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The influences of shear stress on Extracellular Polymeric Substances of activated sludge

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ABSTRACT

Shear stress is one of the key factors in aggregation and detachment of microbial flocs, which will influence the production of Extracellular Polymeric Substances (EPS) as well. In this study, the components and composition of activated sludge EPS, including loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), were analyzed under different shear stresses. To perform this, sequencing batch reactors were set up separately with various shear stresses (represented by superficial upflow air velocity). The results show that shear stress significantly affects EPS production in the SBRs. When the superficial upflow air velocity increased from 2.3 to 9.1 m/h, the contents of total EPS, TB-EPS, and LB-EPS first increased and then decreased. The highest total EPS (70.94 mg COD/gVSS) and LB-EPS (27.96 mg COD/gVSS) contents were obtained under superficial upflow air velocity of 5.7 m/h, while the maximum TB-EPS (45.03 mg COD/gVSS) content was attained when superficial upflow air velocity rose up to 6.8 m/h. The contents of polysaccharides, proteins and humic substances in EPS showed similar trends with an increase in the shear stress. Compared with the variation of protein and humic substance, the variation of polysaccharide was extremely dramatic in this study, especially in LB-EPS. It was also found the contents of EPS (TB-EPS, LB-EPS and total EPS) have a significant linear correlation with the sludge volume index (SVI). The highest correlation coefficient ($R^2 = 0.9522$) showed the closest correlation between LB-EPS contents and SVI. These findings indicate that LB-EPS and polysaccharides rather than TB-EPS and proteins play important roles for the settling properties of activated sludge under various shear stresses.

Keywords: Shear stress; EPS; LB-EPS; TB-EPS; SVI

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1. Introduction

Extracellular Polymeric Substances (EPS) are biosynthetic polymers which are secreted by microbial cells into their environment [1]. Generally, they are found outside the cell surface and in the intercellular space of microbial aggregates. Polysaccharides (PN), proteins (PS), and humic substances (HS) are regarded as the main components of EPS [1,2]. As a complex mixture of macromolecules, EPS among dispersed cells can decrease the repulsive force, increase the proximity, form the bridge, and eventually contribute to adhesion and aggregation [3]. Because of the important role of EPS in cell adhesion, cell aggregation, and microbial aggregates formation, EPS has attracted much attention in recent years.

Hydrodynamic shear stress drives fluid motion and flocs aggregation in bioreactor. As the main energy consumption in biological water treatment processes, hydrodynamic shear stress can affect the production of EPS in microbial aggregates significantly. It was widely reported that high hydrodynamic shear stress in bioreactor led to high amount of EPS [4–6]. Shin et al. [7] found that high shear stress from airflow rates caused high ratio of carbohydrate to protein in the EPS. When the shear stress rose, the content of carbohydrate increased while protein was almost constant. Similar results were shown in aerobic granules reactors [8,9]. In some cases, the increase of EPS was mainly attributed to the stimulation of shear stress on extracellular protein secretion [10,11]. However, excessive shear stress can cause the reduction of EPS. Menniti et al. [12] reported that the EPS contents in a lower shear environment were greater than that in higher shear environment in membrane bioreactor. Celmer et al. [13] demonstrated that increasing of shear stress reduced the thickness of biofilm and caused the decline of EPS in membrane biofilm reactor.

The structure of EPS can be illustrated as two layer model [14]. The inner layer is made up of tightly bound EPS (TB-EPS), and the outer layer consists of loosely bound EPS (LB-EPS). TB-EPS can affect the attachment and aggregation of flocs, which is closely related to the granulation of biological flocs [15,16], while the LB-EPS has strong effect on sludge flocculation and sedimentation [17–20]. Although some researchers have reported the effects of shear stress on EPS in biological water treatment, few results revealed the relationship between shear stress and different types of EPS (TB-EPS and LB-EPS). Therefore, the study aims at (1) evaluating the influences of shear stress on the compositions of TB-EPS and LB-EPS, respectively, and (2) illustrating the relationships between EPS and flocs settling. It is hoped that the results will be useful for optimizing the hydrodynamic conditions in bioreactors.

2. Material and methods

2.1. Reactor set-up and operation

Six identical sequencing batch reactors (SBRs) made of clear acrylic plastic were used in this study. Each reactor (inner diameter 15 cm, height 30 cm) has a working volume of 4 L. SBRs were operated in cycles of 8 h each, which consisted of six stages: filling (10 min), anoxic stage (120 min), aerobic stage (240 min), settle (60 min), withdraw (15 min), and idle (35 min). The same seed sludge and synthetic wastewater was added to the reactors. A magnetic stirrer was used to mix the bioflocs and liquor in anoxic stage. Aeration in aerobic stage was supplied by an air sparger at the bottom of reactor. Shear stress in terms of superficial upflow air velocity was controlled at 2.3, 4.5, 5.7, 6.8, 7.9, and 9.1 m/h by airflow meter. The mixed liquor suspended solid (MLSS) in each SBR was controlled at about 3,000 mg/L. The pH of SBRs was maintained at 7.0 ± 0.5 by adding phosphate buffer. The experiments were conducted at 20 ± 1℃ using a thermostatic bath.

The seed sludge was taken from the secondary clarifier in a local municipal wastewater treatment plant in Jiangning economic development zone, Nanjing City, China. Synthetic wastewater was used in this experiment. The composition of the influent was as follows: sodium acetate 383 mg/L, NH_4Cl 120 mg/L, KH_2PO_4 24 mg/L, $MgSO_4{\cdot}7H_2O$ 10 mg/L, $CaCl_2{\cdot}2H_2O$ 15 mg/L, and trace element solution 0.5 mL/L. The composition of trace element in the feed was: H_3BO_3 2 mg/L, $FeCl_2{\cdot}4H_2O$ 2 mg/L, EDTA 2 mg/L, $ZnCl_2{\cdot}4H_2O$ 0.4 mg/L, $MnCl_2{\cdot}4H_2O$ 0.8 mg/L, $CuCl_2{\cdot}2H_2O$ 0.2 mg/L, $(NH_4)_6MO_7{\cdot}4H_2O$ 1.1 mg/L, $NiCl_2{\cdot}6H_2O$ 1 mg/L.

The reactors were operated over 2 months. During the experimental period, COD and NH_4^+-N concentrations in influent and effluent were measured every day. When the substrates removal efficiencies of COD and NH_4^+-N were stable at higher than 80%, the start-up period (before day 40) was finished. During the end of start-up period, the properties of activated sludges changed little before it came to the steady state. In the steady-state period, activated sludge in the reactors was regularly sampled for investigating the composition and contents of EPS, while the settleability of activated sludge was evaluated by sludge volume index (SVI).

2.2. Analytical methods

Measurements of COD, NH_4^+-N, MLSS, and SVI were determined according to the standard methods [21]. Dissolved oxygen (DO) was measured with a dissolved oxygen meter (HACH-HQ30d, Japan).

Tightly bound EPS (TB-EPS) and loosely bound EPS (LB-EPS) were used to describe EPS fractions. Thus, a chemical extraction method by Liang et al. [22] was introduced. A 10-mL suspension (with approximately 1.0 g sludge) was pretreated using ultrasonic at 20 kHz and 40 W for 30 s. After centrifuging the sample at 4℃ and 2,000*g* for 15 min, the bottom sediments were collected and then resuspended to their original volume using ultra pure water, adding into 0.06 mL formamide (37%) at 4℃. Afterwards, the extraction was carried out in an orbital incubator (20–30 rpm) for 1 h to get the suspension which was then centrifuged (5,000*g*, 15 min, 4℃). The supernatant was collected that to be used for chemical composition analysis of LB-EPS. For the extraction of TB-EPS, the remaining sediment was resuspended into 10-mL container with an extraction buffer (2 mM $Na_2PO_4{\cdot}12H_2O$, 4 mM $NaH_2PO_4{\cdot}H_2O$, 9 mM NaCl, 1 mM KCl, pH 7). 70 g/gVSS of gel CER was added into the suspension at 4℃. The extraction was carried out in an orbital incubator (600 rpm). After 1 h, the suspension was centrifuged (5,000*g*, 15 min, 4℃), and the supernatant was collected to analyze chemical composition of TB-EPS.

As the major components of the EPS matrix in the activated sludge system, polysaccharides, proteins, and humic acid were analyzed in this study as well. The sum of the TB-EPS and LB-EPS was regarded as the total EPS. Polysaccharide was qualified by the Anthrone method [23], while protein and humic acid were determined by the modified Lowry method [24].

3. Results and discussions

3.1. Reactors performance

The substrate (COD and NH_4^+-N) removal was shown in Fig. 1. From the 1st day to the 40th day, substrates of COD and NH_4^+-N were gradually removed. After 40 d of operation, the effluent concentrations kept almost stable on the whole. Substrate removal efficiencies of more than 80% (COD and NH_4^+-N) were observed in all of the reactors. The SVI in reactors were measured as well. During steady-state period (from 41th to 71th day), the settling properties also reached stability represented by constant values of SVI.

3.2. The contents of EPS under different shear stress

Fig. 2 exhibits the contents of TB-EPS, LB-EPS, and total EPS at different shear stress. As shown in Fig. 2, increase in shear stress stimulated EPS production under low shear stress (superficial upflow air velocity ≤ 5.7 m/h). The content of TB-EPS and LB-EPS was 41.07 and 22.69 mg COD/gVSS when the superficial upflow air velocity was 2.3 m/h. When the superficial upflow air velocity became higher to 4.5 m/h, the TB-EPS and LB-EPS also increased (41.67 and 25.05 mg COD/gVSS, respectively). The highest concentration of total EPS (70.94 mg COD/gVSS) and LB-EPS (27.96 mg COD/gVSS) were both obtained under superficial upflow air velocity of 5.7 m/h. Similar results on total EPS production under various shear stresses were reported in aerobic flocs [10], aerobic granules [8], and aerobic biofilm [6]. However, high shear stress (superficial upflow air velocity > 5.7 m/h) turned out to inhibit EPS production. When the superficial upflow air velocity continued to increase higher than 6.8 m/h, the content of TB-EPS, LB-EPS, and total EPS all experienced different levels of decreases. The trends of total EPS response to different shear stresses were in agreement with other observations [11–13]. It should be noted that the highest concentration of TB-EPS (45.03 mg COD/gVSS) appeared later than that of LB-EPS, which might reveal that LB-EPS is more sensitive to shear stress than TB-EPS. It was also observed that as the main fraction of total EPS, TB-EPS did not change that significantly as LB-EPS under various shear stresses. Similar results were reported at different influent conditions [25,26] and operating conditions [27].

EPS are originated from the metabolism or lysis of microorganism [27]. The increasing secretion of EPS is believed to be an important self-protection strategy of microbial cell against unfavorable external conditions. Therefore, limited increases of shear stress enhance the EPS production. Excessive shear stress inhibits the production of EPS, which was probably attributed to the persistent disruption in the microbial community. The variation of LB-EPS contents appeared to be much higher than that of TB-EPS in this study. This may be related to the dynamic structure and spatial distribution of EPS [1]. TB-EPS located in the inner layer is bound tightly to the cell surface, while LB-EPS in the outer layer is more easy to diffuse from the TB-EPS dynamically. Therefore, under the impact of shear stress, the EPS in the outer layer will suffer more than the EPS in the inner layer, presenting more variation.

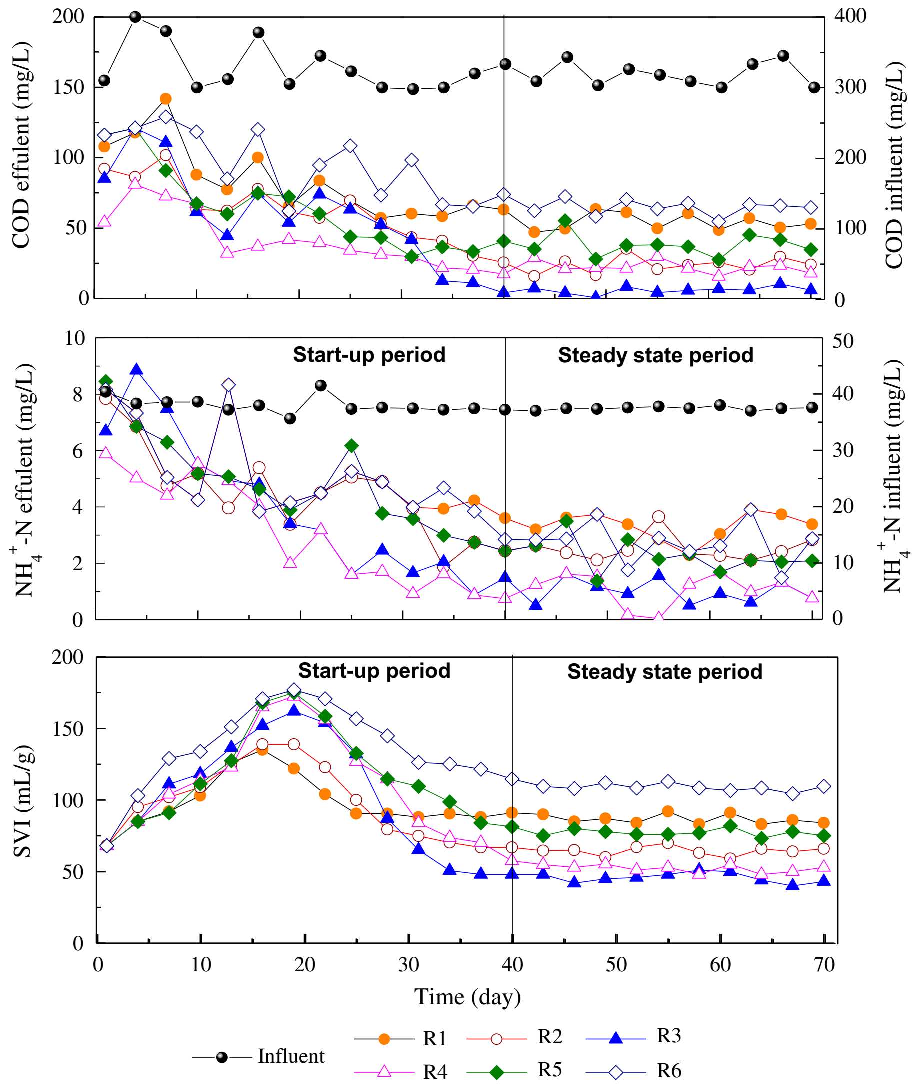

Fig. 1. Performance of rectors under different shear rates.

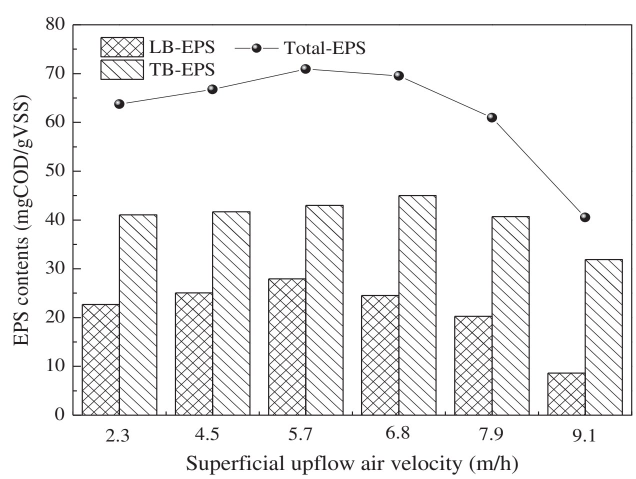

Fig. 2. The contents of EPS under different shear stress.

3.3. The compositions of EPS under different shear stress

The main components of EPS are polysaccharides, proteins, and HS [1,2]. The compositions of total EPS, LB-EPS, and TB-EPS under different shear stress are shown to be different in Fig. 3. It can be seen from Fig. 3(a), proteins make the largest proportion in total EPS, while HS the smallest. Similar results were reported by Fang et al. [28] and Wu et al. [29]. Furthermore, shear stresses have insignificant effects on proteins and HS under low shear stress. For the total EPS, when the shear stress (by superficial upflow air velocity) increased from 2.3 to 6.8 m/h, the contents of proteins changed from 27.30 to 28.73 mg COD/gVSS, meanwhile the contents of humus varied from 14.61 to 15.14 mg COD/gVSS. Further increasing of shear stress brought dropping to the contents of proteins to 25.90 mg COD/gVSS (under the

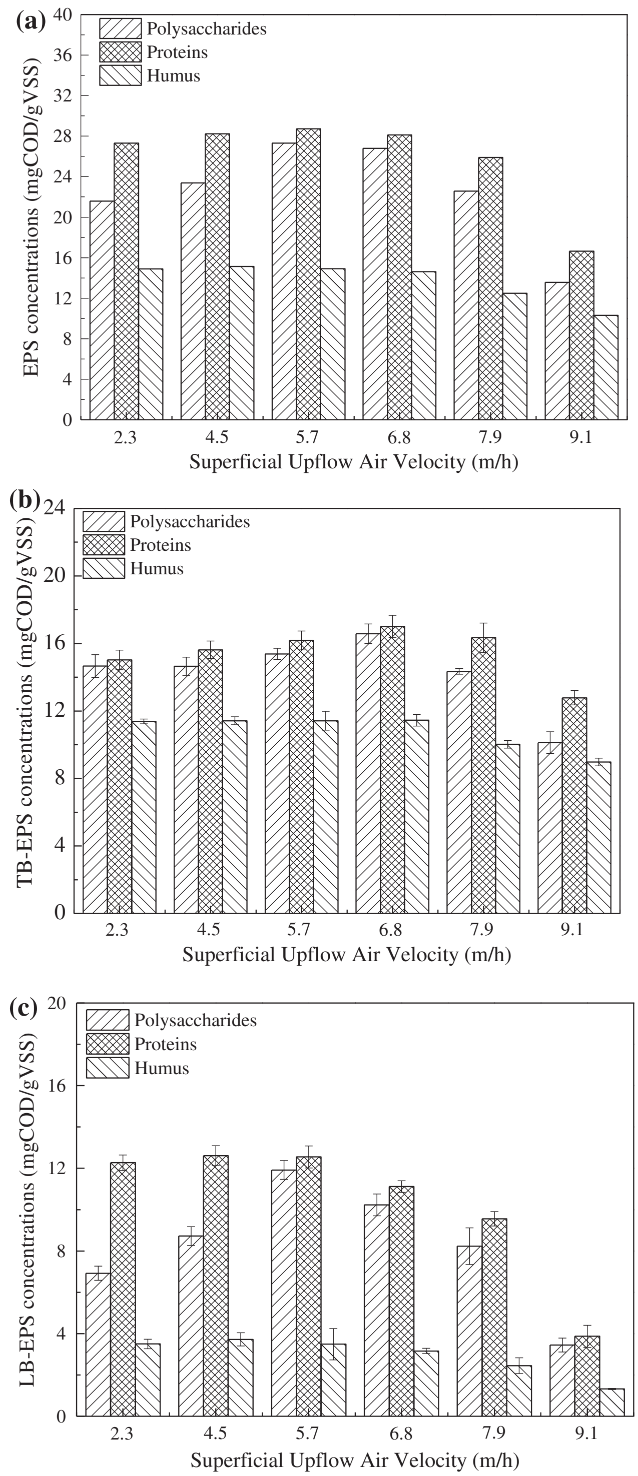

Fig. 3. The compositions of EPS under different shear stress: (a) Total EPS, (b) TB-EPS, and (c) LB-EPS.

superficial upflow air velocity of 7.9 m/h) and 16.65 mg COD/gVSS (under the superficial upflow air velocity of 9.1 m/h). In contrast with proteins and humus, the polysaccharides were significantly affected by shear stress. When superficial upflow air velocity lowed than 5.7 m/h, much more polysaccharide was obtained with the increase of shear stress. After that, the contents of polysaccharide dropped dramatically from 27.3 to 13.57 mg COD/gVSS (with superficial upflow air velocity increasing from 5.7 to 9.1 m/h). The relationships between shear stress and main compositions of total EPS (polysaccharides and proteins) were consistent with the study of Qin et al. [9] and Wang et al. [6] under low shear stress, and in agreement with the study of Celmer et al. [13] and Menniti et al. [12] under high shear stress.

The contents of polysaccharides, proteins, and HS in TB-EPS and LB-EPS were measured for further analysis (Fig. 3(b) and (c)). As illustrated in Fig. 3(b) and (c), the variation trends of TB-EPS and LB-EPS compositions at different shear stress resemble total EPS compositions. The highest content of polysaccharides in TB-EPS (16.57 mg COD/gVSS) and that in LB-EPS (11.92 mg COD/gVSS) were found under superficial upflow air velocity of 6.8 and 5.7 m/h, respectively. The variations of polysaccharides were also extremely higher than that of proteins and HS under different shear stress especially in LB-EPS. These results not only indicated that polysaccharides were more sensitive to shear stress than proteins in EPS, but also proved polysaccharides in LB-EPS were the major changing fraction of total EPS under different shear stress.

Increases of hydrodynamic shear stress can reduce mass transfer resistance between the bulk liquid and bioflocs, which can stimulate the microbial respiration [4,5,30]. Therefore, the components of EPS were lifted with the increasing of shear stress. However, excessive shear stress can detach the microbial aggregates and decrease the bacterial activity [29,31]. Thus the decline of polysaccharides, proteins, and HS were observed under high shear stress. The changing of polysaccharides was higher than that of proteins and HS. This finding is mainly attributed to the spatial distribution of EPS components. It was reported most of the proteins were found in the inner layer of microbial aggregates, while the polysaccharides were present in the outer layer [29,32,33]. The enhanced production of extracellular polysaccharides can be regarded as a prompt response to a variation of environments. As a result, polysaccharides in the outer layer are supposed to give much more feedback than proteins in the inner layer.

3.4. Correlations between EPS and settleability of activated sludge

Fig. 4 illustrates the average SVI of activated sludge in SBRs during steady-state period. It was

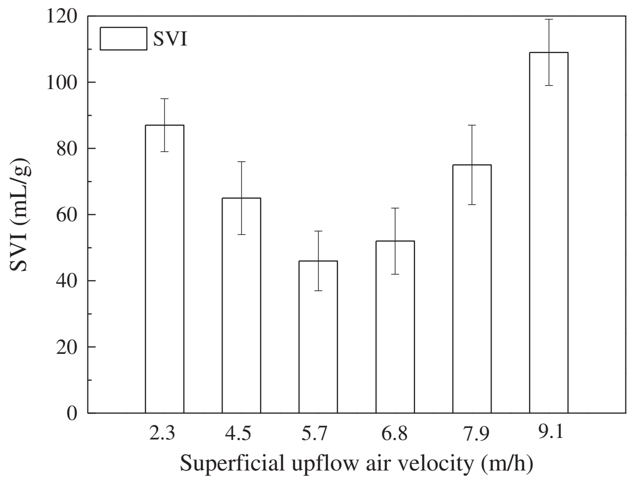

Fig. 4. The SVI of activated sludge under different shear stresses.

shown that the SVI first decreased and then increased with the increasing of shear stress. The roles of EPS in flocs settleability have been noticed by many researchers. However, most of these works focused on relationship between EPS and SVI in aggregates formation period. Only a few publications referred to the relationships of EPS and SVI in steady state period of activated sludge system. Therefore, the SVI and EPS contents were measured under different shear force in steady state. Values of SVI and EPS contents during every steady state were measured three times each and averaged to study the relationships between SVI and EPS (Fig. 5). The contents of TB-EPS, LB-EPS, and total EPS had significant linear correlations with SVI,

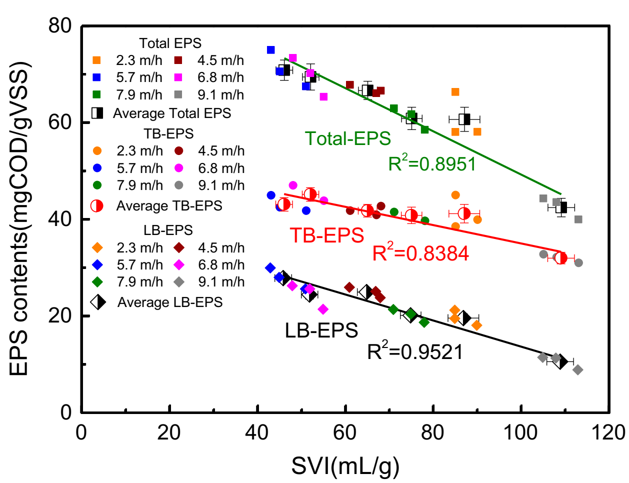

Fig. 5. The relationships between EPS and SVI of activated sludge during steady state period.

respectively. One possible explanation for this phenomenon could be the function of EPS for cell attachment.

As a mixture of polymers, EPS can closely bind with cells through complex interactions, which will enhance and promote the aggregation of bioflocs [1]. Therefore, the sludge settleability improved with the increase of EPS contents. These results differed from that in previous studies. Jin et al. [34] reported that total EPS had a negative effect on sludge settleability in a full scale wastewater treatment plant. Li and Yang [17] revealed there was positively correlation between SVI and LB-EPS content, whereas no correlation could be exhibited between SVI and the TB-EPS content. Ye et al. [35] and Wang et al. [20] not only illustrated the negative linear correlation between SVI and the LB-EPS content, but also exhibited closer linear correlation between SVI and the TB-EPS content. The contradictory results may be caused by different extracted method of TB-EPS, LB-EPS, and total EPS. Based on the comparison of correlation coefficient (R^2), LB-EPS showed the closest correlation ($R^2 = 0.9522$). This finding indicated that LB-EPS probably is more important than TB-EPS as for the settleability of activated sludge.

4. Conclusions

Shear stress, valued by superficial upflow air velocity in this study, has significant effect on EPS production in the anoxic–aerobic SBRs. When the superficial upflow air velocity increased from 2.3 to 9.1 m/h, more EPS, LB-EPS, and TB-EPS were first gained but after a peak, the contents declined. The highest contents of total EPS (70.94 mg COD/gVSS) and LB-EPS (27.96 mg COD/gVSS) were obtained under superficial upflow air velocity of 5.7 m/h, while the maximum content of TB-EPS (45.03 mg COD/gVSS) was attained under superficial upflow air velocity of 6.8 m/h. LB-EPS is more sensitive to shear stress than TB-EPS. The contents of polysaccharides, proteins, and HS in EPS appear similar trends, but the variations of polysaccharides were extremely higher than that of proteins and HS under different shear stress, especially in LB-EPS. Significant linear correlations were found between the contents of EPS (TB-EPS, LB-EPS, and total EPS) and settleability of activated sludge. The highest correlation coefficient ($R^2 = 0.9522$) was obtained between LB-EPS contents and SVI. These results above indicated that LB-EPS and polysaccharides rather than TB-EPS and proteins play important roles in the settling properties of activated sludge under the various shear stresses.

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