Microbial behaviors involved in cake fouling in membrane bioreactors under different solids retention times

Wu, Bing; Yi, Shan; Fane, Anthony Gordon

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1 **Microbial Behaviors Involved in Cake Fouling in Membrane**
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7 **Bioreactors under Different Solids Retention Times**
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10 Bing Wu^{1,*}, Shan Yi², Anthony G. Fane¹
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17 5 ¹Singapore Membrane Technology Centre, Nanyang Technological University, 639798,
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19 6 Singapore
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22 7 ²Department of Civil and Environmental Engineering, University of California, Berkeley, CA
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24 8 94720, USA
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27 9 *Corresponding Author: Bing Wu; Email: wubing@ntu.edu.sg; Tel: 65-6790-2600; Fax: 65-
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4 32 **Abstract**
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7 33 Biomass characteristics and membrane performances in the MBRs operated at a high flux
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9 34 of 30 L/m² hr under different SRTs (10 days, 30 days, and infinity) were monitored.
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11 35 Results showed that more serious cake-fouling happened in the SRT-infinity MBR,
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13 36 which correlated with the activated sludge characteristics such as smaller floc size and
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15 37 greater EPS amount. DGGE analysis indicated that the microbial community shifted in
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17 38 different ways under various SRTs, which also influenced EPS productions in the MBRs.
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19 39 Different microbial communities were developed on the membrane surface at various
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21 40 operating stages and SRTs. Possibly, the activated sludge characteristics (such as MLSS
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23 41 concentration, EPS properties) and hydrodynamic conditions influenced by the SRTs
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25 42 were associated with cake layer development and membrane fouling propensity. Insight
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27 43 into the EPS characteristics and deposition behaviors of bacterial flocs will be crucial to
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29 44 explore appropriate biofouling control strategies in MBRs.
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37 45 *Keywords:* Bound EPS; Extracellular polymeric substances; Flux; Membrane fouling;
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39 46 PCR-DGGE; Shear force
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1. Introduction

The membrane bioreactor (MBR) technology offers several benefits compared to the conventional activated sludge process, such as superior effluent quality, small footprint, and reduced waste sludge production. However, the universal appeal of MBR technology is limited by membrane fouling, which reduces productivity and increases operating and energy costs of MBRs (Han et al., 2005; Ng and Hermanowicz, 2005). In general, membrane fouling is attributed to the larger soluble molecules plugging and narrowing the pores of membranes, or the particles depositing on the membrane surfaces to form a cake layer (Defrance et al., 2000; Bae and Tak, 2005).

Among the potential foulants, of particular interest are extracellular polymeric substances (EPS), which are excreted or autolyzed by microorganisms and are believed to be a major fouling contributor to MBRs. EPS normally attach on the bacterial surface in the flocs (bound EPS), or present dissolved or suspended in the solution (soluble EPS). Based on the distribution of EPS, the mixed liquors are considered to contain two fractions: microbial floc and supernatant including colloids and solutes. Recent studies have attempted to quantify the fouling caused by each fraction of the mixed liquor, such as the suspended solids (i.e. bacterial flocs), colloids, and solutes, although the results are inconsistent. Some researchers have concluded that the resistance of the cake layer formed by microbial floc appeared to determine the overall resistance (Chang and Lee, 1998; Defrance et al., 2000; Lee et al., 2003; Bae and Tak, 2005). However, other researchers have identified the soluble compounds or colloids as prime important factors in causing membrane fouling in MBRs (Wisniewski and Grasmick, 1998; Bouhabila et al., 2001; Chang and Kim, 2005). It suggests that the fouling contributions from various

fractions of the mixed liquor may be variable based on membrane properties, hydrodynamic conditions and physiological characteristics of the activated sludge which were determined by the reactor operating conditions (Bouhabila et al., 2001; Bae and Tak, 2005).

Recently, EPS properties and their contributions to membrane fouling at different SRTs have been investigated (Ng et al., 2006; Al-Halbouni et al., 2008; Malamis and Andreadakis, 2009). Some researchers further characterized the microbial communities, especially those involved in cake layer formation and fouling evolution at the different operating conditions of MBRs (Jinhua et al., 2006; Zhang et al., 2006; Huang et al., 2008; Duan et al., 2009). However, the dynamic microbial communities in the cake layer and in the mixed liquor at different SRTs are poorly investigated. In addition, the factors influencing microbial community structures in the cake layer at various operating stages, especially in the initial phase, remain not well explained.

The object of this study was to investigate microbial diversity, EPS properties, and membrane fouling tendency in the MBRs operated at a high flux (sub-critical flux) under different SRTs. By analyzing the similarity index using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) tools, the relationship between the microbial community shifts, EPS properties and reactor performance was examined and the fingerprints of cake layer community and mixed liquor community were compared. Importantly, the cake-fouling relevant communities at the unstable (i.e. initial period) and stable operation of the MBRs were monitored to explain how the activated sludge characteristics influenced the cake layer development.

2. Materials and methods

2.1. Description of MBR system and operating conditions

Three-parallel MBRs were operated simultaneously at the same hydraulic retention time (HRT) of 12 hr, aeration rate of 3 L/min, and flux of 30 L/m²*hr at a room temperature of 25°C with different SRTs (10 days, 30 days, and infinity). In the each MBR, a U-shape hollow fibre membrane module (Polyacrlonitrile, pore size of 50,000 Dalton, 0.1 m², Blue Star Company, China) was directly submerged in a 3 L cylindrical reactor. Permeate was obtained by a suction pump whose condition (on/off) was controlled by a level switch (i.e., intermittent filtration). Transmembrane pressure (TMP) in the MBR was monitored by the pressure transducers (SMC, Japan), which were connected to a personal computer equipped with data log system (LabVIEW, National Instruments, USA).

The seed sludge was initially from the Jurong Wastewater Treatment and Reclamation Plant (Singapore), which had been acclimatized in the lab-scale MBRs for more than one year. Each reactor was seeded with the same amount of the acclimatized activated sludge (3 g/L) and fed with the synthetic wastewater at an organic loading of 2.28 g COD L⁻¹ day⁻¹. The synthetic wastewater consisted of C₆H₁₂O₆ (500 mg/L), CH₃COONa (500 mg/L), NH₄Cl (300 mg/L), MgSO₄·7H₂O (100 mg/L), KH₂PO₄ (53 mg/L), K₂HPO₄ (107 mg/L), NaHCO₃ (40 mg/L), CaCl₂ (7.5 mg/L), and FeCl₃ (0.5 mg/L).

2.2. Analytical methods

Total organic carbon (TOC) was examined by a TOC analyzer (Shimadzu, Japan). Mixed liquor suspended solids (MLSS) were measured as described by standard methods

(APHA, 1998). Microbial floc size was examined with a laser particle sizer (Malvern, UK). The pellet and supernatant were separated from the mixed liquor sample by centrifuging at 4000 rpm for 10 min. The bound EPS was extracted from the pellet following a method described in the previous study (Wu et al., 2010). The soluble EPS from the supernatant and the bound EPS were analyzed for polysaccharides and protein based on the methods described by Dubois et al. and Bradford, respectively (Dubois et al., 1956; Bradford, 1976).

2.3. DNA extraction, PCR, and DGGE conditions

The membrane modules were cleaned with 0.5~1.0% sodium hypochlorite before use. Two pieces of fibres were cut from a membrane module after working for 48 hr in the MBRs at the different periods of time (Day 3 and Day 120). The fibres were then gently washed with sterile 0.85% NaCl solution to remove any floc loosely attached on the membrane surface. After that, the fibres were put into a sterile tube filled with 50 ml sterile 0.85% NaCl solution and the attached bacteria (i.e., bacteria in the cake layer) were removed from the membrane surface by ultrasonication for 30 sec. Meanwhile, the mixed liquor sample was taken from the MBR and put into a sterile tube, which was ultrasonicated for 30 sec to disperse the flocs.

DNA was extracted by bead beating samples followed by extraction with saturated phenol and chloroform, based on a protocol described previously (Zhuang et al., 2005). The V3 region of bacterial 16S rRNA was amplified by PCR in a thermal cycler (Eppendorf, Germany). A 50 µl mixture contained 1.25 U of *Taq* DNA polymers, 5 µl 10× Buffer B, 4 µl 25 mM MgCl₂, each deoxynucleoside triphosphate at a concentration

of 200 μ M (Promega, USA), 25 pmol of each primer (5'-GCC CGC CGC GCG CGG CGG GCG GGG CGG GGG CAC GGG GGG CCT CCT ACG GGA GGC AGC AG-3' and 5'-ATT ACC GCG GCT GCT GG-3'), and 500 ng of the DNA template (Yi et al., 2006). DGGE analysis was used to analyze the PCR-amplified DNA fragments by the Dcode Universal Mutation Detection System (Bio-Rad, USA). The fragments were separated on polyacrylamide gels (10%, 37.5:1 acrylamide-bisacrylamide, Bio-Rad, USA) with a 30 to 70% linear gradient of denaturant. Gels were run for 15 hr at a constant 90 V maintained at 60°C and photographed by a Gel Imaging System (Kodak, USA). Similarity index of band patterns was calculated by a GelCompar II software (Applied Maths, Kortrijk, Belgium), which was automatically calculated with Dice coefficient by the unweighted pair group method with arithmetic mean algorithms (UPGMA).

3. Results and discussion

3.1. Activated sludge characteristics and reactor performances under different SRTs

The activated sludge had an initial concentration of 3 g/L, F/M of 0.75 day⁻¹, and a median particle size (d_{50}) of about 300 μ m in the three MBRs. After 60-day operation (the reactors approached the steady state), the effluent TOCs in the three MBRs were within 10 mg/L (above 90% removal rate, data not shown), almost regardless of SRTs. The MLSS concentrations were steady at 4.8 $\pm$ 0.6, 8.3 $\pm$ 1.2, and 13.1 $\pm$ 0.7 g/L (Table 1), accordingly, the ratios of F/M kept at about 0.44 $\pm$ 0.06, 0.27 $\pm$ 0.04, and 0.17 $\pm$ 0.05 day⁻¹ for the SRT 10-day, SRT 30-day, and SRT-infinity MBRs, respectively. The median particle sizes (d_{50}) of the activated sludge in the SRT-infinity MBR maintained at 178.0 $\pm$ 16.9 μ m, slightly lower than those in the SRT 10-day and SRT 30-day MBRs

(218.5±43.2 and 243.7±46.2 μm respectively) (Table 1). The dissolved oxygen values clearly decreased with extending SRTs at a fixed aeration rate of 3 L/min, reaching about 6.2, 3.8, and 1.8 mg/L for the SRT 10-day, SRT 30-day, and SRT-infinity MBRs, respectively (Table 1).

Table 1 also shows that the activated sludge in the SRT 30-day MBR had slightly higher EPS concentration (0.85±0.09 g/L) than that in the SRT 10-day MBR (0.78±0.09 g/L), which were nearly half of that in the SRT-infinity MBR (1.31±0.17 g/L). The soluble EPS and bound EPS concentrations at the steady state were monitored as shown in Figure 1. The soluble EPS content was within a range of 0.015~0.045 g/L, only around 1.5~4.5% of the bound EPS. Additionally, the soluble EPS amount dropped with an increase of SRTs, but the bound EPS amount followed an opposite trend. Possibly, the more shear force induced by aeration in the shorter SRT MBR could lead to the release of bound EPS into the supernatant and then generate more soluble EPS (Wang et al., 2009).

At the steady state, the MBRs exhibited a two-stage increase in the transmembrane pressure (TMP) profile at the flux of 30 L/m² hr (Figure 2). Clearly, a higher membrane fouling rate occurred in the SRT infinity MBR (0.0439 kPa/hr), almost 2-fold of SRT 10-day (0.0269 kPa/hr) and 30-day MBRs (0.0201 kPa/hr) (Table 1). Several factors could explain the serious fouling phenomenon at the prolonged SRT, such as the relatively smaller floc size (Pearson's correlation efficient $r = -0.994$; P-value= 0.001, right-tailed Student's t -test) and the greater amount of EPS ($r = 0.912$; P-value= 0.056) at a confidence level of 90%. This suggests that under the high flux of 30 L/m² hr, cake fouling appeared to be a predominant contributor to TMP increase in the MBRs.

3.2. Microbial community shifts and EPS productions under different SRTs

DGGE profiles (Figures 3A-C) show bacterial population shifted with operating time in different ways under various SRTs although the similar band patterns were found in the initial period (Figure 4A, Lanes 1-3) presumably due to the same seed being employed in the three MBRs. By examining the similarity index of the DGGE profile, it was observed that at the beginning of operation (Day 3-10), the microbial community structure in the each reactor shifted rapidly (Figure 5A). When the MBRs reached to the steady state, the change of bacterial population became insignificant in the each MBR (Figures 3A-C; stable similarity index achieved, data not shown). However, the dominant microbial communities in the MBRs were obviously different under the various SRTs (Figure 4B, Lanes 1-3). It should be recalled that the various SRTs were associated with different ratios of food to microorganisms (F/M) and dissolved oxygen concentrations. Either or both of these environmental conditions could probably favor specific bacterial species, as a result, dominant microbial communities displayed different profiles at the various SRTs. The similar observation was also made by Duan and colleagues when the MBRs were operated at the short SRTs (i.e., 3, 5, and 10-day) (Duan et al., 2009).

In the beginning of MBR operation, obvious changes in their EPS productions also occurred in the MBRs, mainly via protein productions (Figures 5A and B). Possibly, the bacteria could induce more enzymes in order to acclimatize to the new living environment and experience metabolic stress. When the MBRs approached the steady state, the change of EPS production in the each reactor was not significant (Figure 5C). This correlated with the other researchers' findings that besides stable membrane permeability, activated sludge characteristics (biomass concentration, particle size) and

organic removal rate, stable microbial community and their metabolic properties (such as EPS production) were also considered as important factors to indicate the stable MBR performance (Miura et al., 2007).

In particular, at the steady state, EPS productions maintained at about 130 ± 19 , 111 ± 10 , and 80 ± 18 mg/ gMLSS for the SRT 10-day, SRT 30-day, and SRT-infinity MBRs, respectively (Figure 5C), which indicates that EPS amount per unit biomass dropped with extending SRTs. The similar observation has been reported by other researchers as well (Malamis and Andreadakis, 2009).

Shorter SRT MBRs had a higher F/M ratio, a higher dissolved oxygen concentration, and a more efficient oxygen transfer rate (due to a decrease of fluid resistance associated with lower MLSS concentration and viscosity) compared to longer SRT MBRs. This surplus substrate and energy utilized by the microorganisms could contribute to form intracellular storage granules and extracellular polymers that accumulated outside of the cells (Liao et al., 2001). For prolonged SRT, it has been reported that biological activity such as specific oxygen uptake rate, specific nitrification rate and specific denitrification rate was decreased (Han et al., 2005). It is evident that a comparatively large amount of cell lysis could occur due to lower microbial growth rates and higher levels of endogenous metabolism at higher SRTs. However, although cell lysis was predominant and contributed greatly to the total EPS production, the limited substrate, and lower dissolved oxygen concentration could decrease the sludge yield coefficient and retard EPS production by microorganisms. Therefore, EPS production as a characteristic of microorganisms is dependent on the dominant bacterial species as well as the living

conditions of the microorganisms, such as F/M and dissolved oxygen. An optimal SRT range with lower EPS production can be expected based on these factors.

3.3. Comparison of microbial communities in the cake layer and in the mixed liquor

The DGGE footprints of the microbial communities attached on the membrane surface (i.e., a cake layer) and suspended in the mixed liquor were compared when the MBRs were operated at different SRTs. After inoculation for 3 days in the MBRs, microbial community structures in the mixed liquor showed a high similarity (Figure 4A, Lanes 1-3, the similarity index of 89%), possibly because the same seed was fed into the reactors and during this short period of time, the microbial communities mainly focused on regulation of their metabolic function to adapt to the new environment. However, apparently, the cake layer harbored diverse microbial species, which insignificantly correlated with the dominant species in the mixed liquor of the MBRs. Also, the microbial communities in the cake layers displayed the different profiles at the various SRTs (Figure 4A, Lanes 4-6).

At the steady state, the PCR-DGGE fingerprints demonstrated that there were distinctly different microbial community structures in the mixed liquors of the MBRs employing various SRTs. However, the bacterial consortia in the cake layer and in the mixed liquor shared numerous common bands in the each MBR (Figure 4B). The difference on the microbial communities in the cake layer and in the mixed liquor at the various stages was proposed to be associated with the force balance (i.e., the relationship between the cross-flow lift force and permeation drag force) on the microbial flocs along the membrane surface (Thomas et al., 2000; Wang et al., 2005).

In the high-flux MBRs, for colloids and particulars (size typically $> 0.2 \mu\text{m}$), the back transport mechanism that limits concentration polarization could be inertial lift or shear induced diffusion described as $J = k_{si} \ln(C_w / C_b) + V_L$ (Belfort et al., 1994). In the formula, k_{si} is the shear induced diffusivity (m/s), C_w and C_b are the species concentrations along membrane wall and in the bulk, and V_L is the inertial lift velocity (m/s). In a crossflow filtration system, if membrane permeation velocity (J) is less than lift velocity (V_L) at the membrane surface, particles would not deposit on the membrane surface, otherwise the deposition of particles occurs (Green and Belfort, 1980).

At the beginning of MBR operation, due to the limited viscosity of the activated sludge (less MLSS and EPS amount), the shear force created by the aeration (aeration intensity of $1.8 \text{ m}^3/\text{m}^2 \text{ hr}$) was more sufficient than the permeation force (the fixed flux of $0.03 \text{ m}^3/\text{m}^2 \cdot \text{hr}$) along the membrane. This possibly led to easy detachment of the cake layer from the membrane surface. As a result, some specific microbial species, which may be not dominant in the mixed liquor, were selectively accumulated on the membrane during 2-day's filtration. Our finding was similar to the report of Zhang and colleagues that microbial species could selectively attach to the membrane at a high tangential shear force during a short period of filtration (4 hr) (Zhang et al., 2006). Other researchers also observed the similar phenomenon that the bacterial community structures on the membrane surface and in the mixed liquor were obviously different at a lower flux ($0.002 \sim 0.015 \text{ m}^3/\text{m}^2 \cdot \text{hr}$) when the membranes had been filtrated for 7~50 days (Jinhua et al., 2006; Huang et al., 2008).

In contrast, at the steady state, increases of MLSS and EPS concentrations, presenting high viscosity, provided a greater barrier. It has been reported that shear force along the

membrane decreased exponentially with increasing sludge viscosity (Meng et al., 2007). Thus, the strong convection force associated with the high flux was possibly more than the shear force at the layer-solution interface, especially in the longer-SRT MBRs. Accordingly, the bacterial flocs could be driven to tightly attach on the membrane surface, which induced the similar microbial communities in the cake layer as those in the mixed liquor after 2-day's filtration. Therefore, the microbial community structures determined by the operating conditions (such as SRT) of MBRs could influence the activated sludge characteristics (such as EPS properties), which were associated with cake layer development and membrane fouling propensity. Further tracing bacterial community developed on the membrane surface with TMP increase and exploring their relationship at different operating stage (e.g., unstable and stable stages) and conditions (e.g., various SRTs, permeate flux) will be proposed to investigate.

4. Conclusions

This study examined the shifts of microbial community and the changes of EPS properties in the MBRs, which displayed different profiles at various SRTs. More serious cake-fouling occurred in the SRT-infinity MBR, due to the smaller floc size and the greater EPS amount. The microbial community developed on the cake layer was possibly determined by the dynamic balance of the shear force and the convection force along the membrane surface, which was affected by the biomass characteristics at the different stages of MBR operation.

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402 **Figure Captions:**

403 **Fig. 1. Soluble EPS (A) and bound EPS (B) concentrations in the MBRs at different**
404 **SRTs. (◇) SRT of 10 day; (□) SRT of 30 day; (○) SRT of infinity.**

405 **Fig. 2. TMP profiles plotted as a function of time at different SRTs (Day 105 to Day**
406 **120). (◇) SRT of 10 day; (□) SRT of 30 day; (○) SRT of infinity.**

407 **Fig. 3. DGGE of PCR-amplified 16S rRNA from the microbial communities in the**
408 **mixed liquor. (A) SRT of 10 day; (B) SRT of 30 day; (C) SRT of infinity.**

409 **Fig. 4. DGGE of PCR-amplified 16S rRNA from the microbial communities in the**
410 **initial phase (A, Day 3) and at the steady state (B, Day 120). (1) In the mixed liquor**
411 **at SRT of 10 day; (2) In the mixed liquor at SRT of 30 day; (3) In the mixed liquor**
412 **at SRT of infinity; (4) In the cake layer at SRT of 10 day; (5) In the cake layer at**
413 **SRT of 30 day; (6) In the cake layer at SRT of infinity.**

414 **Fig. 5. EPS production and similarity index in the initial phase (A), protein**
415 **production in the initial phase (B), and EPS production at the steady state (C).**
416 **White fill represents EPS production and grey fill represents similarity index; (◇)**
417 **SRT of 10 day; (□) SRT of 30 day; (○) SRT of infinity.**

Table 1. Activated sludge characteristics and membrane performances in the MBRs at the steady state.

SRT (Days)	Activated Sludge Characteristics				Membrane performance
	MLSS (g/L)	Median Particle size (μm)	Dissolved Oxygen (mg/L)	EPS concentration (g/L)	Fouling rate (dTMP/dt) (kPa/hr)
10	4.8 $\pm$ 0.6	218.5 $\pm$ 43.2	~6.2	0.78 $\pm$ 0.09	0.0269
30	8.3 $\pm$ 1.2	243.7 $\pm$ 46.2	~3.8	0.85 $\pm$ 0.09	0.0201
Infinity	13.1 $\pm$ 0.7	178.0 $\pm$ 16.9	~1.8	1.31 $\pm$ 0.17	0.0439

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5