

Characteristics of the extracellular products of pure oxygen aerated activated sludge in batch mode

Jia-Ying He, Hong-Ling Zhang, Hong Wang, Ya-Qi Hu and Yong Zhang

ABSTRACT

The effects of pure oxygen aeration on compositional characteristics of soluble microbial products (SMP) and extracellular polymeric substances (EPS) of the activated sludge acclimated in a sequential batch reactor (SBR) were explored in batch mode. The structure of the extracellular products would change with different aeration methods or aeration rates. The proportion of SMP of most oxygen aerated sludge was less than 10%, while that in air aerated sludge was as high as 30–40%.

The proportion of TB-EPS decreased from 56.95% to 30.63%, and the proportion of LB-EPS increased obviously with the increase of oxygen aeration rate. The contents of the protein (PN) and the polysaccharide (PS) of extracellular products with oxygen aeration were significantly different, and the PN was much higher than PS ($p < 0.05$). The zeta potential of each component in activated sludge was negative, gradually decreasing with the progress of biological treatment. The fluorescence peaks in SMP, LB-EPS and TB-EPS with pure oxygen aeration were attributed to tryptophan PN-like and humic acid-like fractions. The results showed that the proportion of the components in the extracellular products could be regulated by adjusting the aeration rate and aeration mode, so as to optimize the treatment process of activated sludge.

Key words | activated sludge, aeration rate, extracellular polymeric substances, pure oxygen aeration, soluble microbial products

HIGHLIGHTS

- SMP with pure oxygen aerated effluent was lower than that of air aeration.
- The proportion of TB-EPS was higher under low aeration rate with pure oxygen aeration.
- PN was much higher than PS with pure oxygen aeration.
- Zeta potential was related to both the total amount of extracellular products and the PN content.

INTRODUCTION

Activated sludge is the most widely used biological process for wastewater treatment around the world (Lin *et al.* 2020). Oxygen supply is the key factor to ensure the normal bacteria metabolism of aerobic activated sludge (Holenda *et al.* 2008). Air aeration is the most commonly used oxygen

supplementation method for activated sludge treatment processes. However, the low oxygen content of the air inevitably reduces the efficiency of the aeration system. The pure oxygen aeration replaces air with oxygen in the aeration system to maintain high DO concentration in the aerobic system, capable of efficiently removing organic pollutants in wastewater (Skouteris *et al.* 2020). Since it was put into commercial use in 1970, it has successfully treated dozens of industrial wastewaters (Zhang *et al.* 2019).

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Jia-Ying He[†]

Hong Wang

Ya-Qi Hu

Yong Zhang (corresponding author)

School of the Environment,

Nanjing Normal University,

Nanjing 210023,

China

E-mail: zy@njnu.edu.cn

Hong-Ling Zhang[†]

Nanjing Institute of Environmental Science,

MEP,

Nanjing 210000,

China

Yong Zhang

Key Laboratory of Virtual Geographic Environment

(Nanjing Normal University), Ministry of

Education,

Nanjing 210023,

China;

State Key Laboratory Cultivation Base of

Geographical Environment Evolution (Jiangsu

Province),

Nanjing 210023,

China

and

Jiangsu Center for Collaborative Innovation in

Geographical Information Resource

Development and Application,

Nanjing 210023,

China

[†]These two authors contributed equally to this paper.

Soluble microbial products (SMP) and extracellular polymeric substances (EPS) are two important secondary metabolites of activated sludge in the process of biodegradation (Kunacheva & Stuckey 2014). Research in SMP is ongoing. Although there is no accurate and unified definition of SMP, the theory that SMP is an organic compound pool released to the environment through substrate metabolism and biomass degradation has been widely accepted (Soh *et al.* 2020). In general, SMP is slowly biodegradable because most of its components are proteins (PN), polysaccharides (PS), amino acids and other high molecular weight substances. SMP is the main component of dissolved COD or TOC in the effluent of biological treatment, which has an important impact on the discharge standard of sewage treatment (Soh *et al.* 2020). Notably, the possibility of SMP degradation under aerobic conditions in biological systems has been reported in recent years. In aerobic biological systems, SMPs produced by autotrophic microorganisms can be consumed by heterogeneous microorganisms as carbon sources (Merkey *et al.* 2009), and the utilization associated products (UAP) produced by heterotrophic organisms can also be reused by themselves (Ni *et al.* 2009).

EPS is a complex heterogeneous polymer with high molecular mass that is secreted outside the cell under certain environmental conditions (Silva *et al.* 2012). It has significant effects on the physical and chemical properties of sludge, such as flocculation, surface charge of sludge, settling performance and dehydration (Liu *et al.* 2010; Wang *et al.* 2013). EPS has a dynamic double-layer structure, which consists of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) to protect cells and create a stable biological environment for microorganisms (Gao *et al.* 2020). The contributions of LB-EPS and TB-EPS to sludge aggregation are different. Sludge cells in the outer region of flocs are entangled by the weak interaction of LB-EPS, but excessive LB-EPS can disrupt cell adhesion and weaken the structure of flocs, resulting in poor separation between sludge and water. TB-EPS is responsible for cell adhesion and adhesion in internal flocculent structure through strong interaction (Liu *et al.* 2010).

SMP and EPS are composed of similar main components, including PS, PN, humic acid, etc. PN and PS, as the main components of extracellular products, play an important role in the physical and chemical properties of activated sludge. PS has a highly branched chemical structure. The chain molecules are intertwined and interlaced into an amorphous network structure. It is an adhesive for capturing and collecting bacteria (Vyrides & Stuckey 2009). Moreover, the surface of PS also has negative and

hydrophilic functional groups such as hydroxyl, carboxyl and phosphate groups. PN possesses amino acid functional groups with positive and hydrophobic properties, such as alanine, cysteine and valine, which can enhance the surface hydrophobicity, reduce the electrostatic repulsion between bacteria and enhance the aggregation of aerobic particles (Tsuneda *et al.* 2003). Therefore, it is very important to understand the dynamic states of these biological products in the biochemical system. However, there are few references about the composition of EPS and SMP in the pure oxygen aeration activated sludge process.

The main purpose of this study was to explore the generation and composition of EPS and SMP of activated sludge in a sequencing batch reactor (SBR) with pure oxygen aeration. The removal efficiency of organic matter, composition and content of EPS and SMP were determined and compared. The characteristics of stratification (LB-EPS and TB-EPS) of EPS in pure oxygen aerated sludge were analyzed in terms of substance concentration, component content, and zeta potential charge. This study aimed to better understand the effect of pure oxygen aeration on the treating process of organic wastewater by activated sludge.

MATERIALS AND METHODS

Wastewater and activated sludge

Synthetic wastewater was employed as the experimental influent with sodium acetate as the single carbon source to avoid the interference of influent substances on PN and PS determinations. The compositions of the synthetic wastewater include 1,000 mg/L of CH_3COONa , 170 mg/L of NH_4Cl , 20 mg/L of KH_2PO_4 , 40 mg/L of NaHCO_3 , 40 mg/L of CaCl_2 , and 164 mg/L of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (Han *et al.* 2019). Activated sludge was obtained from a municipal wastewater treatment plant in Nanjing as seed sludge and pre-cultured in a 150 L plastic container under a normal environment with the synthetic wastewater. The pre-culture process was in sequencing mode and had two cycles a day. Each cycle (5.5 h) consisted of the following steps: feeding (0.5 h), aeration (3 h), settling (0.5 h), and decanting (0.5 h) and idling (1 h). The aeration rate and the solids retention time (SRT) were controlled at 300 L/h and 15 days, respectively. Detailed culture parameters could be found in Zhang *et al.* (2017). After 3 months of consecutive cultivation, the sludge could recover its activity and adapt to the synthetic wastewater, then it was prepared for further experiments.

Reactors and experimental design

Four identical reactors made of Plexiglass were employed as the experimental equipment with the effective volume of each reactor as 7.8 L. Detailed reactor size could be found in [Hu *et al.* \(2019\)](#). In this paper, four oxygen flow rates of 5 L/h, 10 L/h, 15 L/h, and 20 L/h were set for the batch tests. The cultured activated sludge was washed with deionized water for three times and then pumped into the four parallel pure oxygen aeration experimental devices, and the aeration amount was adjusted by rotor flow meters. The four aeration devices were operated simultaneously for 180 minutes. At the same time, the stirrer was kept running in each reactor to prevent sludge sedimentation. The influent COD of the simulated wastewater was controlled at 300–450 mg/L, and the sludge concentration (mixed liquor suspended solids, MLSS) was 2,000 mg/L, approximately. The operating temperature was maintained at 25 ± 2 °C.

EPS and SMP extraction

The extraction method of SMP was as follows: take 50 mL of the sludge mixture from the reactor with a centrifuge tube, centrifuge at 4,000 r/min for 5 minutes, filter the supernatant through a filter paper, and filter through a suction filter through a 0.45- μ m pore membrane ([Li *et al.* 2013](#)). The obtained filtrate solution was then used for SMP test.

EPS was extracted with the low-temperature thermal method ([Yang & Li 2009](#); [Ye *et al.* 2011](#)). Firstly, 40 mL of the sludge mixture was taken from the reactor with a centrifuge tube, centrifuged at 4,000 r/min for 5 minutes. Secondly, the supernatant was decanted, and the sludge was diluted to the original volume of 40 mL with 0.9% NaCl solution. The mixture was heated to 50 °C and stirred for 1 min. Afterwards, the sample was centrifuged again at 4,000 r/min for 10 minutes, filtered through a 0.45- μ m pore size filter, and the filtrate was prepared for LB-EPS determination. Finally, the remaining sludge was replenished to the original volume with 0.9% NaCl solution, heated at 70 °C in a water bath for 30 minutes, then centrifuged at 4,000 r/min for 15 minutes, and passed through a 0.45- μ m filter membrane again. The supernatant was regarded as the TB-EPS of the sludge sample.

Analytical methods

COD and MLSS were determined according to SEPA standard methods ([SEPA 2002](#)). DO concentration was detected

by a dissolved oxygen meter (JPBJ-608, China). Zeta potential of samples was measured by a Malvern zeta analyzer (Mastersizer 2000, USA). The PN contents in EPS and SMP extractions were determined based on the method of modified Lowry method, employing bovine serum albumin as the standard. The PS contents in EPS and SMP extractions were determined by the phenol-sulphuric acid method ([Kim *et al.* 2001](#)). SMP, LB-EPS, and TB-EPS were calculated as the sum of PS and PN of each sample. T-test was employed to test the significance of the results. Statistically significance was defined with $p < 0.05$.

The 3D-EEM spectra of SMP, LB-EPS and TB-EPS samples were measured by a molecular fluorescence photometer (Thermo Scientific, USA) with an excitation wavelength (Ex) ranging from 200 to 400 nm, emission wavelength (Em) ranging from 200 to 500 nm, scanning interval of 5 nm. The excitation and emission slits were 10 nm and the scanning speed was maintained at 1,200 nm·min⁻¹ for all the measurements.

RESULTS AND DISCUSSION

Effect of pure oxygen aerated sludge system on COD degradation

[Figure 1\(a\)](#) shows the degradation process of COD by the pure oxygen aerated sludge at the aeration rate of 5, 10, 15 and 20 L/h, respectively. The specific degradation rates of COD, obtained by dividing the linear fitting slope in [Figure 1\(a\)](#) by the sludge concentration, were 0.823, 0.473, 0.607 and 1.251 mgCOD/(gMLSS·min), respectively. [Figure 1\(b\)](#) shows the change of dissolved oxygen in the reaction system of pure oxygen aeration and air aeration with the same flow (20 L/h). When the flow rate was 20 L/h, the organic matter degradation rate of pure oxygen aeration (1.251 mgCOD/(gMLSS·min)) was much higher than that of air aeration (0.512 mgCOD/(gMLSS·min)). At the end of the reaction, the removal rate of COD in the pure oxygen aerated reactor was as high as 95.06%, which was 11.70% higher than that in the air aerated reactor under the same conditions ([Figure 1\(b\)](#)). Variations of DO concentration were further detected. At the beginning of the reaction, the DO concentration of the two systems was basically the same, both at low levels. However, DO concentration in the pure oxygen aerated reactor increased significantly after 120 minutes, different from the steady state of DO in the air aerated system which was always at a lower level. The DO concentration of pure oxygen aerated

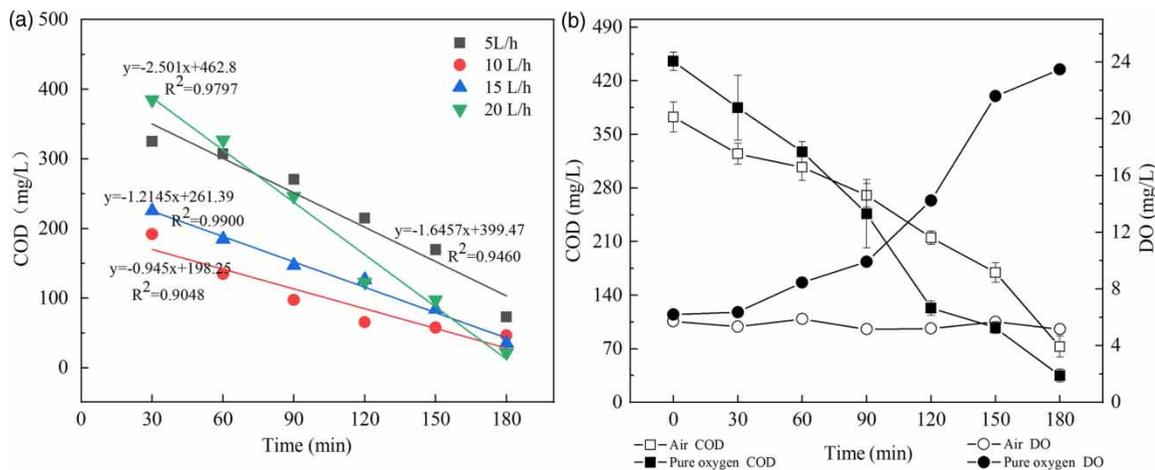


Figure 1 | Effect of pure oxygen aerated sludge system on COD degradation: (a) COD degradation processes with different aeration rates, (b) dynamic changes of COD and DO concentrations of pure oxygen aeration and air aeration (20 L/h).

system was related to the residual COD concentration. Before 120 min, most of the degradable organic matter in the pure oxygen aerated system could be rapidly degraded. After that, the oxygen consumption of pure oxygen aerated sludge decreased quickly, which led to the significant increase of DO concentration. These results indicated that the pure oxygen aeration could provide sufficient oxygen for microorganisms to participate in the biodegradation process under the condition of higher organic load, and thus improve the degradation efficiency. However, the improvement of processing efficiency was limited when the organic load was at a lower level. This rule was similar to the process of organic matter degradation by activated sludge with pressurized aeration (Xu *et al.* 2016). Therefore, these intensified aeration methods would be more economic with high organic load due to their higher degradation rates and lower hydraulic retention time (HRT), which could partially compensate their oxygen supplementation cost.

Effect of pure oxygen aeration on components of activated sludge

Figure 2 shows the changes of SMP, LB-EPS and TB-EPS over reaction time under air aeration and pure oxygen aeration with the aeration rates of 5 L/h and 20 L/h, respectively. In the air aerated reactor (Figure 2), SMP shows a gradual increase trend with the operation time at the low flow of 5 L/h. However, an opposite trend occurred when the air flow was increased to 20 L/h. The content of LB-EPS increased first and then decreased under the two aeration rates. Especially with high flow, the increasing trend was obvious between 60 and 120 minutes. TB-EPS

fluctuated in a small range, and the change was not obvious compared with SMP and LB-EPS.

With regard to the pure oxygen aerated system (Figure 2), when the flow was 5 L/h, the SMP content was significantly reduced, which was adverse to that of the air aeration. SMP was significantly lower than that with air aeration at 20 L/h flow ($p < 0.05$). LB-EPS decreased gradually at low flow and increased gradually under high flow. Compared with LB-EPS, the content of TB-EPS in pure oxygen aerated system was similar to that in the air aerated system, which was always stable, slightly increased at low flow and basically unchanged at high flow.

In general, SMP has poor biodegradability and is difficult to be assimilated or degraded by cells (Zhang *et al.* 2020). In the air aerated system, SMP was released through substrate degradation or biomass metabolism. Because it was difficult to be further degraded, SMP was accumulated in the reactor. In Figure 2, it could be found that SMP with the air flow of 20 L/h was higher than that with 5 L/h in the early stage of the reaction ($p < 0.05$). The reason may be that higher dissolved oxygen would lead to more SMP production, which was mainly due to the increase of UAP content in SMP (Xie *et al.* 2012). The production of UAP depended on the substrate utilization, so more UAP would be produced under the condition of sufficient substrate supply at the early stage (Magbanua & Bowers 2010). However, in the pure oxygen aerated system with sufficient DO, the content of SMP gradually decreased or remained at a low level. It may be because that hyperoxia environment effectively activates the heterotrophic bacteria which could utilize most of UAP in SMP for growth (Merkey *et al.* 2009). From the perspective of SMP generation, pure

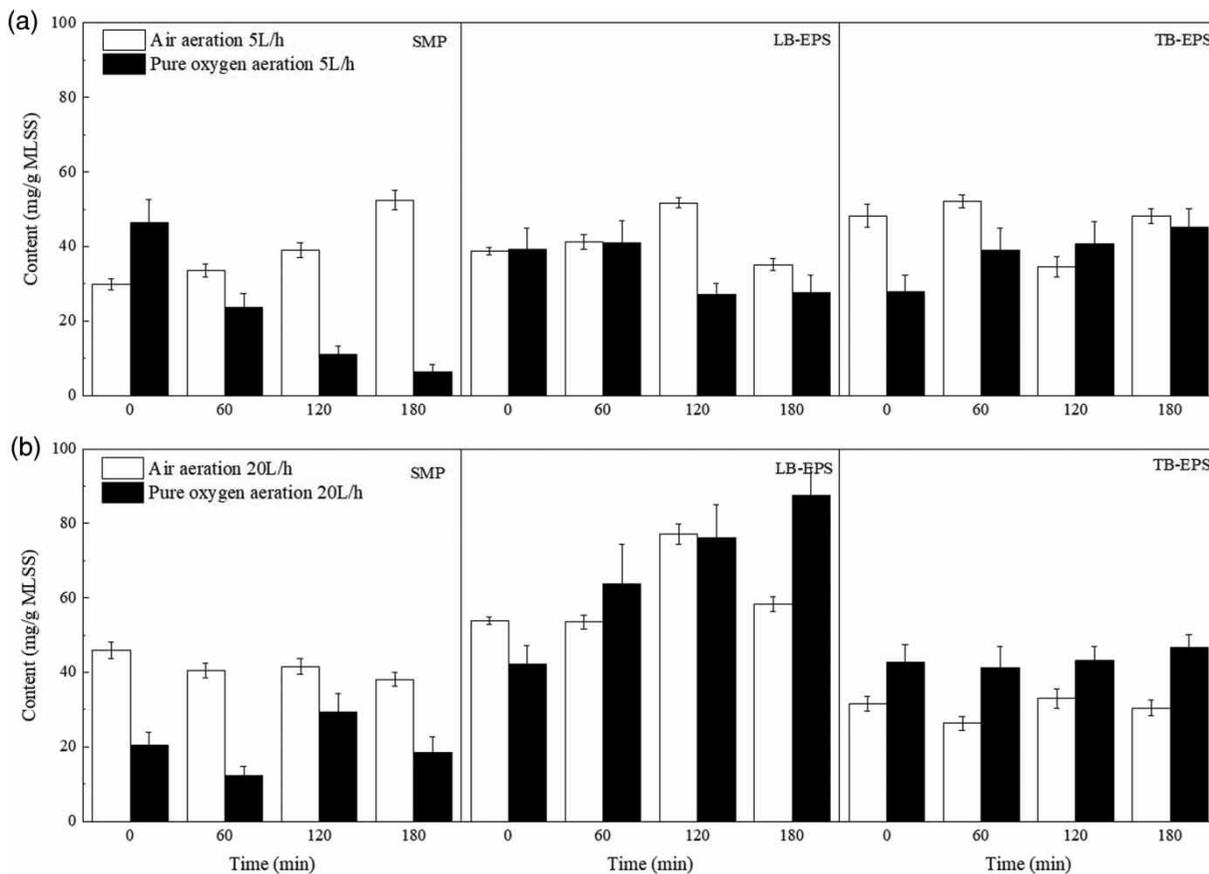


Figure 2 | Changes of extracellular products under different aeration rates: (a) 5 L/h, (b) 20 L/h.

oxygen aeration could reduce effluent SMP and improve effluent water quality of the biological treatments.

EPS plays a crucial role in improving the stability of aerobic sludge, and different physical states of EPS have different effects on the system (Wang *et al.* 2005). Compared with air aeration, the content of LB-EPS increased obviously with the increase of reaction time under high flow of pure oxygen, from 42.2 mg/gMLSS to 87.4 mg/gMLSS. Excessive oxygen transfer impetus would accelerate the metabolism of microorganisms, and thus secrete more EPS (Peng *et al.* 2012). Since LB-EPS has a highly hydrated matrix, a dispersed mucus layer, and no obvious edges, a higher level of LB-EPS can negatively affect bio-flocculation and sludge water separation (Deng *et al.* 2020). TB-EPS, located in the inner layer, is responsible for cell adhesion and attachment in the internal flocculent structure through strong interaction, and plays a role in assisting the cell to exchange material and energy with the surrounding environment (Deng *et al.* 2020). It could be found that compared with LB-EPS, TB-EPS could be relatively stable in the pure oxygen aerated system.

Figure 3 shows the changes of PN and PS of SMP, LB-EPS and TB-EPS of the pure oxygen aerated sludge under condition of different aeration rates. At the end of the 180 min-reaction, both PN and PS of SMP presented an increasing trend with the increase of the aeration rates. It is interesting that, in LB-EPS, PN decreased and increased over time at low (5 L/h) and high (20 L/h) aeration rates, respectively. With the increase of the aeration rate, the PN content of TB-EPS rose firstly, and when the flow exceeded 15 L/h, PN declined.

Table 1 shows PN and PS contents of total EPS in pure oxygen aeration activated sludge. The contents of PS in EPS of the four aeration systems were much lower than that of PN, similar to those of SMP ($p < 0.05$). Li *et al.* (2020) explored the EPS composition of biofilm and found that PN accounted for the largest proportion. Jiang *et al.* (2020) studied EPS composition in activated sludge, and also found that PN was dominant. The protein has a higher concentration of negatively charged amino acids, which is more involved in the electrostatic bond of multivalent cations than PS, and becomes the key to the structural stability of

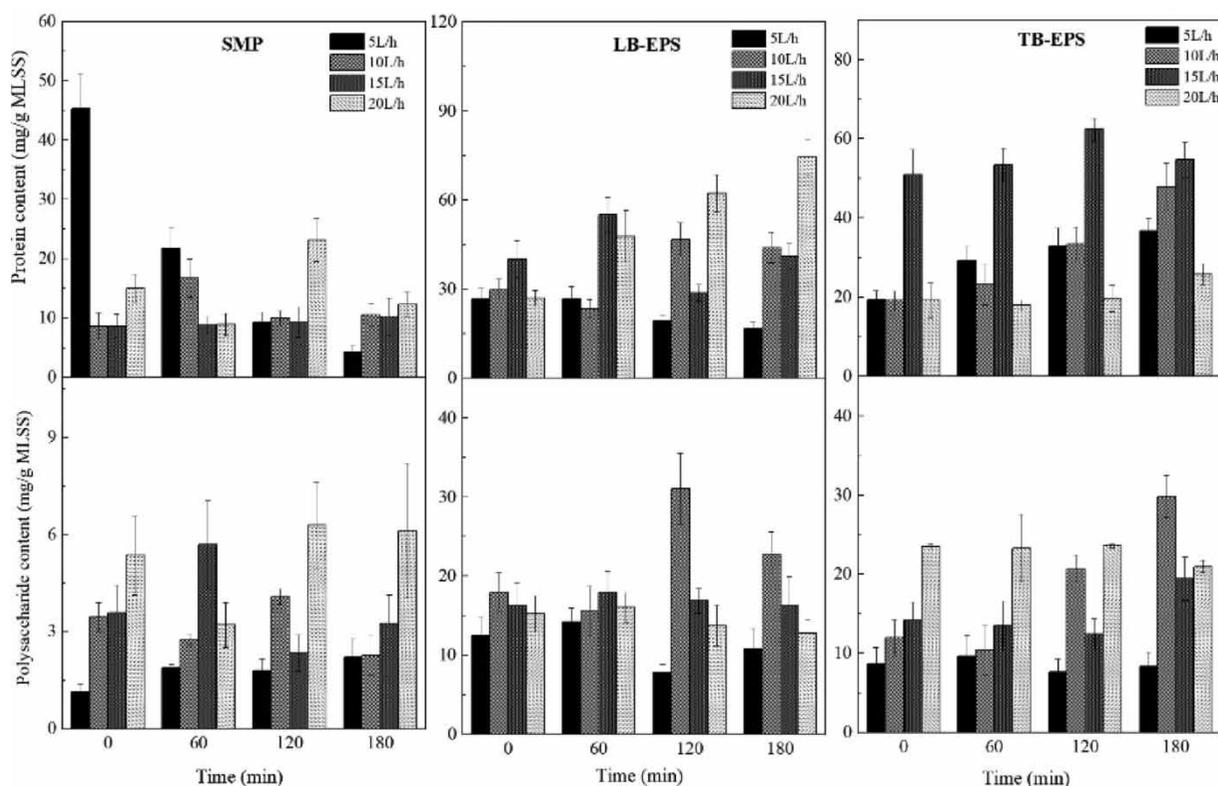


Figure 3 | Changes of PN and PS in the components of activated sludge aerated with pure oxygen: SMP, LB-EPS and TB-EPS.

Table 1 | PN and PS contents of total EPS in pure oxygen aeration activated sludge

Components	Time (min)	Time (min)			
		5 L/h	10 L/h	15 L/h	20 L/h
Protein mg/g MLSS	0	46.00	48.89	91.14	46.12
	60	56.00	46.58	108.37	65.71
	120	52.15	80.00	91.17	81.99
	180	53.50	91.88	95.69	100.39
Polysaccharide mg/g MLSS	0	21.13	29.88	30.45	38.74
	60	23.84	26.09	31.46	39.31
	120	15.56	51.67	29.35	37.41
	180	19.25	52.61	35.74	33.73

activated sludge flocs (D'Abzac *et al.* 2010). But different from SMP, EPS polysaccharide decreased gradually at low flow. Under the condition of medium flow of 10 L/h, the content of PS increased significantly in the later stage of the reaction. At higher flow, the content of PS did not change significantly. It has been reported that properly increasing the aeration intensity can induce the activation of the polysaccharide synthesis channel and increase the PS content in the EPS of the activated sludge, while the increase of the PS content is conducive to the flocculation and sedimentation of the activated sludge (Shin *et al.*

2000). However, conversely, changes in enzyme activity associated with PS biosynthesis also affected the content of PS (Wang *et al.* 2020).

The proportions of the three extracellular products in the activated sludge at different aeration rates are shown in Figure 4. The proportions of the total EPS in the pure oxygen aerated sludge accounted for approximately 90% at all aeration rates (Figure 4(a)), significantly higher than those in the air aerated sludge (Figure 4(b)). Generally, the proportion of TB-EPS decreased with the increase in flow, while the proportion of LB-EPS showed an increasing trend when the flow increased.

With the increase in flow, although the proportion of TB-EPS in pure oxygen aeration system decreased gradually, its content (Figure 3) increased in each system. Ren *et al.* (2019) found that the flow was positively correlated with TB-EPS by exploring the composition characteristics of EPS in the up-flow biofilter under different aeration rates. The main reason for this was that a high aeration rate induced the biofilm to produce more EPS, which could stabilize the biofilm structure and allow it to withstand the shearing force (Abu Bakar *et al.* 2018). Combined with Figures 3 and 4, it was found that the proportion of

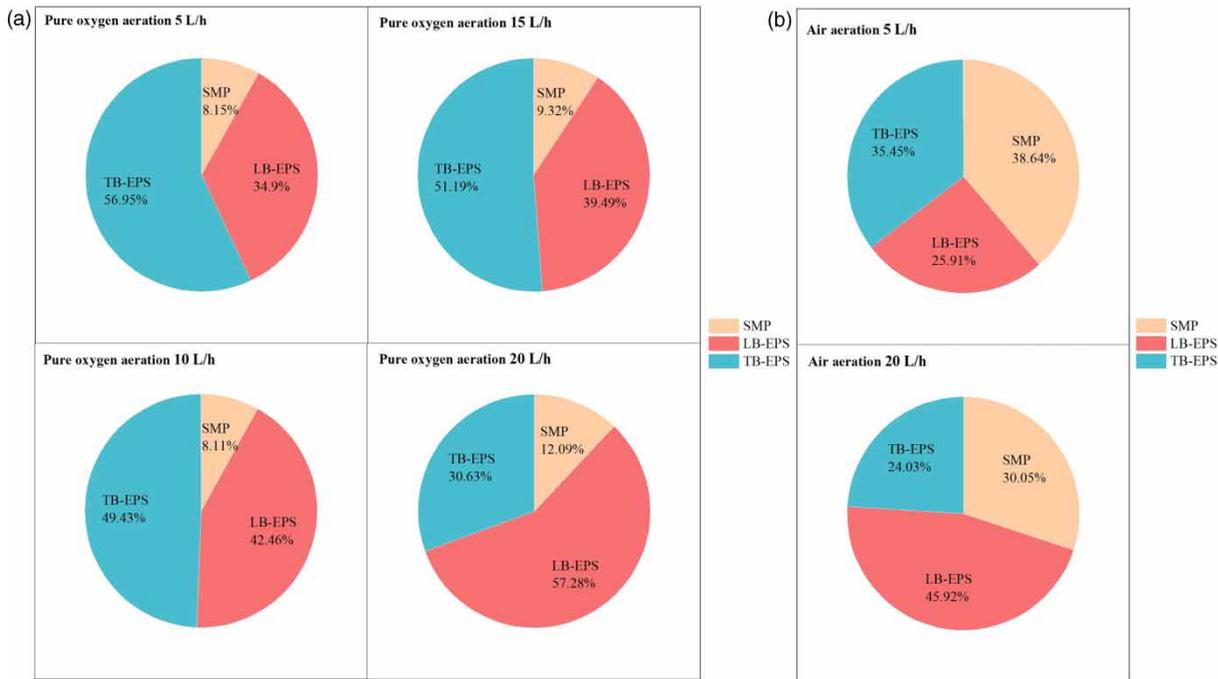


Figure 4 | Proportion of the extracellular products in activated sludge under pure oxygen (a) and air (b) aeration conditions.

LB-EPS of the pure oxygen aerated sludge increased gradually with the increase of aeration rate, but the total content of LB-EPS had no obvious correlation with aeration rate. The result was not accordant with the previous study by Ren *et al.* (2018) reporting that higher aeration rate would cause the decrease of LB-EPS concentration, which is still worthy of further studies.

Some studies have demonstrated that the proportion of LB-EPS and TB-EPS, rather than their quantities, is more important in sludge aggregation (Abu Bakar *et al.* 2018). Compared with the air aerated system (Figure 4(b)), pure oxygen aeration improved the proportion of LB-EPS and TB-EPS in the extracellular products of the activated sludge system, and greatly reduced the proportion of SMP. The results indicated that the activated sludge system with pure oxygen aeration could achieve the effects of improving the stability of aerobic activated sludge and the quality of effluent water.

Effect of pure oxygen aeration on the chargeability of various components of activated sludge

The changes in the zeta potential of SMP, LB-EPS and TB-EPS in activated sludge with different aeration rates over time are shown in Figure 5. The zeta potential values of SMP, LB-EPS and TB-EPS were all negative, indicating

that the microbial metabolites and activated sludge extracellular polymers in the four aeration systems were all negatively charged. This was mainly due to the ionization of functional groups such as carboxyl, sulfate and phosphate in the extracellular products (Sutherland 2001). According to the component analysis (Figure 3), the four pure oxygen aerated systems all contained more protein. The study by Wilen *et al.* (2003) showed that the increase in the amount of polymer materials in sludge and extracted EPS was related to more negative charges. Among them, protein and humic showed the strongest correlation and contributed the most to the negative charges. Most obviously, the initial PN content of SMP (Figure 3) was much higher at the flow of 5 L/h than that of the other three aeration rates. Accordingly, its initial zeta potential was much lower than the other three aeration rates.

It could also be seen from Figure 5 that the zeta potential of SMP, LB-EPS and TB-EPS gradually decreased with the progress of biological treatment, indicating that the negative charges were gradually decreasing. The SMP potentials of the four aeration rates increased at an average of 7.3 mV. The maximal increment was 8.6 mV at the lowest flow of 5 L/h. The potential of LB-EPS increased by 3.5 mV on average, while that of TB-EPS increased by only 1.6 mV. Combined with the content change of each component in Figure 3, it could be shown that, because

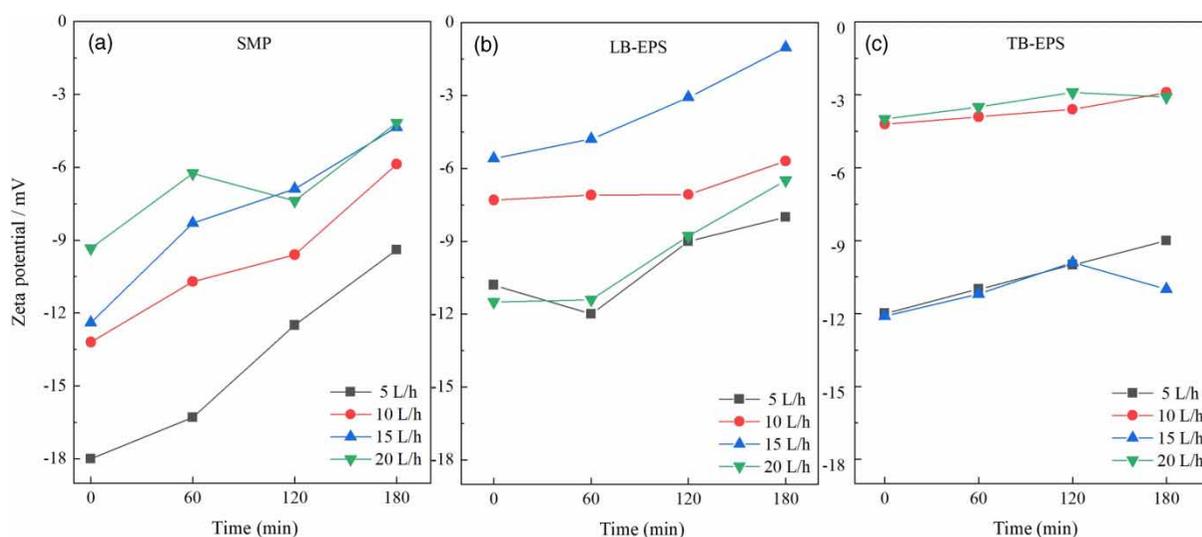


Figure 5 | Zeta potential change of the extracellular products in the pure oxygen aerated system: (a) SMP, (b) LB-EPS, (c) TB-EPS.

the pure oxygen aeration system provided sufficient dissolved oxygen for microorganisms to degrade organic matter, the SMP proteins that were not easily degraded in the outer layer were degraded in large quantities during the reaction, which made the negative charge of SMP decrease rapidly. However, the content change of LB-EPS was less than that of SMP, so its potential change was also smaller than that of SMP. The content of TB-EPS located in the inner layer changed less, and also achieved the minimum variation of the zeta potential. Thus, in pure oxygen aeration system, there was a certain correlation between the change of zeta potential of extracellular products and their contents.

Figure 6 shows the relationship between the content of components of extracellular products and zeta potential. The total amount of TB-EPS increased with the increasing zeta potential. Some studies have shown that increasing EPS concentration could reduce the negative charge on the surface of activated sludge flocs, reduce the repulsive force between flocs and promote sludge flocculation sedimentation (Goodwin & Forster 1985). However, some experiments also showed that there was a positive correlation between the amount of EPS and the negative charge of sludge. (Morgan *et al.* 1990).

The content of PS was low and the influence on zeta potential was not decisive. However, it is generally believed that the increase of PS content will increase the negative charge of the system, because the carboxylic acid and other groups contained in PS could generate negative charge after dissociation (Liao *et al.* 2001). Liao *et al.* (2001) found that the ratio of PN to PS had a negative

effect on the surface charge. This was consistent with the results of Figure 6(c), where the PN content in TB-EPS increased and the negative charge of the system decreased. The reason was that most the amino groups in PN are positively charged and could neutralize the negative charges of carboxyl groups and phosphates. PN could also chelate or ionic bond with metal ions in water, compress the electric double layer, reduce cell zeta potential, and thus promote the collision and aggregation between microbial cells (Sheng *et al.* 2010).

It's interesting that the effects of PN content on zeta potential in TB-EPS (Figure 6(c)) was different with that in SMP/LB-EPS (Figure 6(a) and 6(b)). Similar difference of the effects of the total amount on zeta potential also occurred between TB-EPS and SMP/LB-EPS. SMP and LB-EPS located in the outer layer were in close contact. Some studies believed that there was a conversion relationship between them, and the release of SMP was closely related to the shedding or hydrolysis of EPS (Lapidou & Rittmann 2002). This provides a certain basis for the similarity of SMP and LB-EPS. It was found that the main three-dimensional excitation-emission matrix fluorescence peak of LB-EPS appeared red-shifted and the main peak of TB-EPS blue-shifted as the reaction proceeded. The red shift was related to the presence of functional groups such as carbonyl, hydroxyl, alkoxy, amino and carboxyl groups (Chen *et al.* 2002). Studies have shown that about 25% of amino acids in extracellular products were negatively charged after hydrolysis (Dignac *et al.* 1998). Therefore, the opposite trend of change between the two groups may be related to changes in amino acids.

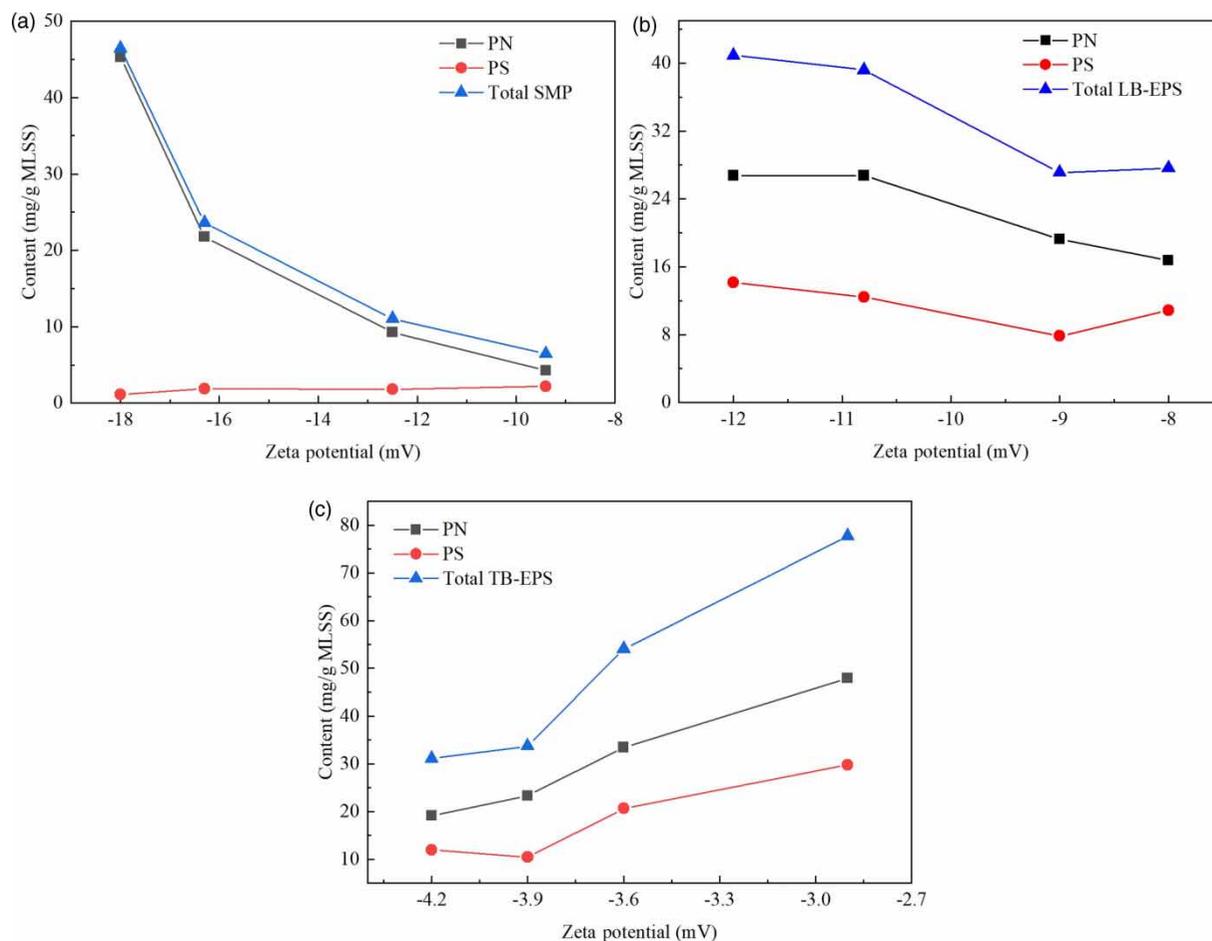


Figure 6 | Variation of component content with zeta potential in pure oxygen aeration system with low flow of 5 L/h: (a) SMP, (b) LB-EPS, (c) TB-EPS.

EEM fluorescence spectra of SMP and EPS

To further explore the effects of different pure oxygen aeration rates on the chemical composition of extracellular products, the molecular characteristics of SMP, LB-EPS and TB-EPS were identified by EEM spectra. As shown in Figure 7, five main Ex/Em peaks (peaks A, B, C, D and E) of the SMP, LB-EPS and TB-EPS were mainly located at 255–294/327–351 nm, 342/435 nm, 301/401–410 nm, 360–361/449–450 nm and 301–322/401–427 nm, which were characterized as tryptophan PN-like fractions (peak A), humic acid-like fractions (peak B, peak C and peak D) and visible humic acid-like fractions (peak E), respectively (Chen *et al.* 2015). The composition of SMP, LB-EPS and TB-EPS were similar. It has been reported that there is a transformation relationship between SMP and EPS (Laspidou & Rittmann 2002).

With the change of aeration rate, the fluorescence peaks of the three extracellular products were shifted to different degrees. Under 10 L/h aeration, the location of peak A in the LB-EPS was shifted by 21 nm along the Em axis compared with low aeration. Peak A in the SMP was also shifted by 4 nm along the Ex/Em axis at 15 L/h. Under 20 L/h aeration, peak A of TB-EPS was shifted by 3 nm. Location of peak A in the LB-EPS appeared more changed than that in the SMP and TB-EPS, which could be attributed to the fact that LB-EPS was more sensitive to aeration rate than SMP and TB-EPS. This was consistent with the results shown in Figure 3. The shift of fluorescence peak in LB-EPS may be related to the decomposition of condensed aromatics and the decomposition of macromolecules into small fragments (Swietlik *et al.* 2004). The change of aeration rate would not only change the fluorescence peak positions of SMP, LB-EPS and TB-EPS, but also affect the fluorescence

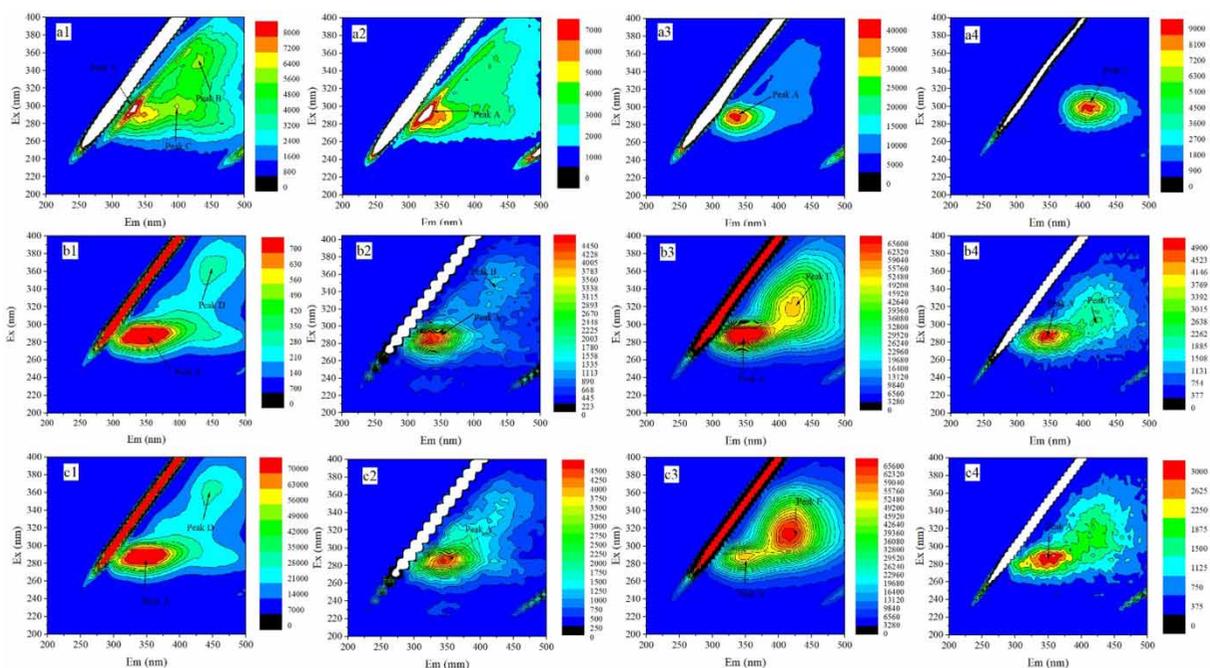


Figure 7 | EEM fluorescence spectra of SMP, LB-EPS and TB-EPS fractions of activated sludge aerated with pure oxygen: a1, a2, a3 and a4 were SMP at 5 L/h, 10 L/h, 15 L/h and 20 L/h pure oxygen aeration rates; b1, b2, b3 and b4 were LB-EPS at 5 L/h, 10 L/h, 15 L/h and 20 L/h pure oxygen aeration rates; c1, c2, c3 and c4 were TB-EPS at 5 L/h, 10 L/h, 15 L/h and 20 L/h pure oxygen aeration rates.

peak intensity. The chemical composition of the three extracellular products would be affected by the aeration rate, but there was no obvious positive and negative correlation, as can be seen from Figure 3.

CONCLUSION

Pure oxygen aeration could increase the degradation rate of organic matter and shorten the reaction time compared with air aeration. SMP accumulation existed in the air aeration system at lower aeration rate, while pure oxygen aeration could effectively reduce SMP. Compared with air aeration, pure oxygen with high aeration rate stimulated the production of LB-EPS of the activated sludge. Different pure oxygen aeration rates also affected the composition of EPS. The production of LB-EPS increased with the increasing aeration rate. The results also showed that the protein content was much higher than polysaccharides in pure oxygen aerated sludge. From the proportion of extracellular products, the proportion of TB-EPS decreased with the increase of aeration, while the proportion of LB-EPS showed the opposite trend. Zeta potential of each component in activated sludge was negative, gradually decreasing with the progress of biological treatment. In

pure oxygen aerated sludge, zeta potential decreased with the increase of PN content and the total extracellular product amount in SMP/LB-EPS, while it was opposite in TB-EPS. The difference may be related to the changes in amino acids in TB-EPS and in SMP/LB-EPS. The fluorescence peaks in SMP, LB-EPS and TB-EPS of activated sludge with pure oxygen aeration were attributed to tryptophan PN-like and humic acid-like fractions.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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