Effect of temperatures and alternating anoxic/oxic sequencing batch reactor (SBR) operating modes on extracellular polymeric substances in activated sludge

Hongwei Sun, Chenjian Cai, Jixue Chen, Chunyu Liu, Guangjie Wang, Xiaoqiang Li and Huanan Zhao

ABSTRACT

In order to investigate the effect of temperatures and operating modes on extracellular polymeric substances (EPS) contents, three sequencing batch reactors (SBRs) were operated at temperatures of 15, 25, and 35 °C ($R_{15\,°C}$, $R_{25\,°C}$, and $R_{35\,°C}$, respectively), with two SBRs operated under alternating anoxic/oxic conditions ($R_{A/O}$ and $R_{O/A}$, respectively). Results showed that higher contents of tightly bound EPS (TB-EPS) and total EPS appeared in $R_{15\,°C}$, while loosely bound EPS (LB-EPS) dominated in $R_{35\,°C}$. In all three kinds of EPS (LB-EPS, TB-EPS and total EPS) assessed, protein was the main component in $R_{15\,°C}$ and $R_{25\,°C}$, while polysaccharides dominated in $R_{35\,°C}$. Moreover, compared with $R_{O/A}$, $R_{A/O}$ was favorable for the production of the three kinds of EPS. Furthermore, three kinds of EPS and their components were augmented during the nitrification process, while they declined during the denitrification process under all conditions except for $R_{35\,°C}$.

Key words | DNA, extracellular polymeric substances (EPS), operating modes, polysaccharide (PS), protein (PN), temperature

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HIGHLIGHTS

- Low temperatures are more favorable for extracellular polymeric substances (EPS) production than high temperatures.
- The anoxic/oxic operating mode generated more EPS than the oxic/anoxic mode.
- EPS were dominated by proteins at 15 and 25 °C, and by polysaccharides at 35 °C.
- The EPS content increased during nitrification and reduced during denitrification.

INTRODUCTION

Extracellular polymeric substances (EPS) play a vital role in biological wastewater treatment because they are the main component of activated sludge. EPS are a kind of microbial metabolite or cell lysis product, which accumulate on the surface of activated sludge flocs and are mainly comprised of proteins (PN), polysaccharides (PS) and nucleic acid (DNA) (Urbain *et al.* 1993; Yang *et al.* 2019). EPS mainly originate from either influent wastewater or bacterial cells and they can be separated into loosely bound EPS (LB-EPS) or tightly bound EPS (TB-EPS) fractions, with LB-EPS and TB-EPS located in the outer and inner layers, respectively (Ramesh *et al.* 2006; Li & Yang 2007; Han *et al.* 2013; Wang *et al.* 2019).

Previous studies have shown that EPS have both positive and negative functions in activated sludge. EPS can remove heavy metals or organic pollutants from wastewater due to their adsorptive capability (Gao *et al.* 2011; Tahir & Yasmin 2019; Wei *et al.* 2019). Higher TB-EPS contents have been reported to noticeably enable EPS to form flocs in activated sludge (Zhang *et al.* 2014; Zeng *et al.* 2019). Moreover, PN enrichment in TB-EPS has positive effects on the sedimentation and dehydration of activated sludge (Li & Yang 2007; Basuvaraj *et al.* 2015; Yang & Li. 2019). EPS can act as a protective barrier to avoid the impacts of toxic external conditions (Miao *et al.* 2018). The negative effects of EPS are that excessive EPS contents, especially

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PS-enriched LB-EPS, can induce poor dewaterability and bioflocculation (Li & Yang 2007; Basuvaraj *et al.* 2015). In addition, EPS are responsible for increased membrane fouling in membrane bioreactors (van den Brink *et al.* 2011; Shi *et al.* 2017; Zhang & Jiang 2019).

The generation of EPS is influenced by various operational factors, such as pH, redox potential, sludge retention time (SRT), carbon source availability, temperature, and operating modes (Guo *et al.* 2013; Wang *et al.* 2013; Geyik *et al.* 2016; Cao *et al.* 2018; Hu *et al.* 2019). Among these factors, temperature is regarded as a key parameter in EPS production with different effects previously reported. Some literature reported that the contents of EPS and PN increased with decreasing temperature (Gao *et al.* 2013; Guo *et al.* 2013; Ge *et al.* 2019; Wang *et al.* 2019). Especially, Gao *et al.* (2013) reported that this phenomenon was caused by variation in microbial community structure, affecting the microbial metabolite components significantly.

In contrast, other studies have found that the contents of EPS, PN and PS all increased with increasing temperature (Cho *et al.* 2018; Muñoz Sierra *et al.* 2018; Zhao *et al.* 2018). Hence, these discrepancies indicate that there is still much information to be obtained regarding how temperature influences EPS generation. Additionally, to date, few studies have investigated the DNA variation in TB-EPS and LB-EPS contents with temperature variation.

Reactor operating modes also play a crucial role in EPS generation. For example, research conducted by Hu *et al.* (2019) found that when the operation mode was converted from anoxic/oxic to completely oxic, the EPS content increased with CH_3COONa as carbon source. Wang *et al.* (2013) indicated that the LB-EPS contents in B-mode sequencing batch reactors (SBRs) (18 h reaction and 6 h idle) were three-fold higher than in S-mode (30 min filling, 18 h reaction, 2 h settling, 1 h withdrawal and 2.5 h idle), with LB-EPS contents decreasing at an increased SRT. However, in-depth studies of the effects of alternating anoxic/oxic modes on LB-EPS, TB-EPS, total EPS and their components in SBRs are still very limited. Moreover, few studies have investigated the variation of EPS and its components during the process of nitrification and denitrification under different temperatures and operating modes.

This study aimed to elucidate (1) the influences of temperature on the contents of the three kinds of EPS (LB-EPS, TB-EPS and total EPS) and their components; (2) the effects of alternating anoxic/oxic operation modes on the contents of the three kinds of EPS and their components; (3) the variation in the three kinds of EPS and their components during the nitrification/denitrification process. This research can be helpful to better understand EPS production under different temperatures and operating modes in SBRs, which provides a better solution for the practical operation of wastewater treatment plants.

MATERIALS AND METHODS

Experimental setup and procedure

Experiments were carried out in five identical lab-scale SBRs (5 L jacketed glass reactors). Two of the SBRs were operated under different operating modes of oxic/anoxic ($R_{O/A}$) and anoxic/oxic ($R_{A/O}$), and were fed with domestic wastewater. The other three SBRs were operated at three different temperatures (15, 25 and 35 °C) and fed with synthetic wastewater. The $R_{O/A}$ cycle consisted of feeding, oxic reaction, anoxic reaction, settling and decanting. The $R_{A/O}$ cycle consisted of feeding, anoxic reaction, oxic reaction, settling and decanting. The operational cycles of the three varying temperature SBRs ($R_{15\,°C}$, $R_{25\,°C}$ and $R_{35\,°C}$) were similar to the cycle applied to the $R_{O/A}$ system. Detailed experimental conditions are shown in Table 1 and Figure 1.

Inoculated sludge and influent contents

The seed sludge for the five SBRs was aerobic activated sludge collected from a local domestic wastewater treatment plant at Lanzhou, China, where the oxidation ditch process was employed. The initial mixed liquor suspended solids (MLSS) concentration was approximately 3,000 mg/L.

The composition of synthetic wastewater was as follows: NH_4Cl (115 mg/L), CH_3COONa (385 mg/L), KH_2PO_4 (26 mg/L) and trace element solution. The trace element solution consisted of $MgSO_4{\cdot}7H_2O$ (5.07 mg/L), $MnSO_4{\cdot}4H_2O$ (0.31 mg/L), $FeSO_4{\cdot}7H_2O$ (2.49 mg/L), $CuSO_4$ (0.25 mg/L), $Na_2MoO_4{\cdot}2H_2O$ (1.26 mg/L), $ZnSO_4{\cdot}7H_2O$ (0.44 mg/L), NaCl (0.25 mg/L), $CaSO_4{\cdot}2H_2O$ (0.43 mg/L), $CoCl_2{\cdot}6H_2O$ (0.41 mg/L) and EDTA (1.88 mg/L).

After a steady condition was achieved, the experiments lasted for 100 days (one cycle per day) under their respective temperature or operating mode. Samples were collected from the end of nitrification (sampling) and denitrification (sampling) under two different operating modes ($R_{A/O}$ and $R_{O/A}$) and three different temperatures ($R_{15\,°C}$, $R_{25\,°C}$, and $R_{35\,°C}$). In order to better understand the variation of EPS and its components during the process of nitrification and denitrification, we conducted a full-cycle sampling every

Table 1 | Operating conditions of SBRs under different temperatures and operating modes

	Substrate concentration (mg/L)		Phase time of the SBR (min)					Operational parameters			
Reactors	COD	NH_4^+-N	One cycle	Filling	Aeration	Anoxic	Sedimentation and withdrawal	MLSS (mg/L)	Temperature (°C)	pH	DO (mg/L)
$R_{15\,°C}$	203	40.7	340	5	180	120	35	3,812	15 ± 1.0	7.5 ± 0.2	0.1 ~ 2.5
$R_{25\,°C}$	203	40.7	250	5	150	60	35	3,728	25 ± 1.0	7.5 ± 0.2	0.1 ~ 2.5
$R_{35\,°C}$	203	40.7	160	5	90	30	35	3,765	35 ± 1.0	7.5 ± 0.2	0.1 ~ 2.5
$R_{O/A}$	163	26	395	5	240	120	30	5,400 ± 400	25 ± 1.0	7.5 ± 0.2	0.1 ~ 2.5
$R_{A/O}$	163	26	395	5	240	120	30	5,400 ± 400	25 ± 1.0	7.5 ± 0.2	0.1 ~ 2.5

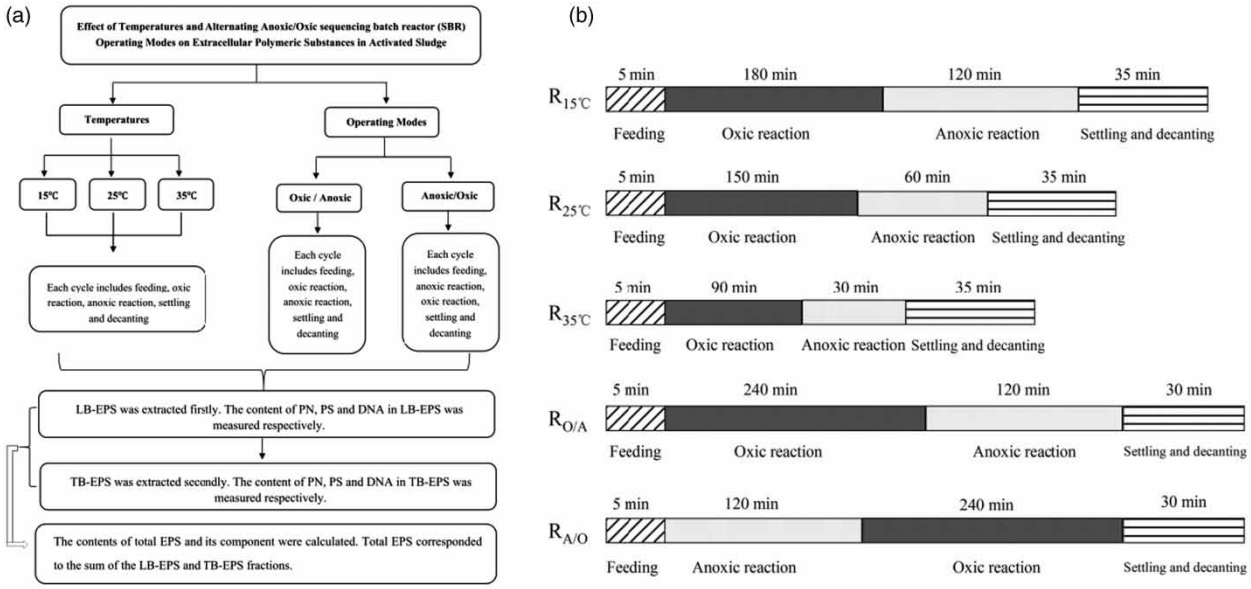

Figure 1 | (a) Experimental design protocol; (b) SBR cycles in a time sequence graph under different temperatures and operating modes.

30 minutes on the 75th day. All the above samples were conducted in duplicate, and their average values are reported.

EPS extraction

The total EPS contents were regarded as the sum contents of LB-EPS and TB-EPS in this work. The method of modified thermal extraction has been reported widely for determining the contents of EPS due to its low level of cell lysis during extraction (Sheng *et al.* 2010). Therefore, this method was used to extract LB-EPS and TB-EPS in the present study.

EPS extraction was performed by first transferring 10 mL of well-mixed sludge liquid to a centrifuge tube. After settling for 30 min, the liquid was centrifuged at 2,100 × *g* and 4 °C for 10 min. Second, the obtained supernatant was passed through a 0.45 μm microporous membrane for analysis. The collected supernatant was regarded as the LB-EPS fraction. Third, to replace the removed supernatant, the same amount of Ringer liquid was added to the residual activated sludge. Fourth, after being mixed and heated at 80 °C for 60 min in a constant temperature water bath, the mixture was centrifuged again at 12,000 × *g* and 4 °C for 10 min. Finally, the obtained supernatant was passed through a 0.45 μm microporous membrane for analysis, with the collected supernatant regarded as TB-EPS.

Analytical methods

Chemical oxygen demand (COD), NH_4^+-N, NO_2^--N, NO_3^--N and MLSS were measured according to the standard methods (APHA 1998). Temperature, pH and dissolved oxygen (DO) were monitored using on-line probes (WTW

Multi 3420, Germany). The nitrite accumulation ratio (NAR) was calculated according to Equation (1) as follows:

$$\text{NAR}\ (\%) = \frac{\text{NO}_2^-\text{-N}}{\text{NO}_2^-\text{-N} + \text{NO}_3^-\text{-N}} \times 100\% \quad (1)$$

With respect to the EPS components analysis, the PS content was measured via the phenol-sulfuric acid method, with glucose used as the standard (Masuko *et al.* 2005). The Lowry method was employed to quantify the PN content, using bovine serum albumin as the standard (Frolund *et al.* 1996). DNA was determined by the method of ultraviolet absorption (Mengistu *et al.* 1994).

RESULTS AND DISCUSSION

Effect of temperature on the contents of different EPS fractions

The influences of temperature on the contents of the three different EPS fractions are presented in Figure 2. A significantly higher percentage of TB-EPS accounting for total EPS was obtained with increasing temperatures, with values of 79.0 ± 6.7% (15 °C), 77.9 ± 5.9% (25 °C) and 70.7 ± 8.9% (35 °C), respectively. In contrast, the proportions of LB-EPS accounting for total EPS exhibited relatively lower levels, with values of 20.9 ± 6.7%, 22.1 ± 5.9% and 29.3 ± 8.9% observed with increasing temperatures of 15, 25 and 35 °C, respectively. These results in combination with previous reported studies demonstrated that LB-EPS content augmentation with increasing temperature may be attributed to the increased temperature providing more appropriate environmental conditions for outer layers' microbial growth, resulting in the enhancement of metabolic activity and accelerated growth rate. Therefore, more macromolecular polymers were secreted, leading to an increase in LB-EPS content. But for TB-EPS, its content decline with increasing temperature may be attributed to inner layers' cell autolysis reduction, thereby reducing the TB-EPS yield. Remarkably, although LB-EPS increased while TB-EPS decreased with increasing temperature, the proportion of TB-EPS accounting for total EPS was larger than that of LB-EPS. Previous studies have also observed that TB-EPS was the main component of total EPS (accounting for between 52.4% and 72.8%) (Miao *et al.* 2018; Du *et al.* 2019).

When assessing the contents of the three EPS fractions under all temperatures, two opposite trends were observed in the present study. As shown in Figure 2(a), the content

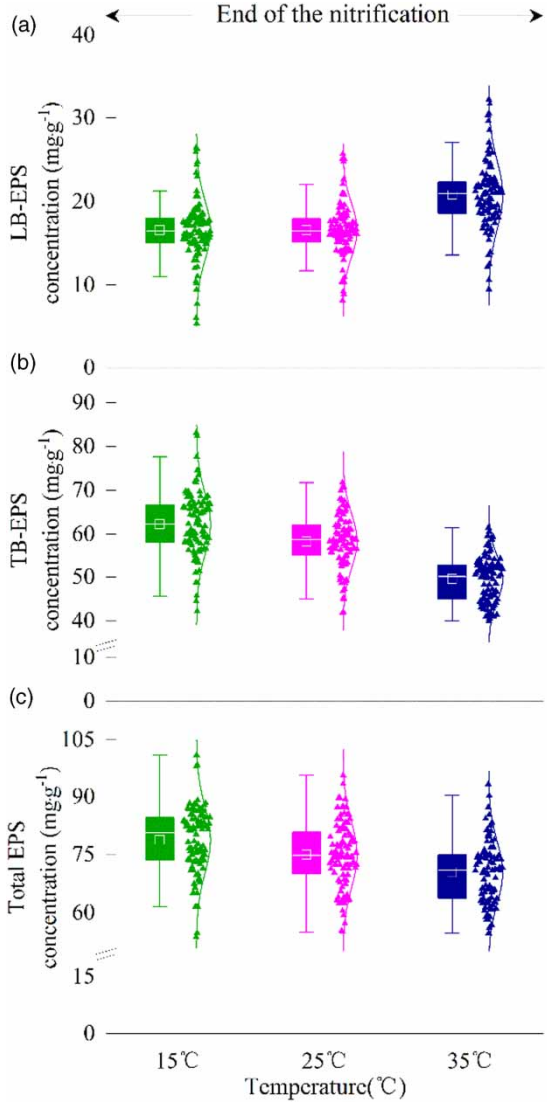

Figure 2 | Effect of temperature on the contents of (a) LB-EPS, (b) TB-EPS and (c) total EPS over 100 cycles at the end of nitrification stage. Note: each box diagram and normal distribution curve consisted of 100 samples (100 cycles). The box diagram is on the left; the top line of box diagram means the maximum value of samples, the bottom line of box diagram means the minimum value of samples and the middle line of box diagram is the average value of samples. The normal distribution curve is on the right.

of LB-EPS increased from 16.5 mg/g MLSS at 15 °C to 21.0 mg/g MLSS at 35 °C. However, the content of TB-EPS and total EPS decreased from 62.3 and 78.8 mg/g MLSS at 15 °C to 50.0 and 71.0 mg/g MLSS at 35 °C, respectively (Figure 2(b) and 2(c)).

The variation in EPS fractions observed in the present study were similar to the findings previously reported in the literature in membrane bioreactor systems treating domestic or synthetic wastewater at temperatures ranging from 5 to 30 °C (Wang *et al.* 2009; Gao *et al.* 2013; Ma

et al. 2013). These results clearly demonstrate that temperature is one of the most important factors affecting EPS generation. In particular, low temperatures are reportedly more conducive to EPS production (Janus & Ulanicki 2010). In the present study, temperature was also found to have an obvious effect on the contents of LB-EPS and TB-EPS. Low temperatures were found to be more conducive to the growth of TB-EPS than LB-EPS. The biological mechanism of EPS formation decreased at increasing temperatures, with high temperatures resulting in cell autolysis reduction, thereby reducing the EPS yield.

Moreover, few studies have reported that the relationship between EPS content and temperature was positive. Among the reported literature, Cho et al. (2018) showed that the EPS content increased with a temperature increase from 15 °C to 35 °C in an anaerobic ceramic membrane bioreactor treating domestic wastewater. This may be attributed to the increased temperature providing more appropriate environmental conditions for microorganism growth, resulting in the enhancement of metabolic activity and accelerated growth rate. Therefore, more macromolecular polymers were secreted, leading to an increase in EPS content.

Effect of operating modes on the contents of different fractions of EPS

The contents of the three EPS fractions under different operating modes are illustrated in Figure 3. The TB-EPS content accounted for more than 80.2% of total EPS in $R_{O/A}$ and $R_{A/O}$ modes at the end of nitrification and denitrification, while LB-EPS accounted for less than 19.8%. Hence, TB-EPS was found to be the dominant component, while LB-EPS was the minor component of the total EPS matrix in activated sludge. Moreover, three kinds of EPS contents at the end of nitrification were found to be higher than that at the end of denitrification both in $R_{A/O}$ and $R_{O/A}$ mode. The main reason for this phenomenon was that exoenzymes hydrolyze PS into glucose, which was utilized by denitrifying bacteria to perform denitrification, resulting in EPS reduction.

In addition, the ratio of $R_{A/O}$/ $R_{O/A}$ in the contents of the three EPS fractions ranged from 1.0 to 1.4 at the end of nitrification and denitrification (Figure 3), indicating that the $R_{A/O}$ mode was more conducive to EPS production in activated sludge. The reason for this could be due to the higher metabolic accumulation of $R_{A/O}$ than $R_{O/A}$. The three kinds of EPS contents were reduced during the anoxic denitrification stage, while they were augmented in the oxic nitrification stage (Figure 6), followed by continuous decline due to their withdrawal. Hence, the three kinds of EPS contents in

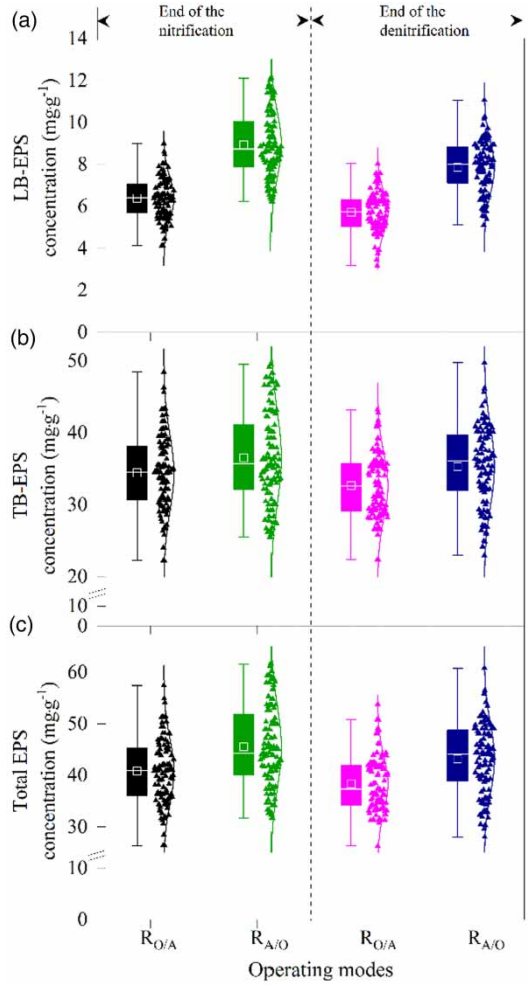

Figure 3 | Effect of operating mode on the contents of (a) LB-EPS, (b) TB-EPS and (c) total EPS over 100 cycles at the end of nitrification and denitrification stages.

$R_{A/O}$ first decreased, then increased and subsequently decreased, whereas the three kinds of EPS contents in $R_{O/A}$ first increased, then decreased continuously.

Effect of temperature on the contents of PS, PN and DNA in different EPS fractions

Quantitative differences in components of different EPS fractions under different reactor temperature conditions are shown in Figure 4. The contents of PN and DNA in LB-EPS (13.1 and 0.4 mg/g MLSS), TB-EPS (28.5 and 5.6 mg/g MLSS) and total EPS (42.5 and 6.0 mg/g MLSS) remained relatively unchanged as the temperature increased from 15 °C to 25 °C. However, both PN and DNA underwent a sharp decline when the temperature continued to increase to 35 °C. This result was consistent with the findings reported by Ge et al. (2019), who observed that the

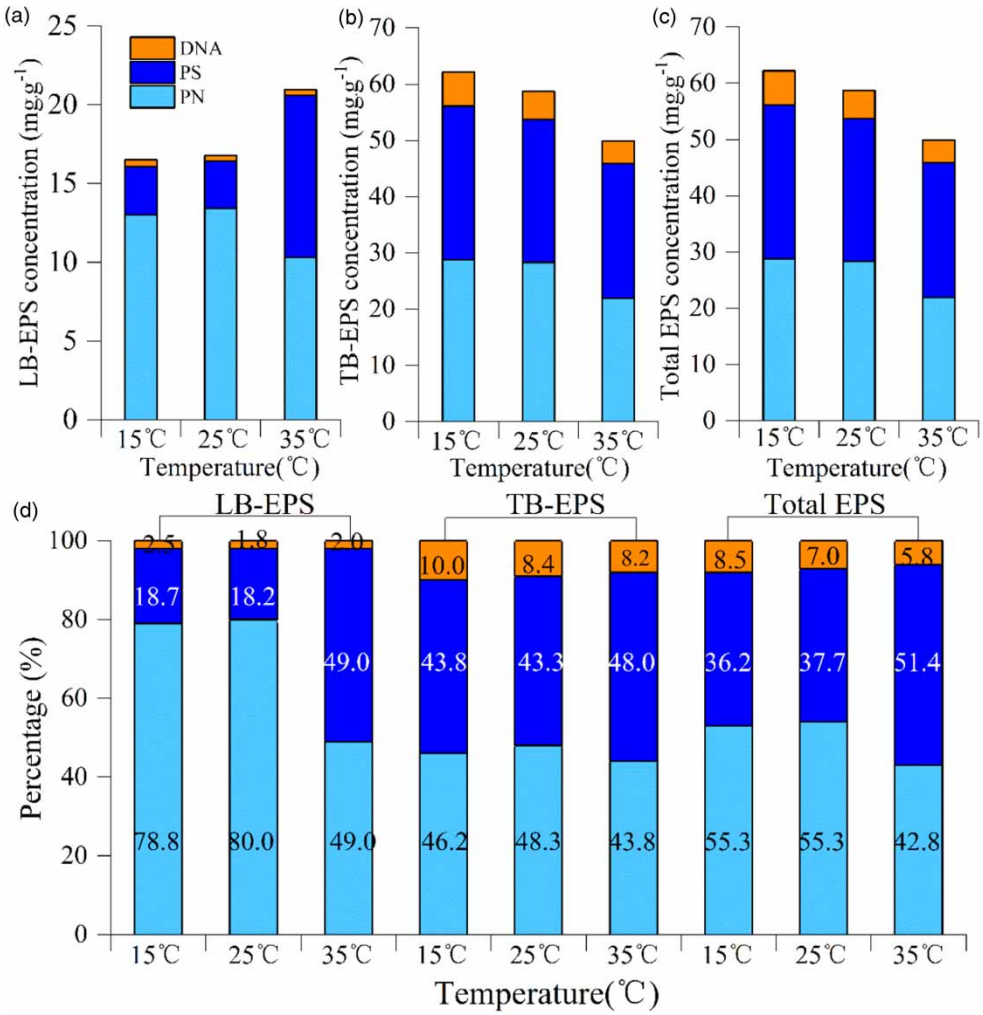

Figure 4 | Effect of temperature on the average contents of DNA, PS and PN over 100 cycles in (a) LB-EPS, (b) TB-EPS and (c) total EPS at the end of nitrification stage. (d) Effect of temperature on the average percentage of DNA, PS and PN over 100 cycles in the three different EPS fractions at the end of nitrification stage.

PN of EPS in raw sludge decreased when the temperature increased from 25 °C to 65 °C.

For PS, a similar pattern of variation was observed in total EPS and LB-EPS, with concentrations initially slightly decreasing from 28.5 and 3.1 mg/g MLSS at 15 °C, respectively, to 28.2 and 3.0 mg/g MLSS at 25 °C, respectively. Contents then increased to 36.5 and 10.3 mg/g MLSS at 35 °C, respectively. These results are in agreement with the reported findings of Gao *et al.* (2013) and Muñoz Sierra *et al.* (2018). In contrast, the PS content of TB-EPS decreased gradually from 27.3 mg/g MLSS at 15 °C to 24.0 mg/g MLSS at 35 °C, which was similar to findings reported by Ping *et al.* (2018). However, previous studies have also obtained opposite results. For example, Wang *et al.* (2010) found that PN and PS contents of EPS in supernatant all increased under lower temperatures (decreased from 26 °C to 8 °C).

Figure 4(d) presents the percentage of PN, PS and DNA components in the three assessed EPS fractions under different reaction temperatures. It can be seen that PN accounted for between 46.2 and 80.0% of the different fractions of EPS at 15 and 25 °C, followed by PS (18.2–43.8%) and DNA (1.8–10.0%), indicating that PN was the main component at these two temperatures. Cho *et al.* (2018) also found that PN accounted for between 65.8 and 72.2% at four different temperatures (15 °C, 20, 25, and 35 °C).

However, a discrepancy was observed when the reactor temperature increased to 35 °C. PS was the dominant component of the three assessed fractions of EPS (48.0–51.4%), while PN was the second largest component (42.8–49.0%) and DNA was the smallest component (2.0–8.2%). Furthermore, we found that although PN and PS abundances changed significantly with temperature variation, their

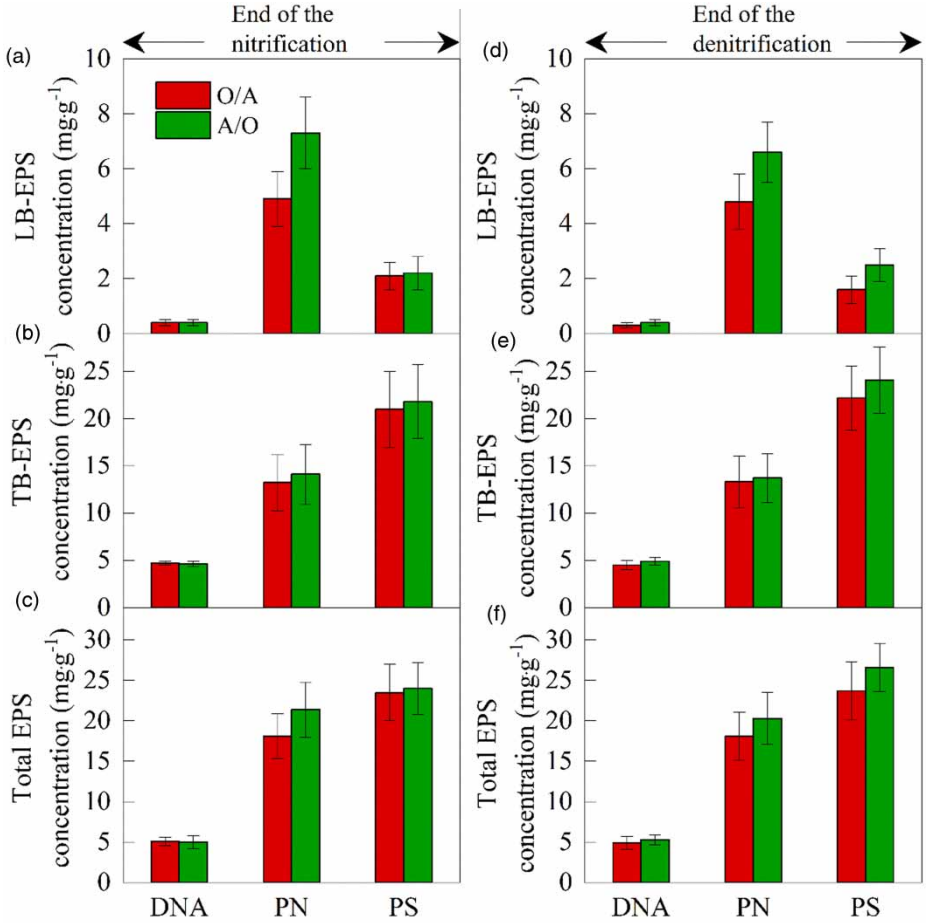

Figure 5 | Effect of operating modes on the average contents of DNA, PN and PS over 100 cycles in LB-EPS, TB-EPS and total EPS at the end of nitrification and denitrification stages.

overall sum changed negligibly with increased temperature, remaining stable in the range of 90.0–98.2% of total EPS. This was due to microbial catabolism, with PN and PS utilized by microbes in the biological reaction process, while at the same time microbes produced more PN and PS due to increased anabolism.

Different components were dominant with increase in the reactor temperature from 15–25 °C to 35 °C, due to nitrification and nitritation. $R_{15\,^{\circ}C}$ and $R_{25\,^{\circ}C}$ reactors maintained the nitrification stage, with NAR values less than 6.3% and 25.1%, respectively. This indicates that heterotrophic bacteria, ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) coexisted in activated sludge. The $R_{35\,^{\circ}C}$ reactor maintained the nitritation process with a stable NAR value of 92.4%, suggesting that heterotrophic bacteria and AOB were the dominant microbes in activated sludge (Ge *et al.* 2015). Therefore, different temperatures resulted in differences in the reactor microbial community, inducing different PN, PS and DNA proportions in the three fractions of EPS.

In addition, it can be pointed out that the cycle time of $R_{15\,^{\circ}C}$, $R_{25\,^{\circ}C}$, and $R_{35\,^{\circ}C}$ was 340, 250 and 160 minutes, respectively. This was attributed to the three reactors possessing three different nitrification rates. In detail, the three-temperature experiments were based on two aspects. One was that the same initial substrate concentrations were used in three reactors. The other was that the three reactors can finally achieve complete nitrification and denitrification. Under these two preconditions, the three reactors were operated at three temperatures. The nitrification rate increased with the increasing temperature, resulting in the high temperature reactor having the shortest cycle time.

Effect of operating mode on the contents of PS, PN and DNA in different fractions of EPS

Quantitative differences in components of the three EPS fractions under two different operating modes are shown in Figure 5. At the end of the nitrification stage, the PN

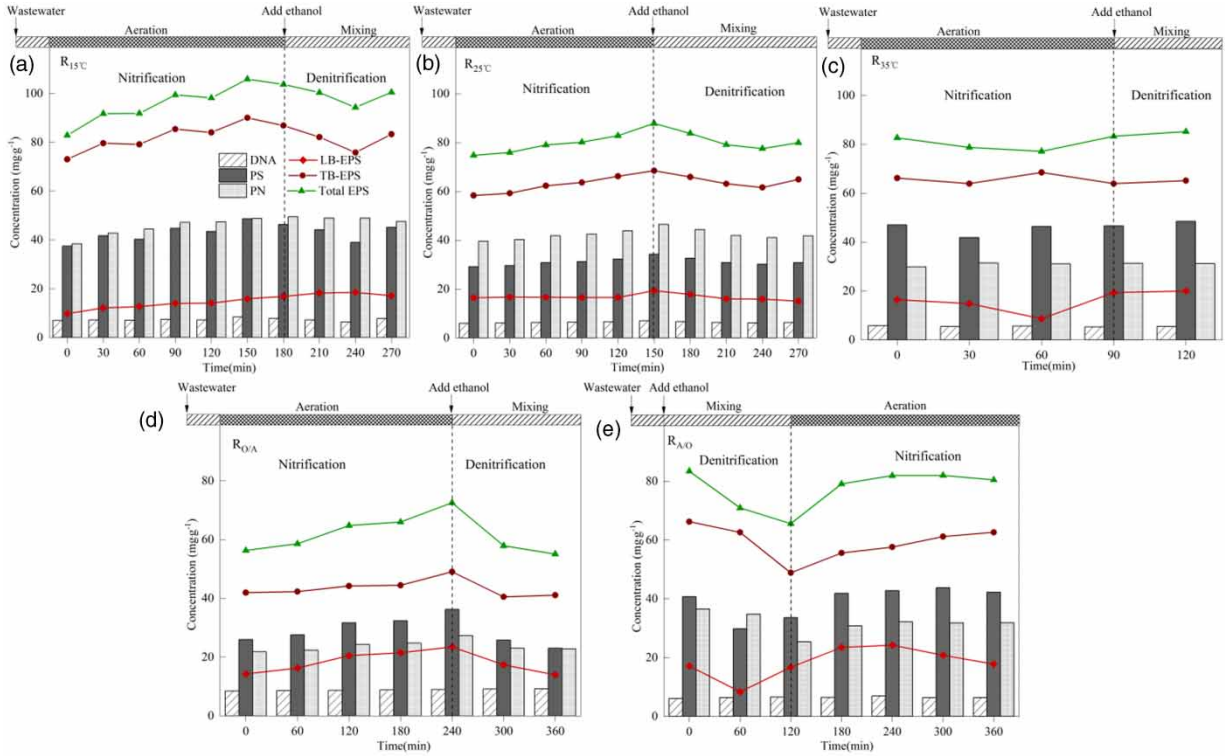

Figure 6 | Variation in three kinds of EPS and their components during a typical cycle (75th cycle) under different temperatures and operating modes: (a) $R_{15\,°C}$; (b) $R_{25\,°C}$; (c) $R_{35\,°C}$; (d) $R_{O/A}$; (e) $R_{A/O}$.

content of total EPS and LB-EPS in the $R_{A/O}$ system was 1.2 and 1.6-fold higher than in the $R_{O/A}$ system, respectively. At the end of the denitrification stage, the PS content of LB-EPS in the $R_{A/O}$ system was 1.5-fold higher than in the $R_{O/A}$ system. Therefore, the operating modes had significant effects on the contents of PS and PN in EPS. Although the contents of PN, PS and DNA in the $R_{A/O}$ reactor were higher than in the $R_{O/A}$ reactor, there was no obvious relationship observed between the operating mode and the dominant component in all three fractions of EPS.

In addition, the dominant components in the three fractions of EPS were significantly different. In total EPS and TB-EPS, PS accounted for 49.9% and 54.9%, PN accounted for about 39.7% and 33.5%, and DNA accounted for 10.5% and 11.6%, respectively, showing that PS was the dominant component. There have been relatively few previous studies reporting that PS is the main component of EPS. Wang *et al.* (2018) reported that PS was the dominant EPS fraction, accounting for 56.0% of EPS in activated sludge in an aspartic acid system using the model substrates of Mono-Pros. Wei *et al.* (2017) also found that PS was the most abundant component, accounting for 69.1% during the nitritation phase in a reactor operated under alternating anoxic and aerobic conditions.

For LB-EPS, PN was the most significant component. It accounted for 70.3%,which was in good agreement with the previous findings of various studies (de Oliveira *et al.* 2018; Han *et al.* 2018a; Dasgupta *et al.* 2019). Nevertheless, opposite results have also been reported. Shao *et al.* (2019) found that the LB-EPS component of suspended flocs and biofilms contained almost no PN content during nitrification and nitritation processes. This phenomenon was probably caused by increasing ammonia concentrations (from 100 mg/L to 800 mg/L), allowing more cations to be neutralized by negatively charged amino acids in LB-EPS (Laspidou Chrysi & Rittmann Bruce 2002). Basuvaraj *et al.* (2015) reported that TB-EPS was found to contain an abundance of PN, whereas the PS content of LB-EPS was larger in lab-scale systems. This was probably due to differences in the abundance of *Zoogloea*, which were rare in their research but were abundant in the present study.

The change in EPS fractions and components in a typical SBR cycle

In order to further investigate the variation of EPS and its components, a typical reactor cycle (75th cycle) was assessed during nitrification and denitrification. As shown

in Figure 6, three kinds of EPS and their components augmented during the nitrification process and then declined during the denitrification process in $R_{15\,°C}$, $R_{25\,°C}$, $R_{A/O}$ (25 °C) and $R_{O/A}$ (25 °C). The variation in three kinds of EPS and their components during denitrification were consistent with the findings of Ding *et al.* (2019), when the temperature was controlled to 25–27 °C. Stable and significant variation was not obtained in the $R_{35\,°C}$ system, implying that the variation in three kinds of EPS and their components was not influenced by operating mode but by operating temperature.

Moreover, the variation in three kinds of EPS and their components during nitrification and denitrification may be attributed to the change in DO in the external environment (Wei *et al.* 2017; Ding *et al.* 2019). Activated sludge was transferred from an oxic to an anoxic state, which triggered the absence of lipid carriers, playing a crucial role in the release of exo-polysaccharides and resulting in EPS reduction (Morgan *et al.* 1990). In addition, EPS serve as an energy source and are utilized by denitrifying bacteria in the anoxic process, further resulting in EPS reduction (Hoa *et al.* 2003).

While there are a limited number of studies reporting the variation in three kinds of EPS and their components during nitrification and denitrification, similar research has assessed EPS in simultaneous nitrification and denitrification (SND) processes. For example, Kong *et al.* (2016) found that the variation in EPS and its components was related to the relative abundance of nitrifying bacteria, such as AOB and NOB. It was found that the relative abundance of nitrifying bacteria and some denitrifying bacteria reduced, causing a decline in EPS contents during the SND process. Similar results were also reported by Han *et al.* (2018b), who found that the PN and PS content of EPS was reduced in the SND process. Therefore, it is important to further explore the relationship between variations in EPS and its components in reactor nitrification and denitrification stages.

CONCLUSIONS

The results of this study demonstrate that both temperature and operating mode exerted significant effects on the EPS content. Lower temperatures (15 °C) enhanced the contents of total EPS and TB-EPS, especially the PN and DNA contents, while reducing the PS content. Higher temperatures (35 °C) increased the content of LB-EPS, especially the PS content. In addition, higher contents of LB-EPS, TB-EPS, total EPS and their components were secreted in the $R_{A/O}$ system. Furthermore, in both $R_{A/O}$ and $R_{O/A}$ systems, PS dominated in TB-EPS and total EPS fractions, while PN dominated in the LB-EPS fraction.

Additionally, in the $R_{15\,°C}$, $R_{25\,°C}$, $R_{O/A}$ and $R_{A/O}$ reactor systems, three kinds of EPS and their components showed a consistent pattern of variation during the processes of nitrification and denitrification. Higher contents were secreted during the nitrification process, while lower contents were secreted during the denitrification process. In contrast, the contents of three kinds of EPS and their components fluctuated in the $R_{35\,°C}$ system.

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DATA AVAILABILITY STATEMENT

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

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