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# Role of extracellular polymeric substances on nutrients storage and transfer in algal-bacteria symbiosis sludge system treating wastewater

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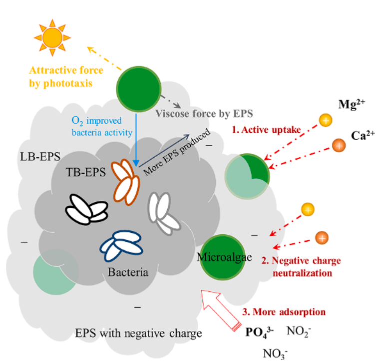
## HIGHLIGHTS

- First study to reveal the role of EPSs on nutrients storage and transfer in ABSS.
- The EPS in ABSS system performed better than that of control for nutrients storage.
- $Ca^{2+}$ and $Mg^{2+}$ uptake by microalgae partially neutralized electronegativity of EPSs.
- Microalgae led to an increase of both EPS content and PSs compose.
- Both increased adsorbability and content of EPS contributed to nutrients storage.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

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## ABSTRACT

This study reported the role and significance of extracellular polymeric substances (EPSs) on nutrients storage and transfer in an algal-bacteria symbiosis sludge (ABSS) system for wastewater treatment, and the novel algae-based sequencing batch suspended biofilm reactor (A-SBSBR, Ra) was selected as model of ABSS system. Results showed that compared to conventional SBSBR, the EPS of Ra performed better storage for $NO_2^-$-N, $NO_3^-$-N, total phosphorus and $PO_4^{3-}$ -P, with increase ratios of 43.7%, 36.0%, 34.1% and 14.7% in sludge phase and 174.0%, 147.4%, 150.4% and 122.0% in biofilm phase, respectively. The analysis of mechanisms demonstrated that microalgae active transport and uptake for divalent cations could enhance their local concentrations around ABS flocs and partially neutralized negative charge of EPSs, and more anions related to nutrients were absorbed in EPSs. Moreover, $O_2$ produced by microalgae photosynthesis enhanced bacteria activity and improved the production of EPSs in both sludge and biofilm phases.

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## 1. Introduction

Recently, the algal-bacteria symbiosis system has been proved as a potential popularization and application prospects for advanced wastewater treatment (Meng et al., 2020; Saravanan et al., 2021; Wang et al., 2021), attributing to its high-efficiency for nutrients removal and recovery but low energy consumption and carbon dioxide ($CO_2$) emissions (Ji et al., 2018; Tang et al., 2018a, 2016). Unfortunately, poor settleability of microalgae have limited the spread of algal-bacteria symbiosis system (Medina and Neis, 2007; Tang et al., 2018b, 2016). Focusing on above limitations, many efforts have been attempted to solve or alleviate them such as chemicals additions (Ryu et al., 2018; Guo et al., 2015; Yu et al., 2015) and biological flocculants (Tang et al., 2018b; Anbalagan et al., 2016; Dhaouefi et al., 2019). Compared to adding chemicals, settlement improvement of microalgae by sludge adsorption is environment friendly without residual chemical floc's potential pollution to either microalgae or water body (Saravanan et al., 2021; Guo et al., 2015). Moreover, the key operation conditions for AS system can overlap parameters needed for microalgae, such as temperature (18–30 °C) (Venkata Mohan et al., 2015), pH (7–9) (Pérez et al., 2017; Hidaka et al., 2017), and demand ratio between total nitrogen (TN) and phosphorus (TP) (8–45 g TN/g TP) (Cuellar-Bermudez et al., 2017). Thus, the algal-bacteria symbiosis sludge (ABSS) system, the mixed flocs of microalgae and AS, have the advantages of AS and traditional algal-bacteria symbiosis systems.

However, there still exists some key limiting factors for long-term operation of ABSS system to treat wastewater. Firstly, microalgae cells can be damaged under a higher hydraulic disturbance strength (>10 W/$m^3$ water), and then the ABSS structure is destroyed (Souza et al., 2019). Secondly, the growth rate of microalgae can be inhibited with solar decrease caused by high turbidity of sludge and wastewater, leading to lack of luminous energy for microalgae utilization (Meng et al., 2019; Tang et al., 2018b). Thirdly, the aeration process needed in AS system can restrain the photosynthetic efficiency of microalgae by product inhibition (Zhang et al., 2020a; Saravanan et al., 2021). In addition, the multiplication rate of bacteria is more rapid than that of microalgae (2–4 times, such as *Pseudomonas* sp. in comparison with *Chlorella* sp.), resulting in a different retention time demand (Manheim et al., 2019).

In order to possibly alleviate the problems above, a novel algae-based sequencing batch suspended biofilm reactor (A-SBSBR) has been built successfully in the previous studies of the authors (Tang et al., 2018a, 2018b). The suspended carriers (i.e., the density of carriers is lower than that of water) have been added into an ABSS system based on sequencing batch reactor to form A-SBSBR (Tang et al., 2018a). Compared to conventional ABSS system, the floating carriers in A-SBSBR have different effects and roles with or without aeration. On one hand, floating carriers can get more optical energy for microalgae enrichment because of density difference between carriers and sludge at non-aeration period. On the other hand, carriers float on the top of water and sludge sinks to the bottom of the reactor without shading on carriers' surface. Moreover, floating carriers achieve sufficient substance exchange with wastewater, microalgae and bacteria at aeration period (Tang et al., 2018a). In addition, AS and microalgae biomass can be regulated and balanced by suspended sludge discharge (bacteria are predominant) and adherent biofilm carriers renewed (microalgae biomass is primary), respectively (Tang et al., 2018a, 2018b).

In AS system, microorganisms are wrapped in extracellular polymeric substances (EPSs), which are existed as microbial secretions and mainly composed of proteins (PNs) and polysaccharides (PSs), accounting for 50–72% of EPSs (He et al., 2016; Sheng et al., 2010). EPSs play important roles in maintaining structure stability and formation of sludge flocs, as well as transferring and storing nutrients and energy substrate, etc (Lin et al., 2014; Li and Yang, 2007; Sheng et al., 2010; Yan et al., 2015; Zhu et al., 2020). Generally, EPSs can be regarded as a dynamic double-layered structures, including the loosely bound EPSs (LB-EPSs) and the tightly bound EPSs (TB-EPSs), and TB-EPSs surround

**Table 1**
Basic operation parameters of Ra and Rc.

| Common parameters for Ra and Rc | | | | | | | | Extra parameters only for Ra | | |
|---|---|---|---|---|---|---|---|---|---|---|
| T (°C) | Operation cycle (h) | | Aeration rate($m^3$ air/h) | Average DO (mg/L) | HRT (h) | SRT (d) | MLSS(mg/L) | Algae inoculation rate ($w_{algae}/w_{sludge}$) | Illumination time (h) | Illumination intensity (lux) |
| 24 ± 2 | Fill | 0.5 | 0.08 | 2.16–2.47 | 12 | 20 | 3000 | 1:4 | 12 (from 9:00 to 21:00) | 6000 |
| | Aeration | 4 | | | | | | | | |
| | Settling | 1 | | | | | | | | |
| | Withdraw | 0.5 | | | | | | | | |

**Table 2**
Synthetic ingredients of simulated domestic wastewater.

| Name | Content (mg/L) | Name | Content (mg/L) | Name | Content (mg/L) | Name | Content (mg/L) |
|---|---|---|---|---|---|---|---|
| Glucose | 200 | $K_2HPO_4{\cdot}3H_2O$ | 38 | $H_3BO_3$ | 2.86 | $CuSO_4{\cdot}5H_2O$ | 0.08 |
| Starch | 200 | $MgSO_4{\cdot}7H_2O$ | 50 | $MnCl_2{\cdot}4H_2O$ | 1.86 | $Co(NO_3)_2{\cdot}6H_2O$ | 0.05 |
| $NaHCO_3$ | 300 | $CaCl_2$ | 5 | $ZnSO_4{\cdot}7H_2O$ | 0.22 | | |
| $NH_4Cl$ | 155 | | | $Na_2MoO_4{\cdot}2H_2O$ | 0.39 | | |

the cells (Li and Yang, 2007; He et al., 2021). Till now, many researches have focused on the effects of EPSs for nutrients or other pollutants removal during wastewater treatment process (Li et al., 2021; Wang et al., 2020b), proving that the content, compose and proportion of EPSs are key factors to keep the activities of microorganisms (Xu et al., 2020; Sheng et al., 2010; Yan et al., 2015; Yu et al., 2021). Thus, it is hypothesized that EPSs can also make a certain effect on nutrients storage and transfer during wastewater treatment in ABSS system.

As EPSs may also play a crucial role in nutrients transfer in an ABSS system, but the key functions of them in realizing nutrients storage and transfer among microalgae, bacteria and substrate have not been reported. Whether the interaction between microalgae and bacteria could affect the characteristics of EPSs or not is also unclear. This study selected A-SBSBR as model to investigate the role of EPSs on nutrients storage and transfer in ABSS system, which contains both sludge phase and biofilm phase. And the storage types and contents of nutrients in EPSs of either sludge and biofilm phase were clarified, as well as the mass transfer process. Meanwhile, microalgae in biofilm and sludge phases obtained different light condition, so the content discrepancy of EPSs in two phases related to the changes of microalgae growth were identified. Based on the results, the possible mechanisms for nutrients storage and transfer in EPSs of ABSS system were proposed. The outcome of this study will establish some fundamentals that permit on the exploration of novel ABSS system for the advanced wastewater treatment and further practical application of ABSS system, as well as the role of EPSs in nutrients removal and symbiosis in ABSS system.

## 2. Materials and methods

### 2.1. Experimental set-up and reactor operation

The bench-scale experiments were conducted in 2 cylindrically glass-made SBRs with the depth of 60.0 cm, the diameter of 15.0 cm and the working volume of 8.0 L. The control group was inoculated by AS, named Rc. And the experiment group was inoculated by the mixture of AS and microalgae with weight ratio of 4/1 (Tang et al., 2018a), named Ra. The ratio of AS to microalgae biomass could achieve a stable ABSS system. The detailed operation parameters were listed in Table 1. Algae biomass was put into SBR to form algae-SBR (A-SBR) and then 150 suspended carriers were put into A-SBR to form A-SBSBR after stable operation of former. When the ABS biofilm was formed and carriers with biofilm were replaced periodically, the A-SBSBR (Ra) was built successfully and then got into operation for 80 days (from Day 70–150). It is noting that all carriers could be circularly utilized, which was beneficial for accommodation and rapid growth of microorganism.

### 2.2. Influent and inoculum

The synthetic ingredients of simulated domestic wastewater were listed in Table 2. The theoretical and actual concentrations of $NH_4^+$-N, TN, $PO_4^{3-}$-P, TP and COD were 40, 40, 5, 5 and 400 mg/L and 42.4, 43.3, 4.8, 4.9 and 377.4 mg/L, respectively. The inoculum of aerobic sludge and algae were both obtained from the engaged and spare secondary sedimentation tank of a local wastewater treatment plant, Harbin, China, respectively. The detailed cultivation methods for the inoculums of microalgae and aerobic sludge were the same as previous studies (Tang et al., 2018a, 2016).

### 2.3. Analytical methods

#### 2.3.1. Extraction and analysis of EPSs

The heat method was employed to extract EPSs (He et al., 2016; Li and Yang, 2007). And the specific details were shown as a flow chart in Supplementary Materials.

#### 2.3.2. Microalgae biomass analysis

Chlorophyll *a* (Chl-a) content and related measurement methods were based on its linear relationship with microalgae biomass under stable environment conditions. And its concentration was measured at four wavelengths: 750 nm, 663 nm, 645 nm and 630 nm by ultraviolet spectrophotometry (Tang et al., 2018b). According to the main microalgae community structure and species in ABSS system (Wang and Wang, 1984), the relational expression between Chl-a and microalgae biomass can be listed as follows:

$$b_a = 204 \times b_{Chl-a} \tag{1}$$

where $b_a$ is the content of microalgae biomass (mg/L) and $b_{Chl\text{-}a}$ is the content of Chl-a (μg/L).

Considering that the Chl-a content is affected by the quantities of selected biofilm carrier, so the total biomass of microalgae ($B_a$, mg/L) in biofilm can be expressed by equation as follows:

$$Ba = 204 \times bChl - a \times N/n \tag{2}$$

where $N$ is the total quantities of carriers and $n$ is the quantities of selected carriers for Chl-a analysis. Thus, the contents of microalgae biomass in biofilm ($M_a$, mg) can be calculated by the following equation:

$$M_a = 204 \times bChl - a \times N/n \times V \tag{3}$$

where $V$ is the Chl-a extraction volume (L).

According to the above equation, the total quality of bacteria in biofilm ($M_s$, mg) can be obtained, the details are listed as follows:

$$M_s = M_T - M_a = M_{T1} - M_{T2} - 204 \times bChl - a \times N/n \times V \tag{4}$$

where $M_T$ is the total quality of biofilm (mg). $M_{T1}$ and $M_{T2}$ are the qualities of carriers with biofilm and without biofilm, respectively.

#### 2.3.3. Chemical analysis

The measurements of $PO_4^{3-}$-P, TP, TN, $NH_4^+$-N, $NO_3^-$-N and $NO_2^-$-N were conducted by the Standard Methods (CEPB, 2002). And the contents of PSs and PNs were determined by sulfuric acid-phenol method (DuBois et al., 1956) and Lowry method (LOWRY et al., 1951), respectively. As microorganism biomass in biofilm existed as attached form, the nitrogen contents in EPSs of biofilm phase (mg N/g organic biomass in biofilm, mg N/g VSS) was firstly convert to volume content (mg N/L water, nitrogen quality in EPSs of biofilm divided by effective volume of system (8 L)). The contents of calcium ion ($Ca^{2+}$) and magnesium ion ($Mg^{2+}$) were determined by inductively coupled plasma optical emission spectrometry (Optima 8300, PerkinElmer Co., Ltd., USA). EPS is the outermost layer of sludge flocs and microorganisms are wrapped in it (Sheng et al., 2010; Li and Yang, 2007). Thus, the variation of sludge surface property can represent the characteristics of EPSs. In order to measure Zeta potential of EPSs in sludge and soluble microbial products (SMPs) in supernatant, the dilute solution of mixed liquor suspended solids at aeration period and the liquid supernatant at settling period

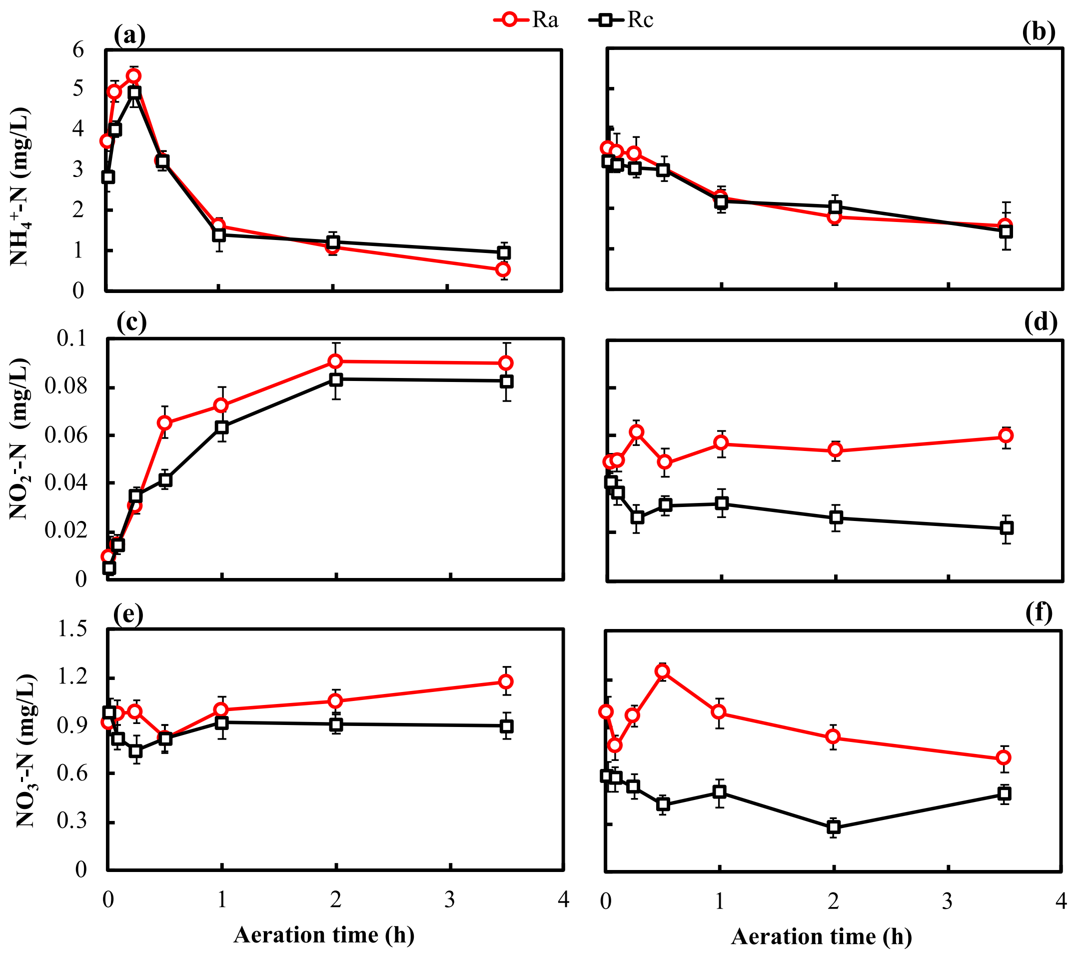


**Fig. 1.** Contents of $NH_4^+$-N, $NO_2^-$-N and $NO_3^-$-N in EPSs of sludge phase in Ra and Rc at aeration stage. (a), (c) and (e) LB-EPSs; (b), (d) and (f) TB-EPSs.

were sampled and then measured by a zeta potential analyzer (Zetasizer 3000, Malverin, England). The specific surface area of sludge was measured by laser particle size analyzer (MasterSizer 2000, Malvern, England).

## 3. Results and discussion

### 3.1. Nitrogen transformation and distribution in EPSs

#### 3.1.1. Variations of inorganic nitrogen in EPSs

Inorganic nitrogen conversion and mass transfer process in EPS of sludge phase at aeration period are shown in Fig. 1, the variation trends of three inorganic nitrogen, including $NH_4^+$-N, $NO_2^-$-N and $NO_3^-$-N, were similar in Ra and Rc. For content of $NH_4^+$-N, it peaked within 30 min and then deceased gradually, and was stable in 2 h in LB-EPS. Compared to $NH_4^+$-N, the contents of $NO_2^-$-N and $NO_3^-$-N in LB-EPS increased slowly (Fig. 1(c) and (e)), indicating that $NH_4^+$-N (the only nitrogen type) was mainly adsorbed at first and then gradually oxidized to $NO_2^-$-N and $NO_3^-$-N in LB-EPS. For TB-EPS, the contents of $NH_4^+$-N showed deceased trends firstly and then became stable, whereas the contents of both $NO_2^-$-N and $NO_3^-$-N were relatively stable, suggesting that TB-EPS was mainly served as a site for inorganic nitrogen transfer rather than conversion. These results proved that the EPSs in sludge phase of Ra and Rc showed similar characteristics and three types of inorganic nitrogen were affected by same action mode, which was possible due to EPSs produced by bacteria rather than microalgae (Zhang et al., 2020b; Martins et al., 2011). In addition, from Fig. 1, it can also be found that although the variation trends were similar, the contents of three inorganic nitrogen in Ra and Rc were different. The contents of $NO_2^-$-N and $NO_3^-$-N in Ra were higher than that in Rc, whereas the contents of $NH_4^+$-N showed an opposite change. Compared to Rc, $NO_2^-$-N and $NO_3^-$-N contents in Ra increased by 43.7% and 36.0%. The possible reason was that more positive ions may be adsorbed in EPSs and neutralize the electronegativity of EPSs, and then the contents of both $NO_2^-$-N and $NO_3^-$-N were increased, inversely, the contents of $NH_4^+$-N, positively charged, were decreased in Ra. It's worth noting that the opposite effects on changes of $NH_4^+$-N and $NO_2^-$-N (or $NO_3^-$-N) were consistent with their electrical property. As the difference between Ra and Rc was the existence and growth of microalgae,

**Table 3**
The contents of inorganic nitrogen in EPSs of sludge and biofilm phases for Ra and Rc at the end of aeration stage.

| Name | Phase | $NH_4^+$-N (mg/L) | | $NH_4^+$-N (mg/g EPS) | | $NO_2^-$-N (mg/L) | | $NO_2^-$-N (mg/g EPS) | | $NO_3^-$-N (mg/L) | | $NO_3^-$-N (mg/g EPS) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LB-EPS | TB-EPS | LB-EPS | TB-EPS | LB-EPS | TB-EPS | LB-EPS | TB-EPS | LB-EPS | TB-EPS | LB-EPS | TB-EPS |
| A-SBSBR | Sludge | 0.51 | 1.67 | 6.10 | 8.90 | 0.1 | 0.06 | 1.14 | 0.34 | 1.23 | 0.73 | 14.87 | 3.93 |
| | Biofilm | 0.18 | 0.53 | 7.39 | 6.72 | 0.05 | 0.04 | 1.99 | 0.45 | 0.48 | 0.44 | 19.32 | 5.55 |
| C-SBSBR | Sludge | 0.98 | 1.52 | 10.02 | 9.59 | 0.09 | 0.03 | 0.97 | 0.20 | 0.97 | 0.51 | 9.70 | 3.20 |
| | Biofilm | 0.18 | 0.25 | 10.35 | 5.64 | 0.01 | 0.01 | 0.81 | 0.24 | 0.2 | 0.13 | 11.16 | 2.98 |

**Table 4**
The contents of organic nitrogen in EPSs of sludge and biofilm phases for Ra and Rc at the end of aeration stage.

| Name | Phase | Organic nitrogen (mg/L) | | Organic nitrogen (mg/g EPS) | |
|---|---|---|---|---|---|
| | | LB-EPS | TB-EPS | LB-EPS | TB-EPS |
| A-SBSBR | Sludge | 9.36 | 15.47 | 110.94 | 82.30 |
| | Biofilm | 3.62 | 9.45 | 146.30 | 120.40 |
| C-SBSBR | Sludge | 11.60 | 14.25 | 118.57 | 90.04 |
| | Biofilm | 2.68 | 5.82 | 149.87 | 129.33 |

microalgae may improve cations adsorbing in EPSs and then influence the electronegativity of EPSs.

In order to further confirm conjecture of algae influence on the electrical property of EPSs, the contents of inorganic nitrogen in EPSs of biofilm phase were also analyzed. Moreover, the content of each nitrogen form in per gram EPS (mg N/g EPS) is shown in Table 3. For either total EPSs or unit mass of EPSs, the contents of $NO_2^-$-N and $NO_3^-$-N in Ra were all higher than that in Rc, but the contents of $NH_4^+$-N showed opposite change in biofilm phase. Compared to Rc, the contents of $NO_2^-$-N increased by 75.0% and 66. 7% in LB- and TB-EPSs of Ra, and $NO_3^-$-N increased by 23.6% and 67.6%, respectively. And the content improvements of $NO_2^-$-N and $NO_3^-$-N in total EPSs of biofilm in Ra were 174.0% and 147.4% higher than that in Rc.

The variations of inorganic nitrogen contents in EPSs proved that the introduction of microalgae could affect both content and distribution of inorganic nitrogen in EPSs of either sludge or biofilm phase. On one hand, microalgae could be adsorbed to EPSs and increased local $O_2$ content of EPSs by photosynthesis (Tang et al., 2016; Wang et al., 2020a), and then the conversion rate of $NH_4^+$-N may be improved, resulting in a higher difference of $NH_4^+$-N in EPSs of Ra (Fig. 1(a)). On the other hand, microalgae could take in metal ions such as $Ca^{2+}$ and $Mg^{2+}$ from liquid phase (Suresh Kumar et al., 2015; Liu et al., 2017), contributing to neutralization of negative charge in EPSs, and then more negatively charged ions such as $NO_2^-$-N and $NO_3^-$-N were stored in EPSs.

### 3.1.2. Variations of organic nitrogen in EPSs

The storage changes of organic nitrogen in EPSs are shown in Table 4. For unit mass of EPSs (mg organic nitrogen/g EPSs), the content of organic nitrogen in Ra was lower than that in Rc of either sludge or biofilm phase. For sludge phase, the content of organic nitrogen in unit mass in LB- and TB-EPSs of Ra was decreased 6.4% and 8.6% compared to that of Rc, respectively. For biofilm phase, the decrease ratio was 2.4% and 6.9%, respectively in LB- and TB-EPSs. For total EPSs (mg organic nitrogen/L), similar results were observed in sludge phase, but an opposite result was found in biofilm phase. Although the contents of organic nitrogen in EPSs were obviously higher than that of inorganic nitrogen, the changes of inorganic nitrogen still could reflect the removal nutrients, including transfer and storage, from wastewater. This is because $NH_4^+$-N was the primary nitrogen source in wastewater in present study. Even though some kinds of organic nitrogen were the main nitrogen source in water, these organic compounds were firstly bio-degraded to $NH_4^+$-N. As inorganic nitrogen existed much more than organic nitrogen in wastewater used in this work, the contents of organic nitrogen in EPSs can represent the organic matters such as PNs secreted by microorganisms (Yan et al., 2015; Sheng et al., 2010), which reflected some characters variation of EPSs. According to Table 4, the content difference of organic nitrogen between Ra and Rc was likely due to the variation of total EPS content and PNs ratio in EPSs, which would be discussed in the following section.

To sum up, transformation and storage variation of inorganic nitrogen in EPSs greatly influenced nitrogen removal in wastewater. And the addition of microalgae to AS system to form ABSS system could improve both conversion rate of $NH_4^+$-N and storage content of $NO_2^-$-N and $NO_3^-$-N in EPSs. Moreover, microalgae growth may affect ratios of organic nitrogen in EPSs by changing bacteria activity and its extracellular secretions characters. And the characters variation of EPSs would be further investigated in subsequent section.

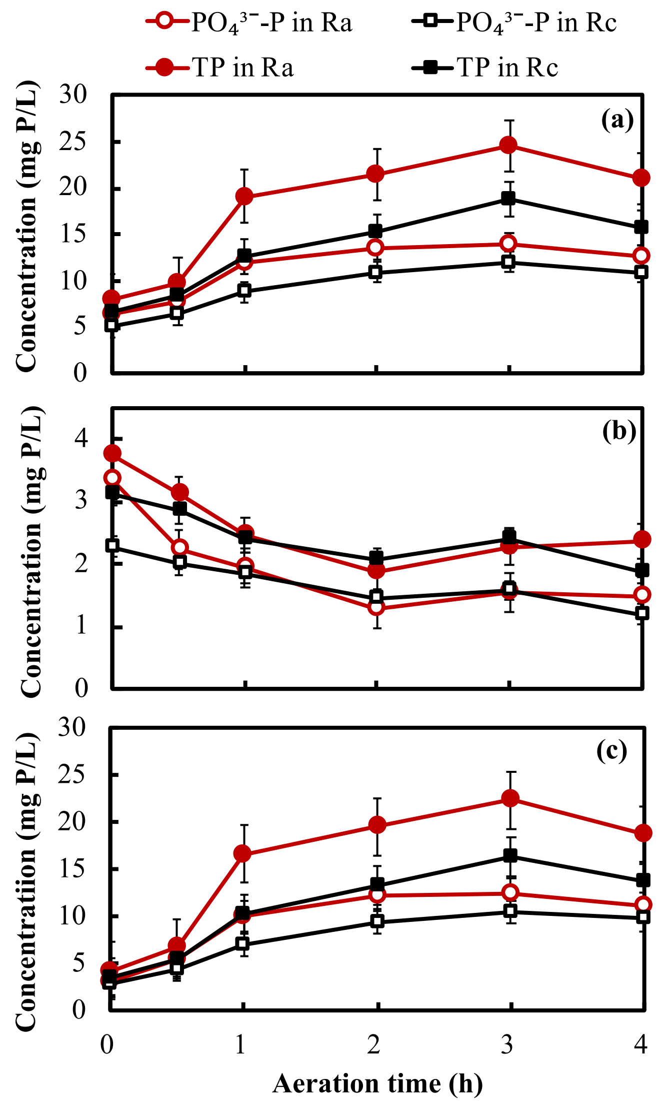


**Fig. 2.** Variations of TP and $PO_4^{3-}$-P in EPSs of sludge phase in Ra and Rc. (a) total EPSs, (b) LB-EPSs and (c) TB-EPSs.

### 3.2. Phosphorus transformation and distribution in EPSs

As shown in Fig. 2, the change trends of TP and $PO_4^{3-}$-P in EPSs of sludge phase in Ra and Rc were similar. The contents of TP and $PO_4^{3-}$-P firstly decreased with time going on and then exhibited stable in LB-EPSs with values of 2.4 and 1.5 mg/L in Ra and 1.9 and 1.2 mg/L in Rc, respectively (Fig. 2(b)). For TB-EPSs, the contents of TP and $PO_4^{3-}$-P firstly increased to 22.4 and 12.4 mg/L and then decreased to 18.7 and 11.1 mg/L in Ra, correspondingly, they firstly increased to 16.4 and 10.5 mg/L and then decreased to 13.8 and 9.8 mg/L in Rc, respectively (Fig. 2(c)). As TP and $PO_4^{3-}$-P contents in TB-EPSs was much more than those in LB-EPSs, phosphorus transfer trends in total EPSs was similar to TB-EPS (Fig. 2(a)). Compared to Rc, the contents of TP in total, LB- and TB-EPSs of sludge phase in Ra were increased by 34.1%, 25.4% and 35.3%, and the corresponding contents of $PO_4^{3-}$-P were increased by 14.7%, 23.7% and 13.5%. These results suggested that the TB-EPSs showed more significant phosphorus storage ability and contributed to major TP of total EPSs in either Ra or Rc, but EPSs of Ra showed a better phosphorus storage than that of Rc (Sheng et al., 2010; Wei et al., 2011). It's worth noting that the difference between TP and $PO_4^{3-}$-P in LB-EPSs

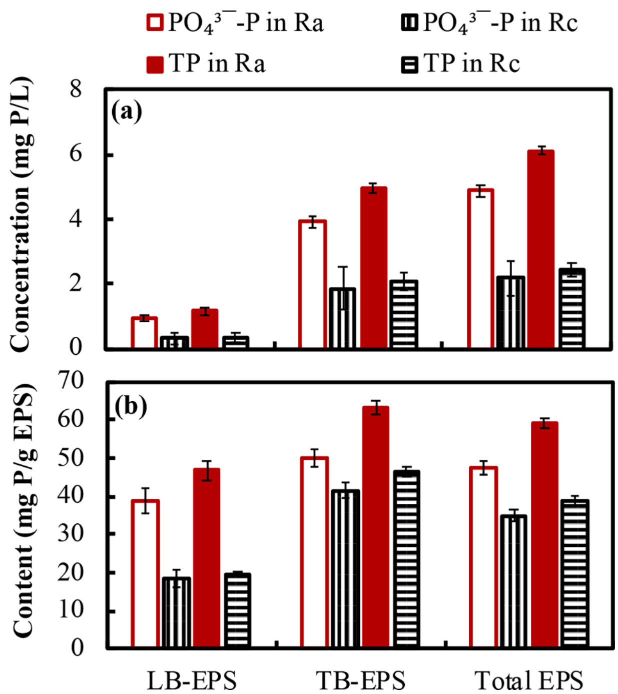


**Fig. 3.** Variations of TP and $PO_4^{3-}$-P in EPSs of biofilm phase in Ra and Rc at the end of aeration stage. (a) phosphorus in EPSs and (b) phosphorus in per unit mass of EPSs.

of Ra (0.88 mg/L) was close to that of Rc (0.69 mg/L) at the middle and end of aeration period. It indicated that $PO_4^{3-}$-P storage improvement was the main cause to TP enrichment in EPSs of Ra.

Similar results were also observed in the case of biofilm phase. At the end of aeration stage, TP contents in LB-, TB- and total EPSs of Ra were increased by 231.1%, 136.8% and 150.4%, respectively compared to that of Rc. And for $PO_4^{3-}$-P content, they increased by 191.7%, 109.8% and 122.0% in LB-, TB- and total EPSs of Ra (Fig. 3(a)). The possible reason can be attributed to the content increase of either EPSs or phosphorus in unit mass of EPSs. The phosphorus contents in unit mass of EPSs in Ra and Rc are shown in Fig. 3(b). For TP, they increased by 29.04%, 70.65% and 22.07% in unit mass of total, LB- and TB-EPSs in Ra compared to Rc, and the corresponding $PO_4^{3-}$-P contents increased by 14.43%, 50.31% and 8.14%. The content increase of phosphorus in unit mass of EPSs contributed to the enhanced phosphorus storage in EPSs of Ra. Moreover, whether the content of EPS could cause phosphorus content increase or not in Ra would be discussed in the next section. Based on above results, it can be concluded that phosphorus increment was mainly caused by $PO_4^{3-}$-P and its increase amount was more significant in LB-EPSs of biofilm in Ra.

These results suggested that the introduction of microalgae improved phosphorus storage in EPSs of sludge phase by promoting $PO_4^{3-}$-P adsorption in Ra. As $PO_4^{3-}$-P had negative charge, its content changes may be attributed to the same reason as $NO_2^-$-N and $NO_3^-$-N, which also carried negative charge. Moreover, the content increment of $PO_4^{3-}$-P in EPSs of either sludge or biofilm phase in Ra was much more than that of $NO_2^-$-N and $NO_3^-$-N, which may be related to different intake order of microalgae for $PO_4^{3-}$-P, $NO_2^-$-N and $NO_3^-$-N. Microalgae can excessively take in $PO_4^{3-}$-P over its actual demand and store phosphorus in the form of polyphosphates (poly-P) in cell (Solovchenko et al., 2016). Differently, both $NO_2^-$-N and $NO_3^-$-N were not priority nutrients for microalgae compared to either $NH_4^+$-N or $CH_4N_2O$ (Abdel-Raouf et al., 2012). Moreover, the difference between TP and $PO_4^{3-}$-P was probably attributed to the accumulation of organophosphorus such as phospholipid and nucleic acid fragments, which were also the components of EPSs (Yan et al., 2015; Nguyen et al., 2019).

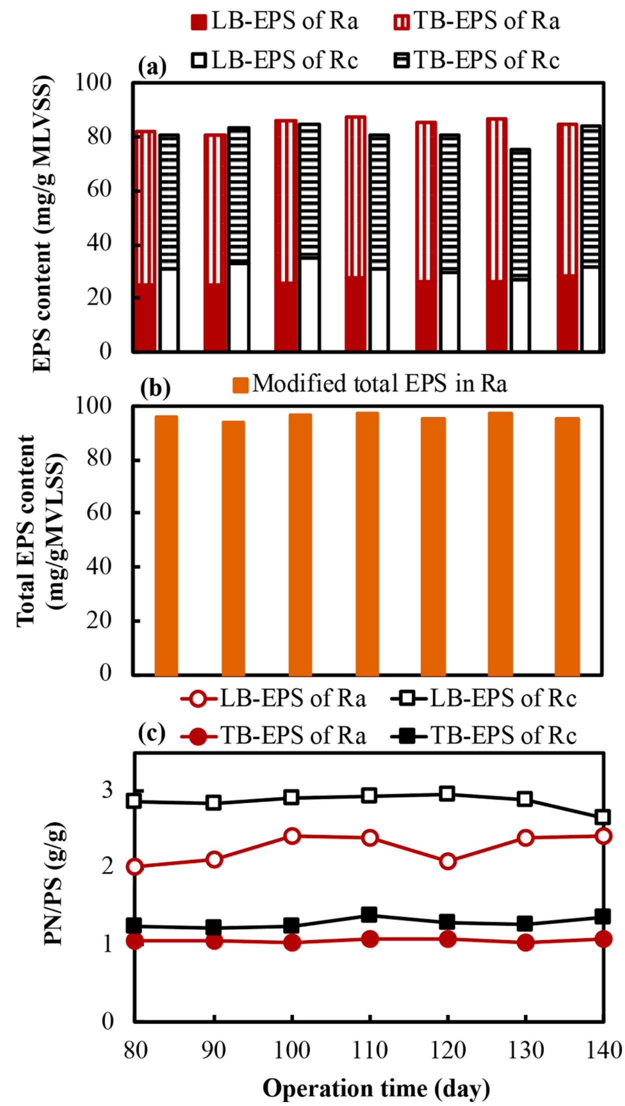


**Fig. 4.** Content and compose of EPSs in sludge phase of Ra and Rc. (a) measured data, (b) modified data and (c) the ratios between PNs and PSs.

### 3.3. *The characteristic variations of EPSs*

#### 3.3.1. *Content and compose variations of EPSs*

The contents of total EPSs in Ra and Rc were analyzed, as well as the ratios between PNs and PSs in LB- and TB-EPSs. The detailed variations of EPSs in sludge and biofilm phases are presented in Figs. 4 and 5. As shown in Fig. 4(a), the contents of total EPS in sludge phase of Ra was higher than that of Rc. Specially, EPSs was mainly produced by bacteria (Zhang et al., 2020b), and microalgae growth in Ra led to the increased MLVSS but little secretion of EPSs compared to bacteria with increase of both MLVSS and EPSs. Thus, the content of EPSs in sludge phase of Ra was needed to be modified according to Eq. (4), and the result is shown in Fig. 4(b), it was found that the contents of total EPSs in sludge phase of Ra had increase ratio of 18.4% compared to that of Rc. In addition, the composes of LB- and TB-EPSs were also changed in Ra, with an obvious decreased ratio between PNs and PSs compared to that in Rc (Fig. 4(c)). The increase of total EPSs and decrease of PNs/PSs ratios were positively related to the activities of bacteria (Huang et al., 2015), proving that the introduction of microalgae may increase the activity of AS.

As shown in Fig. 5(a) and (b), the modified content of EPSs in biofilm phase of Ra was significantly higher than that in Rc, with an increase ratio of 293.2%. And the PN/PS in LB- and TB-EPSs of Ra were all lower

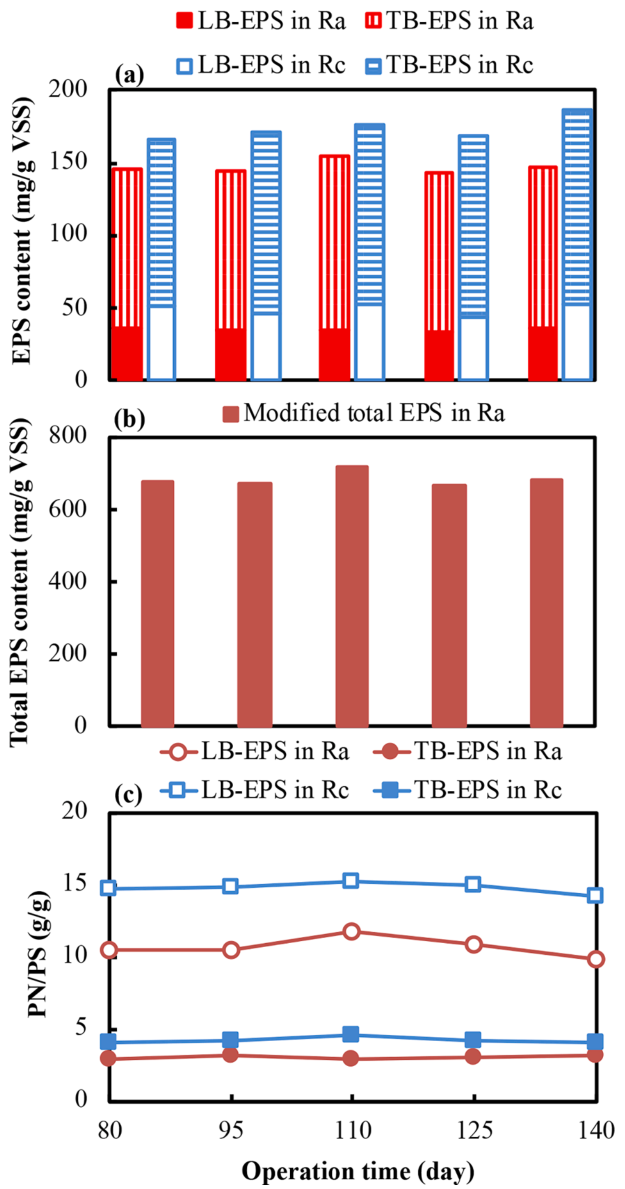


**Fig. 5.** Content and compose of EPSs in biofilm phase of Ra and Rc. (a) measured data; (b) modified data and (c) the ratios between PNs and PSs.

than that of Rc. As most of the organic nitrogen were existed as PNs, a lower content of organic nitrogen in EPSs of Ra (unit mass) mainly attributed to a lower PN/PS in either sludge or biofilm phase of Ra. However, the EPS in biofilm of Ra was much more and the total organic nitrogen in EPSs of biofilm in Ra was still more than that of Rc (Table 4). Thus, the content increase of total EPSs was another possible reason for nutrients storage improvement in EPSs of Ra.

Moreover, the content ratio between microalgae biomass and total organic biomass in biofilm was much higher than that in sludge phase, correspondingly, the increment comparison of EPSs in above two phases of Ra exhibited the same trend. In other words, more enrichment of microalgae in ABSS system could lead to more accumulation of EPSs produced by bacteria. This result may be attributed to the following two reasons. On one hand, $O_2$ produced by microalgae photosynthesis could promote the activities of aerobic bacteria and then the production of EPSs may be enhanced (Tang et al., 2016; Wang et al., 2020a). The contents of microalgae biomass showed positive correlation with the contents of EPSs. On the other hand, the microalgae phototaxis provided an external force to separate microalgae from sludge flocs, which was a relatively opposite force compared to viscous force contributed by EPSs (Meng et al., 2019; Sheng et al., 2010). And more EPSs provided more viscous force to keep balance with attractive force by light source on microalgae. Thus, more microalgae could be adsorbed by more EPSs, which is one of the most important factors to keep the stability of ABSS system.

**Table 5**
Variation comparisons of zeta potential and divalent cation Ra and Rc.

| | Sludge flocs of Ra | Sludge flocs of Rc | Supernatant of Ra | Supernatant of Rc | Influent |
|---|---|---|---|---|---|
| Zeta potential (mV) | −15.21 | −17.44 | −18.87 | −21.96 | / |
| Specific surface areas ($m^2$/g) | 0.08 | 0.05 | / | / | / |
| $Ca^{2+}$ (mg/L) | / | / | 0.17 | 0.22 | 0.48 |
| $Mg^{2+}$ (mg/L) | / | / | 0.55 | 0.62 | 1.62 |

Sampling time: Day 120; parallel sample quantity: 3.

In addition, the increased contents of nutrients were more in LB-EPSs, rather than in TB-EPS. This result may be due to adhesion location of microalgae in EPSs. According to ABSS formation process, stable AS was mixed with microalgae (Tang et al., 2018a, 2018b), and microalgae cells firstly contacted with LB-EPSs to be adsorbed. Meanwhile, microalgae cells exhibited phototaxis, an ability to orient themselves toward light sources to aid photosynthesis (Yu et al., 2019). This attractive force related to phototaxis was opposite to viscose force contributed by EPSs (Saravanan et al., 2021; Tang et al., 2018b). Besides, lightproof characteristic of EPSs was also against microalgae photosynthesis. Thus, microalgae cells were likely to locate in LB-EPSs when the ABSS system was formed and operated steadily. Based on above, it can be induced that the characteristics of LB-EPSs in Ra may be more significantly influenced by microalgae.

### *3.3.2. Key characteristic variations of EPSs*

The specific surface areas of sludge and zeta potentials of EPSs in Ra and Rc are shown in Table 5. Zeta potentials of SMP and EPSs in Ra and Rc were all negative, but the corresponding absolute values in Ra were lower than that in Rc. Specifically, SMP was mainly released from EPSs of either sludge or biofilm phase, and the surface charge of EPSs was mainly affected by LB-EPSs. Zeta potential represented the stability and settleability of colloid, and a lower absolute value of zeta potential meant less charge of EPSs (Su et al., 2014; Yousefi et al., 2020). Thus, the EPSs in Ra had less negative charge than that in Rc. In order to further confirm the reason for zeta potential variations, the concentration variations of $Ca^{2+}$ and $Mg^{2+}$ were observed in Ra and Rc. According to the ingredients of synthetic wastewater, the main inorganic cations in influent contained sodium ions ($Na^+$), $Ca^{2+}$ and $Mg^{2+}$. On one hand, $Ca^{2+}$ and $Mg^{2+}$ had more positive charge, and were more easily adsorbed by EPSs. On the other hand, $Ca^{2+}$ and $Mg^{2+}$ were essential elements for either cell wall synthesis of microalgae or formation of chlorophyll (Ouyang et al., 2018; Meng et al., 2020), which was more possibly taken in microalgae cells through active transport. As shown in Table 5, the concentrations of $Ca^{2+}$ and $Mg^{2+}$ in effluent of Ra were less than that of Rc, i.e., more $Ca^{2+}$ and $Mg^{2+}$ were moved to solid phase (sludge and biofilm) in Ra. As the pH of influent was neutral and the main anions was bicarbonate radical ions ($HCO_3^-$), the reduction of $Ca^{2+}$ and $Mg^{2+}$ was mainly due to the enhanced adsorption of EPSs by microalgae addition rather than precipitation. It meant that microalgae growth in ABSS system neutralized the electronegativity of EPSs. Moreover, the results of specific surface area also indeed proved above conclusion. Specific surface areas of sludge flocs in Ra was larger than that in Rc, indicating

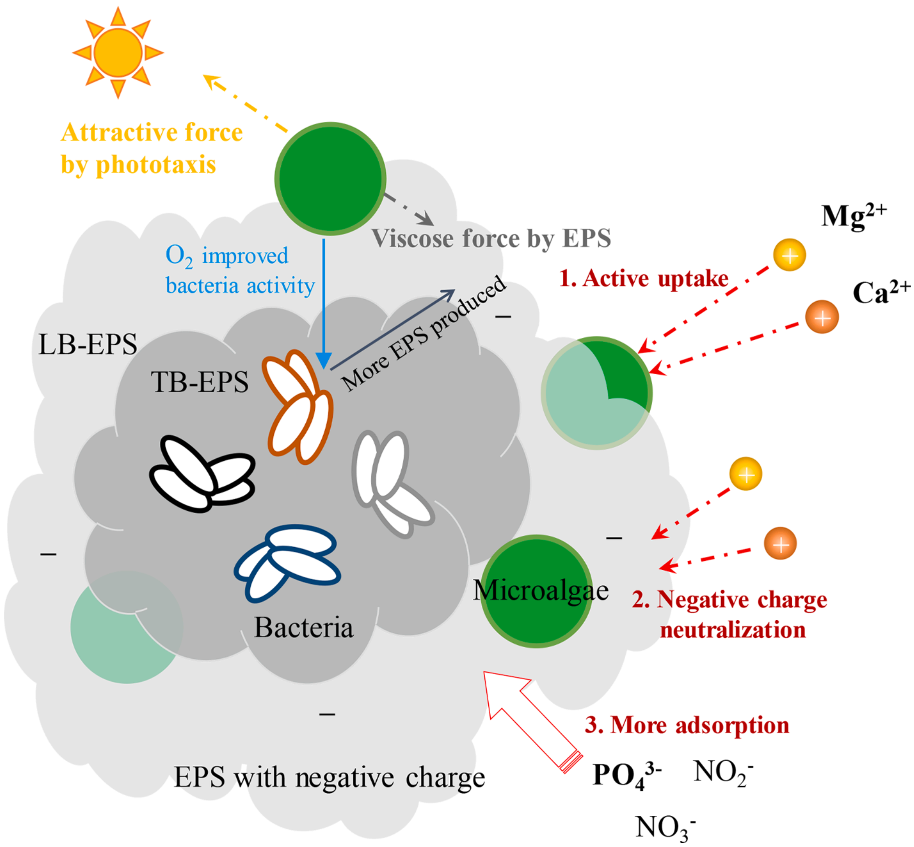


**Fig. 6.** The possible mechanisms for nutrients storage and transfer in EPSs of ABSS system.

that sludge in Ra had a larger contact area and laid a foundation for faster substance exchange with wastewater.

### 3.4. The mechanisms for nutrients storage and transfer in EPSs of ABSS system

Based on the findings of this work, the possible mechanisms for nutrients storage and transfer in EPSs of ABSS system were stated (Fig. 6). The EPS in either sludge or biofilm phase of ABSS system was still produced by bacteria, but the addition and growth of microalgae caused some function and characteristic changes of EPSs. Especially, the transfer, conversion and storage of nutrients in EPSs were changed. Firstly, microalgae could actively take in $Ca^{2+}$ and $Mg^{2+}$ for their own cell wall synthesis and chlorophyll formation (Ouyang et al., 2018; Meng et al., 2020). And active transport of $Ca^{2+}$ and $Mg^{2+}$ led to local concentrations increased in EPSs (Table 5). Secondly, EPSs around microalgae cells with negative charges showed more opportunities to get in touch with ions with positive charges, mainly $Ca^{2+}$ and $Mg^{2+}$, so the negative charges of EPSs were partially neutralized and the EPS of Ra showed lower zeta potential (Table 5). Thirdly, anions such as $PO_4^{3-}$, $NO_3^-$ and $NO_2^-$ in influent were absorbed more by EPSs in Ra (Figs. 1–3 and Table 3). In addition, more EPSs were secreted (Figs. 4 and 5), which may due to the improved bacteria activity influenced by more $O_2$ produced by microalgae photosynthesis (Tang et al., 2018; Wang et al., 2020a). More EPSs brought more viscous force to balance more opposite force by microalgae phototaxis, which may be one of the main foundations for higher ratio of microalgae and bacteria in biofilm phase. This study would provide some foundations for the development of microalgae domestication, novel wastewater treatment process based on ABSS developing and related wastewater pretreatment.

## 4. Conclusion

This work selected A-SBSBR as model to study the role of EPSs on nutrients storage and transfer in ABSS system treating wastewater. The related nutrients, including $NO_2^-$-N, $NO_3^-$-N, TP and $PO_4^{3-}$ -P in EPSs of Ra increased by 43.7%, 36.0%, 34.1% and 14.7% in sludge phase and 174.0%, 147.4%, 150.4% and 122.0% in biofilm phase. Partial electrical neutralization of EPSs contributed by microalgae's active uptake for $Ca^{2+}$ and $Mg^{2+}$ and increased EPSs content due to more $O_2$ production by microalgae photosynthesis were the possible mechanisms for the enhanced nutrients storage and transfer in EPSs of ABSS system.

### CRediT authorship contribution statement

**Cong-Cong Tang:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft. **Xinyi Zhang:** Visualization, Investigation, Writing - original draft. **Zhang-Wei He:** Investigation, Validation. **Yu Tian:** Supervision, Writing - review & editing. **Xiaochang C. Wang:** Project administration.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2021.125010.

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