Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 21(2009) 1329–1335
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Effect of sludge retention time on sludge characteristics and membrane fouling of membrane bioreactor

OUYANG Ke, LIU Junxin*

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China. E-mail: oyk10220000@yahoo.com.cn

Received 22 December 2008; revised 15 February 2009; accepted 24 February 2009

Abstract

Three identical membrane bioreactors (MBRs) were operated over 2 years at different sludge retention time (SRT) of 10 d, 40 d and no sludge withdrawal (NS), to elucidate and quantify the effect of SRT on the sludge characteristics and membrane fouling. The hydraulic retention times of these MBRs were controlled at 12 h. With increasing SRT, the sludge concentrations in the MBRs increased, whereas the ratio of volatile suspended solid to the total solid decreased, and the size of sludge granule diminished in the meantime. A higher sludge concentration at long SRT could maintain a better organic removal efficiency, and a longer SRT was propitious to the growth of nitrifiers. The performance of these MBRs for the removal of COD and NH_4^+-N did not change much with different SRTs. However, the bioactivity decreased as SRT increase. The measurement of specific oxygen uptake rates (SOUR) and fluorescence *in situ* hybridization (FISH) with rRNA-targeted oligonucleotide probes testified that SOUR and the proportion of the bacteria-specific probe EUB338 in all DAPI-stainable bacteria decreased with increasing SRT. The concentrations of total organic carbon, protein, polysaccharides and soluble extracellular polymeric substance (EPS) in the mixed liquor supernatant also decreased with increasing SRT. The membrane fouling rate was higher at shorter SRT, and the highest fouling rate appeared at a SRT of 10 d. Both the sludge cake layer and gel layer had contribution to the fouling resistance, but the relative contribution of the gel layer decreased as SRT increase.

Key words: membrane bioreactor; sludge retention time; extracellular polymeric substances; wastewater treatment

DOI: 10.1016/S1001-0742(08)62422-5

Introduction

Membrane bioreactor (MBR) is commonly regarded as a promising technology for wastewater treatment and reclamation. By replacing the settling tank with a membrane filtration device, the MBR method shows many advantages over the conventional activated sludge (CAS) process, such as an excellent effluent quality and complete retention of microorganisms within the bioreactor to increase biomass concentration.

Sludge retention time (SRT) is an important factor affecting the performance of bioreactors, as SRT can have significant influence upon biomass properties in a MBR system. A long SRT is considered as an advantage of the MBR, because it can reduce the amount of sludge produced so as to save the cost for the sludge handling and disposal. In fact, many MBR researchers have operated their systems with longer SRT, since a higher biomass concentration, which was derived from longer SRT, gave rise to a higher treatment efficiency. Nevertheless, at long SRT the accumulation of dead or inactive microorganisms occurs in the MBR, and thus affects the sludge activity (Huang *et al*., 2001; Han *et al*., 2005). Attention has been given to the relationship between SRT and extracellular polymeric substances (EPS) formation in recent studies (Cho *et al*., 2005; Masse *et al*., 2006). Ng *et al*. (2006) operated MBRs at SRTs of 3, 5, 10 and 20 d, and found the concentrations of total organic carbon, proteins, and carbohydrates in the mixed liquor supernatant increased with decreasing SRT. In this study, the longest SRT was 20 d, while in real practice SRT might be much longer. However, Lee *et al*. (2003) ran three lab-scale submerged MBRs at SRT of 20, 40, and 60 d at a constant permeate flux, and no significant change in EPS was reported as SRT increased from 20 to 60 d.

Membrane fouling is a major constraint for the application of MBR in wastewater treatment, because it increases operating costs and capital investment. Therefore, the influence of SRT on membrane fouling is a relevant issue to be tackled. Membrane fouling is an inevitable phenomenon of all membrane processes (Kimura *et al*., 2005), but there were contradictory reports in literature about the effect of SRT on membrane fouling of submerged MBRs. Several studies stated that long SRT would increase the membrane fouling rate. Han *et al*. (2005), running the bioreactors at SRTs of 50, 70 and 100 d, clearly showed that at prolonged SRT membranes were fouled more severely and it was difficult to control membrane fouling with air scour. Lee *et al*. (2003) also observed that the overall fouling resistance

* Corresponding author. E-mail: jxliu@rcees.ac.cn

increased in the MBRs as SRT prolonged. However, other authors observed an opposite trend. Ahmed *et al*. (2007), operating their MBRs at SRTs of 20, 60 and 100 d, found that membrane fouling at shorter SRTs was rapid and the fouling rate declined with an increasing SRT. Ng *et al*. (2006) had also demonstrated that long SRT reduced the membrane fouling rate.

To date, it can be seen that the information about the effect of SRT on the characteristics of sludge and the membrane fouling in MBR varied from one study to another. In most of available publications, MBRs were operated at either an extremely long (Masse *et al*., 2006) or an extremely short SRT (Ng and Hermanowicz, 2005). Some studies were based on synthetic wastewater. Many researchers used only one MBR to study the effect of SRT by varying the SRT sequentially over the entire operation duration (Yoon *et al*., 2004). However, such approach might be inappropriate, as the operation performance and the characteristics of sludge could be influenced by the operation history and may not be affected solely by the SRT. Moreover, domestic wastewater characteristics can fluctuate with time too.

The objective of this study was to investigate the effects of SRT on the characteristics of sludge and the membrane fouling in MBR, using three different MBRs systems operated in parallel for domestic wastewater treatment. The trans-membrane pressure (TMP), mixed liquor suspended solids (MLSS), sludge production rates, specific oxygen uptake rate, particle sizes, and membrane fouling were measured at different SRTs.

1 Methods and materials

1.1 Experimental system and operational conditions

Three laboratory-scale submerged MBRs were used in this study (Fig. 1). The volume of each bioreactor was 35 L, and a water level sensor was used to keep a constant liquid level in the bioreactor. The membrane modules were made of hollow fibers of polyvinylidene fluoride (PVDF) with a mean pore size of 0.22 μm (Motimo, China), and

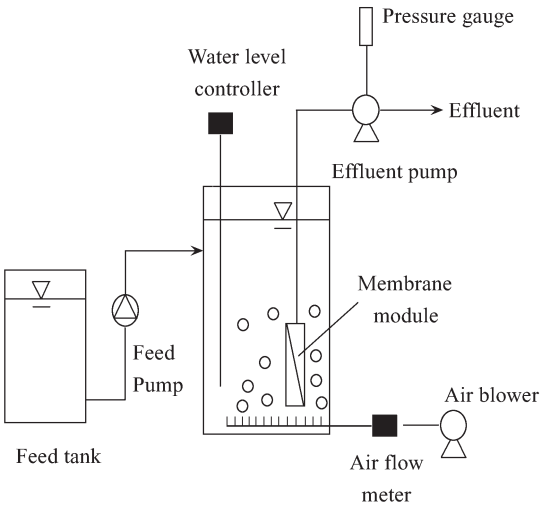

Fig. 1 Schematic structure of membrane bioreactor (MBR).

were all of uniform hydrophilicity. The effective filtration area of each membrane module was 0.30 m^2. The three MBRs were operated over a period of two years at a SRT of 10 d, 40 d and no sludge withdrawal (NS), respectively, to investigate the influence of different SRTs on sludge characteristics and membrane fouling. The hydraulic retention times (HRT) of these MBRs were all controlled at 12 h. The operating flux of membranes was maintained at 9.7 L/(m^2·h). A suction mode of 5 min "on" and 1 min "off" was adopted. Aeration was done through an air diffuser installed directly beneath the membrane module for transferring oxygen to microorganisms, mixing the liquor and cleaning the membrane. The air flow rates in three MBRs were maintained at 4 L/min. The experimental wastewater came from a domestic sewer and was fed into every MBR system by feed pumps. The raw wastewater characteristics are shown in Table 1. The seed sludge was obtained from the aeration tank of a local sewage treatment plant.

Table 1 Characteristics of the test wastewater

Item	COD_{Cr}	BOD_5	NH_4^+-N	PO_4^{3-}-P
Index range (mg/L)	85–750	55–300	45.6–186.3	3.1–10.5
Mean ± SD (n = 32)	324 ± 105	172 ± 65	69.7 ± 28.5	5.1 ± 2.2

1.2 Analytical methods

COD, NH_4^+-N, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solid (MLVSS) were analyzed according to the standard methods (Chinese SEPA, 2002). Total organic carbon (TOC) was measured using a TOC analyzer (TOC-Shimadzu, Japan). The particle size distribution was determined with a laser particle size analyzer (Mastersize 2000, UK).

EPS, consisting of polysaccharides, proteins, lipids and nucleic acids (Lee *et al*., 2003), were characterized in terms of soluble or bound polysaccharide and protein, since polysaccharides and proteins are considered as the most important constituents of EPS (Khongnakorn *et al*., 2005; Bai and Leow, 2002). Extraction of soluble and bound EPS in mixed liquor was carried out according to the methods of Lei and Zhou (2006), and Liu and Fang (2002). Proteins were determined by Lowry's method with bovine serum albumin (BSA) as a standard (Lowry *et al*., 1951). Polysaccharides were measured by the phenol-sulfuric acid method with glucose as a standard (Dubois *et al*., 1956).

The metabolic activity of the sludge in the MBRs was examined by the specific oxygen uptake rates (SOUR), which were measured with a membrane oxygen electrode (Cellox 325, WTW, Germany). The dissolved oxygen concentration was recorded as a function of time. According to the method described earlier (Witzig *et al*., 2002), the membrane oxygen electrode was inserted in a flask, sealed with a rubber stopper to prevent oxygen loss. The sludge was mixed with a magnetic stirrer. The DO concentration in the flask was recorded by the membrane

oxygen electrode at real-time.

The bacterial activity was quantified by the fluorescence *in situ* hybridization (FISH) method with rRNA-targeted oligonucleotide probes. The activated sludge samples extracted from the MBRs were fixed for 3 h with 4% paraformaldehyde, and then stored in a 1:1 mixture of phosphate-buffered saline (PBS, pH 7.4) and ethanol at –20°C. Before hybridization the samples were dispersed into individual cells by ultrasonication. The hybridization and washing procedures were performed in accordance to Manz *et al*. (1994). The 16S rRNA-targeted oligonucleotide probes used in this study was EUB338 which could quantify members of the domain bacteria with a sufficiently high rRNA content. Usually, cells with low rRNA contents were often associated with dormant, starved or very slowly growing states with a low metabolic activity (Witzig *et al*., 2002). Only the EUB probe-positive cells were considered as active (Li *et al*., 2006). The total bacterial number was determined by 4,6-diamidino-2-phenylindole (DAPI) staining. After the hybridization with EUB338, the slides were incubated in the dark with DAPI staining solution (1 μg/mL, 0.9 mol/L NaCl and 20 mmol/L Tris/HCl, pH 7.2) for 10 min, washed with distilled water, and dried in air. The slides were mounted with antifade reagents. Fluorescent hybridized cells were examined using an epifluorescent microscope (Axioskop2 mot plus, Zeiss Corp., Germany) equipped with a cooled CCD camera (AxioCam MRm, Zeiss Corp., Germany). All image processing and analysis were performed with the aid of a standard software package provided by Zeiss (Axio Vision 4.1) (Li *et al*., 2006).

1.3 Evaluation of membrane fouling

Based on the Darcy's law, the degree of membrane fouling was calculated as follows (Bae and Tak, 2005):

$$R = \frac{\Delta P}{\eta J} \tag{1}$$

$$R_t = R_m + R_f \tag{2}$$

$$R_f = R_c + R_g \tag{3}$$

where, R is the filtration resistance, ΔP (Pa) is the difference of trans-membrane pressure, η (Pa·s) is the viscosity of the permeate, J ($m^3/(m^2 \cdot h)$) is the membrane permeate flux, R_t (m^{-1}) is the total filtration resistance, R_m (m^{-1}) is the intrinsic membrane resistance, R_f (m^{-1}) is the fouling resistance, R_c is the cake layer resistance caused by sludge cake layer formed on the membrane surface, R_g is the fouling filtration resistance accounting for pore plugging and adsorption of foulants onto the membrane surface by colloids and solutes in the supernatant. R_m was measured in deionized water. R_f was calculated from R_t and R_m. In order to evaluate R_g, supernatant samples were taken after 2 h of gravitational sedimentation of the activated sludge mixed liquor, then filtration tests were performed with the supernatant samples, and R_g was calculated using Eq. (1). R_c was calculated from Eq. (3). All membrane modules were washed before filtration experiments.

2 Results and discussion

2.1 Performance of MBRs and characteristics of sludge at different SRTs

Three MBRs were initially inoculated with the activated sludge from a conventional wastewater treatment plant. After 80 d, all of the MBRs reached a steady state, and COD_{Cr} and NH_4^+-N removals of about 90% were achieved. The effluent quality of the MBRs was excellent during the steady operation, as the COD_{Cr} and NH_4^+-N concentrations in effluents of the MBRs were lower than 50 mg/L and 8 mg/L, respectively. The experimental results showed that the performance of these MBRs for COD_{Cr} and NH_4^+-N removal obviously was not affected by SRTs. The NH_4^+-N removal efficiency increased slightly (Table 2). As shown in Table 2, with increasing SRT the sludge concentration increased, the ratio MLVSS/MLSS decreased, and the sludge yield decreased. In fact, the long SRT and membrane retention brought a high sludge concentration in the MBR. Under such a low loading operation, the substrate/biomass ratio (F/M) was only 0.06 kg COD/(kg SS·d) in the MBR without sludge withdrawal, which was much lower than in the activated sludge process. The majority of cells in MBR were in an endogenous respiration state instead of a physiological state for growth under the low loading operation condition. Hence, it resulted in a low sludge yield. On the other hand, the accumulation of inert substances resulted in a decrease of the MLVSS/MLSS in the MBR at longer SRT. The experimental results in Table 2 indicated that the influence of SRT on the characteristics of the sludge in the MBR was quite obvious, but its impact on the MBR performance was insignificant.

Another change of the sludge characteristics was the difference of the sizes of sludge granules (Fig. 2). The initial size of sludge granules in the three MBRs was uniform, 163 μm, because the same seed sludge was introduced. It is known that the granule size diminished with the extension of runtime, and was affected by the SRT. After an operation period of 300 d, the sludge granule sizes in the MBRs at SRT of 10 d, 40 d and NS were 69.3, 64.5, 36.2 μm, respectively. The floc size of sludge in the MBRs was interrelated with a bonding force among bacteria cells and a hydraulic shear force due to an aeration. A longer SRT operation could bring a lower F/M ratio, and the accumulation of inert substances in the MBR reduced the

Table 2 Treatment efficiency and sludge characteristics in MBRs at different sludge retention times (SRTs)

SRT	10 d	40 d	NS
COD_{Cr} removal (%)[a]	91.2 ± 9.3	88.2 ± 8.6	90.0 ± 12.3
NH_4^+-N removal (%)[a]	89.6 ± 11.5	91.7 ± 8.3	92.0 ± 8.9
MLSS (g/L)[b]	2.0 ± 0.6	5.6 ± 1.5	7.4 ± 2.0
MLVSS/MLSS (%)[b]	78.6 ± 10.5	69.6 ± 9.3	62.6 ± 12
Sludge yield (kg SS/(kg COD·d))	0.26	0.16	0.12

[a] Data are expressed as mean ± SD (n = 30); [b] data are expressed as mean ± SD (n = 27).
MLSS: mixed liquor suspended solids; MLVSS: mixed liquor volatile suspended solids. NS: no sludge withdrawal.

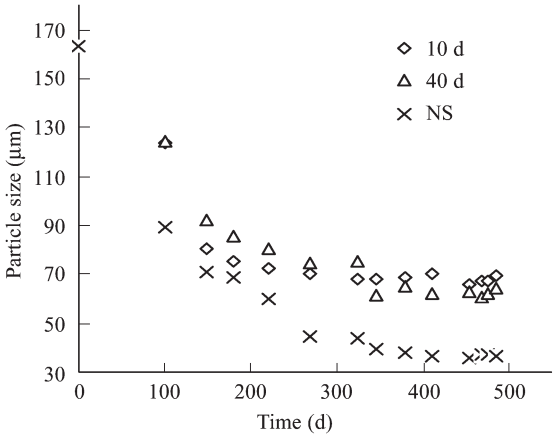

Fig. 2 Particle size distribution of sludge at different SRTs.

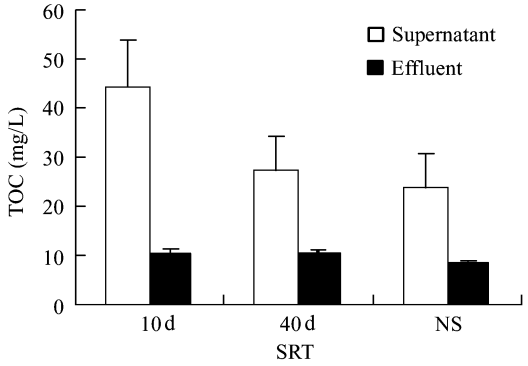

Fig. 3 Concentrations of total organic carbon (TOC) in supernatant and effluent at different SRTs.

Table 3 Protein and polysaccharide concentrations in the supernatant and effluent at different SRTs

SRT	Polysaccharide (mg/L)		Protein (mg/L)	
	Supernatant	Effluent	Supernatant	Effluent
10 d	31.55 ± 7.12	10.32 ± 3.57	34.63 ± 7.28	17.45 ± 4.23
40 d	12.46 ± 3.76	9.21 ± 2.37	14.53 ± 3.45	12.97 ± 4.85
NS	9.31 ± 2.49	6.56 ± 3.47	13.46 ± 4.12	7.39 ± 3.16

Data are expressed as mean ± SD (n = 12).

Table 4 Bound-EPS in flocs attached on the membrane surface at different SRTs

SRT	Polysaccharide (mg/g SS)	Protein (mg/g SS)
10 d	59.44 ± 19.86	35.23 ± 10.26
40 d	41.32 ± 15.43	31.08 ± 8.47
NS	42.14 ± 12.25	30.9 ± 6.51

Data are expressed as mean ± SD (n = 6).

bonding force. In the presence of a strong shear force, the sludge floc size diminished (Defrance and Jaffrin, 1999).

As the membrane kept the nitrifying microorganisms in the reactors at complete sludge retention, these autotrophic nitrifiers could proliferate without any loss. Consequently, a higher nitrification rate could be achieved in MBRs than in the conventional biological treatment plants (Chang *et al*., 2002). In spite of a diminished VSS/MLSS ratio, the high sludge concentration at long SRT can maintain a steady COD removal rate, and the long SRT is propitious to the growth of nitrifiers, which resulted in an increase in NH_4^+-N removal rate.

2.2 Characteristics of EPS

In this study, the EPS was represented by the sum of polysaccharides and proteins. The EPS can be sub-divided into three fractions: EPS bound to microbial flocs in the mixed liquor, EPS bound to microbial flocs adhered on the membrane, and EPS in the water phase. Membrane fouling is expected to be caused exclusively by the soluble EPS in the water phase and the bound EPS attached on the fouled membrane surface (Evenblij *et al*., 2005). The soluble EPS concentrations in mixed liquor supernatant and the bound-EPS contents in microbial flocs attached on the fouled membrane surface are shown in Tables 3 and 4, respectively. The concentrations of polysaccharides and proteins in the mixed liquor supernatant and effluent were found to decrease with increasing SRT. The TOC concentrations in the mixed liquor supernatant and effluent also displayed a similar trend as the polysaccharides and proteins (Fig. 3).

The data in Table 3 and Fig. 3 show that the concentrations of polysaccharides, proteins and TOC in the mixed liquor supernatant were always higher than those in the effluents at the three SRTs. This indicates that polysaccharides and proteins were accumulated in the MBRs. Although the protein concentration was higher than polysaccharide concentration in the supernatant (Table 3), the content of polysaccharides was higher than that of proteins in the bound-EPS in flocs attached on the membrane surface (Table 4). This is an interesting phenomenon. The membrane rejection of polysaccharides was more serious than that of proteins. The fouled membrane surface could have created an additional resistance for the soluble EPS to permeate the membrane. The passage of polysaccharides and proteins across the membrane could be at different rates, thereby resulting in different rejection efficiencies (Ng *et al*., 2006). This might be a possible cause. However, the actual mechanism of this phenomenon, which can be very challenging to elucidate, has yet to be identified. In addition, the EPS, especially polysaccharides, plays a crucial role in bioaggregate formation, because the polysaccharide fibrils can serve as a support of various compounds and cells (Meng *et al*., 2006; Walker and Bob, 2001). Hence, the concentration of polysaccharide would have a clear effect on the size of microbial flocs. The smaller the concentration of the polysaccharides was, the smaller the sludge floc size was. That was validated by the results in Fig. 2 and Table 3. As SRT increase, the concentrations of polysaccharides and proteins decreased. That was beneficial to the breakage of sludge flocs and resulted in a reduction of sludge particle size as SRT increase.

2.3 Bioactivity at different SRTs

The oxygen consumption rate can be taken as an equivalent indicator for the evaluation of the sludge metabolic activity. In this study, the SOUR of sludge in the MBR at a SRT of 10 d was 13.6 mg O_2/(gVSS·h), whereas it

decreased to 9.0 mg O_2/(gVSS·h) in the MBR without sludge withdrawal (Fig. 4). The experimental results indicated that the impact of SRT on the metabolic activity of sludge was obvious. The decrease of the SOUR can be correlated with the F/M ratio in the MBRs and the intercepting action of membrane. A low F/M ratio would cause a limiting supply of nutrient for microorganism growth, and could result in a low sludge yield (Table 2). The experimental results in Table 2 and Fig. 4 show that the F/M ratio was 0.17 kg COD/(kg SS·d) in the MBR at SRT of 10 d, and the corresponding sludge yield was 0.26 kg SS/(kg COD·d), whereas the F/M ratio was 0.06 kg COD/(kg SS·d) in the MBR without sludge withdrawal, corresponding to a decreased sludge yield of 0.12 kg SS/(kg COD·d). The decrease of the sludge activity was consistent with the reduction of the sludge yield. The efficient membrane interception was another cause resulting in the SOUR decrease, because a large amount of inert biomass was retained in the MBR. In order to quantify the sludge bioactivity in the MBRs, the total bacterial number was counted by DAPI staining, and the active cells were examined by the EUB probe as described in Section 1.2. The EUB/DAPI ratio was used to assess the overall physiological state of bacteria. Figure 4 shows the changes of the EUB/DAPI ratio with the SRT. About half of the DAPI-stained cells gave a clear fluorescence signal at the SRT of 10 d. However, the EUB/DAPI ratio decreased rapidly to 21%, when the SRT increased from 10 d to without sludge withdrawal in the whole experimental duration (exception for sampling). This implied that almost 80% of the bacteria in the MBR at a long SRT were inactively. This also indicated that membrane interception played an important role in the accumulation of dead or inert cells.

2.4 Membrane fouling at different SRTs

A dynamic layer is gradually formed on the membrane during the MBR operation, and consequently membrane fouling occurs. The dynamic layer includes a cake layer formed by sludge particles intercepted and/or adsorbed by membrane and a gel layer formed by the organic solutes and EPS. In general, the degree of membrane fouling is expressed by the membrane resistance. According to the Eq. (1), the total filtration resistance of membrane (R_t) can be calculated from the monitored trans-membrane pressure (TMP). Figure 5 shows the experimental results of the trans-membrane pressure of the MBRs at different SRTs. The results showed that membrane fouling at shorter SRT operation was rapid, and the fouling rate declined with increasing SRT. The highest fouling rate was found at a SRT of 10 d in the MBR, and the lowest fouling rate appeared in the MBR without sludge withdrawal. These observations were inconsistent with some previous studies that suggested MBRs operated under a prolonged SRT tend to have a higher fouling potential (Lee *et al*., 2003). This might be explained by the fact that the MLSS concentrations in all three reactors were very low, and the MLSS concentration was only 7.4 g/L in the case of "without sludge withdrawal". At higher MLSS concentrations, the effect may change. The membrane fouling can be caused not only by microbial floc, but also by supernatants (Bai and Leow, 2002). Hence, the soluble EPS concentration in the mixed liquor supernatant plays an important role in membrane fouling. A high concentration of soluble EPS may enhance membrane fouling (Kimura *et al*., 2005). This study shows that the soluble EPS concentration increased at short SRT, and the corresponding membrane fouling rate increased synchronously.

Besides the soluble EPS in the mixed liquor supernatant, the sludge cake layer formed by sludge particles or floc is also an important fraction resulting in membrane fouling. In order to evaluate the contribution of these two fractions to membrane fouling, the mixed liquor from the MBRs was microfiltered as described by Defrance *et al*. (2000). According to Eqs. (1)–(3), the total filtration resistance (R_t) of the membrane, the cake layer resistance (R_c) and the gel layer resistance (R_g) caused by polymers, fragments of lysed cells and dilute matter are shown in Fig. 6. It is clear that both R_c and R_g have contribution to the fouling resistance. R_c and R_g decrease with increasing SRT, and R_g has a larger declining range than R_c. When SRT increased from 10 to 40 d, the decline of R_g was obvious, and then its decline was slow. The decline trend of R_g was similar to that of EPS in the mixed liquor supernatant in Table 3. On the other hand, the contributions of R_c and R_g to total fouling resistance were identical at SRT of 10 d, however,

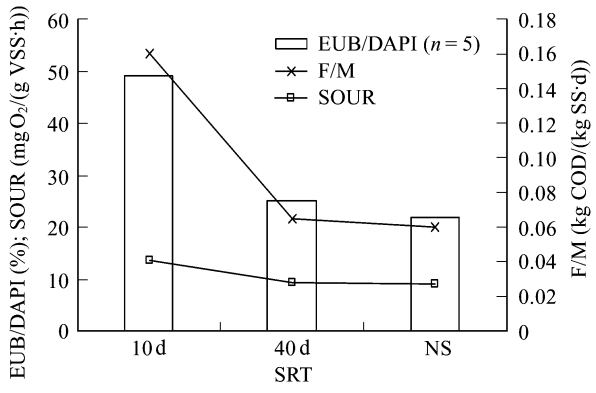

Fig. 4 Bioactivity at different SRTs. F/M: substrate/biomass ratio. EUB/DAPI: the active cells/the total cells, SOUR: the specific oxygen uptake rates.

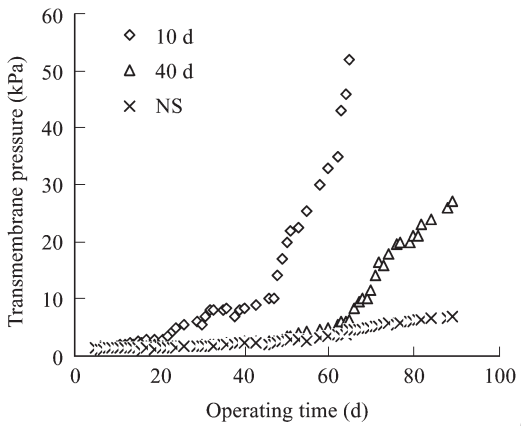

Fig. 5 Trans-membrane pressure at different SRTs.

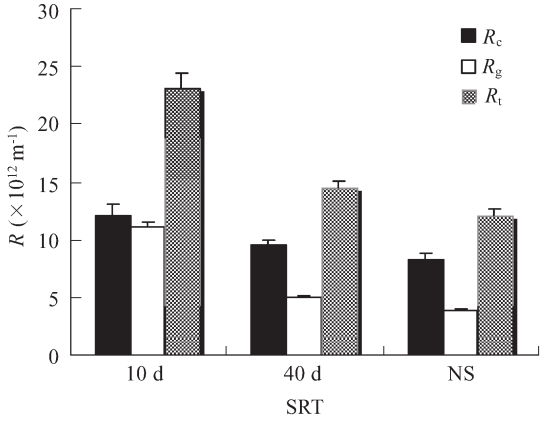

Fig. 6 Resistances of various fractions from the mixed liquor at different SRTs.

the relative contribution of R_g decreased with decreasing SRT.

3 Conclusions

In this study three MBRs were operated at SRTs of 10 d, 40 d, and NS to elucidate the effect of SRT on sludge characteristics and membrane fouling. The following specific conclusions were obtained:

(1) With increasing SRT, the sludge bioactivity characteristics, SOUR and EUB/DAPI ratio in the MBRs decreased. However, the removals for COD_{Cr} and NH_4^+-N were not significantly affected by the SRTs. The high sludge concentration due to the complete rejection by membrane enhanced the overall performance of the MBRs, and resulted in a decrease of the F/M ratio and the sludge yield.

(2) The soluble EPS in the mixed liquor supernatant is an important factor influencing membrane fouling. The experimental results show that the protein concentration was higher than the polysaccharide concentration in the mixed liquor supernatant, whereas the polysaccharide content was higher than the protein content in the flocs attached to the membrane surface. With increasing SRT, their contents decreased, hence, the filtration resistance of the membrane also decreased.

Acknowledgments

This study is financially supported by the National Natural Science Foundation of China (No. 50678170).

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