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Microbial Community Developments and Biomass Characteristics in Membrane Bioreactors under Different Organic Loadings

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Abstract

Microbial community developments and biomass characteristics (concentration, particle size, extracellular polymeric substances (EPS), and membrane fouling propensity) were compared when three MBRs were fed with the synthetic wastewater at different organic loadings. Results showed that the bacterial communities dynamically shifted in different ways and the EPS displayed dissimilar profiles under various organic loadings, which were associated with the ratios of food to microorganism and dissolved oxygen levels in the MBRs. The membrane fouling tendency of biomass in the low-loading MBR (0.57 g COD/L day) was insignificant different from that in the medium-loading MBR (1.14 g COD/L day), which was apparently lower than that in the high-loading MBR (2.28 g COD/L day). The membrane fouling propensity of biomass was strongly correlated with their bound EPS contents, indicating cake layer fouling (i.e., deposition of microbial flocs) was predominant in membrane fouling at a high flux of 30 L/m^2 hr.

Keywords: Denaturing gradient gel electrophoresis; Dissolved oxygen; Extracellular polymeric substances; Membrane fouling; Ratio of food to microorganism

1. Introduction

In membrane bioreactors (MBRs), extracellular polymeric substances (EPS) are believed to be primarily responsible to membrane fouling. The operating conditions (e.g., sludge retention time (SRT), hydraulic retention time (HRT), aeration) of MBRs and the addition of flocculants in MBRs have been proved to affect EPS characteristics, which further lead to different membrane fouling profiles (Meng et al., 2007; Phattaranawik et al., 2007; Malamis and Andreadakis, 2009; Ji et al., 2010; Wu et al., 2011).

Since MBRs have been applied to treatment of a wide range of industrial and municipal wastewater with variable nutrient inputs, the influence of organic loading on MBR performance has been also paid more attention. Several studies have pointed out that the differences in the nutrient sources could influence physiological properties of biomass and chemical compositions of the extracted exopolymers in the various activated sludge processes (Huang et al., 1994; Boyette et al., 2001; Sponza, 2003). In MBRs, the changes of biomass characteristics could further have an effect on the membrane performances. For example, compared to the constant loadings, less membrane fouling occurred in the MBRs with the variable loadings at steady state due to lower contents of polysaccharides in the supernatant (Zhang et al., 2010).

In detail, the changes of organic loadings can cause variations in the ratios of food to microorganism (F/M) or dissolved oxygen (DO) levels, which are two fundamental parameters associated with microbial behaviors. Accordingly, biomass characteristics (e.g., concentration, particle size, viscosity, floc structure) are also influenced by the variable microbial properties. Importantly, as the excreted and autolysis substances of

microorganisms, EPS that strongly depend on microbial community and activity, tend to display various characteristics under different organic loadings.

In recent years, researchers have examined microbial consortia developed in MBRs under different substrate conditions using the molecular biological techniques such as DNA sequencing, fluorescent in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE) (Stamper et al., 2003; Chen and LaPara, 2006; LaPara et al., 2006; Miura et al., 2007; Huang et al., 2008). For example, analysis of microbial communities and functions in the MBRs treating low-strength wastewater revealed that the substrate composition could be a primary factor influencing bacterial community (Chen and LaPara, 2006; LaPara et al., 2006). A similar phenomenon was also observed by Miura and his colleagues (Miura et al., 2007).

Despite these efforts, so far, the detailed comparison of microbial communities and EPS properties in the MBRs with different organic loadings have not been well examined, especially their relationship with membrane performances. The objects of this study were to analyze microbial community developments using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) tools and to explore the potentially influencing factors on EPS properties. In addition, the membrane fouling tendencies of the biomass derived from the MBRs with various organic loadings were evaluated.

2. Methods

2.1. MBR description and operating conditions

Three parallel MBRs had a working volume of 3 L with a submerged hollow fibre membrane module (Polyacrylonitrile, hydrophilic, 50,000 Dalton, 0.1 m^2, Blue Star

Company, China). Permeate was obtained by a suction pump whose condition (on/off) was controlled by a level switch. The initial seed sludge (from the Jurong Wastewater Treatment and Reclamation Plant, Singapore) was acclimated to the simulated municipal wastewater (Table 1, as the substrate composition in the low-organic-loading MBR). The MBRs were operated at SRT of 30 days, HRT of 12 hr, aeration rate of 3 L/min, and flux of 30 L/m^2 hr at a room temperature of 25°C for 60 days (2×SRT). After that, the three MBRs with the similar acclimated activated sludge (~5 g/L) were operated with different organic loadings, whose compositions were summarized in Table 1. The low-organic-loading MBR (hereafter defined as LOMBR), medium-organic-loading MBR (hereafter defined as MOMBR), and high-organic-loading MBR (hereafter defined as HOMBR) were operated at the same above-mentioned conditions.

2.2. Analytical methods

Total organic carbon (TOC) was monitored using a TOC analyzer (Shimadzu, Japan). Mixed liquor suspended solids (MLSS) were measured as proposed by standard methods (APHA, 1998). Microbial floc size was examined with a laser particle sizer (Malvern Mastersizer, UK). The pellet and supernatant were separated from the mixed liquor sample (10 mL) by centrifuging at 4,000 rpm for 10 min. The bound EPS was extracted from the pellet following a method described previously (Wu et al., 2010). In detail, after the supernatant was removed, the microbial floc was dispersed in distilled water to keep the total volume of 10 mL. Then 12 μL of formaldehyde (37%) was added and kept for 1 hr at 4°C, after which 0.8 mL NaOH (1 M) was added and kept for 3 hr at 4°C. After that, the microbial floc solution was centrifuged (13,200 rpm, 20 min, 4°C) to remove the suspended solids. The soluble EPS in the supernatant and the bound EPS extracted from the pellet were

analyzed by examining polysaccharides and protein amounts. Polysaccharides concentration was determined according to the phenol-sulfuric acid method with glucose as a standard by measuring the absorbance at 490 nm using a spectrometer (Jasco V-550, Japan) (Dubois et al., 1956) and the protein concentration was determined by binding of Bradford reagent to the protein with bovine serum albumin (BSA) as standard, which causes an absorption maximum of the dye at 595 nm (Bradford, 1976). In this study, the sum of the polysaccharides concentration (mg/L) and protein concentration (mg/L) was defined as the total EPS concentration (mg/L).

2.3. Membrane fouling assay

To study the membrane fouling propensity of biomass in the MBRs, a membrane fouling assay was set up as shown in Figure 1. The biomass samples were taken from the MBRs at Day 48, 56, 63, 69, 70. A membrane (Polyethersulfone, hydrophilic, 50,000 Dalton, 0.001 m^2, Millipore, USA) was put in a 110 mL of filtration cell. Non-commercial polyacrylonitrile membrane with pore size of 50,000 Dalton was available except 40,000 Dalton. The trial experiments indicated that the polyethersulfone membrane had an almost similar filterability as a polyacrylonitrile membrane (40,000 Dalton). Thus, polyethersulfone membranes were used in this study with regard to the same pore size as the polyacrylonitrile membrane modules in the MBRs.

The biomass sample (20 mL) was placed in the filtration cell and the volume was maintained by replenishing with distilled water from a reservoir (a capacity of 2 L). This could avoid the effect of increasing biomass concentration during the filtration on the fouling resistance. The operating pressure was maintained at about 103 kPa by regulating a

valve connecting with a nitrogen gas cylinder. The transmembrane pressures were monitored by two pressure transducers (Cole-palmer, USA). The permeate flowrate was fixed at 0.03 L/hr using a pump (Masterflex, Cole-palmer, USA) and the accurate permeate flux was determined by weighting the permeate on an electronic balance (Mettler-Toledo, Switzerland). The two pressure transducers and balance were connected to a personal computer equipped with data log system (LabVIEW, National Instruments, USA). The total fouling resistance was calculated based on the equation $R_t = \Delta P/\mu J$, in which, R_t means the total resistance (m^{-1}), ΔP means the transmembrane pressure (kPa), μ means the permeate viscosity (Pa s), and J means permeate flux (L/m^2 hr). Here, $R_t = R_m + R_{ci}$, in which, R_m is the clean membrane hydraulic resistance (m^{-1}) and R_{ci} is the sum of cake layer and irreversible resistances of the biomass (m^{-1}). Before the membrane fouling assay, all membranes were rinsed by floating skin-side down in distilled water to remove impurities from the membrane surfaces. The membrane resistances (R_m) were then measured using distilled water. Membrane fouling rate (m^{-1}/min) is defined as the change of R_{ci} (m^{-1}) within a 60 min of filtration. The specific fouling resistance (α, m kg^{-1}) is calculated by the R_{ci} at 60 min (m^{-1}) divided by the amount of the biomass (kg, MLSS concentration multiplied by 20 mL of sample volume) filtered onto the membrane surface (m^2).

2.4. DNA extraction, PCR, and DGGE conditions

The biomass from the MBRs was mixed with saturated phenol (pH 8.0, Sigma-Aldrich, USA) and glass beads ((BioSpec Products, USA), followed by bead-beating using a Mini BeadBeater (BioSpec Products, USA). The DNA in the aqueous layer achieved by centrifugation (5 min at 5,000×*g*) was further extracted by phenol: chloroform (4:1) mixture and then chloroform as described previously (Kowalchuk et al., 1997; Zhuang et al., 2005).

The V3 region of bacterial 16S rRNA gene (~ 194 bp) was amplified by PCR in a thermal cycler (Eppendorf, Germany). A 50 μL mixture contained 1.25 U of *Taq* DNA polymerase, 5 μL 10× Buffer B, 4 μL 25 mM $MgCl_2$, each deoxynucleoside triphosphate at a concentration of 200 μM (Promega, USA), 25 pmol of each primer (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3' and 5'-ATT ACC GCG GCT GCT GG-3'), and 500 ng of the DNA template (Muyzer et al., 1993; 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%, Bio-Rad, USA) with a 30 to 70% gradient of denaturant. Gels were run for 15 hr at a constant 90 V maintained at 60°C. After that, the gel was stained with ethidium bromide (Sigma-Aldrich, USA) for 20 min and destained in 1×TAE buffer (Bio-Rad, USA) for 1 hr. The gel was photographed by a gel imaging system (Kodak, USA). Similarity index of band patterns was calculated by comparing the two band profiles at the target day and the first day using 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. MBR performances

The three MBRs were operated under the same substrate loading (as that in the LOMBR) for 60 days (2×SRT) to achieve the similar acclimated activated sludge. Then the MBRs were fed with different organic loadings (Table 1) and operated for another 80 days (>

2×SRT, Day 0-Day 80). During the operation, the effluent TOCs in the MBRs were within 10 mg/L (above 90% removal rate, data not shown), almost regardless of organic loadings.

3.2. Biomass characteristics

3.2.1. MLSS and particle size

After 45-day operation, MLSS concentrations in the MBRs maintained at the constant values of 2.2±0.2, 4.0±0.4, and 8.4±0.5 g/L for the low-, medium-, and high-organic-loading MBRs, respectively (Table 2). Accordingly, the F/M ratios kept relatively constant within a range of 0.24~0.32 g COD/g MLSS day (Figure 2A and Table 2). This suggests that once the organic loading has been fixed, the biomass concentration freely evolves until the F/M ratio approaches a constant value which only relates to the SRT (Pollice et al., 2004). In addition, with the variations of biomass concentrations, the DO amounts in the MBRs evolved differently at a fixed aeration rate (3 L/min). After about 45 days, the DO arrived at a level of 6.9, 5.4, and 0.6 mg/L for the low-, medium-, and high-organic-loading MBRs, respectively (Table 2).

The acclimated activated sludge had an initial median particle size of ~130 μm (Figure 2B). In the low-organic-loading MBR, the median particle sizes maintained at 159±23 μm after 45-day operation. The greater sizes of biomass flocs occurred in the medium- and high-organic-loading MBRs, which were kept at 386±33 μm and 195±23 μm after 45-day operation, respectively. Interestingly, there was a rising trend of biomass size in the high-carbon-loading MBR initially, but after 45 days a sharp drop was observed. At higher organic loadings, biomass concentration raised, which consumed more dissolved oxygen and led to higher viscosity (potentially affecting oxygen transfer). This situation could result

in the aerobic condition converting temporarily to the anoxic condition in the high-organic-loading MBR (i.e., the low DO average of about 0.6 mg/L). The change of the reactor conditions could influence microbial community compositions (discussed in the section 3.2.2) and their metabolisms (discussed in the section 3.2.3), which further affected biomass characteristics such as particle size.

3.2.2. Bacterial community shift

DGGE analysis was performed to produce genetic fingerprints that could provide information on the compositions and changes of the microbial communities. The DGGE profiles (Figures 3A-C) show the microbial population shifted with operating time in different ways under various organic loadings. It is evident that some species could propagate in the suitable conditions and their competitors not favored to the available substrate (i.e., F/M ratio) and/or electron acceptor (i.e., DO level) conditions could disappear from the MBRs. After about 50 days, the change of bacterial population became insignificant in the each MBR (Figures 3A-C, the data from day 56 to day 80 were not shown), which corresponded to the stable effluent quality and biomass characteristics (e.g., MLSS, particle size).

At steady state, the dominant microbial communities in the MBRs fed with various organic loadings were significantly different although the similar initial activated sludge was employed (Figures 3D and E). This was possibly attributed to the fact that the DO evolved to different levels in the MBRs fed with various organic loadings. For example, lower DO levels occurred in the MBRs fed with higher organic loadings, which could

provide limited electron acceptors to the microorganisms. Thus, this situation may tend to promote the presence and propagation of anoxic/anaerobic bacteria.

3.2.3. EPS production

In this study, the sum of polysaccharides and proteins (both containing soluble and bound contents) was considered to represent the total EPS. In addition, EPS concentration (i.e., described also as content or amount, mg/L) was defined as a factor to be associated with membrane fouling (discussed in the section 3.2.4), while EPS production (mg/gMLSS, equals to EPS concentration, mg/L, divided by biomass concentration, g/L) was thought as a parameter to indicate the microbial activity.

The initial trend for the EPS production in the each MBR showed an increasing profile, which were loosely associated with the microbial population shift indicated as the DGGE similarity index (it was achieved by comparing the two band profiles at the target day and the first day) (Figure 4A). EPS production approached a peak of ~163 mg/gMLSS on day 14 for the low-organic-loading MBR. The highest EPS productions in the medium- and high-organic-loading MBRs happened on the day 8, approximately ~117 and ~99 mg/gMLSS, respectively. After that, EPS productions dropped gradually and stabilized at 102±12, 62±6, and 58±5 mg/gMLSS for low-, medium-, and high-organic-loading MBRs, respectively (Figure 4A). Correspondingly, the similarity indices dramatically dropped within the first 10 days, and then fluctuated insignificantly.

In detail, obvious fluctuations in polysaccharides (Figure 4B) and protein productions (Figure 4C) in the each MBR occurred in the beginning of the operation. After about 40 days, polysaccharides production reached 53±9, 34±9, and 26±8 mg/gMLSS for the low-,

medium-, and high-carbon-loading MBRs, respectively. It suggests a lower level of polysaccharides production at a higher organic loading. The biomass in the low-organic-loading MBR produced more protein than those in the medium- and high-organic-loading MBRs, both of which achieved comparable protein productions.

The phenomenon on EPS production was possibly associated with the change of F/M ratio (in the initial phase) and DO level (at steady state) in the MBRs. Initially, the MBRs fed with different organic loadings had various F/M ratios since the same amount of acclimated activated sludge was employed. This situation may favor the presence of desirable microorganisms and to discourage the growth of unsuitable microorganisms (i.e., significant variations on microbial communities in the three MBRs). Besides population changes, metabolic function regulations (i.e., bacterial activity) may also arise as a result of adaptation of the microbial communities to the conditions (Zhuang et al., 2005). The favored bacteria could flourish in the new living environment, possibly by excreting enzymes to tolerate the metabolic stress or by converting the excess source and energy to extracellular substances (Figures 4B and C, more protein and polysaccharides produced in the initial period of operation).

Subsequently, the variation of biomass concentration could result in a change of DO in each MBR at a fixed aeration rate. When the biomass concentrations in the three MBRs developed to the different constant values (i.e., more biomass in a higher-loading MBR) at steady state, the DO showed dissimilar levels (Table 2). Although at this stage the F/M ratios were within a range of 0.24~0.32 g COD/g MLSS day, the different DO levels appeared to create various living conditions (e.g., limited available electron acceptors in a

higher-loading MBR) for the microbial communities, which could influence their metabolisms such as EPS productions.

To test this conclusion, further experiments on the effect of dissolved oxygen on EPS production were performed. Purified oxygen was introduced with the air (still keeping the total aeration rate of 3 L/min) into the high-organic-loading MBR at day 80 to improve the dissolved oxygen value to about 6 mg/L. As a result, the EPS production increased from about 58±5 to 91±2 mg/gMLSS within 2 days. This result was also substantiated by other researchers that at a high oxygen concentration, EPS production was higher than that at a low oxygen concentration (Wilshusen et al., 2004; Yun et al., 2006).

The results also implies that the stable microbial community and their metabolic properties (such as EPS production), corresponding with steady TOC removal rates, biomass concentration, and particle size, were considered as important factors to indicate the stable MBR performance (Miura et al., 2007; Wu et al., 2011)

3.2.4. Membrane fouling tendency

In the MBRs, membrane fouling propensity is decided by the biomass characteristics (e.g., concentration, particle size, EPS amount, viscosity), and hydrodynamic conditions (e.g. filtration flux, shear force) that are dependent on biomass properties. For example, higher biomass and EPS concentrations in the MBRs operated with higher organic loadings were considered to increase viscosity, which inevitably influenced hydrodynamic conditions (e.g., shear force) along the membranes. To simplify the influencing factors and to examine the individual contribution of biomass characteristics to the membrane fouling, the biomass samples were taken from the MBRs and their membrane fouling tendencies were tested in

the filtration cell (Figure 1). The rationale for these short-term fouling tests was to apply a standard constant flux (30 L/m^2 hr which was same as that employed in the three MBRs) filtration protocol to biomass samples.

When the stable MBR performances were achieved (i.e., stable effluent quality, MLSS, particle size, and EPS production), the biomass samples (on day 48, 56, 63, 69, and 70, n=5) were taken from the MBRs. The averaged values and standard deviations of the membrane fouling tendencies derived from the samples are plotted as the results and error bars in Figure 5. It shows that the biomass in the MBRs fed with higher organic loadings had higher membrane fouling rates. The specific fouling resistances that were calculated by normalizing the biomass amount were also compared. Apparently, the specific fouling resistances followed a dependent response to organic loading level.

In the MBRs, as secreted or lysed substances from microorganisms, EPS molecules or EPS matrix formed with the immobilized cells can be easily attached on the membrane surface due to their physico-chemical properties. Therefore, EPS are the potentially major sources to increase total resistance by pore blocking or cake formation, and ultimately result in membrane fouling in a MBR system (Rosenberger and Kraume, 2002; Malamis and Andreadakis, 2009).

The EPS concentration in the medium-organic-loading MBR (1.14 g COD/ L day) was slightly higher than that in the low-organic-loading MBR (0.57 g COD/ L day) (insignificantly increased by 26%, *P*-value =0.222; paired Student's *t*-test). Correspondingly, the average specific fouling resistance and membrane fouling rate of biomass derived from the medium-organic-loading MBR were insignificantly higher than those of the low-

organic-loading MBR by 32% (*P*-value =0.138) and 85% (*P*-value =0.072), respectively (Figure 5). This suggests that when the organic loadings fed to MBRs were within a reasonable range (i.e., from 0.57 to 1.14 g COD/L day), biomass characteristics (e.g., EPS concentration and membrane fouling tendency) could not be affected obviously. This finding corresponds well with the conclusions from other researchers (Nagaoka et al., 1998). However, compared to biomass in the medium-organic-loading MBR (1.14 g COD/ L day), the biomass in the high-organic-loading MBR (2.28 g COD/ L day) had significantly higher EPS concentration (1.72-fold, *P*-value =0.000), specific fouling resistance (2.53-fold, *P*-value =0.018), and membrane fouling rate (10.50-fold, *P*-value =0.023).

Further, statistical analysis indicates that at a high flux of 30 L/m^2 hr, the specific fouling resistance (a membrane fouling index excluding the effect of biomass amount) was strongly correlated with EPS content of biomass (Pearson's correlation coefficient r =0.979; *P*-value =0.004), but not the particle size (r =-0.292; *P*-value =0.63). More specifically, the specific fouling resistance was significantly influenced by the bound EPS content (extracted from the microbial flocs, r =0.981; *P*-value =0.003) rather than the soluble EPS content (r =0.514, *P*-value =0.376) in the MBRs. This suggests that cake layer formation (i.e., the deposition of the microbial flocs) was predominant in causing membrane fouling at a high flux of 30 L/m^2 hr. The similar observation was achieved in the previous study (Wu et al., 2011). In addition, the protein contents (r =0.998; *P*-value =0.000) in the MBRs could significantly determine the development of membrane fouling compared to the polysaccharides contents (r =0.688; *P*-value =0.199).

4. Conclusions

This study investigated the biomass characteristics when the MBRs were fed with different organic loadings. In the initial operation period, a variation of F/M ratios happened in the each MBR, which led to significant changes in bacterial community and activity (i.e., EPS production). At steady state, the MBRs fed with various substrate loadings achieved similar F/M ratios, but different DO levels which displayed a great influence on dominant bacterial population and EPS production. In addition, serious membrane fouling easily happened when the MBR was fed with an organic loading over a moderate range due to greatly higher EPS concentration, especially the bound EPS and protein contents. It implies that organic loading fed to MBRs is a factor to decide the sustainable operation of MBRs. Increasing organic loading within an optimal range not only improves MBR treatment capability, but also benefits membrane fouling control (i.e., avoiding serious membrane fouling) to indirectly reduce MBR operation cost.

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Figure Captions:

Fig 1. Schematic of the membrane fouling assay (dead-end mode).

Fig 2. The effect of organic loading on the F/M ratio (A) and median particle size of biomass (B).

Fig 3. DGGE patterns of bacterial communities in the MBRs operated with different organic loadings. (A) Low-organic-loading MBR; (B) Medium-organic-loading MBR; (C) High-organic-loading MBR; (D) Day 0; (E) Day 56. The DNA template in gel E was the same as that in gel A, B, or C. The PCR amplification, gel preparation, staining and destaining processes may influence the band darkness and position in the gels.

Fig 4. Similarity indices of microbial communities and EPS productions (A), polysaccharides productions (B), and protein productions (C) under different organic loadings.

Fig 5. The effect of organic loading on the membrane fouling tendency. The averaged values and standard deviations of the membrane fouling tendencies derived from the samples (on day 48, 56, 63, 69, and 70) are plotted as the results and error bars.

TABLE 1. Compositions of synthetic wastewater fed to the MBRs.

Compound	Concentration (mg/L)		
	LOMBR	MOMBR	HOMBR
Glucose	250	500	1000
CH_3COONa	250	500	1000
NH_4Cl	150	300	600
KH_2PO_4	26.5	53	106
K_2HPO_4	53.5	107	214
$NaHCO_3$	20	40	80
$FeCl_3$	0.25	0.5	1.0
$MgSO_4 \cdot 7H_2O$	50	100	200
$CaCl_2$	3.75	7.5	15.0
Organic loading (g COD/L day)	0.57	1.14	2.28

*** COD represents chemical oxygen demand.**

Table 2. MBR performances and biomass characteristics at steady state.

	Dissolved Oxygen (mg/L)	MLSS (g/L)	F/M ratio (gCOD/ gMLSS day)	Median Particle size (μm)	EPS production (mg/g MLSS)
LOMBR	6.9	2.2±0.2	0.24-0.29	159±23	102±12
MOMBR	5.4	4.0±0.4	0.26-0.32	386±33	62±6
HOMBR	0.6	8.4±0.5	0.26-0.29	195±23	58±5

*** The data presented here were obtained after 45-day' MBR operation.**

Table 3. EPS distribution and composition in the MBRs at steady state.

	Distribution (mg/L)		Composition (mg/L)		Total EPS (mg/L)
	Soluble EPS	**Bound EPS**	**Polysaccharides**	**Protein**	
LOMBR	13.6±8.9	151.1±40.1	96.2±15.5	68.5±35.3	164.7±41.7
MOMBR	25.0±15.4	272.2±6.7	191.0±7.6	104.6±26.4	291.8±26.5
HOMBR	24.2±18.3	583.4±64.4	210.1±64.2	387.2±112.0	603.5±73.1

*** The data presented here were obtained after 45-day' MBR operation.**

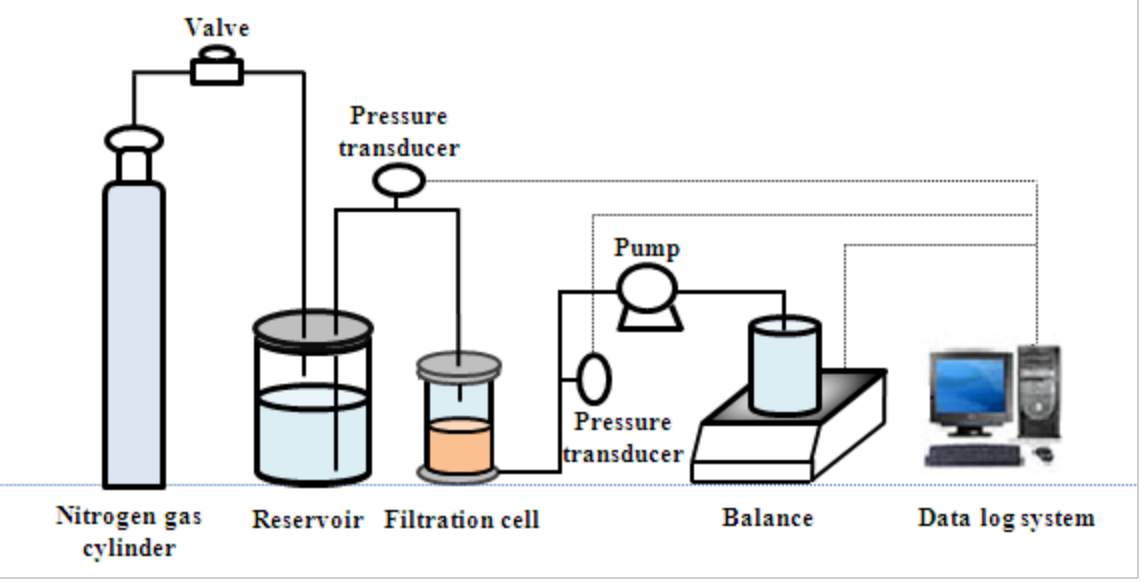
Valve
Pressure transducer
Pump
Pressure transducer
Nitrogen gas cylinder
Reservoir
Filtration cell
Balance
Data log system

FIGURE 1.

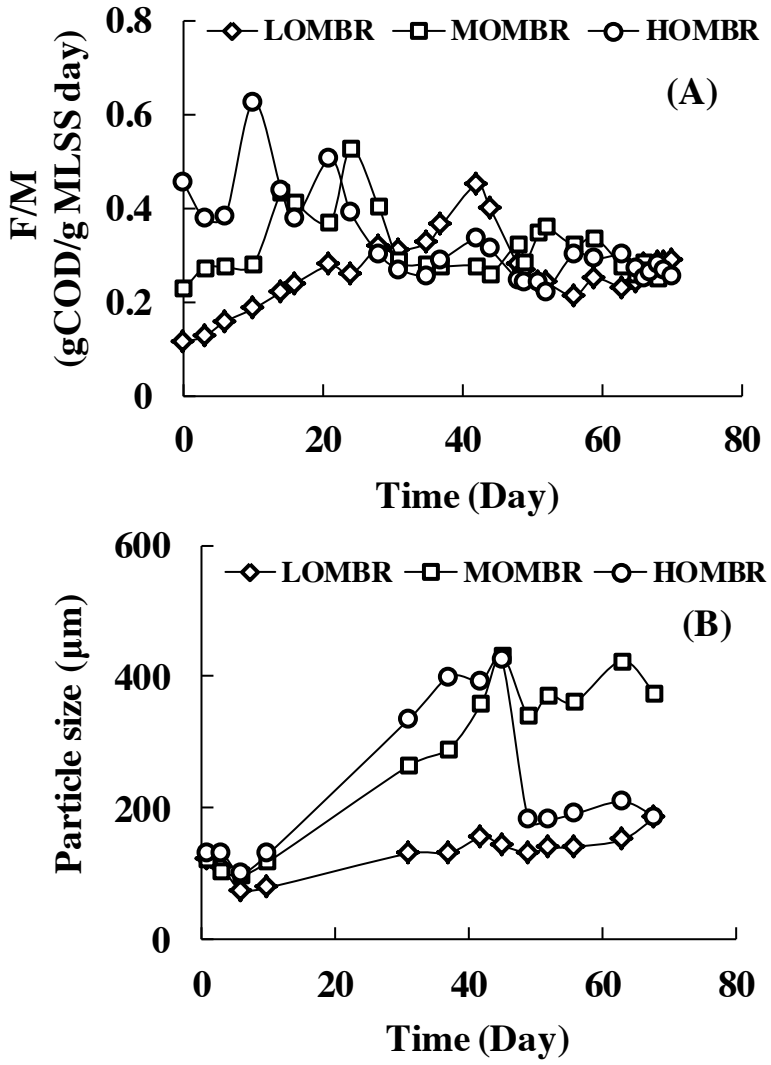
LOMBR
MOMBR
HOMBR
(A)
F/M
(gCOD/g MLSS day)
0.8
0.6
0.4
0.2
0
0
20
40
60
80
Time (Day)
LOMBR
MOMBR
HOMBR
(B)
Particle size (μm)
600
400
200
0
0
20
40
60
80
Time (Day)

FIGURE 2.

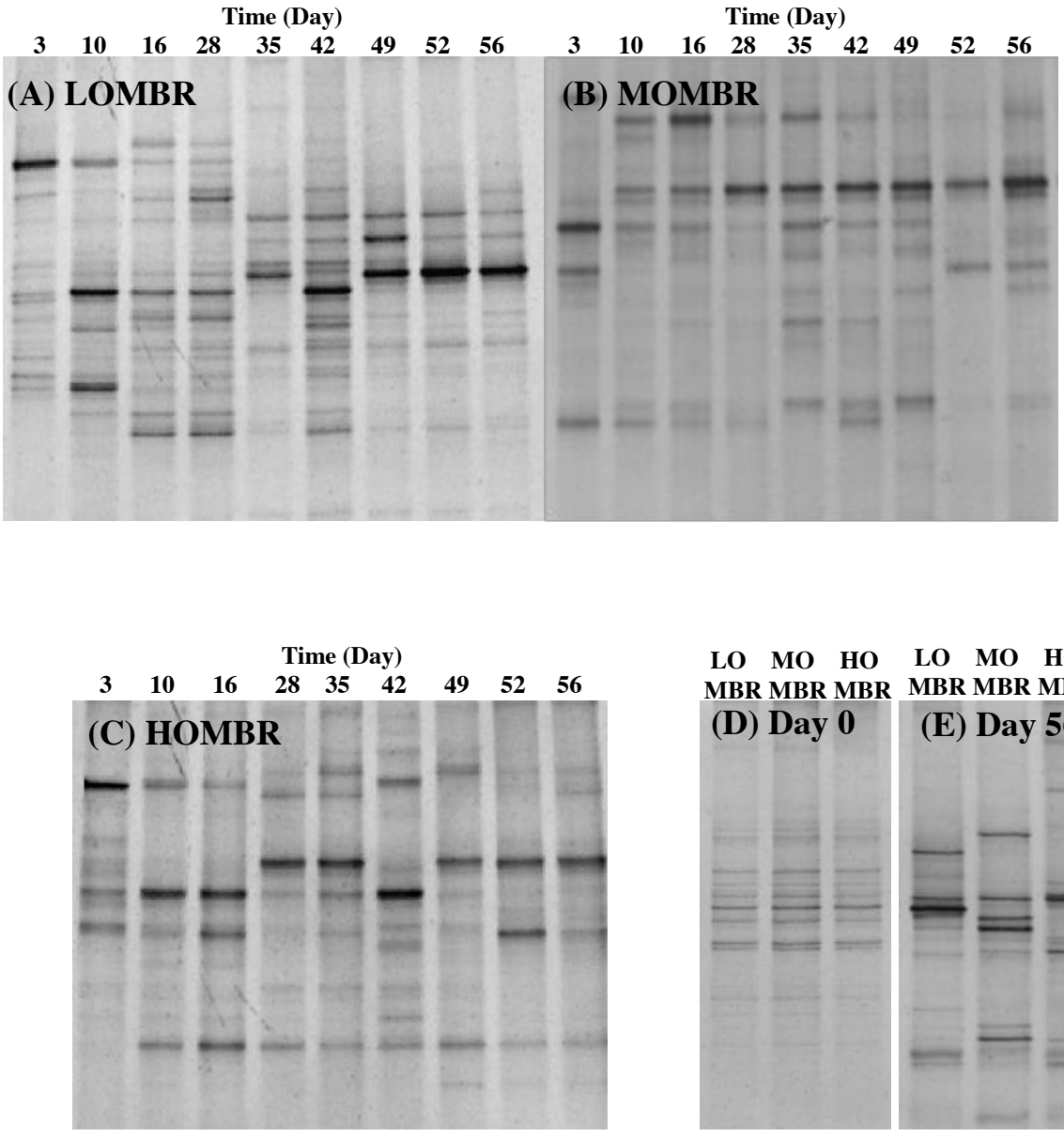
Time (Day)
3 10 16 28 35 42 49 52 56
(A) LOMBR
Time (Day)
3 10 16 28 35 42 49 52 56
(B) MOMBR
Time (Day)
3 10 16 28 35 42 49 52 56
(C) HOMBR
LO MBR
MO MBR
HO MBR
(D) Day 0
LO MBR
MO MBR
HO MBR
(E) Day 56

FIGURE 3.

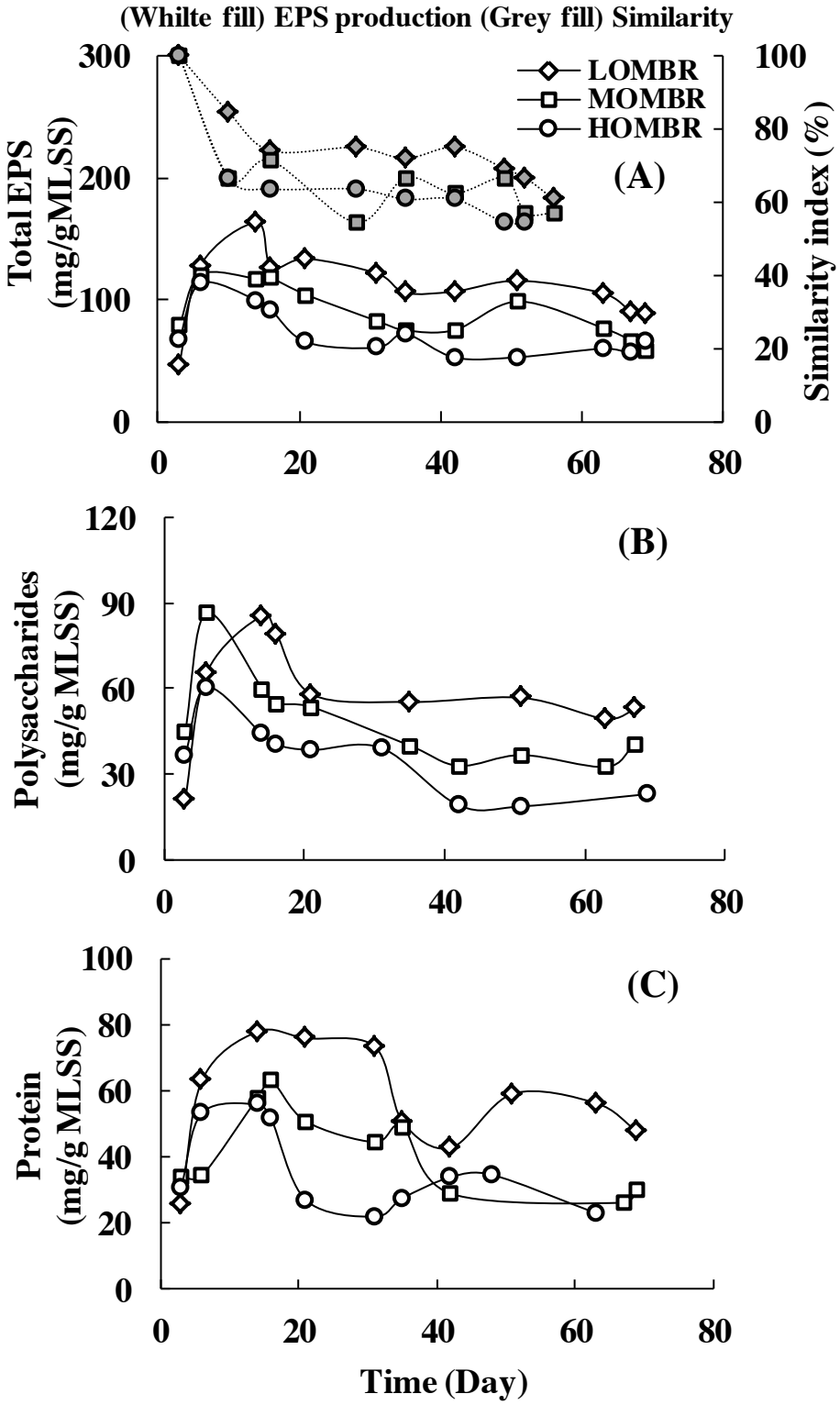
(Whilte fill) EPS production (Grey fill) Similarity
LOMBR
MOMBR
HOMBR
(A)
Total EPS (mg/gMLSS)
Similarity index (%)
(B)
Polysaccharides (mg/g MLSS)
(C)
Protein (mg/g MLSS)
Time (Day)

FIGURE 4.

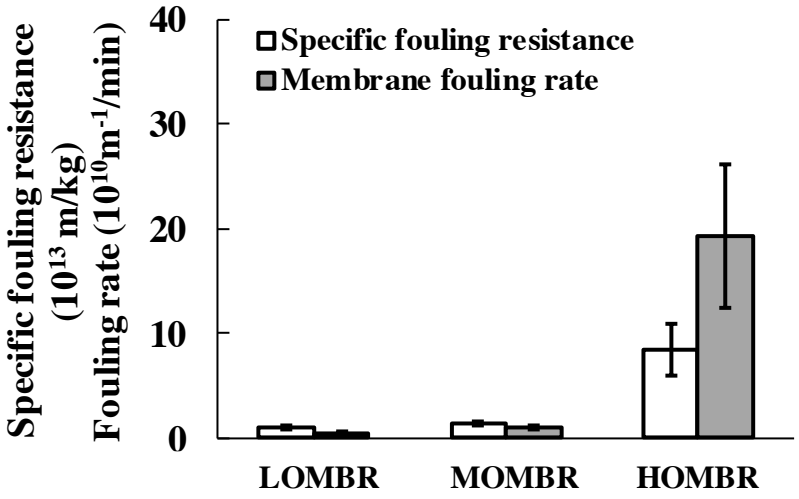
Specific fouling resistance
(10^13 m/kg)
Fouling rate (10^10 m^-1/min)
40
30
20
10
0
Specific fouling resistance
Membrane fouling rate
LOMBR
MOMBR
HOMBR

FIGURE 5.