

# Biofiltration of trimethylamine, dimethylamine, and methylamine by immobilized *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1

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## Abstract

A biofilter using granular activated carbon with immobilized *Paracoccus* sp. CP2 was applied to the elimination of 10–250 ppm of trimethylamine (TMA), dimethylamine (DMA), and methylamine (MA). The results indicated that the system effectively treated MA (>93%), DMA (>90%), and TMA (>85%) under high loading conditions, and the maximum degradation rates were 1.4, 1.2, and 0.9 g-N kg<sup>-1</sup> GAC d<sup>-1</sup>. Among the three different amines treated, TMA was the most difficult to degrade and resulted in ammonia accumulation. Further study on TMA removal showed that the optimal pH was near neutral (6.0–8.0). The supply of high glucose (>0.1%) inhibited TMA removal, maybe due to substrate competition. However, complete TMA degradation was achieved under the co-immobilization of *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1 (~96%). Metabolite analysis results demonstrated that the metabolite NH<sub>4</sub><sup>+</sup> concentrations decreased by a relatively small 27% while the metabolite NO<sub>2</sub><sup>-</sup> apparently increased by heterotrophic nitrification of *Arthrobacter* sp. CP1 in the co-immobilization biofilter.

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**Keywords:** Activated carbon; Ammonia; Degradation; Immobilization; Nitrification

## 1. Introduction

Amines, especially trimethylamine (TMA), are a major source of odor in air streams at composting facilities, and they are difficult to degrade (Zhu et al., 1997). TMA emissions can also be found in fish-meal manufacturing plants (Sandberg and Ahring, 1992; Kim et al., 2001), wastewater treatment plants (Shieh and Keenan, 1986), landfills, livestock farms, and in hog manure (Leson and Winer, 1991; Cao et al., 1997; Chang et al., 2004).

In recent years, waste gas biotreatment technology has proven to be effective at treating odorous and toxic compounds. The key factor in biological treatment is the use of microorganisms (Chung et al., 2004, 2007). In previous studies, *Arthrobacter* (Schlegel, 1992), *Aminobacter*

(Raymond and Plopper, 2002), *Hyphomicrobium* (Tuhela et al., 1998), and *Paracoccus* sp. (Kim et al., 2001, 2003) were reported to have successfully degraded TMA in water, but few reports have focused on the investigation of the metabolic pathways of TMA. Kim et al. (2003) indicates that TMA is initially oxidized to dimethylamine (DMA) in *Paracoccus* sp., and TMA N-oxide is a metabolic intermediate of TMA. DMA is further oxidized to methylamine (MA). Finally, MA is completely oxidized to NH<sub>3</sub> under aerobic conditions. Recently, Chang et al. (2004) used activated sludge as the microbial source to conduct the removal of TMA gas. Greater than 90% removal efficiencies were achieved at an inlet loading of less than 27 mg-N h<sup>-1</sup>, but a long gas residence time of 5.3 min and the accumulation of metabolite NH<sub>3</sub> (lead to alkalization) posed obstacles to the biofilter application. Ding et al. (2007) also used activated sludge microorganisms to eliminate 19–57 ppm of TMA at a residence time of 60 s. Although the TMA removal has obtained reasonable

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efficiency, the  $\text{NH}_3$  accumulation raises the pH of the biofilter due to lack of nitrification (Chen et al., 2005). Additionally, some pathogenic organisms present in the activated sludge may escape into the surrounding environment and complicate the ultimate disposal of the medium (Dumontet et al., 2001).

Previous reports have shown that  $\text{NH}_3$  is easily oxidized by autotrophic nitrifiers *Nitrosomonas* (Chung et al., 2007) and *Nitrosospira* sp. (Mahmood and Prosser, 2006), but they would lose superiority in the nitrification processes because of their low growth rates (Hunik et al., 1992). In contrast, the heterotrophic nitrifier *Arthrobacter* sp. is a promising potential microorganism for biofilter application because its biomass concentrations are  $10^4$ – $10^5$  times greater than those of the autotrophs (Prosser, 1989). Chung et al. (2004) demonstrated that a granular activated carbon (GAC) biofilter using *Arthrobacter* sp. as an inoculant strain was more effective (>99%) in eliminating  $\text{NH}_3$  than different biofilter types under similar conditions (Kapahi and Gross, 1995; Yani et al., 1998), and  $\text{NH}_3$  is easily converted to organic N (cellular N) during  $\text{NH}_3$  assimilation (Chung et al., 2004). Nevertheless, information on utilizing microorganisms to simultaneously remove the TMA and  $\text{NH}_3$  present in air streams is still scant.

TMA, DMA, and MA are major nitrogen-containing waste gases emitted from the composting process at composting facilities. In this study, a GAC biofilter inoculated with *Paracoccus* sp. CP2 was developed to eliminate different amine concentrations, with particular focus on the degradation of TMA. The maximum degradation rate ( $V_m$ ) and half-saturation constant ( $K_s$ ) of *Paracoccus* sp. CP2 were each investigated using a Michaelis–Menten equation. Among different amine treatments, TMA was difficult to degrade and had a low affinity for *Paracoccus* sp. CP2. The removal efficiencies decreased in the following order: MA > DMA > TMA. To completely degrade TMA and reduce  $\text{NH}_3$  emissions, the system used co-immobilized *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1. A satisfactory result for TMA removal (>95%) was then achieved.

## 2. Materials and methods

### 2.1. Microorganism cultivation and immobilization procedure

The amine degrader, *Paracoccus* sp. CP2, and heterotrophic ammonia oxidizer, *Arthrobacter* sp. CP1, were isolated from swine wastewater and grown in nutrient broth at 26 °C. In all experiments, an inflow medium was provided from a nutrient tank which contained (in  $\text{g l}^{-1}$ )  $\text{KH}_2\text{PO}_4$  5.4,  $\text{K}_2\text{HPO}_4$  10.5,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  0.2, and Fe(III)-citrate 0.01. The final pH of the medium was adjusted using 0.1 N NaOH or HCl. In this study, GAC was used as the support material with a uniform size of 4.5 mm and bulking density of  $0.48 \text{ g cm}^{-3}$ . *Paracoccus* sp. CP2 or *Arthrobacter* sp. CP1, each grown in 100-ml nutrient broth for 2 d, were harvested by centrifugation (8000 g for 10 min). The cell

pellets were separately drawn and put into a 10-l polyvinyl chloride tank containing 7 l of nutrient broth for microbial growth. Before the biofilter experiments, about 2.0 kg of GAC were separately mixed with the above solution for biofilm development. During the immobilization process, fresh broth was replaced every 3 d until  $10^8$ – $10^9$  cells  $\text{g}^{-1}$  GAC were achieved for both *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1. After 4 weeks, the single or mixed cell-laden GAC in the tank was transferred to the biofilter. In addition, all materials and implements were maintained in aseptic conditions during the above operation.

### 2.2. Apparatus and amine removal for continuous operation

To investigate the capacity of GAC without any microorganisms to adsorb amine, the Bed Depth Service Time (BDST) experiment, as described by Chung et al. (2005), was performed in the laboratory. A lab-scale biofilter made of glass (12 cm  $\phi$   $\times$  40 cm height) was operated at room temperature ( $26 \pm 2$  °C) using a heating blanket. The depth and weight of GAC in the biofilter were 20 cm and 1.1 kg, respectively. A perforated sieve plate of polyvinyl chloride material was fitted to the bottom of the biofilter to allow the solution to flow out. The column wall contained three sampling ports, including one at the middle for GAC sampling, one at the crown, and one underneath to measure gas concentrations of the inlet and outlet. MA, DMA, and TMA gas were separately fed into the biofilter using 0.64-cm outer diameter Teflon tubing and fittings. The inlet amine concentrations were controlled with a syringe pump (Cole-Parmer, USA) and the flow rate of pure air with a mass flow controller (Sierra Instruments, USA). Pure air was supplied from a cylinder as the oxygen source. It first passed an air filter (pore size, 0.22  $\mu\text{m}$ ) and then flowed downward through the top of the biofilter. The inflow medium in a 10-l tank located at the bottom of the biofilter was introduced at the top of the biofilter using a peristaltic pump at a rate of  $5 \text{ l min}^{-1}$  for 2 min, six times a day to maintain the moisture of the biofilter and supply nutrient to the microorganisms. The medium volume was maintained at 7 l by periodically adding distilled water. The peristaltic pump was connected to a spray nozzle located on the top of the biofilter to uniformly spray the medium on the biofilter bed surface. In continuous biofilter experiments, moisture content changes have been insignificant, with the average distribution in the range of 38–40%. To estimate the operating performance of the biofilter, 10–250 ppm of MA, DMA, and TMA were separately introduced to the biofilter system at various empty bed residence times (EBRTs) in the range of 30–60 s. The EBRT was defined as the packing volume of GAC in biofilter divided by the air flow rate.

### 2.3. Analytical methods

The inlet and outlet concentrations of MA, DMA, and TMA were analyzed by gas chromatograph (Perkin–Elmer,

USA) equipped with a Stabilwax-DB column (RESTEK, USA) and a flame-ionization detector.  $\text{NH}_3$  gas concentrations were measured with a portable ammonia-monitoring device (ATTAIN, Japan). The pH value in the leachate was determined by analysis of circulating liquids using a pH meter. To determine the moisture content in the GAC, about 0.5 g of it were weighed and dried over a 24-h period at  $103 \pm 0.5$  °C. In addition, 1.0 g of GAC was periodically withdrawn, mixed with 10 ml of distilled water, and vortexed for 3 min. The suspension solutions were assembled to analyze the pH value of GAC, the metabolic products (e.g. organic nitrogen, ammonium, nitrate and nitrite), and the number of microorganisms. The gram staining method was used to determinate Gram-negative (*Paracoccus* sp. CP2) and Gram-positive bacteria (*Arthrobacter* sp. CP1) by direct microscopic counts, as reported by Binnerup et al. (1998). The number was expressed in cells  $\text{g}^{-1}$  GAC. In addition, we also periodically used a traditional plate-counting method to identify the colonies of the predominant microorganisms by the procedures of total DNA extraction, polymerase chain reaction (PCR) amplification of 16S rDNA, and sequencing and comparing results with those in the GenBank database by using the BLAST (Basic Local Alignment Search Tool) server of the National Center for Biotechnology Information (Pizarro et al., 1996). Organic nitrogen was determined by the Kjeldahl method (Chung et al., 2004). Ammonium concentration in solution was determined using an ion-specific electrode. Nitrate and nitrite concentrations in solution were measured by ion chromatography (Dionex 4500i). Amine concentrations in solution were measured with a 1.0- $\mu\text{l}$  direct injection by gas chromatography.

### 3. Results and discussion

#### 3.1. System performance in terms of amine removals

A biofilter using GAC as the support material with immobilized *Paracoccus* sp. CP2 was applied to the separate treatment of 10–250 ppm of MA, DMA, and TMA at the EBRTs of 30–60 s. Fig. 1 shows the profiles of inlet/outlet concentrations and removal efficiencies of MA, DMA, and TMA at various EBRTs during 180-d operation. According to the calculation from the BDST experiment (Chung et al., 2005), the theoretical saturation time for GAC ranged from 8 to 15 d for a single amine at an inlet concentration of  $100 \pm 5$  ppm and an EBRT of 30 s. However, physical adsorption (100% removal) took only 6–8 d. It was conjectured that the accumulation of  $\text{NH}_3$  metabolite would in theory shorten the saturation time because of the physical adsorption of  $\text{NH}_3$  gas by GAC as described by Chung et al. (2005). In fact, no  $\text{NH}_3$  emission was detected at any outlet of the biofilter during the first 4 days, and then an increasing concentration of  $\text{NH}_3$  was observed in the biofilter exhaust air (data not shown). After theoretical saturated adsorption of GAC (8–15 d), dynamic equilibrium between adsorption and bio-

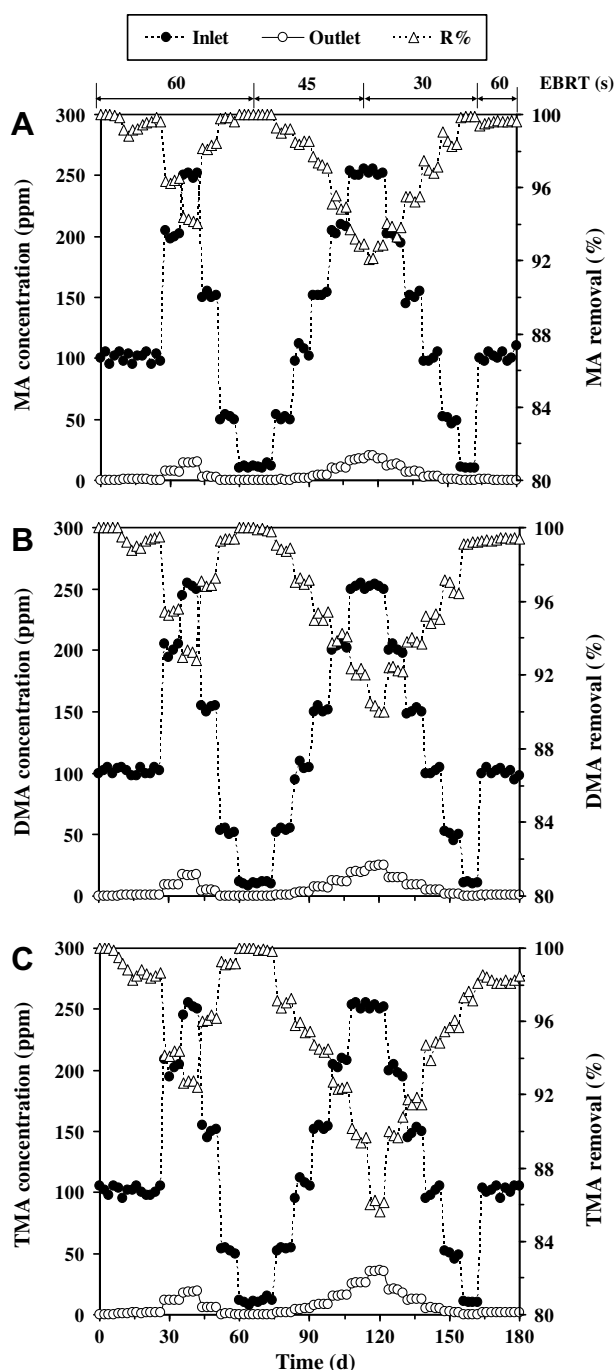


Fig. 1. Profiles of inlet/outlet concentrations and removal efficiencies at various EBRTs for MA (A), DMA (B) and TMA (C) removals during 180-d operation.

degradation was separately achieved. Steady-state efficiency (98–99%) could be carried out for  $100 \pm 2.9$  ppm of MA, DMA, and TMA removals at an EBRT of 60 s on 8th–26th day. The overall removal efficiencies were effectively maintained at 98–100% at an inlet concentration ranging from 10 to 100 ppm. The removal efficiencies of MA, DMA, and TMA immediately dropped to 6%, 7% and 9% when the inlet concentration increased to 250 ppm on 36th–42nd day. The removal efficiencies of

MA and DMA dipped by 2–3% while TMA noticeably decreased by 7% from 92% to 85% when the EBRT was reduced by 2-fold from 60 s to 30 s at an inlet concentration of 250 ppm between 116th and 122nd day. Nearly 99–100% removal efficiencies with differences of only 1% could be maintained at different EBRTs (30–60 s) with low inlets ( $\sim 10$  ppm). These results demonstrated that TMA was more difficult to degrade than MA and DMA. The reason was presumably that the maximum degradation rate of *Paracoccus* sp. CP2 had already been reached in this system for TMA removal, and the kinetics of contaminant degradation conformed to zero-order reactions at high inlet concentrations (Van-Lith et al., 1997; Schroeder, 2002). Chang et al. (2004) used activated sludge as a microbial source to remove TMA gas. The efficiency exceeded 90% at low loading conditions ( $<27$  mg-N  $h^{-1}$ ) and an EBRT of 5.3 min. Compared with this study and with a similar inlet loading, the GAC biofilter inoculated with *Paracoccus* sp. CP2 eliminated TMA more effectively ( $>92\%$ ) and at a shorter EBRT of 45 s.

The values of  $V_m$  and  $K_s$  of *Paracoccus* sp. CP2 for different amine compounds were evaluated using the Michaelis–Menten equation as described by Chung et al. (2000).  $V_m$  and  $K_s$  of MA were calculated from the regression equation to be  $1.4$  g-N  $kg^{-1}$  GAC  $d^{-1}$  and 30 ppm. The values of  $V_m$  and  $K_s$  were  $1.2$  g-N  $kg^{-1}$  GAC  $d^{-1}$  and 54 ppm for DMA. The  $V_m$  of TMA was  $0.9$  g-N  $kg^{-1}$  GAC  $d^{-1}$ ,  $K_s$  being 64 ppm. Ding et al. (2007) used a sludge and compost biofilter to separately eliminate TMA gas. The removal efficiencies achieved were 60% and 80% in sludge and compost biofilters, respectively, and the values of  $V_m$  were  $9.1$  and  $9.3$  g-TMA  $m^{-3}$   $h^{-1}$ , respectively. In contrast, the immobilized *Paracoccus* sp. CP2 biofilter used in this study achieved a very high  $V_m$  of  $80$  g-TMA  $m^{-3}$   $h^{-1}$  ( $0.9$  g-N  $kg^{-1}$  GAC  $d^{-1}$ ). Additionally, the system achieved the highest  $V_m$  for MA degradation as well as the highest affinity because a decrease in  $K_s$  suggested an enhancement in biomass affinity as shown in the following order: MA (30 ppm)  $>$  DMA (54 ppm)  $>$  TMA (64 ppm). In other words, TMA was the most difficult among three amine treatments to degrade in the biofilter. Hence, we further evaluated the TMA removal at different operating conditions in this system, and *Arthro-bacter* sp. CP1 was co-immobilized in the biofilter to realize a more effective and complete degradation of TMA.

### 3.2. Effect of pH change on TMA removal

Acidification or alkalization has often been an obstacle to traditional biofilters in waste gas treatment (Yang and Allen, 1994). Hence, maintaining constant pH in the biofilter system is an important operating factor affecting the adsorption of TMA by GAC and biodegradation. To estimate the biofilter response to pH variations, the inlet concentration of TMA was controlled at 150 ppm at an EBRT of 60 s, and the average removal efficiencies were calculated over a 30-d experiment for every pH change (5.0–9.0) as

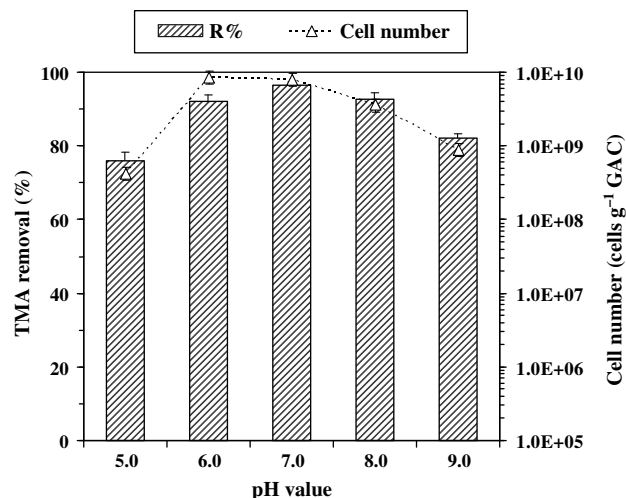


Fig. 2. Effect of pH change on TMA removal efficiency and cell number in the biofilter. The error bars indicate the standard deviation.

shown in Fig. 2. Approximately 92–96% removal efficiencies for TMA were achieved in the range 6.0–8.0, and cell numbers reached  $3\text{--}8 \times 10^9$  cells  $g^{-1}$  GAC. When the pH rose from 8.0 to 9.0, the removal efficiency decreased by 10% from 92% to 82%. In contrast, the efficiency significantly decreased to 76% when the pH was lowered to 5.0. Although pH changes did not result in significant decreases in cell numbers ( $10^8\text{--}10^9$  cells  $g^{-1}$  GAC), the TMA biodegradation was inefficient at low and high pH, and a decrease in removal efficiency was observed. The optimal pH in the biofilter for TMA removal ranged from 6.0 to 8.0, with especially good results at pH 7.0. Neutral pH resulted in maximal enzyme activities for TMA degradation by *Paracoccus* sp. CP2, as reported previously (Chang et al., 2004).

### 3.3. Effect of glucose concentration on TMA removal

In general, organic substrates can be used as a carbon and energy source for heterotrophic bacterial growth. Hence, we further evaluated TMA removal relative to the supply of glucose enhancing and stimulating bacterial metabolism and growth in the biofilter. To find its optimal concentration, the inlet concentration of TMA was controlled at 150 ppm at an EBRT of 60 s, and the average removal efficiencies were calculated over a 30-d period under different glucose concentrations. Fig. 3 shows the effect of glucose concentration on TMA removal efficiency and cell number in the biofilter. The results indicated that as the glucose concentration increased, the number of *Paracoccus* sp. CP2 increased slightly from  $5.5 \times 10^9$  to  $1.2 \times 10^{10}$  cells  $g^{-1}$  GAC. The average removal efficiency of TMA was 95–96% at a low glucose concentration (0.01%). However, when glucose concentrations increased to 0.1% and 1.0%, the removal efficiency plunged to 86% and 75%. TMA removal efficiency was strongly affected at high glucose concentrations ( $>0.1\%$ ) by substrate competition because *Paracoccus* sp. CP2 primarily used glucose



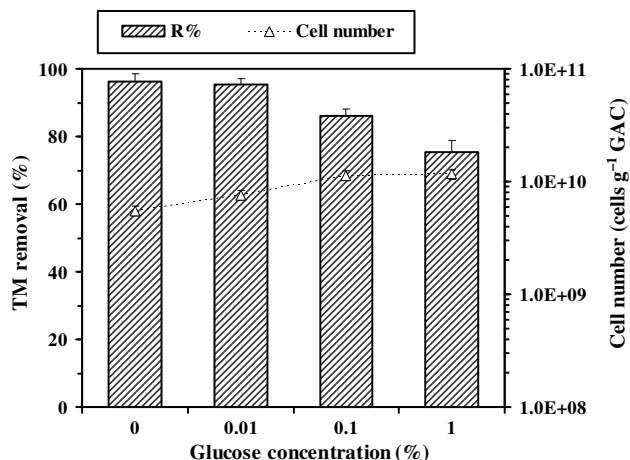


Fig. 3. Effect of carbon source on TMA removal efficiency and cell number in the biofilter. The error bars indicate the standard deviation.

as a carbon and energy source for its growth. Thus, substrate competition will likely be a potential problem in managing the efficacy of biodegradation in the biofilter (Hernandez et al., 1991).

#### 3.4. Effect of long-term shutdown on biofilter performance

Most manufacturing and treatment processes in various industries must undergo long shutdown periods because of process changes or holiday breaks (Escalas et al., 2003). Hence, in this study, we evaluated the effect on the biofilter's TMA removal, as well as on the microorganisms, of an 11-d shutdown period (5th–15th day). The results are shown in Fig. 4. The removal efficiency was almost 96% on average, and *Paracoccus* sp. CP2 numbered nearly  $3.4 \times 10^9$  cells  $g^{-1}$  GAC before shutdown. Although the common air and inflow medium were continuously provided, no TMA gas was introduced into the biofilter during the shutdown period. The removal efficiency achieved 82%

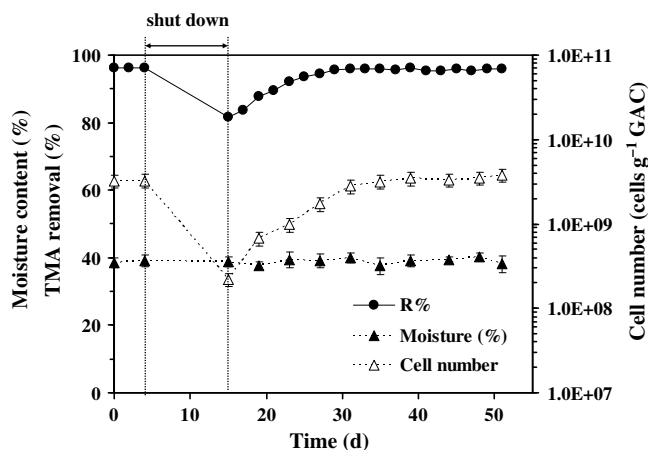


Fig. 4. Changes of TMA removal efficiency, cell number and moisture content in the biofilter in the time period before and after the shutdown. The error bars indicate the standard deviation.

when 150 ppm of TMA was fed again at an EBRT of 60 s on 15th day. Interestingly, the cell number of *Paracoccus* sp. CP2 slipped to  $2.2 \times 10^8$  cells  $g^{-1}$  GAC. The difference in the cell number might be because TMA absorbed on GAC or because polysaccharide in biofilms supplied for the growth of microorganisms was impermanent (Murray and Zinder, 1987). This suggestion was confirmed by an increase of  $NH_3$  metabolite (data not shown). The system recovered its original high efficiency (>95%) after 18 d. The results of this study demonstrated that the *Paracoccus* sp. CP2 biofilter using GAC as the packing material effectively treated TMA emissions from discontinuous sources, and its resilience could match that of other biofilters (Sercu et al., 2005; Qi and Moe, 2006).

#### 3.5. Effect of co-immobilization of *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1 on TMA and $NH_3$ removals

The previous results indicate that *Paracoccus* sp. CP2 successfully eliminated TMA gas in this study. Metabolite  $NH_3$ , however, cannot be degraded simultaneously, and  $NH_3$  accumulation or emission may result in obstacles to biofilter application (Chang et al., 2004; Ding et al., 2007). Chung et al. (2004) showed that a lab-scale biofilter inoculated with *Arthrobacter* sp. CP1 was more effective (>99%) at treating  $NH_3$  than other biotreatment systems under similar operating conditions (Kapahi and Gross, 1995; Yani et al., 1998). It was also effective at eliminating  $NH_3$  gas in the presence of additional organic carbon sources (Chung et al., 2005), and some reports have proved that *Arthrobacter* sp. could directly use TMA as a carbon and energy source (Schlegel, 1992). Hence, for completely degrading TMA and reducing  $NH_3$  emissions simultaneously, the effectiveness of co-immobilized *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1 was evaluated. This system was operated at an EBRT of 45 s with an inlet concentration of  $250 \pm 10$  ppm TMA and initial pH 7.0 by adding 0.01% of glucose as an auxiliary carbon source for heterotrophic nitrification of *Arthrobacter* sp. CP1. The average efficiencies of TMA and emission concentrations of TMA and  $NH_3$  were calculated for 60 d after the dynamic equilibrium between adsorption and biodegradation, as shown in Fig. 5. The removal efficiency of TMA reached  $88 \pm 4\%$ , and TMA and  $NH_3$  emissions in exhaust gases were  $29 \pm 8$  ppm and  $205 \pm 14$  ppm when *Paracoccus* sp. CP2 ( $4.6 \times 10^9$  cells  $g^{-1}$  GAC) was used as the sole inoculant in the biofilter. However, when *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1 were co-immobilized on GAC in the biofilter by adding 0.01% glucose, efficiencies of 95% were achieved, and TMA and  $NH_3$  emissions plummeted to  $11 \pm 5$  ppm and  $15 \pm 4$  ppm, respectively. Although average TMA removal efficiencies of 94% could be reached by adding 0% of glucose under the co-immobilization of *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1,  $NH_3$  emission was as high as  $184 \pm 15$  ppm, which was 12-fold higher than under the addition of 0.01% glucose, while TMA emission was  $16 \pm 3$  ppm. Therefore, adding 0.01%

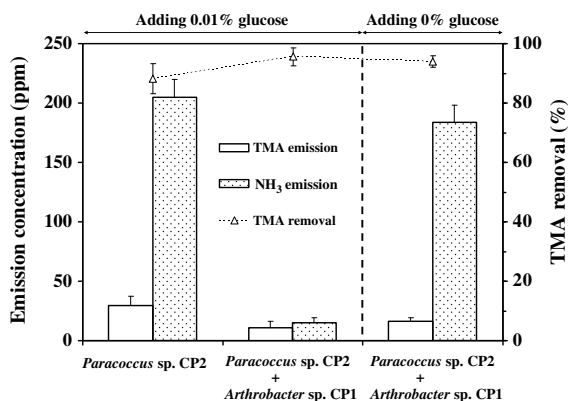


Fig. 5. Effect of co-immobilization of *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1 on biofilter performance by adding 0% and 0.01% glucose. The error bars indicate the standard deviation.

glucose in the system using co-immobilized *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1 was necessary to improve NH<sub>3</sub> and TMA (7%) removal, especially NH<sub>3</sub> removal (76%).

To understand the metabolites of TMA biodegradation under the addition of 0.01% glucose, a mass balance from the accumulation of nitrogen (the difference between inlet and outlet) was conducted and shown in Fig. 6. Since amine concentrations were always less than 0.1% of total nitrogen in this system, the data were neglected (data not shown). When *Paracoccus* sp. CP2 alone was used to degrade TMA, the main metabolites in this system were: NH<sub>4</sub><sup>+</sup> (58.7 ± 1.0%), organic-N (37.1 ± 2.2%), NH<sub>2</sub><sup>-</sup> (3.9 ± 0.8%), and NH<sub>3</sub><sup>-</sup> (0.3 ± 0.1%). These results indicated that both microbial ammonification and assimilation were the main metabolic pathways of TMA. Also, nitrification at low TMA level was responsible for the NH<sub>3</sub> metabolism. Some reports have indicated that *Paracoccus* sp. is capable of oxidizing NH<sub>3</sub> to NH<sub>2</sub><sup>-</sup> by heterotrophic nitrification (Crossman et al., 1997; Stouthamer et al., 1997). A slight amount of the NH<sub>3</sub><sup>-</sup> present in this system might have

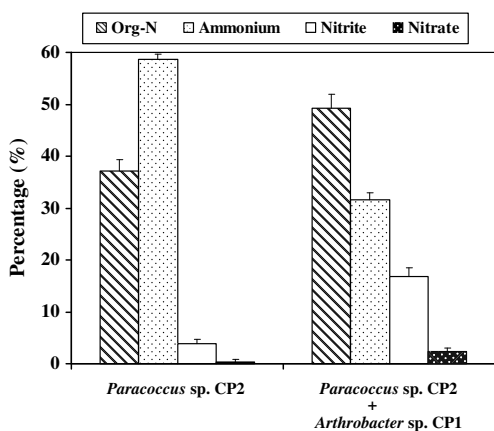


Fig. 6. Profile of the percentage of nitrogen metabolites in this system under the addition of 0.01% glucose. The error bars indicate the standard deviation.

resulted from the chemical oxidation of NH<sub>2</sub><sup>-</sup> (Chung et al., 2005). In contrast, the metabolite NH<sub>4</sub><sup>+</sup> decreased by 27% from 58% to 31% in the co-immobilization biofilter while NH<sub>2</sub><sup>-</sup> increased to 16% by the heterotrophic nitrification of *Arthrobacter* sp. CP1, all of which is in agreement with the results from Schlegel (1992).

#### 4. Conclusions

This study details an application of a GAC biofilter inoculated with *Paracoccus* sp. CP2 for amine biodegradation in an air stream. The biofilter is confirmed to effectively reduce 10–100 ppm of MA, DMA, and TMA with greater than 98% removal efficiencies at an EBRT of 60 s. Among the three amine treatment processes at an EBRT of 60 s, TMA has both a relatively lower affinity ( $K_s = 64$  ppm) and degradation rate ( $V_m = 0.9$  g-N kg<sup>-1</sup> GAC d<sup>-1</sup>) for *Paracoccus* sp. CP2, resulting in removal efficiency of more than 93%, and the optimum pH is 6.0–8.0. Finally, the biofilter with co-immobilized *Paracoccus* sp. CP2 and *Arthrobacter* sp. CP1 can effectively degrade TMA (>95%). In addition, NH<sub>3</sub> produced by TMA biodegradation can also be successfully eliminated and oxidized to NO<sub>2</sub><sup>-</sup> by heterotrophic nitrification of *Arthrobacter* sp. CP1.

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#### References

- Binnerup, S.J., Højberg, O., Sørensen, J., 1998. Gram characteristics determined on single cells and at the microcolony level of bacteria immobilized on polycarbonate membrane filters. *J. Microbiol. Meth.* 31, 185–192.
- Cao, N., Du, J., Chen, C., Gong, C.S., Tsao, G.T., 1997. Production of fumaric acid by immobilized *Rhizopus* using rotary biofilm contactor. *Appl. Biochem. Biotech.* 63, 387–394.
- Chang, C.T., Chen, B.Y., Shiu, I.S., Jeng, F.T., 2004. Biofiltration of trimethylamine-containing waste gas by entrapped mixed microbial cells. *Chemosphere* 55, 751–756.
- Chen, Y.X., Yin, J., Wang, K.X., 2005. Long-term operation of biofilters for biological removal of ammonia. *Chemosphere* 58, 1023–1030.
- Chung, Y.C., Huang, C., Tseng, C.P., Pan, J.R., 2000. Biotreatment of H<sub>2</sub>S and NH<sub>3</sub>-containing waste gases by co-immobilized cells biofilter. *Chemosphere* 41, 329–336.
- Chung, Y.C., Lin, Y.Y., Tseng, C.P., 2004. Operational characteristics of effective removal of H<sub>2</sub>S and NH<sub>3</sub> waste gases by activated carbon biofilter. *J. Air Waste Manage.* 54, 450–458.
- Chung, Y.C., Lin, Y.Y., Tseng, C.P., 2005. Removal of high concentration of NH<sub>3</sub> and coexistent H<sub>2</sub>S by biological activated carbon (BAC) biotrickling filter. *Bioresour. Technol.* 96, 1812–1820.
- Chung, Y.C., Ho, K.L., Tseng, C.P., 2007. Two-stage biofilter for effective NH<sub>3</sub> removal from waste gases containing high concentrations of H<sub>2</sub>S. *J. Air Waste Manage.* 57, 337–347.
- Crossman, L.C., Moir, J.W., Enticknap, J.J., Richardson, D.J., Spiro, S., 1997. Heterologous expression of heterotrophic nitrification genes. *Microbiol.-UK* 143, 3775–3783.

- Ding, Y., Shi, J.Y., Wu, W.X., Yin, J., Chen, Y.X., 2007. Trimethylamine (TMA) biofiltration and transformation in biofilters. *J. Hazard. Mater.* 143, 341–348.
- Dumontet, S., Scopa, A., Kerje, S., Krovacek, K., 2001. The importance of pathogenic organisms in sewage and sewage sludge. *J. Air Waste Manage.* 51, 848–860.
- Escalas, A., Guadayol, J.M., Cortina, M., Rivera, J., Caixach, J., 2003. Time and space patterns of volatile organic compounds in a sewage treatment plant. *Water Res.* 37, 3913–3920.
- Hernandez, B.S., Higson, F.K., Kondrat, R., Focht, D.D., 1991. Metabolism of and inhibition by chlorobenzoates in *Pseudomonas putida* P111. *Appl. Environ. Microb.* 57, 3361–3366.
- Hunik, J.H., Meijer, H.J.G., Tramper, J., 1992. Kinetics of *Nitrosomonas europaea* at extreme substrate, product and salt concentrations. *Appl. Microbiol. Biot.* 37, 802–807.
- Kapahi, R., Gross, M., 1995. Biofiltration for VOC and ammonia emissions control: odor control. *Biocycle* 36, 87–88.
- Kim, S.G., Bae, H.S., Lee, S.T., 2001. A novel denitrifying bacterial isolate that degrades trimethylamine both aerobically and anaerobically via two different pathways. *Arch. Microbiol.* 176, 271–277.
- Kim, S.G., Bae, H.S., Oh, H.M., Lee, S.T., 2003. Isolation and characterization of novel halotolerant and/or halophilic denitrifying bacteria with versatile metabolic pathways for the degradation of trimethylamine. *FEMS Microbiol. Lett.* 225, 263–269.
- Leson, G., Winer, A.M., 1991. Biofiltration: an innovative air pollution control technology for VOC emission. *J. Air Waste Manage.* 41, 1045–1054.
- Mahmood, S., Prosser, J.I., 2006. The influence of synthetic sheep urine on ammonia oxidizing bacterial communities in grassland soil. *FEMS Microbiol. Ecol.* 56, 444–454.
- Murray, P.A., Zinder, S.H., 1987. Polysaccharide reserve material in the acetotrophic methanogen *Methanosarcina thermophila* strain TM-1: accumulation and mobilization. *Arch. Microbiol.* 147, 109–116.
- Pizarro, J., Jedlicki, E., Orellana, O., Romero, J., Espejo, R.T., 1996. Bacterial populations in samples of bioleached copper ore as revealed by analysis of DNA obtained before and after cultivation. *Appl. Environ. Microbiol.* 62, 1323–1328.
- Prosser, J.I., 1989. Autotrophic nitrification in bacteria. *Adv. Microb. Physiol.* 30, 125–181.
- Qi, B., Moe, W.M., 2006. Performance of low pH biofilters treating a paint solvent mixture: continuous and intermittent loading. *J. Hazard. Mater.* 31, 303–310.
- Raymond, J.A., Plopper, G.E., 2002. A bacterial TMAO transporter. *Comp. Biochem. Phys. B* 133, 29–34.
- Sandberg, M., Ahring, B.K., 1992. Anaerobic treatment of fish meal process waste-water in a UASB reactor at high pH. *Appl. Microbiol. Biot.* 36, 800–804.
- Schlegel, H.G., 1992. *Allgemeine Mikrobiologie*. Thieme Verlag Stuttgart, New York.
- Schroeder, E.D., 2002. Trends in application of gas-phase bioreactors. *Rev. Environ. Sci. Biotechnol.* 1, 65–74.
- Sercu, B., Nunez, D., Van-Langenhove, H., Aroca, G., Verstraete, W., 2005. Operational and microbiological aspects of a bioaugmented two-stage biotrickling filter removing hydrogen sulfide and dimethyl sulfide. *Biotechnol. Bioeng.* 90, 259–269.
- Shieh, W.K., Keenan, D.J., 1986. Fluidized bed biofilm reactor for wastewater treatment. *Biochem. Eng.* 33, 132–168.
- Stouthamer, A.H., De-Boer, A.P., Van-Der-Oost, J., Van-Spanning, R.J., 1997. Emerging principles of inorganic nitrogen metabolism in *Paracoccus denitrificans* and related bacteria. *Anton. Leeuw. Int. J. G.* 71, 33–41.
- Tuhela, L., Robinson, J.B., Tuovinen, O.H., 1998. Characterization of chemotactic responses and flagella of *Hyphomicrobium* strain W1-1B. *J. Bacteriol.* 180, 3003–3006.
- Van-Lith, C., Leson, G., Michelson, R., 1997. Evaluating design options for biofilters. *J. Air Waste Manage.* 47, 37–48.
- Yang, Y., Allen, E.R., 1994. Biofiltration control of hydrogen sulfide 1. Design and operational parameters. *J. Air Waste Manage.* 44, 863–868.
- Yani, M., Hirai, M., Shoda, M., 1998. Ammonia gas removal characteristics using biofilter with activated carbon fiber as a carrier. *Environ. Technol.* 19, 709–715.
- Zhu, J., Bundy, D.S., Li, X.W., Rashid, N., 1997. Reduction of odor and volatile substances in pig slurries by using pit additives. *J. Environ. Sci. Heal. A* 32, 605–619.