

Effect of solids retention time on membrane fouling in membrane bioreactors at a constant mixed liquor suspended solids concentration

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Abstract. Membrane fouling at different solids retention times (SRT) (7, 12 and 20 days) was studied under well-controlled conditions in a laboratory-scale aerobic submerged membrane bioreactor under constant biomass concentration using a synthetic high strength wastewater. An increase in SRT was found to improve membrane performance and this correlated to changes in the total production of bound extracellular polymeric substances (EPS), and the composition and properties of bound EPS using X-ray photoelectron spectroscopy (XPS) and Fourier transform-infrared spectroscopy (FTIR) and floc sizes. A larger amount of total bound EPS was found at the lowest SRT (7 days) tested but the ratio of proteins (PN) to carbohydrates (CH) in bound EPS increased with an increase in SRT. Similarly, the quantity of soluble microbial products (SMP) decreased with an increase in SRT and the SMP PN/CH ratio increased with an increase in SRT. SMP concentrations positively correlated to the percentage of membrane pore blocking resistance. The quantity of total bound EPS and total SMP positively corresponded to the membrane fouling rate, while the PN/CH ratio in the bound EPS and SMP negatively correlated to the membrane fouling rate. The results show that both the quantity and composition of bound EPS and SMP and floc sizes are important in controlling membrane fouling.

Keywords: membrane fouling; surface properties; solids retention time; extracellular polymeric substances; soluble microbial products

1. Introduction

Solids retention time (SRT) is one of the key operational parameters affecting membrane fouling (Le-Clech *et al.* 2006, Huang *et al.* 2011). SRT is related to the growth rate of activated sludge, which influences biomass properties including particle size, mixed liquor suspended solids (MLSS) concentration, bound extracellular polymeric substances (EPS) and soluble microbial products (SMP), and thus membrane performance (Le-Clech *et al.* 2006).

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Membrane fouling is the major obstacle that limits the wide application of membrane bioreactors (MBRs) for wastewater treatment (Gasmi *et al.* 2015, Nam *et al.* 2015). Among the various factors that affect membrane fouling (Gasmi *et al.* 2015, Nam *et al.* 2015), SRT is a dominant factor that controls membrane fouling through the changes of sludge properties. Previous studies on the effect of SRT on membrane performance in MBRs indicate there is little consensus on the role SRT plays (Ahmed *et al.* 2007, Annop *et al.* 2014, Grelier *et al.* 2006, Lee *et al.* 2003, Van Den Broeck 2012). Some studies observed improved membrane performance at longer SRTs of 2-10 days (Trussell *et al.* 2006), 8-80 days (Nuengjamnong *et al.* 2005), 10-40 days (Liang and Song 2007), and 20-60 days (Wang *et al.* 2010). On the other hand, other investigations have found the reverse trend. Lee *et al.* (2003) reported that the prolonged SRT ranging from 20 days to 40 days and 60 days could increase the membrane fouling resistance. Han *et al.* (2005) also reported that membrane fouling and sludge viscosity increased when the SRT increased from 30 to 100 days. Furthermore, Annop *et al.* (2014) found that an increase in SRT (15, 30 and 60 days) in an anaerobic membrane bioreactor led to poorer membrane performance. A careful review of these studies indicates that mixed liquor suspended solids (MLSS) concentration was different at different SRTs, which may affect membrane performance as well.

It is well known that MLSS is an important factor affecting the membrane fouling as microbial flocs and supernatant containing colloids and solutes are the two main fractions of MLSS. Each fraction plays a role in membrane fouling. Different membrane fouling behaviors are anticipated at different MLSS concentrations even with the same sludge (Rosenberger *et al.* 2005). Therefore, it is desirable to eliminate the influence of MLSS on membrane performance while studying the effect of SRT and focusing the physiological state (cell age or growth rate) only. Furthermore, practically, it is desirable for the MLSS to be maintained at a constant level in commercial pilot-plant or full-scale MBRs (Wu *et al.* 2013). When changes in influent flow rate and COD concentration occur, MLSS may be wasted accordingly to maintain the same MLSS concentration, which results in changes in SRTs (Wu *et al.* 2013). However, there was only limited study on the effect of SRT on MBR performance at the same MLSS concentration (Wu *et al.* 2013). The objective of this research was to clarify the effect of SRT on biological performance and membrane fouling at the same MLSS concentration by regulating the feed COD. At the same MLSS, membrane fouling rate will be affected only by the changes in sludge properties caused by different physiological states (cell age or growth rate) of sludge but not the MLSS under different SRTs. Therefore, a better controlled study was designed to investigate the effect of SRT on membrane fouling. The effects of three different SRTs (7, 12, and 20 days) on the membrane fouling behaviours were evaluated at the same MLSS concentration. The relationship between the evolution of membrane fouling and sludge properties at different SRTs were also established. The membrane flux, fouling resistance, surface composition of sludge, bound EPS, SMP, and particle size (PSD) were monitored. The surface properties of sludge samples were characterized using novel analytical technique, like X-ray photoelectron spectroscopy (XPS), and Fourier transform infrared (FTIR) spectroscopy.

2. Materials and methods

2.1 Experimental set up and operating conditions

The schematic diagram of the MBR set-up for this study is the same as shown in the study by

Table 1 Operational conditions of the aerobic MBR

	Day 1-117	Day 118-269	Day 270-390
Three solid retention time (days)	7	12	20
Influent COD concentration (g COD/L)	5.07±0.16	3.52±0.10	2.46±0.10
Hydraulic retention time (hour)	36	36	36
Food to microorganisms ratio (F/M) (kg COD/kg MLSS·d)	0.42±0.01	0.31±0.01	0.21±0.01
Operation pH	6.9±0.1	6.9±0.1	6.9±0.1
Operation temperature (°C)	35	35	35

Hao and Liao (2015). The submerged aerobic MBR system consists of a 6.0 L working volume bioreactor with flat-sheet microfiltration membranes (SINAP Membrane Science & Technology Co. Ltd., Shanghai, China) installed. The flat sheet membranes were made of polyvinylidene fluoride (PVDF) materials with a pore size of 0.3 μm and a molecular weight cut off (MWCO) of 70,000 Da. Coarse air bubble diffusers (2.6 liter per minute (LPM) on each side of the membrane module) were installed under the membrane module in order to provide air scouring for membrane fouling control and oxygen for biodegradation. Furthermore, finer aeration (1.2 LPM on each side of the membrane module) was also used to maintain the dissolved oxygen (DO) level larger than 2 ppm. A magnetic stirrer (Thermolyne Cimarec, Model S47030) was used at the bottom of the reactor to improve sludge mixing. The temperature of the MBR was maintained constant at mesophilic temperature of $35\pm 1^\circ\text{C}$ by means of warm water jacket. The pH was monitored by a pH electrode (Thermo Scientific, Beverly, MA), and automatically adjusted to 6.9 ± 0.1 by a pH regulation pump using 0.5 M NaOH solution.

The feed was automatically pumped into the bioreactor by a peristaltic pump (Masterflex Model 7520-50, Barnant Co., USA), which was controlled by a liquid level sensor (Madison Co., USA) and a controller (Flowline, USA). The major composition of feed included glucose, nitrogen (NH_4Cl) and phosphorus (KH_2PO_4) in a proportion of chemical oxygen demand (COD): N: P=100:5:1 and other trace metals ($\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 5.07 mg/L; $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$, 2.49 mg/L; $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$, 1.26 mg/L; $\text{MnSO}_4\cdot 4\text{H}_2\text{O}$, 0.31 mg/L; CuSO_4 , 0.25 mg/L; $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$, 0.44 mg/L; NaCl, 0.25 mg/L; $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$, 0.43 mg/L; $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$, 0.41 mg/L). The system was semi-continuously fed with the synthetic high strength wastewater which was stored at 4°C . The membrane operational cycles were 4 min of operation (on) and 1 min of relaxation (off). A vacuum gauge was installed between the membranes and suction pump to monitor the variation of the trans-membrane pressure (TMP). The instant membrane flux was maintained at $7 \text{ L/m}^2\cdot\text{h}$. The main operating conditions are summarized in Table 1. New membranes were used for each operation cycle, in order to maintain the same initial membrane conditions for SRT of 12 and 20 days.

2.2 Analytical methods

2.2.1 Water quality measurement

The influent synthetic industrial wastewater, permeate and mixed liquor were sampled periodically from the system. The influent, permeate and supernatant COD was measured using COD test vials (Thermo Scientific COD test kits, 0-1500 mg/L) and mixed liquor suspended solids (MLSS) was analyzed according to Standard Methods (APHA 2005). Supernatant COD was

determined after centrifuging the mixed liquor for 20 min at 18700×g.

2.2.2 Particle size distribution measurement and analysis

The particle size distribution (PSD) measurements of bulk sludge were routinely conducted. The PSD was determined by a Malvern Mastersizer 2000 instrument (Worcestershire, UK) with a detection range of 0.02-2000 μm . Each sample was automatically measured three times with a standard deviation of 0.1-4.5%.

2.2.3 Morphology of sludge and textual of cross-section of fouled membranes

Bulk sludge were routinely taken out from the MBR and viewed for morphology under a conventional optical microscope (COM) (Olympus IX 51 Inverted Microscope, Olympus America Inc., Melville, NY). The textual of cross-section of cake layers in wet form was measured by using microtome slicing technique (Histostate Microtome, Model: 855, Reichert Scientific Instruments Division of Warner-Lambert Technologies Inc., NY, USA) and COM. A cross-section of the cake layer in wet form was observed by a COM to investigate the physical structure of sludge cake layers formed on membrane surface. In order to prevent the structure and thickness of cake layer from change, the cake layer was saturated with 0.85% NaCl aqueous solution and then frozen at -22°C before being fixed on to a sample stage using optimal cutting temperature (O.T.C) compound (Sakura Finetechnical Co. Ltd., Tokyo, 103, Japan) (Hao *et al.* 2016).

2.2.4 Bound extracellular polymeric substances (EPS) extraction and measurement

The bound EPS of bulk sludge was extracted using cations exchange resin (CER) (Dowex Marathon C, Na^+ form, Sigma-Aldrich, Bellefonte, PA) method (Frolund *et al.* 1997). 100 mL of the bulk sludge from the MBR was centrifuged (IEC MultiRF, Thermo IEC, Needham Heights, MA, USA) at 18,700×g for 20 min at 4°C . The sludge pellets were re-suspended to their original volume using a buffer consisting of 2mM Na_3PO_4 , 4mM NaH_2PO_4 , 9mM NaCl and 1mM KCl at pH 7. The sludge was then transferred to an extraction beaker filled with buffer and the CER (80 g/g-MLSS). The extraction was lasted for 2 hours at 4°C . The EPS was determined as the sum of proteins (PN) and carbohydrates (CH) and were measured colorimetrically by the methods of Lowery *et al.* (1951) and DuBois *et al.* (1956), respectively. The total bound EPS was represented by adding the concentrations of bound CH and PN.

2.2.5 Surface composition of sludge by X-ray photoelectron spectroscopy (XPS)

The surface concentrations of elements (C, O, N and P) in bulk sludge were examined by XPS. The wet sludge samples were freeze-dried at -35°C for one week. The freeze-dried sludge samples were ground to a powder before being analyzed by a ThermoFisher Scientific K-Alpha XPS Spectrometer equipped with monochromatic AlK α X-ray source with a spot source of 400 μm (ThermoFisher, E. Grinstead, UK). Charge compensation was also provided. A survey spectrum was taken at low resolution (PE-150 eV). High resolution spectra were taken of C1s regions (PE-25 eV). Data processing was performed using the software (Advantages) associated with the equipment.

2.2.6 Soluble microbial products (SMP) measurement

The supernatant from centrifugation of MLSS was filtered by 0.45 μm membrane filters (Millipore) for SMP analysis. The PN and CH of SMP were measured using the methods of Lowery *et al.* (1951) and DuBois *et al.* (1956), respectively. The total SMP is the sum of the

concentrations of soluble CH and PN.

2.2.7 Membrane resistance

Membrane resistances were evaluated using Darcy's law as shown below

$$R_t = \frac{\Delta P}{J \times \eta_T} = R_m + R_f + R_c \quad (1)$$

$$\eta_T = \eta_{20^\circ\text{C}} \cdot e^{-0.0239(T-20)} \quad (2)$$

where R_t is the total filtration resistance (m^{-1}), J represents the permeate flux ($\text{m}^3/\text{m}^2\text{h}$), ΔP is the trans-membrane pressure difference (Pa), and η_T is the permeate dynamic viscosity (Pa·s). T is the permeate temperature in $^\circ\text{C}$.

R_t was corrected to 20°C to compensate for the dependence of viscosity on temperature. The experimental procedure to determine each resistance value is as follows: R_t is total membrane resistance (m^{-1}) and calculated from the filtration data at the end of operation in mixed liquor, R_m is the intrinsic membrane resistance and evaluated by the clean water flux of tap water, R_c is fouling layer Resistance (m^{-1}). R_f is fouling resistance due to irremovable adsorption and pore blocking (m^{-1}). When fouling occurred, membrane surfaces was cleaned by removing the fouling layer with a wet sponge and tap water, and then the membrane was submerged in tap water for flux and TMP measurement to find R_f .

2.3 Statistical analysis

An analysis of variance (ANOVA) was employed to identify whether there is significant difference between treatment means when evaluating membrane fouling and bound EPS/SMP concentration and composition under different SRTs. The difference was considered statistically significant at a 95% confidence interval ($p < 0.05$). The student t-test also was applied to analyze the content of surface chemical composition of bulk sludge. The paired p values were calculated for the differences between SRT of 7 and 12 day; 12 day and 20 day; and 7 day and 20 day. Data sets were considered statistically different if the p values were less than 0.05.

3. Results and discussion

3.1 Overall COD removal efficiency

As shown in Fig. 1, the operating periods reflected the 3 SRT stages studied (7, 12 and 20 days). In order to avoid the influence of various MLSS on biological and membrane performance, the MLSS for each of the SRTs was maintained at approximately 7580 ± 289 mg/L by regulating the feed COD concentration. The influent COD loading was 5070 ± 165 mg COD/L, 3517 ± 103 mg COD/L and 2460 ± 100 mg COD/L for SRTs of 7 days, 12 days and 20 days stages, respectively. During Day 1-44, membrane leakage occurred and the COD removal efficiency was only 81%. At steady-state operation, the permeate COD values remained between 10 mg/L and 69 mg/L corresponding to the removal efficiency larger than 98%. A steady-state permeate COD of 39.9 ± 15.5 mg/L, 43.7 ± 18.7 , 24.2 ± 7.1 mg/L was achieved for SRT of 7, 12 and 20 days, respectively. Similarly, a supernatant COD of 326.9 ± 60.8 , 85.2 ± 44.3 , 53.9 ± 20.1 mg/L was found

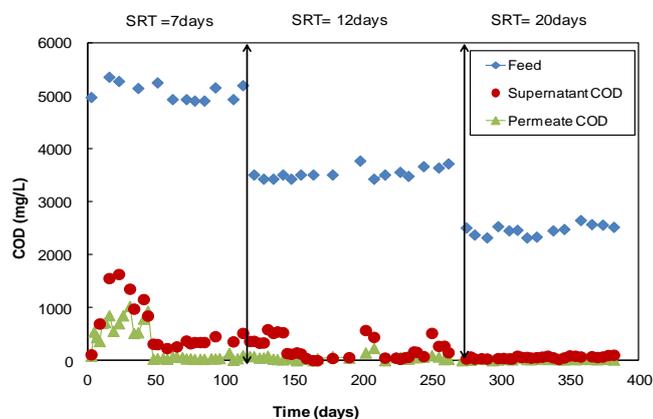


Fig. 1 Variation of the influent, supernatant and permeate COD

for an SRT of 7, 12 and 20 days, respectively. It is clear that the removal efficiency of COD increased and supernatant COD decreased with an increase in SRT. Meanwhile, the supernatant COD values were higher than the permeate COD indicating the presence of a large quantity of colloidal particles and soluble microbial products (SMPs) in the sludge suspension that retained by the membrane and fouling layers. This could be the explanation of the higher supernatant COD at SRT=7 days stage that leads to much faster fouling rate. Membrane leakage occurred on day 200 and day 250, which resulted in the deterioration in COD degradation: both permeate and supernatant COD jumped to high values and the COD removal efficiency dropped, as shown in Fig. 1. The findings indicate that SRT had only limited influence on COD removal efficiency, under steady-state operation, in this study but significant difference in supernatant COD existed under different SRTs.

3.2 Trans-membrane pressure (TMP)

The change in TMP of the MBR with time is illustrated in Fig. 2. The first period (day 1-117) of operation at an SRT of 7 days was characterized by severe membrane fouling and rapidly increased TMP. Physical cleaning with a wet sponge was necessary and carried out daily for flux recovery. The black block from day 45 to 130 in Fig. 2 was due to the overlaps of TMP jump lines caused by serious membrane fouling each day. During the second period of operation at an SRT 12 days, the first 21 days (day 118 to 139) involved a transition to steady-state operations beginning day 140 through to day 269 before transitioning to the third period. A subtle fluctuation of TMP was initially observed during the first 21 days followed by a relatively low and stable TMP period ranging from 21 to 24 days operation before a TMP jump. For the SRT of 20 days (day 270 to 390), an extended stable and low TMP period of operation (ranging from 29 to 41 days) was achieved in each membrane operation cycle. A three-stage TMP profile was observed for SRT of 12 and 20 days: a rapid increase in TMP in the first few hours followed by a flat and low and stable TMP stage (weeks) and eventually by a TMP jump. The TMP profile exhibited the order of membrane fouling rate from high to low: SRT 7 days > SRT 12 days > SRT 20 days. This indicates that a longer SRT tends to result in lower membrane fouling potential. This result was obtained at the same MLSS and thus eliminated the influence of MLSS concentration. Thus, the change of membrane fouling rate can be correlated to changes in sludge properties caused by

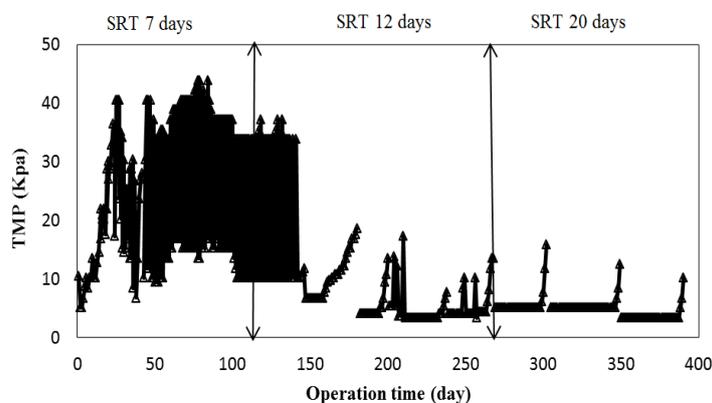


Fig. 2 TMP profile at different SRTs (the black mark between days 45 and 130 is due to the overlaps of daily TMP jump lines caused by serious membrane fouling for these days)

Table 2 A series of resistances for the filtration of activated sludge at the end of filtration cycles under different SRTs

SRT	Resistances (m^{-1})				Cake layer resistance ratio (%)
	R_m	R_c	R_f	R_t	$R_c/(R_c+R_f)$
7 days	$(1.88\pm 0.24)\times 10^{12}$	$(3.29\pm 0.06)\times 10^{13}$	$(19.1\pm 7.3)\times 10^{11}$	$(3.75\pm 0.14)\times 10^{13}$	94%
12 days	$(1.88\pm 0.18)\times 10^{12}$	$(1.73\pm 0.53)\times 10^{13}$	$(7.41\pm 1.69)\times 10^{11}$	$(1.85\pm 0.73)\times 10^{13}$	96%
20 days	$(2.42\pm 0.52)\times 10^{12}$	$(1.22\pm 0.26)\times 10^{13}$	$(3.93\pm 0.20)\times 10^{11}$	$(1.49\pm 0.31)\times 10^{13}$	99%

*Sample mean \pm relative error, number of measurements: $n=2$ for SRT 7 days; $n=3$ for SRT 12 days; $n=2$ for SRT 20 days

physiological age but not MLSS. The change in membrane fouling rate under different SRTs might be explained by the fact that the membrane fouling could be caused by the high concentration of soluble organic matter and bound EPS. SMP have been found as the major foulants in the operation of MBRs for wastewater treatment (Drews *et al.* 2008, Paul and Hartung 2008, Rosenberger *et al.* 2006). A higher concentration of SMP or soluble EPS may attribute to faster membrane fouling rate (Kimura *et al.* 2005).

3.3 Fouling resistance and textual of cross-section of cake layers

The operational cycle for each operation condition was usually defined as the period between two physical and chemical cleaning procedures and resistance analysis was conducted at the end of each run. The total filtration resistance (R_t), the cake layer resistance (R_c), and resistance caused by pore blocking and adsorption (R_f) are shown in Table 2. In our study, greater fouling occurred at SRT 7 days which resulted in the rapid rise of TMP and daily physical cleaning with a wet sponge was necessary. The high percentage (94-99%) of cake layer resistance suggests that cake layer formation was the dominant mechanism of membrane fouling during this study. This is consistent with the microscopic observation of microtome sliced membrane, as shown in Fig. 3. Cake layer was observed on membrane surface at the end of each operation cycle for all SRT tested. Thus, physical cleaning of cake layer using a wet sponge was required to maintain membrane

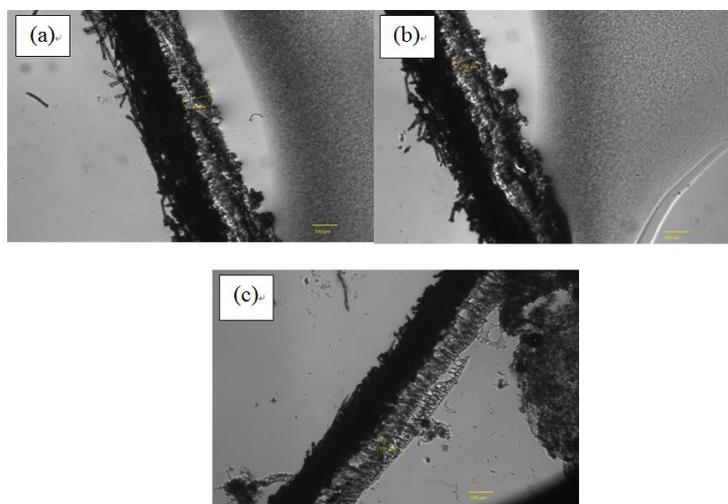


Fig. 3 Cross-sectional morphology of wet fouled membranes at different SRTs (a) SRT=7days; (b) SRT=12 days; and (c) SRT=20 days

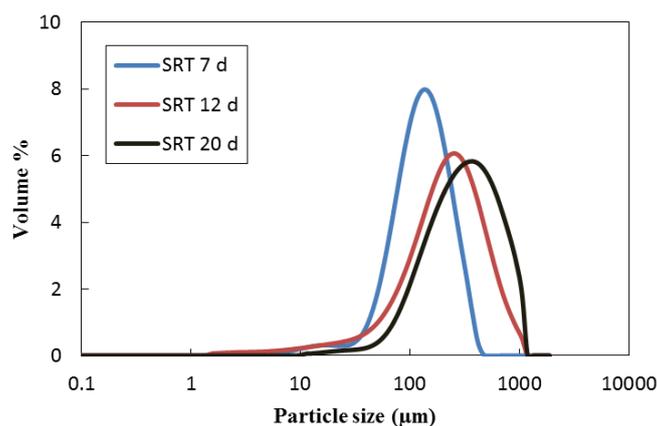


Fig. 4 Particle size distribution of bulk sludge at different SRTs

performance, and chemical cleaning was not required. The cake layer thickness was approximately 130-150 μm at the end of membrane filtration cycles under all SRTs tested. To explain the lower membrane fouling rate at higher SRTs, it could be reasonably hypothesized that either the cake layer formation rate was lower at higher SRTs or the cake layer structure, such as porosity, would be more favourable for membrane filtration at higher SRTs. More importantly, R_c at SRT 20 days represented over 99% of the total fouling resistance (R_t) and it was higher than R_c values observed at SRT 7 and 12 days. This result suggests that less irremovable fouling at prolonged SRT. The observed lower membrane fouling rates at higher SRTs would be explained by the changes in sludge properties, such as physical structure, bond EPS, and SMPs in later sections.

3.4 Particle size distributions (PSD) and morphology of sludge flocs

The PSD of bulk sludge at different SRTs are shown in Fig. 4. A significantly larger proportion

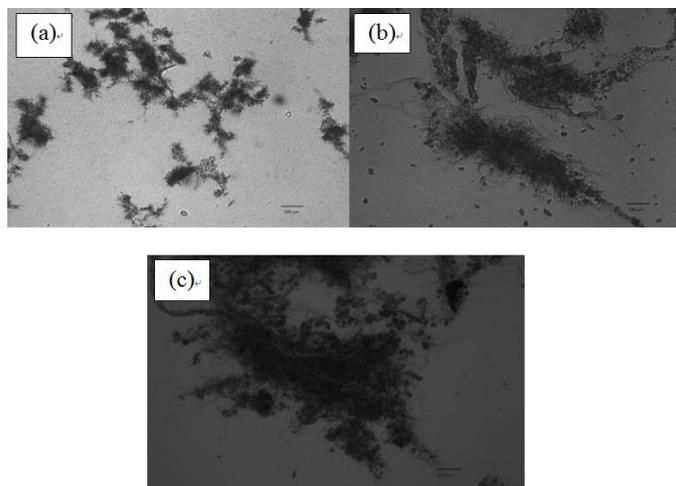


Fig. 5 Morphology of sludge flocs at different SRTs (a) SRT=7 days; (b) SRT=12days; (c) SRT=20 days

of smaller particles were present at the shorter SRT of 7 days. The particle size of bulk sludge increased as the SRT increased. This is consistent with the microscopic observation as shown in Fig. 5. Irregular shapes of sludge flocs were observed at all SRTs tested. The larger sludge floc sizes at the higher SRTs (12 and 20 days) were due to the backbones of filamentous microorganisms (level 0-2, based on the classification of Jenkins *et al.* 2003) for the formation of large flocs. SRTs would affect floc macrostructure by influencing the relative proportion of floc-forming bacteria and filamentous bacteria in the floc (Maharajh 2010). Studies show that SRTs below 12 days produced irregularly shaped pin-point flocs (Maharajh 2010). The results from this study indicate that a balanced floc-forming and filamentous bacteria microbial community were developed to produce large flocs at SRTs 12 and 20 days.

Small particles have a stronger tendency to accumulate on the membrane surface to form a compact and dense cake layer which results in poorer filtration (Lim and Bai 2003). On the other hand, it is well-known that cake layer formed by large flocs would have a higher porosity and thus reduce filtration resistance and enhance membrane filtration (Cao *et al.* 2015). The results are consistent with the findings of Meng *et al.* (2006) in that the presence of a small amount of filamentous microorganisms would enhance membrane filtration. The change in PSD provides an explanation for the changes in membrane fouling rate at different SRTs.

3.5 Bound and soluble EPS

Bound EPS are biopolymers of biological origin attached on floc or cell surfaces. It contains both tightly bound EPS and loosely bound EPS (Li and Yang 2007). PN and CH are usually considered as dominant components representing the total amount of bound EPS. Fig. 6(a) presents the comparison of bound EPS content in the bulk sludge. Statistical analysis using ANOVA confirmed that differences in PN, CH and total bound EPS were all statistically significant (ANOVA, $p < 0.05$) among different SRTs. PN, CH and total bound EPS concentration decreased with an increase in SRT. PN were observed as the predominant compounds in bound EPS compared to CH. On average, the total bound EPS were $61.01(\pm 5.16)$, $30.32 (\pm 3.45)$ and

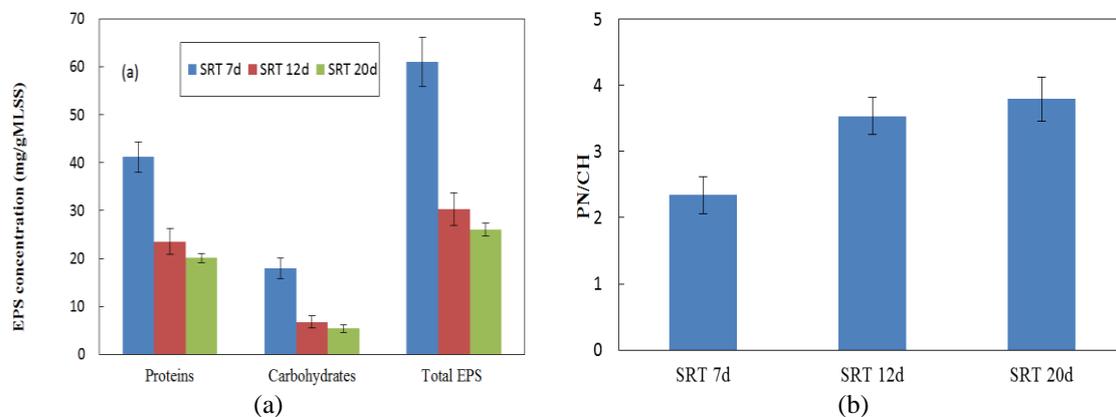


Fig. 6 Comparison of bound EPS of bulk sludge at different SRTs (ANOVA, $p < 0.05$, number of measurements: $n=6$ for each SRT)

26.07 (± 1.73) mg/g MLSS for SRTs of 7, 12 and 20 days, respectively. The results show the same tendency of change of bound EPS in SRT as observed by Lee *et al.* (2003) and Meng *et al.* (2007). It is generally believed that the bound EPS is related to growth rate and is produced in proportion to substrate utilization rate (Laspidou and Rittmann 2002). At shorter SRTs, the larger amount of bound EPS produced is likely owing to a greater proportion of C was not converted for growth. The excess carbon substrates were converted into bound EPS and more PN were produced and thus resulted in more excretion of intracellular polymers (Lee *et al.* 2003). Meng *et al.* (2007) also reported that a larger amount of bound EPS were produced at a higher organic loading or F/M ratio. In contrast, the biomass at a higher SRT has a lower metabolism rate, consumes less food, and generates less microbial products. Therefore, a shorter SRT with a higher organic loading rate or F/M ratio led to a larger amount of bound EPS. It is generally believed that the quantity and composition of bound EPS would play an important role in cake layer formation by modifying the surface interactions between sludge flocs and membrane surfaces or fouling layers (Lin *et al.* 2014, Wang *et al.* 2013). Previous investigations revealed that the bound EPS are controlling factor for membrane fouling in MBRs (Laspidou and Rittmann 2002, Lee *et al.* 2003, Lin *et al.* 2014, Meng *et al.* 2007, Wang *et al.* 2013). Therefore, the findings from this study were in agreement with studies that greater bound EPS content led to faster fouling rates.

The change in the ratio of PN to CH (PN/CH) in bound EPS with respect to SRT is shown in Fig. 6(b). An increase in SRT led to an increase in PN/CH ratio. The change in the ratio of PN/CH indicates the difference in EPS composition and concentration at different SRTs and is consistent with the finding of XPS results (differences in element C and N surface concentration at different SRTs as shown in later sections) from this study. Similar trends in the ratio of PN/CH in bound EPS have been reported by Liao *et al.* (2001) and Wu *et al.* (2013). On the one hand, the higher PN/CH ratios at higher SRTs might enhance cake layer formation rate, due to hydrophobic interactions. It is likely that it is the cake layer structure (porosity) rather than the cake layer formation rate that determines membrane filtration at different SRTs. The larger floc sizes at higher SRTs (Figs. 3 and 4) would likely form a cake layer with a higher porosity and thus reduce membrane filtration resistance (Cao *et al.* 2015). Meng *et al.* (2006) found that PN has more tendencies to attach on membrane surface and cause membrane fouling. The larger amount of PN in bound EPS might explain the higher fouling rate at the SRT of 7 days. Furthermore, a negative

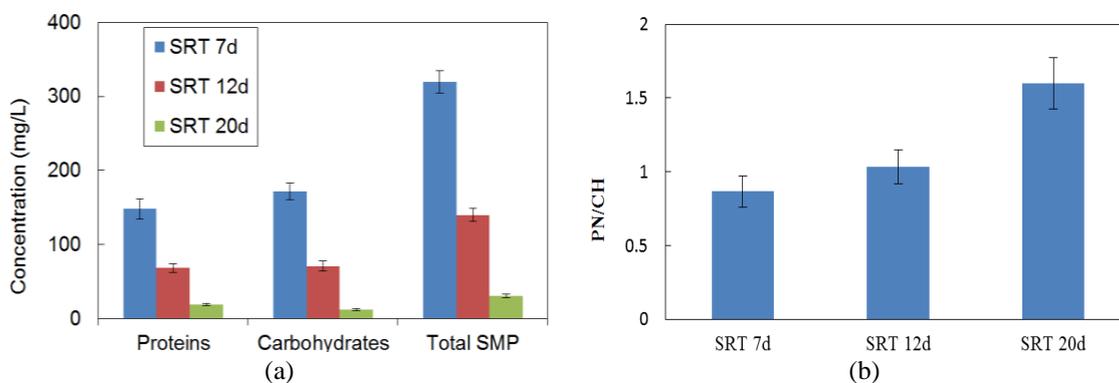


Fig. 7 SMP concentrations at different SRTs (ANOVA, $p < 0.05$, number of measurements: $n=4$ for SRT 7d, $n=4$ for SRT 12d, $n=6$ for SRT 20d)

relationship exists between the PN/CH ratio and membrane fouling rate. As bound EPS composition significantly affects sludge flocculation (Meng *et al.* 2006), it is likely that the lower PN/CH ratio in bound EPS led to a change in the bound EPS composition and thus EPS properties and sludge flocculating ability, which are responsible for the higher propensity of membrane fouling at the short SRT (7 days).

Soluble microbial products (SMP), namely soluble EPS, are biopolymers released from the microbial aggregates into the water phase (Flemming and Wingender 2001, Rosenberger and Kraume 2002, Wang *et al.* 2013). SMP include biomass-associated products (BAP) and substrate-utilization-associated products (UAP) (Laspidou and Rittmann 2002). As shown in Fig. 7, CH were found as the predominant fraction and accounted for more than half of the SMP concentration for SRT 7 days. However, their fraction changed when SRT increased to 20 days. The ANOVA results indicated that SRT had a significant impact on the SMP production (ANOVA, $p < 0.05$) (Fig. 7). The total SMP, soluble PN and CH contents in SMPs at three SRTs exhibited the similar trend which typically dropped with an increase in SRT. Considering the fact that glucose is an easily biodegradable compound, CH in SMP are mainly polysaccharides in BAP and UAP. SMP might have different role in membrane fouling, as compared to bound EPS. Li *et al.* (2013) found that bound EPS played an important role in fouling development at stage I, while SMP were the major contributors to the self-accelerating fouling at stage II. SMP would be more likely to cause pore blocking and/or gel formation, while bound EPS affect cake layer formation (Lin *et al.* 2014, Wang *et al.* 2013, Wang and Wu 2009). Hong *et al.* (2014) found out that gel layer would be easily formed and thus significantly increases filtration resistance at a high SMP concentration. This is supported by the positive correlation between the pore blocking/adsorption resistances (6%, 4% and 1% of the sludge filtration resistance at SRTs of, 7, 12 and 20 days, respectively) (Table 2) and SMP concentrations (319.5 ± 15.4 , 140 ± 9.1 and 31.04 ± 2.42 mg SMP/L at SRTs of, 7, 12 and 20 days, respectively) (Fig. 7). Clearly, SMP concentration had a significantly positive correlation to pore blocking/adsorption and/or gel layer fouling potential. Higher SMP concentration derived from shorter SRT would enhance membrane fouling and induce a faster TMP increase. The PN/CH ratios in SMPs (ANOVA, $p < 0.05$) were also compared to provide more information on characteristics of SMP. The lower PN/CH ratio at a shorter SRT might probably be explained by the fact that the CH was originated from the original substrates and metabolism products. Similar to the negative correlation between PN/CH ratio in bound EPS

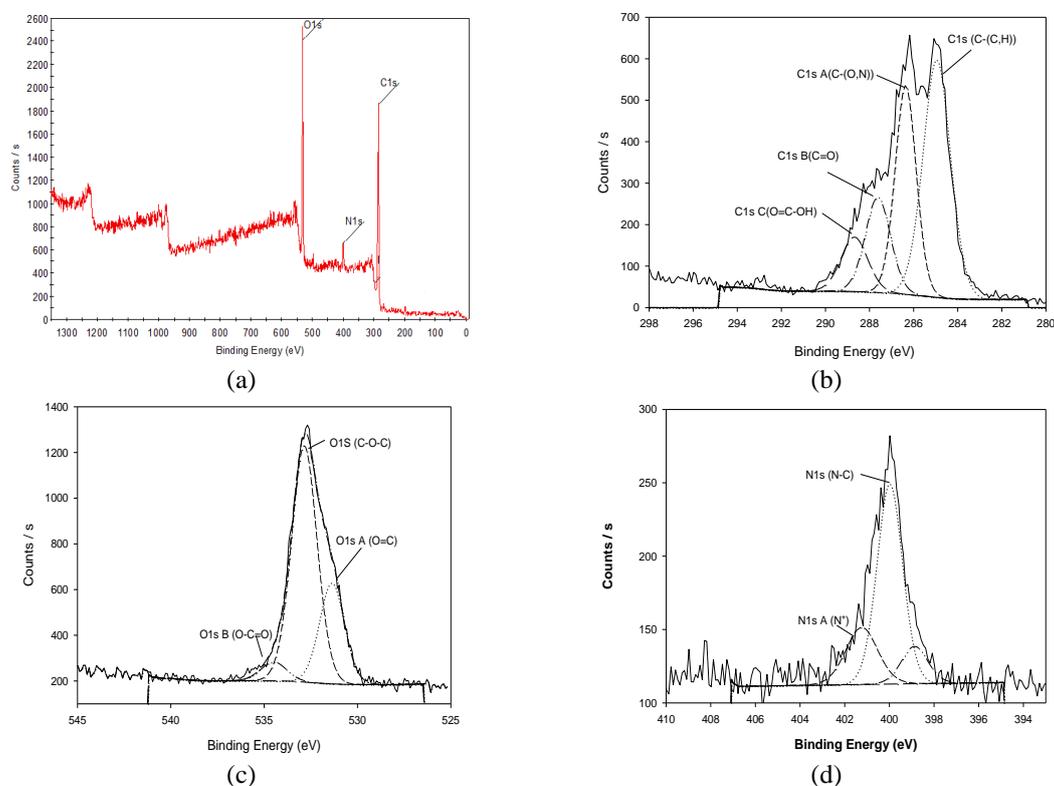


Fig. 8 Representative XPS spectra of bulk sludge at an SRT 12 days: (a) whole spectra, (b) C1s spectra, (c) O1s spectra and (d) N1s spectra

and membrane fouling, a lower PN/CH ratio in SMP corresponded to a higher membrane fouling rate. This result is consistent with the findings of Huang *et al.* (2011) and indicates that not only the quantity but also the composition of SMP play an important role in membrane fouling.

On the other hand, the fouling potentials of SMP in effluents were monitored to determine the changes of SMP composition after filtration. It appears that the concentration of SMP in effluent was 319.5 ± 15.4 , 140.6 ± 9.1 and 31.04 ± 2.4 mg/L at an SRT of 7, 12 and 20 days (ANOVA, $p < 0.05$) respectively, and was significantly lower than those in supernatants (Fig. 7). The results suggest a significant amount of SMP with a colloidal size smaller than $0.45 \mu\text{m}$ were retained by the membrane (pore size: $0.3 \mu\text{m}$) and cake layers and accumulated on membrane surface and/or inside pore surfaces and thus caused biopolymer adsorption and pore blocking fouling. This indicates that the SMP from sludge suspension are more prone to foul the membrane. Therefore, the fouling control strategies can be conducted by operating MBR at longer SRT which greatly minimizes the content of PN and CH of SMP.

3.6 Surface composition of sludge by XPS analysis

The surface chemical composition of bulk sludge at different SRTs were analysed by XPS. XPS has been widely used to study the surface functional groups of materials, including bacteria, and each peak corresponds to electrons with a characteristic binding energy from a particular

Table 3 Surface composition of the sludge at different SRTs determined by XPS: average atom fraction (%)*

Element component	Significant difference					
	SRT 7d	SRT 12d	SRT 20d	SRT 7d and 12d	SRT 12d and 20d	SRT 7d and 20d
Total C	66.29±0.14	64.41±0.17	65.35± 0.51	Y(0.000)	Y(0.030)	Y(0.003)
C-(O,N)	27.22±2.44	26.11±0.99	27.70± 1.28	N(0.780)	N(0.669)	N(0.768)
C-(C,H)	23.37±0.39	22.53±2.93	23.05± 3.18	N(0.650)	N(0.845)	N(0.873)
C=O	10.09±1.11	8.22±1.50	9.10± 1.38	N(0.873)	N(0.498)	N(0.389)
O=C-OH	5.61±0.13	7.76±1.95	5.57± 0.07	N(0.199)	N(0.204)	N(0.976)
Total O	25.08±1.64	26.58±2.82	25.30±0.34	N(0.469)	N(0.479)	N(0.826)
C-OH and C-O-C	6.27±0.93	6.79±0.51	6.93±0.18	N(0.544)	N(0.477)	N(0.220)
O=C	16.84±1.40	16.67±0.73	16.90±0.73	N(0.865)	N(0.724)	N(0.949)
O-C=O	3.14±0.81	3.91±0.80	1.17±0.29	N(0.985)	N(0.097)	N(0.294)
Total N	5.65±0.11	5.02±0.30	6.25±0.10	Y(0.040)	Y(0.006)	Y(0.002)
N-C	3.49±0.78	3.21±0.63	4.51±0.43	N(0.471)	Y(0.028)	N(0.118)
N+	1.40±0.13	1.15±0.08	1.18±0.19	N(0.488)	N(0.344)	N(0.526)

*Sample number $n=3$ under each SRT tested. Y means significant difference and N means no significant difference at a 95% confidence interval

element (Badireddy *et al.* 2008, Dengis and Rouxhet 1996, Dufrene *et al.* 1997, Hao *et al.* 2016, Omoike and Chorover 2004). As shown in Fig. 8 for a representative XPS spectra of bulk sludge at an SRT of 12 days, the major peaks are the C1s (284.8 eV), O1s (532.0 eV) and N1s (400.0 eV) and a minor peak is associated with P2p (133.4 eV) (Badireddy *et al.* 2008). The C peaks (C1s, C1sA, C1sB, C1sC) was resolved into four component peaks: C bound only to C and H, C-(C,H) at a binding energy of 284.8 eV; C singly bound to O or N, C-(O, N) at a binding energy of 286.3 eV; C bound to O making two single bonds or one double bond, C=O or O-C-O at a binding energy of 288.0 eV; and a weak bond at 289.0 eV from O=C-OH and O=C-OR, commonly found in carboxyl or ester group (Badireddy *et al.* 2008, Hao *et al.* 2016). The O peaks (O1s, O1sA, and O1sB) could be related to two bonds: O-C bond at a binding energy of 532.7 eV, and O=C bonds at a binding energy of 531.4 eV. The N peaks (N1s and N1sA) contain two different bonds: N-C bond at a binding energy of 400.12 eV and N-H bonds at a binding energy of 402.10 eV (Badireddy *et al.* 2008, Hao *et al.* 2016). All XPS spectra of bulk sludge from different SRTs showed similar pattern of peaks with only differences in the relative intensity of peaks.

Concentrations of surface elements C, O, N under different SRT conditions were summarized in Table 3. The atomic fraction of C1s, O1s, N1s and P2p on sludge surface is 64-67%, 24-29%, 4-6.5%, and 0.5-0.8%, respectively. A significant difference in the quantity of C and N was observed among SRT of 7, 12 and 20 days (ANOVA, $P<0.05$). No significant difference (ANOVA, $P>0.05$) in the quantity of O was observed between each SRT condition. There are 0.5-0.8% atom fraction composed of P on sludge surface and no significant difference in element P was observed among different SRTs (ANOVA, $P>0.05$). Since XPS detects the surface elements concentrations in a few nm in depth on floc surface, the XPS results are related to the results of bound EPS. It is well known that the bound EPS consist of PN, CH, nucleic acids, lipids and humic acids, which are located at or outside the cell surface. CH and PN are the dominant components in bound EPS (Li

and Yang 2007, Liao *et al.* 2001). PN in bound EPS are the primary source for the elemental N. The P could be from the nucleic acids in bound EPS. The larger atom fraction of C, O, N and smaller fraction of P suggests that the presence of a large amount of CH and PN and a small amount of nucleic acids. Therefore, the XPS results confirmed the results of chemical analysis of bound EPS (Fig. 6). Previous studies (Badireddy *et al.* 2008, Dengis and Rouxhet 1996, Dufrene *et al.* 1997, Hao *et al.* 2016, Omoike and Chorover 2004) revealed that the C-(C, H) bonds might originate from lipids or from amino acid side chains. Polysaccharides or CH contain hydroxide and acetal or hemiacetal building blocks. PN and uronic acids in CH might contain carboxylate and carboxyl function groups. The amide may represent peptidic bonds in PN. The ammonium might be a counter-ion of surface negative sites and the protonated amine could be due to basic amino acids (Dengis and Rouxhet 1996). There were relative higher N concentrations at SRT 7 and 20 days, as compared to that at SRT 12 days (Table 3). The relative higher total N concentration at SRT 7 days might be explained by the higher PN concentration in bound EPS (Fig. 6). Furthermore, the relative higher total N concentration at SRT 20 days might be related to the relative higher PN/CH ratio in EPS (Fig. 6). The higher PN/CH ratio in bound EPS would lead to an increase in the atom fraction of elemental N on sludge surface. The lowest element N concentration at an SRT of 12 days could be related to the fact that a transition in sludge properties appeared between SRT of 9 and 12 days existed (Liao *et al.* 2001).

4. Conclusions

This study focused on clarification of the effect of physiological state (e.g., SRT) on sludge properties and their role in membrane fouling by designing an experiment at the same MLSS and using novel surface analytical technique (XPS), which emphasized the effect of growth rate and eliminated the effect of MLSS. A summary of the conclusions obtained from this study is listed below.

- At the same MLSS, membrane performance was improved with an increase in SRT.
- Surface analysis by XPS indicates significant difference in surface concentrations of elements C and N on sludge surface at different SRTs, which suggest different compositions in bound EPS at different SRTs.
- The total bound EPS and total SMP quantity decreased but the ratio of PN/CH in bound EPS/SMP increased with an increase in SRT.
- SMP concentrations positively correlated to the pore blocking resistance, implying SMP played an important role in controlling biopolymer adsorption and pore blocking fouling mechanism.
- The larger floc sizes at higher SRTs correlated to an improved membrane performance.
- The total bound EPS of bulk sludge and total SMP in supernatant positively correlated to the fouling rate, while the ratio of PN/CH in both bound EPS and SMP negatively correlated to fouling rate, indicating that both the quantity and composition of bound EPS and SMP have significant effect on membrane fouling.

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