Chemosphere 258 (2020) 127146

Contents lists available at ScienceDirect

Chemosphere

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

Aerobic degradation of high tetramethylammonium hydroxide (TMAH) and its impacts on nitrification and microbial community

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HIGHLIGHTS

- Aerobic TMAH degradation achieved with high TMAH concentration up to 6854 mg L^{-1}.
- Aerobic specific TMAH degradation rates followed Monod-type kinetics.
- Lag time for the onset of nitrification highly correlated with initial TMAH.
- Potential aerobic TMAH-degraders includes *Mycobacterium* sp. and *Hypomicrobium* sp.
- TMAH may inhibit nitrification by inactive expression of *amo*A gene.

ARTICLE INFO

Article history:
Received 29 February 2020
Received in revised form
15 May 2020
Accepted 18 May 2020
Available online 23 May 2020

Handling Editor: Veeriah (Jega) Jegatheesan

Keywords:
tmm gene
Messenger ribonucleic acid (mRNA)
Nitrification
Inhibition

ABSTRACT

Tetramethylammonium hydroxide (TMAH) was often used as developer in the high-tech industries. Information regarding biological treatment of high TMAH-containing wastewater is limited. This study investigated aerobic degradation of high TMAH, its impacts on nitrification, and microbial community in a sequencing batch reactor (SBR). The initial TMAH concentrations of SBR gradually increased from 200 to 4666 mg L^{-1} (equivalent to 31 to 718 mg-N L^{-1}) to enrich microbial community for aerobic TMAH degradation and nitrification. The results indicated that the aerobic specific TMAH degradation rates followed the Monod-type kinetics with a maximum specific TMAH degradation rate of 2.184 mg N $hour^{-1}$ g volatile suspended solid $(VSS)^{-1}$ and the half-saturation coefficient of 175.1 mg N L^{-1}. After TMAH degradation and ammonia release, the lag time for the onset of nitrification highly correlated with initial TMAH fed for the SBR. According to the microbial community analysis using next generation sequencing (NGS), potential aerobic TMAH-degraders including *Mycobacterium* sp. and *Hypomicrobium* sp. were enriched in the aerobic SBR. The results of real-time quantitative polymerase chain reaction (qPCR) and reverse transcript (RT)-qPCR indicated that *Hyphomicrobium* sp. may be able to utilize both TMAH and its degradation intermediates such as trimethylamine (TMA), while *Thiobacillus* sp. can only utilize TMAH. The qPCR and RT-qPCR results suggested that TMAH may inhibit nitrification by inactive expression of *amo*A gene and the intermediates of TMAH degradation may compete ammonia monooxygenase (AMO) enzyme with ammonia for nitrification inhibition.

1. Introduction

High-tech industries, including semiconductor and thin-film transistor liquid crystal display (TFT-LCD), grow rapidly and become important industries in Taiwan. During the manufacturing processes, huge amount of organic solvents was used and generated organic wastewater. Tetramethylammonium hydroxide (TMAH), an often used organic solvent as developer in the high-tech industries, is corrosive and toxic to human health. High concentrations of TMAH-containing wastewater can be treated using physical/chemical processes such as catalytic oxidation, ion exchange, or reverse osmosis, but implementation of these treatment technologies is generally very costly (Hirano et al., 2000; Shibata et al., 2006; Yang et al., 2015). Therefore, biological processes were considered most cost-effective for TMAH-containing wastewater treatment.

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https://doi.org/10.1016/j.chemosphere.2020.127146
0045-6535/

Biodegradation of TMAH has been evaluated under aerobic, anaerobic, and anoxic condition (Chang et al., 2008; Lei et al., 2008). Up to 10,000 mg L^{-1} of TMAH was treated in a lab-scale up-flow anaerobic sludge blanket (UASB) system with biogas production, but high concentration of ammonia was also found in the effluent (Chang et al., 2008). Methanogenic treatment of TMAH-containing wastewater in full-scale UASB (Hu et al., 2012; Whang et al., 2015) and the methanogens responsible for TMAH degradation have been evaluated (Whang et al., 2015; Hu et al., 2012). Other studies have shown aerobic systems, including aerobic sequencing batch reactor (SBR) and membrane reactor (MBR) systems, can be used for treating lower TMAH (200–330 mg L^{-1}) and ammonia released from TMAH degradation, which cannot be achieved in anaerobic systems (Lei et al., 2008; Wu et al., 2008). Furthermore, some aerobic microorganisms from soil and activated sludge have been found degrading and utilizing TMAH as carbon source for growth, such as *Pseudomonas aminovorans*, *Nocardia* sp., *Mycobacterium* sp., *Paracoccus kocurii*, *Mcobacterium* sp., *Hypomicrobium* sp., and *Pseudomonas* strains. (Anthony, 1982; Ghisalba et al., 1985; Ohara et al., 1990; Urakami et al., 1990; Tanaka, 1994). *Mcobacterium* sp. could grow on 8 wt% of TMAH, while *Paracoccus kocurii* could utilize 10 mM of TMAH, trimethylamine (TMA), dimethylamine (DMA), and mono methylamine (MMA) (Urakami et al., 1990; Tanaka, 1994).

TMAH is a methylated amine compound and methylated amines can be found frequently in marine environments as trimethylamine oxide and in soil environments as degradation products of pesticide (Anthony, 1982). Aerobic TMAH degradation has a similar degradation pathway with other methylated organic compounds, in which the methyl groups from methylated amines are oxidized to formaldehyde, then latter the formaldehyde can be assimilated (Lidstrom, 2006). The proposed pathway for aerobic TMAH degradation is presented in Fig. 1 that TMAH is first degraded to TMA through demethylation pathway by tetramethylammonium monooxygenase (Anthony, 1982). Then TMA is further oxidized to DMA and formaldehyde. TMA oxidation can be divided into two different pathways: first oxidation catalysed by trimethylamine dehydrogenase leads to direct oxidation of TMA to DMA. The second oxidation catalysed by trimethylamine monooxygenase (encoded by tmm gene) is firstly oxidizing trimethylamine to trimethylamine N-oxide (Chen, 2012), and then subsequently degraded to DMA. Then DMA is oxidized to MMA and formaldehyde by dimethylamine monooxygenase. Methylamine is further degraded into ammonia and formaldehyde by three different routes, first direct degradation from methylamine to ammonia which involves the methylamine dehydrogenase (MADH), second by conversion of methylamine into N-methylglutamate then to methylene-H_4F via N-methylglutamate dehydrogenase, then third by blue copper amine oxidases (Chistoserdova and Lidstrom, 2013). Although aerobic biological treatment for low TMAH-containing wastewater has been reported, the information for aerobic treatment for high TMAH concentrations is unavailable, presumably due to the inhibitory effects on aerobic TMAH-degrading and nitrifying communities at high TMAH concentrations (Lei et al., 2008; Wu et al., 2008).

This study was motivated to evaluate aerobic degradation of TMAH at high concentrations and its impacts on nitrification. The aerobic TMAH-degrading microbial community was enriched using an SBR and acclimated with gradually increased TMAH. The effects of high TMAH concentrations on aerobic TMAH degradation and nitrification were evaluated. Microbial community and population dynamics in the aerobic SBR treating high concentrations of TMAH were investigated using molecular tools including NGS, real-time qPCR and reverse transcript qPCR techniques.

2. Material and method

2.1. Reactor operation and chemical analytical methods

An SBR was used for evaluating aerobic degradation of TMAH and its effect on further nitrification (Fig. 2). The SBR with 8 L working volume was seeded with sludge from a full-scale TFT-LCD wastewater treatment plant. TFT-LCD wastewater from the full-scale TFT-LCD wastewater treatment plant was used as the

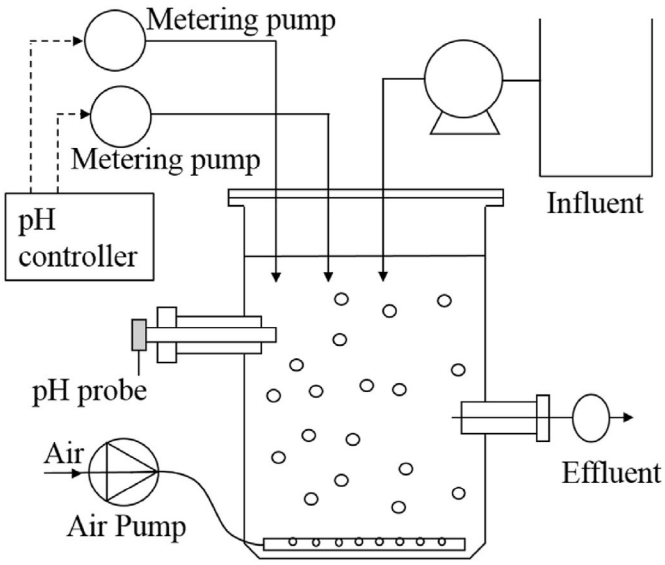

Fig. 2. Schematic of aerobic SBR fed with TMAH.

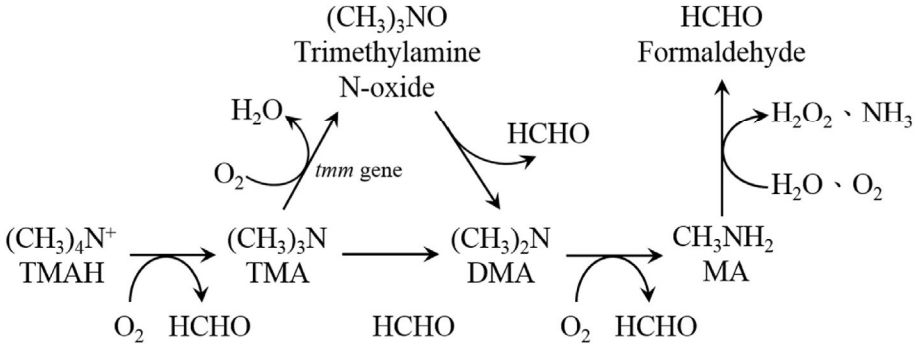

Fig. 1. Oxidization of TMAH to formaldehyde (Modified from Anthony, 1982).

influent feed of the aerobic SBR. The influent wastewater was stored in 4 °C and warmed to room temperature before being fed into the aerobic SBR. Stock TMAH (NMP-Ultra II, Taiwan Maxwave Co., Ltd) was added into the wastewater to achieve a high concentration of TMAH in the influent. The SBR was equipped with an air pump to provide aerobic condition and a pH meter to control the pH around 6.8 to pH 7.4.

A reaction run started with wastewater containing predetermined TMAH concentrations fed into the SBR to reach a total working volume of 8 L. After TMAH completely degraded and ammonia completely oxidized, the test run was finished and aeration was stopped to allow the sludge settled down. After sludge settled down by gravity, 4 L supernatant was withdrawn and the following test run began. Two phases of operation were conducted in this study. In the first phase, initial TMAH was gradually increased from 200 to 4666 mg L^{-1} (equivalent to 31 to 718 mg-N L^{-1}) in the 9 test runs to evaluate the aerobic TMAH degradation kinetics and effects of TMAH on further nitrification. In the Phase 2, 5 test runs were conducted to evaluate the long term effect of high initial TMAH concentrations (2662 to 6854 mg L^{-1}, equivalent to 410 to 1.054 mg N L^{-1}) on nitrification.

Samples collected from the SBR for measurements of ammonia, nitrite, and nitrate, TMAH, and TMAH-intermediate species were first filtered through a 0.22 μm membrane filter to remove biomass in the samples to avoid biological reaction before further analyses. Analyses of nitrogen-containing compounds, mixed liquor suspended solids (MLSS), and mixed liquid volatile suspended solids (MLVSS) were performed based on the Standard Methods by APHA (1998). Analyses of TMAH, TMA, DMA, and MMA were performed according to Mrklas et al. (2003) by using an ion chromatography (ICS-1000, Dionex, USA) with CG-3 and CS-15 as guard and analytical columns, respectively.

The specific TMAH degradation rate was calculated using the data collected from the SBR operation. The dependences of specific TMAH degradation rate on initial TMAH concentrations were evaluated using the Monod type kinetic expression as the following equation (Grady et al., 1999):

$$q = \frac{q_{max} \times S}{K + S} \quad (1)$$

where q is specific TMAH degradation rates (mg N $hour^{-1}$ g VSS^{-1}); q_{max} is maximum TMAH degradation rates (mg N $hour^{-1}$ g VSS^{-1}); S is TMAH concentration (mg N L^{-1}); K is half-saturation coefficient for TMAH (mg N L^{-1}).

The Gompertz function were also applied to calculate the lag time for nitrate production and maximum nitrate production rates (Fang et al., 2007). The equation could be expressed as the following equation:

$$S(t) = S(0) - P \times \exp\left\{ -\exp\left[\frac{R_m \times e}{P} \times (\lambda - t) + 1\right]\right) \quad (2)$$

where S(t) and S(0) were the nitrate concentrations at time 0 and t during the experiment; P was the nitrate production potential; Rm was the potential maximum nitrate production; and λ was the potential lag time.

2.2. DNA and RNA extraction and qPCR

Sludge samples were collected during the whole operation duration of Run 5 in Phase 2 to understand the dynamic of microorganism through the whole test run. The sludge samples were stored in −80 °C freezer before being applied to DNA and RNA extraction. The DNA and RNA extraction were carried out using Fast-DNA SPIN kits for soil (MP Biomedicals) and RNeasy Mini Kit (Qiagen, Valencia, CA, USA), respectively, with homogenizer FastPrep-24 Classic (MP Biomedicals), and following the manufacturer's instructions. After extraction, DNA was purified using QIAquick PCR Purification Kit, while RNA was purified using RNase-Free DNase Set (Qiagen) and immediately applied to the reverse-transcription reaction using the BluePrint RT Reagent Kit (TaKaRa Bio, Otsu, Japan) with random hexamer following the manufacturer's instructions. Both DNA and complementary DNA (cDNA) were stored at −20 °C before applying to further analysis.

The qPCR analyses targeting on total bacteria, TMAH-degrading microorganisms (*Hyphomicrobium* sp. and *Thiobacillus* sp.), functional gene of TMAH degradation (trimethylammine monooxygenase gene, tmm), and functional gene of ammonia-oxidizing bacteria (AOB) (ammonia monooxygenase gene, *amo*A) were conducted as *duplicates* by following the procedures in previous studies (Fierer et al., 2005; Chen, 2012; Fukushima et al., 2013). Primers used in this study are listed in Table 1. The LightCycler 480 (Roche) was used for qPCR analysis with LightCycler 480 SYBR Green I Master (Roche) and 0.2 μM of primers.

2.3. Sample preparation, library construction, and next generation sequencing analysis

Sludge samples collected from the SBR at the end of the two phases were analyzed by the Welgene Biotech Co., Ltd. (Taiwan) for next generation sequencing analysis (NGS). DNA extraction was carried out using WelPrep DNA kit (Welgene Biotech., cat no. D001), according to the instruction from the manufacturer. The extracted DNA quality was checked with optical density (OD) 260/280 in the rage of 1.8–2.0. The PCR was conducted with bacterial specific-primers S17 (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′) and A21 (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′), which

Table 1
Primers used for qPCR.

Primer	Sequence (5′-3′)	Specificity	References
amoA-1F	GGG GTT TCT ACT GGT GGT	AOB *amoA* gene	Rotthauwe et al. (1997)
amoA-2R	CCC CTC KGS AAA GCC TTC TTC		
Hypho F	GGC GGT GAC AAT GCG CAG C	*Hyphomicrobium* 16s rRNA	Fukushima et al. (2013)
Hypho R	ATT CAC CGC GCC ATG CTG ATG		
Thio F	CCT CAC GTT ATT CGA GCG G	*Thiobacillus* 16s rRNA	Hayes et al. (2010)
Thio R	ACG CAC TCT AGA CTG CCA		
tmm F	ATG TAY CGY TAY CTV TGG TC	*tmm* gene	Chen (2012)
tmm R	GTG AAC CAC TGR TCC TGC AT		
Eub338	ACT CCT ACG GGA GGC AGC	Total Eubacteria 16s rRNA	Fierer et al. (2005)
Eub518	ATT ACC GCG GCT GCT GG		

targeting on 16 S rRNA gene V3—V4 region. The TapeStation (Agilent Technologies) was used to check the amplified DNA size. Then the NGS was conducted using the Illunima Miseq platform. Nextera XT Index Kit was applied to attach the DNA samples to indices and Illumina sequencing adapters. After the library construction, samples were mixed with MiSeq Reagent Kit v3 (600-cycle), loaded to Miseq cartridge, and then placed to the instrument. Automated cluster generation and 2 × 300 bp paired-end sequencing run were then performed.

2.4. Operational taxonomic unit (OTU) analysis and annotation

After the sequencing analyses, the products were filtered to obtain the qualified reads. The total reads were merged by Flash (Magoč and Salzberg, 2011). Then Usearch was used to remove the low quality sequence and the chimera sequence and to construct OTUs using an identity threshold of 97% (Edgar, 2013). All the OTU sequences were classified to taxa, using Mothur and SILVA databases (Wang et al., 2007; Yilmaz et al., 2013). Finally, the rank abundance and diversity were calculated by using Qiime (Caporaso et al., 2010).

3. Results and discussion

3.1. Kinetics of aerobic TMAH biodegradation and nitrification

To evaluate the effects of TMAH on aerobic TMAH degradation kinetics and further nitrification, initial TMAH concentrations were gradually increased from 200 to 4666 mg L^{-1} (equivalent to 31 to 718 mg-N L^{-1}) in 9 test runs in the first phase of SBR operation. Aerobic TMAH degradation releases ammonia (Anthony, 1982), and ammonia can be further oxidized to nitrate by nitrifying microorganisms. In the Phase 1, TMAH was completed degraded and ammonia increased as TMAH degraded in the 9 test runs. Then released ammonia was then oxidized to nitrate, but a lag time for ammonia oxidization and nitrate production was observed in the test runs with higher nitial TMAH concentrations. In Innocenzi's study (2019), 77—99% of TMAH removal could be achieved with an initial TMAH concentration about 700 mg L^{-1} (equivalent to about 100 mg-N L^{-1}), but removal efficiency decreased in the later test experiments. In addition, accumulation of ammonia indicated that almost no nitrification occurred in Innocenzi's study (2019). Kinetic parameters, including specific TMAH degradation rate, lag time of nitrate production, and maximum specific TMAH degradation rate, are summarized in Table 2. The results indicated that completely aerobic degradation of high TMAH concentrations could be achieved even with initial concatenation as high as 4666 mg L^{-1} (equivalent to 718 mg N L^{-1}). Hu et al. (2012) indicated that substrate inhibition of TMAH was observed at the TMAH concentration

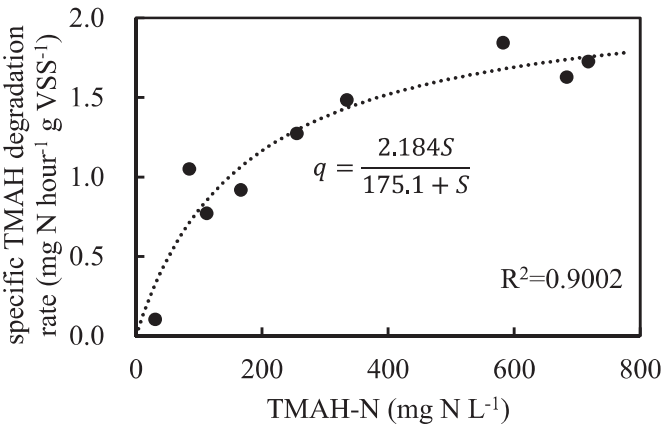

Fig. 3. Specific aerobic TMAH degradation rates under different initial TMAH concentrations following the Monod-type kinetics.

higher than 150 mg L^{-1}, but no substrate inhibition for TMAH was found in the current study, presumably due to a gradual acclimation with increased TMAH applied in the SBR. The dependence of the specific aerobic TMAH degradation rates on initial TMAH concentrations were evaluated using the Monod type kinetic expression as shown in Fig. 3. Results showed that the specific TMAH degradation rate increased as initial TMAH concentration increased and the trend followed the Monod-type kinetics well. The maximum specific TMAH degradation rate was calculated as 2.184 mg N $hour^{-1}$ g VSS^{-1} (equivalent to 14.196 mg TMAH $hour^{-1}$ g VSS^{-1}), and the half-saturation coefficient for TMAH was 175.1 mg N L^{-1}. The maximum specific aerobic TMAH degradation rate obtained in this study was higher than that (8.8 mg TMAH $hour^{-1}$ g VSS^{-1}) in Hu's study (2012). The higher maximum aerobic specific TMAH degradation rate obtained in this study may result from gradual acclimation with increased TMAH concentrations from 200 to 4666 mg L^{-1} for the TMAH-degrading microorganisms in the SBR. The sludge used in Hu's study (2012), however, was obtained from an aerobic tank with influent TMAH concentration less than 100 mg L^{-1}, which was much lower than the concentrations used in the current study.

The lag time for nitrification and maxima specific nitrate production rate under different initial TMAH concentrations are presented in Fig. 4. Delay of nitrification (ammonia oxidization and nitrate production) occurred under 550 mg L-1 initial TMAH (equivalent to 85 mg N L-1), and the extent of delay increased as the initial TMAH concentration increased. Lin et al. (2016) also showed an increase in the initial TMAH level (up to 500 mg L-1) gradually decreased the activity of AOB. In Fig. 4, a linear correlation was found between the lag time of nitrate production and initial TMAH

Table 2
Kinetic parameters of aerobic TMAH degradation and nitrification under different initial TMAH concentration (Phase 1).

Run	Initial TMAH	Initial TMAH-N	Specific TMAH degradation rate	Lag time for nitrate production	Maxima specific nitrate production rate	R^2 of Gompertz eq. fitting	VSS
Unit	mg L^{-1}	mg N L^{-1}	mg N $hour^{-1}$ g VSS^{-1}	Hour	mg N $hour^{-1}$ g VSS^{-1}	–	mg L^{-1}
1	200	31	0.10	0	0.36	0.872	5540
2	550	85	1.05	20.2	0.13	0.980	4750
3	730	112	0.77	17.5	0.57	0.991	4850
4	1082	167	0.92	60.7	0.24	0.986	4140
5	1660	255	1.27	51.4	0.62	0.995	4140
6	2177	335	1.49	81.6	0.70	0.998	4130
7	3787	583	1.85	133.9	1.49	0.994	4510
8	4443	671	1.60	132.6	2.21	0.999	4370
9	4666	718	1.73	144	1.40	0.997	4570

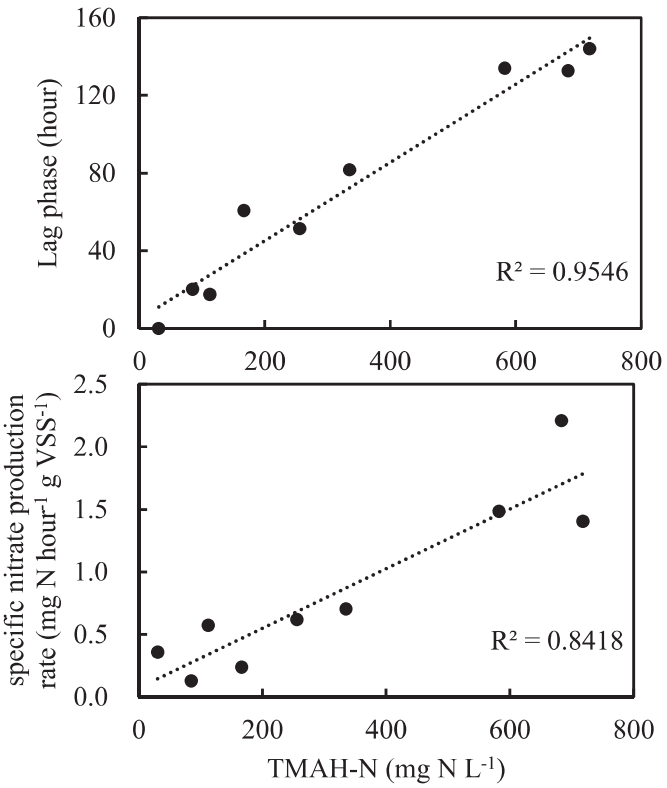

Fig. 4. Linear correlation of lag time for nitrification and maximum specific nitrate production rate under different initial TMAH concentration.

concentration indicating that a high TMAH concentration inhibited the onset of nitrification. However, the increase of initial TMAH concentration didn't adversely impact on specific nitrate production rate. The specific nitrate production rate increased with increased initial TMAH concentration, and a linear correlation was found.

3.2. Long term effect of high TMAH on nitrification under high concentration

Based on the results obtained in the Phase 1 operation, severe TMAH inhibition on nitrification was found at high initial TMAH concentration. In the Phase 2 operation, high concentrations of TMAH between 2662–6854 mg L^{-1} TMAH (equivalent to 410–1054 mg N L^{-1}) were fed in the SBR to evaluate the long-term effect of high TMAH concentrations on nitrification. The profile of TMAH, ammonia, nitrate, nitrite, SS, and VSS are presented in Fig. 5. Results indicated that TMAH could be completely degraded even with initial TMAH as high as 6854 mg L^{-1}. Specific TMAH degradation rates maintained above 1.3 mg N $hour^{-1}$ g VSS^{-1} throughout the Phase 2 operation. For the first 3 runs in the Phase 2 operation, ammonia released as soon as TMAH was degraded, and then was oxidized to nitrate with a lag time. In the Runs 4 and 5, a longer lag-phase for ammonia oxidation was observed.

In the Run 5, potential intermediate products of TMAH degradation, including TMA, DMA, and MMA, were analyzed to obtain a better understanding of aerobic TMAH degradation. The TMA was produced as high as 233 mg-N L^{-1} during the end of TMAH degradation and was further degraded within one day. Both DMA and MMA were below detection limits through the whole test run. The low concentration of TMA observed in the SBR might be due to the fast utilization of TMA by the microorganisms enriched in the SBR. This result agreed with previous studies that no or low concentrations of intermediate species (TMA, DMA, and MMA) were detected during degradation of 100–200 mg-N L^{-1} of TMAH under either aerobic or anaerobic conditions (Lei et al., 2008; Chen et al., 2016).

3.3. Microbial population diversity

Sludge samples collected from the SBR at the end of the two phases were analyzed for microbial community analysis using the NGS method. Fig. 6 presents the microbial community structures found in the two phases at phylum and genus levels, respectively, with abundance high than 1%. The Illumina sequencing of 16s rRNA gene identified 27 phyla from the sludge samples in the aerobic SBR. Among these phyla, 9 phyla covered 98.4% and 98.2% of total sequences from the Phases 1 and 2 samples, respectively. Proteobacteria was the most abundant phylum (44.4% and 27.3% in the Phases 1 and 2 respectively), followed by Actinobacteria (22.3% and 23.19%, in the Phases 1 and 2 respectively), Acidobacteria (14.8% and 19% in Phase 1 and 2, respectively). Microorganisms, which reported to have TMAH degradation ability, such as *Aminobacter aminovorans* (Urakami et al., 1992), *Methylobacterium* sp.(Urakami et al., 1993), *Paracoccus* sp. (Ohara et al., 1990), and *Hypomicrobium* sp. (Chistoserdova and Lidstrom, 2013), *Pseudomonas* sp.(Ghisalba et al., 1985) were belong to Proteobacteria phylum, while *Mycobacterium* sp. (Urakami et al., 1990) were belong to Actinobacteria phylum. Based on the sequencing results, it was evident that the aerobic SBR successfully enriched the TMAH-degrading microorganisms. Potential TMAH-degrading microorganisms found in the SBR included *Hypomicrobium* (25.2% in Phase 1 and 1.8% in Phase 2, respectively), *Mycobacterium* (11.5% and 10.9% in Phase 1 and 2, respectively), and other microorganisms with lower abundance included *Methylobacteriaceae*, *Paracoccus*, *Thiobacillus*, *Methylocaldum*, *Nocardia*, and *Methylophilis*. In a previous study, Wu et al. (2008) reported enrichments of *Hyphomicrobium* sp., *Rhodobacter* sp., and *Methyloversatilis* sp. in an aerobic MBRs and *Thiobacillus* sp., *Nitrosomonas* sp., *Thauera* sp., and *Azoarcus* sp. in anaerobic-aerobic SBRs treating both dimethyl sulfoxide (DMSO) and TMAH. However, Wu's study (Wu et al., 2008) employed the cloning method for microbial identification which can only identify dominant microorganisms. As a result, non-dominant TMAH-degrading microorganisms might not be detected by the cloning method. In the present study, the NGS method was used to investigate both dominant and non-dominant microorganisms presented in the SBR. In addition, ammonia-oxidizing bacteria (*Nitrosomonas* sp. and *Nitrosospira* sp.) and nitrite-oxidizing bacteria (*Nitrospira* sp.) were also detected in the SBR although their abundance was with minor proportion. Zhang et al. (2010) also found that AOB 16 S rRNA gene abundance was 1–3 order of magnitude less than total bacteria.

3.4. Dynamic of microbial abundance and activity

The abundance (DNA level) and activity (RNA level) dynamics of total bacteria, functional microorganism (*Hyphomicrobium* sp. and *Thiobacillus* sp.), and functional genes (amoA gene of AOB and tmm gene) during a full cycle of TMAH biodegradation were investigated using real-time qPCR and RT-qPCR analyses. Fig. 7 presents the concentration profile of nitrogen-related compounds and folds of change of functional microorganism and genes on DNA and RNA level observed in the Run 5 of the Phase 2 operation. The results indicated that abundance of total bacteria (DNA level) varied within two folds through the whole run. Distinct dynamics of *Hyphomicrobium* sp. and *Thiobacillus* sp., methylamine-utilizing bacteria (Lommen et al., 1990; Gorren et al., 1995; Chistoserdova and

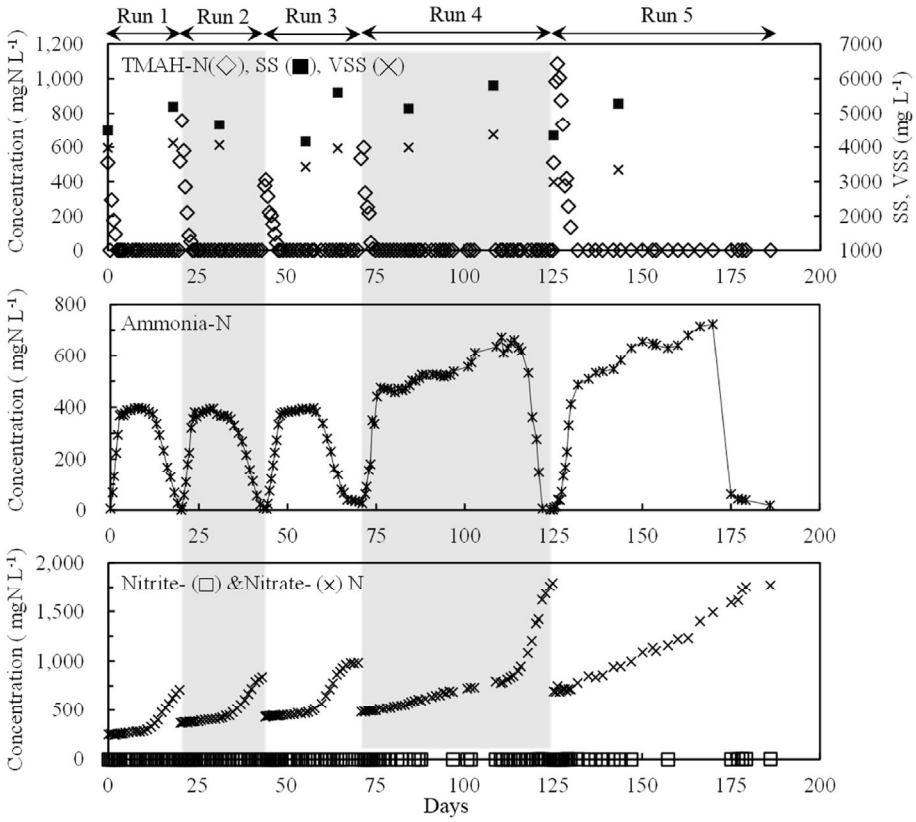

Fig. 5. Profile of TMAH degaration and nitrification in aerobic SBR under high TMAH concentrations.

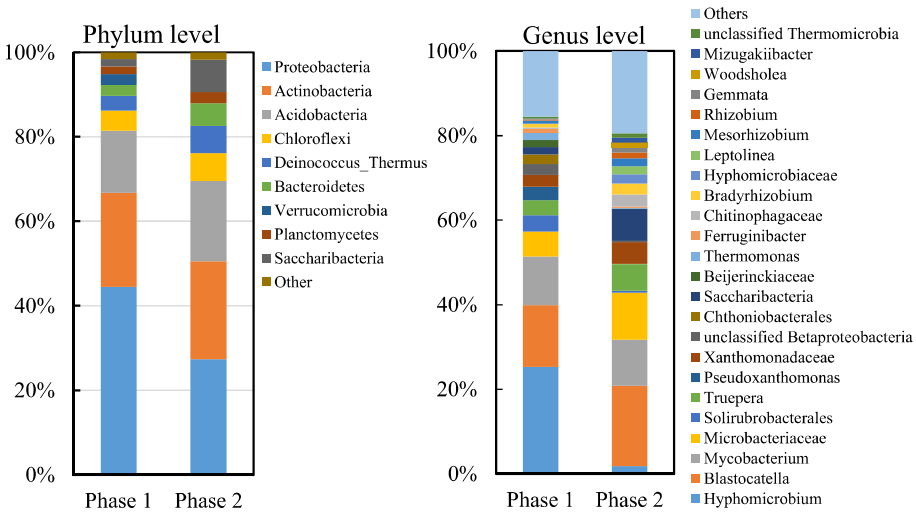

Fig. 6. Microbial community structure of two phases in phylum and genus level.

Lidstrom, 2013), were observed in the Run 5. The dynamics and activity of *Hyphomicrobium* sp. and *Thiobacillus* sp. were assumed to correlate with TMAH degradation. Abundance and expression of 16 S rRNA for *Hyphomicrobium* sp. decreased slightly (0.26–0.72) in the first 3 days of the test run, but during Days 50–54 the abundance increased 3.8 times and expression of 16 S rRNA decrease 3 order of magnitude. Abundance and expression of 16 S rRNA for *Thiobacillus* sp. decreased at Day 1, and then from Days 1.1–3.6 increased significantly with 13 times and 550 times on abundance and expression of 16 S rRNA, respectively, which was consistent with TMAH degradation. After depletion of TMAH, abundance of *Thiobacillus* sp. increased about 7.7 and 11.3 times at Days 6.7 and 45 higher than that observed at Day 0 of the test run. The 16 S rRNA expression of *Thiobacillus* sp. gradually decreased and wasn't detected after Day 49. It is presumed that *Thiobacillus* sp. in the SBR maybe mainly utilize TMAH, but not TMA, DMA, and MMA. As a result, the dynamic of *Thiobacillus* sp. was consistent with TMAH degradation. *Hyphomicrobium* sp., however, could utilize TMAH,

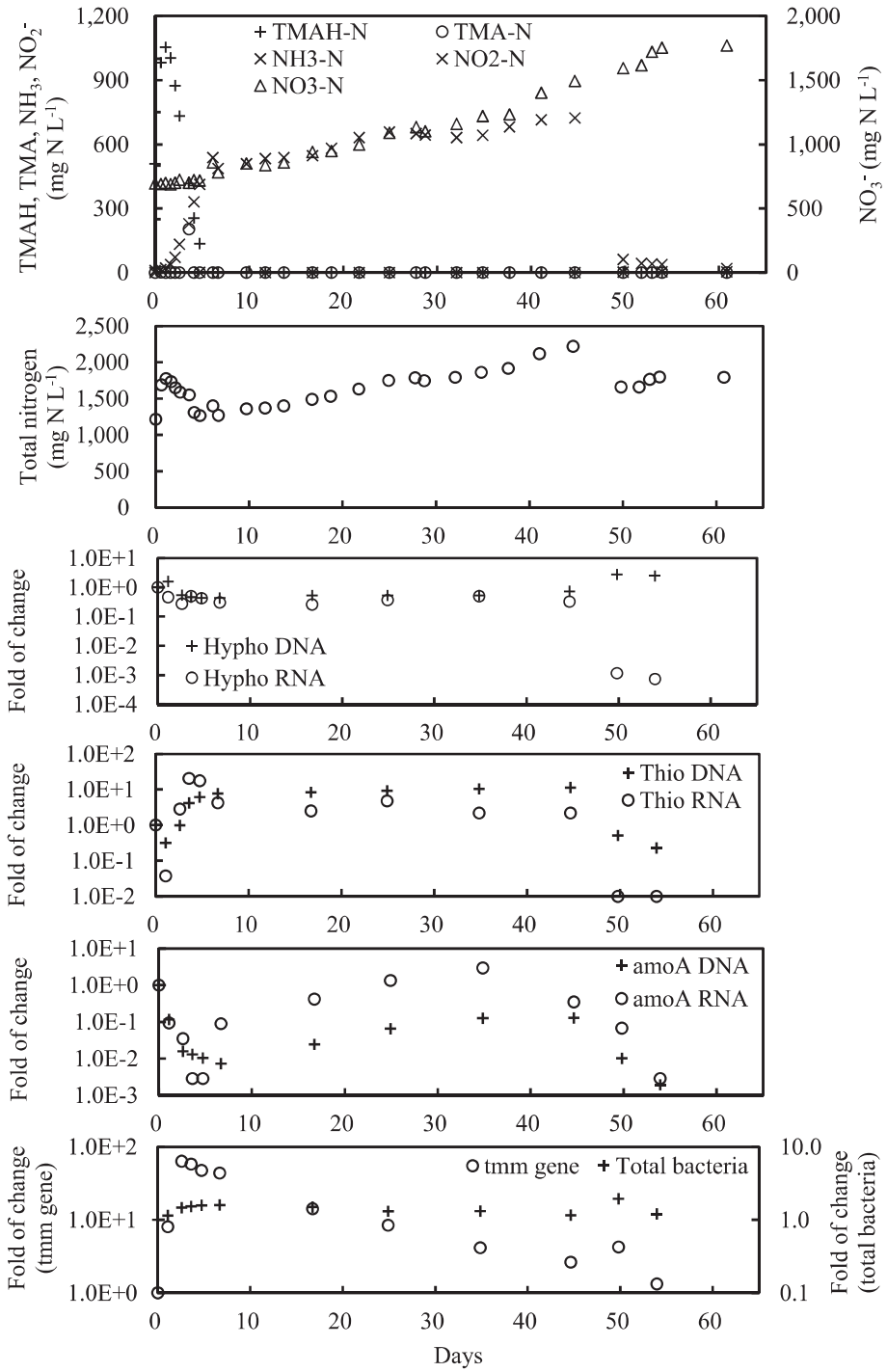

Fig. 7. Total Eubacteria, AOB, *Hyphomicrobium*, *Thiobacillus*, and tmm gene dynamics during TMAH degradation (Run 5 of Phase 2). Total nitrogen was calculated as sum of TMAH-N, TMA-N, ammonia, nitrite, and nitrate nitrogen.

TMA, and DMA (Meiberg et al., 1980), resulting in stable abundance and activity along the test run.

Trends of abundance and expression of *amo*A mRNA gene for AOB were found similar in the test run. Both DNA and RNA level of *amo*A gene decreased during the first 5 days of the run, suggesting an inhibitory effect of TMAH on AOB. After TMAH depletion on Day 6.7, the RNA level of *amo*A gene started to increase with observed nitrate production, while the DNA level of *amo*A gene increased later. Both DNA and RNA level of *amo*A gene maintained at relatively high level until the end of the test run (Day 45), then decreased at Day 49 with depletion of ammonia.

The tmm gene encoded trimethylamine monooxygenase enzyme which is responsible for TMA oxidation. Its abundance increased about 60 times in the first 3 days of the run, and then gradually decreased after Day 7. TMA was only detected at Day 3.6, and then was oxidized completed. However, the gene expression level was not detected through the whole test run.

3.5. Inhibition of TMAH on nitrification

It is known that ammonia oxidation can be inhibited by many organic compounds. Inhibitors and inactivators of AMO and their mechanisms have been discussed by Arp and Stein (2003). The inhibition mechanism consists of interfering with enzyme, which is reversible by removing inhibitors. The inactivated mechanism consists of structural destruction of the enzyme, such as the formation of a covalent bond. It is irreversible by removing inactivators. The results of the Phase 1 showed that TMAH inhibited the onset of nitrification, but not specific nitrate production rate. RT-qPCR also showed that *amo*A gene started expression after depletion of TMAH, which suggestion TMAH may inhibit nitrification by inhibit expression of *amo*A gene (Arp and Stein, 2003). However, the results of the Run 5 in the Phase 2 showed that even *amo*A gene started expression, the nitrate production didn't increase significantly and ammonia accumulated in the system. Ammonia was significantly oxidized about 40 days after TMAH depletion. It is suggested that intermediate product of TMAH degradation may compete AMO enzyme with ammonia to inhibit nitrification (Arp and Stein, 2003).

4. Conclusion

Aerobic degradation of high concentrations of TMAH was demonstrated using an SBR. The specific aerobic TMAH degradation rates followed the Monod-type kinetics with a maximum specific TMAH degradation rate of 2.184 mg N hour^{-1} g VSS^{-1} and the half-saturation coefficient of 175.1 mg N L^{-1}. Delay of nitrification occurred at TMAH higher than 550 mg L^{-1}, and the lag time for the onset of nitrification highly correlated with initial TMAH fed for the SBR. Potential aerobic TMAH-degraders including *Mycobacterium* sp. and *Hypomicrobium* sp. were enriched in the aerobic SBR. *Hyphomicrobium* sp. may be able to utilize both TMAH and its degradation intermediates such as TMA, while *Thiobacillus* sp. can only utilize TMAH. The qPCR and RT-qPCR results suggested that TMAH may inhibit nitrification by inactive expression of *amo*A gene and the intermediates of TMAH degradation may compete AMO enzyme with ammonia for nitrification inhibition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to acknowledge the financial support from the Ministry of Science and Technology of Taiwan (MOST 105-2221-E-006 -009 -MY3, 106-2621-M-006-009-MY3, 108-2811-M-006-545) and the partial financial support from the Ministry of Education of Taiwan through the Top University Project Grant to the National Cheng Kung University (NCKU).

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