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The effect of organic loading on bacterial community composition of membrane biofilms in a submerged polyvinyl chloride membrane bioreactor

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ABSTRACT

The effect of organic loading on bacterial community composition of membrane biofilms was investigated using a submerged polyvinyl chloride membrane bioreactor. The low and high loadings were set at 0.33 and 0.52 gCOD/(gVSS d), respectively. The results showed that membrane fouling occurred earlier and faster under the high loading conditions. Denaturing gradient gel electrophoresis (DGGE) analysis revealed that the similarity of bacterial community in the membrane biofilms between the two loadings was 0.67, higher than that in the mixed liquors (0.52–0.55), which indicated that some specific bacteria were selected preferentially on the membranes. Clone library analysis of the membrane biofilms indicated that *Betaproteobacteria* and *Bacteroidetes* under the high loading were 54.72% and 19.81%, respectively. Microarray results further confirmed that the two bacteria were the dominant microorganisms in the high loading biofilm. The severe membrane fouling may be aroused mainly by the enrichment of the two bacteria under the high loading.

1. Introduction

Membrane bioreactors (MBRs) have been widely used for wastewater treatment and reuse, as they provide several highlighted advantages such as perfect effluent, small footprint, high biodegradation efficiency and less sludge production (Fu et al., 2009). However, membrane fouling and membrane price are two major obstacles for the wider application of MBRs (Meng et al., 2009). Membrane fouling significantly shades the overall membrane performance, increases operating costs and shortens membrane life (Miura et al., 2007). Additionally, the large-scale use of MBRs requires a significant decrease in price of the membranes.

Recent researches have clearly shown that biofilm formation induces membrane fouling (Miura et al., 2007; Huang et al., 2008a,b). Although the biofilms have been extensively investigated by visual techniques such as scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), and atomic force microscopy (AFM) to characterize membrane biofouling, bacterial communities involved in the fouling process was not considered systematically (Wang et al., 2008; Zhang et al., 2008). Even though a few studies have focused on the bacterial community in the membrane biofilms (Ivnitsky et al., 2007; Huang et al., 2008a,b; Miura et al., 2007), the information on structure, diversity, and dominant species identity of the membrane biofilm communities in MBRs is still limited. Therefore, a better understanding of the bacterial community in the attached biofilms is necessary to reveal the mechanism of membrane biofouling and to control it in MBRs. Food/microorganism (*F*/*M*) ratio, which ultimately controls biomass characteristics, has confirmed to be one of the most important operating parameters affecting on fouling propensity (Trussell et al., 2006; Huang et al., 2008a,b). In other words, a combined study on bacterial community in MBRs and the effect of *F*/*M* ratio are very necessary.

Polyvinyl chloride (PVC), as a synthetic promising plumbing material, has been extensively used in homes and distribution systems (Heim and Dietrich, 2007). PVC fibers, for example, have been exploited as ultrafiltration membranes (UF) in surface water treatment (Guo et al., 2009). Even though PVC membrane has seldom been used in MBRs for wastewater treatment, it would be a powerful alternative for the wider application of MBRs considering its excellent performance in wastewater treatment and cheap price.

In this study, a submerged MBR equipped with PVC hollow-fiber UF membranes was applied to investigate the effect of *F*/*M* on bacterial community composition in the membrane biofilms. For this purpose, the MBR were operated at a low *F*/*M* ratio of 0.33 gCOD/(gVSS d) and a high *F*/*M* ratio of 0.52 gCOD/(gVSS d). Polymerase chain reaction-denaturing gradient gel electrophoresis

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(PCR-DGGE), phylogenetic analysis and 16S rRNA clone library were used to analyze the bacterial community composition of the membrane biofilms, to determine what kind of bacteria responsible for the biofouling in MBRs during the long-term operations. Finally, high-density universal 16S rRNA microarray technology was applied to check the general species distribution and dominant microorganisms in the high loading biofilm.

2. Methods

2.1. MBR and operating conditions

The MBR used in this study consisted of an anoxic (AN, 4 L) tank and an aerobic (AO, 8 L) tank. The membrane module was installed in the AO tank. The MBR was fed with synthetic wastewater. Wastewater quality and operational conditions were shown in Table 1. The MBR was operated at a low *F*/*M* of 0.33 gCOD/(gVSS d) for 84 days. Then the fouled membrane module was replaced with a new one for the sequent 42-day operation at a high *F*/*M* of 0.52 gCOD/(gVSS d). The reactor was seeded with aerobic activated sludge from a local municipal wastewater plant (Quyang wastewater treatment plant, Shanghai, China), and operated preliminarily for around 1 month for acclimatization. The MBR was equipped with hollow-fiber PVC UF membrane modules with a total surface area of 0.25 m^2. The nominal membrane pore size was 0.01 μm (Litree Company, Suzhou, China). The outside and inner diameters of the fibers were 1.45 and 0.85 mm, respectively. Permeate was collected inside the capillary fibers and extracted from the membrane at a constant sub critical permeate flux (6 L/m^2 h) by the peristaltic pumps. Filtration was carried out with an intermittent suction cycle of 9 min on and 3 min off. Air was supplied continuously under the membrane modules at a flow rate of 0.6 m^3/h over the whole operation. Compared to conventional activated sludge systems, the aeration intensity in MBRs was strong to achieve membrane scouring and bacterial growth. Sludge wasting was carried out daily to maintain mixed liquor suspended solid (MLSS) at constant levels in the MBR. The evolution of membrane fouling was indicated by the increase in transmembrane pressure (TMP) using a vacuum meter (YN-60, Shanghai Weiken). In order to alleviate membrane fouling, the membrane modules were backwashed weekly using MBR permeate, and maintained by on-line washing every month using a solution of sodium hypochloride (200 ppm). When the TMP increased above 50 kPa, the membrane modules were taken out for physical and/or chemical cleaning. Physical cleaning was carried out by spraying pressurized water on the membrane surfaces, and chemical cleaning was conducted by submerging the membrane modules in solutions of sodium hypochloride (2000 ppm) overnight and citric acid (pH 2) for 2 h. The concentrations of the MLSS in the reactor were maintained at low levels. This may resulted from the less organic compounds in the feed wastewater and intense aeration in the MBR. The experiments were conducted at 25 ± 2 °C.

Table 1
Water quality parameters and operational conditions of the MBR.[a]

Operational parameters	Run 1 (low *F*/*M*)	Run 2 (high *F*/*M*)
Feed COD (mg/L)	187.9 ± 28.1	345.2 ± 28.5
Feed NH_4^+—N (mg/L)	24.9 ± 4.7	25.6 ± 6.0
Feed TN (mg/L)	26.9 ± 4.8	34.6 ± 5.7
Effluent COD (mg/L)	10.5 ± 3.6 (94.4%)	13.08 ± 5.58 (96.2%)
Effluent NH_4^+—N (mg/L)	0.8 ± 0.9 (96.9%)	0.8 ± 0.7 (96.9%)
Effluent TN (mg/L)	8.9 ± 4.8 (66.9%)	8.0 ± 3.2 (77.0%)
Aeration intensity (m^3/h)	0.60	0.60
DO in the MBR tank (mg/L)	6.6 ± 0.3	6.3 ± 0.5
DO in the anoxic tank (mg/L)	<0.3	<0.2
pH in the MBR tank	7.60–8.2	7.5–8.3
SRT (d)	20	20
HRT (h)	8.0	8.0
MLSS (mg/L)	2602 ± 203	3202 ± 459
Average organic loading (gCOD/(gVSS d))	0.33	0.52

[a] Numbers are means ± standard deviations, and the corresponding average removal efficiencies are indicated in parentheses.

2.2. Raw wastewater and chemical analysis

Raw wastewater was prepared every 2 days referring to Liu et al. (2005). The compositions of raw wastewater were listed in Table 2. The water quality fluctuation was attributed to the slight sedimentation of starch and the degradation of organic compounds in the wastewater storage tank.

Raw wastewater and MBR permeate were collected every 2 days, and analyzed for full set of conventional performance parameters such as chemical oxygen demand (COD) and ammonia–nitrogen (NH_4^+—N) (Supporting Information text). MLSS and mixed liquor volatile suspended solids (MLVSS) were measured according to Chinese NEPA standard methods (2002). Extracellular polymeric substances (EPS) were extracted from the mixed liquors according to a modified thermal treatment method described previously (Wang et al., 2009). EPS concentrations were quantified as DNA, carbohydrates and proteins. DNA concentrations were determined by ultraviolet spectrophotometer. Carbohydrate concentrations were measured using anthrone method with glucose as a standard, and proteins were quantified using bicinchoninic acid assay (BCA) with bovine serum albumin as a standard.

2.3. DNA extraction and PCR amplification

Membrane biofilm and the parallel mixed liquor samples for bacterial analysis were taken simultaneously from the MBR at the end of each operation (Fig. 1). The biofilm samples were taken from different locations on the fouled membrane modules and formed a composite sample (300 mm length in term of membrane). Then the fouled membrane module was cleaned chemically and repaired with membrane labels for other study. The hollow-fiber membranes were cut into pieces (about 10 mm) using a sterile steel knife, then the biofilms were detached from the fouled membrane by an ultrasonic instrument (SK3300-35 KHz, China). To more effectively release the bacterial cells sticking to the membranes, an additional bead-beating step (2 × 30 s) was included before DNA extraction (Huang et al., 2008a,b). Prior to DNA extraction, all the attached and suspended cell pellets were rinsed twice with TENP buffer (50 mM Tris, 20 mM EDTA, 100 mM NaCl, 0.01 g/mL PVP, pH 10) to remove humic substances, then resuspended with a sodium phosphate buffer (120 mM, pH 8.0) and

Table 2
Compositions and concentrations of raw wastewater.[a]

Compositions	Run 1 (low *F*/*M*)	Run 2 (high *F*/*M*)
Glucose (mg/L)	175	250
Corn starch (mg/L)	175	250
Urea (mg/L)	64	64
Peptone (mg/L)	28	28
KH_2PO_4 (mg/L)	52.8	52.8
$MgSO_4 7H_2O$ (mg/L)	9	9
$MnSO_4 7H_2O$ (mg/L)	6	6
$FeSO_4$ (mg/L)	0.3	0.3
$CaCl_2$ (mg/L)	8	8
$NaHCO_3$ (mg/L)	120	120

[a] Raw wastewater COD = (100–400) mg/L, BOD_5/COD = 0.52–0.65, NH_4^+—N = (10—40) mg/L, pH 6.0–8.5.

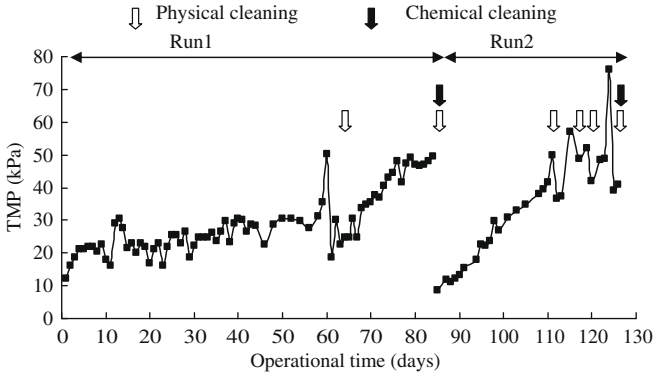

Fig. 1. Changes in TMP during the operations.

washed twice. Finally, the total genomic DNA was extracted using a Fast DNA spin kit (EZ-10, BIO BASIC INC., Canada) and further purified using a DNA Gel Purification Kit (UNIQ-10, Shanghai Sangon Co., Ltd., China) as described in the manufacturer's instructions. For DGGE analysis, a nested PCR was performed on the purified DNA using bacterial universal primers 27f and 1492r along with F357-clamp and R518 (Supporting Information text).

2.4. DGGE fingerprints and statistical analysis

DGGE was performed with a D-code System (Bio-Rad, USA) according to the standard method (Muyzer et al., 1993). The details were indicated in the Supporting Information text. Specific DGGE bands were manually excised from the gel, re-amplified, cloned and sequenced as described previously (Xia et al., 2008). All nucleotide sequences were determined by Generay Co., Ltd. (Shanghai, China). DGGE images were processed with Smartview software (Supporting Information text).

2.5. 16S rRNA gene-cloning and phylogenetic analysis

The nearly full-length 16S rRNA genes from extracted DNA were amplified with the bacterial universal primers 27f and 1492r for building cloning library (Duan et al., 2009). Triplicate PCR products were pooled to minimize bias, purified with a QIAquick PCR purification kit (QIAGEN), and cloned using a pTG19-T TA cloning kit (Generay, China). Four clone libraries were built with DNA extracted from membrane biofilm and suspended mixed liquor samples taken from the MBR at major fouling time points (Fig. 1). Ampicillin and x-gal were used to screen colonies from plasmids. Positive clones were identified and amplified by the primer pairs M13 using the same program for 16S rRNA amplification above. For each sample, approximately 100 positive clones were selected for sequencing. Chimeric sequences were identified as described (Huang et al., 2004) and excluded from subsequent analysis. All the sequences were compared to the known sequences for phylogenetic analysis. Operational taxonomic units (OTUs) were defined as groups in which the sequence similarity was more than 97% (Huang et al., 2008a,b). Phylogenetic trees were constructed by neighbor-joining method with the Clustal X software package. Bootstrap resampling analysis for 1000 replicates was performed to estimate the confidence of tree topologies. The 16S rRNA gene sequences from this study have been deposited in National Institutes of Health (NIH) genetic sequence database (GenBank) under accession numbers GU257488 to GU257893.

2.6. High-density 16S rRNA microarray

High-density 16S rRNA microarray (G2, designed by Lawrence Berkeley National Laboratory and synthesized by Affymetrix Inc.), where 506, 944 immobilized probes were fixed on a 1 cm^2 silicon chip targeting to 8935 clusters of 16S rRNA, was used to check the species distribution and dominant microorganisms in the high loading biofilm. The average number of replicated probes chosen for each cluster was 24 for precision control. The clusters were considered as in one OTU on the basis of less than 3% sequence divergence. The phylochips were hybridized to a mixture of 16S rRNA gene fragments that were amplified from the total DNA. After sufficient hybridization, washing and staining, the chips were scanned with a laser for detection of hybridization locations by fluorescence (Supporting Information text). The phylochip results were analyzed by Affymetrix software (GeneChip microarray analysis suite, version 5.1).

3. Results and discussion

3.1. The performance of MBR

The MBR was operated at a low *F*/*M* of 0.33 gCOD/(gVSS d) for 84 days. Then the fouled membrane module was replaced with a

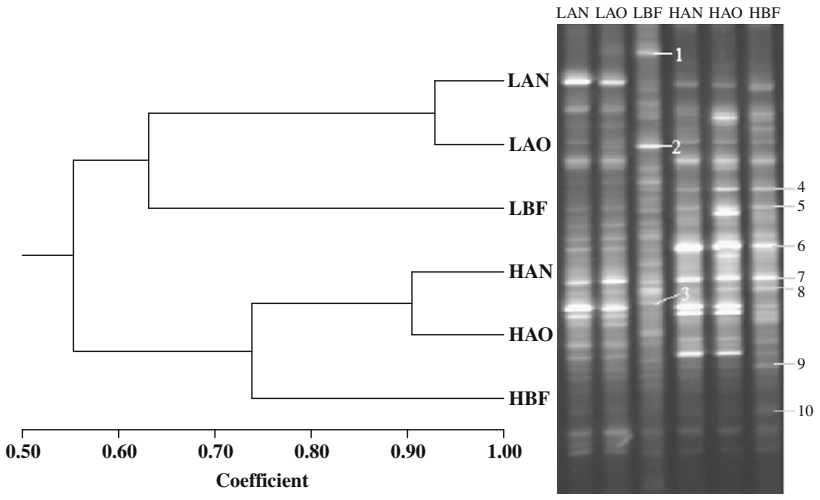

Fig. 2. DGGE fingerprints and cluster analysis of membrane biofilm and mixed liquor samples. LAN, LAO-the anoxic and aerobic sludge samples under the low loading conditions; HAN, HAO-the anoxic and aerobic sludge samples under the high loading conditions; LBM, HBM-the low and high loading membrane biofilms.

new one for the subsequent 42-day operation at a high *F*/*M* of 0.52 gCOD/(gVSS d). During the operations, the MBR removed nearly 90% of the organic compounds and 95% of NH_4^+—N. It can be seen from Table 1 that the increase of substrate loading had no negative impact on the removals of COD and NH_4^+—N. In addition, the average total nitrogen (TN) removal efficiency increased

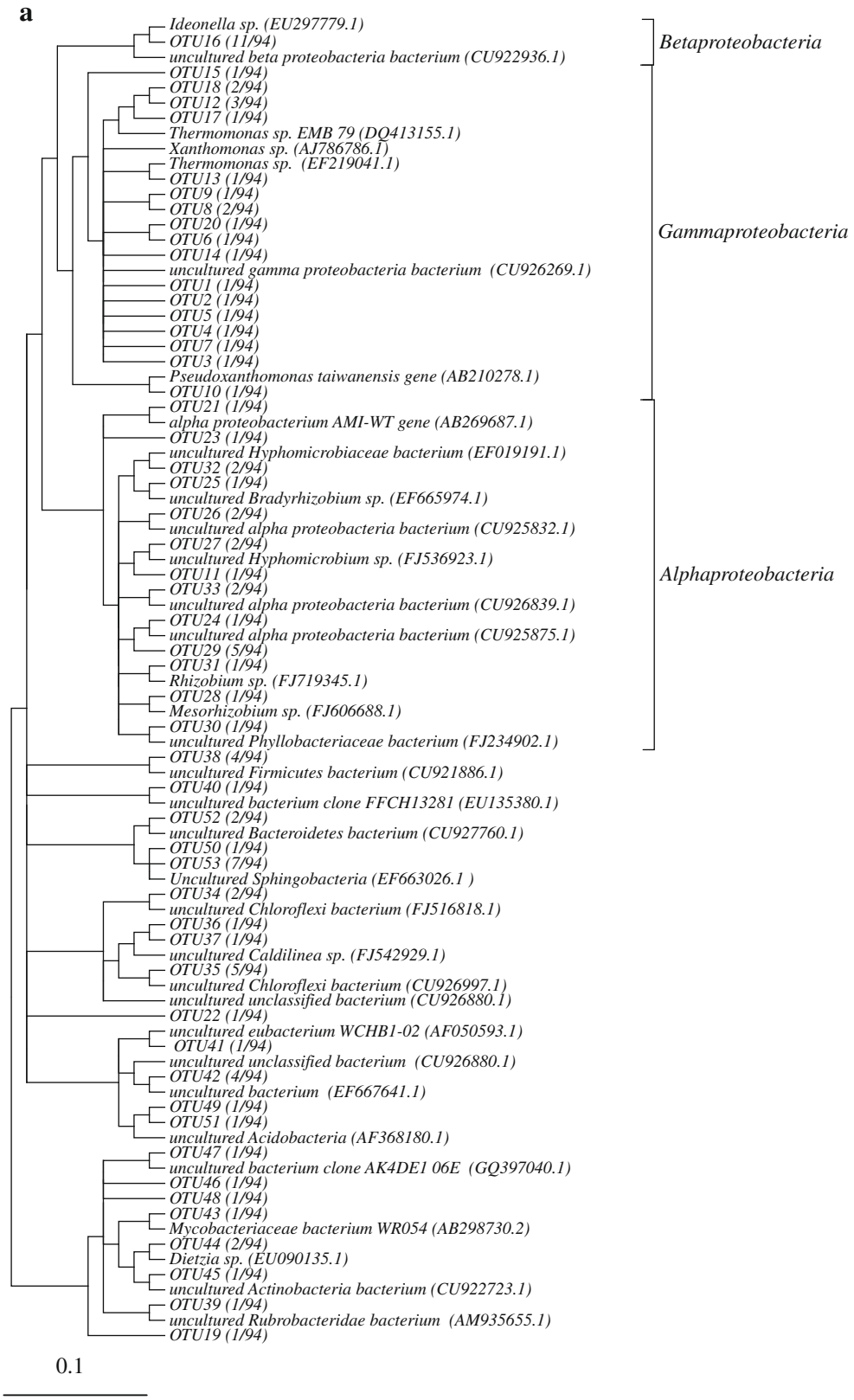

Fig. 3. Phylogenetic trees of the membrane biofilm samples. (a) Phylogenetic tree of the low loading biofilm and (b) Phylogenetic tree of the high loading biofilm. The numbers of clones for each OTU are indicated in parentheses.

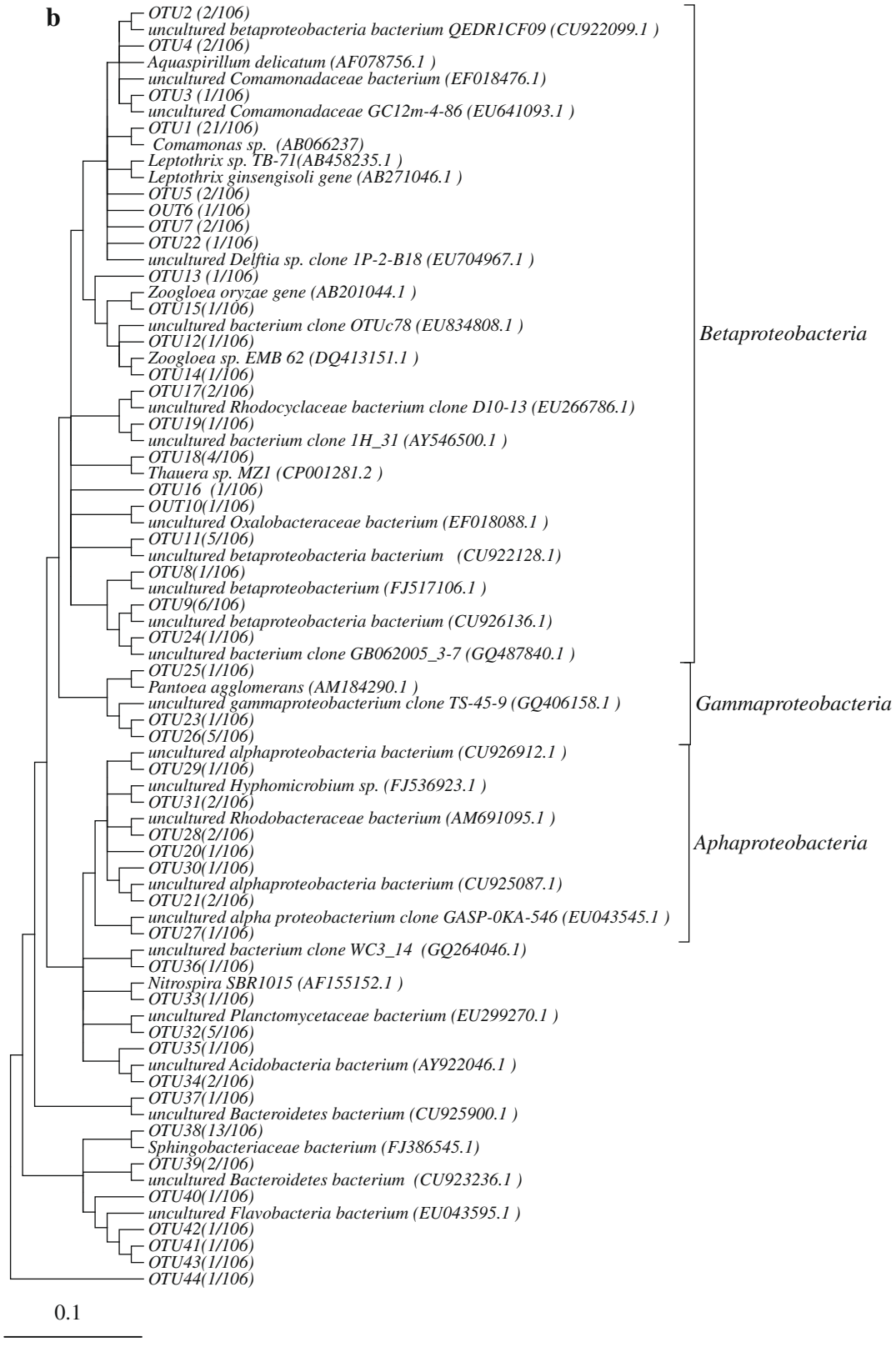

Fig. 3 (*continued*)

from 66.9% to 77.0% when the *F*/*M* changed from 0.33 to 0.52 gCOD/(gVSS d). Once the TMP reached above 50 kPa, the membrane modules were taken out for physical cleaning to remove the sludge cake deposited on the membrane surface. However, the membrane surface was still covered by a thick, brown and homogeneous biofilm after physical cleaning. The TMP still increased rapidly with operation time, demonstrating the occurrence of irreversible membrane fouling even after repeated physical cleaning in the high loading stage (Fig. 1). Visual inspections showed that the considerably thick biofilm layers and faster bio-

film development correlated well with the faster fouling rate in the high loading stage (Fig. 1). The result clearly indicated that the biofilm formation was associated with irreversible membrane fouling, which was further confirmed by the fluorescence in situ hybridization (FISH) results (Supporting Information text and Fig. S1).

By contrast, the low loading operation did not exhibit a significant TMP increase over the whole period. This may be attributed to the lower EPS levels in the reactor (Supporting Information Tables S1) and the bacterial community. The formation of EPS was growth-related and produced in direct proportion to substrate utilization (Laspidou and Rittmann, 2002). Thus the increase of *F*/*M* would induce the generation of more EPS. In this study, the high *F*/*M* was connected with high EPS concentration and severe membrane fouling. Similar results were reported by Meng et al. (2007). Furthermore, *F*/*M* may have an important impact on the diversity, composition and dominant species of bacterial community in the membrane biofilms.

3.2. DGGE fingerprint

DGGE fingerprint was used to reveal the bacterial communities of the biofilm and mixed liquor samples from different major fouling time points in the MBR (Fig. 1). DGGE separations were conducted in triplicate to assess the reproducibility, exhibiting highly similar gels. Only one of the replicates was shown in this paper as an example.

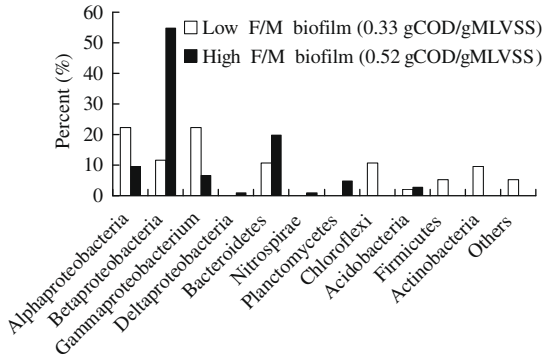

Fig. 4. Phylogenetic distribution of the OTUs in the two membrane biofilm clone libraries.

According to the DGGE profile and clustering analysis (Fig. 2), the membrane biofilm communities were significantly different from the corresponding suspended communities in the MBR at both tested conditions. Under the low loading conditions, the bacterial community in the AN tank showed a greater similarity (Cs = 0.93) to that in the AO tank, but the bacterial community in the biofilm showed only 0.64 similarity to that in the matched mixed liquors in the same aerobic basin. The similar synchronism was also observed during the high loading operation. This finding indicated that some specific groups of bacteria were preferentially selected on the membrane surfaces.

It can be seen from DGGE profile (Fig. 2) that *F*/*M* ratio had a significant impact on the bacterial communities in the mixed liquors and membrane biofilms in the MBR. The suspended bacterial communities in the low loading operation showed 0.52 (AO tank) to 0.55 (AN tank) similarity to that in the high loading operation, while the membrane biofilm communities in the two different loading operations indicated 0.67 similarity. It was interesting that the similarity of bacterial community in the membrane biofilms was higher than that in the mixed liquors between the two different loading operations. These provided further evidence that certain bacteria were selected preferentially on the membranes. In addition, the DGGE profile presented that the bacterial diversity of the membrane biofilms was complex as well as that of the mixed liquors in the MBR, which was further supported by the traditional Shannon diversity indices (Supporting Information Tables S2).

Several dominant DGGE bands were excised successfully from the gels, re-amplified, purified, cloned and sequenced for phylogenetic identities (Fig. 2). Phylogenetic analysis demonstrated that most of the dominant species responsible for biofouling were affiliated to *Proteobacteria* and *Bacteriodetes* (See the details in Supporting Information text). More information of the bacterial species was elucidated by the subsequent 16S rRNA clone library and microarray analyses.

3.3. Analysis of the 16SrRNA clone library

Two 16S rRNA clone libraries were constructed to identify potentially dominant species in the membrane biofilms. Each clone

Table 3
The 20 dominant species detected by high-density 16S rRNA microarray in the high *F*/*M* membrane biofilm.

Phylum	Class	Order	Family	Subfamily	pf[a]	Intensity[b]
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*sf*_1	1	5199
Proteobacteria	*Gammaproteobacteria*	*Legionellales*	*Unclassified*	*sf*_1	0.941	5092
Proteobacteria	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	*Sf*_3	1	4979
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Sf*_1	1	4812
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Sf*_1	1	4769
Proteobacteria	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sf*_1	0.909	4738
Firmicutes	*Clostridia*	*Unclassified*	*Unclassified*	*Sf*_7	1	4704
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Alcaligenaceae*	*Sf*_1	1	4650
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Sf*_1	1	4569
Proteobacteria	*Alphaproteobacteria*	*Unclassified*	*Unclassified*	*Sf*_6	0.955	4526
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Sf*_1	0.962	4525
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Sf*_1	1	4469
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Sf*_1	0.986	4468
Proteobacteria	*Betaproteobacteria*	*Methylophilales*	*Methylophilaceae*	*Sf*_1	0.933	4464
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Sf*_1	1	4464
Proteobacteria	*Alphaproteobacteria*	*Rhodobacterales*	*Unclassified*	*Sf*_5	0.958	4452
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Sf*_1	1	4449
Proteobacteria	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Sf*_1	1	4448
Bacteroidetes	*Sphingobacteria*	*Sphingobacteriales*	*Unclassified*	*Sf*_3	0.917	4430
Acidobacteria	*Acidobacteria*	*Acidobacteriales*	*Acidobacteriaceae*	*Sf*_14	0.923	4428

[a] The value of "pf" means the fraction of scored sub-tests (probe pairs) that gave a positive response for the OUT, the higher the number, the more reliable the results.
[b] The average hybridization intensity across all probe pairs was scored in an OTU, representing the comparative abundance of the group.

library included about 100 randomly selected clones. All clones were grouped into different OTUs on the basis of more than 97% sequence similarity within an OTU. In total, 53 and 44 OTUs were obtained from the low and high loading biofilm clone library, respectively. The estimated coverage ratios of the low and high loading biofilm clone library were 60.64% and 74.53%, respectively.

Fig. 3a provides the phylogenetic tree of the membrane biofilm under the low organic loading conditions. Sequence analysis of 94 clones from the low loading biofilm revealed a total of 53 OTUs belonging to 10 different phyla. *Proteobacteria* was the largest group, accounting for 56.38% of total clones. The second dominant groups were *Bacteroidetes* and *Chloroflexi*, both accounting for 10.64%. The next frequently detected phylum was *Actinobacteria*, occupying about 9.57%. Other minor lineages detected included *Acidobacteria*, *Firmicutes*, etc. Among the *Gammaproteobacteria* in the biofilm, the clones related to *Thermomonas* sp. were frequently detected (detection frequency of 8.51%). It was also identified as one of the dominant species in DGGE separation. Thus *Thermomonas* sp. probably was one of the particularly important bacterial species in the formation of membrane biofilm in the low loading operation.

Fig. 3b presents the phylogenetic tree of the membrane biofilm under the high organic loading conditions. Sequence analysis of 106 clones from the high loading biofilm indicated eight distinct phyla. Similar to the low loading biofilm, *Proteobacteria* dominated the high loading biofilm clone library, occupying 71.70% of total clones. Although *Betaproteobacteria* were enriched significantly in the high loading biofilm, occupying 54.72% of the library, *Alpha-* and *Gamma-Proteobacteria* were obviously less favored in the membrane habitats. In particular, *Bacteroidetes* were highly abundant in the high loading biofilm, accounting for 19.81%, while it was only 6.48% in the matched suspended mixed liquor. The next frequently detected phylum was *Planctomycetes*, 4.72% approximately. Members belonging to *Nitrospirae* and *Acidobacteria* were also identified. Compared with the low loading membrane biofilm, *Betaproteobacteria* and *Bacteroidetes* were significantly enriched in the high loading membrane biofilm (Fig. 4). Also, membrane fouling occurred earlier and faster under the high loading conditions (Fig. 1), thus the two groups of bacteria may play a major role in the development of membrane biofilm fouling.

It was noticeable that the *F*/*M* ratio greatly affected the dominant species selected on the membrane surfaces. These differences would further influence the progress of biofouling in the MBR. It was interesting that *Bacteroidetes* were significantly enriched in the both membrane biofilms compared to the matched suspended mixed liquors. Moreover, *Bacteroidetes* were further enriched from 10.64% of the low loading biofilm to 19.81% of the high loading biofilm. Simultaneously, *Betaproteobacteria* (54.72% of total clones) had absolute dominance over others in the high loading biofilm. The significant enrichment of *Betaproteobacteria* and *Bacteroidetes* may be associated with the severe membrane fouling under the high loading conditions. The high *F*/*M* supported a more active biomass which had a high biofilm formation potential, and these bacterial activities maintained consistently higher EPS concentrations in the mixed liquors. Furthermore, the soluble EPS could serve as readily available substrates for the bacteria attached on the membranes, thus promoting faster biofilm fouling. In addition, similar to the DGGE results, there was only limited overlap among the clone OTUs within the matched attached and suspended clone libraries, thee were only 5 OTUs in common from a total of 99 OTUs under the low loading conditions, and 6 OTUs in common from 108 OTUs under the high loading conditions.

These comprehensive phylogenetic analyses revealed that some specific bacteria (i.e. *Bacteroidetes and Betaproteobacteria*) were responsible for the membrane biofilm fouling (Chen et al., 2004; Pang and Liu, 2007; Zhang et al., 2006). This implied that these specific bacterial groups should be focused upon, rather than the total bacterial community presented in the mixed liquors, to effectively control biofouling in MBRs.

3.4. High-density universal 16S rRNA microarray

High-density universal 16S rRNA microarray was performed to check the bacterial community composition of the high loading biofilm. The total OTU number detected by microarray was 1460 based on less than 3% sequence divergence. These OTUs belonged to 40 different phyla or candidate divisions. *Proteobacteria* was confirmed to be the largest phylum (798 OTUs). The second and third groups were *Firmicutes* (196 OTUs) and *Bacteroidetes* (107 OTUs), respectively. Other dominant lineages detected included *Actinobacteria* (60 OTUs), *Acidobacteria* (45 OTUs), *Chloroflexi* (37 OTUs), etc. Some minor phyla and candidate divisions (no more than 9 OTUs) such as *Chlorobi*, *OP10*, *TM7* were also found in the high loading biofilm. Nevertheless, the high number of OTUs in a certain phylum may not reflect the real abundance in the reactor in terms of dominating bacteria. It may be that a few species with high abundance dominated the populations, thus in principle determined the properties of the biomass, not a large number of different bacteria in very low amounts (Choi et al., 2006). So hybridization intensity was used to assess the species abundance of the microorganisms.

The average hybridization intensity across all probe pairs was scored in an OUT to represent the comparative abundance of the group, and the 20 dominant bacteria of the high loading biofilm were listed in Table 3. The results showed that *Proteobacteria* (specifically *Betaproteobacteria*, 12 strains) were the largest group, possessing 17 of total 20 strains, with 1 *Bacteroidetes*, 1 *Acidobacteria* and 1 *Firmicutes*. Within the *Betaproteobacteria*, the predominant members were mainly associated with the order *Burkholderiales* (11 strains) and *Rhodocyclales* (1 strain). A strain of *Xanthomonadaceae*, which was detected as dominating species in DGGE separation, was also identified by microarray. Among the *Alphaproteobacteria,* one strain related to *Sphingomonadaceae* was found as one of the most abundant species. It has been considered as the key microorganism contributing to biofouling in a reverse osmosis membrane system for freshwater purification (Pang et al., 2005). More importantly, *Betaproteobacteria* and *Bacteroidetes* were also identified as dominant species, indicating that they probably played an important role in the development of the earlier and faster membrane fouling under the high loading conditions.

Microarray analysis also suggested that the bacterial community of membrane biofilms in MBR was far more complex and diverse than expected, thus the dominant species would be quite different with the changes of feed water, process configurations, membrane materials, and operating conditions. The corresponding data available in the literatures were listed in Table 4. Miura et al. (2007) suggested that *Betaproteobacteria* probably played an important role in the formation of membrane biofilms treating municipal wastewater. Pang et al. (2005) reported that the genera related to *Sphingomonas* were the major species in the biofilms formed on the membrane surfaces in a reverse osmosis system treating potable water. In this study, *Betaproteobacteria* and *Bacteroidetes* were found to be important in the development of membrane fouling. The better understanding of these dominant species in the membrane biofilms would provide profound insights into the mechanism of biofouling, the rational application of cleaning protocols and the development of strategies for hindering biofouling in MBRs.

Table 4
Specific bacterial groups responsible for membrane biofilm fouling.

References	Process	Membrane type	Wastewater	Operational conditions	Dominant bacteria
Miura et al. (2007)	Full-scale MBRs	Polyethylene hollow-fiber microfiltration membrane	Municipal wastewater	Aeration = 3.5 or 5.0 m^3/h, permeate flux = 16.7 L/(m^2·h), MLSS = 20 g/L, HRT = 3.6 h	*Betaproteobacteria*
Huang et al. (2008a,b)	Laboratory-scale MBRs	Flat sheet microfiltration membrane	Municipal wastewater	Aeration = 1.2m^3/h, permeate flux = 15 or 30 L/(m^2 h) SRT = 8 or 30 days	*Alphaproteobacteria*, *Betaproteobacteria* and *Bacteroidetes*
Xia et al. (2008)	Pilot-scale MBR	Polyethylene hollow-fiber microfiltration membrane	Bathing wastewater	Permeate flux = 16.7 L/(m^2 h) MLSS = 4–10 g/L, HRT = 8 h	*Pseudomonas* sp., *Ochrobactrum anthropi* sp., and *Enterobacter* sp.
Ivnitsky et al.(2007)	Cross-flow nanofiltration	Polyamide tubular nanofiltration membranes	Tertiary wastewater effluent and synthetic wastewater	Pressure = 5 bar, recirculation rate = 132.5 L/h, temperature = 20, 25 or 34 °C, filtration duration = 8–24 days	*Betaproteobacteria* and *Gammaproteobacteria*
Pang et al. (2005)	Reverse osmosis system	Reverse osmosis membrane	Potable water	Pressure = 15–18 bar, permeate flux = 6 m^3/h, recovery = 75%	*Sphingomonas* organisms
Pang and Liu (2007)	Reverse osmosis system	Reverse osmosis membrane	MBR effluent	–	*Rhizobiales* organisms

4. Conclusions

This study focused on the effect of organic loading on membrane fouling and bacterial community composition of membrane biofilms in a submerged polyvinyl chloride MBR. The results showed that membrane fouling occurred earlier and faster under the high loading conditions. Bacterial community analyses suggested that some specific bacteria were selected preferentially on the membranes. The results of clone library and microarray further revealed that *Bacteroidetes* and *Betaproteobacteria* may play an important role in the development of membrane fouling during the long-term operations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2010.03.082.

References

Chen, C.-L., Liu, W.-T., Chong, M.-L., Wong, M.-T., Ong, S.L., Seah, H., Ng, W.J., 2004. Community structure of bacterial biofilms associated with membrane-based water purification processes as revealed using a polyphasic approach. Appl. Microbiol. Biotechnol. 63, 466–473.

Chinese NEPA, 2002. Water and Wastewater Monitoring Methods, fourth ed. Chinese Environmental Science Publishing House, Beijing, China

Choi, H., Zhang, K., Dionysiou, D.D., Oerther, D.B., Sorial, G.A., 2006. Effect of activated sludge properties and membrane operation conditions on fouling characteristics in membrane bioreactors. Chemosphere 63, 1699–1708.

Duan, L., Andrade, I.M., Huang, C.-L., Xia, S.Q., Hermanowicz, S.W., 2009. Effects of short solids retention time on bacterial community in a membrane bioreactor. Bioresource Technol. 100, 3489–3496.

Fu, Z.M., Yang, F.L., Zhou, F.F., Xue, Y., 2009. Control of COD/N ratio for nutrient removal in a modified membrane bioreactor (MBR) treating high strength wastewater. Bioresource Technol. 100, 136–141.

Guo, X., Zhang, Z., Fang, L., Su, L., 2009. Study on ultrafiltration for surface water by a polyvinyl chloride hollow fiber membrane. Desalination 238, 183–191.

Heim, T.H., Dietrich, A.M., 2007. Sensory aspects and water quality impacts of chlorinated and chloraminated drinking water in contact with HDPE and cPVC pipe. Water Res. 41, 757–764.

Huang, L.N., Zhou, H., Zhu, S., Qu, L.H., 2004. Phylogenetic diversity of bacteria in the leachate of a full-scale recirculating landfill. FEMS Microbiol. Ecol. 50, 175–183.

Huang, L.N., Wever, H.D., Diels, L., 2008a. Diverse and distinct bacterial communities induced biofilm fouling in membrane bioreactors operated under different conditions. Environ. Sci. Technol. 42 (22), 8360–8366.

Huang, X., Wei, C.-H., Yu, K.-C., 2008b. Mechanism of membrane fouling control by suspended carriers in a submerged membrane bioreactor. J. Membr. Sci. 309, 7–16.

Ivnitsky, H., Katz, I., Minz, D., Volvovic, G., Shimoni, E., Kesselman, E., Semiat, R., Dosoretz, C.G., 2007. Bacterial community composition and structure of biofilms developing on nanofiltration membranes applied to wastewater treatment. Water Res. 41, 3924–3935.

Laspidou, C.S., Rittmann, B.E., 2002. A unified theory for extracellular polymeric substances, soluble bacterial products, and active and inert biomass. Water Res. 36, 2711–2720.

Liu, R., Huang, X., Xi, J.Y., Qian, Y., 2005. Microbial behaviour in a membrane bioreactor with complete sludge retention. Process Biochem. 40, 3165–3170.

Meng, F.G., Shi, B.Q., Yang, F.L., Zhang, H.M., 2007. Effect of hydraulic retention time on membrane fouling and biomass characteristics in submerged membrane bioreactors. Bioprocess Biosyst. Eng. 30, 359–367.

Meng, F.G., Chae, S.R., Drews, A., Kraume, M., Shin, H.S., Yang, F.L., 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. Water Res. 43, 1489–1512.

Miura, Y., Watanabe, Y., Okabe, S., 2007. Membrane biofouling in pilot-scale membrane bioreactors (MBRs) treating municipal wastewater: impact of biofilm formation. Environ. Sci. Technol. 41, 632–638.

Muyzer, G., Dewaal, E.C., Uitterlinden, A.G., 1993. Profiling of complex bacterial populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16s ribosomal-RNA. Appl. Environ. Microbiol. 59, 695–700.

Pang, C.M., Liu, W.-T., 2007. Community structure analysis of reverse osmosis membrane biofilms and the significance of Rhizobiales bacteria in biofouling. Environ. Sci. Technol. 41, 4728–4734.

Pang, C.M., Hong, P.Y., Guo, H.L., Liu, W.-T., 2005. Biofilm formation characteristics of bacterial isolates retrieved from a reverse osmosis membrane. Environ. Sci. Technol. 39, 7541–7550.

Trussell, R.S., Merlo, R.P., Hermanowicz, S.W., Jenkins, D., 2006. The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater. Water Res. 40 (14), 2675–2683.

Wang, Z.W., Wu, Z.C., Yin, X., Tian, L.M., 2008. Membrane fouling in a submerged membrane bioreactor (MBR) under sub-critical flux operation: membrane foulant and gel layer characterization. J. Membr. Sci. 325, 238–244.

Wang, Z.W., Wu, Z.C., Tang, S.J., 2009. Extracellular polymeric substances (EPS) properties and their effects on membrane fouling in a submerged membrane bioreactor. Water Res. 43, 2504–2512.

Xia, S.Q., Guo, J.G., Wang, R.C., 2008. Performance of a pilot-scale submerged membrane bioreactor (MBR) in treating bathing wastewater. Bioresource Technol. 99, 6834–6843.

Zhang, K., Choi, H., Dionysiou, D.D., Sorial, G.A., Oerther, D.B., 2006. Identifying pioneer bacterial species responsible for biofouling membrane bioreactors. Environ. Microbiol. 8 (3), 433–440.

Zhang, G.J., Ji, S.L., Gao, X., Liu, Z.Z., 2008. Adsorptive fouling of extracellular polymeric substances with polymeric ultrafiltration membranes. J. Membr. Sci. 309, 28–35.