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ScienceDirect
Bioresource Technology 99 (2008) 6834–6843
BIORESOURCE TECHNOLOGY

Performance of a pilot-scale submerged membrane bioreactor (MBR) in treating bathing wastewater

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Received 24 October 2007; received in revised form 25 January 2008; accepted 26 January 2008
Available online 24 March 2008

Abstract

Bathing wastewater was treated by a pilot-scale submerged membrane bioreactor (MBR) for more than 60 days. The results showed that the removal rates of main pollutants of wastewater such as COD_{Cr}, LAS, NH_4^+–N and total nitrogen (TN) were above 93%, 99%, 99%, and 90%, respectively. The results of denaturing gel gradient electrophoresis (DGGE) and fluorescent in situ hybridization (FISH) indicated that the bacteria were stable. The abundant nitrobacteria intercepted by the membrane led to the high removal rate of ammonia and TN. FISH and 16S rDNA gene sequence analysis revealed that some specific phylogenetic group of bacteria, the *Pseudomonas* sp. *Ochrobactrum anthropi* sp. and *Enterobacter* sp. probably played a major role in the development of the mature biofilms, which led to the severe irreversible membrane biofouling.
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Keywords: Bathing wastewater; MBR; Molecular-based methods; Membrane fouling

1. Introduction

Linear alkylbenzene sulphonates (LAS) are the most used surfactants in the world for their excellent characteristics of emulsification and penetration. It has been demonstrated that these compounds are not mineralized under anaerobic conditions because methanogenesis is inhibited (Krueger et al., 1998). Both the wastewater from LAS product factories and the wastewater from the LAS users are harmful to the environment (Kristian et al., 2001). As a kind of typical surfactants wastewater, bathing wastewater has lower pollution but contains higher LAS than municipal wastewater. There are many public bathrooms in China so that a great amount of bathing wastewater, which is an ideal wastewater to be reclaimed, is produced.

Membrane bioreactors (MBRs), which permit a very long sludge retention time (SRT), high mixed liquor suspended solids (MLSS) and low F/M ratio due to the interception of the membrane, have been found to be advantageous over the conventional activated sludge systems in terms of system stability and compactness (Cicek, 2003; Zhang et al., 2003). The smaller footprint, higher quality effluent and better control (Le-Clech et al., 2003) over biological conditions provide MBRs with significant benefits over other biological wastewater treatment processes. Due to the stringent discharge criteria, increasing space constraints and desired flexibility for future expansion and upgrade, MBRs have gained considerable interest in wastewater reuse.

MBRs can produce much higher quality effluent through retaining soluble microbial products (SMPs) in the system (Gao et al., 2004). However, the major drawbacks of MBRs are membrane fouling and subsequent membrane cleaning costs. Previous studies had focused on various factors affecting the membrane fouling in MBRs, including MLSS concentration (Germain et al., 2005), various constituents of activated sludge (Laure et al., 2000), extracellular polymeric substance (EPS) (Lesjean et al., 2005; Cho and Fane, 2002; Ji and Zhou, 2006),

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doi:10.1016/j.biortech.2008.01.044

supernatant chemical oxygen demand (COD_{Cr}) (Nuengjamnong et al., 2005), food/microorganisms (F/M) ratio (Kimura et al., 2005), sludge characteristics (Itonaga et al., 2004; Kimura et al., 2005), and the amount and composition of microbial products (Yamato et al., 2006; Rosenberger et al., 2006; Ng et al., 2006; Fan and Zhou, 2007). Unified and well-described mechanisms of membrane biofouling that can lead to the development of appropriate strategies to control membrane biofouling are not currently available. This is partly due to the diverse range of operating conditions, membranes, and feed water matrices (mostly synthetic media were used) that have been employed. In addition, only a few studies have focused on microbial aspects, such as microbial colonization, biofilm formation, and microbial community structures on the membrane surfaces in MBRs (Chen et al., 2004; Zhang et al., 2006).

There are very limited studies on the analysis of aerated MBRs in treating the bathing wastewater systemically and hence the objective of the paper was to study the performance for treating bathing wastewater (from students' public bathroom in Tongji University of China) in a continuously aerated MBR. The other major focus of this work related to membrane fouling. The membrane fouling was analyzed through the changes of trans-membrane pressure (TMP) and scanning electron microscope (SEM) of membrane. Molecular-based methods were used to identify key bacteria responsible for membrane fouling during the operation.

2. Methods

2.1. Pilot-scale equipment

The main body of the pilot-scale equipment consists of an anoxic (AN) tank and an aerobic (AO) tank (Fig. 1). The MBR has a total volume of 210 L, in which the volume of AN tank and the AO tank are 70 L and 140 L, respectively. Fixed packing was put into the AN tank to improve the hydrolyzation effect. The biofilm will form on the fixed packing gradually, the microorganisms on which could hydrolyze the pollutants (Grebenchikova et al., 2002). Operation of the pilot-scale MBR was carried out at the Tongji University students' public bathing wastewater treatment station. The MBR was operated with the bathing wastewater. The MBR was equipped with hollow-fiber micro filtration (MF) membrane modules made of polyethylene that had a total surface area of 3 m^2 and a nominal pore size of 0.4 μm (Mitsubishi Rayon, Tokyo, Japan). Intermittent filtration was done (12 min filtration and 3 min pause) in the constant flow rate (0.4 m^3/m^2·day) mode of operation using suction pumps. For submerged aerobic MBRs, intermittent suction is effective for suppression of fouling (Liu et al., 2000; Gui et al., 2002). The concentration of the mixed liquor suspended solid (MLSS) in the reactor was maintained at about 4–10 g/L. In the end of the first stage, the MLSS concentration was 4 g/L. In the last stage the MLSS concentration increased to 10 g/L. But it was still not high for the MBR, the reason may be that the influent was short of the substrate and the microorganisms in reactor were in endogenesis respiration for the scarceness of food (the COD and other nutrients' concentrations in bathing wastewater were less than those of the municipal wastewater).

2.2. Wastewater quality

The COD_{Cr}, BOD_5, total nitrogen (TN), NH_4^+–N, NO_3^-–N, NO_2^-–N, LAS, total phosphorus (TP), SS and turbidity of the bathing wastewater were 99–206 mg/L, 40–60 mg/L, 22–49 mg/L, 21–47 mg/L, 1–7 mg/L, <1 mg/L, 2.8–8 mg/L, 0.5–0.8 mg/L, 75–240 mg/L, and 90–220 NTU, respectively. The bathing wastewater had high LAS concentration, low carbon strength and phosphorus concentration

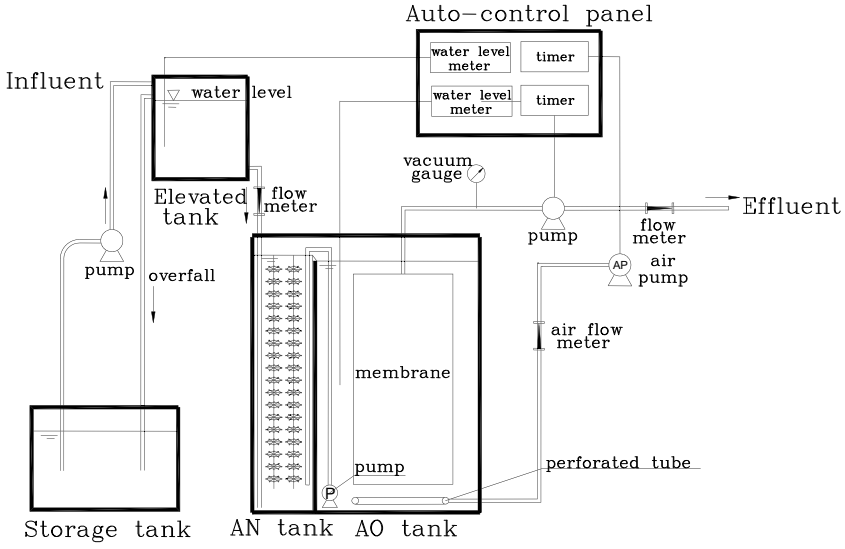

Fig. 1. The sketching of pilot-scale MBR.

(phosphorus limit in detergents), and NH_4^+–N was the main component of total nitrogen (TN).

2.3. Analytical methods

Wastewater parameters, including COD, BOD_5, TN, NH_4^+–N, NO_3^-–N, NO_2^-–N, LAS, TP and SS of the influent, AN liquor, supernatant liquor (AO liquor) and effluent of MBR, were analyzed according to Chinese NEPA (1997) standard methods. The samples of AN liquor and supernatant liquor were all obtained by filtrating the mixed liquor through filter papers with the pore size of 0.45 μm. The turbidity was measured using a turbidimeter (2100N, HACH, USA).

The membrane fouling was observed with SEM (XL-30, Philips, Netherlands). TMP values were measured using an online pressure sensor (EH50, RUIPU Incorporated, Beijing, China). Most of the results were taken from the average of duplicate samples.

2.4. PCR-DGGE and FISH analysis

Culture independent molecular-based methods (i.e., polymerase chain reaction-denaturing gel gradient electrophoresis (PCR-DGGE), fluorescence in situ hybridization (FISH) and 16S rDNA gene sequence analysis) were used to analyze the microbial community structures of mixed liquor and biofilms which attached to the membrane surfaces in the MBR.

2.4.1. DNA extraction

Total DNA of activated sludge was extracted using a Fast DNA Spin kit (Shenergy Color, China) as described in the manufacturer's instructions.

Total DNA extraction of biofilm in the membrane surface:

Several hollow-fiber membranes were cut from the modules, and then the hollow-fiber membranes were cut into about 10 mm pieces using a sterile cutter knife. At last, biofilm was detached from these treated membrane surface by an ultrasonic instrument. Then the samples were centrifuged at 10,000 rpm for 5 min. The subsequent steps were the same as those in total DNA extraction of activated sludge.

Nucleic acids concentrations were measured spectrophotometrically.

2.4.2. 16S rDNA and amoA gene fragment PCR amplification

The primer pair 338f, P518r (Xia et al., 2005a,b) and the other one *amoA*1F, *amoA*2R (Hornek et al., 2006) were used for amplification of the V3 region part of 16S rDNA genes and the *amoA* gene, respectively. Each 50 μl PCR reaction mixture contained 25 ng of extracted template DNA, 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1 mM $MgCl_2$, 0.1 mM dNTP, 0.5 mM both primers, and 0.5 U Taq DNA polymerase (Takara, China). The PCR reactions were performed with Mycycler (Bio-rad, CA, USA). Amplification of the first round was carried out under the following conditions: an initial denaturation at 94 °C for 10 min followed by 30 cycles consisting of denaturation at 94 °C for 45 s, primer annealing at 60 °C (V3 region part of 16 S rDNA genes) or 57 °C (*amoA* gene) for 45 s, and primer extension at 72 °C for 45 s. A final extension step was conducted at 72 °C for 10 min.

For DGGE analysis, the second round of PCR were performed in the same program as the first round except for the attachment of a 40 bp gc-clamp at the 5′ end of the forward primers (Sofia et al., 2004; Xia et al., 2005a,b). The PCR products were analyzed with 1.5% agarose gel to confirm that the fragments were about 240 bp (V3 region part of 16S rDNA genes) and 465 (*amoA* gene) bp.

2.4.3. DGGE profiling

DGGE of the PCR product was performed with the D-code System (BioRad, CA, USA) according to previous research (Xia et al., 2005a,b). The PCR products of the second round were loaded onto 8% (w/v) (for 16S rDNA gene) and 6% (w/v) (for *amoA* gene) polyacrylamide gels. The polyacrylamide gels were made with a denaturing gradient ranging from 35 to 60% (for 16S rDNA gene) and 30 to 55% (for *amoA* gene). Electrophoresis was conducted using a 1.0 × TAE buffer at 150 V and 60 °C for 5 h.

Two staining solutions were used after electrophoresis. One was $AgNO_3$ solution: the gels were soaked for 15 min in fixation buffer (30% ethanol, 5% acetic acid) and then incubated in 0.3% $AgNO_3$ solution for 20 min, washed in deionized (DI) water 5 min for two times, kept in the 2.5% sodium bicarbonate and 0.1% formaldehyde until the bands appeared clearly, finally the staining process was stopped with 10% acetic acid. The other was ethidium bromide (EB) solution: the gel was stained with a 1.0 × TAE buffer with EB (0.5 mg L^{-1}) for 20 min and rinsed with DI water. Then the gel was scanned using an electrophoresis UV (for gel stained by EB solution) and visible (for gel stained by $AgNO_3$ solution) analyzer (FR-110, Shanghai, China) to acquire the DGGE band image.

2.4.4. Sequencing of DGGE fractions

The gel fraction with targeted DNA was cut off and transferred to a micro centrifuge tube. Thirty microliter DI water was added and the product was stored overnight at 4. The product was used as a PCR template and amplified. The PCR products were sequenced by Sangon Company (Shanghai, China) and the sequences were examined in blastn (NCBI, USA) for similarity comparison.

DGGE products were analyzed with Smartview, and biodiversity of the samples was calculated with the Shannon–Wiener index (H') formula, as described in the following equation (Shannon and Weaver, 1963)

$$H' = -\sum_{i=1}^{s}(P_i)(\ln P_i)$$

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

2.4.5. FISH analysis

Biofilm biomass of (0.2 g) or 250 μL activated sludge samples were centrifuged in 1 × PBS (phosphate buffered saline) three times at 10,000 rpm for 3 min, the supernatant was discarded every time. Then the samples were fixed with 4% paraformaldehyde for 3 h at 4 °C. After being rinsed with 1 × PBS solution, samples were immobilized on gelatin coated slide glass, and then dehydrated by successive ethanol solution 50%, 80%, 100% each for 3 min, dried in the air. The mixture of 9 μL hybridization buffer and 1 μL fluorescent probes (EUB338, Nso190, and Nit3) and competitive probes (NEUB338, CNit3) was hybridized with the fixed samples. Then the slides were incubated in a pre-warmed moisture chamber at 46 °C controlled by a hybridization oven (Thermo HBSNSRS220, USA) for 3 h. Next the slides were dipped into the washing solution at 48 °C for 15 min. After being rinsed in DI water (ice bath) once, dried in the air, the slides were analyzed with fluorescence microscope equipped with a digital camera (Nikon ECLIPSE TE2000-U, Japan). The composition of hybridization and washing solution was referred to Schramm's work (Schramm et al., 1998). The information of oligonucleotide probes EUB338, Nit3 and Nso190 along with the different concentration of NaCl and formamide correspondingly referred to Coskuner's et al. (2005) and Egli's et al. (2003) work (listed in Table 1).

3. Results and discussion

3.1. Operation of MBR

3.1.1. Inocula and runs

The inoculated sludge was from the recycle sludge tank of the east municipal wastewater treatment plant (EMWTP) located in Shanghai, China. The original biomass concentration in the reactor was about 2 g L^{-1} MLSS. Then the MBR was aerated for two days without discharge. Subsequently, the glucose, phosphate and other nutrients were fed along with the bathing wastewater into the MBR to make the sludge acclimation. During this stage, the amount of the extra nutrients decreased to zero step by step till day 13. From day 14, the bathing wastewater was fed continuously into the MBR without extra nutrients.

It took about 10 days in this stage till the biomass concentration reached about 4 g/L and stable. There were three stages in total, the first stage was adaptable stage (3rd day–13th day); the second stage was steady stage (14th day–33rd day); and the third stage was mature stage (34rd day–62nd day).

3.1.2. Pollution removal

(1) COD_{Cr} removal

In the first stage, system adapted the quality of wastewater gradually while the COD_{Cr} concentration of effluent decreased. In the second stage, the COD_{Cr} concentration of effluent fluctuated with the influent and was relatively stable. In the third stage, activated sludge in the system became mature and the EPS in AO tank accumulated (some were intercepted by membrane) (Namjung et al., 2007), so the COD_{Cr} of supernatant liquor rose gradually. But the COD_{Cr} concentration of effluent was still steady due to membrane interception (Fig. 2). This is in accordance with the result of Elif's et al. (2007). The COD_{Cr} removal rate was 91–93% when the COD_{Cr} concentration of influent fluctuated at 99–206 mg L^{-1}. The COD value of the AN tank effluent fluctuated with the influent and the average COD removal ratio ranged from 20% to 30%, indicating a part of COD was removed in anoxic stage.

(2) LAS removal

The hydrolyzation of the partial LAS happened in AN Tank. In AO tank, the hydrolyzed products and residual LAS were removed further (Fig. 3). In the first stage, the effluent was not satisfactory. In the second stage, the system adapted to the influent quality and the effluent was stable. In the mature stage, the sludge in two tanks became mature and they adapted to the influent quality further and the effluent was more satisfactory.

EPS is believed to originate from different sources (Nidal et al., 2003). And lysis of bacterial cells is a main reason for this. In our MBR system, due to the effects of endogenesis respiration for the scarceness of the substrate, continuous aeration mode and long SRT (Li et al., 2007), a part of the bacteria in MBR were lysed gradually. In the mature stage, sludge in two tanks became mature and

Table 1
Oligonucleotide probes used for FISH and corresponding hybridization and washing conditions

Probe	Sequence(5′–3′)	Fluorescence label	Target organisms	Competitor	Formamide (%)	NaCl (mM)
EUB338	GCTGCCTCCCGTAGGAGT	FAM	Bacteria	NEUB338	20	900
Nso190	CGATCCCCTGCTTTTCTCC	Cy3	Ammonia-oxidizing bacteria (AOB)β-Proteobacteria	–	50	28
Nit3	CCTGTGCTCCATGCTCCG	HEX	Nitrobacteria	CNit3	40	56

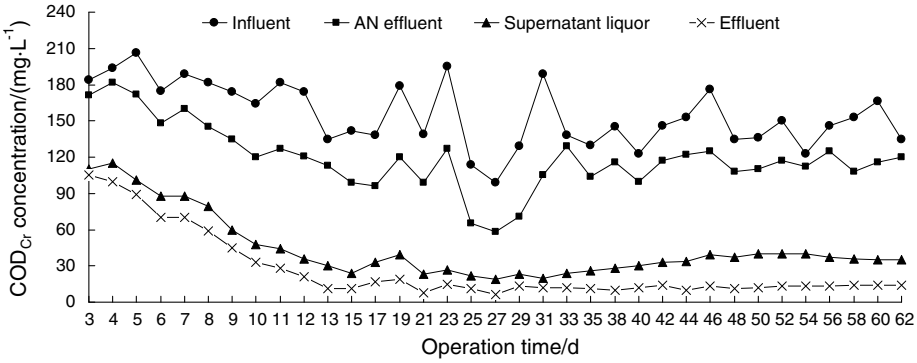

Fig. 2. Variations of COD_{Cr}.

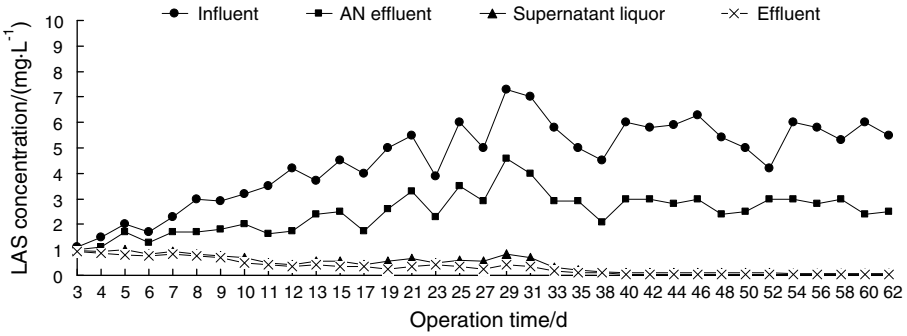

Fig. 3. Variations of LAS.

EPS of AO tank's sludge became more than before, so the surface of membrane was adhered by more organic matters (bio-cake layers (Jeison and van Lier, 2006; Hong et al., 2007)). Then part of LAS could be adsorbed by the organic matters and decomposed by the microorganisms on the membrane surface when permeation. As a result, the effect of microorganisms and organic matters (bio-cake layers on the membrane surface) on LAS removal was more crucial than membrane. Thus the LAS removal efficiency was the highest in the three stages. However, the fouling of membrane was severe due to the same reason. In mature stage, the LAS removal efficiency rose distinctly to 99% as the LAS concentration of influent fluctuated at 2.8–8 mg L^{-1}.

Nearly half of LAS was removed in the AN tank, especially in the mature stage. The fixed packing (biofilm on them) played a role in removing the LAS.

(3) Nitrogen removal

The sludge in AO tank was recycled to the AN tank with the sludge recycle ratio of 100–150% to enhance the TN removal. As shown in Fig. 4, in the first stage, the NH_4^+–N removal and TN removal were not distinct because the amounts of nitrobacteria and denitrobacteria were low. In the second stage, the nitrobacteria and denitrobacteria of the activated sludge increased gradually and the NH_4^+–N removal was better than before. In the mature stage, the NH_4^+–N and TN removal ratios were above 99% and 90%. The advantage of MBRs to provide better retention of slow growing microorganisms (like nitrobacteria) (de Silva et al., 1998) enhanced the nitrogen removal.

3.2. Microbial community structures analysis

3.2.1. DGGE profiles of all bacterial communities in 16S rDNA (staining by $AgNO_3$)

The DGGE profiles of all bacterial communities in 16S rDNA V3 region were shown in Fig. 5. Each sample exhibited distinct DGGE patterns. The bacterial community structures of the samples in three stages were different from the inoculated samples. The activated sludge in MBR changed compared with the conventional activated sludge system (CAS) in the EMWTP. In the first stage, the sludge was adapting to the wastewater so that the adapted bacteria won in the competition and the others disappeared. So there were only a few bands in the treatment system. In the second stage, the dominant microorganisms had grown up and the bands were relatively abundant. In the third stage, the microorganisms were mature, so the bands became even more abundant than the second stage.

Shannon–Wiener index showed that the genus of bacteria in inoculated sludge were abundant (The indexes of the samples of inoculated activated sludge, 1st stage, 2nd stage and 3rd stage were 2.689, 1.892, 2.493 and 2.511, respectively). The index was the minimum in the first stage, indicating that the bacteria were affected by the quality of the influent. In the latter two stages, the bacteria had adapted the water quality and had become more stable, so the index

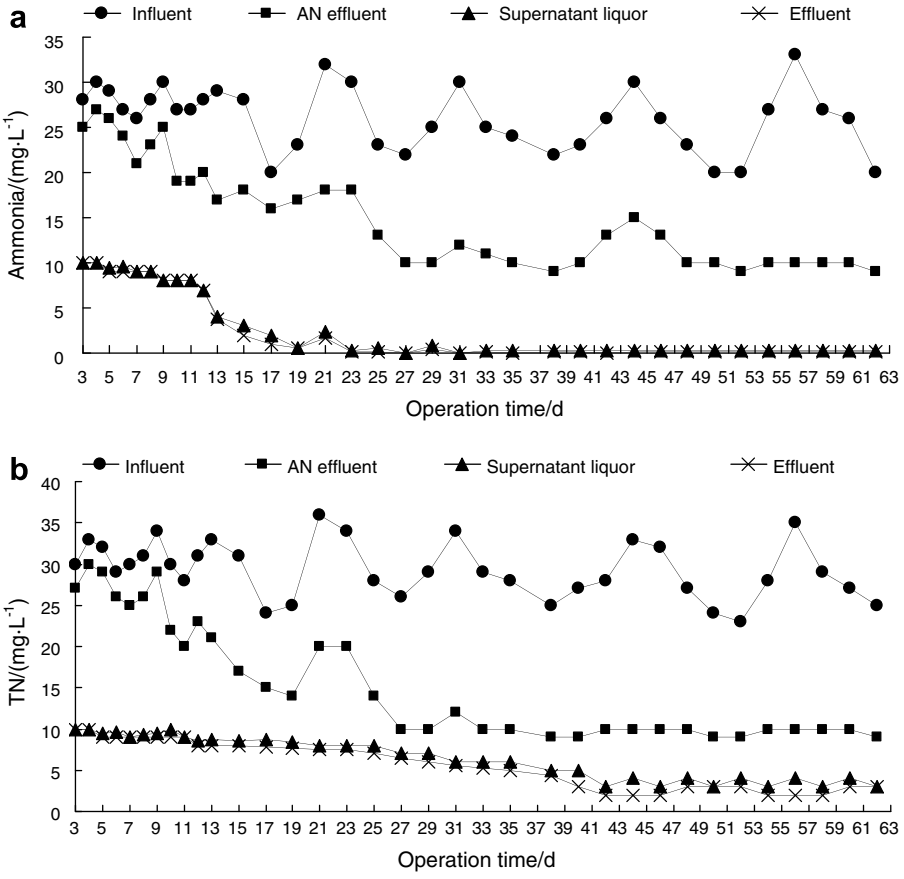

Fig. 4. Variations of ammonia (a) and TN (b).

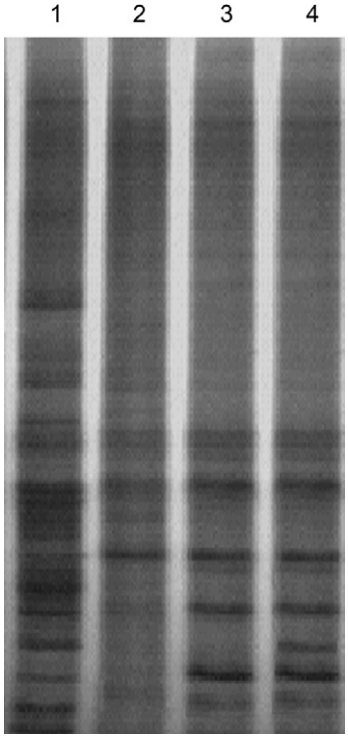

Fig. 5. DGGE band profiles of all bacterial communities (amplified 16S rDNA fragments) from mixed liquor samples. The labels of 1–4 at the top of the lanes represent samples of inoculated activated sludge, 1st stage, 2nd stage and 3rd stage, respectively.

was higher than the first stage. As could be seen from the DGGE profiles, the third stage had the higher diversity than other stages, which showed that abundant genus of all bacteria in mature MBR.

The results indicated that after adapted to the influent, the bacteria were stable. The diversity of microbial composition was high; the influence of wastewater quality fluctuation on dominant species was negligible, so the system exhibited an excellent capability of resisting the shock loading. The good effluent quality of system also proved the stabilization of bacterial function.

3.2.2. DGGE profiles of amoA gene communities (staining by EB)

The band patterns obtained by DGGE profiles of the *amoA* gene communities (Fig. 6) differed completely from that of DGGE profiles of all bacterial communities (Fig. 5). The band intensities of *amoA* DGGE profiles were generally less complex than DGGE profiles of all bacterial communities and increased with operation time.

In CAS system, more organic carbon compounds meant more electron donors to heterotrophs. They could grow quickly with these organic carbon compounds. At the same time, more DO was consumed. Thus the autotrophs like the ammonia-oxidizing bacteria (AOB) and the nitrite-oxidizing bacteria (NOB) were restricted during the struggle with heterotrophs for the scarceness of DO (Nogueira

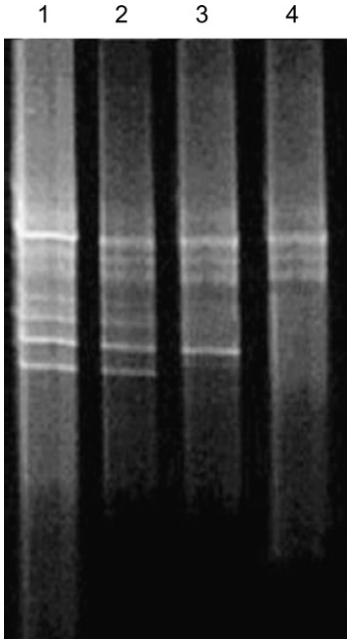

Fig. 6. DGGE band profiles of *amoA* gene fragments from mixed liquor samples in the MBR. The labels of 4, 3, 2, and 1 at the top of the lanes represent samples of inoculated activated sludge, 1st stage, 2nd stage and 3rd stage, respectively.

et al., 2002; Peng and Zhu, 2006). In MBR system, DO was superfluous and could not restrict them. The DGGE separation results of *amoA* gene fragments showed that more than 7 kinds of gene express forms (Fig. 6a 1st and 2nd lane) existed in the mature stage, which indicated the diversity of AOB. This phenomenon was also verified by the nitrification effects shown in Fig. 4a.

Shannon–Wiener index showed that the genus of bacteria in inoculated sludge were not abundant. (The indexes of the samples of inoculated activated sludge, 1st stage, 2nd stage and 3rd stage were 1.051, 1.393, 1.613 and 1.609, respectively). The generation time of nitrobacteria such as AOB and NOB were long (Xia et al., 2005a,b; Li et al., 2006), so the CAS could not make these bacteria enrichment. As seen in Fig. 6b, the index of every stage was gradually increased. That is to say, the nitrobacteria gradually increased due to the interception of membrane. What is more, ammonia in the wastewater was suitable for more genus of AOB.

3.2.3. Quantitative analysis of nitrobacteria (by FISH)

Microbial nitrification (oxidation of NH_4^+–NO_3^- via NO_2^-) was done by two groups of bacteria, AOB and NOB. The fractions of AOB and NOB in the MBR biomass were monitored by FISH with two probes (in Table 1). The probe of Nso190 was used to target at the β-proteobacteria AOB, and Nit3 probe was used to identify the presence and distribution of nitrobacteria like NOB in the system.

The population of nitrobacteria increased gradually with time. The average fractions of AOB and NOB at different stages accounted for 3.4%, 5.8%, 9.1%, 9.9%, and 2.6%, 3.5%, 4.7%, 5.2% of the total bacteria. In the biotreatment systems, the proportion of AOB and NOB was different (Xia et al., 2005a; Li et al., 2006), which depended on the system character and the operational conditions. In this system, the AOB and NOB could account for 9.9% and 5.2% of all organisms. Generally AOB could survive at elevated ammonia concentrations more than NOB because of its lower ammonia inhibition constants (Mota et al., 2005). The larger quantity of AOB than NOB was because that the ammonia was the major component of the total nitrogen of the wastewater.

FISH results showed there were a lot of nitrobacteria (interception of the membrane) in the system, which resulted in the high removal of ammonia and total nitrogen.

3.3. Fouling of membrane

TMP variation and SEM of membrane in different stages were measured. At the same time, the microbial community structures of the biofilm on membrane surface were also analyzed by FISH and DGGE.

3.3.1. TMP variation and SEM analysis

The membrane fouling of the MBR was demonstrated by an increase of TMP. The TMP increased from 5 kPa to 35 kPa during more than 60 days of operation. MBR was cleaned chemically by mixed solution of NaClO and NaOH (effective Cl was 3000 mg L^{-1}) at the 60th day.

On day 25 and day 37, the membrane modules were physically cleaned by removing the sludge cake deposited on the membrane surfaces. But the TMP still increased gradually with time, indicating occurrence of irreversible membrane fouling even after physical cleaning. After the chemical cleaning, the TMP dropped dramatically and the filtration capacity of the membrane was almost recovered fully, which indicated that the organic matters were removed by the chemical clean agent on the surface of membrane, as demonstrated in the SEM.

New hollow-fiber membrane surfaces were porous and free of particles in SEM image. SEM images of fouled membrane surfaces were taken before and after physical cleaning on day 37. Before physical cleaning, the membrane pores were not clear and some organics and microorganisms deposited on the membrane surfaces. After physical cleaning, the membrane pores were relatively visible though some microorganisms were still present on the membrane surface. It was microorganisms and organics that contributed to membrane biofouling. The organics were removed a lot but some microorganisms still lived actively on the membrane surfaces after physical cleaning, which indicated that some bacteria seemed to have piled to promote cell-to-cell interaction or adhesion to the membrane surfaces. As a result, the TMP did not drop abruptly after physical cleaning and rose again after several days.

On day 60, the chemical cleaning was performed and there were no activated microorganisms on the membrane

surface. After chemical cleaning, the chemical clean agent removed the primary fouling of the membrane and the microorganisms were killed. But the cleaned membrane was not recovered as the new membrane, because there were still organic pollutants (e.g. EPS) remaining as irreversible fouling.

3.3.2. Microbial community structures in the biofilm of membrane

Fig. 7 shows that the DGGE images of 16 s rDNA V3 region for the biofilm attached to the membrane surface in different days. The bacterial community structures of samples were similar. Three main kind of bands appeared regularly with the operation time. In the anaphase there were also several distinct bands, indicating that the biofilm on the membrane surface was more effective than before. Consequently the fouling of membrane became more serious as seen in the TMP variation. The three gene sequences from the bands were sequenced by Sagon Company (Shanghai, China) and were compared with the NCBI BLASTN system. The probable bacteria species were listed in Table 2.

At the same time, biofilm forming process on the membrane surface was also analyzed using FISH (EUB338) (The green radiation meant the amount of the microorganisms, and the radiation became brighter, the microorganism's vigor was higher). At the beginning of the operation, the green radiation was dark, which meant only a few bacteria attached to the membrane surface because of the continuous aeration. But gradually the preponderant bacteria accumulated on the membrane surface (the green radiation became brighter in the steady stage). At the prophase of the mature stage, the amount of preponderant bacteria that could adhere to the membrane was the largest, so the green radiation was the brightest. At the anaphase of the mature stage, the bacteria grew old and lacked vigor, thus the green radiation was not brighter than in the prophase of this stage.

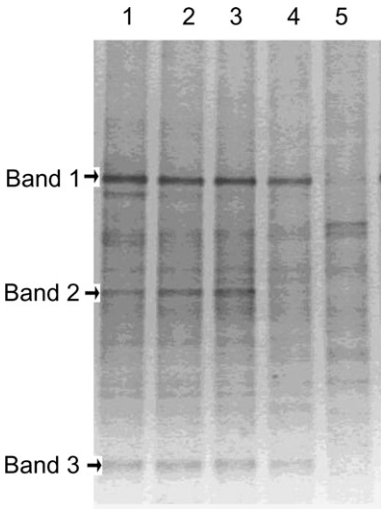

Fig. 7. DGGE band profiles of amplified 16S rDNA fragments from biofilm samples in the membrane of MBR. The labels of 5, 4, 3, 2, and 1 at the top of the lanes represent samples of activated sludge of beginning stage (2nd day), adapted stage (1st stage), steady stage (2nd stage), the prophase of the mature stage (3rd stage) and the anaphase of the mature stage (3rd stage), respectively.

From the FISH and 16S rDNA gene sequence analysis, it was concluded that there were several kinds of bacteria which could adhere to the surface of membrane to induce severe membrane biofouling. In this study, the bacteria species that played a major role in development of the mature biofilms were like to be *Pseudomonas* sp. *Ochrobactrum anthropi* sp. and *Enterobacter* sp. (Table 2).

4. Conclusions

Bathing wastewater was treated by a pilot-scale submerged membrane bioreactor (MBR) for more than 60 days.

The results of the bathing wastewater experiment indicated that the removal rates of main pollutants of wastewater such as COD_{Cr}, LAS, NH_4^+–N and TN were above 93%, 99%, 99% and 90%, respectively. PCR-DGGE of all bacterial communities in 16S rDNA V3 region of activated sludge in MBR showed that each sample exhibited distinct DGGE patterns. The influence of wastewater quality fluctuation on dominant species was negligible. The band patterns obtained by DGGE profiles of the *amoA* gene communities differed completely from that of DGGE profiles of all bacterial communities. The DGGE separation results of *amoA* gene fragments showed that more than seven kinds of gene express forms existed in the mature stage, indicating the diversity of AOB. FISH analysis of activated sludge in MBR indicated that the high removal rate of ammonia and TN was due to the abundant nitrobacteria intercepted by the membrane.

The observation (by SEM and FISH) confirmed that there were certain bacteria on the membrane surface which caused the biofouling of membrane. The bacteria species were like to be *Pseudomonas* sp. *O. anthropi sp*. and *Enterobacter* sp., respectively.

Table 2
Sequences length and closest phylogenetic affiliation of three bands in DGGE

Band	Sequences length	Phylogenetic relationship		
		Species	Accession no.	Similarity
1	154	*Pseudomonas* sp.	Query: lcl| 4903Request ID: CR5JCXUN016	152/154 (98%)
2	166	*Ochrobactrum anthropi* sp.	Query: lcl|8746Request ID: CP7B3ZRE012	166/166 (100%)
3	169	*Enterobacter* sp.	Query: lcl|22211Request ID: CR612EDG01R	168/169 (99%)

Acknowledgements

This study was financially supported by the New Century Excellent Talent Scholarship of China (NCET-05-0387), the Doctorial Unit Scholarship of China (20050247016) and the Sino-Danish Scientific Cooperation Program under grant number S14NNP28.

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