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# Effects of short-time aerobic digestion on extracellular polymeric substances and sludge features of waste activated sludge

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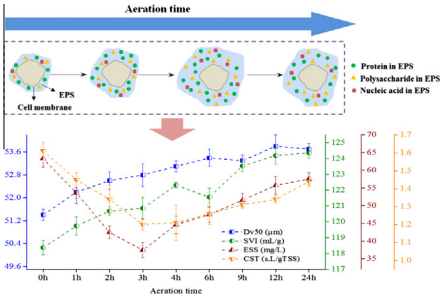
## HIGHLIGHTS

- Effects of short-time aerobic digestion on EPS and sludge features were ascertained.
- Both EPS concentration and sludge floc size increased with extending digestion time.
- Sludge settleability gradually became poor during the aerobic digestion process.
- Short-time aerobic digestion could promote sludge flocculability and dewaterability.
- Sludge features showed close relations to proteins and polysaccharides in EPS.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Aerobic digestion is an important stabilization process for waste activated sludge. However, extended aerobic digestion consumes substantial oxygen and increases the running cost (oxygen demand and sludge production) and $CO_2$ emission. To tackle these issues, this study investigated the effects of short-time aerobic digestion (STAD) on microbial extracellular polymeric substances (EPS), sludge features, and their correlations. The levels of proteins and polysaccharides in EPS consistently increased as the aerobic digestion time increased. Nucleic acid in EPS increased only within the first 4 h and then decreased. With an extended digestion time, sludge floc size (Dv50) increased by 4.67%, and the sludge volume index (SVI) increased by 5.06%, indicative of a deteriorating settleability. The sludge after STAD exhibited better flocculability and dewaterability than that after the prolonged aerobic digestion. Proteins and polysaccharides in the EPS significantly correlated with Dv50 ($R^2 = 0.97$, $P = 0.00$ and $R^2 = 0.92$, $P = 0.00$, respectively), SVI ($R^2 = 0.88$, $P = 0.00$ and $R^2 = 0.93$, $P = 0.00$, respectively), effluent suspended solids (ESS, $R^2 = 0.68$, $P = 0.01$ and $R^2 = 0.82$, $P = 0.00$, respectively) and the normalized capillary suction time (CST, $R^2 = 0.66$, $P = 0.01$ and $R^2 = 0.78$, $P = 0.00$, respectively). No significant correlation

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was found between nucleic acid and these sludge features. These results indicated that proteins and polysaccharides in EPS may govern sludge floc size, settleability, flocculability and dewaterability. Interestingly, good sludge flocculability and dewaterability were observed when the proteins and polysaccharides in EPS were lower than 21.55 and 12.27 mg/g VSS, respectively.


## 1. Introduction

Activated sludge process is a common biological process used in municipal and industrial wastewater treatment plants (WWTPs) [1]. One of the drawbacks of this technology is that a significant amount of waste activated sludge (WAS) is produced during the process. According to the China National Information Infrastructure (CNII), the yield of wet sludge (water content 80%) in urban sewage treatment plant reached 33.59 million tons per year, that is, 92 thousand tons per day [2]. WAS generally contains pathogenic organisms, toxic organic substances and heavy metals, and inorganic nutrients such as phosphate and ammonium, which threats the environment and public health [3]. Therefore, it is imperative to develop effective disposal processes for WAS to meet the stringent environmental regulations [4].

Anaerobic and aerobic digestion processes are the two main biological treatment methods for the stabilization and reduction of WAS [5]. Anaerobic digestion is commonly used in large-sized WWTPs to convert WAS to biogas (e.g., methane or hydrogen) or value-added products (e.g., organic acids) [6]. On the other hand, aerobic digestion is applied in medium-sized and small-sized WWTPs for economic considerations [7]. As a promising process for sludge treatment, aerobic digestion has been received extensive attention in recent years due to the short sludge retention time, fast degradation rate and efficient pathogen inactivation [8]. In aerobic digestion, organic matter is oxidized and products such as carbon dioxide, nitrate and phosphate are generated with release of heat [9]. In addition, aerobic digestion of activated sludge is known as a stabilization process in which biologically stable products are produced and both the sludge mass and volume are reduced. Up to the end of 2014, 1808 WWTPs were in operation in China and over 70% of them were medium- and small-size plants with the processing capacities of wastewater lower than 10 thousand tons per day [2]. Accordingly, aerobic digestion process could be a better choice for the treatment of WAS in WWTPs due to its economy and practicability.

Previous studies reported that aerobic digestion could significantly affect the WAS properties or features that influence settleability, flocculability, dewaterability and the downstream treatment efficiency. Murthy and Novak [10] found that aerobic digestion caused poor dewatering properties and increased biopolymer content. Murthy and Novak [11] also found that aerobic digestion reduced sludge dewaterability due to the increase of dissolved extracellular polymeric substances (EPS). Murthy and Novak [10] reported that the increase of dissolved EPS during aerobic digestion was mainly caused by the increase of proteins, similar to anaerobic digestion. Moreover, the increase of dissolved EPS could also be attributed to carbohydrates [9]. Thus, the dewaterability of aerobically digested sludge may depend on the interplay of dissolved EPS and carbohydrates, which remains elusive so far. Liu et al. [12] have employed the classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory to describe the *Rhodopseudomonas acidophila* flocculation, and they reported that the surface characteristics of *R. acidophila* could be changed and its flocculability might be improved by manipulating the content and component of bacterial EPS. Most of the studies focused on the effects of EPS on WAS properties in long-time aerobic digestion. A large amount of oxygen required by the long-time digestion will increase the cost of sludge treatment and disposal. Short-time aerobic digestion (STAD), which can markedly decrease the demand of oxygen, would be a more economically viable sludge treatment. However, it is difficult to apply the STAD process to the sludge treatment when few characteristics of the process are known.

To understand the characteristics of the STAD process for the sludge treatment, this study investigated the effects of STAD on EPS and WAS features, including flocs size, settleability, flocculability and dewaterability, and further analyzed the correlations between EPS and these sludge features.

## 2. Materials and methods

### 2.1. Sludge samples and aerobic digestion reactor

The WAS samples used in the aerobic digestion system were the secondary settling tank return sludge from a full-scale municipal WWTP in Shanghai, China. Anaerobic–anoxic–aerobic process was applied in the WWTP with a capacity of 60,000 $m^3$/d. After gravity concentration of the sludge, its main parameters were as following: pH 6.8–7.5, total suspended solids (TSS) 9.0 ± 1.0 g/L, and the ratio of volatile suspended solids to TSS (VSS/TSS) 65 ± 8%. Sludge samples were stored at 277.15 K and used within 2 d.

A 6.0-L (Φ12 cm × 53 cm) plastic cylindrical barrel was used as the aerobic digestion reactor and placed on an accurate strengthen electronic stirrer (JJ-1, Changzhou, China). The effective volume of the reactor was 5.0 L. The sludge in the reactor was well mixed at 300 rpm. The reactor was continuously aerated from the bottom through a microporous aeration disk to maintain dissolved oxygen (DO) of 2–3 mg/L. Schematic of the reactor can be found in Fig. 1. The experiments were carried out under room temperature

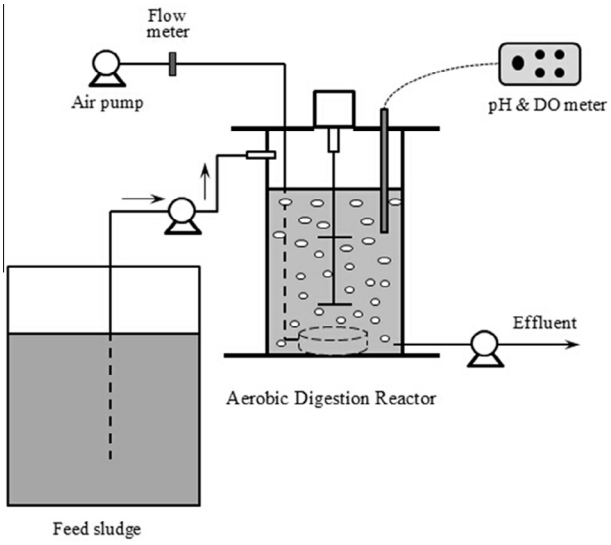


**Fig. 1.** Schematics of the aerobic digestion process.

(298.15 ± 2 K), and the pH of the sludge suspension was in the range of 6.8–7.5. Sludge samples collected from the reactor at different aerobic digestion times were subjected to immediate characterization without storage. TSS and VSS were measured according to Standard Methods [13]. All analyses were carried out in triplicate.

### 2.2. Extraction and analyses of EPS

Extraction of EPS was carried out with ultrasonication followed by centrifugation. The ultrasonic reactor was equipped with a transducer (20 kHz, diameter of 13 mm, model, company, address) according to a previous study [14]. During ultrasonic treatment (power density 2.7 kW/L, pulse 4 s), the tip of the transducer was immersed at about 10 mm deep into 100-ml sludge sample for 2 min, and the temperature was maintained at about 298.15 K. The treated sludge samples were then centrifuged twice at 10,800*g* and 277.15 K for 10 min each. The supernatant was collected for the EPS analysis.

Protein, polysaccharide and nucleic acid in the EPS were measured by a UV/VIS spectrophotometer (HACH, DR2800) following the colourimetric methods. Briefly, protein was measured using the Bradford method with bovine serum albumin as the standard [15]; polysaccharide was measure using the phenol–sulfuric acid method with glucose as the standard [15] and nucleic acid was measured using the diphenylamine colorimetric method with calf thymus deoxyribonucleic acid as the standard [15].

### 2.3. Flocs size analysis

The size distributions of the WAS flocs were determined by a Mastersizer 3000 Laser Diffraction Particle Size Analyzer (Malvern, UK) with a 300-mm lens, which enabled the measurement of particle size, ranging from 0.01 to 3500 μm. The sludge samples were filtered through a 0.45-μm black polycarbonate membrane filter (25 mm diameter, Schleicher & Schuell, German). The filtered sludge was continuously recycled with a peristaltic pump and exposed to a 4-mW He–Ne (wavelength 632.8 nm) and 10-mW LED (wavelength 470 nm) laser (MN300RB, Shenzhen Optlaser Technologies, China). Each sample was measured three times with a low standard deviation (⩽0.5%). The average size of the flocs was calculated based on the medium diameter (Dv 50).

### 2.4. Determining the sludge settleability, flocculability and dewaterability

The settleability of the sludge was evaluated by measuring the settleability volume index (SVI) [16]. A 100-ml graduated cylinder was filled with mixed liquor and the settling time was 30 min.

For the evaluation of flocculability, sludge suspension was slowly mixed in a beaker at a rotational speed of 50 rpm for 3 min on an electric mixer (DJ6CS, Shanghai Yiheng Scientific instrument Co., Ltd, China). After 30 min of sedimentation, the supernatant was collected to measure the concentration (g/L) of effluent suspended solids (ESS), which indicated the performance of the sludge flocculation and effluent clarification.

The dewaterability of the activated sludge was determined by measuring the capillary suction time (CST) with a CST instrument and a Whatman No. 17 chromatography grade paper as detailed elsewhere [13]. Mikkelsen and Keiding [9] reported that for a specific sludge, the CST was dependent on the concentration of the suspended solids. In the present study, the CST values were divided by the initial TSS for normalization and then expressed in the unit of s L/g-TSS. The tests were conducted in triplicate to achieve a standard deviation of 5% or less.

### 2.5. Statistical analysis

Statistical analyses were performed with SPSS software for Windows (SPSS, Chicago, Illinois). The Pearson's correlation coefficient, $R^2$, was used to estimate the linear correlation between two parameters. Quadratic regressions were also used to estimate the quadratic parabola correlation between two parameters. Correlations were considered statistically significance at a 95% confidence interval ($P < 0.05$).

## 3. Results and discussion

### 3.1. Effects of the STAD process on EPS of WAS

The effects of the STAD process on the EPS of the WAS are shown in Fig. 2. The concentrations of both proteins and polysaccharides in the EPS increased with the aerobic digestion time. After 9 h of aerobic digestion, the concentrations of proteins (23.9 mg/g VSS) and polysaccharides (14.6 mg/g VSS) reached peak values, and were 48.3% and 48.5% higher than those from the original WAS, respectively. Therefore, the STAD process promoted the excretion of proteins and polysaccharides, which was in good agreement with previous findings [17]. The nucleic acid concentration in the EPS increased within the first 4 h and then decreased. In the EPS, proteins were the predominant component with a proportion of 50.5–54.3%, followed by polysaccharides (29.0–33.3%). Nucleic acids accounted for a small proportion of the EPS (12.4–19.4%). The predominance of proteins in the EPS might be related with the presence of a large quantity of exoenzymes [18]. The degradation and uptake of readily biodegradable organic pollutants in the supernatant might lead to a high level of exoenzymes in EPS matrix [19].

The total EPS concentration increased from 31.47 to 44.32 mg/g VSS with extending aerobic digestion time from 0 h to 4 h, and then reached equilibrium. In the early stage of the STAD process, microorganisms grew using the organic pollutants in the supernatant, and thereby enhancing the excretion of EPS [20]. In the later stage, however, the amount of organic pollutants became limited and microorganisms consumed EPS through endogenous respiration [20]. Thus, a balance between excretion and consumption of EPS was achieved in the later stage of the STAD process.

### 3.2. Effects of the STAD process on sludge features of WAS

Table 1 shows that the flocs size increased when extending the aerobic digestion time. The initial flocs size was about 51.4 μm and

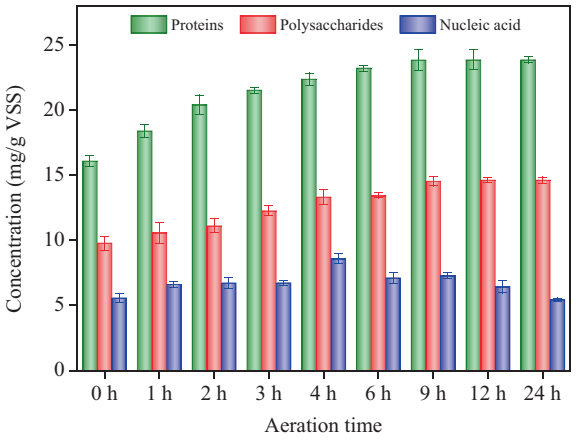


**Fig. 2.** Effects of the STAD process on EPS of WAS.

**Table 1**
Features of the sludge from the aerobic digestion reactor at different aerobic digestion times.

| Parameter | Unit | Activated sludge sample at different aerobic digestion time | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 h | 1 h | 2 h | 3 h | 4 h | 6 h | 9 h | 12 h | 24 h |
| Dv50 | μm | 51.4 ± 0.2 | 52.2 ± 0.4 | 52.6 ± 0.3 | 52.8 ± 0.5 | 53.1 ± 0.2 | 53.4 ± 0.3 | 53.5 ± 0.2 | 53.8 ± 0.5 | 53.7 ± 0.2 |
| SVI | ml/g | 118.38 ± 0.46 | 119.75 ± 0.58 | 120.68 ± 0.27 | 120.86 ± 0.68 | 122.32 ± 0.18 | 121.56 ± 0.56 | 123.54 ± 0.37 | 124.20 ± 0.56 | 124.37 ± 0.36 |
| ESS | mg/L | 63.35 ± 2.54 | 53.56 ± 1.46 | 42.46 ± 1.76 | 37.45 ± 2.17 | 44.65 ± 2.43 | 47.45 ± 2.12 | 51.56 ± 1.89 | 55.73 ± 2.47 | 57.56 ± 1.74 |
| CST | s L/g TSS | 1.61 ± 0.0.05 | 1.45 ± 0.04 | 1.34 ± 0.08 | 1.20 ± 0.03 | 1.21 ± 0.10 | 1.26 ± 0.06 | 1.31 ± 0.02 | 1.34 ± 0.02 | 1.44 ± 0.02 |

**Table 2**
Linear correlation between EPS and SVI.

| SVI = *m* EPS + *n* | | SVI (ml/g) | Units of EPS | $R^2$ | References |
|---|---|---|---|---|---|
| *m* | *n* | | | | |
| *Positive slope* | | | | | |
| +3.46 | +14.90 | 69–315 | mg glucose/L MLSS | 0.79 | [22] |
| +16.29 | +75.65 | 99–203 | g EPS/100 g SS | 0.45 | [23] |
| +57.60 | −27.34 | 65–400 | g EPS/100 g SS | 0.67 | [1] |
| *Negative slope* | | | | | |
| −0.95 | +50.4 | 30–50 | mg EPS/g VSS | 0.98 | [16] |
| −19.03 | +647.0 | 175–345 | g glucose/100 g SS | 0.76 | [24] |
| No clear correlation | | 60–85 | | | [25] |
| No clear correlation | | 182–189 | | | [26] |

slightly increased to 53.8 μm after 12 h of aerobic digestion. The increase of flocs size could be due to the growth and reproduction of microorganisms in the STAD reactor. According to a previous study on the change of flocs size during aerobic digestion [8], average diameter of the flocs decreased to half of its primary value (from 125 to 65 μm) during the first 4 d of the performance. The floc diameter then increased to 90–98 μm on day 6–8, and constantly reduced afterwards. The flocs size reached 56 μm on the 22nd day and 22 μm on the 40th day of aerobic digestion. In contrast, the present study showed relatively stable sludge flocs size in the STAD, possibly because the aerobic digestion process only lasted for 12 h and the content of nutrients from the sludge supernatant were very low.

During the aerobic digestion process, the SVI value showed a slight increase, and reached 124.37 ml/g after 24 h of aerobic digestion, which is congruent with the stable flocs size observed above as SVI varies with the floc size [8]. Accordingly, the settleability of the sludge in the STAD reactor did not change significantly during the STAD process. The ESS value decreased from 63.35 mg/L at 0 h (untreated WAS) to 37.45 mg/L at 3 h, indicating that the sludge flocculability was enhanced. Further extending the aerobic digestion time, however, reduced sludge flocculability as the ESS value increased to 57.56 mg/L after 24 h of treatment.

The sludge dewaterability was improved after aerobic digestion as the average normalized CST decreased from 1.61 s L/g TSS, to 1.20 s L/g TSS after 3 h of digestion. However, CST increased to 1.44 s L/g TSS after 24 h of digestion, which means STAD could better improve the sludge dewaterability as opposed to the long time aerobic digestion. Some previous studies also reported poorer dewatering properties with aerobic digestion [10,21], because of the increase of dissolved EPS (proteins or carbohydrates) in sludge [10,21].

### 3.3. Correlations between EPS and sludge features in the STAD system

#### 3.3.1. Person correlations between EPS and flocs size

Fig. 3(a)–(d) compares the Pearson correlations between the proteins, polysaccharides, nucleic acid and total EPS of the aerobic digested sludge and the sludge floc size. The floc size correlated well with the concentration of protein ($R^2$ = 0.97, $P$ = 0.00), polysaccharide ($R^2$ = 0.95, $P$ = 0.00), and total EPS ($R^2$ = 0.92, $P$ = 0.00). There was no significant correlation between floc size and the concentration of nucleic acid ($R^2$ = 0.02, $P$ = 0.72). Therefore, proteins and polysaccharides in EPS may play the dominant roles in the sludge floc size as well as other properties such as settleability.

During the aerobic digestion process, the increased concentrations of proteins and polysaccharides led to increased thickness of EPS layer and thus increased sludge floc size [21]. However, although the DNA concentration increased with the increase of sludge biomass, the floc size did not increase as the DNA concentration increased.

#### 3.3.2. Person correlations between EPS and sludge settleability

The Pearson correlations between the proteins, polysaccharides, nucleic acid and the total EPS of the aerobic digested sludge and the sludge settleability were shown in Fig. 4. SVI correlated well with proteins ($R^2$ = 0.88, $P$ = 0.00), polysaccharides ($R^2$ = 0.93, $P$ = 0.00), and the total EPS ($R^2$ = 0.71, $P$ = 0.00). Similar to floc size, there was no significant correlation between SVI and nucleic acid ($R^2$ = 0.02, $P$ = 0.72). Results showed that proteins and polysaccharides contents in EPS would have more significant effects on the settleability of activated sludge. Some studies showed that sludge settled faster with lower EPS [1,16,22–26], whereas some others reported the opposite as shown in Table 2. Poxon and Darby [27] mentioned that EPS contained high proportion of bound water, and thus increased EPS might lead to more bound water into the aggregates, producing highly porous sludge flocs with a lower density, which results in poor sludge settleability. In addition, the increasing EPS concentration enhanced the cell surface charge due to the negative charge of EPS, which caused the increase of repulsive forces among cells and the decrease of activated sludge settleability [5].

#### 3.3.3. Person correlations between EPS and sludge flocculability

Due to the complex nature of the microbial communities, sludge does not always flocculate efficiently [12]. As a result, the suspended solid level may increase in the effluent and, in many cases, exceed the discharge requirements [28]. The correlations

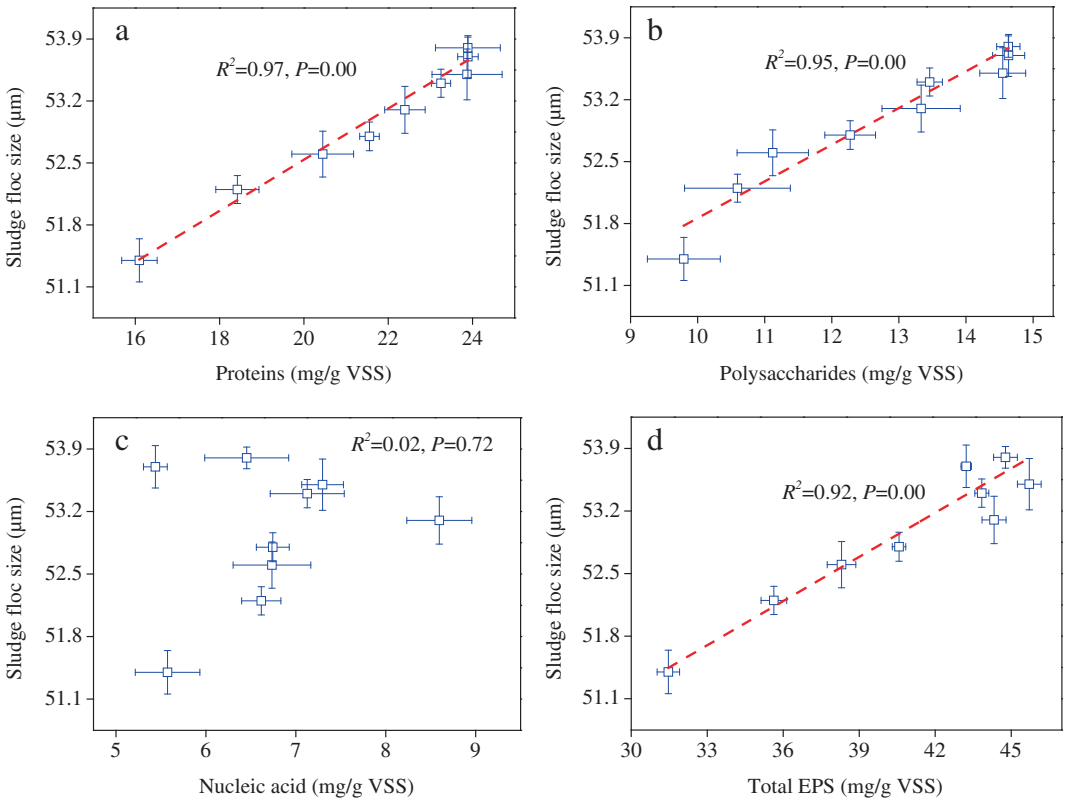


**Fig. 3.** Pearson correlations between (a) proteins, (b) polysaccharides, (c) nucleic acid, (d) total EPS and sludge floc size.

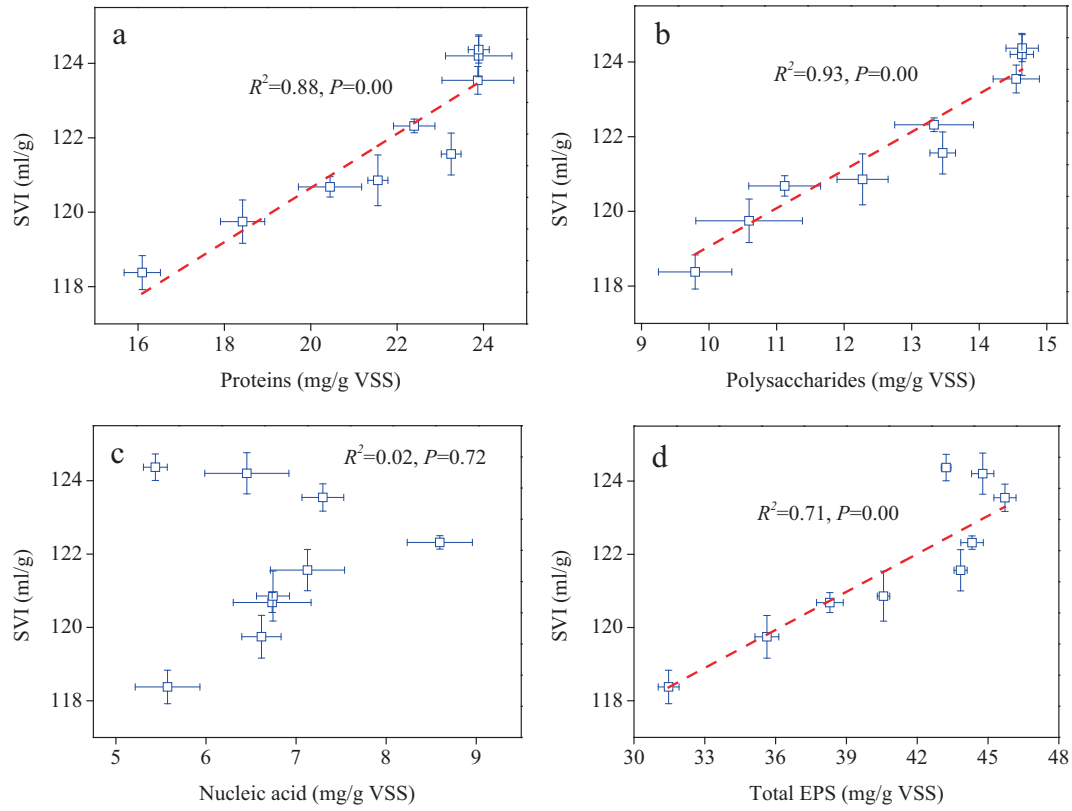


**Fig. 4.** Pearson correlations between (a) proteins, (b) polysaccharides, (c) nucleic acid, (d) total EPS and sludge settleability evaluated by the SVI.

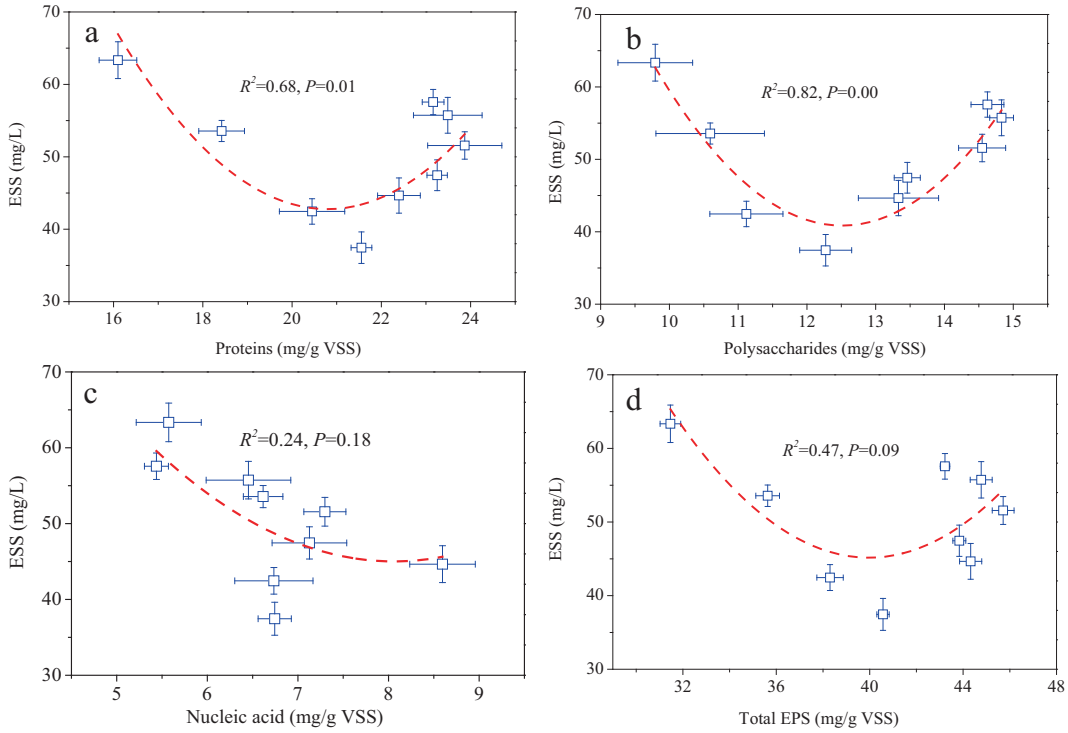


**Fig. 5.** Pearson correlations between (a) proteins, (b) polysaccharides, (c) nucleic acid, (d) total EPS and sludge flocculability evaluated by the ESS. The red dotted lines are quadratic fitting curves. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

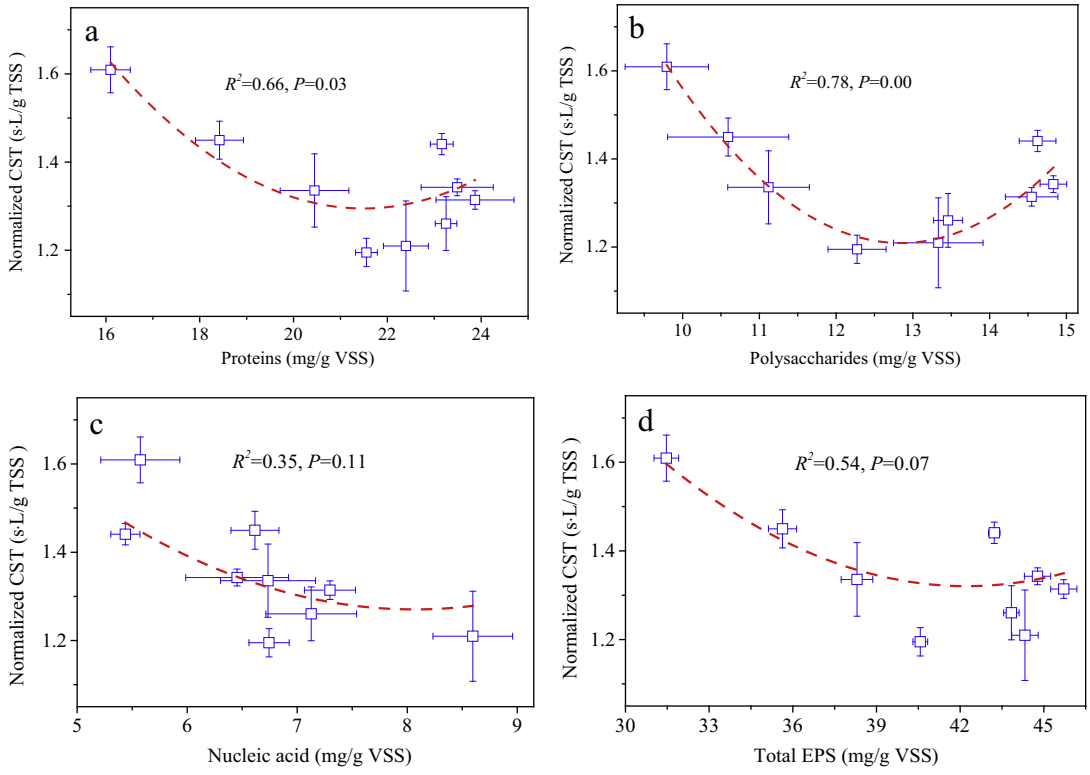


**Fig. 6.** Pearson correlations between (a) proteins, (b) polysaccharides, (c) nucleic acid, (d) total EPS and sludge dewaterability as evaluated by the Normalized CST. The red dotted lines are quadratic fitting curves. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between EPS and sludge flocculability after STAD can be analyzed with the correlations between EPS features and ESS shown in Fig. 5. ESS correlated well with the concentration of protein ($R^2$ = 0.68, $P$ = 0.01) and polysaccharide ($R^2$ = 0.82, $P$ = 0.00), but not with that of nucleic acid ($R^2$ = 0.24, $P$ = 0.18) and total EPS ($R^2$ = 0.47, $P$ = 0.09). It is worth noting that good sludge flocculation

with adequate effluent clarification was observed when the concentrations of protein and polysaccharide are lower than 21.55 and 12.27 mg/g VSS, respectively. Beyond these concentrations, the effluent quality degenerated and the ESS increased. These results support the finding that the presence of a large amount of EPS exerts negative effects on sludge flocculation [12,28,29]. The excessive EPS can deteriorate the attachment between cells and weaken the aggregated structure, which can result in greater cell detachment from the sludge flocs in a turbulent environment and thus cause worse effluent quality. Most of the previous studies didn't notice the better sludge flocculability with the increasing of EPS concentration. According to the DLVO theories, the total energy of adhesion is the result of the van der Waals attractive forces and the generally repulsive interactions, and the cells could aggregate when the total energy barrier in the DLVO curves could be overcame [12]. Under low EPS concentration, the increasing of EPS content is beneficial for the cell to overcome the total energy barrier, resulting in better sludge flocculability [12].

*3.3.4. Person correlations between EPS and sludge dewaterability*

EPS is considered to be a significant factor affecting sludge dewaterability [28]. Fig. 6(a)–(d) depict the correlations between the proteins, polysaccharides, nucleic acid and total EPS of the aerobic digested sludge and the sludge dewaterability (normalized CST). The normalized CST again showed good correlations with the concentration of protein ($R^2 = 0.66$, $P = 0.03$) and polysaccharide ($R^2 = 0.78$, $P = 0.00$), but not with that of nucleic acid ($R^2 = 0.35$, $P = 0.11$) and with total EPS ($R^2 = 0.54$, $P = 0.07$). The flocculability increased with the concentration of proteins and polysaccharides when they were lower than 21.55 and 12.27 mg/g VSS, respectively, and started to increase at higher concentration. Houghton et al. [30] found that the dewaterability of activated sludge increased with the concentration of EPS until reaching the maximum level of about 35 mg/g SS, beyond which the dewaterability decreased. It was concluded that the increase of dewaterability with EPS at low concentration was due to the enhancement of flocculation. Flocculation resulted in increasing flocs size, thereby improving the sludge dewaterability. On the other hand, EPS above 35 mg/g SS would increase the amount of surface water bound by EPS, and thus would lower the sludge dewaterability.

## 4. Conclusions

In this study, the effects of STAD on EPS and sludge features of WAS were studied, and the correlations between EPS and sludge features were discussed. STAD could promote the production of EPS. Proteins and polysaccharides in EPS have more significant effects on the settleability, flocculability and dewaterability of activated sludge. Under the low concentration of EPS, the increasing of EPS content performed better sludge flocculability and dewaterability. These results promote our understanding of the STAD characteristics, and provide some theoretical evidences for the STAD application to the sludge treatment.

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