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# Influence of operating conditions on extracellular polymeric substances and surface properties of sludge flocs

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ABSTRACT

Extracellular polymeric substances (EPSs) originating from the metabolism or lysis of microorganisms play important roles in biological wastewater treatment. It is necessary to understand the correlation between EPSs and the reactor operation and sludge-retention time (SRT) of the activated-sludge process to gain efficient operational strategies. Laboratory-scale completely mixed activated-sludge processes were used to grow the activated sludge with starch or glucose. Two modes of reactor operation, the S-mode and B-mode, were investigated. The results showed that polysaccharides and proteins were the main constituents in the EPSs. The percentage of polysaccharides (PSs) and proteins (PNs) in the loosely bound EPSs (LB-EPSs) and tightly bound EPSs (TB-EPSs) were 18–90% and 18–85%, respectively. The LB-EPS content was related to the operation modes and SRT. The LB-EPS contents in B-mode were triple that of S-mode, and the LB-EPS yield of sludge decreased at an increased SRT. The polysaccharides and proteins in the LB- and TB-EPSs were both dependent on the operation modes and SRT.



## 1. Introduction

Extracellular polymeric substances (EPSs) are metabolic products that accumulate on the surface of bacterial cells and are the representative components of the flocs in the activated-sludge process. Recently, some researchers have focused on extracting the EPSs, measuring the EPS content, and analysing the relative content of chemical components (Ge, Deng, Wang, Ma, & Liu, 2007; Ren, Xie, & Xing, 2010; Wolfstein & Stal, 2002). EPSs are composed of loosely bound EPSs (LB-EPSs) and tightly bound EPSs (TB-EPSs) (Li & Yang, 2007; Ramesh, Lee, & Hong, 2006). A variety of organic substances, such as polysaccharides (PSs), proteins (PNs), DNA, humic acid, and uronic acid were found in EPSs. The main chemical composition consisted of polysaccharides in the EPSs produced by bacteria growing in pure cultures, such as *Pseudomonas* sp. strain 1.15 (Cescutti, Toffanin, Pollesello, & Sutherland, 1999). Conversely, more recent studies suggested that proteins, rather than polysaccharides, were the predominant constituents in the EPSs extracted from mixed-culture systems, such as activated-sludge flocs, anaerobic granulation sludge, and biomembranes (Liu & Fang, 2003; Shih, Chen, & Wu, 2010; Yuan et al., 2011; Zhong & Wang, 2010).

Furthermore, some operating parameters were found to influence the formation of EPSs, such as the SRT, dissolved oxygen, and nutrient level (Liu & Fang, 2003; Orr, Zheng, Campbell, Mcdouqall, & Seviour, 2009; Papinutti, 2010; Wang et al., 2012). Past research showed inconsistent results for the relationship between the SRT and EPS formation. Some published results have indicated that the amount of EPSs increased at an increased SRT, whereas some researchers have reported the opposite results (Geesey, 1982; Wingender, New, & Flemming, 1999). Liao, Allen, Droppo, Leppard, and Liss (2001) suggested that the EPS content was independent of the SRT. The chemical nature of the wastewater may also influence the EPS constituents. Urbain, Block, and Manem (1993) reported that the EPS constituents varied widely among sludges obtained from seven municipal wastewater treatment plants. Considerable variations even occurred in the EPSs collected from the same plant but at different sampling times.

Analysing the EPS content and its chemical composition is often considered the basis of understanding the significant role of EPSs in natural aquatic ecosystems and biological wastewater treatment systems. For a biological treatment process, the SRT is an important operational factor in the removal of pollutants and in the minimisation of the amount of excess sludge (Choi, Lee, Lee, & Kim, 2008). Therefore, the main goal of this study was to investigate the effect of the operation mode and SRT on the formation, composition and matrix of EPSs and to compare the results with the samples from municipal wastewater treatment plants.

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**Table 1**
Operational conditions of the SBRs.

| SBR | B1 | B2 | B3 | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|---|---|
| Operation mode | | B-mode | | | S-mode | | | S-mode | |
| Culture | | Starch | | | Starch | | | Glucose | |
| Influent amount (L/d) | 0.32 | 0.16 | 0.08 | 1 | 1 | 1 | 1 | 1 | 1 |
| Influent COD (mg/L) | 6000 | 5300 | 5800 | 2000 | 900 | 500 | 2000 | 900 | 500 |
| MLSS (mg/L) | 1900 ± 140 | 2010 ± 170 | 2080 ± 170 | 2042 ± 150 | 2180 ± 160 | 2200 ± 150 | 2070 ± 96 | 2250 ± 98 | 2160 ± 112 |
| F/M (gCOD/gMLSS d) | 0.63 | 0.26 | 0.14 | 0.63 | 0.26 | 0.14 | 0.63 | 0.26 | 0.14 |
| SRT (d) | 5 | 10 | 20 | 5 | 10 | 20 | 5 | 10 | 20 |

F/M: food-to-microorganism ratio.

## 2. Materials and methods

### *2.1. Sludge samples*

Seed samples of microbial flocs were obtained from a full-scale activated-sludge treatment system: Stanley municipal sewage plant in Hong Kong, P.R. China. A total of six bench-scale sequencing batch reactors (SBRs; jacketed glass reactors of 1.6 L) were operated at two modes of operation and three different sludge retention times (SRTs; 5 d, 10 d, and 20 d). The cycle of three reactors (named S-mode) consisted of a 30 min fill, 18 h reaction, 2 h settle, 1 h withdrawal periods and 2.5 h idle for a total cycle time of 24 h. The other three SBRs (named B-mode) consisted of an 18 h reaction and 6 h idle, also for a total cycle time of 24 h. The reactors were fed synthetic wastewater, starch or glucose, respectively. The composition and concentrations of micronutrients in the feed were: $MgSO_4{\cdot}7H_2O$, 5.07 mg/L; $FeSO_4{\cdot}7H_2O$, 2.49 mg/L; $Na_2MoO_4{\cdot}2H_2O$, 1.26 mg/L; $MnSO_4{\cdot}4H_2O$, 0.31 mg/L; $CuSO_4$, 0.25 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.

The operating conditions included a temperature of 20 ± 2.0 °C, a pH of 7 ± 0.2 and a dissolved oxygen level of between 2.5 and 5.5 mg $O_2$/L.

### *2.2. Sludge sample pretreatment*

The sludge samples were rapidly transported and placed at a temperature of 4 °C for 1.5 h, and the floating debris was removed. A 50 mL aliquot of the mixed sample was centrifuged at a speed of 6000 × *g* for 5 min to finish sludge concentration. The sample was then washed twice at a temperature of 4 °C, with centrifugation at a speed of 2000 × *g* for 15 min to remove the EPS from the water. The buffer solution was made of 2 mM $Na_3PO_4$, 4 mM $NaH_2PO_4$, 9 mM NaCl and 1 mM KCl with a pH of 7.

### *2.3. Extraction and analysis of EPS*

The dewatered sludge pellet was resuspended in the buffer solution with several glass beads, sonicated at 20 kHz for 2 min, shaken horizontally at 150 rpm for 10 min, and sonicated again for an additional 2 min. The liquor was centrifuged at 8000 × *g* for 10 min to separate the solids and supernatant. The collected supernatant was regarded as the LB-EPS fraction of the sludge sample. The residual sludge pellet was resuspended in buffer solution, sonicated for 3 min, heated at 80 °C for 30 min, and then centrifuged at 12,000 × *g* for 20 min to collect the supernatant (Ge et al., 2007). The collected supernatant was regarded as the TB-EPS fraction of the sludge sample.

The analyses of the COD, MLSS and VSS were performed in accordance with standard methods (APHA, 1998). Both the LB-EPS and TB-EPS extractions were analysed using a TOC analyser (Shimadzu TOC-$V_{CPN}$ Total Organic Carbon Analyzer). The carbohydrate content in the EPS was measured using the anthrone method (Daniels, Hanson, & Philips, 1994), with D-glucose as the standard. A modified Lowry method (Daniels et al., 1994) was used to quantify the protein in the EPS using bovine serum albumin (BSA) as the standard. The pH and dissolved oxygen (DO) level in the reactor were determined using a pH meter and a DO meter, respectively. All of the sample analyses were performed in triplicate. The microbial observation was performed using a scanning electron microscope (SEM, S440, Leica, Wetzlar, Germany).

## 3. Results and discussion

### *3.1. Operating conditions of the SBRs and EPS production for all samples*

The sludge samples were fed with starch and glucose synthetic wastewater in the SBRs for 180 days. The conditions of the reactor operation are shown in Table 1. The stable operation of these SBRs was obtained with a high biomass retention and pollutant removal performance.

The range of the LB-EPS and TB-EPS production of the activated floc samples from the lab-scale cultivation and wastewater treatment plant is shown in Table 2. The total content of the EPSs varied from 6% to 17%. The LB-EPSs represented a small proportion of the total EPSs. LB-EPS levels varying within the range of 0.65–16.5% were found in the sludge samples at the different modes of operation, SRTs and culture conditions. However, the TB-EPS contents appeared to be much higher (>80% EPS) than the LB-EPS in this study. The PN/PS ratio varied with the operating conditions. The EPS content and composition of activated-sludge floc found in this study (Table 2) is consistent with other investigations (Frølund, Palmgren, Keiding, & Nielsen, 1996; Urbain et al., 1993). As shown in Table 2, the marked differences between each type of floc indicate the importance of the wastewater chemistry and operational variables in affecting the EPS content and composition.

**Table 2**
Qualitative analysis of EPS from all sludge samples.

| Sludge sample | LB (mg/gMLSS) | | | TB (mg/gMLSS) | | |
|---|---|---|---|---|---|---|
| | PS | PN | TOC | PS | PN | TOC |
| Starch culture | 0.31–13.9 | 0.52–6.68 | 0.41–7.88 | 33.3–116.9 | 28.1–111.9 | 45.9–80.2 |
| Glucose culture | 0.29–2.10 | 0.58–2.49 | 0.37–1.85 | 23.1–89.8 | 36.1–73.0 | 49.3–69.4 |
| Sewage treatment plant | 0.58–1.17 | 1.03–1.79 | 0.62–1.57 | 17.5–33.4 | 33.1–62.8 | 30.6–50.0 |

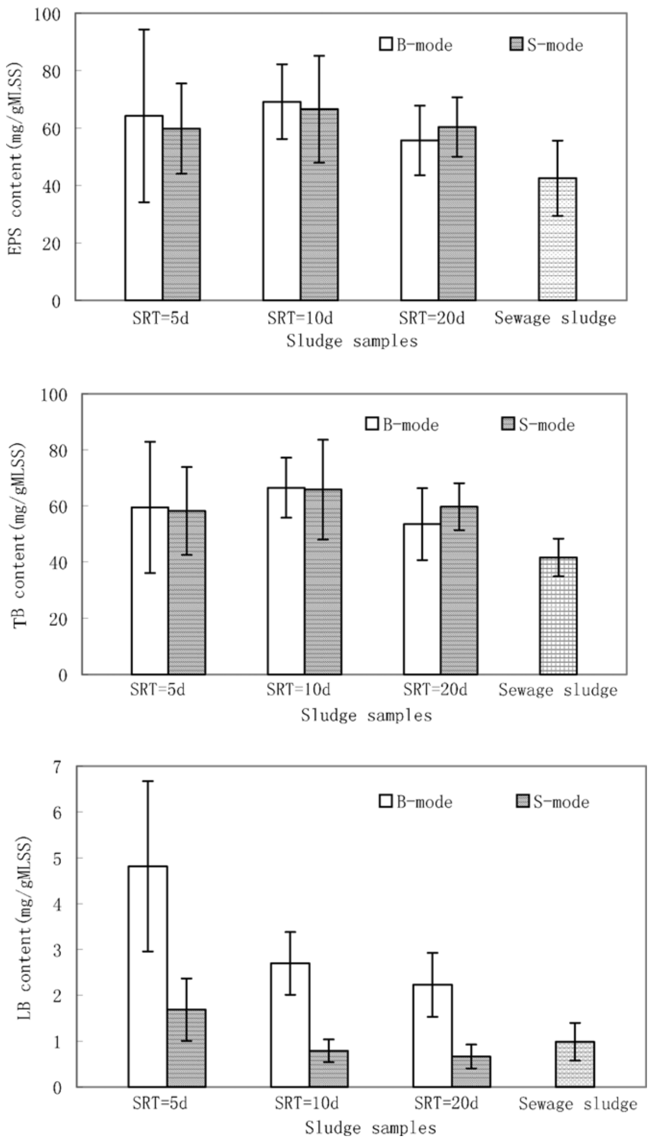


**Fig. 1.** Changes of EPS, TB-EPS and LB-EPS concentration by different operational modes and SRTs.

### 3.2. Effect of the operating conditions and SRT on the EPS content and composition

The influence of the modes of reactor operation and SRT on the EPS content is shown in Fig. 1a. No simple relationship between the modes of reactor operation and EPS content was observed in the sludge sample cultured with starch. The total quantities of EPSs were in the range of 40–80 mg/g MLSS, which did not change notably at a different SRT. When the SRTs were 5 d, 10 d and 20 d, the average value of the EPS productivity in B-mode was 64.3, 69.2 and 55.7 mg/g MLSS, respectively, and the EPS productivity in S-mode was 59.8, 65.8 and 60.4 mg/g MLSS, respectively.

The flocs matrix is likely to have a dynamic double-layered EPS structure of loosely bound EPSs (LB-EPSs) diffused from the tightly bound EPSs (TB-EPSs) that lie in the interior of the sludge flocs (Li & Yang, 2007; Poxon & Darby, 1997; Ramesh et al., 2006; Wingender et al., 1999). The results showed that more than 80% of the EPSs were TB-EPSs, and similar results were also observed for the content variation of the TB-EPSs and total EPSs under the different operating conditions. However, the LB-EPS content changed consistently with the mode of the reactor operation. The concentration of the LB-EPSs from the sludge in B-mode with metabolite accumulation was triple that of S-mode without metabolite accumulation and the LB-EPS content decreased at an increased SRT.

As is well known, metabolic products accumulate more readily in the outer layer of the cells in sludge in B-mode than in S-mode, thus increasing the yield of LB-EPSs. EPSs are a class of macromolecular materials secreted from the cells of microorganism. The low molecular weight polymers are mainly produced by bacteria in the logarithmic growth phase, which are representative components of the loosely bound EPSs (LB-EPSs). Conversely, high molecular weight polymers, the components of tightly bound EPSs (TB-EPSs), are chiefly formed in the stationary and decline phases. Therefore, the LB-EPS content decreased, and the TB-EPS content increased at increased SRTs.

### 3.3. Effect of the operating conditions and SRT on the PS and PN of EPSs

#### 3.3.1. Effect of the operating conditions and SRT on the PS and PN of LB-EPSs

The changes in the PS and PN components of sludge samples are shown in Fig. 2a and b. The PN was the principal component, and the PS was the second component of the EPS matrix in the flocs from the sewage treatment plant (Fig. 2a and b), which was consistent with the results of previous studies (Bura, Cheung, Liao, Finlayson, & Lee, 1998; Sponza, 2003). Therefore, in this investigation, only the PS and PN of the EPSs were compared. As presented in Fig. 2a and b, the change in the operating conditions and SRT resulted in considerable changes in the PS and PN of the EPSs, and the trends were similar to the influence of the operating condition and SRT on the LB-EPS. The PS and PN of the LB-EPS matrix were both higher in B-mode than in S-mode (Fig. 2a and b), and the contents of the two components both decreased at an increased SRT.

In addition, as shown in Fig. 2c, the PN/PS ratio changed with the variations of the operating mode and SRT. The PN/PS ratios of the LB-EPSs were higher in S-mode than in B-mode and increased with an increase in the SRT. A significant proportion of the metabolic products of the microorganism in the reactors cultured with starch can be present in the form of PSs. The accumulation of the metabolic products of the flocs was lower in S-mode than in B-mode, which resulted in the decline of the relative PS content in the samples from S-mode. Thus, the PN/PS ratio was higher in S-mode than in B-mode (Fig. 2c).

#### 3.3.2. Effect of the operating conditions and SRT on the PS and PN of TB-EPSs

The content and trend of the PS and PN variation in the sludge TB-EPSs from the reactors under different operating conditions and the SRT are illustrated in Fig. 3a and b. The PS productivity of the TB-EPSs was higher in the flocs grown in B-mode than in S-mode, and the PS content gradually declined with an increasing SRT, which was similar to the variation of the PS content of the LB-EPS at pervious discussion (shown in Section 3.3.1). However, a similar change was not observed for the PN in the TB-EPSs for which the levels were lower in B-mode than in S-mode and declined in early running, followed by slight growth, as reported by Liao et al. (2001).

The PN content of the TB-EPS was lower in the sludge samples from the B-mode reactors, whereas the PS content of the TB-EPS was the opposite. Hence, the PN/PS ratios of the TB-EPS from the flocs in B-mode were predictably lower than from the sludge grown in the S-mode reactor. When the SRT was shifted from 5 d to 20 d, the PN/PS ratio declined in the first five days and then resumed increasing again. Similar results were also revealed by previous research (Liao et al., 2001).

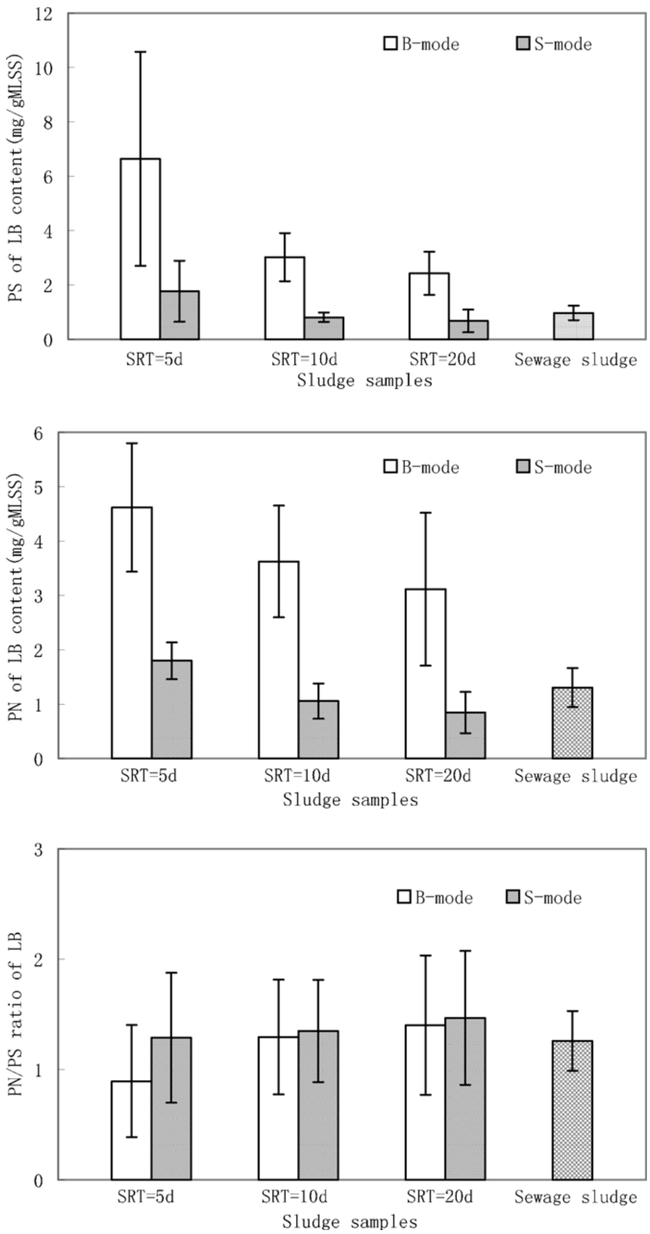


**Fig. 2.** Influence of modes of operation and SRT on chemical compositions of LB-EPS.

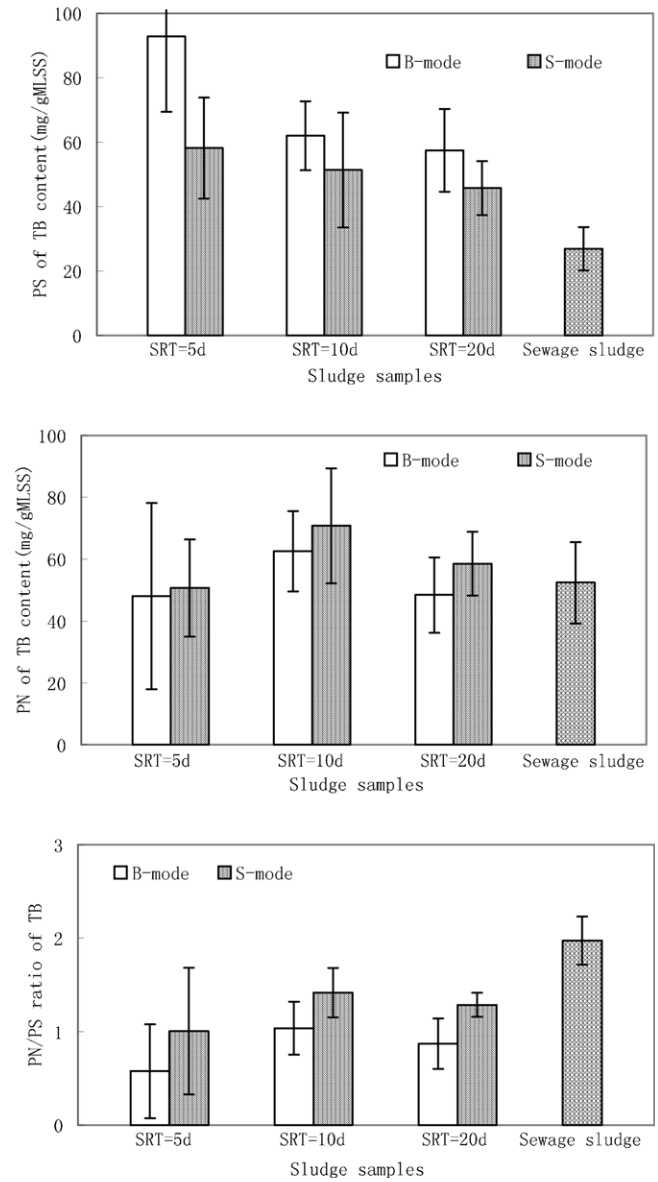


**Fig. 3.** Influence of modes of operation and SRT on chemical compositions of TB-EPS.

### 3.4. Effect of the SRT on sludge surface characteristics

SEM was used to observe the EPS variation in the sludge regularly. Fig. 4 illustrates the SEM observation of the flocs operated in S-mode with different SRTs (5 d, 10 d and 20 d) and carbon sources (glucose and starch). The magnification of SEM was 8.00 kX in this study.

As shown in Fig. 4a, cocci were the predominant bacteria in the sludge matrix cultured with glucose at an SRT of 5 d. These bacteria appear similar to Sarcina, with a mean diameter of approximately 0.6 μm. The sludge flocs were fixed and dewatered before the SEM observation. EPS, a dominant bridging mechanism between the cellular, bioorganic and inorganic floc components, is a product of active secretion, cell surface material shedding, cell lysis, and sorption from the surroundings (Morgan, Forster, & Evison, 1990). In Fig. 4a (SRT = 5 d), the EPSs were mainly responsible for the slimy substance covering the cell surface of the bacteria. The quantity of EPSs was relatively low from the cells with smooth surfaces and relatively high from the cells with rough surfaces on which tightly bound, spherical cell-surface particles were found.

The SEM observations showed that the microbial cells inside the flocs were cross-linked by the EPS (Fig. 4), forming a polymeric network with pores and channels. These results were in agreement with previous studies (Jorand, Guicherd, Urbain, Manem, & Block, 1994; Leppard, 1992; Ravella et al., 2010).

During the operation, other species of microorganisms grew together in sludge flocs, including cocci and bacilli, at an SRT of 10 d (Fig. 4a). When the SRT reached 20 d, the cocci were still the predominant bacteria, whereas the cell number of sarcinas decreased, and the monococci abundance increased. Some bacilli and spindle-shaped bacteria were found at an SRT of 20 d.

The SEM observations for the EPS matrix from the samples grown on starch were similar to the sludge cultured with glucose. The types of bacteria became more diversified in the sludge flocs fed with starch (Fig. 4b). Except for cocci and bacilli, strep bacteria and spiral bacteria were found in the SEM images at an SRT of 20 d (Fig. 4b). The bacterial population changed at the different SRTs, and the EPS production also changed with different bacteria.

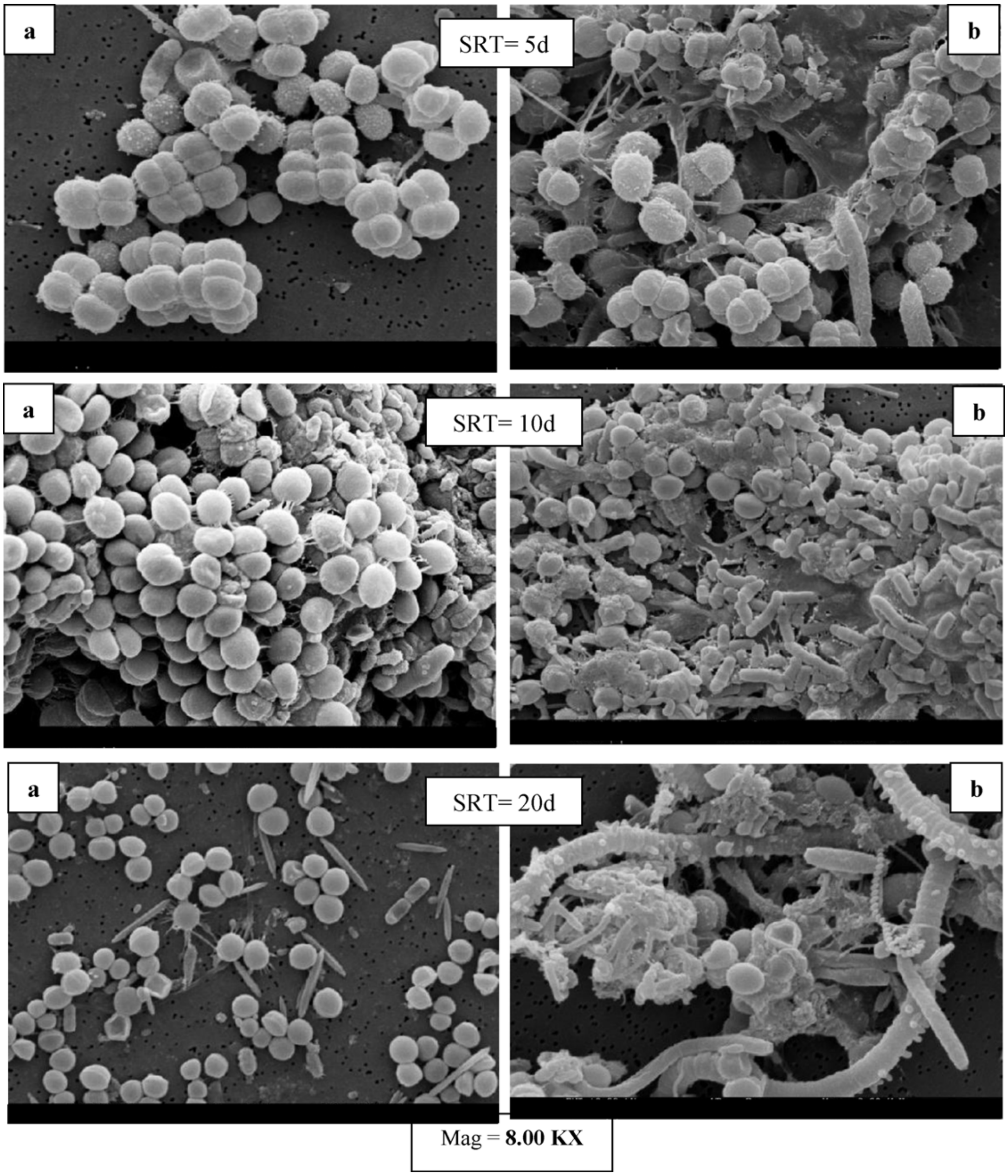


**Fig. 4.** SEM observation of sludge with different SRT and nutrients in S-mode: (a) glucose; (b) starch.

## 4. Conclusions

This article presents a comparative study of EPSs and its chemical constituents and the surface properties of activated sludge under different operating conditions and SRTs.

The results of this study revealed that the PS and PN were the two predominant constituents of the EPS matrix from all of the sludge samples. In general, the amount and composition of EPSs are influenced by the microbial species, growth stage, limitation of the substrate, dissolved oxygen, ionic strength and other conditions. The modes of reactor operation and SRT can induce changes between the physiological state and the endogenous respiration state and affect the formation of an EPS and its components (Pollice, Laera, Saturno, & Giordano, 2008). In this report, the LB-EPS content was influenced by the operating mode and SRT, whereas the TB-EPS content was not. However, the PS and PN in the LB-EPSs and TB-EPSs were both affected by the operating mode and SRT. SEM observations indicated that the carbon source and SRT induced changes in the types of microbes found, thus influencing the EPS quantity and its components.

Further studies are necessary to examine the correlation between the LB-EPS and TB-EPS fractions and other sludge properties (e.g., flocculation, settleability and dewaterability).

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