Bioresource Technology 100 (2009) 3489–3496

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effects of short solids retention time on microbial community in a membrane bioreactor

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ARTICLE INFO

Article history:
Received 12 November 2008
Received in revised form 24 February 2009
Accepted 27 February 2009
Available online 28 March 2009

Keywords:
Membrane bioreactor
Short solid retention time
Microbial community
PCR–DGGE
Clone library

ABSTRACT

Effects of operating lab-scale nitrifying membrane bioreactors (MBR) at short solids retention times (SRT = 3, 5 and 10d) were presented with focus on reactor performance and microbial community composition. The process was capable of achieving over 87% removal of ammonia and 95% removal of chemical oxygen demand (COD), almost regardless of SRT. The denaturing gradient gel electrophoresis (DGGE) analysis shown that bacterial communities evolved in time in a similar way at different SRT. The results of clone library analysis indicated that *Betaproteobacteria* was the dominant bacterial group in all the reactors but there were significant difference of species for different SRT with higher species diversity at longer SRT. Ammonia and COD removal efficiencies were not correlated with the number of bacterial species or their diversity.

1. Introduction

Membrane bioreactors (MBR) are becoming widely applied for biological wastewater treatment, as they offer several advantages, such as space reduction, high biodegradation efficiency, variable feed characteristics, and often less sludge production (Seo et al., 2004; Van der Roest et al., 2002). MBR also tend to operate at long solids retention time (SRT) frequently creates serious problems. High aeration rates are required to provide adequate oxygen supply due to decreased mass transfer efficiency (characterized as α) and effective membrane scouring is difficult to achieve because of increased mixed liquor viscosity. MBR operation at low SRT may be a prudent option for facilities that wish to avoid problems with aeration, reduce energy requirements, and use waste biosolids for the production of renewable resources (energy). A better understanding of structure and dynamics of bacterial community in lower SRT may help to optimize the operating conditions of MBR.

There are very few data available concerning MBR operation at low SRT. Previous studies found MBR can acquire better removal efficiency when compare with conventional activated sludge at the same short SRT (Ng and Hermanowicz, 2005). They also found that MBR was capable of achieving excellent quality effluent, almost independent of SRT (Ng and Hermanowicz, 2005; Huang et al., 2001). However, there are only few reports on microbial communities in MBR and the effects of SRT. Previous studies about the bacterial communities in lower SRT have been mostly focused on using some methods such as PCR–DGGE (Zhang et al., 2006a,b), phospholipid fatty acid analysis (Cicek et al., 2001) and respiratory quinone profile (Ahmed et al., 2007). Despite these efforts, the development of structure and diversity of bacterial community in MBRs treating municipal wastewater are not well understood and more molecular-based techniques have not been applied to low SRT.

The goal of this research was to characterize the evolution of bacterial communities over time in pilot-scale MBRs and to relate the structure of microbial community to the performance of the reactors. For this purpose, the MBRs were operated in parallel at SRT of 10, 5 and 3 days. Population changes were monitored by PCR–DGGE. Total bacterial concentrations were quantified by Real-Time PCR. Finally, 16S rDNA clone library and phylogenetic analysis were used to investigate bacterial communities in the three bioreactors.

2. Methods

2.1. Bench-scale submerged membrane bioreactors

Each of three MBRs consisted of a cylindrical tank with the operating volume of 4 L and diameter of 14.5 cm. A hollow fiber membrane module with an effective filtration area of 0.03 m^2

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doi:10.1016/j.biortech.2009.02.056

and a nominal pore size of 0.4 μm (Sterapore LHEM03334, Mitsubishi Rayon, Japan) was used. An aerator was installed underneath the membrane module to supply oxygen to the reactor and to control membrane fouling by hydraulic shear force and agitation. In order to maintain membrane flux, the aeration rate for each MBR was maintained at 27.6 L/min, resulting in the dissolved oxygen concentration close to the saturation (about 9 mg/L).

Synthetic wastewater, mainly composed of acetate and corn starch as carbon sources, was used to provide consistent influent feed composition. The influent (total influent COD of 182 ± 30 mg/L) contained 505.4 mg/L Sodium Acetate, 30 mg/L Corn Starch, 50 mg/L Yeast Extract, 133.75 mg/L NH_4Cl, 30.8 mg/L KH_2PO_4, 71 mg/L $MgSO_4 \cdot 7H_2O$, 19.3 mg/L $CaCl_2 \cdot 2H_2O$, 17.4 mg/L $FeSO_4 \cdot 7H_2O$, 0.07 mg/L $CuCl_2 \cdot 2H_2O$, 0.126 mg/L $MnCl_2 \cdot 4H_2O$, 0.132 mg/L $ZnSO_4 \cdot 7H_2O$, 0.03 mg/L$Na_2MoO_4 \cdot 2H_2O$, 0.0248 mg/L H_3BO_3 and 0.0332 mg/L KI. The reactors were inoculated with activated sludge from the San Jose/Santa Clara Water Pollution Control Plant (San Jose, CA). A sample of activated sludge collected from the aeration tank was settled for 2 h and the supernatant was discarded. In order to provide the same inoculum in all experiments, settled sludge was mixed with glycerol (15% v/v) and stored at −20 °C. At the start of each experimental run, a portion of the frozen activated sludge was thawed within 30 min, diluted threefold with deionized water to 8 L, then aerated in a vessel and feed continuously with stock solution at a flow rate of 3 L/d in a batch-fed mode to restore its viability. After 48 h, a 4-L volume of the revitalized sludge was used as an inoculum to achieve an initial mixed liquor suspended solids (MLSS) concentration of 2000 mg/l.

All MBRs were operated at the same HRT of 6 h. The desired SRT was maintained by wasting excess sludge daily from the reactors. The influent was continuously fed into the MBRs and the effluent was continuously withdrawn through the submerged membrane. The experiments were conducted at room temperature at approximately 20 °C.

2.2. Analytical methods for water quality parameters

All water quality parameters were tested every two days. Standard Methods 2540D/E (APHA et al., 1998) were used for analysis of MLSS and mixed liquor volatile suspended solids (MLVSS). Total chemical oxygen demand (COD), total nitrogen, and ammonia–nitrogen were measured according to manufacturer's instructions using Hach Method 8000, 8039, and 8008, respectively.

EPS concentrations were measured as TOC, DNA, carbohydrates and proteins using a cation exchange resin (CER) (Sigma–Aldrich, PA, USA) extraction method (Frolund et al., 1996). The exchange resin (70 g of CER/g VSS) was added to a 50 mL sample and mixed at 600 rpm using a single blade paddle for 2 h. Total organic carbon (TOC) were analyzed with a Shimadzu TOC analyzer. DNA concentrations were determined by ultraviolet spectrophotometer. Carbohydrate concentrations were quantified using anthrone method (Jenkins et al., 1993) with dextrose used as a standard. Proteins were quantified using the binochoninic acid protein assay kit (Sigma–Aldrich Product Code BCA-1).

2.3. Sampling and DNA extraction

The biomass in the MBRs was sampled periodically throughout a 40-day period, with more frequent sampling during the first 2 weeks. The samples were centrifuged for 5 min at 1000 g, the supernatant was decanted and the pellet was resuspended in Tris–EDTA buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). All samples were immediately frozen after resuspention and stored at −20 °C until DNA extraction.

DNA was extracted from the samples with a Fast DNA Spin Kit (Qiagen, CA, USA) as described in the manufacturer's instructions. To minimize variations in DNA extraction, templates used for Real-Time PCR quantification were prepared by mixing the DNA that was extracted in triplicate for each sample.

2.4. PCR amplification

The 16S rRNA genes were amplified from the DNA extracts using universal primers 27F and 1492R (Chandler et al., 1997). PCR amplifications were carried out in a total volume of 50 μl in 0.2-mL tubes using a DNA thermocycler (Eppendorf, Germany). The PCR mixture contained 1.25 U of Taq polymerase (Promega, WI, USA), 1× PCR buffer, 2 mM $MgCl_2$, 0.5 μmol of each primer, each deoxynucleoside triphosphate at a concentration of 200 μM, and 40 ng of template DNA. The temperature cycling conditions were as follows: after pre-incubation at 94 °C for 2 min, 25 cycles of 94 °C for 2 min, 52 °C for 45 s, and 72 °C for 3 min; finally 72 °C for 12 min.

For DGGE analysis, a nested PCR was performed on the PCR products obtained from previously described primers with a second primer 357-GC F and 518R (Muyzer et al., 1993). The cycling program was the same as before except 30 cycles were performed instead of 25 and the annealing temperature was 55 °C. The sizes of PCR products were assessed by Agilent 2100 Bioanalyzer (Agilent, CA, USA).

2.5. DGGE

DGGE was performed using a D-Code System (Bio-Rad, CA, USA) maintained at a constant temperature of 60 °C in 1× TAE buffer. PCR amplicons were loaded onto 8% (w/v) polyacrylamide gels (37.5:1, acrylamide/bisacrylamide) using a denaturing gradient ranging from 30 to 55% denaturant (100% denaturant was 7 M urea and 40% formamide in the 1× TAE buffer). Gels were run at 75 V for 16 h and stained with silver nitrate.

Specific PCR–DGGE bands were manually excised from the gel, suspended in 30 mL sterile water and incubated overnight at room temperature. The 5 μl supernatant was used as a template for PCR performed with primers 338F and 518R under the same conditions as described above. The PCR products were then purified with QIAquick PCR Purification Kit (Qiagen, CA, USA). All nucleotide sequences were determined at DNA Sequencing Facility at the University of California, Berkeley. Shannon–Wiener diversity index (H′) was used to evaluate the structural diversity between microbial communities of different SRT (Shanno, 1963) as:

$$H' = -\sum_{i=1}^{s}(p_i)(InP_i)$$

where H' is the Shannon biodiversity index, P_i is the ratio of one specific group of bacteria to the total microorganisms, and s is the total number of microbial species in the samples.

2.6. 16S rRNA gene-cloning and phylogenetic analysis

The biomass samples taken after 40 days of MBR operation were used to build cloning library. The PCR products were ligated into a PCR 2.1-TOPO vector and transformed into TOP 10 *Escherichia coli* competent cells following the manufacturer's instructions (Invitrogen, CA, USA). Ampicillin and x-gal were used to screen for colonies with plasmids. For each sample, approximately 100 clones were analyzed. Positive clones were identified by PCR amplification with the primer pairs M13 using the same program as for 16S rDNA amplification. The RFLP (Restriction Fragment Length Polymorphism) was used to analyze the clones. The PCR products were digested using 10 units of restriction enzyme *Msp*I (NEB, MA, USA). Digested PCR products were separated using

2% agarose gel electrophoresis, and stained with ethidium bromide. Clones demonstrating identical restriction patterns were considered to be the same operational taxonomic units (OTUs). At least two representative clones from each group were chosen for sequencing. All the sequences were compared with similar sequences of the reference organisms by performing a BLAST search in the NCBI database. Phylogenetic trees were constructed by the neighbor-joining method with the CLUSTAL W software package (Thompson et al., 1994).

The diversity of microbial communities was determined by Shannon–Wiener index using the same method as DGGE analysis. Additionally, species richness and equitability index were calculated by the following equation (Stamper et al., 2003):

$$\text{Species richness}(d): d = (S-1)/\log N$$

$$\text{Equitability index}(\text{EI}): \text{EI} = H'/\log S$$

where S is the number of species, N is number of individuals, H' is Shannon–Wiener index of diversity.

2.7. Real-Time quantitative PCR assays

Real-Time quantitative PCR was carried out using an ABI StepOnePlus 7500 Real-Time PCR systems (ABI, CA, USA). Quantification of the 16S rRNA gene of total bacteria was performed using the primers 1055F and 1392R (Harms et al., 2003). Each PCR mix (total volume of 20 μL) for all triplicate Real-Time PCR assays consisted of 10 μl Fast SYBR Green master mix (ABI, CA, USA), 0.625 μmol of forward and reverse primers, and approximately 5 ng of DNA template. The program for fast Real-Time PCR was as follows: 20 s at 95 °C, followed by 40 cycles consisting of 3 s at 95 °C, and 30 s at 60 °C. All PCR runs included a negative control reaction and a positive control reaction using HPLC-grade H_2O without template and a previously amplified template, respectively. An external standard curve was generated with serial dilutions of a known copy number of the bacterial 16S rRNA gene as described previously (Okano et al., 2004).

3. Results and discussion

3.1. MBR performance

General performance of the MBR at different SRT is summarized in Table 1. The performance of NH_4^+—N removal efficiencies were high and stable with average values of 89.5%, 86.9% and 87.9% at different SRT. The COD removal efficiencies were higher than 95% when the influent COD load was 0.27 kg/m^{3}d in each reactor. Some reports on nitrification in MBRs suggested that NH_4^+—N removal efficiencies improve with increasing SRT. Fan et al. (1996) showed that NH_4^+—N removal efficiencies increased from 94% to 99% in a side-stream MBR as the SRT increased from 5d to 10d. However, other researchers (Huang et al., 2001) indicated that the MBR process was capable of achieving high removals efficiency of both COD and NH_4^+—N, almost independently of SRT (5–40d). They found that SRT did not significantly affect nitrification in the MBR systems. Membrane filtration improved performance of the system due to complete suspended solid retention and also because of retention of protein and polysaccharides from the sludge supernatant.

As expected, MLSS and MLVSS concentrations decreased as SRT was shortened. However, the VSS/SS ratios increased from 77% to 86% as the SRT decreased from 10d to 3d. This increase suggested more active biomass although VSS is an imperfect measure of active biomass. Cicek and co-workers (2001) found that the overall enzymatic activity of biomass did not change significantly at most SRTs except at the lowest SRT where the activity was the highest. Liang and collaborators (2007) also found that the metabolic activity of biomass in an MBR slightly increased as SRT decreased. Inert biomass (i.e., metabolic products mainly from endogenous respiration) increased at long SRTs (Huang et al., 2001).

EPS concentrations were quantified as TOC, proteins, DNA and carbohydrates and expressed as a fraction of MLVSS (normalized concentrations). The normalized EPS concentrations for three reactors are presented in Fig. 1. In this work, the EPS concentration per unit of VSS increased with decreasing SRT. However, the reactor operated at longer SRT has higher MLVSS concentration, making the total EPS content higher than in the reactors at lower SRT. The carbohydrate EPS (EPS_c) and protein EPS (EPS_p) concentrations normalized by MLVSS were presented as a function of the SRT for all reactors along with the EPS_p/EPS_c ratio. The EPS_p concentration was higher than the EPS_c concentration in all reactors for all tested conditions. The EPS_p/EPS_c ratio in the pilot-scale MBR decreased with decreasing SRT. Similar results were reported in other studies (Lee et al., 2003; Liao et al., 2001).

3.2. DGGE analyzes

The PCR–DGGE was used to compare the changes in the composition of the microbial community with time in the three reactors and to compare the reactors. DGGE profiles of three replicates from each reactor indicated good reproducibility. Only one of the replicates is shown in this paper as an example.

The changes in the bacterial community structure during operation of three MBRs are shown in Fig. 2. The DGGE band pattern for each MBR showed similar changes. Some bands like 1 and 7 were consistently present at all conditions though their intensity varied in different periods. Some bands like 3, 8 and 9 disappeared during reactors' operation. Band 8 disappeared after 20 days while bands 3 and 9 disappeared after 10 days in all three reactors. Some bands like 12, 16 and 18 appeared and some bands like 4 became weak by the end of the operation. It was observed that the speed at which some bands appeared and disappeared increased as SRT decreased. Band 2 disappeared within 20 days in the reactor with SRT = 10d,

Table 1
Performance of the MBRs operated at different SRT.

SRT	10d	5d	3d
Ammonia influent (mg/L)	35 ± 5	35 ± 5	35 ± 5
Ammonia effluent (mg/L)	3.7 ± 2.3	4.6 ± 2.7	4.2 ± 2.9
Ammonia removal efficiency	89.5%	86.9%	87.9%
COD influent (mg/L)	182 ± 30	182 ± 30	182 ± 30
COD effluent (mg/L)	6.9 ± 5.8	8.1 ± 8.8	8.9 ± 6.4
COD removal efficiency	96.2%	95.6%	95.1%
MLSS (mg/L)	1064 ± 299	575 ± 199	303 ± 69
MLVSS (mg/L)	822 ± 147	480 ± 103	260 ± 43
SVI (ml/g)	136 ± 12	150 ± 19	259 ± 81

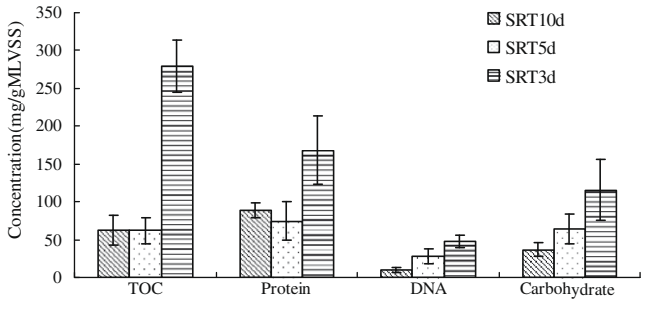

Fig. 1. The TOC, protein, DNA, carbohydrate concentration values of EPS at different SRTs.

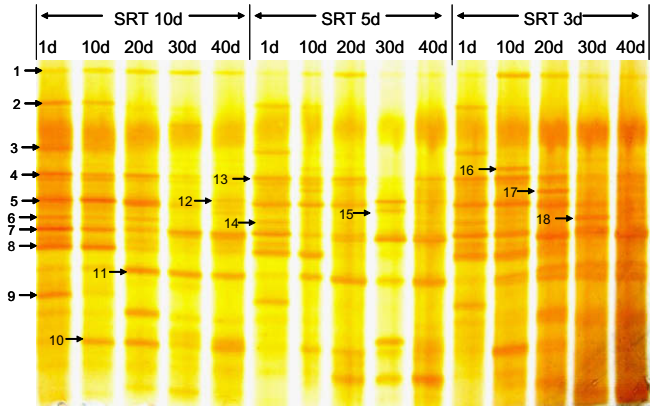

Fig. 2. Denaturing gradient gel electrophoresis profiles of universal bacteria gene dynamic change with operated time. Band position was highlighted with a numbered arrow. Treatment: MBR operated at SRT 10d, SRT 5d and SRT 10d.

while it became faint in only 10 days and disappeared completely in 20 days in the reactor operated at SRT of 5d. However, at SRT of 3d it disappeared completely within 10 days. Similarly, band 11 appeared and became prominent at different times depending on the SRT. The SRT affects the capabilities and performance of biological treatment systems. For example, it determines the types of microorganisms that can grow in a bioreactor. If the operating SRT is shorter than the minimum SRT associated with microorganisms responsible for a particular biochemical transformation, these microbes will be wasted from the bioreactor faster than they grow, a stable population will not develop, and the particular biochemical transformation will not cease to function.

The differences in microbial community structure of the three MBRs after the same length of operation are shown in Fig. 3. It was found that the biomass at SRT of 10d had more bands, especially after 20 days of operation. At this time, bands 22, 23 and 24 just appeared in this system and band 21 was more intense at 10-d SRT compared to other two SRTs. This band was weakened and perhaps disappeared altogether after next 10 days of operation (30 days total).

Bacterial diversity as determined by Shannon–Wiener index (H') decreased as SRT decreased. The H' values for the SRT 3d, 5d and 10d were 1.42, 1.75 and 2.68, respectively (Table 2). These values describe the diversity and their changes can be attributed to the same reasons as discussed above. In general, the composition of the feed stream (nutrients) is the primary driving force affecting the composition of a bacterial community (Chen and LaPara, 2006). In our study the feed was composed only of a few constituents while the microbial population was quite complex. The observed complexity of the bacterial communities exceeded the complexity of the feed. Thus, we infer that the bacterial community contained functionally redundant populations that evolved dynamically as has also been suggested for bacterial communities in other ecosystems (Konopka et al., 1999; Yin et al., 2000).

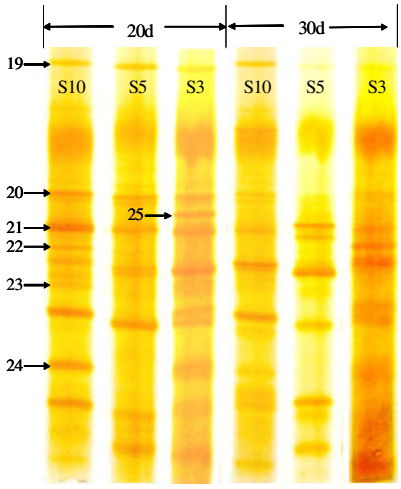

Fig. 3. Comparison of universal bacteria community in MBR operated at different SRT using denaturing gradient gel electrophoresis. Band position was highlighted with a numbered arrow. Treatment: MBR operated at SRT 10d (S10), SRT 5d (S5) and SRT 3d (S3).

Table 2
The species richness, equitability index and diversity of microbial communities in the three reactors.

Index	3d	5d	10d
Determined from DGGE			
Shannon–Wiener index (H')	1.42	1.75	2.68
Determined from phylogenetic analysis			
Shannon–Wiener index (H')	2.44	3.17	3.32
Species richness (d)	10.00	13.50	16.50
Equitability index (EI)	1.85	2.19	2.19

The bands were excised and amplified for nucleotide sequence analysis. Some bands failed to provide useful sequence data because of the DGGE specific limitation such as the detection of heteroduplex molecules (when two similar but different strands joint together) or when one species have more than one rDNA sequence-producing band. *Acinetobacter* sp. (band 13) was detected in all MBRs. It has been reported that *Acinetobacter* was one of the most predominant species in the MF membrane fouling layer. *Acinetobacter* caused the most severe fouling in MBR under relatively static condition in spite of its hydrophilic property (Choi et al., 2006). This suggested that *Acinetobacter* tends to be sticky, and thus attaches well to the medium but has less resistance to shear forces. It was noticed that *Acinetobacter* could play a very important role in early bacterial surface colonization in MBRs, which leads to the development of mature biofilms (biofouling layers) on the membranes (Choi et al., 2006).

The other detected bacteria included *Myxococcales* (band 1), *Flavobacterium* (band 5), *Spirosoma* (band 6), *Aquaspirillum* sp. (band 7), *Runella* sp. (band 10), *Zoogloea* sp. (band 11), *Nitrosomonas* (band 12), *Nitrospira* sp. (band 15) and *Nannocystineae bacterium* (band 25). Phylogenetic analysis suggested that about half of the bacteria were affiliated with *Bacteriodetes, Proteobacteria,* and also with *Nitrospirae* – an important group of nitrifying bacteria in wastewater treatment.

3.3. Cloning library

Three clone libraries were constructed with 100 clones consisting of 1143-bp insert of 16S rRNA gene fragments from three reactors. RFLP analysis was used to analyze samples from each reactor to identify potentially dominant bacterial community members. Representative RFLP results are shown in Electronic Supplementary material in the online version of this article.

For the reactor operated at SRT of 3d, the clones were classified into 21 OTUs based on the restriction patterns. The largest fraction (21%) of the clones exhibited the highest levels of similarity (98 to 100%) to "*uncultured bacterium clone:0102*", an unclassified species near *Deltaproteobacteria* and *Nitrospira* sp. in the phylogenetic tree (Fig. 4). The next largest fraction (17%) of the clones exhibited the highest levels of similarity (97–100%) with *Aquaspirillum* sp. (in the *Betaproteobacteria* subdivision) and another 17% of clones were very similar (93–99%) to *Myxobacterium*, which belongs to *Deltaproteobacteria.*

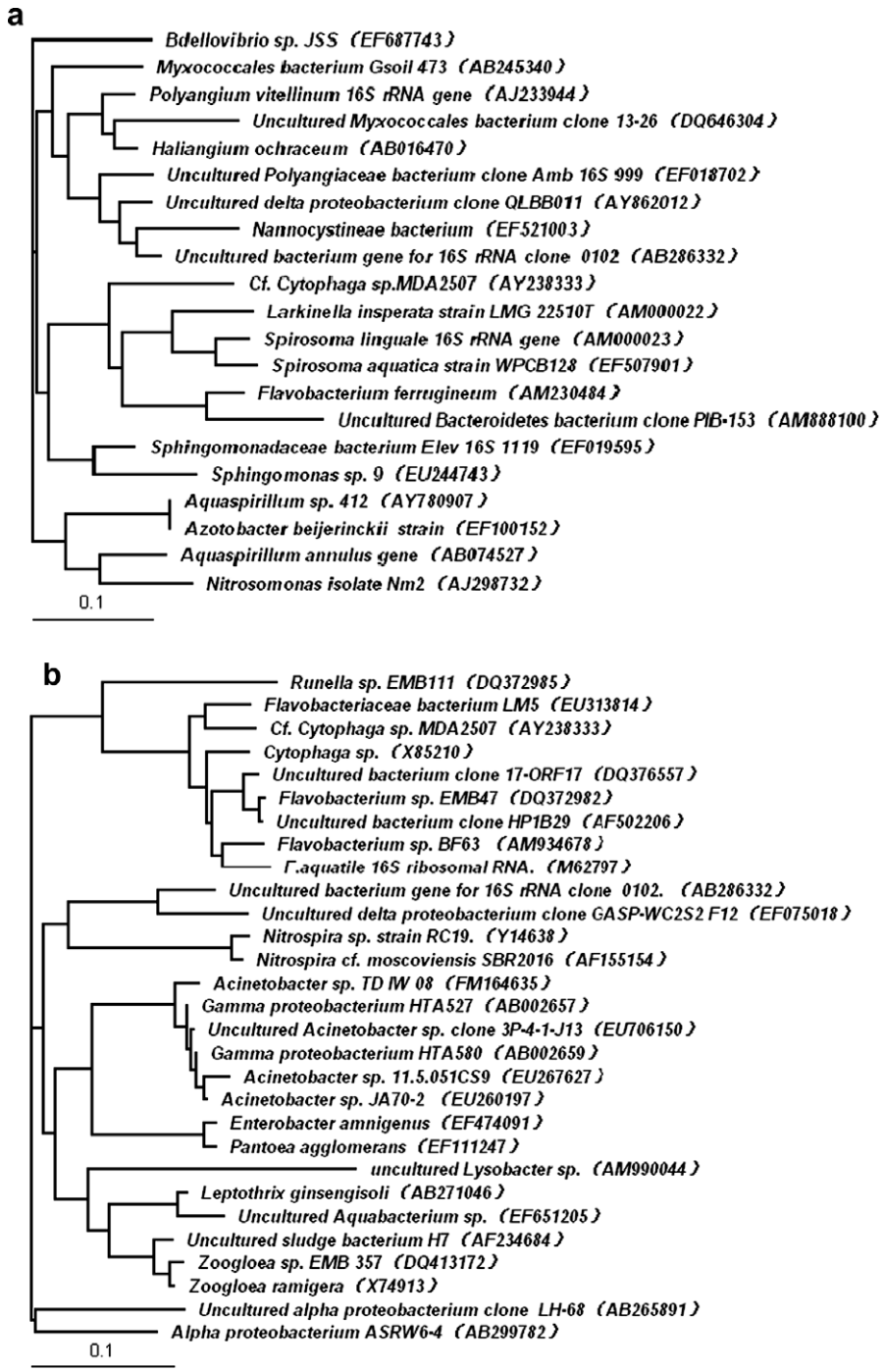

Fig. 4. Phylogenetic relationships among universal bacterial sequences retrieved from the three reactors. The tree was constructed using the neighbor-joining algorithm with the Kimura parameter in the PHYLIP software package. Designation of the clones in bold includes the following information: accession number in the GenBank and the Scale bar represents 0.1 substitutions per nucleotide position. (a) Phylogenetic tree for SRT 3d; (b) Phylogenetic tree for SRT 5d; and (c) Phylogenetic tree for SRT 10d.

In the reactor operated at SRT of 5d, we identified 28 OTUs classified from 100 clones. The "*uncultured bacterium clone:0102*" was also the dominant bacterial species (8.8% of the total population). The same percent of the clones exhibited the highest levels of similarity (94–99%) to *Zoogloea* sp. which are related to *Betaproteobacteria*. Another 7% of clones were very similar (97–99%) to *Flavobacterium* sp., which belongs to *Bacteroidetes*.

The clone library at SRT of 10d has 33 OTUs. The main bacteria include *Aquaspirillum* sp. (9.8%), "*uncultured bacterium clone:0102*" (6.6%), *Myxobacterium* and *Nitrospira* sp. These four species belong to *Betaproteobacteria*, unclassified bacteria, *Deltaproteobacteria* and *Nitrospirae*, respectively. It is apparent from the phylogenetic analysis that the SRT has a tremendous impact on nitrification because it affects the ability of autotrophic nitrifying bacteria or nitrifiers such as *Nitrosomona* and *Nitrobacter* to strive in the reactor. It has been shown that the maximum specific growth rates for nitrifiers are considerably lower than those for heterotrophic bacteria. Hence nitrifiers grow slower than the heterotrophic bacteria. We also found more *Nitrospirae* at longer SRT in this study.

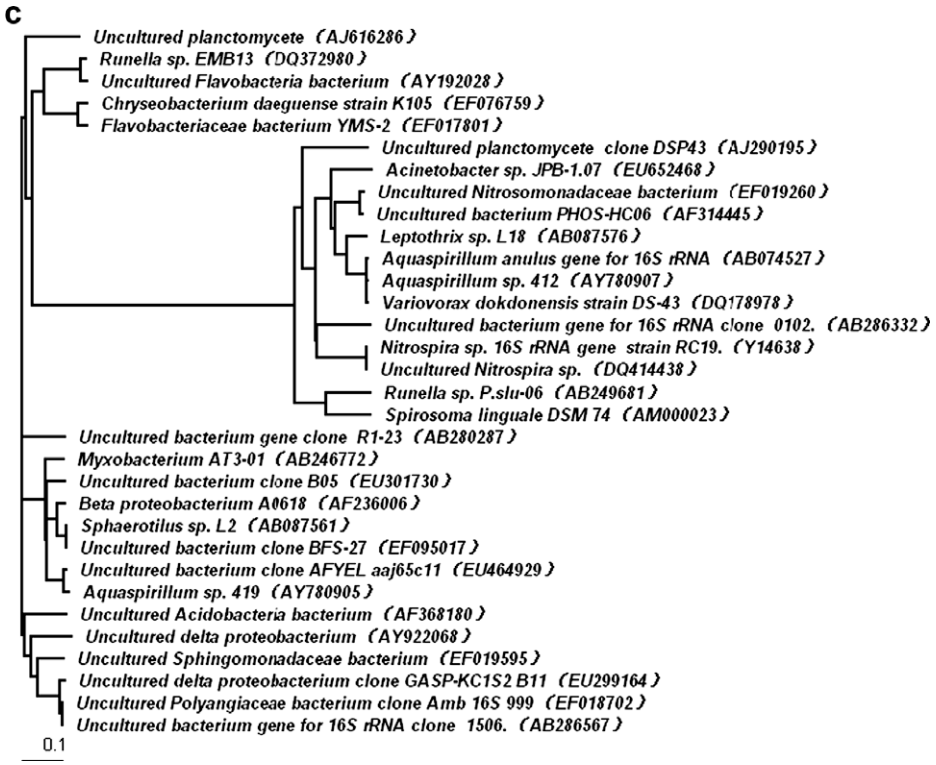

Fig. 4 (*continued*)

The values of indices characterizing the community (species richness, equitability index, and diversity) in the three reactors are shown in Table 2. All indices support our observation that microbial communities became more diverse at longer SRT. It has been assumed that the increases in SRT could enhance the development of slow growing microorganisms since they are able to consume macro-molecules (polysaccharides, carbohydrates, and protein) as substrates and produce less biopolymers (Masse et al., 2006). Despite these differences, operational performance of all three reactors (measured by COD removal or nitrification) was not different suggesting that the increased microbial diversity compensated for lower growth (and metabolic) rates or that some species present at long SRT performed specialized functions not captured by conventional operational parameters.

The phylogenetic relationship of all representative clones in each library was assessed by constructing phylogenetic trees (Fig. 4). For SRT of 3d *Deltaproteobacteria* and *Betaproteobacteria* were the dominant groups, which constituted 42% and 37% of the whole microbial community, respectively. *Bacteroidetes* was the next group and constituted 10%. For SRT of 5d *Gammaproteobacteria* and *Betaproteobacteria* were the largest groups at 26% and 23%. *Bacteroidetes* and *Deltaproteobacteria* were also large populations at 19% and 14%, respectively. In the reactor operated at SRT of 10d *Betaproteobacteria* was the dominant group constituting 39%. *Bacteroidetes* and *Deltaproteobacteria* were again large fractions occupying 15% and 11%, respectively. *Betaproteobacteria* were dominant group in all the reactors. Similar dominance by this group was also found in other studies using the Quinone profile in a membrane bioreactor fed different carbon sources (Ahmed et al., 2008). This group was also found in other wastewater treatment bioreactors. Bramucci et al. (2003) analyzed the bacterial community in an industrial activated sludge process using DGGE and clone library techniques and reported that *Betaproteobacteria* accounted for the major proportion of the community. Niu et al. (2006) found *Betaproteobacteria, Bacteroidetes* and *Gammaproteobacteria* were dominant groups in the natural circulation system by using a 16S rRNA clone library. Snaidr et al. (1997) found that *Betaproteobacteria* was the main group in the aeration basin of a municipal sewage plant.

In our work, although all three reactors had the same dominant groups like *Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria* and *Bacteroidetes*, the phylogenetic analysis showed some significant differences in the presence of bacterial species between reactors. For example, *Sphaerotilus* sp. was detected at the SRT of 10 d but was not found in other two reactors. It has been known that this bacterial group can cause such problems as pipe clogging and foaming of activated sludge in wastewater treatment due to its ability to attach to solid surfaces by entanglement or using an adhesive basal element on one end of the filament (Pellegrin et al., 1999). Chudoba (1985) indicated that long SRT favored filamentous growth because of their low specific growth rates.

The clone library-based approach is generally restricted by the selected clone numbers and sludge sampling and it is possible that some bacteria remain undetected. For example, we could not find *Acinetobacter sp.* in clone library, but the DGGE results showed that this species was present in the reactors.

3.4. Real-Time quantitative PCR

Real-Time PCR was used to examine dynamic changes of the total bacterial 16S rRNA gene copies in the three MBR (Fig. 5). After inoculation, the bacterial numbers were around 3.75×10^7 copy numbers per microliter of mixed liquor (biomass) in the three reactors. There was no significant difference between three reactors for the first 8 days of operation. After this time, the numbers of gene copies were higher at longer SRT. After 20d, there were 8.07×10^9, 1.33×10^{10} and 1.58×10^{10} copies per microliter of mixed liquor for SRT of 3d, 5d and 10d, respectively. The bacterial

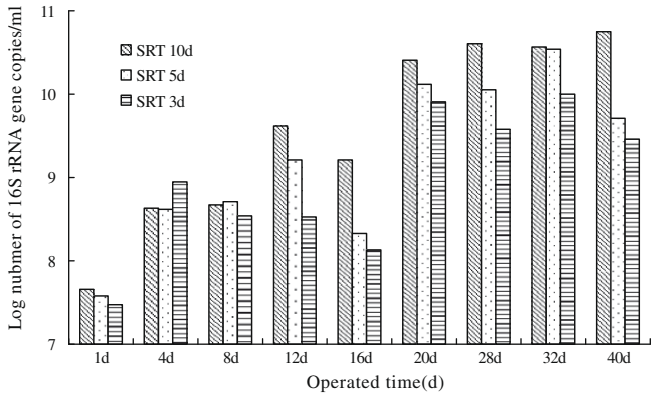

Fig. 5. Bacterial 16S rRNA gene copy number change in MBR with operating time at different SRT.

numbers noticeably increased during the first 20 days in all reactors, and stabilized after that time. We suggest that certain microorganisms, chosen for their abilities to decompose the chemical components of synthetic wastewater, would, when coupled with the MBR, significantly improve the stability and efficiency of this system after this stage.

4. Conclusions

This study compared the performance and microbial community composition in MBR treating the same synthetic wastewater operated over low range of SRTs (3, 5 and 10 days). All three MBRs were able to achieve very good COD (about 95%) and NH_4^+—N (87–90%) removal efficiencies regardless of the SRT values. The ratio of the active biomass to that of the total biomass (MLVSS/MLSS) decreased with increasing SRT, indicating that the increased sludge age could decrease the microbial activity in the MBR.

Several molecular methods were used to analyze the microbial community. In all reactors the bacterial species numbers and gene copy numbers increased as SRT increased. The same bacterial groups such as *Betaproteobacteria* dominated biomass at all SRT. However, microbial diversity was much higher at longer SRT despite a very simple composition of the feed. The increased of diversity was not captured by the conventional performance parameters (such as removal efficiency).

Acknowledgements

The senior author (Liang Duan) thanks the China Scholarship Council for the financial support in Berkeley. Professor Lisa Alvarez-Cohen is gratefully acknowledged for providing some of the specialized facilities. Ivan Moreno Andrade was supported by a postdoctoral fellowship from the UC Mexus program. Chun-Lin Huang was supported by a grant from the Government Scholarship Ministry of Education, Chinese Taiwan government.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2009.02.056.

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