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Continuous deodorization and bacterial community analysis of a biofilter treating nitrogen-containing gases from swine waste storage pits

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Abstract

A biofilter inoculated with *Arthrobacter* sp. was applied to the simultaneous elimination of trimethylamine (TMA) and ammonia (NH_3) from the exhaust air of swine waste storage pits. The results showed that the biofilter achieved average removal efficiencies of $96.8 \pm 2.5\%$ and $97.2 \pm 2.3\%$ for TMA and NH_3, respectively. A near-neutral pH (7.3–7.4) was maintained due to the accumulation of acid metabolites and the adsorption of alkaline NH_3. Low moisture demand, low pressure drop and high biofilm stability in the system were other advantages. After long-term operation, the bacterial community structure showed that at least twenty-five bands were explicitly detected by a denaturing gradient gel electrophoresis (DGGE) method. However, the inoculated *Arthrobacter* sp. still maintained a dominant population (>50%). *Paracoccus denitrificans*' presence in the biofilter could play an important role in oxidizing NH_3 and reducing nitrite by heterotrophic nitrification and anaerobic denitrification.
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1. Introduction

In order to increase waste handling flexibility, swine wastes need to be separated by a solid–liquid separator (Su et al., 1997; Zhu et al., 2001). However, nitrogen-containing gases like trimethylamine (TMA), dimethylamine (DMA), methylamine (MA) and ammonia (NH_3) are often emitted from swine waste storage pits and cause a nuisance in the vicinity of these swine facilities. Among the nitrogen-containing waste gases, TMA and NH_3 are major odor components, and they are colorless, have low threshold limits, and are potentially toxic to humans (Guest and Varma, 1992; Wright et al., 2005; Bai et al., 2006).

In earlier literature, the use of microorganisms to degrade TMA has been established in the field of waste water treatment, but less is known about the application of waste gases (Warren et al., 1997). Recently, Chang et al. (2004) used an aerobic biofilter containing entrapped mixed microbial cells from activated sludge to reduce TMA gas. The removal efficiency was higher than 90% at inlet loading below 27.2 mg-N/h, but a long gas residence time of 5.3 min and the accumulation of NH_3 metabolite resulted in obstacles for the application. On the other hand, biofiltrations applied to the reduction of NH_3 emissions from swine facilities have shown removal efficiencies of approximately 8.4% in a biochip filter (Martens et al., 2001), 41–94% in a biotrickling filter (Melse and Mol, 2004) and 64–93% in wood chip biofilters (Sheridan et al., 2002). A combination of granular activated carbon (GAC) as packing material and *Arthrobacter* sp. as inoculant has been shown more effective (>99%) in treating NH_3 (Chung et al., 2004) than different types of laboratory-scale biofilters under similar conditions (Kapahi and Gross, 1995; Yani et al., 1998). Although NH_3 or TMA removal

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by biofiltration has obtained efficiency to a certain extent, the removal of a single compound under a constant operation condition is impractical for field application. Besides, there are still many unknowns, including the removal performance of the system and diversity of the bacterial comunity on a molecular level during a long-term field operation. Amann et al. (1995) have indicated that up to 99% of the microorganisms which are active in biofilters are not culturable by traditional cultivation methods. In recent years, molecular methods have been successfully used to analyze the bacterial community structure in various environments. Among them, the DGGE technique is a powerful tool that can analyze the bacterial community on a molecular level (Muyzer et al., 1993; Cebron and Garnier, 2005; Chen and LaPara, 2006).

To overcome and examine mixed waste gas emissions and track the succeeding changes in the bacterial community in the biofilter during a long-term operation, we simultaneously removed two major nitrogen-containing wastegases TMA and NH_3 during short gas residence times by using a GAC biofilter inoculated with *Arthrobacter* sp. Extensive research was conducted to evaluate the performance of the biofilter during a long-term operation, including aspects like removal efficiency, shutdown effect, pressure drop and metabolites. In addition, DGGE was used to understand the distribution and composition of bacterial communities and provided direct evidence to demonstrate that the inoculated *Arthrobacter* sp. was dominant in this biofilter during long-term operation.

2. Methods

2.1. Microorganism cultivation and medium preparation

The heterotrophic nitrifier *Arthrobacter* sp. was isolated from swine waste water, and some elementary experiments in the laboratory were effective for TMA and NH_3 removal (data not shown). *Arthrobacter* sp. was enriched in nutrient broth at 26 °C, and the broth contained yeast extract 5 g/L, tryptone 10 g/L and dextrose 2 g/L. In long-term experiments, the inflow medium was used and contained glucose 1.0 g/L, KH_2PO_4 5.4 g/L, K_2HPO_4 10.5 g/L, $MgCl_2 \cdot 6H_2O$ 0.2 g/L and Fe(III)-citrate 0.01 g/L. The pH was finally adjusted to 7.0 using 0.1 N NaOH or HCl. The phosphate buffer capacity was calculated as 54.6 mM/pH.

2.2. Immobilization procedure

GAC was used as the support material with a uniform size of 4.5 mm, bulking density of 0.48 g/cm^3 and specific surface area of 1050 BET-m^2/g. *Arthrobacter* sp. was grown in 10-L nutrient broth for one day before being harvested by centrifugation at 7000 rpm for 15 min. The pellets were put into a 300-L PVC tank containing 100 L of nutrient broth for bacterial growth. About 105 kg of GAC were mixed with the above solution for bacterial attachment. Fresh broth was added every three days until the number reached about 3.4×10^8 CFU/g-GAC. The cell-laden GAC was then packed into the biofilter. In addition, all materials and implements were maintained in aseptic conditions as far as possible during the above operation.

2.3. Apparatus and odor removal for continuous operation

In this study, our objective was the removal of odor components TMA and NH_3 because they were the main source of odor from the swine waste storage pits. To investigate the capacity of GAC to adsorb TMA and NH_3, respectively, GAC without any microorganisms the bed depth service time (BDST) experiment, as described by Chung et al. (2005), will be performed in laboratory.

Fig. 1 shows a schematic diagram of the GAC biofilter designed to reduce TMA and NH_3 emissions, and this system was set up in a swine farm in the city of Hsinchu in Taiwan. One PVC column (48 cm $\phi \times 120$ cm of working height) was packed with cell-laden GAC and supported by a perforated sieve plate at the bottom of the column. The packed volume and weight of GAC were 217 L and 104.2 kg, respectively. The column wall contained three sampling ports, including one at the middle for GAC sampling, one at the crown and one underneath for measuring gas concentrations of the inlet and outlet. Air was extracted

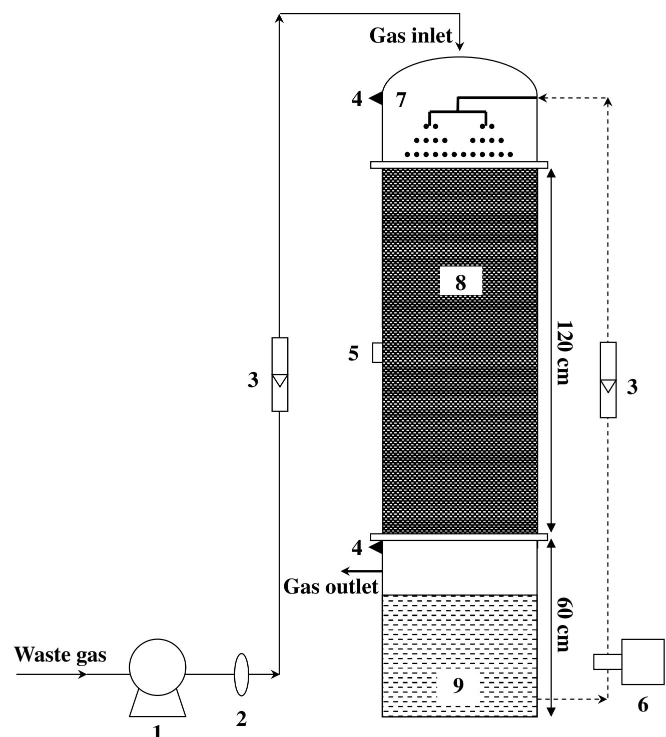

Fig. 1. A schematic diagram of the GAC biofilter: (1) exhaust fan; (2) air filter; (3) flow meter; (4) gas sampling port; (5) GAC sampling port; (6) peristaltic pump; (7) sprinkler zone; (8) the biofilter bed inoculated with *Arthrobacter* sp.; (9) nutrient tank.

and pumped with an exhaust fan. It first passed an air filter (pore size of 0.16 mm) to leach nonessential impurities and then flowed downward through the top of the biofilter. The flow meter and valve were used to monitor and control the gas flowing through the system. An inflow liquid medium (see medium preparation) stored in a nutrient tank (48 cm $\phi \times 60$ cm of working height) located at the bottom of the biofilter bed was re-circulated by a peristaltic pump at 8 L/min for 10 min, six times a day. The medium volume was maintained at 70 L by periodically adding distilled water. The peristaltic pump was connected to a spray nozzle located on the top of the biofilter to uniformly spay the medium on the surface of the biofilter bed. 0.1% of glucose as carbon source was added once every two weeks. For estimating the system performance, waste gases were supplied to the system at various empty bed gas residence times (EBRT) in the range of 20–45 s.

2.4. DNA extraction

GAC (0.5 g) mixed with 5 ml of sterile water were vortexed for 3 min, and the supernatant was centrifuged at 7000 rpm for 15 min. The pellet was treated by enzymatic digestions (lysozyme and proteinase K), sodium dodecyl sulfate (SDS) treatment and freeze-thaw cycles followed by phenol-chloroform extractions as described by Ausubel et al. (1988).

2.5. Polymerase chain reaction (PCR) amplification

PCR primers for 968fGC (5′-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC-3′) and 1401r (5′-CGG TGT GTA CAA GAC CC-3′) were used to amplify the segment of eubacterial 16S rDNA from nucleotide 968 to 1401 for the numbering in *E. coli* sequence (Brosius et al., 1981). PCR amplification reaction was performed using an MJ Research PTC-200 Peltier thermal cycler at a final volume of 50 μL containing 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTP, 25 pmol of each primer, 0.5 U of *Taq* DNA polymerase (Amplitaq Gold, Perkin–Elmer) and 10–50 ng of DNA. The thermal cycle was performed by use of 35 cycles of 1 min at 94 °C, 1 min at 58 °C and 1 min at 72 °C, followed by a final step at 72 °C for 10 min.

2.6. DGGE and phylogenetic analysis

DGGE was performed by using a mutation detection system (Bio-Rad D-Code System). Gels were made in a denaturant gradient (45–60%) with 8% (wt/vol) polyacrylamide gels (Bio-Rad). The electrophoresis was run and stained as described by Nakatsu et al. (2000). Gel images were captured using a Bio-Rad gel documentation system and analyzed using the Bio-Rad image program (Quantity One 4.5.0) for qualitative analysis. DNA fragments were cut from DGGE gels, and diffused into the water as described by Kinnunen and Puhakka (2004). One microliter of the eluate was used as template in a PCR process with the primers 968f (without GC clamp) and 1401r and the PCR program described above. The purified products were sequenced by an ABI-Prism model 377 automatic sequencer (Perkin–Elmer, USA). Sequences were compared with the GenBank database by using the BLASTN facility of the National Center for Biotechnology Information (NCBI) in an effort to search for the closest evolutionary relative. Matrices of evolutionary distances were calculated and drawn by using neighbor-joining methods and the Clustal W program (Thompson et al., 1994). Phylogenetic trees were constructed from the evolutionary distance using Tree View software.

2.7. Scanning electron microscopy (SEM)

GAC samples were individually taken out on the 327th and 523th days, and treated with glutaraldehyde 3% solution buffered with 0.1 M sodium phosphates to fix the cells. This was followed by dehydration in ethanol, critical point drying with a critical point dryer, mounting on aluminum stubs using double-sided tapes and then sputter-coating with gold (Chung et al., 2004). Micrographs were obtained from a Hitachi S4500 scanning electron microscope.

2.8. Analytical methods

TMA gas concentrations were analyzed in a Clarus 500 gas chromatograph (Perkin–Elmer, USA) equipped with a 0.32 mmID Stabilwax-DB column (RESTEK, USA) and a flame-ionization detector (FID). NH_3 gas concentrations were measured with a portable ammonia-monitoring device (ATTAIN, Japan). The pressure drop was measured across the biofilter using a U-tube water manometer, and the unit was expressed as mm-H_2O/m-biofilter height. The pH value in leachate was determined by the analysis of circulating liquids using a pH meter. To determine the moisture content in GAC, about 0.5 g were weighed and dried over a 24-h period at 103 ± 0.5 °C. In addition, 2.0 g of GAC was periodically withdrawn, mixed with 20 mL of distilled water, and vortexed for 3 min. The suspension solutions were then assembled to analyze the pH value of GAC, the metabolic products (e.g. nitrate, nitrite, ammonium and organic nitrogen) and the number of *Arthrobacter* sp. using Hagedorn and Holt selective medium (Hagedorn and Holt, 1975). The total cell number was expressed as the number of colony forming units per gram dry GAC (CFU/g-GAC). Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations in solution were measured by ion chromatography (Dionex 4500i). Ammonium (NH_4^+) concentration in solution was determined using an ion-specific electrode. Amine concentrations in solution were measured with 1.0 μL direct injection by GC. Organic nitrogen was determined by the Kjeldahl method (Chung et al., 2004).

3. Results and discussion

3.1. System performance in terms of TMA and NH_3 removals

The profile of removal efficiencies and inlet concentrations for TMA at various EBRTs during the 523-day operating period is indicated in Fig. 2. According to the calculation from the BDST experiment (Chung et al., 2005), the theoretical saturation time of GAC was about 6 days for single TMA gas by estimating actual inlet concentration and gas flow rate. However, 100% removal efficiency took 22 days. This showed that TMA removal was not only adsorbed by GAC, but also mainly oxidized by *Arthrobacter* sp. or other microbes in the biofilter. After theoretical saturated adsorption of GAC (the 6th day), a dynamic equilibrium was achieved between adsorption and biodegradation, and steady-state efficiency (97%) could be performed for 0.4–4.5 ppm of TMA (loading: 31–308 mg-N/m^3/h) on the 60th–187th day. Even though the biofilter was manipulated at an EBRT of 20 s, TMA removal efficiencies, maintained at 91.9–93.5% (92.9 $\pm$ 0.4% on average), were acceptable on the 348th–424th day (34–251 mg-N/m^3/h). TMA removal ranged from 91% to 100% with an overall average of 96.8 $\pm$ 2.5% with inlet concentration below 5.2 ppm (loading below 355 mg-N/m^3/h) during 523 days. A report in the literature on the use of an aerobic biofilter to treat TMA gas has shown that TMA removal is higher than 90% at inlet loading below 27.2 mg-N/h. However, a long residence time of 5.3 min is needed (Chang et al., 2004). Compared with those results, the GAC biofilter eliminated TMA emissions more effectively at shorter EBRTs.

Fig. 3 shows the profile of removal efficiencies and inlet concentrations for NH_3 during operating periods. According to the calculation, the saturated adsorption time by GAC alone was about 11 days. In fact, 100% removal took 25 days. After dynamic equilibrium, nearly 96% removal could be maintained at an EBRT of 30 s on the 25th–187th day (22–594 mg-N/m^3/h), except during the shutdown and restart-up periods (the 46th–73th day). When the NH_3 inlet suddenly rose to 7.2 ppm (604 mg-N/m^3/h) on the 196th day, the efficiency fell perceptibly 5.1% from 95.7% to 91.6% at an EBRT of 25 s. However, when 6.7 ppm of NH_3 (404 mg-N/m^3/h) was introduced at an EBRT of 35 s on the 263th day, the removal efficiency (98%) did not significantly change. A similar situation was also found on the 288th day. When the biofilter was conducted at the short EBRT (20 s), the system was still effective in treating NH_3 emissions with an average removal efficiency of 94.2 $\pm$ 0.7% on the 348th–424th day (33–537 mg-N/m^3/h). These results indicated that as flow rate increased, residence time decreased, and NH_3 gas should not have had sufficient time to diffuse into the biofilm and be readily biooxidized. NH_3 removals ranged from 88% to 100% with an overall average of 97.2 $\pm$ 2.3% at inlet concentrations below 8.5 ppm (loading below 604 mg-N/m^3/h) in the 523-day operation. Previous reports on reducing NH_3 emission from swine facilities have shown that the removal efficiencies were approximately 8.4% in a biochip filter (Martens et al., 2001), 41–94% in a biotrickling filter (Melse and Mol, 2004) and 64–93% in wood chip biofilters (Sheridan et al., 2002). In this study, the GAC biofilter has better performance and stability for NH_3 removal based on a long-term evaluation of field operations.

In addition, in order to meet the ambient air standards at 0.02 and 1.0 ppm for TMA and NH_3, optimal operating conditions are needed for better overall performance. In this study, we found that TMA outlet would exceed the standard level of 0.02 ppm when the biofilter was conducted at an EBRT of 35 s. However, the system demonstrated high removal efficiency in treating NH_3, and its

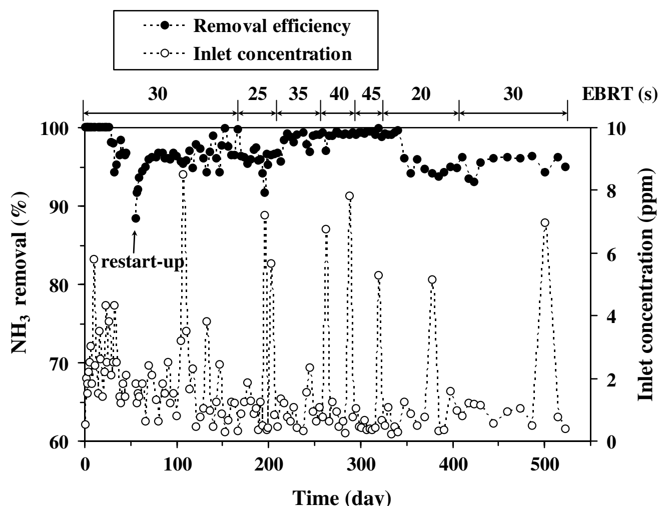

Fig. 3. Profile of removal efficiencies and inlet concentrations for NH_3 at various EBRTs during operating periods.

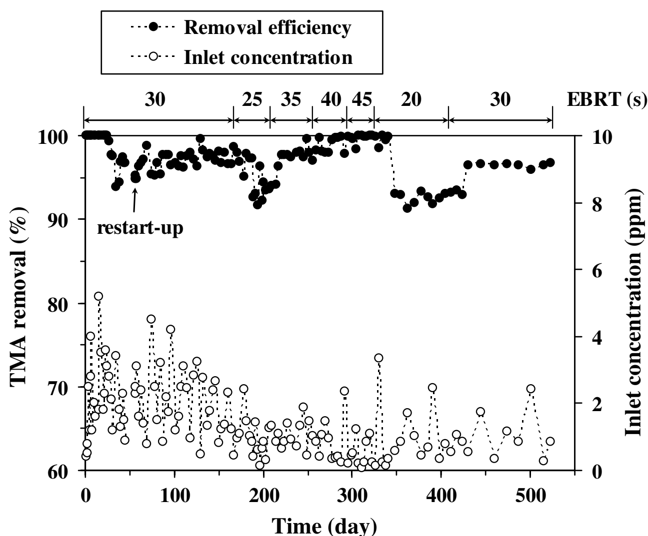

Fig. 2. Profile of removal efficiencies and inlet concentrations for TMA at various EBRTs during operating periods.

outlet concentrations were lower than 1.0 ppm during the short EBRT (20 s). An EBRT of at least 40 s must be controlled, and the biofilter could simultaneously meet both standard levels of ambient NH_3 and TMA for air quality regulation. When the biofilter was not located in residential areas, it could meet both standards at an EBRT of less than 40 s because the odor would be diluted by diffusion. In contrast to TMA removal, NH_3 is less sensitive to changes in EBRT. Inlet loadings of NH_3 are higher than TMA, but NH_3 is easier to treat than TMA in this system, as a previous report has noted (Chang et al., 2004).

3.2. Effect of shutdown on biofilter ability

The biotreatment systems in many manufacturing industries are exposed to discontinuous emissions of pollutants because of either producing process rotation or weekend shutdown. An acclimation period may be necessary when the biosystem is restarted (Deshusses et al., 1996; Swanson and Loehr, 1997). The same situation also occurred on the swine farm. Hence, to examine the influence of shutdown on the biofilter without liquid medium or air including waste gases is very important. In this study, a 10-day shutdown (the 46th–55th day) was carried out, and results are shown in Figs. 2 and 3. Removal efficiencies of TMA and NH_3 were nearly 96% on average, and *Arthrobacter* sp. was approximately 5.62×10^8 CFU/g-GAC before shutdown. The number of *Arthrobacter* sp. dropped to 1.28×10^8 CFU/g-GAC when lacking carbon (Glucose and TMA) and nitrogen sources (NH_3). However, when mixed waste gases were introduced into the biofilter again, TMA and NH_3 removal was only reduced by 1.9% and 8.3%, respectively. The slight difference in the bacteria number might be because NH_3, TMA or Glucose absorbed on GAC could be impermanently supplied to the predominant *Arthrobacter* sp. or other microbes during a 10-day shutdown. This could be confirmed by an increase of metabolites (NO_2^-) (data not shown). A greater than 94% TMA removal was still maintained even after administration of a 10-day shutdown. Further, the system required 4–18 days to recover its original high efficiency (96%) for TMA and NH_3 when waste gas was introduced to the biofilter with a similar inlet loading as before. In general, the time required for biofilter reacclimation after shutdown is proportional to the duration of the shutdown (Kinney et al., 1999). Chen et al. (2005) also found that a biofilter required several days or even as long as 3 months to recover its preshutdown performance in treatment of NH_3 after longer shutdown periods (>7 days). These results show the GAC biofilter is able to cope effectively with TMA and NH_3 emissions from discontinuous sources, and its resilience can at least match that of other biofilters.

3.3. Changes of pH value, moisure content and pressure drop

A neutral pH can be attributed to a dynamic equilibrium between the accumulation of acid metabolites and the adsorption of NH_3, and it is required to maintain the optimal activity of *Arthrobacter* sp. and operational stability for NH_3 removal (Chung et al., 2005). The stable pH can be considered advantageous since this is optimum for most biofilter operations (Leson and Winer, 1991; Devinny et al., 1999). In this study, the pH was measured once a week. A slight alkalinity phenomenon was observed during the first 15 days of operation, and pH in the GAC and leachate gradually increased from 6.83 to 7.55 and 7.01 to 7.92, respectively, demonstrating the absorption of TMA and NH_3. After the adsorption period, the biodegradation activity progressively achieved a dynamic equilibrium. Therefore, TMA and NH_3 might be utilized by the predominant *Arthrobacter* sp. within the biofilm and decomposed them to nitrite with the production of H^+, reducing the pH of the system. A slow decrease in pH values was observed on the 56th day while a near-neutral pH was stably maintained (7.3–7.4) for a long-term (data not shown).

In general, the pressure drop across a biofilter depends on the air flow rate, characteristics of the packing media (like particle size, moisture content) and the height of the biofilter. Pressure drop increases with operating time due to aging and compacting of the media and to large biomass accumulations (Morgan-Sagastume et al., 2001). Since pressure drop contributes to treatment costs, its relationship to the moisture content of media is crucial to effectively operating the biofilter. Fig. 4 shows the change of moisture content and pressure drop in the biofilter during operating periods. The average distributions of moisture content were $41.7 \pm 0.6\%$. This suggested that the moisture content was less sensitive to changes of EBRT because of GACs stable water-holding capacity or an intermittent sprinkling of water. In fact, a moisture content above

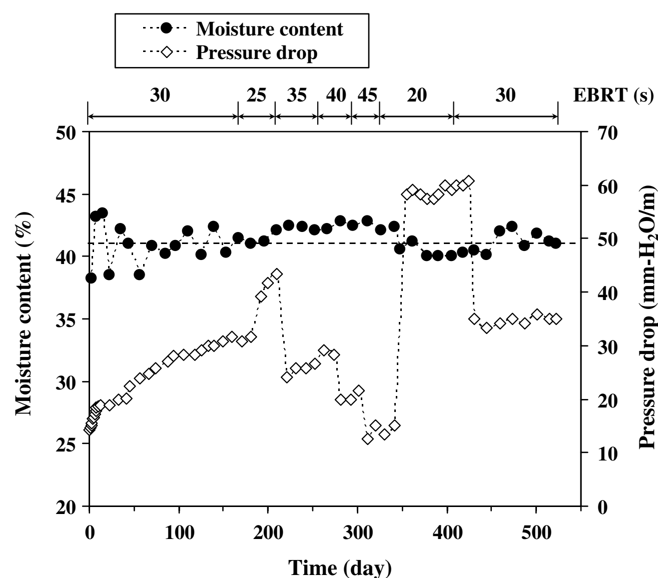

Fig. 4. Changes of moisture content and pressure drop in the biofilter during operating periods. Dashes (—) indicates the average value of moisture content.

40% was suggested as optimal for biofilters seeking to maintain biological activity (Leson and Winer, 1991). Thus, the GAC biofilter had a lower moisture demand than other biofilters (Sercu et al., 2003; Pandey et al., 2006).

The changes in pressure drop across the biofilter were checked weekly (Fig. 4). The pressure drop tended to increase with operating time due to an increase in the compact intensity of the biofilter bed by gas compression or gravity and reached a steady state (31.3 ± 0.5 mm-H_2O/m) on the 150th day. When EBRT decreased to 25 and 20 s, pressure drops increased to 41 and 59 mm-H_2O/m, respectively. However, when an EBRT of 30 s was established again on the 431th day, pressure drop immediately fell to 35 mm-H_2O/m (near the 150th day). The biomass in this system had accumulated (5.6×10^9 CFU/g-GAC) after 523 days of operation, but the change of pressure drop varied only slightly (3–4 mm-H_2O/m) at the same EBRT (30 s). The pressure drop was approximately in the range of 14–61 mm-H_2O/m during long-term operation, and no sudden change (at the same EBRT) in pressure drop occurred across the biofilter. A similar variation occurred in our previous study, and no additional increase in pressure drop was observed (Chung et al., 2004). This demonstrated that the GAC had good mechanical strength that led to negligible bed compression and short-circuiting during operating periods, and this corresponded well with an operational limit (below 300 mm-H_2O/m) for biofilters (Huang and Shen, 1998). An intermittent sprinkling of water was also helpful in reducing pressure drop. The pressure drop across the GAC in the biofilter was apparently minimal (14 mm-H_2O/m) under a higher air velocity (96 m/h), and might be energy saving as compared with a compost biofilter (110 mm-H_2O/m for air velocity of 75 m/h) (Morgan-Sagastume et al., 2003).

3.4. TMA and NH_3 metabolites analysis

Substrate and metabolite accumulations in biofilters often affect the instability of pH and cause acidification or alkalinity (Chung et al., 2004). To understand TMA and NH_3 metabolites after biodegradation, an analysis of mass balance for nitrogen was conducted to demonstrate the pH stability of this system after a 523-day operation. The main metabolites in the GAC bed/leachate were: organic-N (30.82%/18.58%), NH_4^+–N (9.59%/12.30%), NO_2^-–N (7.63%/8.51%) and NO_3^-–N (0.02%/0.04%). These species in the GAC bed and leachate contained 48.06% and 39.47% of total nitrogen according to the mass balance from the accumulation of nitrogen (the difference between inlet and outlet) in the biofilter. Since amine concentrations were always less than 0.01% of total nitrogen in this system, the data were neglected (data not shown). The determined metabolites only accounted for 87.49% (48.06% + 39.47%) of total nitrogen conversion with respect to water-losing or nitrogen gas production during operating periods. In addition, no N_2O production occurred in the overall biodegradation process. These results confirmed that bacterial assimilation (49.4%) was the main metabolic pathway of TMA and NH_3. Chang et al. (2004) have shown that TMA yields hydroxide ion (OH^-) in aqueous solution, and hydrogen ions (H^+ formed by CO_2 production can neutralize hydroxide ions to maintain a stable pH. Moreover, ammonia adsorpted by GAC could neutralize the acidity (H^+ from metabolite production and consequently maintain a near-neutral pH.

3.5. Bacterial observation by SEM

The SEM analysis for biofilm development onto the GAC drawn from the biofilter was carried out on the 327th and 523th days, respectively. The cells were directly attached to the surface of GAC on the 327th day, and the biofilm was apparently observed to spread throughout the surface of GAC by the connection of extracellular secretions (Trinet et al., 1991). The presence of attached cells morphologically similar to *Arthrobacter* sp. (short rod) was observed, and it was the dominant pattern. Other morphologies also appeared in SEM images (data not shown). After long-term operation (523-day), the well structure of biofilm and cells were maintained, and no clogging or disintegration phenomenon was found. In the meantime, greater than 95% removal efficiencies for TMA and NH_3 and 3.1×10^9 CFU/g-GAC for *Arthrobacter* sp. were obtained by the Hagedorn and Holt selective medium (Hagedorn and Holt, 1975). Based on the variations in pressure drop, the biofilm formation did not acutely cause a clogging phenomenon in the biofilter.

3.6. Successive changes and analyses in DGGE profiles

The traditional plate-counting method used to quantify microorganisms might underestimate the actual number of bacteria since only cultivable, viable cells are detected on the agar plates used, thus possibly constituting only a part of the total population present in biofilters (Amann et al., 1995; Devinny et al., 1999). To understand the distribution of all the bacterial communities and their evolution in biofilm during the operating periods in this study, the PCR-DGGE was used to analyze bacterial compositions, including both the culturable and nonculturable microbes.

Fig. 5 shows the profile of DGGE bands generated after PCR amplification and subjected to the PCR-DGGE analysis during 523-day operation. Individual lanes contain 16S rDNA PCR products from total DNA extracts at different operating times (12, 108, 210, 327, 431 and 523 days). Lanes 2–6 show community fingerprints similar to each other but unique compared to lane 1. The complexity of DGGE bands increased with increasing operating time. However, the band V (*Arthrobacter* sp.) was consistantly a dominant population in the bacterial consortium throughout the biofilter. DGGE bands indicated that the relative intensity of band V accounted for 97.9% of all bands at 12 days (Table 1) and consisted of at least 12 bands. The intensity of band V was reduced approximately

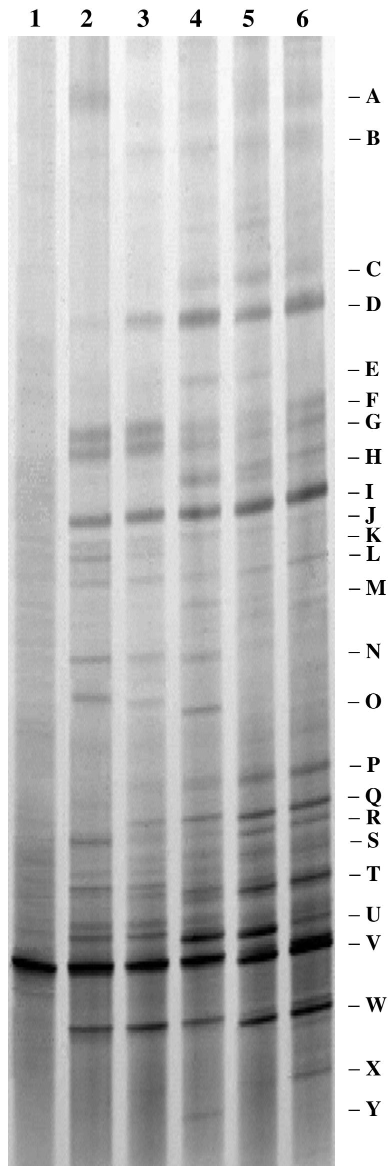

Fig. 5. Profile of DGGE bands generated after PCR amplification of 16S rDNA fragments at different times. Lane 1: 12-day; 2: 108-day; 3: 210-day; 4: 327-day; 5: 431-day; 6: 523-day operation.

20% on the 108th day of operation due to the production of other bands (at least 17 bands), especially bands F (2.6%), G (2.5%), I (4.1%) and W (5.4%). After 210 days of operation, band V decreased to 66.1%, and band W increased up to 14.1%. At least 21 bands on the DGGE profile were detected during operating periods. Inoculated *Arthrobacter* sp. (band V) still maintained a dominant population of more than 50% after the 523-day period. In the meantime, a stable and effective performance (95% removal) in treating TMA and NH_3 emissions was achieved. Colby and Zatman (1973) proved that *Arthrobacter* sp. could grow with TMA as carbon and energy sources, and Schlegel (1992) indicated that *Arthrobacter* sp. was also able to catalyze heterotrophic nitrification of nitrogen-containing organic compounds.

Twenty-five discernible bands (A–Y) were individually identified as members of different eubacterial phyla by comparison with the GenBank database. The most intense band V was identified and showed homology to *Arthrobacter* sp. with a similarity of 99% grouped with the phylum *Actinobacteria*. Eight other bands (D, E, J, P, S, T, X and Y) also belonged to the phylum *Actinobacteria,* and their closest relatives showed homology to *Micrococcus luteus* (97%), *Corynebacterium glutamicum* (96%), *Salana multivorans* (98%), *Cellulosimicrobium cellulans* (96%), *Cellulosimicrobium funkei* (96%), *Promicromonospora* sp. (93%), *Terrabacter terrae* (96%) and *Terrabacter* sp. (94%). Nine bands (A, B, C, F, G, H, K, N and O) were clustered within the phylum *Firmicutes*, namely *Staphylococcus* sp. (99%), *Staphylococcus aureus* (99%), *Staphylococcus caprae* (98%), *Bacillus anthracis* (98%), *Bacillus cereus* (97%), *Bacillus* sp. (93%), *Aerococcus sanguinicola* (99%), *Lactobacillus rhamnosus* (98%) and *Lactobacillus casei* (98%). Six bands (I, M, Q, R, U and W) were clustered within the phylum *Proteobacteria*, namely *Paracoccus denitrificans* (99%), *Xanthomonas axonopodis* (96%), *Pseudomonas putida* (94%), *Pseudomonas* sp. (93%), *Dechloromonas hortensis* (95%) and *Comamonas* sp. (96%). Only band L (*Flavobacterium aquatile*, 97% similarity) showed a deeply rooted branch not closely related to any other band, and it was classified in the phylum *Bacteroidetes* with weaker intensity (0.1–0.4%). The band that migrated between band B and C was not successfully purified or identified. Most bands were present in lower proportions of less than 3% and did not show any drastic change by DGGE analysis. However, the intensities of band I, T and W were individually increased as the semi-abundant bands with 7.1%, 5.6% and 14.5% while the band V on the DGGE profile was decreased on the 523th day of operation. According to the above results, the majority of the sequences were from aerobic bacteria. This was expected since the metabolic activity of microorganisms is mostly aerobic. However, anaerobes represented a minor fraction of the sequences analysed and included: *S. multivorans* (band J), *L. rhamnosus* (band N) and *L. casei* (band O). *Lactobacillus* strains are likely to come from the gastrointestinal tract of swine, and the species is known to be capable of microaerophilic growth (Pandey et al., 1994). In this study, dissolved oxygen (DO) levels in the biofilter were never below 5.0 mg/L during operating periods, which means that the oxygen transfer rate exceeded the oxygen uptake rate by bacterial respiration (data not shown). Certainly, these conditions are not suitable for the growth of *Lactobacillus*. Therefore, we hypothesized that they should be inactive in this aerated system even though the intensities of these bands at certain time points slightly increased (Fig. 5).

Previous reports do not find any *Bacillus* sp. in aerobic processes using traditional culture techniques (Hensel et al., 1989; LaPara et al., 2000). In this study, we used the DGGE method based upon more direct analysis of 16S rDNA to confirm the existence of *Bacillus* sp. in the

Table 1
Relative abundance of DGGE bands during 523-day operation

Bands[a]	Percent intensity of total intensity for operation times (days)[b]					
	12	108	210	327	431	523
A	0.0	1.2	0.2	0.1	0.1	0.2
B	0.0	0.1	0.5	0.2	0.1	0.3
C	0.0	0.0	0.0	0.4	0.2	0.2
D	0.0	0.2	1.8	1.2	1.4	0.8
E	0.0	0.0	0.0	0.6	0.1	0.0
F	0.0	2.6	2.1	0.8	0.4	0.4
G	0.0	2.5	2.2	0.9	0.3	0.4
H	0.1	0.0	0.0	0.2	0.3	0.3
I	0.2	4.1	3.2	2.1	2.8	7.1
J	0.2	0.2	0.1	0.2	0.1	0.1
K	0.1	0.3	0.1	0.0	0.0	0.0
L	0.1	0.2	0.4	0.2	0.1	0.2
M	0.2	0.0	0.0	0.2	0.0	0.1
N	0.1	0.4	0.7	0.7	0.0	0.0
O	0.0	0.8	0.6	1.1	0.0	0.0
P	0.0	0.0	0.2	0.7	1.3	2.2
Q	0.2	1.1	0.8	0.9	3.2	3
R	0.0	0.4	0.1	0.2	2.5	2.1
S	0.3	0.0	0.1	5.8	2.7	2.3
T	0.2	1.3	2.3	5.3	5.6	5.6
U	0.4	1.8	4.2	14.5	8.7	3.5
V	97.9	77.4	66.1	55.2	58.6	54.1
W	0.0	5.4	14.1	7.2	11.3	14.5
X	0.0	0.0	0.2	0.0	0.2	2.6
Y	0.0	0.0	0.0	1.3	0.0	0.0

[a] DGGE bands correspond to A–Y bands on the DGGE profile in Fig. 5.
[b] Percent intensities were calculated from the intensity of the bands on each DGGE profile.

biofilter, and the most abundant species were *B. anthracis* and *B. cereus*, especially on the 108th day.

Fontana et al. (2005) have shown that *Staphylococcus* sp. (band A–C) can reduce nitrate to nitrite. In addition, we found that *P. denitrificans* (band V) existed in the biofilter. Some reports have proven that *P. denitrificans* is capable of oxidizing NH_3 to nitrite by nitrification and reducing nitrite to nitrogen gas by denitrification (Uemoto and Saiki, 1996; Crossman et al., 1997; Stouthamer et al., 1997). This direct evidence demonstrated that *P. denitrificans* existed in the system and could denitrify nitrite to nitrogen gas in the deeper biofilm zones. However, the possibility of other microbes in the biofilter also having the capability of degrading TMA and NH_3 or playing a part in the co-metabolism of them remains unclear.

4. Conclusions

Effective control of low concentrations of TMA and NH_3 in exhaust air from swine waste storage pits is an important process because the two-odor components are toxic to animals and have low threshold limits. In this study, the GAC biofilter has demonstrated that it can effectively reduce TMA and NH_3 emissions (more than 96%) during a 523-day operating period. This system could simultaneously meet both standard levels of ambient TMA and NH_3 for air quality regulation at an EBRT of more than 40 s. In addition, NH_3 is less sensitive to changes in EBRT as compared with TMA removal. No significant acidification or clogging phenomenon occurred. The bacterial community of the biofilm in the biofilter has a complex diversity, but inoculated *Arthrobacter* sp. remains the predominant population in the bacterial consortium during operating periods. The results indicate that the GAC biofilter is an effective technology for simultaneous removals of TMA and NH_3 in the field.

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