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Zhang Jinsong[ab]; Chua Hwee Chuan[a]; Zhou Jiti[b]; A. G. Fane[a]
[a] Institute of Environmental Science and Engineering, and School of Civil & Environmental Engineering, Nanyang Technological University, Innovation Centre, Singapore [b] School of Environmental and Biological Science and Technology, Dalian University of Technology, China

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Zhang Jinsong
Institute of Environmental Science and Engineering, and School of Civil & Environmental Engineering, Nanyang Technological University, Innovation Centre, Singapore and School of Environmental and Biological Science and Technology, Dalian University of Technology, China

Chua Hwee Chuan
Institute of Environmental Science and Engineering, and School of Civil & Environmental Engineering, Nanyang Technological University, Innovation Centre, Singapore

Zhou Jiti
School of Environmental and Biological Science and Technology, Dalian University of Technology, China

A. G. Fane
Institute of Environmental Science and Engineering, and School of Civil & Environmental Engineering, Nanyang Technological University, Innovation Centre, Singapore

Abstract: The submerged membrane bioreactor (sMBR) is being increasingly applied for municipal and industrial wastewater treatment. This paper examines the role of sludge retention time (SRT), an important operating parameter of the MBR as it affects the biological characteristics of the sludge and therefore influences

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Address correspondence to A. G. Fane, Institute of Environmental Science and Engineering, and School of Civil & Environmental Engineering, Nanyang Technological University, Innovation Centre, Block 2, Unit 237, 18 Nanyang Drive, 637723, Singapore. Tel.: + 65 67943801; E-mail: agfane@ntu.edu.sg

membrane fouling. Well controlled runs were carried out at short SRT (10 days) and moderate SRT (30 days) in a 30 L submerged MBR equipped with KUBOTA flat-sheet membranes. At steady operation, the mixed liquor suspended solids (MLSS) stabilized at approximately 5–6 g/L and 8.5–10 g/L for SRT 10 and 30 days respectively. The DGGE profiles suggested a shift in the dominant bacterial community with the prolonged SRT. The soluble microbial products (SMP) were 9.3 mg/L and 5.4 mg/L at the SRTs of 10 days and 30 days respectively. The total amount of extracellular polymeric substance (EPS) extracted from the floc and the supernatant was approximately constant at the two SRTs under the same organic loading rate. However, the polysaccharide concentration in the supernatant was about 100% higher for the SRT of 10 days than that for 30 days. The viscosity of the biomass increased with the prolonged SRT, while the estimated average air induced water velocity was similar for the two SRTs. The results of flux stepping tests showed that the membrane fouling rate (dTMP/dt) at SRT 10 days was always higher than that at 30 days at each flux step. Similarly, long term experimental runs at a constant flux of 20 $L/m^2 \cdot h$ clearly showed more severe membrane fouling for the SRT of 10 days than that at 30 days. This implies that fouling is more influenced by the concentration of SMP and Polysaccharides than the MLSS.

Keywords: SMBR, sludge retention time, membrane bio-fouling intensity extracellular polymeric substance (EPS)

INTRODUCTION

The MBR is becoming increasingly popular for wastewater treatment due to its advantages of high permeate quality, small footprint and independent control of solids and hydraulic retention times compared to the conventional activated sludge plants. Without the limitations of settling, the sludge concentration can be high (or low F/M), giving better protection against shock loading. Slow-growing bacteria can also be retained in the MBR.

We have recently proposed an MBR fouling "road map" (1) and identified a wide range of fouling factors. These include the factors that influence the feed as well as membrane properties and system hydrodynamics. One of the most important operational factors that influences the properties of the mixed liquor is the feed to microorganism ratio (F/M). At steady state, the F/M ratio is inversely related to the SRT. The SRT will influence MLSS, the biomass diversity, the supernatant organics, content of EPS, the colloids etc., all of which could foul the membrane.

MBRs have been operated with SRTs ranging from 5 days to complete sludge retention (2). A long SRT is conducive to an increase of sludge concentration and thus a lower organic loading, which has the advantages of a smaller footprint and low sludge production. Some previous studies reported that the membrane fouling propensity in the MBR was higher at longer SRT (3, 4), while others suggested that higher sludge concentration resulted in less fouling at longer SRT and lower F/M ratio (5). These conflicting results

imply that membrane fouling is affected by the state of the biomass at different SRTs and further study is necessary to confirm the trend.

The most apparently different characteristic at different SRTs is the MLSS concentration, and its effect on membrane fouling is not clear. Some researchers reported that fouling increased with increasing sludge concentration (6, 7), while others suggested that the higher sludge concentration resulted in lower fouling under certain conditions (8–10). The mixed liquor consists of two main fractions: microbial flocs and supernatant containing colloids and macro solutes. Each fraction has its own physicochemical and biological properties, which affect membrane fouling (11). This implies that fouling is related not only to the sludge quantity but also other parameters in the MBR. Under constant flux operation, the permeate flux is usually controlled below the apparent "critical flux" and deposition of large floc particles on the membrane is not significant. If big flocs interact with the membrane surface it would not be serious below the critical flux, so from this perspective, high MLSS concentration should not affect membrane fouling.

However, high MLSS conditions might affect microbial behavior, particle size distribution and rheological properties of the biomass which could result in membrane fouling differences. Therefore, the effect of SRT on biodiversity, dissolve oxygen (DO), EPS, SMP, particle size distribution, and viscosity of the activated sludge must be examined at the same time as fouling studies. The structure of the microbial communities growing in MBRs fed simple synthetic wastewater may also be important. DNA extraction, PCR, and the denaturing gradient gel electrophoresis (DGGE) techniques can be applied to explore and analyze the bacterial community structures in bio-treatment of wastewater. With these techniques, the biodiversity of biomass at different SRTs in the MBR system can be identified (12).

With regard to particle size, Huang et al. (11) reported mean particle sizes of 14.8, 48.2, and 30.6 μm for SRTs of 5, 20, and 40 days respectively in a submerged MBR treating domestic wastewater. Their explanation for the smaller particle size at a lower SRT was the higher shear resulting in the breakup of biomass flocs. Lee et al. (13) reported that the floc size distribution was similar for SRT of 20, 40, and 60 days in a sMBR treating synthetic wastewater. The mean floc size increased slightly with increasing SRT, being 5.2, 6, and 6.6 μm for SRTs of 20, 40, and 60 days.

It is recognized that liquid velocity will affect membrane fouling in the MBR. The rheological property of the biomass has a significant effect on the sludge fluidity in air-induced two phase flow systems and results in different water velocities (14, 15). In addition, the viscosity of the mixed liquor affects the shear stress on the membrane surface which is important in preventing particle deposition. It is observed that the shear stress was reported to be low at a low biomass concentration (2–4 mg/L), but increased approximately five times with increase in biomass concentration to 12 g/L (16). However, this has not been thoroughly investigated.

Soluble microbial products (SMP) and extracellular polymeric substance (EPS) in the mixed liquor of MBRs are known to foul the membrane and cause flux decline (17). SMP are defined as the soluble organic compounds that result from feed metabolism (usually with biomass growth and biomass decay) in the supernatant of mixed liquor, and its concentration was found to decrease with prolonging SRT (18). EPS is a comprehensive term for different classes of macromolecules such as polysaccharides, proteins, nucleic acids, (phospho) lipids, and other polymeric compounds. Part of EPS disperses in the supernatant of the mixed liquor but the major portion of EPS is associated with the biofloc. Chang et al. (19) reported that the attached EPS decreased with increasing SRT of 3, 8, and 33 days and membrane fouling increased with increasing EPS. However, Lee et al. (13) reported the total EPS concentration to be independent of SRT for SRTs of 20, 40, and 60 days. These authors observed that the protein to carbohydrate ratio increased with increasing SRT, as did the filtration resistance for short term tests. Taken together, the results suggest decreasing EPS content of floc with increasing SRT up to 20 days after which the concentration is relatively stable, but beyond 20 days, the protein to carbohydrate ratio may be increased. Rosenberger et al. (15) reported increasing suspended EPS concentrations always resulted in decreasing sludge filterability in eight MBRs equipped with submerged modules, wastewater treating either municipal, domestic, or industrial wastewater.

The purpose of this paper is to explore and describe the effect of SRT, at carefully controlled steady-state conditions, on membrane fouling, as well as the understanding its effect on microbial biodiversity, particle size, EPS properties and the rheology of the mixed liquor.

MATERIALS AND METHODS

Experimental Setup

The experimental MBR system (Fig. 1) comprised of a bioreactor (30 L aerated tank) with submerged flat sheet microfiltration (MF) modules (Kubota, 0.125 m^2 each panel and pore size of 0.2 μm). The concentrated simulated municipal wastewater was continuously pumped into the bioreactor at a constant rate, while tap water was provided as a supplement to the bioreactor through a solenoid valve controlled by a level sensor, which maintained a constant level in the bioreactor. In order to operate with a constant flux, the I-FIX software and a WAGO programmable logic controller (PLC) have been used. When a certain flowrate was set, the permeate flow meter provided a feedback signal to the computer and the pump speed adjusted accordingly to keep the flowrate constant. Each channel between the flat sheet membrane modules had separate air diffusers at the bottom. The transmembrane pressure (TMP) was recorded using a Cole-Parmer high accuracy ($\pm$0.13 kPa) pressure transducer in the suction line.

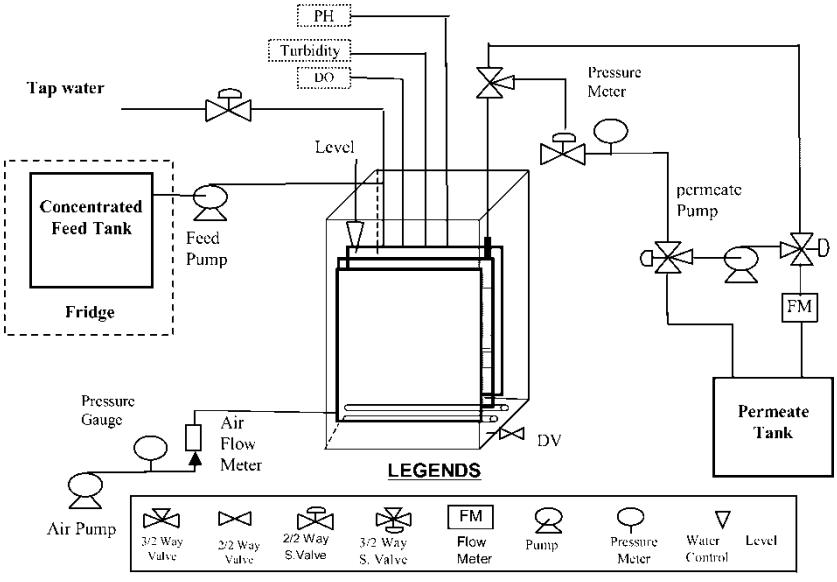


Figure 1. Schematic of the laboratory-scale MBR set-up.

Experimental Conditions

The two experimental runs at 10 and 30 days SRT were conducted under steady state conditions one after the other using the same bioreactor. Figure 2 shows the MLSS changes during the operating period which started with a 30 day start-up to steady state at SRT 10 days (for 35 days – run 1) followed by a transition of 30 days to a SRT 30 days for about 110 days(run 2). Table 1 shows the operating conditions for each run. Excess sludge was discharged on a daily basis from the reactor tank to achieve the desired SRT.

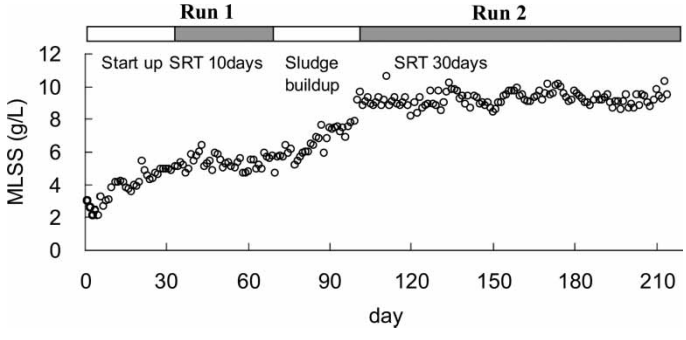


Figure 2. MLSS concentration in the MBR during the operating period.

Table 1. Operating conditions of MBR

	Run 1	Run 2
SRT (days)	10 days	30 days
HRT (hour)	6 hours	6 hours
Operation time (days)	35 days	110 days
Reactor temperature (C)	24–26 C	24–26 C
DO in the biomass solution (mg/L)	3–4	1.2–1.7
Aeration intensity ($m^3/m^2 \cdot h$)	0.75	0.75
Permeate flux ($L/m^2 \cdot h$)	20	20
pH	7–8	7–8

The bioreactor was seeded with sludge from the aeration tank of a local wastewater treatment plant and then fed with simulated municipal wastewater at an organic load of 0.6–0.7 kg TOC/m^3 day. The composition and concentration of the simulated sewage is shown in Table 2. Figure 3 shows that the TOC removal was in the range 93.3–97.1%. The membrane module was washed with tap water and then submerged in 5–10% NaClO solution for 8 hours when the TMP increased by 5–10 kPa.

Analytical Material and Methods

Analytical methods from the "Standard Methods for the Examination of Water and Wastewater" were adopted for the measurement of mixed liquor suspended solids (MLSS) in the bioreactor (20). The supernatant samples were prepared by centrifuging the mixed liquor sample from the bioreactor twice at 4000 rpm for 10 minutes at 4°C. The particle size distribution of the biomass was measured by a particle sizer (MALVERN Mastersizer HYDRO2000SM). The EPS extraction method followed that reported by Zhang et al. (21, 22). Supernatant EPS was physically extracted, without adding any chemical extractant, simply by centrifugation (4000 G) at 4 deg C for 10 minutes and

Table 2. Composition and concentration of the synthetic wastewater

Nutrient	mg/L
Glucose, anhydrous	250
Meat extract	30
peptone	50
KH_2PO_4	7
$MgSO_4$	7
$FeSO_4$	4
$CH_3COONa \cdot 3H_2O$	200

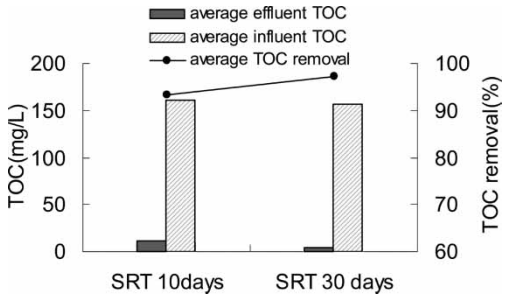


Figure 3. TOC removal performances at different SRTs.

followed by high-speed centrifugation (20000 G) for 20 minutes; the pellet (biofloc) of the sample was resuspended with distilled water and pellet EPS extraction followed the "formaldehyde plus NaOH extraction method." The total EPS was the sum of the supernatant EPS and pellet EPS. The rheological characteristics of the biomass were measured by a RheoStress1 Rheometer (Thermo Hakke). TOC was measured by the SHIMADHU TOC-VCSH. DNA was extracted using the protocol described by Tay et al. (23) and amplified by PCR (24). DGGE analysis of DNA fragments utilized the BIORAD (Decode, USA) gradient gel electrophoresis system and was based on the protocol of Watanabe et al. (24) and Luxmy et al. (25). Flow velocities were estimated by particle image velocimetry (PIV) in a simulate vessel. PIV is a whole field noninvasive measurement technique that allows the investigation of spatial flow structures in both steady and unsteady flows. This technique is suitable for use in the study of flow velocities. More details of the PIV technique applied to membranes can be found elsewhere (26).

RESULTS AND DISCUSSION

MLSS and F/M at SRTs of 10 days and 30 days

The experimental runs lasted 35 and 110 days for the SRTs of 10 and 30 days respectively. As shown in Fig. 2, the MLSS concentrations at the SRTs of 10 and 30 days increased and stabilized at approximately 5 g/L (4.5–6 g) and 9.2 g/L (8.5–10 g) respectively. Figure 4 shows that the corresponding F/M ratios were 0.12 and 0.07 g TOC/g · MLSS · day at the SRTs of 10 and 30 days respectively.

Dominant Bacteria in the Biomass at SRTs of 10 Days and 30 Days

DGGE was performed in order to provide information on the bacterial diversity within the MBR community. The DGGE profiles (Fig. 5) reveal

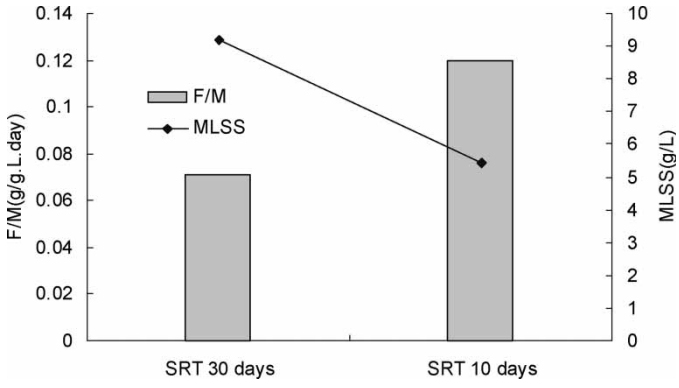
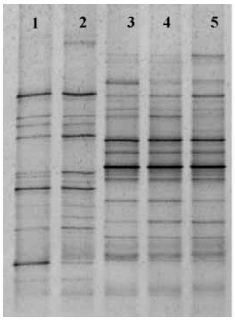


Figure 4. Organic loading rates at two stable SRT conditions.

that the band pattern for the 10 days SRT samples was similar regardless of sampling time, suggesting a stabilized community. This was also the case for the SRT of 30 days. However, differences of the band patterns between the two SRTs were clear, suggesting a shift in the dominant bacterial community. Different dominant bacterial communities at different SRTs imply differences in bacterial metabolism and potential membrane fouling propensity.

Particle Size Distributions at SRTs of 10 Days and 30 Days

Figure 6a illustrates the sludge particle size distributions at the two SRTs, which can be characterized by normal distributions. In Fig. 6a, it can be observed that the particle size became larger at the longer SRT of 30 days. All the sludge particles were smaller than 200 μm. The mean



1. the 20th day sample from biomass at SRT 10 days
2. the 30th day sample from biomass at SRT 10 days
3. the 60th day sample from biomass at SRT 30 days
4. the 70th day sample from biomass at SRT 30 days
5. the 80th day sample from biomass at SRT 30 days

Figure 5. DGGE analysis of activated sludge samples at the SRT of 10 days and 30 days.

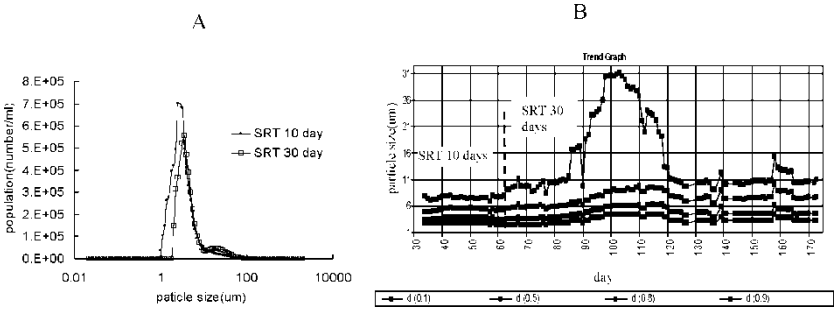


Figure 6. Particle size at SRT of 10 days and 30 days.

particle sizes in terms of population (d50) at the SRTs of 10 and 30 days were 3.6 and 5.6 μm respectively. In Fig. 6b, d(0.1), d(0.5), and d(0.9) are the sizes below which 10%, 50%, 90% of the distribution fall. Figure 6b. shows that d(0.1), d(0.5), and d(0.9) increased at the longer SRT of 30 days, implying larger particles. Figure 7 shows the biofloc number concentration in the mixed liquor at different SRTs. The total particle number concentration of the biomass at the SRT of 30 days was higher than that at the SRT of 10 days. This was due to the higher MLSS concentration at the SRT of 30 days. However, taking the particles based on d(0.5) for SRT 10 days as the standard of the smaller particles in the biomass, the result is the opposite. In other words, the number of smaller particles for the SRT of 30 days was less for the SRT 10 days. This may be significant as smaller particles could affect membrane fouling more seriously. In the submerged MBR system, an increase in particle size can, in principle, result in higher permeate flux. This is because the shear-induced diffusion increases as particle size increases

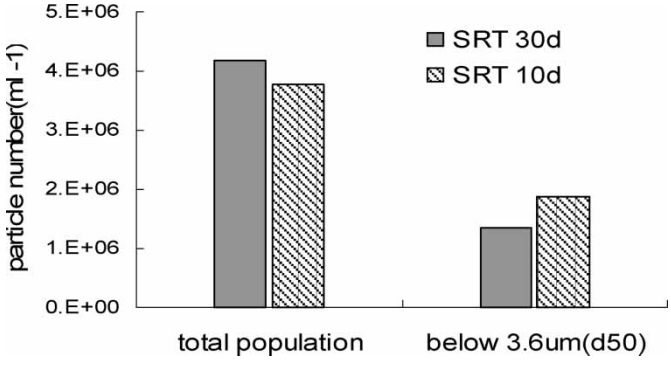


Figure 7. The particle population at the SRT of 10 days and 30 days.

(27). The smaller fraction of poly-disperse particles deposits on the membrane preferentially.

SMP and EPS Compositions

Figure 8 shows the TOC of the MBR effluent and reactor supernatant at the two SRTs. Under steady state conditions, the supernatant TOC were approximately 9.3 mg/L and 5.4 mg/L at the SRT of 10 days and 30 days respectively. The effluent TOC of the MBR were approximately 4.6 mg/L and about 2.4 mg/L at the SRTs of 10 days and 30 days representing TOC retentions of about 56% and 51% respectively.

EPS has been recognized as a major foulant in MBRs and is associated with the floc but also present in the supernatant. Figure 9a shows that the total amount of EPS were similar at 0.28 ± 0.1 g/L and 0.31 ± 0.1 g/L at the SRTs of 10 and 30 days and Figure 9b shows that the EPS production rates were 0.051 and 0.035 g/g MLSS respectively. Figures 10a and 10b show the concentrations of protein and polysaccharide in the supernatant. The supernatant protein fluctuated with time and the concentrations were similar for both the SRTs of 10 and 30 days. However, the polysaccharide concentrations in the supernatant showed a significant difference, with the polysaccharide concentration in the supernatant at the SRT of 10 days about double that at 30 days. This raised supernatant polysaccharide may explain the differences in membrane fouling rate described later.

Figure 10c and 10d show the protein and polysaccharide distribution in the supernatant and in the cell pellet. Up to 97% of the total EPS were associated with the cell pellets, suggesting the main fraction of EPS were adhered to the biofloc. Even though the EPS was associated with biofloc, it could still contribute to membrane fouling as flocs have been observed to attach and detach on the membrane (1).

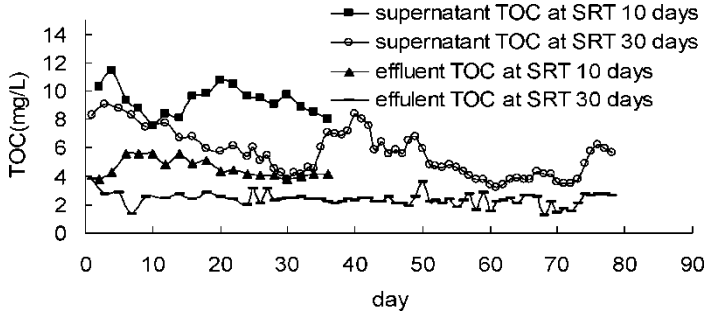


Figure 8. TOC of supernatant and effluent at the SRT of 10 days and 30 days.

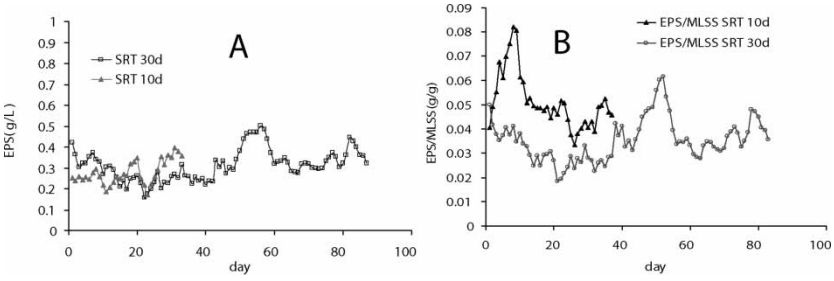


Figure 9. EPS concentration and distribution at the SRT of 10 days and 30 days.

Rheological and Hydrodynamic Characteristics

The rheological properties of the mixed liquor and hydrodynamic conditions will affect the shear stress, which is the main weapon in preventing deposition of foulant on the membrane surface. Figure 11 shows the viscosity characteristics of the mixed liquor. The MBR mixed liquor exhibited non-Newtonian behavior at the two SRTs, and the viscosity decreased as the shear rate increased. The results show that the viscosity and shear stress were always higher for the SRT of 30 days mixed liquor than that for 10 days.

In order to estimate the water velocity in the upriser of the MBR tank, a simulation experiment was performed. Glycerol solution was used to simulate the different viscosities of the MBR mixed liquor, where the viscosities of the mixed liquor for SRTs of 10 days and 30 days were

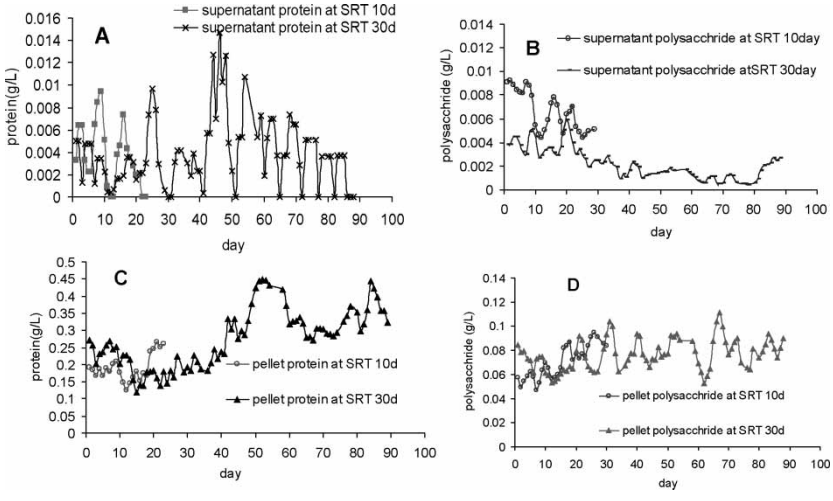


Figure 10. Protein and polysaccharide distribution in supernatant and in pellet (floc).

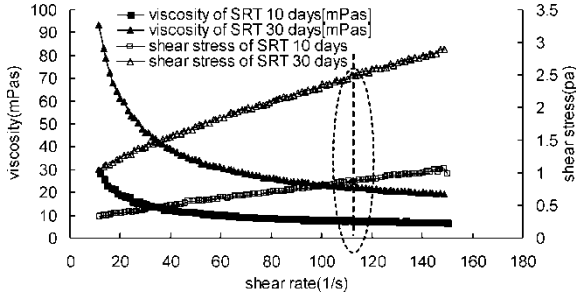


Figure 11. Biomass viscosity and shear stress at different shear rates.

approximately 7–15 mPas and 18–35 mPas respectively for shear rates higher than 60/s. Hence, the corresponding viscosities of the glycerol solutions were simulated at 9.6 mPas and 20.14 mPas respectively. Table 3 shows the hydrodynamic conditions of the simulated glycerol solutions in the MBR tank. Using particle image velocimetry (PIV), two dimensional velocity vectors were captured in a plane in the downcomer field. The average air lift water velocity in the riser of the tank was then calculated to be 0.079 m/s and 0.08 m/s at the SRTs of 30 days and 10 days respectively. Hence, the liquid velocities were assumed similar even though the viscosity for the SRT of 30 days was approximately 3 times higher than that for 10 days. (Using the lift water velocity as the reference of the tangential linear velocity in the viscosity measurement, the relative shear rate is 114/s). From Fig. 11, at this shear rate, the viscosities of the SRT 10 days and 30 days sludge were 7.72 mPas and 21.86 mPas respectively. The shear stress for SRT 10 days and 30 days sludge are then estimated to have been about 0.9 Pa and 2.54 Pa at a shear rate of 114/s respectively. Based on this analysis, although the viscosity was different at the two SRT conditions, the average air induced water velocity was very similar at low viscosity conditions.

Thus we can presume the average shear rate was similar on the membrane surface at the two SRT sludge conditions, but the estimated shear stress of the

Table 3. Water velocity measured with simulated mixture of glycerol and water

Viscosity	Average liquid velocity (air 5 L/min)
Simulated high viscosity (20.14 mPas) SRT 30 days	0.079 m/s
Simulated low viscosity (9.6 mPas) SRT 10 days	0.08 m/s

mixed liquor at SRT 30 days was about 3 times higher than that of SRT 10 days. Higher surface shear stress should provide better fouling control at SRT 30 days. The short-term and long-term tests described below confirm the lower fouling at SRT days.

Flux Stepping Tests

The flux stepping method was used to determine the apparent "critical flux." After each flux step the membrane was cleaned with 0.6% NaClO solution in order to maintain a constant clean water permeability.

Figure 12a and 12b show the TMP and flux against time for flux-stepping experiments at the SRTs of 10 days and 30 days. The results are compared in Fig. 13 which shows that as the flux increased, the rate of change of transmembrane pressure (dTMP/dt) increased. Importantly, the dTMP/dt for the SRT of 10 days was always higher than that for 30 days. From Fig. 13, we estimate that the "critical flux" was about $10\,L/m^2 \cdot h$ at the SRTs of 10 days and $20\,L/m^2 \cdot h$ at the 30 days.

Long Term Experiments

The long term TMP profiles in Fig. 14 were obtained during the 'steady-state' operating periods shown in Fig. 2. A constant flux of $20L/m^2 \cdot h$ was maintained at the SRTs of 10 days and 30 days. The flux level of $20\,L/m^2 \cdot h$ is around the "critical flux" of the sludge at the SRT of 30 days, while it is above "critical" at the SRT of 10 days. It should be noted that a flux of $20\,L/m^2 \cdot h$ is typical of an MBR. It can be seen that the TMP at the SRT of 10 days increased faster than that at the SRT of 30 days. For the SRT of 10 days, the TMP increased gradually at a rate of approximately 0.7 kPa/day for 8 days, followed by a rapid increase in TMP ("TMP jump"). The dTMP/dt at the SRT of 30 days increased slowly at a rate of approximately 0.24 kPa/day without exhibiting a rapid TMP rise

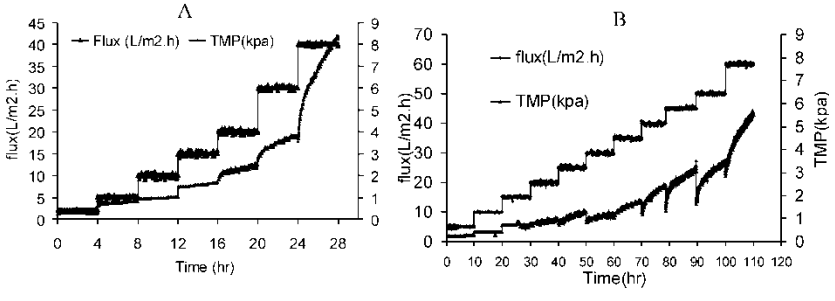


Figure 12. Flux stepping test at the SRT of 10 days and 30 days.

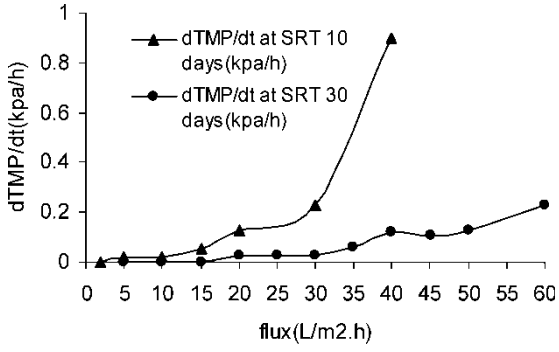


Figure 13. dTMP/dt vs. flux at the SRT of 10 days and 30 days.

after 25 days of operation (This phenomenon is described in previous work (1)). The flux stepping experiments show that the "critical flux" increased with prolonged SRT, and the long term experiments confirmed the result in long term filtration.

These carefully controlled experiments clearly show that in an MBR operated at a typical flux with a short SRT of 10 days, the fouling is worse that for a prolonged SRT of 30 days. The greater fouling tendency at SRT 10 days was observed even though the MLSS was significantly lower at 5–6 g/L compared to 8.5–10 g/L. The worse fouling at SRT 10 days could be explained by the significantly higher polysaccharide content of the mixed liquor supernatant or by the increased population of small biofloc. We observed different population structures by DGGE, but we cannot directly link that back to the different fouling properties. Another factor that may have given the SRT 30 days MBR lower fouling is the apparently greater shear stress at the membrane surface imposed by the air induced flow.

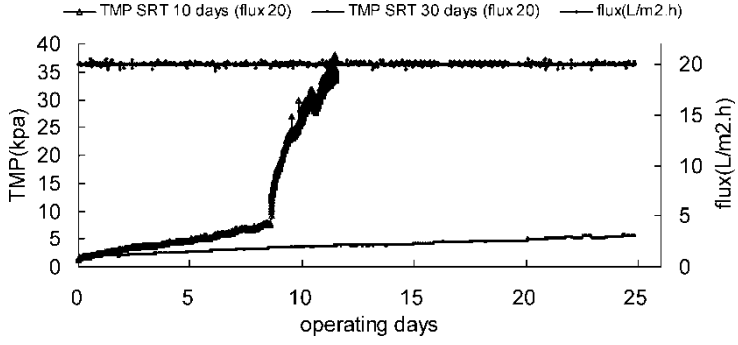


Figure 14. Long term TMP profile at flux 20 $L/m^2 \cdot h$ at the SRT of 10 days and 30 days.

CONCLUSIONS

This paper investigated the biodiversity, physical, and biochemical characteristics of biomass in submerged MBRs at low and prolonged SRT and their effect on membrane fouling under constant flux operation.
The conclusions are as follows:

i. the fouling propensity of the biomass at SRT of 10 days was greater than at SRT of 30 days; this was evident from both short-term (flux-stepping) and long term tests;
ii. in long term tests at "steady state," at a flux of $20\,L/m^2 \cdot h$, the SRT 10 days MBR showed a TMP rise of 0.7 kPa/day and a TMP jump after 8 days, whereas the SRT 30 days MBR showed a TMP rise of only 0.24 kPa/day and no TMP jump for up to 25 days;
iii. the greater fouling tendency for the SRT 10 day MBR occurred even though its MLSS was about 5–6 g/L compared with 8.5–10 g/L for the SRT 30 day MBR;
iv. the factors most likely to have contributed to the greater fouling for SRT 10 days are the larger population of small particles in the MLSS and the significantly higher polysaccharide EPS in the supernatant;
v. the lower shear stress estimated for the SRT 10 day MBR may also have played a role.

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