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Biological treatment of TMAH (tetra-methyl ammonium hydroxide) in a full-scale TFT-LCD wastewater treatment plant

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ABSTRACT

This study evaluated biological treatment of TMAH in a full-scale methanogenic up-flow anaerobic sludge blanket (UASB) followed by an aerobic bioreactor. In general, the UASB was able to perform a satisfactory TMAH degradation efficiency, but the effluent COD of the aerobic bioreactor seemed to increase with an increased TMAH in the influent wastewater. The batch test results confirmed that the UASB sludge under methanogenic conditions would be favored over the aerobic ones for TMAH treatment due to its superb ability of handling high strength of TMAH-containing wastewaters. Based on batch experiments, inhibitory chemicals present in TFT-LCD wastewater like surfactants and sulfate should be avoided to secure a stable methanogenic TMAH degradation. Finally, molecular monitoring of *Methanomethylovorans hollandica* and *Methanosarcina mazei* in the full-scale plant, the dominant methanogens in the UASB responsible for TMAH degradation, may be beneficial for a stable TMAH treatment performance.

1. Introduction

The amount of pollutants produced during manufacturing processes of TFT-LCD (thin-film transistor liquid crystal display) substantially increases due to an increasing production of the opto-electronic industry in Taiwan. Organic solvents used in TFT-LCD manufacturing processes account for more than 33% of the total organic wastes present in wastewater. The main components of these organic solvents are composed of the stripper (dimethyl sulfoxide (DMSO) and monoethanolamine (MEA)), developer (tetra-methyl ammonium hydroxide (TMAH)) and chelating agents. These compounds are recognized as slow-biodegradable organic compounds and very few information is available regarding their biological treatability (Chang et al., 2008; Park et al., 2001). In a previous study (Lei et al., 2010), it was found that MEA can be treated without difficulties under aerobic, anoxic, and anaerobic conditions, while a higher DMSO degradation rate can be achieved under anaerobic condition with the presence of external electron donors.

TMAH, a toxic and corrosive alkaline chemical, is widely used in the semiconductor and opto-electronic industries as developer (Hirano et al., 2001). It is a highly toxic compound and caused a few deaths in Taiwan (Wu et al., 2008). One way to treat this kind of wastewater is oxidation treatment. Hirano et al. (2001) developed a process which combined pyrolysis and catalytic oxidation to treat TMAH wastewater. Although this method avoids the production of noxious compounds like NO_x or NH_3, the cost of this method is still much higher than the biological ones and makes it difficult to be employed in full scale.

The treatment of TMAH-containing wastewaters using activated sludge treatment is usually difficult (Urakami et al., 1990). However, this can be achieved by some methylotroph and some *Paracoccus* spp., *Kluyveromyces delphensis, Bacillus circus, Acinetobacter* sp. (Anthony, 1982; Urakami et al., 1990). According to the proposed pathway for aerobic TMAH degradation, tetra-methyl ammonium ion is first degraded to trimethylamime, and then to dimethylamine, methylamine and finally to ammonia. Formaldehyde is produced in each of the reactions and can be quickly oxidized to carbon dioxide and water under aerobic conditions. In addition to aerobic conditions, methanogenic degradation of TMAH has been reported (Tanaka, 1994). Methanogens are strictly anaerobic archaea capable of producing methane as the end-product during anaerobic conversion of organic compounds (Ferry, 1993). An isolated methanogen has been grown on TMAH and degrade TMAH to methane gas and ammonium (Tanaka, 1994). In a recent study, Chang et al. (2008) successfully enriched

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anaerobic TMAH-degrading sludge under methanogenic conditions using a lab-scale up-flow anaerobic sludge blanket (UASB) fed with TMAH-containing wastewater. Their results indicated that the toxicity effects of TMAH up to 10,000 mg/L on enriched UASB sludge was negligible (Chang et al., 2008). Although anaerobic processes involving diverse microbes for industrial wastewater treatment have been successfully applied in full-scale for decades, their application to TMAH treatment at full-scale has not been evaluated. Furthermore, information for microbial community of methanogenic TMAH treatment processes is also quite limited at this time.

In this study, biological treatment of TMAH in a full-scale methanogenic UASB bioreactor followed by an aerobic bioreactor was evaluated. Batch experiments were conducted to evaluate aerobic and methanogenic TMAH biodegradation kinetics, and possible adverse effects on methnogenic TMAH degradation caused by potential inhibitors present in TFT-LCD wastewater. Finally, microbial community of methanogenic TMAH treatment processes was also evaluated using molecular methods.

2. Methods

2.1. Description of full-scale wastewater treatment bioreactors

The full-scale bioprocess for TMAH-containing wastewater treatment investigated in this study included an UASB bioreactor followed by a conventional activated sludge (CAS) system, as shown in Fig. 1. The influent wastewater of the UASB contained mainly TMAH, some domestic wastewater, and some other chemicals such as surfactants used during TFT-LCD manufacturing. The capacity of the UASB was about 1000 m^3/d and the hydraulic retention time (HRT) was 15 h. The influent and effluent TMAH concentrations of UASB were averaged around 1200 and 100 mg/L, respectively. During this study, the full-scale UASB was able to perform up to 92% of TMAH degradation efficiency and achieve a more than 90% in gas composition for methane. The CAS bioreactor with a total volume of 1000 m^3 treated 1000 m^3/d of wastewater effluent from the UASB. The CAS bioreactor, with an average biomass concentration of 2000 mg VSS/L, was operated at hydraulic retention time (HRT) and solids retention time (SRT) of 1 and 20 days, respectively. The influent COD of the CAS bioreactor varied between 400 and 1000 mg/L, depending on the TMAH removal efficiency of the UASB. Based on determined average TMAH loading of the UASB, two phases were defined in this study. In Phase I, the average influent TMAH was 1528 mg/L, while the average influent TMAH in Phase II was 1144 mg/L.

2.2. Batch experiments for TMAH biodegradation

A series of batch tests were performed in order to study biodegradation of TMAH under aerobic and methanogenic conditions. For aerobic batch tests, sludge was taken from the CAS bioreactor, while for methanogenic batch tests sludge was taken from the UASB. For the aerobic batch experiment, 800 mL of examined mixed-liquor-suspended solids (MLSS) were centrifuged at 10,000g for 10 min. The supernatant was discarded, and the solids were resuspended in a 1 L flask containing 800 mL of the nutrient medium with composition same as that previously described (Lei et al., 2010). The initial biomass concentrations in batch experiments were controlled at around 2000 mg/L. During the experiment, the MLSS in the flask was mixed using a magnetic stirrer and aerated for aerobic condition experiment to maintain the DO concentration above 3 mg/L. The pH of the batch reactor was controlled with a pH controller at 7 ± 0.1 by addition of 0.1 MHCl or 0.1 MNaOH. The batch tests were carried out in an incubator maintained at 27 ± 2 °C. Samples were frequently taken throughout the batch experiments for the determination of TMAH.

Methanogenic batch experiments were conducted to evaluate the effects of TMAH concentration and potential inhibitory chemicals present in TFT-LCD wastewater on TMAH degradation activity of the UASB sludge. Batch tests were conducted in 1L serum bottles sealed with a rubber stopper. The total liquid volume was 400 mL. UASB sludge and predetermined concentrations of TMAH (1000 mg/L for inhibitor batch tests) and potential inhibitors such as sulfate, surfactants, and DMSO were mixed using a shaker at 150 rpm and incubated at 35 ± 1 °C. The pH was frequently monitored and maintained at 7.0 ± 0.2 during experiments. Batches without inhibitors were conducted as control experiments, demonstrating TMAH degradation performance of UASB sludge. For each experiment, duplicated batches were conducted and samples were taken frequently for analyses.

2.3. Analytical methods

The composition of biogas in the headspace was analyzed using a gas chromatograph (China GC 8900, Taipei, Taiwan) equipped with a thermal conductivity detector (TCD). A 2 m stainless column packed with Hayesep Q (60/80 mesh) was installed in a 60 °C oven. The operational temperatures of the injection port, the oven, and the detector were all set at 60 °C. Nitrogen was used as the carrier gas at a flow rate of 15 mL/min. The concentration of TMAH was analyzed by an ion chromatography (IC) (Mrklas et al., 2003). The IC used was ICS-1000 (Dionex, California, USA) equipped with a IonPacCG-18 column as the guard and cation analytical column, a CSRS 3002-mm self-regenerated suppressor, and a conductivity detector. A 3 mM of methanesulfonic acid was used as the eluent at a flow rate of 0.25 mL/min. The pH, ORP, COD and volatile suspended solids (VSS) were measured according to standard methods (APHA, 1995).

2.4. Genomic DNA extraction and polymerase chain reaction (PCR) amplification

The UltraClean Soil DNA Isolation kit (Mo Bio Laboratories, Solana Beach, CA) was used to obtain genomic DNA from the batch

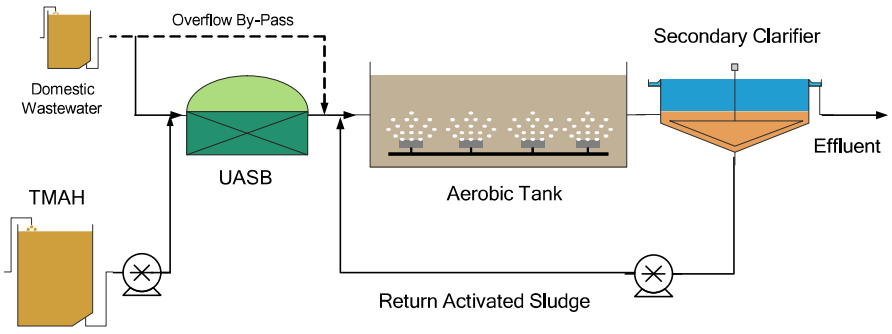

Fig. 1. Schematic of the full-scale bioprocess for TMAH-containing wastewater treatment.

sludge. A specific primer set including a forward primer mlas (5′-GGTGGTGTMGGGDTTCACMCARTA-3′) and a reverse primer mcrA-rev (5′-CGTTCATBGCGTAGTTVGGRTAGT-3′) was used for amplifying a 480 base pair (bp) fragment of *mcrA* functional gene of methanogen (Steinberg and Regan, 2008). The *mcrA* gene, encoding the alpha subunit of methyl coenzyme M reductase, is thought to be highly conserved and specific for methanogens (Ferry, 1993).Expression of the *mcrA* gene has been used as an indicator of the methanogenic activity of the methanogen population as this enzyme catalyzes the terminal step in the pathway for methanogenesis. Furthermore, the mcrA gene was also used as a phylogenetic marker to enumerate the methanogen population (Steinberg and Regan, 2008). Taq DNA Polymerase Master Mix RED (Ampliqon, Copenhagen, Denmark) was used as the taq of PCR reaction. The thermal profile used for the amplification was: an initial denaturation at 95 °C for 3 min, 5 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 45 s, and extension at 72 °C for 30 s with a ramp rate of 0.1 °C/s from the annealing to extension temperature, 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 30 s followed by a final extension at 72 °C for 10 min.

2.5. Terminal restriction fragment length polymorphism (T-RFLP) analysis

To perform the T-RFLP analysis for the mcrA functional gene of methanogen, the forward primer mlas was labeled at the 5′ end with 6-hexachlorofluorescein (6-HEX) and the thermal condition was same as the PCR condition described in the previous section. Purified PCR products were digested with FatI restriction endonuclease for 2 h and digestion temperature was 37 °C. Digested samples were analyzed by capillary electrophoresis at the Nucleic Acid Analysis and Synthesis Core Laboratory of the National Cheng Kung University in Tainan, Taiwan, to determine the size of fragments using an ABI Prism 377 automated sequencer (Perkin-Elmer Corp., Wellesley, MA, USA). The relative abundance of each T-RF was determined by calculating the ratio between the height of each peak and the summed height from all peaks in one sample. The peaks with relative abundance with <1% or smaller T-RF length (<50 bp) were neglected in this study, due to high background noise.

2.6. Clone library construction and analysis

PCR products of unlabeled primer set of mlas with mcrA-rev were purified by electrophoresis in a 1.5% (wt/vol) agarose gel, recovered using a gel purification kit (Gene-Spin TM 1-4-3 DNA extraction kit), and used in the construction of the mcrA functional gene clone libraries. The purified PCR products were ligated into the pGEM-T Easy Vector System (Promega, Madison, WI, USA) and transformed into DH5a *Escherichia coli* competent cells following the manufacture's protocol (Invitrogen).Clones were randomly selected and re-amplified with mlas and mcrA-rev primer set. PCR products of the clones were digested with FatI restriction endonuclease to find the corresponding to each observed T-RFLP results. The resulting fragments were analyzed on ethidium bromide stained agarose gels. Selected clones were incubated with nutrient broth in 10 mL tube one day. The tubes were centrifuged at 10,000g for 1 min and kept the cells with discarding the supernatant. Plasmids of clone cells were extracted by Wizard^{R} Plus minipreps DNA purification system (Promega, Madison, WI,USA). DNA sequencing reactions were performed using ABI3100 and 3730 capillary sequencers (Applied Biosystems) and sequencing was performed at the National Cheng Kung University in Tainan, Taiwan. Sequences of the clones were searched on the NCBI GenBank database for homologous sequences and their identity using the BLAST.

3. Results and discussion

3.1. Full-scale bioprocess performance for treatment of TMAH-containing wastewater

The influent TMAH and effluent COD concentrations of the full-scale bioprocess during this study are summarized in the Fig. 2. In general, the UASB was able to perform a satisfactory TMAH degradation efficiency, but the effluent COD of the aerobic bioreactor seemed to increase with an increased TMAH in the influent wastewater. In Phase I, the average effluent COD of 388 mg/L corresponded to an average influent TMAH of 1528 mg/L, while in Phase II the average effluent COD of 302 mg/L corresponded to an average influent TMAH of 1144 mg/L. In order to achieve a stable water quality of the treated effluent, better understanding of the treatment capacity of the individual bioreactors is necessary, and thus optimization of the full-scale bioprocess can be attained.

3.2. TMAH biodegradation under aerobic and methanogenic conditions

The results of specific TMAH degradation rates observed at different TMAH concentrations under aerobic and methanogenic conditions are presented in Fig. 3. As shown in Fig. 3, the aerobic sludge was able to degrade TMAH with a maximum specific

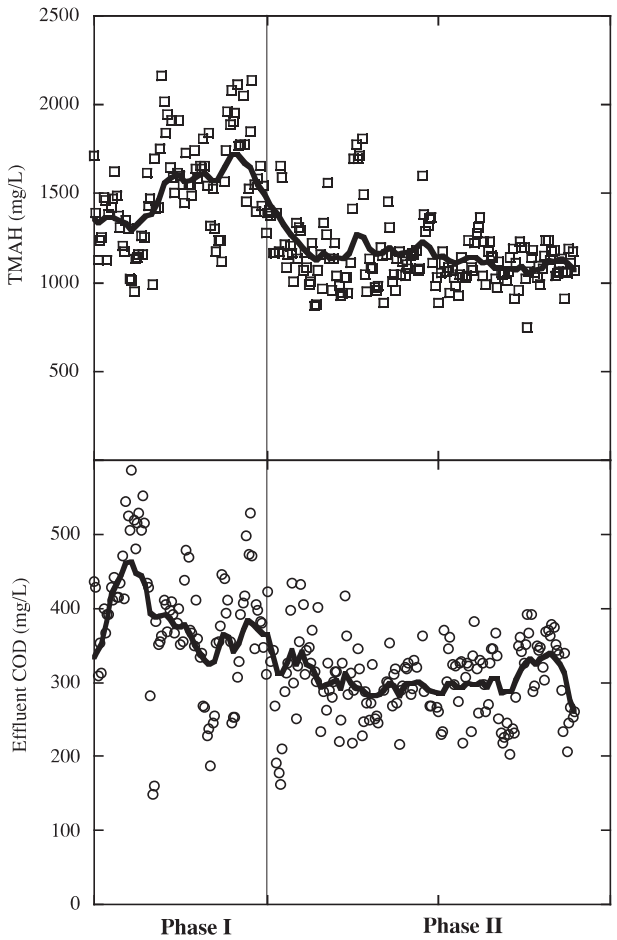

Fig. 2. Influent TMAH (upper) and effluent COD (bottom) concentrations of the full-scale bioprocess. The lines represent moving average of measured data.

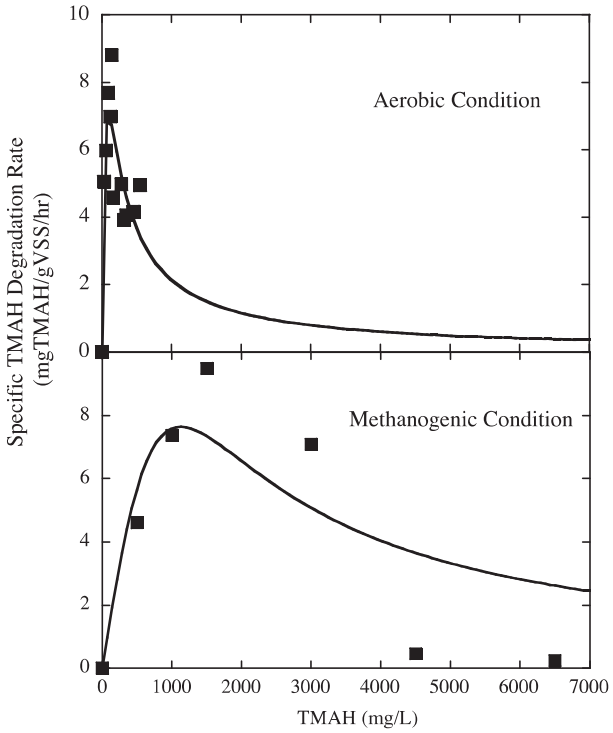

Fig. 3. Specific TMAH degradation rates observed at different TMAH concentrations under aerobic (upper) and methanogenic (bottom) conditions. The lines represent regression curves following Haldane-type kinetic expression.

degradation of 8.8 mg TMAH/gVSS/h at initial TMAH of 145 mg/L, but inhibition on the specific TMAH degradation rate occurred at TMAH concentrations higher than 150 mg/L. Furthermore, complete inhibition with respect to TMAH degradation was observed in the beginning of batch experiments with TMAH concentrations higher than 450 mg/L, although the aerobic sludge could gradually degrade TMAH after 5 h of inhibition. Biological treatment of TMAH-containing wastewater is usually difficult in activated sludge systems (Urakami et al., 1990), but complete TMAH degradation can be possible by methylotrophs (Hampton and Zatman, 1973; Anthony, 1982) and *Paracoccus* spp. (Ohara et al., 1990). Lei et al. (2010) have confirmed that aerobic degradation of TMAH could be achieved in aerobic and anoxic–oxic sequencing batch reactors, but aerobic conditions were more effective compared to anoxic ones. By using the acclimated aerobic sludge, a maximum specific degradation rate of 8.1 mg TMAH/gVSS/h was obtained in Lei et al. (2010) without significant inhibitory effect up to 300 mg/L of TMAH. It is suggested that acclimation of aerobic TMAH-degrading community is necessary to reduce a sudden inhibition at an unexpectedly high TMAH concentration.

Under methanogenic conditions, the UASB sludge was able to attain a specific degradation rate of 9.5 mg TMAH/gVSS/h despite that the initial TMAH concentration was as high as 1500 mg/L. Additionally, the specific degradation rate of the UASB sludge only slightly decreased to 7.1 mg TMAH/gVSS/hat the initial TMAH concentration as high as 3000 mg/L. Therefore, the UASB sludge under methanogenic conditions would be favored over the aerobic ones for TMAH treatment due to its potential of handling a higher strength of TMAH-containing wastewaters. Indeed, previous study (Chang et al., 2008) demonstrated that anaerobic TMAH-degrading sludge enriched in a lab-scale UASB was able to perform more than 95% of TMAH removal efficiency at volumetric loadings around 7.30 kg TMAH/m^3/day. However, at the initial TMAH concentration >4500 mg/L, the specific TMAH degradation rate of the investigated full-scale UASB sludge dramatically decreased to less than 0.5 mg TMAH/gVSS/h, due presumably to an inhibition on the UASB sludge at a high TMAH concentration. Accordingly, the UASB sludge under methanogenic conditions seemed to be promising for treatment of high strength TMAH-containing wastewaters, but a maximum concentration allowed for methanogenic TMAH biodegradation should be determined using the full-scale sludge. Furthermore, in addition to the high TMAH concentration, unexpected sources having a negative impact on methanogenic TMAH degradation also need to be clarified in order to avoid an unstable water quality of the treated effluent as depicted in Fig. 2.

3.3. Effects of potential inhibitors on methanogenic THAM biodegradation

Batch experiments were conducted to evaluate potential inhibition that might encounter during methanogenic TMAH degradation in the full-scale UASB bioreactor. In this study, a modified Gompertz equation (Fang et al., 2007) was employed to calculate the kinetic parameters of the TMAH biodegradation under methanogenic condition. The equation could be expressed as follows:

$$S = S_0 - A\exp\{-\exp[R_m * e * (\lambda - t)/A + 1]\}$$

where S_0 and S were the TMAH concentrations at time 0 and t during the experiment, A the degradation potential, R_m the potential maximum degradation rate, and λ the potential lag phase. The results of best-fit kinetic parameters for methanogenic batch experiments are summarized in Table 1.

Compared to the batch without potential inhibitor present, addition of Surfactant 1 (2000–10,000 mg/L) caused no apparent lag period for TMAH degradation but a slight decrease in specific TMAH degradation rate, while addition of Surfactant 2 (4000–20,000 mg/L) caused a slight increase of lag period but a noticeable decrease in specific TMAH degradation rate. For the batches added with either ST801 (2000–10,000 mg/L) or ST823 (5000–20,000 mg/L), considerable inhibition on TMAH biodegradation was observed, resulting in a longer lag period and a lower specific TMAH degradation rate. These surfactants were occasionally used in TFT-LCD manufacturing process and can be present in TFT-LCD wastewater. It is suggested that the presence of these surfactants, especially ST801 and ST823, at concentrations higher than 1000 mg/L should be avoided to secure stable methanogenic TMAH degradation.

In the presence of sulfate, two sets of batch experiments, Sulfates I and II, were conducted using the UASB sludges taken during the Phases I and II, respectively. For the Sulfate I batches, addition of sulfate (150–1500 mg/L) caused a substantial lag period and significantly reduced specific TMAH degradation rate, especially at concentrations >300 mg/L. For the Sulfate II batches, inhibition on TMAH biodegradation caused by sulfate addition (150–1500 mg/L), however, was much less severe. The clear mechanism for inhibitory effect of sulfate on methanogenic TMAH degradation is not clear at this time, but its possible toxic effects on methanogenic activity have been examined (Lin et al., 2001) and reviewed previously (Chen et al., 2008). In anaerobic bioreactors with the presence of suitable organic substrates, sulfate can be reduced to sulfide by the sulfate reducing bacteria (SRB), resulting in two possibilities of inhibition during sulfate reduction. Primary inhibition is due to competition for common organic and inorganic substrates by the SRB, which reduces methane production (Harada et al., 1994). Secondary inhibition results from the toxicity of produced sulfide from sulfate reduction to various microbial groups in anaerobic bioreactors including acidogens, acetogens, and methanogens (Anderson et al., 1982; Oude Elferink et al., 1994; Colleran et al., 1995, 1998).In the current study, the observed different inhibitory effects of sulfate on TMAH biodegradation are likely due to the difference in microbial community

Table 1
Summary of methanogenic TMAH degradation experiments with addition of different potential inhibitors.

Inhibitor	Concentration (mg/L)	q (mg-TMAH/gVSS/h)	λ (h)	R_m (mg-TMAH/h)	A (mg/L)	R^2
ST801	0	16.5	0.5	250	894	0.995
	2000	7.28	2.0	107	924	0.931
	4000	3.41	2.8	74.3	805	0.925
	8000	3.00	7.5	79.7	752	0.978
	10,000	1.45	12.9	10.1	891	0.978
ST823	0	17.0	1.0	267	818	1.00
	5000	5.97	9.5	112	1277	0.970
	8000	2.69	10.3	61.6	1100	0.996
	10,000	2.55	8.6	48.3	1160	0.969
	20,000	1.75	10.2	57.7	1099	0.948
Surfactant 1	0	9.04	2.1	283	879	0.993
	2000	7.38	1.9	294	715	1.00
	5000	7.00	1.2	284	779	0.999
	10,000	7.93	2.1	252	875	0.988
Surfactant 2	4000	6.50	2.5	201	870	0.999
	10,000	4.21	3.7	150	910	0.995
	20,000	2.31	2.9	65.4	972	0.998
Sulfate I	0	6.88	5.4	762	1002	0.967
	150	2.78	10.5	355	876	0.984
	300	0.44	42	23.6	1237	0.946
	660	0.51	22	15.8	1296	0.944
	1000	0.22	16.6	8.0	1169	0.886
	1500	0.21	21.1	8.1	1141	0.905
Sulfate II	0	9.43	3.4	173	1113	0.989
	150	9.21	3.3	157	1201	0.987
	300	9.15	3.1	149	1188	0.994
	600	7.63	10.2	385	1652	0.980
	1500	7.75	10.2	385	1680	0.986
DMSO	0	7.72	1.2	178	794	0.991
	100	6.57	1.4	192	762	0.990
	240	6.20	3.3	477	744	0.994
	500	6.05	2.8	281	747	0.999
	750	5.52	2.9	275	713	0.992
	1000	5.77	2.9	291	730	0.980

structures of SRB or methanogens present in the Phases I and II, respectively. It is speculated that a lower SRB population in the Phase II sludge produced less sulfide in the Sulfate II batch experiments, resulting in a lower inhibition on TMAH biodegradation.

3.4. Microbial community structures of methanogens in UASB sludge

Cloning and sequencing of functional gene, *mcrA*, were performed to investigate methanogen community diversity of the UASB sludge. The results of sequence analysis with the neighbor-joining phylogenetic tree, as shown in Fig. 4, suggests that *Methanosarcina*, *Methanomethylovorans*, and unclutured methanogens were the major methanogenic populations found in the UASB bioreactor. Out of the 73 clones retrieved from the year 2007 sample (Phase I), 20 of them were phylogenetically related to *Methanosarcinamazei* (27%) and 17 were related to *Methanomethylovorans hollandica* (23%), leaving the rest of 36 clones related to Methanogenic archaeon DCM1 (GQ339873) (49%), one clone sequence of uncultured Methanomicrobiales. The diversity and distribution of methanogens in the year 2010 sample (Phase II) were similar to those of the year 2007 sample, except for the appearance of *Methanosaeta concilii*.

The methanogenic population dynamics of the UASB was evaluated with the *mcrA*-based T-RFLP as shown in Fig. 5.The T-RFLP profiles of the year 2007 and 2008 samples were dominated by the T-RFs at 281, 330, 356, and 484 bp, while the T-RFs at276, 468, and 484 bp became dominant in the year 2010 samples. According to the expected T-RF signatures predicted by the results of retrieved sequences shown in Fig. 4, the T-RFLP results suggest that *Methanosarcina mazei* (281 bp), *Methanomethylovorans hollandica* (330, 356, and 484 bp) were the major methanogen populations in the UASB during 2007 to 2008, while in the 2010 samples *Methanosarcina mazei* (276 bp), *Methanolobus zinderi* (468 bp), and *Methanomethylovorans hollandica* (484 bp) became dominant.

Methanosarcina species including *Methanosarcina mazei* have been reported for their ability of utilizing methylated amines such as trimethylamine, dimethylamine, and methylamine as carbon source for growth (Ferry, 1993). In addition, *Methanomethylovorans hollandica*, isolated from freshwater sediments, was capable of utilizing methylamines as the carbon source for growth (Lomans et al., 1999; Simankova et al., 2003). It is likely that the high concentration of TMAH-containing wastewater enriched *Methanomethylovorans hollandica* and *Methanosarcina mazei* in the full-scale UASB investigated in this study. On the other hand, their occurrence and dominance in the UASB may be important to maintaining a stable TMAH biodegradation performance of the UASB.

3.5. Engineering significance

For treatment of TMAH-containing wastewaters, biological processes are feasible as demonstrated with the full-scale plant presented in this study. Although the biological process can be an economical option for TMAH treatment, optimization in operation is necessary for these bioprocesses in order to reach a stable performance. For the aerobic bioprocess like the CAS, complete TMAH biodegradation can be achieved but an apparent limitation on the maximum TMAH concentration allowed does exist. By examining the full-scale CAS sludge with biomass concentration of 2000 mg/L, reduction of the specific TMAH degradation occurred at the TMAH concentration higher than 150 mg/L, although the

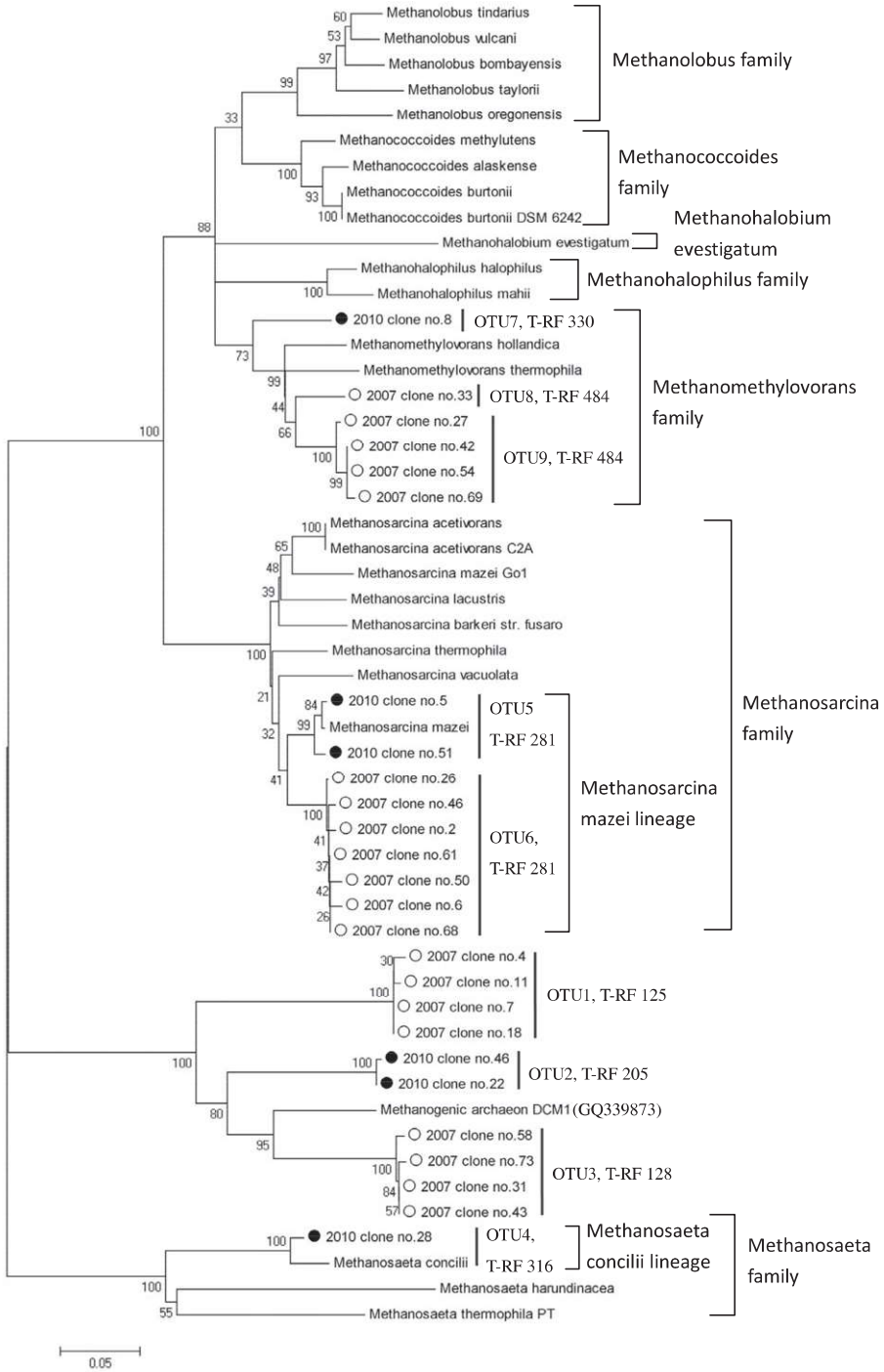

Fig. 4. Neighbor-joining tree based on *mcrA* gene sequences. Unrooted phylogenetic tree was generated using the neighbor-joining method and bootstrap tests were performed with 1000 replicates. The scale bar represents 5% sequence divergence. Symbol: 2007 sample, ○; 2010 sample, ●.

aerobic sludge could gradually degrade TMAH after several hours of inhibition. With an acclimated aerobic sludge (Lei et al., 2010), the inhibitory effect of a higher TMAH concentration (up to 300 mg/L) may be reduced, but the aerobic bioprocess is not recommended for a high strength wastewater containing TMAH more than 1000 mg/L. With the UASB bioprocess, the methanogenic community was able to achieve a stable TMAH removal efficiency even at an influent TMAH concentration of 1000 mg/L. Although the acclimated methanogenic community is capable of treating a higher concentration of TMAH-containing wastewater, a steady feed with a gradual increase in TMAH loading is recommended for the methanogenic sludge since the methanogens are slow growing microorganisms. Additionally, although the detailed mechanisms are not clear at this time, inhibitory chemicals such as surfactants and sulfate should be avoided in the methanogenic process in order to maintain its TMAH treatment performance. On the other hand, granulation of methanogenic consortium is important to the stable operation of anaerobic high rate biological systems, which are closely related to nutritional and environmental factors such as trace metal ions, temperature, seed sludge, wastewater characteristics, carriers, and hydraulic properties of the reactors environment (Khanh et al., 2011; Rajakumar et al.,

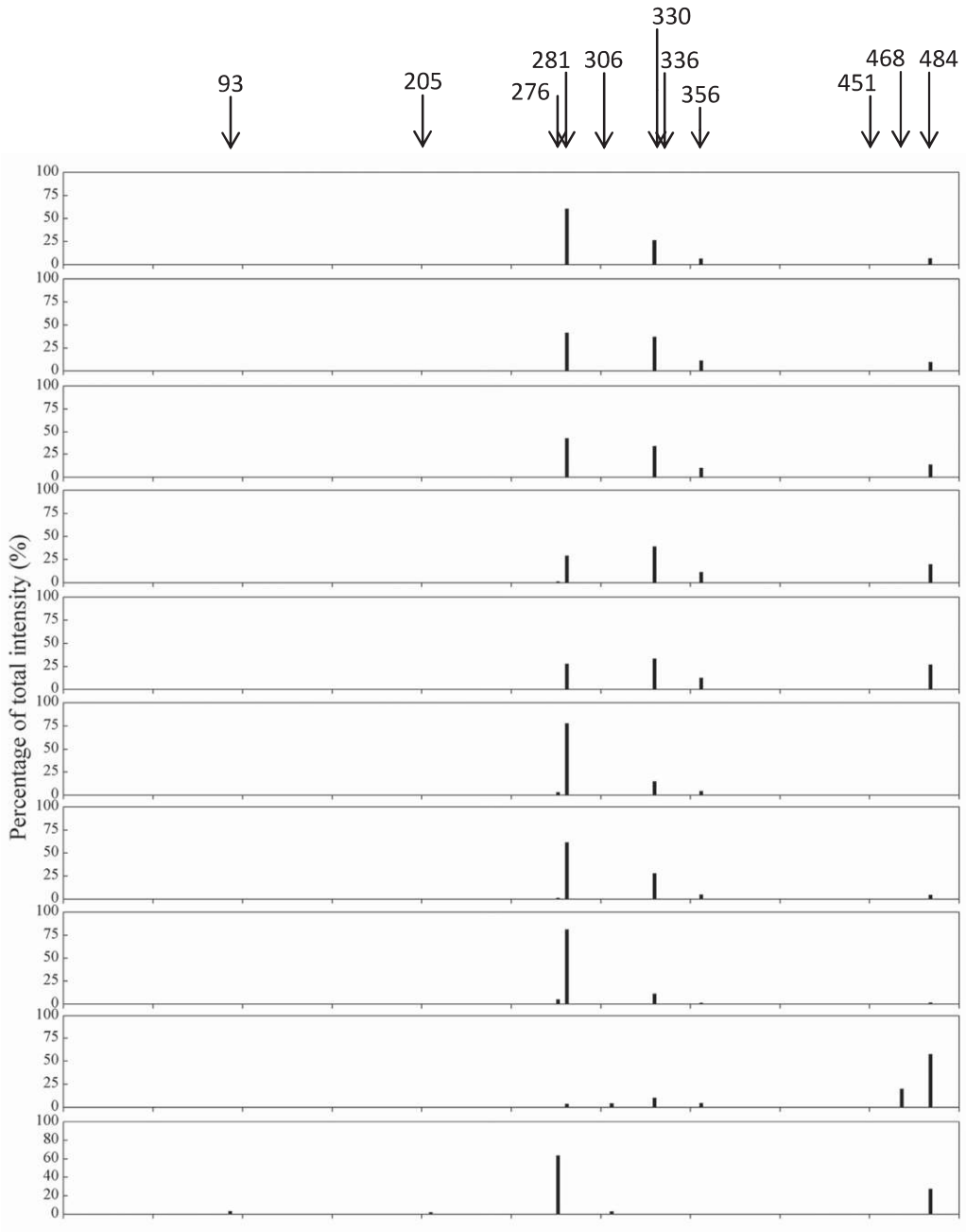

Fig. 5. *mcrA*-based T-RFLP results for the methanogenic population dynamics in the UASB.

2012). The formation and stability of granules in the full-scale UASB treating TMAH can also be alternatives to successful operation, and this warrants future investigations.

4. Conclusions

By investigating a full-scale wastewater treatment plant, this study demonstrates that the UASB sludge under methanogenic conditions would be favored over the aerobic ones for TMAH treatment due to its superb ability of handling high strength of TMAH-containing wastewaters. Inhibitory chemicals present in TFT-LCD wastewater such as surfactants and sulfate should be avoided to secure stable methanogenic TMAH degradation. Regular monitoring on *Methanomethylovorans hollandica* and *Methanosarcina mazei*, the dominant methanogens in the UASB responsible for TMAH degradation, may be beneficial for maintaining a stable TMAH treatment performance.

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