2 c and 2d), which would retain water and then deteriorate sludge filterability (Liu *et al.*, 2016a).

The Fe (II)-oxone conditioning could increase filterability for mixed sludge (No. 7 vs. No. 2; No.8 vs. No.3), secondary sludge (No. 17 vs. No. 15) and anaerobic sludge (No. 20 vs. No. 19) as indicated by the decreased CST values. Similarly, Fe (II)-oxone conditioned sludge samples showed higher zeta potential values than raw sludge samples. Decreases in DOC, protein and polysaccharide in total EPS (the total EPS referred to the sum of SB EPS, LB EPS and TB EPS) were also observed after Fe (II)-oxone conditioning. Kalogo *et al.* (2008) also concluded Fe (II)-activated peroxymonosulfate oxidation can effectively improve sludge filterability with the release of EPS - bound water induced by the destruction and degradation of EPS. It was noted combined ultrasonic and Fe (II)-oxone conditioning did not show better sludge filterability than the single Fe (II)-oxone conditioning (No. 13 vs. No.7). Similarly, combined thermal and Fe (II)-oxone conditioning also did not show superiority in improving sludge filterability than the single Fe (II)-oxone conditioning (No. 18 vs. No.17). This may be due to more organic compounds were released with extra thermal or ultrasonic pretreatments.

3.2. Correlation between CST and sludge physicochemical properties

Table 2 shows the correlation between CST, and physicochemical properties of sludge, including TS, VS, zeta potential, pH, and distribution, abundance, composition of three EPS fractions. A strong negative correlation was found between CST and zeta potential ($R = -0.7525$, $p < 0.01$). It was generally believed that increase of zeta potential will protonate negatively charged functional groups in sludge, reduce the electrostatic repulsion, re-agglomerate sludge particles (Zhang *et al.*, 2015b), and ultimately result in better sludge filterability (Liu *et al.*,

2016a). Additionally, it can be seen from Table 2, a moderate correlation can be seen from CST and pH values, and a lower pH value would result in better filterability, as it would tend to release more H^+ , thus neutralizing the negative charged particles and improving zeta potential value (Liao *et al.*, 2002). Strong correlations were found between CST and protein content in loosely bound EPS (PNL) and polysaccharide in soluble extracellular polymeric substance (PLS). Moderate correlations were found between CST and dissolved organic carbon in all EPS fractions (soluble EPS (DS), loosely bound EPS (DL), and tightly bound EPS (DT)). Moderate correlations were also found between CST and protein content in soluble EPS (PNS) and tightly bound EPS (PNT), as well as polysaccharide in loosely bound EPS (PLL) and tightly bound EPS (PLT). However, there were no significant correlations between CST and TS (VS) values. Mikkelsen and Keiding (2002) investigated different types of sludge from 10 wastewater treatment plants, and also concluded the fraction of EPS in all types of sludge was the most important parameter with respect to sludge structure and filterability rather than initial TSS and VSS content. In terms of reducing the concentrations of macromolecular compounds that deteriorated sludge filterability, the hybrid ultrasonic-acid pretreatment (No. 11) was more effective than the acidification treatment alone (No. 9), with detailed description in Xiao *et al.* (2016). While the hybrid US-Fe (II)-oxone treatment (No. 13 vs. No. 7) and thermal-Fe(II)-oxone treatment (No. 18 vs. No.17) were less effective than the Fe(II)-oxone treatment alone in removing macromolecular compounds, this may be due to the release of intracellular organic compounds by prior ultrasonic and thermal treatments (Fig. 2). However, alkaline (No. 10 vs. No. 1), ultrasonic (No. 4 vs. No. 1), and thermal (No. 16 vs. No. 15) treatments resulted in more intracellular organic compounds released compared to the raw sludge. Moreover, the hybrid ultrasonic-alkaline treatment can release more organic compounds than the alkaline or ultrasonic

treatment alone (No.12. vs. No.10 or No.4). In terms of removing organic compounds that deteriorated sludge filterability, the treatment methods, i.e. acidification, Fe (II)-oxone, and hybrid US-acid treatment can be applied.

3.3. PCA analysis

Since the sludge filterability indexes showed multicollinearity among EPS constituents as indicated by Pearson's analysis shown in Table 2. PCA was conducted to investigate the relationship between sludge filterability and EPS characteristics based on 20 observations and nine variables (DS, DL, DT, PNS, PNL, PNT, PLS, PLL, and PLT). The PCA results showed that of the 9 components of EPS characteristics, the first component (PC1) accounted for about 59.24% and the second component (PC2) accounted for about 25.46% of the total variables in the data set (Table 3). These two components together accounted for about 84.70% of the total variables and the rest of the 7 components only accounted for about 15.3%. Therefore, only correlations showing significant coefficients according to the level of significance, namely PC1 and PC2, were considered in the biplot shown in Fig.3.

From the biplot (Fig. 3), no independent or inverse relations were observed. Moreover, no parameters were independent of all the others, and the majority of the parameters were highly correlated (Fig. 3 & Table 2). Normal correlations were noted among parameters 3 (DT), 6 (PNT) and 9 (PLT), parameters 2 (DL), 5 (PNL), and 8 (PLL), as well as parameters 1 (DS), 4 (PNS) and 7 (PLS). All these correlations can be further confirmed by the Pearson's correlation results in Table 2. Regardless of pretreatment methods, operation conditions and initial physiochemical properties, strong positive correlations were found among DOC, PN and PL in each respective

fraction of EPS. This indicated chemical composition of organic compounds (i.e. DOC, PN and PL) in each layer of EPS was similar and highly correlated for all sludge samples.

With respect to the first PC (PC1), the most positive influencing variables were DOC, protein and polysaccharide in LB EPS, with relatively higher eigenvector projection values (eigenvector is a non-zero vector that does not change its direction when linear transformation is applied to it, and each point in Fig. 3 can be represented as a vector pointing from the center of Fig. 3 to that point) of 0.3665-0.3866, than those in TB EPS (0.2985-0.3205) and SB EPS (0.2986-0.3287). The relatively higher eigenvector projection values indicated the sludge filterability was more correlated with key organic compounds in LB EPS. Higher LB EPS content was generally regarded to deteriorate sludge dewaterability (Zhen *et al.*, 2012), as it had higher bound water content and thus was more hydrated than SB EPS and TB EPS (Li and Yang, 2007). LB EPS was an important constituent of bound EPS, surrounding the sludge micro-flocs (Yuan *et al.*, 2011). The collapse or removal of LB EPS would result in the break-up of sludge matrix and the release of bound water to free water, thus improving sludge filterability. The key organic compounds in LB EPS correlated to sludge filterability shall be discussed later. With respect to the second PC (PC 2), the most positively influencing variables are DOC, protein and polysaccharide in TB EPS. As 59.24% of the variances can be explained by PC 1 rather than PC2 (only 25.46%), therefore, the characterization of key organic compounds by LC-OCD-OND was focused on the most influencing parameter in PC1 rather than PC2, and their correlation to sludge filterability shall be discussed later.

3.4. LC- OCD-OND analysis

LC-OCD-OND result shows both hydrophilic DOC (HI DOC) and hydrophobic DOC (HB DOC) were present in LB EPS of all the samples analysed (Table 4), indicating EPS was amphoteric (Sheng *et al.*, 2010). Additionally, HI DOC was more dominant than HB DOC. Imai *et al.* (2002) also observed the predominance of hydrophilic organic matters over hydrophobic compounds in effluents (sludge, wastewater and their mixture) from sewage treatment plants. Compared to other components, contents of low molecular weight (LMW) protein (molecular weight less than 20 kDa), building blocks and LMW neutrals (molecular weight less than 350 Da) were higher in the sludge samples.

A multiple linear regression model was used to indicate the numerical relationship of the above mentioned fractionized organic compounds in LB EPS and sludge filterability as shown in Eq. 1.

$$\text{CST} = -122.52 + 7.02 \text{ HB DOC} + 754.21 \text{ HI DOC} + 5666.58 \text{ Biopolymer} - 6409.47 \text{ HMW PN} + 2.33 \text{ LMW PN} - 754.22 \text{ Building blocks} - 754.30 \text{ LMW neutrals} - 756.09 \text{ LMW acids} - 6434.74 \text{ HMW PL} - 10.02 \text{ LMW PL} \quad (\text{Eq.1})$$

An analysis of variance (ANOVA) was used to determine the significance of the multiple linear model and the estimated parameters as shown in Table S1 (refer to Supplementary material). The regression coefficient R^2 (0.8197) indicates a good agreement between the experimental and modelled results (Eq.1), but the p values for estimated parameters ($\text{Pr} > |t|$ indicates the p-value is the two-tailed probability computed using t distribution) were insignificant at 95% confidence level (p-value > 0.05).

To better describe the relationship between sludge filterability and fractionized organic compounds, the model was modified with a step-wise selection method. As shown in Table S2

(refer to Supplementary material), the ANOVA results indicated that the coefficients for LMW PN, LMW neutrals, and LMW acids were significant with p values ($Pr > F$ indicates the p-value is associated with the F statistic of a given effect and test statistic) less than 0.05. However, the estimated value for “intercept” was insignificant with p-value of 0.8619 (> 0.05). Therefore, the model was further modified with the deletion of “intercept” item in the modelled equation with “NOINT” function in SAS software. After modifying the “intercept” item, a model was established between sludge filterability and LMW PN, LMW neutrals and LMW acids. As shown in Table S3(refer to Supplementary material), a regression coefficient (R^2) of 0.8735 was achieved (Eq. 2), which indicated adequate satisfaction between experimental and modelled results (Baek and Cadwallader, 1995). The p values of all estimated parameters in Eq. 2 were significant at 95% confidence level (p-value < 0.05). Moreover, the residuals were random and independently distributed (Fig. S1a) (refer to Supplementary material), and followed a normal probability distribution (Fig. S1b) (refer to Supplementary material).

The LMW PN defined in this study was defined as protein with molecular weight less than 20 KDa (Huber *et al.*, 2011). PN had a more strong water binding capacity, thus can worsen sludge filterability (Shao *et al.*, 2009). LMW neutrals referred to mono-oligosaccharides, alcohols, aldehydes and ketones with molecular weight less than 350 Da based on the definition by Huber *et al.* (2011), and they belong to chromatographable DOC (CDOC), which represents the hydrophobic fraction. Higher hydrophobicity was associated with a relatively poor compressibility and settleability, and then affected sludge filterability (Jin *et al.*, 2003). Therefore, the increase of LMW PN and LMW neutrals would increase CST value, and then deteriorated sludge filterability (Eq. 2). In contrast, increased LMW acids concentration would reduce CST values and increase filterability, and this may because more LMW acids were able to

donate protons during the process of dissociation and thus increased the zeta potential, reduced the repulsive electrostatic interactions and agglomerated smaller particles into larger ones and in turn increased sludge filterability (Liu *et al.*, 2016a). It was noted protein with molecular weight less than 20 kDa, mono-oligosaccharides, alcohols, aldehydes and ketones with molecular weight less than 350 Da, and mono-protic organic acid with molecular weight less than 350 Da in loosely-bound EPS were the determining factors affecting sludge filterability. This model can be used to evaluate sludge filterability, and the sludge types are not limited to mixed primary and secondary sludge, waste activated sludge, anaerobic digested sludge, anaerobic digestate from food/kitchen waste, textile dyeing sludge, etc. The key parameters related to this model are low molecular weight protein, low molecular weight neutrals and low molecular acids, hence, as long as above parameters are available, the model can be recalibrated according to various organic wastes.

$$\text{CST} = 1.99 \text{ LMW PN} + 2.73 \text{ LMW neutrals} - 2.75 \text{ LMW acids} \quad (\text{Eq. 2})$$

4. Conclusions

This study attempted to elucidate the role of key organic compounds in EPS in sludge dewatering process for 20 different types of sludge. CST was used to evaluate sludge filterability, and statistical analysis methods, i.e. Pearson's correlation, PCA and multiple linear regression were used to extract the key organic compounds in EPS. The following conclusions can be drawn:

1. CST values showed strong negative correlation with zeta potential and significant positive correlations with pH, DOC, PN and PL concentrations in all EPS fractions (SB, LB and TB);
2. Based on PCA results, DOC, PN, and PL in LB EPS showed more influencing impacts on sludge filterability than the related components in SB EPS and TB EPS;
3. The relationship between the fractionized key organic compounds in LB EPS by LC-OCD-OND and sludge filterability can be modelled with a numerical equation:
$$\text{CST} = 1.99 \text{ LMW PN} + 2.73 \text{ LMW neutrals} - 2.75 \text{ LMW acids}.$$

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Table.1. Physicochemical properties of sludge samples

Sample label	Type	Treatment method	TS (g L ⁻¹)	VS (g L ⁻¹)	pH	Zeta (mV)	d _{0.50} (μm)
1	Mixed	-	12.09	9.54	6.70	-12.45	49.46
2	Mixed	-	18.64	13.22	6.04	-15.33	50.69
3	Mixed	-	40.21	33.51	5.65	-15.20	87.77
4	Mixed	0.4 W mL ⁻¹ US	11.44	8.95	6.70	-14.43	15.07
5	Mixed	1 W mL ⁻¹ US	16.55	11.75	5.97	-16.67	7.29
6	Mixed	1 W mL ⁻¹ US	19.88	16.38	6.14	-19.63	5.10
7	Mixed	Fe(II)-oxone	16.25	13.53	3.59	-3.83	47.94
8	Mixed	Fe(II)-oxone	42.20	30.58	4.13	-5.48	92.95
9	Mixed	acidification	12.23	9.58	2.00	0.58	54.76
10	Mixed	alkaline	11.91	8.77	10.00	-23.23	53.02
11	Mixed	US-acid	12.42	9.86	2.00	0.88	33.71
12	Mixed	US-alkaline	11.93	8.64	10.00	-26.00	28.70
13	Mixed	US and Fe(II)-oxone	14.59	12.09	3.43	-4.24	25.06
14	Mixed	US and Fe(II)-oxone	24.48	15.54	3.19	-2.74	74.86
15	Secondary	-	14.64	11.55	6.33	-13.43	128.38
16	Secondary	Thermal	15.07	11.16	7.51	-20.05	90.06
17	Secondary	Fe(II)-oxone	16.75	11.48	3.21	-5.40	125.73

18	Secondary	Thermal and Fe(II)-oxone	15.22	9.84	7.18	-14.05	88.30
19	AD	-	16.92	10.92	7.55	-23.40	47.70
20	AD	Fe(II)-oxone	15.19	10.98	6.93	-11.70	49.09

“-” denotes there was no pretreatment; “US” denotes ultrasonication; “AD” denotes anaerobic digested sludge; “d_{0.50}” denotes median particle size.

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Fig.1. The schematic diagram of this study.

Fig.2. SCST values (a), contents of different EPS fractions (b), and their protein (c) and polysaccharide (d) concentrations. 1: raw mixed sludge; TS 12.09 g L⁻¹; 2: raw mixed sludge; TS 18.64 g L⁻¹; 3: raw mixed sludge; TS 40.21 g L⁻¹; 4: mixed sludge; 0.4 W mL⁻¹ US; TS 11.44 g L⁻¹; 5: mixed sludge; 1 W mL⁻¹ US; TS 16.55 g L⁻¹; 6: mixed sludge; 1 W mL⁻¹ US; TS 19.88 g L⁻¹; 7: mixed sludge; Fe(II)-oxone; TS 16.25 g L⁻¹; 8: mixed sludge; Fe(II)-oxone; TS 42.20 g L⁻¹; 9: mixed sludge; acid; TS 12.23 g L⁻¹; 10: mixed sludge; alkaline; TS 11.91 g L⁻¹; 11: mixed sludge; US-acid; TS 12.42 g L⁻¹; 12: mixed sludge; US-alkaline; TS 11.93 g L⁻¹; 13: mixed sludge; US and Fe(II)-oxone; TS 14.59 g L⁻¹; 14: mixed sludge; US and Fe(II)-oxone; TS 24.48 g L⁻¹; 15: raw secondary sludge; TS 14.64 g L⁻¹; 16: secondary sludge; thermal; TS 15.07 g L⁻¹; 17: secondary sludge; Fe(II)-oxone; TS 16.75 g L⁻¹; 18: secondary sludge; thermal and Fe(II)-oxone; TS 15.22 g L⁻¹; 19: raw anaerobic digested sludge; TS 16.92 g L⁻¹; 20: anaerobic digested sludge; Fe(II)-oxone; TS 15.19 g L⁻¹.

Fig.3. Correlation circles of measured parameters in EPS characteristics obtained by PCA (PC1 & PC2).