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Short Communication

Purification of waste gas containing high concentration trimethylamine in biotrickling filter inoculated with B350 mixed microorganisms

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ABSTRACT

A biotrickling filter packed with ceramic particles and seeded with B350 microorganisms was applied to remove trimethylamine (TMA) from gaseous waste. A 100% removal efficiency (RE) was obtained when the empty bed residence time (EBRT) was larger than 110 s at an inlet concentration of 0.30 mg/L. Maximum elimination capacity (EC) was 13.13 g m^{-3} h^{-1} (RE = 64.7%) at 55 s of EBRT. TMA concentrations <0.20 mg/L at 83 s of EBRT did not affect the REs (100%). Maximum EC was 13.95 g m^{-3} h^{-1} (RE = 78.1%) at a TMA concentration of 0.42 mg/L. Approximately 53.1% of the carbon in TMA was completely mineralized. Bacterial community analysis in the bioreactor revealed more than 21 species in a stable state. Based on all these results, biotrickling filter inoculated with B350 microorganisms is deemed highly capable of ridding waste gas of TMA.

1. Introduction

Trimethylamine (TMA) is a toxic, nitrogen-containing, flammable organic gas with a low odor threshold of 0.2 μg/m^3 (Yaws, 2001). It can be emitted from fish-meal manufacturing plants (Kim et al., 2001) and swine waste storage pits (Ho et al., 2008). Besides its malodorous property and chronic harmful effects on humans, TMA can also inhibit the synthesis of macromolecules and has teratogenic effects on animal embryos (Guest and Varma, 1992). Unlike dimethylamine and methylamine, TMA is biodegraded with great difficulty and results in ammonia accumulation (Ho et al., 2008). Therefore, TMA removal from gaseous waste is important in the field of environmental engineering.

Among biological technologies, biotrickling filtration has been proven effectively in purifying various odorous compounds (Arellano-García et al., 2010; Cox and Deshusses, 2002; Smet et al., 1998) and has attracted considerable interest because of its superiority in terms of mineralization efficiency (Smet et al., 1998). This efficiency results from the liquid mobile phase in the bioreactor, wherein pH, humidity, and mineral nutrient can be better controlled via the cycled trickling liquid. The biotrickling filter with microbes can cost-effectively convert contaminants into harmless end-products under optimal conditions. Microorganisms thus play a major role in the successful biological treatment of gaseous contaminants.

Although TMA could be degraded by various microorganisms in aqueous solution (Liffourrena et al., 2010), till now, there are few reports on the degradation of gaseous TMA using biotrickling filter (Swanson and Loehr, 1997) and much less seeded with B350. Furthermore, reports on the acclimatization of this group of microorganisms and on the characterization of the stable-state microbial community in a biotrickling filter for TMA treatment are scarce.

In the present work, the B350 group of bacteria was seeded in a biotrickling filter to remove TMA gas. The dependence of the production of CO_2 (P_{CO_2}) on elimination capacities (ECs) and the relationship between nitrogen metabolites of TMA and ECs were investigated to confirm the superiority of this system in removing TMA. The stable-state bacterial community in bioreactor during TMA treatment was also analyzed using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE).

2. Methods

2.1. Microorganisms and culture medium

The B350 group, containing 28 species of microorganisms and various enzymes, was purchased from Bio-System Co., USA. The TMA was from Acros, Geel, Belgium, and all other reagents (A.R.) were from Guangzhou Chem. Reagent Co., Inc., China. Mineral medium (MM) was used as the culture medium (the recipe is listed in the Supporting Information).

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2.2. Apparatus and TMA removal process

All experiments were performed in a biotrickling filter, the configuration and system setup of which were reported in our previous work (Wan et al., 2011). The total height and volume of the packing ceramic particles were 600 mm and 9.23 L, respectively. After the filter was seeded with B350, TMA-containing waste gas was continuously fed into the bioreactor. MM was periodically trickled from the top of reactor at a rate of 7.5 L/h for 10 min each time, 16 times a day. After start-up, REs and ECs of TMA were evaluated by changing gas flow rate (from 200 to 800 L/h, corresponding to EBRT from 166 to 42 s) and inlet concentration (from 0.10 to 0.60 mg/L). The detailed analytical methods to determine gaseous TMA, CO_2 and the various N species in the recirculating liquid are shown in the Supporting Information.

2.3. Biofilm and bacterial community analysis

The biofilm mass (expressed in mg/g of dry ceramic particles) was determined by the weight loss of 50 g of bacteria-supported ceramic particles (Arnaiz et al., 2007). Optical microscopy and scanning electron microscopy (SEM) were employed to observe biofilm formation on the ceramic particles. Biofilm thickness was calculated according to An et al. (2010).

DNA was extracted for PCR amplification as follows. A total of 10 g of ceramic particles were sampled and mixed with 30 mL sterile water. The mixture was shaken at 120 rpm for 30 min and the supernatant was centrifuged at 7000 rpm for 15 min to get bacterial pellets. DGGE was then performed using a mutation detection system, and the sequencing of DGGE fragments were performed at a DNA sequencing facility. The obtained sequences were used to build bacterial community with a phylogenetic tree using neighbor-joining method on free program Mega 4.0. The evolutive distance was based on Kimura 2-parameter model (shown in Supporting Information).

2.4. Calculation

The performance of the biotrickling filter was evaluated in terms of two independent parameters, RE and EC, as described in our previous paper (Wan et al., 2011). P_{CO_2} (g m^{-3} h^{-1}) was calculated according to Elmrini et al. (2004):

$$P_{CO_2} = Q(C_{CO_2,out} - C_{CO_2,in})/(1000V) \quad (1)$$

where $C_{CO_2,in}$ and $C_{CO_2,out}$ are CO_2 concentrations (in mg/L) measured at inlet and outlets of reactor, respectively; Q is gas flow rate (L/h); and V is packing volume (m^3).

3. Results and discussion

3.1. Bioreactor start-up

The start-up performance of biotrickling filter (days 1–23) is shown in Fig. S1. On day 1, 1.10 mg/L gaseous TMA was fed into bioreactor at an EBRT of 110 s. A high RE of 96.9% was obtained due to the adsorption of the packing and medium liquid. However, on day 2, RE decreased to 54.3% as inlet concentration decreased to 0.53 mg/L. When concentrations were maintained at about 0.5 mg/L (days 6–9), REs increased steadily, indicating gradual formation of biofilm. Finally, REs increased to 100% on day 17 when concentration was kept at 0.30 mg/L at EBRT 110 s, indicating that 17 days was enough for the start-up of the biotrickling filter inoculated with B350.

Optical microscopy and SEM were employed to obtain biofilm formation information. As shown in Figs. S2a and S2b, the coarse and porous surface of raw ceramic particle is observed, which is favorable to bacteria immobilization. Comparatively, abundant rod and zoogloea bacterial biofilm were successfully developed onto ceramic particle (Figs. S2c and S2d). Biomass determination (Fig. S3) proved that no bacteria initially grew on ceramic particle, and that bacteria gradually grew with the start-up time. Biomass increased to 2.75, 7.67, and 39.62 mg/g, corresponding to biofilm thicknesses of 70.1, 185.8, and 754.5 μm on the days 10, 30, and 60, respectively. The results indicated that B350 was immobilized on packing and that biotrickling filter was successfully started up with a high TMA degradation ability.

3.2. Bioreactor performance in terms of TMA removal

The effect of the EBRT on the performance of biotrickling filter seeded with B350 was investigated at a fixed concentration of 0.30 mg/L (Table S1). Total REs of 100% were achieved for EBRTs larger than 110 s, and more than 50% of TMA was degraded after passing layers 1–3. When the EBRT was shortened, the total REs decreased gradually to 64.7% and sharply to 49.6% at EBRTs of 55 and 47 s, respectively. Obviously, the inlet load exceeds the degradation ability of B350 in such a short EBRT. Bioreactor performance was also evaluated in terms of EC with various EBRTs (Fig. S4). As the EBRT decreased from 166 to 55 s, the total ECs of layers 1–3 increased from 4.82 to 7.68 g m^{-3} h^{-1}. Further decreases of the EBRT to 47 and 42 s led to decreases in the total ECs of layers 1–3 of 7.66 and 6.58 g m^{-3} h^{-1}, respectively. Similarly, for layers 4–6, the maximum total EC was achieved at an EBRT of 55 s. However, layers 1–3 played more important roles because more than half of the total TMA was eliminated. The ECs plotted against the inlet loads are illustrated in Fig. S4b. REs of 100% were achieved with inlet loads less than 9.96 g m^{-3} h^{-1}. When the EBRT was decreased to 55 s, the inlet load increased to 20.30 g m^{-3} h^{-1}, and accordingly, total ECs increased and peaked at 13.13 g m^{-3} h^{-1} (RE = 64.7%). As the EBRT was further decreased to 42 s, the EC slightly decreased to 11.10 g m^{-3} h^{-1} (inlet load = 26.00 g m^{-3} h^{-1} and RE = 42.7%). Both RE and EC achieved in the present work are higher than in the report of Ding et al. (2007), where in a compost and sludge biofilter for TMA treatment, the maximum REs achieved were only 60% and 81%, and the maximum ECs were only 9.1 and 9.3 g m^{-3} h^{-1}, respectively.

The effect of TMA concentration (0.12–0.60 mg/L) on bioreactor performance was also investigated at an EBRT of 83 s (Table S2). REs decreased with increased inlet concentration. Total REs of 100% were obtained on layers 3 and 6 at concentrations of 0.12 and 0.20 mg/L, respectively. When the concentrations were increased to 0.31 and 0.60 mg/L, total REs dropped slightly at first to 97.9% and then swiftly to 48.4%. At higher inlet concentrations (0.12–0.31 mg/L), our system could eliminate TMA more effectively (>97.9%) compared to the results in another report (Ho et al., 2008). Bioreactor performance was also evaluated in terms of TMA ECs for various inlet concentrations at EBRT 83 s (Fig. 1). Total ECs always increased with increased inlet concentrations. Total EC was only 5.04 g m^{-3} h^{-1} at a concentration of 0.12 mg/L (inlet load = 5.04 g m^{-3} h^{-1}). As concentration increased to 0.42 mg/L (inlet load = 18.13 g m^{-3} h^{-1}), total EC increased to 14.15 g m^{-3} h^{-1} (Fig. 1a). When concentrations were less than 0.20 mg/L (Fig. 1b), 100% REs were achieved. However, total EC decreased to 12.55 g m^{-3} h^{-1}, corresponding to an abrupt RE drop of 48.4% when concentration was further increased to 0.60 mg/L (inlet load = 25.93 g m^{-3} h^{-1}).

Generally, REs always decreased with decreased EBRTs and increased inlet concentrations. The probable reason for this is that REs are controlled by both mass transfer of TMA from air to biofilm (diffusion limitation) and biodegradation process (reaction limitation) in bioreactor (Jin et al., 2005). Longer EBRTs resulted in higher

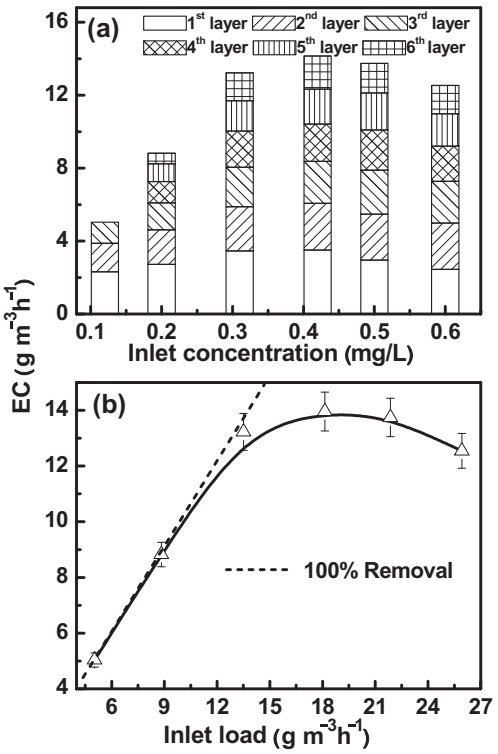

Fig. 1. Changes in TMA ECs at different inlet concentration with fixed EBRT.

REs because of the adequate time for organic molecules in the biofilm to undergo complete biodegradation. Thus, overall REs here were controlled only by the diffusion limitation. On the other hand, a reaction limitation may have occurred with a shorter EBRT. When the EBRT was short, the inlet loads and the TMA transfer rate from air to the biofilm increased. The time was not sufficient for the microbes to degrade excessive TMA. Consequently, ECs initially increased to the maximum level and then either remained constant or decreased, whereas REs dropped gradually because of the limited contact time between the biofilm and TMA. Similarly, increased inlet concentration at a fixed EBRT resulted in increased TMA transfer rate from air to biofilm. A higher concentration gradient improved mass transfer in the bioreactor and resulted in a reaction limitation, wherein bacterial activity became a limiting factor for TMA removal. In addition, high inlet concentration might enhance biomass production, and then excessive biomass might increase the thickness of the biofilm, decrease the porosity of the packings, and block the air flow into the bioreactor. On the contrary, the EC first increased at the diffusion limitation range and remained at almost the maximum level at the reaction limitation range. In the present study, increased inlet load had no significant inhibition on TMA biodegradation, indicating that a biotrickling filter seeded with B350 was highly capable of removing TMA from gaseous waste.

3.3. TMA metabolites

P_{CO_2} is an important indicator of the mineralization degree of organics. It was thus measured because the microbes will ultimately convert TMA to H_2O and CO_2. Assuming complete mineralization of TMA (ignoring biomass generation), the stoichiometric reaction can be described as follows:

$$C_3H_9 + xO_2 \rightarrow yCO_2 + zH_2O + HNO_3 + w \text{ biomass}, \quad (2)$$

Fig. 2a shows a linear relationship between P_{CO_2} and ECs from both theoretical and experimental data, and slope values of 2.24 and 1.19, respectively, were obtained. This means that CO_2 masses produced per mass of eliminated TMA were approximately 2.24 (theoretically) and 1.19 (experimentally). The experimental slope was 53.1% of the theoretical slope, indicating that about 53.1% of TMA carbon was completely mineralized and that 46.9% remained as biomass, intermediates, or other forms.

Kim et al. (2001) reported that TMA could initially oxidize to dimethylamine by way of TMA N–oxide, then to methylamine by enzymes during biodegradation, and then finally oxidized to formaldehyde and NH_3 under aerobic conditions. However, only one intermediate, dimethylamine, was detected in the gas phase in the present study (Fig. S5). Thus, to best understand the metabolites of TMA, nitrogen accumulation was investigated dynamically in the recirculating liquid. As shown in Fig. 2b, the amount of ammonia–N increased from 5.87 to 12.90 g from days 15 to 30 as TMA–N fed into the biotrickling filter increased from 8.48 to 17.52 g, respectively. Ammonia–N initially decreased slightly to 12.51 g, then dramatically to 8.99 g, when TMA–N increased from 22.38 to 27.73 g on days 40 and 50, respectively. This is due to the formation of nitrate and nitrite by nitrification of the microorganisms. Nitrate–N particularly exhibited a gradual accumulation from 2.12 to 14.44 g from days 15 to 50. In addition, 69.2%, 73.6%, 55.9%, and 32.4% of TMA–N were converted into ammonia–N, whereas 25.0%, 19.8%, 25.8%, and 52.1% were converted into nitrate–N on days 15, 30, 40, and 50, respectively. Nitrite–N in the recirculating liquid was notably less than 0.1% during the operation period, indicating rapid transformation of nitrite–N into nitrate–N.

3.4. Bacterial community analysis by PCR-DGGE

The traditional plate-counting method might underestimate the actual bacterial number because only cultivable and viable cells are

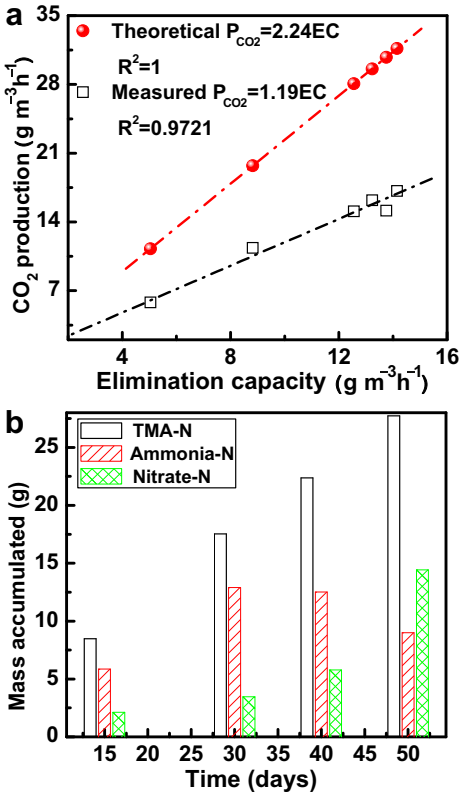

Fig. 2. (a) Theoretical and measured P_{CO_2} versus EC. (b) Analysis of nitrogen element balance.

detected on agar plates (Amann et al., 1995), and SEM only reflects the shape and size of dominant microorganisms. PCR-DGGE was thus employed to analyze the microorganism community at stable state in the bioreactor. Fig. 3a shows the gel-purified PCR products of DNA extraction from the biofilm. The corresponding gene fragment was approximately 240 bp, which is suitable for DGGE analysis. Based on DGGE profiles (Fig. 3b), the bacterial community was highly affected by the accumulation and selective growth of B350 onto the packings as well as the TMA biodegradation process. Among the 28 initial species in B350, more than 21 bands (species) could be detected at stable state in the bioreactor. This suggests that the bacterial community underwent dramatic changes during biofilm formation and maintenance processes due to their different attachment abilities onto the packings and the loss of some low immobilized bacteria from the reactor. Some microbes may have not propagated properly and were thus not detected in the DGGE gel because of their very low concentrations (Yang et al., 2011). Among the detected bands, six dominated the bacterial community at stable state in the bioreactor.

Bands A1–6 were thus excised from the DGGE gel. Their nucleotide sequences were determined (see Supporting Information) and compared with 12 strains based on the nucleotide sequences alignment and phylogenetic tree analyses (Figs. S6–S12). The analyses indicated that the predominant populations represented by bands A1, A5, and A6 have 100% sequence similarity with *uncultured bacterium* clone PPSB-U1 (EU138870), Cday32-37 (HQ011776), and (FJ406571), respectively. Minor populations represented by bands A2, A3, and A4 have 93%, 100%, and 95% sequence similarity with *uncultured bacterium* (FM956738), clone K37 (EU834755), and clone G30-48 (HQ132203), respectively. In summary, although the bacterial community was clearly very sensitive to the TMA fed as the sole carbon source, a new bacterial community could be re-established at stable state in the biotrickling filter, which is the reason for the high REs and ECs of TMA achieved in the present study.

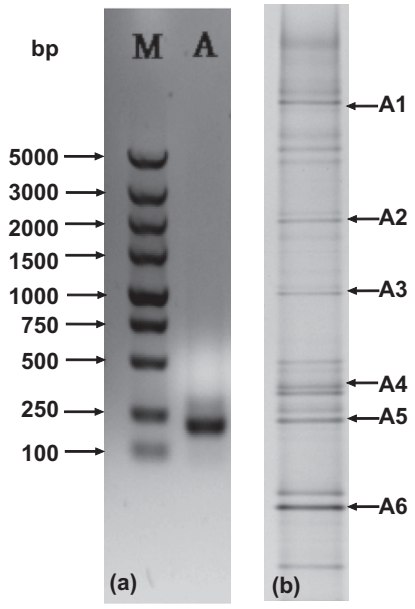

Fig. 3. (a) Agarose gel electrophoresis of PCR product from biofilm DNA. Line M: DNA marker DS 5000; line A: product from biofilm DNA. (b) PCR-DGGE profile of the bacterial at stable state in the biotrickling filter.

4. Conclusions

A biotrickling filter inoculated with B350 excellently purified TMA-containing waste gas. The maximum EC of 13.13 g m^{-3} h^{-1} was obtained at an EBRT of 55 s, and 100% REs were maintained at concentrations <0.20 mg/L at an EBRT of 83 s. TMA could be partially decontaminated into CO_2, ammonia, and nitrate. The stable-state bacterial community in the biotrickling filter was dominated by six species, namely, *uncultured bacterium* clone PPSB-U1 (EU138870), Cday32-37 (HQ011776), (FJ406571), (FM956738), K37 (EU834755), and G30-48 (HQ132203). The high performance of the bioreactor was attributed to these populations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.03.059.

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