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Microbial populations analysis and field application of biofilter for the removal of volatile-sulfur compounds from swine wastewater treatment system

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Abstract

A biofilter packed with granular activated carbon (GAC) was applied to eliminate volatile-sulfur compounds (VSC) emitted from solid–liquid separation tank in swine wastewater treatment system. Hydrogen sulfide, methanethiol, dimethyl disulfide, and dimethyl sulfide were effectively reduced to 96–100% at gas residence times of 13–30 s. Elemental sulfur and sulfate are their primary oxidation metabolites. Regarding odor, an average of 86% reduction was achieved at short residence time (13 s). In addition, bioaerosol emissions could also be effectively reduced by 90% with the biofilter. Advantages of the system include low moisture demand, low pressure drop, and high biofilm stability. Further characterization of bacterial populations of the activated carbon samples using the fluorescent in situ hybridization (FISH) technique revealed that *Pseudomonas* sp. remained the predominant community (56–70%) after long-term evaluation of 415 days.

Keywords: Hydrogen sulfide; Methanethiol; Dimethyl sulfide; Dimethyl disulfide; FISH

1. Introduction

During the last decades, three-step piggery wastewater treatment (TPWT) system, which consists of solid/liquid separation, anaerobic and aerobic procedures, has been successfully introduced to treat swine wastewater [1]. However, malodorous gases are most usually emitted from solid–liquid separation tank in swine wastewater treatment system. The most frequently studied odorants are ammonia (NH_3) and volatile-sulfur compounds (VSC) containing hydrogen sulfide (H_2S), methanethiol (MT), dimethyl sulfide (DMS), and dimethyl disulfide (DMDS) because of their toxicities and lower odor thresholds [2,3].

In recent years, biofiltration in treating low concentration air pollutants has been extensively investigated because of their low capital and operating costs. The field application of biofilters in reducing NH_3 gas has also been investigated, and NH_3 emission has been successfully treated [4,5]. In contrast, most reports regarding the reduction of these VSC have only been undertaken in laboratory-scale biofilters under constant operating conditions [6,7]. Until now, little research have been carried out on the field application of biofilters to treat complicated and mixed VSC since it is hindered by high complexity and instability.

The previous reports have shown that H_2S is easily biodegraded due to its high solubility [8]. For other relatively insoluble and slowly biodegradable VSC, longer gas residence times (>120 s) were required to obtain high removal efficiency (>90%) [9]. Although several types of packing media such as compost [10] and peat [11] in biofilters have been used for VSC reduction in the laboratory and optimal performances (∼90%) have been achieved, the major constraint on biofilter application is frequent media replacements as a result of aging or deterioration [12].

In the last decade, GAC has been used successfully as a good packing material because of the advantage of rapid pollutant adsorption followed by slow release for biodegradation by microorganisms, and a higher removal capacity was achieved in our previous studies [13,14]. However, the GAC system lacked a long-term evaluation in the field applications for VSC reduction. Therefore, the objective of this study is to examine the performance of the biofilter using GAC with immobilized *Pseudomonas* sp. to treat VSC emission from solid–liquid separation

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tank in swine wastewater treatment system at gas residence times of 13–30 s. The FISH method was used to further characterize bacterial populations and bioaerosol reductions. Operational parameters such as gas residence time and inlet concentration of VSC were conducted because the fluctuations of VSC concentration were usually associated with the nature of field operation. Other measured parameters such as pH, metabolites, pressure drop, and moisture content were monitored. The results of FISH study could enhance our understanding of bacterial diversity of biofilms in the biofilter and provide a better basis for understanding the performance and stability of VSC, odor or bioaerosol reduction after long-term evaluation of 415 days.

2. Materials and methods

2.1. Microbial culture and medium

Pseudomonas sp. was isolated from piggery wastewater and enriched in nutrient broth at 26 °C. *Pseudomonas* sp. could be a potential microorganism to utilize some VSC from the laboratory experiments (data not shown). Because a better VSC removal efficiency was obtained when *Pseudomonas* sp. grew on glucose medium compared with other carbon sources (sucrose, fructose, and molasses), glucose was used as a carbon source for microbial growth and VSC removals in this study. In all experiments, an inflow medium containing glucose 10.0 g/L, KH_2PO_4 4.08 g/L, K_2HPO_4 5.22 g/L, NH_4Cl 0.4 g/L, $MgCl_2{\cdot}6H_2O$ 0.2 g/L, and Fe(III)-citrate 0.01 g/L was supplied and stored in the nutrient tank. The final pH was adjusted to 7.0 by using 0.1 N NaOH or HCl.

2.2. Immobilization procedure

GAC with a particle size of 4.5 mm was used as the packing material, and its detailed characteristics were described by Chung et al. [13]. *Pseudomonas* sp. was grown in 10-L nutrient broth for 2 days and was harvested by centrifugation ($8000 \times g$ for 10 min). The pellets were resuspended with 100-L nutrient broth and mixed with about 70-kg of GAC in a 200-L PVC tank. Fresh broth was added once every 3 days until the bacterial count reached nearly 10^8–10^9 CFU/g dry GAC, and then the cell-laden GAC was packed into the biofilter. In addition, all materials and equipments were maintained in aseptic conditions during the above experimental period.

2.3. Experimental setup and operation

The design of the biofilter is shown in Fig. 1. It was installed on a swine farm in the Miaoli County of Taiwan. Two PVC columns (diameter 0.48 m, height 0.5 m) connected in series were packed with GAC immobilized *Pseudomonas* sp. and supported by a perforated sieve at the bottom of each column. The packed volume and weight of GAC in each column was 72.3 L and 34.7 kg, respectively. The column wall contained two types of sampling ports namely GAC and gas samplers. Malodorous gases was exhausted by a fan from solid–liquid separation tank in swine wastewater treatment system and then flowed downward

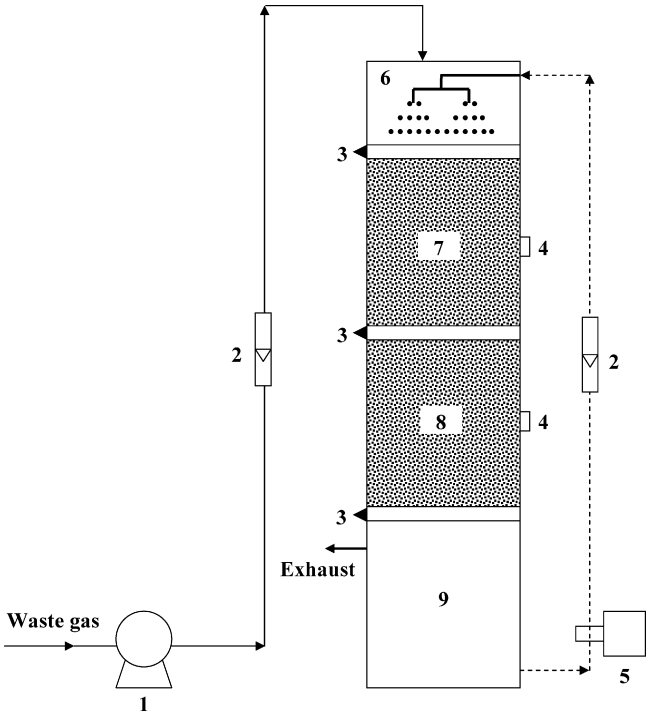

Fig. 1. A schematic diagram of the biofilter: (1) exhaust fan; (2) flow meter; (3) gas sampler; (4) GAC sampler; (5) peristaltic pump; (6) sprinkler zone; (7 and 8) column 1 and column 2 of the biofilter inoculated with *Pseudomonas* sp.; (9) nutrient tank.

through the top of the biofilter; gas flow rate was controlled by a flow meter. The inflow medium stored in a nutrient tank (diameter 0.48 m, height 0.6 m) located at the bottom of the biofilter was recycled by a peristaltic pump at 5 L/min for 10 min, six times a day. The peristaltic pump was connected to a spray nozzle located at the top of the biofilter to spray the medium uniformly on the GAC surface. The solution volume was periodically maintained at 70 L by adding distilled water and fresh inflow medium, and 0.1% of glucose was supplied once weekly. To estimate the operating performance of the biofilter for VSC removals, waste gases were supplied to the system at various empty bed gas residence times (EBRTs) in the range of 13–30 s. The EBRT was defined as the volume of the packed bed section divided by the gas flow rate.

2.4. Analytical methods

Hydrogen sulfide and methanethiol concentrations were analyzed by using a gas chromatograph (GC) coupled to a flame photometric detector (FPD) and a 30-m HP-1 column (Hewlett Packard, USA) with detection limit of 20 ppb [15]. Dimethyl sulfide and dimethyl disulfide concentrations were measured by GC coupled to a flame ionization detector (FID) and a 50-m Ultra-2 column (Hewlett Packard, USA) with detection limit of 50 ppb [16]. To avoid a superposition by a temporal effect, the measurements of pressure drop and moisture content of GAC were performed 2 h after liquid recirculation had occurred. The pressure drop across the biofilter was measured using a U-tube water manometer in mm-H_2O/m-filter height. GAC moisture

was determined after weighing and drying at 103 ± 0.5 °C. The pH value in GAC and leachate was determined using an electronic pH meter. To analyze the metabolites, 1 g of GAC was withdrawn from the biofilter, mixed with 10 mL of distilled water, vortexed for 3 min, and the GAC was withdrawn to analyze the elemental sulfur. Elemental sulfur concentration was analyzed by reacting it with cyanide to produce thiocyanate, which was then quantified as $Fe(SCN)_6^{3-}$ [17]. The residual suspension solutions were analyzed for other sulfur-containing components. Sulfate was measured by ion chromatography. Sulfide was determined using an ion-specific electrode. Sulfite was determined by titration using a standard potassium iodide–iodate titrant and a starch indicator [17]. The odor concentrations in inlet and outlet air of the biofilter were analyzed by an olfactometer with six repetitions and expressed as odor unit per m^3 air (OU/m^3).

2.5. Fluorescent in situ hybridization (FISH)

GAC samples collected from the biofilter at different operation times were fixed and analyzed according to the protocol described by Amann et al. [18]. Table 1 lists the oligonucleotide probes, their specificities and sequences used for FISH in this study. These probes targeting important and major microbial groups found in almost all environmental samples were commercially synthesized, and 5′ labeled with fluorescein isothiocyanate (FITC), cyanine 3 (Cy3) or cyanine 5 (Cy5). FISH images were captured with a cooled charged coupled device (CCD) camera (BX-50, Olympus). At least 20 microscopic fields were analyzed using the aforementioned oligonucleotide probes targeting important microbial groups in the biofilter, and then the percentages and standard deviations were calculated from the total number of cells stained by probe EUBmix. The number was expressed in units of total microbial cells (TMC) per gram dry GAC (TMC/g dry GAC).

2.6. Bioaerosol

The inlet and outlet air of the biofilter was collected and forced through a 500-mL flask with 200-mL sterile saline solution (0.9%) for 24 h at 4 °C [14]. The solution was harvested by centrifugation (8000 × *g* for 10 min), and FISH analysis was performed immediately. The percentage of FISH detectable cells was determined as the fraction of bacterial cells responding to individual probes over the total amount of EUBmix. The bioaerosol counts were reported as TMC/m^3.

2.7. Scanning electron microscopy (SEM)

GAC samples were withdrawn from the biofilter, and then fixed and dehydrated as described by Chung et al. [14]. Micrographs were obtained from a Hitachi S4500 scanning electron microscope.

3. Results and discussion

3.1. VSC removal efficiency

H_2S, MT, DMS, and DMDS have been detected and identified as the main malodor compounds emitted from solid–liquid separation tank in swine wastewater treatment system as a result of their exceptionally low thresholds [2]. For examining the biofilter performance in the course of the VSC removal at short residence times, operating periods were designed for six phases by changing various EBRTs, which included 0–147 days (20 s), 148–197 days (16 s), 198–256 days (23 s), 257–307 days (27 s), 308–352 days (30 s), and 353–415 days (13 s).

Fig. 2 shows the profiles of inlet concentrations and removal efficiencies of VSC during the 415-day operation. The individual concentration often varied significantly with time. According to the calculation from the bed depth service time (BDST) experiment [23], theoretical saturated-adsorption times of GAC were approximately 67 days (H_2S), 51 days (MT), 22 days (DMDS), and 8 days (DMS) for single gas on the basis of estimating real inlet concentration and gas flow rate. During the 415-day operation, 0.1–1.3 ppm of H_2S (inlet loading rate: 0.03–0.48 $g/m^3/h$) (Fig. 2A), 0.2–9.7 ppm of MT (inlet loading rate: 0.07–2.49 $g/m^3/h$) (Fig. 2B), and 0.4–7.1 ppm of DMDS (inlet loading rate: 0.26–5.03 $g/m^3/h$) (Fig. 2C) were introduced into the biofilter with an average of 0.7, 2.5, and 2.1 ppm, respectively. Hundred percent removals were effectively achieved in column 1 throughout the operation even

Table 1
A list of oligonucleotide probes used in this study

Probe	Specificity	Sequence (5′–3′)	Reference
EUB338[a]	Most *Bacteria*	GCTGCCTCCCGTAGGAGT	[18]
EUB338–II[a]	Other *Bacteria* not detected by EUB338	GCAGCCACCCGTAGGTGT	[19]
EUB338–III[a]	Other *Bacteria* not detected by EUB338	GCTGCCACCCGTAGGTGT	[19]
PSE	*Pseudomonas* sp.	GATCCGGACTACGATCGGTTT	[20]
ALF968	*α-Proteobacteria*	GGTAAGGTTCTGCGCGTT	[21]
BET42a	*β-Proteobacteria*	GCCTTCCCACTTCGTTT	[18]
GAM42a	*γ-Proteobacteria*	GCCTTCCCACATCGTTT	[18]
HGC69a	*Actinobacteria*	TATAGTTACCACCGCCGT	[18]
LGC354A[b]	*Firmicutes*	TGGAAGATTCCCTACTGC	[22]
LGC354B[b]	*Firmicutes*	CGGAAGATTCCCTACTGC	[22]
LGC354C[b]	*Firmicutes*	CCGAAGATTCCCTACTGC	[22]

[a] EUB338, EUB338–II and EUB338–III were used in the mixture called EUBmix.
[b] LGC354A, LGC354B and LGC354C were used in the mixture called LGCmix.

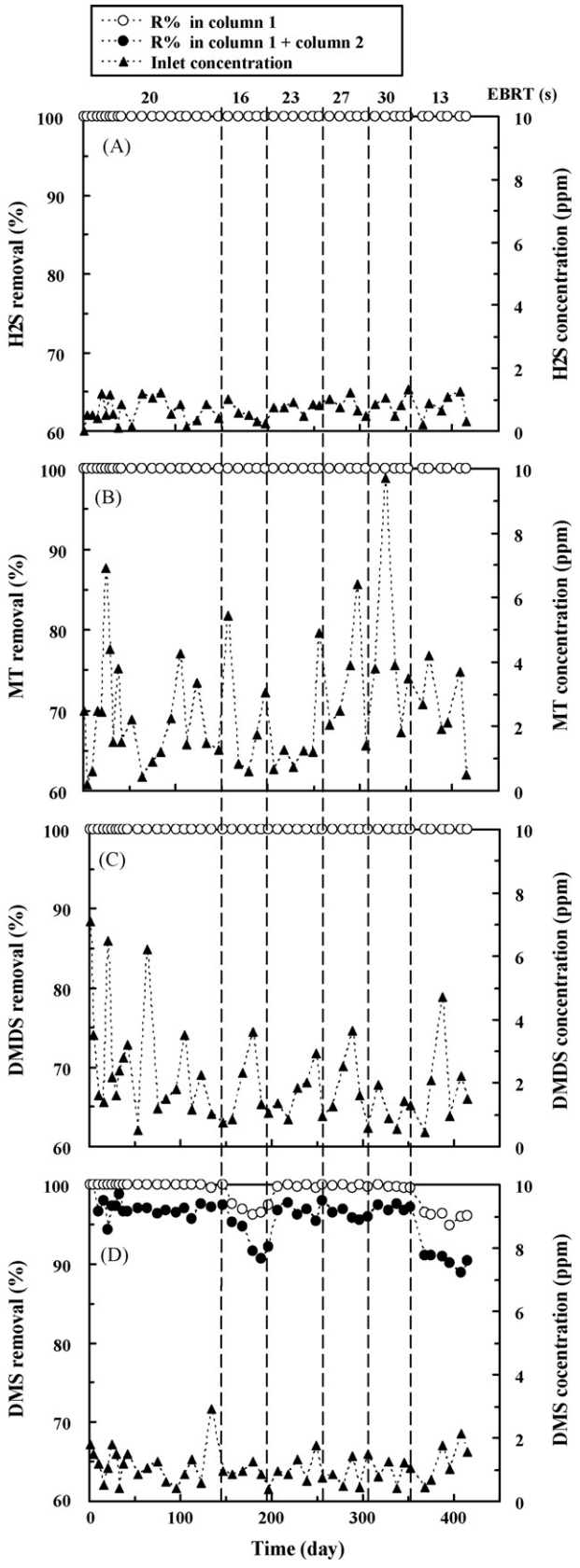

Fig. 2. Profiles of inlet concentrations and removal efficiencies at various EBRTs for H_2S (A), MT (B), DMDS (C), and DMS (D) removals during 415 days.

when an EBRT of 13 s was administered. The result showed that besides being adsorbed by GAC, most of the compounds were biodegraded in the biofilter. In addition, the biotreatment was also 100% effective in treating 0.2–1.8 ppm of DMS (elimination capacity: 0.19–0.84 g/m^3/h) before 64 days, as shown in Fig. 2D. After theoretical saturated adsorption of GAC (8 days), a dynamic equilibrium was achieved between adsorption and biodegradation. Steady-state efficiency (∼99%) could be carried out for 0.4–2.9 ppm of DMS removal at an EBRT of 20 s on the 64th–147th days (elimination capacity: 0.20–1.35 g/m^3/h). When EBRT decreased to 16 s, the removal efficiency decreased by 3% from 99 to 96% with an average inlet loading rate of 0.50 g/m^3/h on the 148th–197th days. As residence times increased to 23–30 s, removal efficiencies improved to 99–100%. However, DMS removal immediately reduced by 4% from 99% to 95% at an inlet concentration ranging from 0.5 to 2.2 ppm (inlet loading rate: 0.315–1.504 g/m^3/h) at lower EBRT (13 s). Apparently, the gas residence time strongly influenced the removal efficiency of DMS because of mass-transfer limit or slower biodegradation [24,25].

Some reports have indicated that when treating a mixture of VSC, the degradation rates decreased in the following order: H_2S > MT > DMDS > DMS [26,27]. The DMS was often difficult to degrade or DMS degraders were strongly inhibited by the presence of other VSC [28]. In fact, in this study, at least 95% removal efficiency was obtained for DMS with an average inlet concentration of 1.1 ppm during the 415-day operation. Apparently, the variable input concentrations did not lead to poor removal efficiencies of DMS. Previous studies of biofilters under similar inlet loading (0.06–0.50 g/m^3/h) for the removal of VSC reported 72% in a compost/wood chip biofilter [29], 91% in a compost/biosolid biofilter [30], 91% in a wood chip biofilter [31], and 95% in a biotrickling filter [32]. Thus, the biofilter used in this study achieved high VSC removal (95–100%) due to its combination of characteristics of GAC adsorption and microbial biodegradation [13,14,25]. Finally, the maximum elimination rates of individual compounds in the biofilter were not determined because the maximum removal capacities could not be reached.

3.2. Odor reduction

Fig. 3 shows the profile of odor concentrations and removal efficiencies at various EBRTs (13, 23, and 30 s). The odor concentrations in the inlet air of the biofilter varied between 6200 and 7800 OU/m^3. The concentrations in the outlet air ranged from 200 to 850 OU/m^3. The biofilter reduced the odor concentrations by more than 86% at an EBRT of 13 s, and excelled by operating at an EBRT of 30 s (approximately 97%). Previous reports on reducing odor emission from swine facilities and composting plants have shown that the deodorization efficiencies were nearly 40% in a pellet/bark biofilter [33], 45% in a biofilter [32], 67% in a wood chip biofilter [29], 81% in a compost/wood chip biofilter [29], 82% in a biotrickling filter [32], and 83% in a biochip filter [33] with similar inlet odor concentrations. In contrast, the biofilter used in this study was highly effective in treating odor emissions.

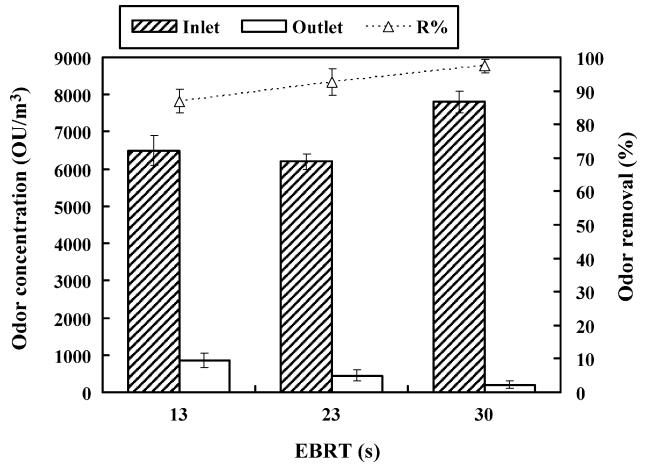

Fig. 3. Profile of odor concentrations and removal efficiencies. The error bars indicate the standard deviation.

3.3. Sulfur mass balance

To understand the metabolites and characteristics of the VSC biodegradation, the analysis of sulfur mass balance was performed on the 415th day, and the percentages of sulfur metabolites are shown in Fig. 4. The percentages of elemental sulfur, sulfate, sulfide and sulfite were 32.5, 12.5, 4.7, and 1.2% in column 1 (total 50.9%); 15.1, 6.2, 1.6, and 1.0% in column 2 (23.9%), and 8.9, 10.2, 1.3, and 0.6% in leachate (21.0%), respectively. Total sulfur compounds accumulated in the biofilter was approximately 372.5 g-S, and these metabolites accounted for 95.8% (50.9% + 23.9% + 21.0%) of total sulfur conversion by calculating concentration variations between the inlet and outlet of these VSC at various EBRTs. The remaining 4.2% of the conversion product was assumed to be due to water loss or bacteria accumulation [34]. Moreover, since MT, DMS, and DMDS concentrations adsorbed on GAC and absorbed in liquid solutions were always less than 0.1% of total sulfur in the biofilter, their accumulations were neglected (data not shown). The average ratios of elemental sulfur, sulfate, and sulfide in column 1 were greater than twice that in column 2 except for sulfite, which had a similar amount. The VSC degradation mainly occurred in column 1 (>90%), but some metabolites of VSC were easily washed out from column 1 by the inflow medium and then accumulated in column 2 in the down-flow biofilter. Besides, higher sulfate concentration (10.2%) was also detected in leachate because it easily accumulated in leachate via the water recycling system.

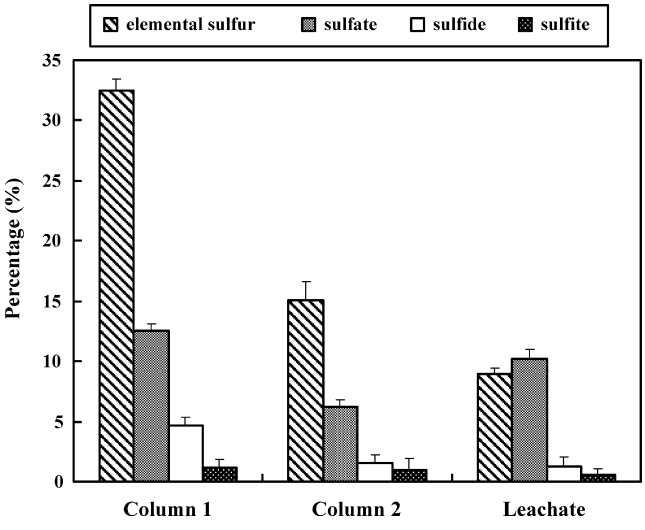

Fig. 4. Profiles of the percentages of sulfur metabolites in column 1, column 2, and leachate of the biofilter after 415 days. The error bars indicate the standard deviation.

In this study, elemental sulfur (56.5%) and sulfate (28.9%) were the primary oxidation metabolites after 415 days operation. The mechanism of VSC degradation has been extensively investigated for VSC-degrading microorganisms *Hyphomicrobium* sp. [35,36] and *Thiobacillus* sp. [28,36,37]. These reports indicated that DMDS and DMS are initially converted to MT. MT is further oxidized to H_2S. Finally, H_2S is completely oxidized to sulfate without providing an additional carbon source. Recently, the studies have demonstrated that *Pseudomonas* sp. cannot utilize DMDS or MT as carbon sources, but it has the ability to oxidize DMDS and MT as sulfur sources on a glucose medium [38]. The VSC metabolic pathway of *Pseudomonas* sp. is similar to that of *Thiobacillus* sp. [28]. Elemental sulfur accumulation in the degradation of VSC in the presence of glucose can also be illustrated from an energy perspective, and our result was in agreement with that of Zhang et al. [39]. Previous studies have shown that H_2S is mainly oxidized to elemental sulfur (90%) and sulfate (9%) because *Pseudomonas* sp. utilized glucose as the main carbon and energy source [14]. In addition, acidification has often been an obstacle to traditional biofiltration technology for treating VSC emissions due to the accumulation of acidic metabolites [10], whereas the stable pH fluctuation (6.8–7.4) was maintained because the major metabolite formed was neutral elemental sulfur, and a stable buffer capacity (33 mM/pH) was kept in this system by periodically providing fresh inflow medium. A similar fluctuation also has been found in our previous research [14,40]. The possibility of element sulfur accumulation in the biofilter affecting the pressure drop needs further investigation.

3.4. Changes of moisture content and pressure drop

The downward gas flow has successfully proven superior to up-flow. However, an inflow medium was easily accumulated in the inlet side of enclosed biofilters resulted in clogging or the formation of anaerobic zones by continuous spraying when no air was introduced into this system (intermittent shutdown) or a low gas flow rate was controlled [24]. Fig. 5 shows the profile of moisture content and pressure drop at various EBRTs. GAC moisture contents from column 1 to column 2 showed an insignificant gradient change in the axial direction of the biofilter. These results indicated that an intermittent sprinkling of water was helpful in controlling moisture at the whole column and reducing the clogging phenomenon. Also, it can substantially short the difference between the moisture content of column 1 and column 2 because of both the force of gravity and a high downward gas flow, as reported by Chung et al. [40].

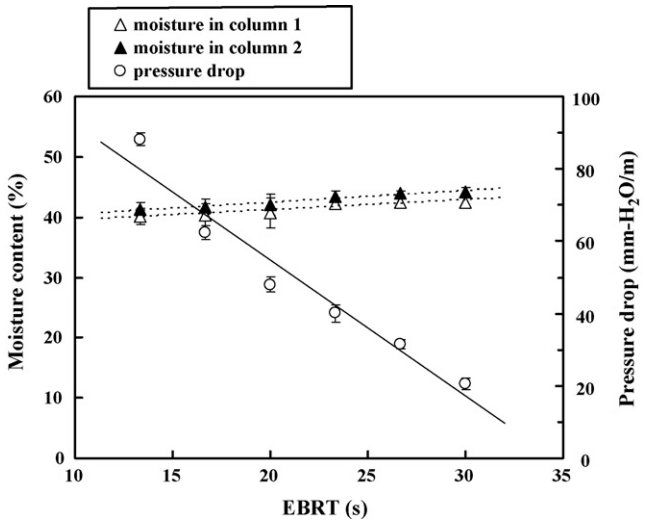

Fig. 5. Effect of gas residence time on moisture content and pressure drop. The error bars indicate the standard deviation.

However, the overall moisture content was still higher than 40% even when the EBRT reached 13 s. The result corresponded with the operational recommendation for the optimal moisture content in biofilters from Leson and Winer [41], and was less than 65% of what a traditional compost biofilter requires to maintain microbial growth [42]. In contrast to other media moistures of field applications with nearly 69% in a wood chip biofilter [4] and 50% in a compost/wood chip biofilter [29], the biofilter had a lower moisture demand and might be energy saving.

Pressure drop across the biofilter was monitored weekly, and the average value was calculated over at least 40 days every EBRTs (13–30 s) in all experiments. Fig. 5 shows that pressure drop increased with decreasing residence time, and they have a good linear correlation ($R^2 = 0.9572$). The pressure drop ranged from 20 to 88 mm-H_2O/m was always acceptable, and no sudden change (SD 0.4–2.3%) in pressure drop occurred at the same EBRT. The result maybe attributed to good mechanical strength of GAC, which led to negligible bed compaction and avoided short-circuiting. Similar results were shown in our previous laboratory study [14], and corresponded with the operational guideline for biofilters (below 250 mm-H_2O/m) [43]. When pressure drop exceeds the accepted level, packing media for biofilters must be replaced or treated [41]. Thus, the biofilter neither required extra backwashing treatment to avoid clogging nor frequent media replacement during long-term operation. The pressure drop mainly depended on gas flow rate, and it was apparently not affected by elemental sulfur accumulation in this system.

3.5. Bioaerosol analysis by FISH

Amann et al. [18] have indicated that up to 99% microorganisms which are active in the environment are not cultivable by using traditional cultivation methods. For the accurate investigation of these bioaerosol constituents, the FISH method with selected probes was applied in the present study to characterize their concentrations and percentages. The result was useful to evaluate the actual bioaerosol emissions and reduction efficiencies by comparing the biofilter inlet and outlet concentrations. Total bioaerosol concentrations in the biofilter inlet varied from 4.1×10^5 to 1.7×10^6 TMC/m^3 by probe EUBmix staining, and the microbial compositions were 40% (*Firmicutes*), 19% (*γ-Proteobacteria*), 15% (*α-Proteobacteria*), 10% (*β-Proteobacteria*), 9% (*Actinobacteria*), and 7% (others) on the 256th day. Thirty-eight percent (*Firmicutes*), 18% (*γ-Proteobacteria*), 16% (*α-Proteobacteria*), 11% (*Actinobacteria*), 10% (*β-Proteobacteria*), and 7% (others) were obtained on the 415th day. The amount of bioaerosol detected in this study is in agreement with the result from Seedorf et al. [44], although higher concentrations have also been reported [45]. The outlet concentrations were approximately $(3.8 \pm 0.4) \times 10^4$ and $(4.6 \pm 0.7) \times 10^4$ TMC/m^3 when the biofilter was continuously operated for 256 days (EBRT = 23 s) and 415 days (13 s). The bioaerosol reductions were effectively achieved at least 90 and 98% at an EBRT of 13 and 23 s, respectively. The data showed that these emissions in the biofilter outlet were lower than those released from a peat biofilter [46] and a compost biofilter [33] because many microbes originally existed in the peat or compost.

The percentage of bacterial communities in bioaerosol of the biofilter outlet is shown in Fig. 6. The percentages of their distributions were 36% (*Firmicutes*), 28% (*γ-Proteobacteria*), 13% (*α-Proteobacteria*), 9% (*β-Proteobacteria*), 8% (*Actinobacteria*), and 6% (others) on the 256th day. Thirty-four percent (*Firmicutes*), 23% (*γ-Proteobacteria*), 17% (*α-Proteobacteria*), 10% (*Actinobacteria*), 8% (*β-Proteobacteria*), and 8% (others) were detected on the 415th day. The community distributions between the 256th and 415th days were highly similar except for *α-Proteobacteria* and *γ-Proteobacteria*. In addition, 6–8% of these communities could not be targeted by these probes. In this study, these microbial distributions were not influenced by environmental factors. For further analysis of the amount of inoculated *Pseudomonas* sp. in bioaerosol, the specific probe (PSE) was used to identify the genus *Pseudomonas*. One to three percent (1.3×10^4 to 2.2×10^4 TMC/m^3) of total bioaerosol

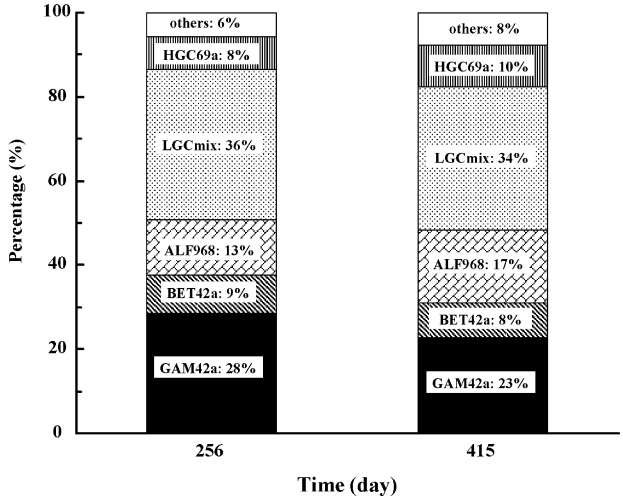

Fig. 6. Profile of bacterial community composition in bioaerosol. All values are presented as percentage of the relative cell area of individual probe against probe EUBmix.

amount determined was *Pseudomonas* sp. in the biofilter inlet. They were released from the outlet exhaust with 4.2×10^3 and 7.8×10^3 TMC/m^3 on the 256th and 415th days, respectively. The results suggested that the biofilter provided good immobilization efficiency for inoculated *Pseudomonas* sp., which could effectively enhance VSC removal. Since the bioaerosol distribution revealed a high similarity (~95%) between the inlet and outlet if we disregarded the existence of *Pseudomonas* sp., it showed that the biofilter had no selectivity in removing bioaerosol. Hence, using the biofilter should be able to effectively obviate the environmental risk of bioaerosol emission [33,46].

3.6. Bacterial community analysis in the biofilter by FISH

Fig. 7 shows the percentage of bacterial community composition and their evolution in biofilms to be directly compared to individual probes at different operating times. After 32 days, the total amount of bacteria in column 1 was approximately 5.4×10^8 TMC/g dry GAC, and the ratios of their distributions were found to be 91.9% (*Pseudomonas* sp.), 2.2% (*Firmicutes*), 1.4% (*α-Proteobacteria*), 1.2% (*β-Proteobacteria*), 1.1% (*γ-Proteobacteria*), 0.8% (*Actinobacteria*), and 1.4% (others), respectively (Fig. 7A). The amount was 8.2×10^8 TMC/g dry GAC in column 2 on the 32nd day with 95.2% (*Pseudomonas* sp.), 1.1% (*Firmicutes*), 0.9% (*γ-Proteobacteria*), 0.8% (*β-Proteobacteria*), 0.7% (*α-Proteobacteria*), 0.5% (*Actinobacteria*), and 0.8% (others), as shown in Fig. 7B. After 415 days, the average ratios of *Firmicutes*, *α-Proteobacteria*, and *γ-Proteobacteria* dramatically increased from 1.7 to 10.8, 1.1 to 7.9, and 1.0 to 5.9%, respectively. *Actinobacteria*, *β-Proteobacteria*, and others slightly increased by 2–4%. The ratio of the dominant *Pseudomonas* sp. gradually decreased with increasing operative time (32–415 days), and varied from 91.9 to 56.4% (4.6×10^9 TMC/g dry GAC) in column 1 and 95.2 to 70.8% (6.8×10^9 TMC/g dry GAC) in column 2. Distribution ratio of these non-inoculated bacteria obviously increased with increasing operative time because most of the bioaerosol (90–98%) containing non-inoculated *Pseudomonas* sp. accumulated in the biofilter. For further isolation of the predominant *Pseudomonas* strains, GAC samples withdrawn from this biofilter were diluted in sterile water and inoculated onto the cetrimide selective medium at 26 °C for the growth of total *Pseudomonas* sp. [23]. After 3 days, about 100–200 colonies were identified and differentiated between the inoculated and non-inoculated *Pseudomonas* sp. by 16sDNA sequence analysis [47]. The sequence results indicated that the inoculated *Pseudomonas* sp. still remained the predominant population with greater than 95%

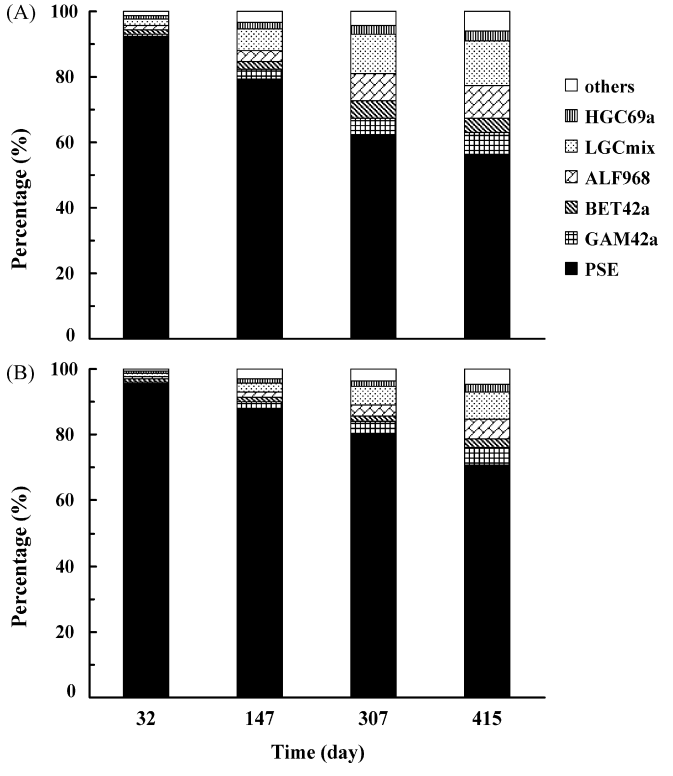

Fig. 7. Profiles of bacterial community composition of GAC samples in column 1 (A) and column 2 (B) of the biofilter at different operating times.

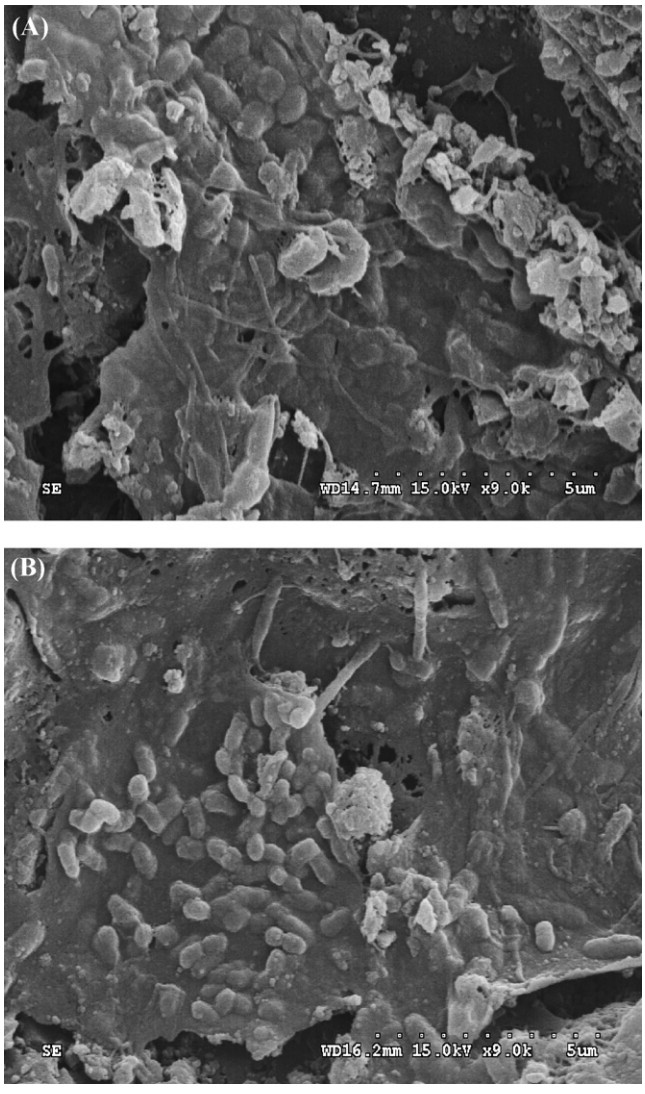

Fig. 8. Micrographs of microorganisms grown on the GAC surface after 415 days. GAC samples withdrawn from column 1 (A) and column 2 (B) of the biofilter by SEM observation.

of total *Pseudomonas* strains after long-term operation (data not shown) even if ratios of other bacterial distribution increased with increasing time.

In addition, a morphological diversity was further observed by SEM analysis, and rod-shaped bacteria (like *Pseudomonas* sp.) were shown to be the dominant pattern even though other morphologies were also observed on SEM images, as shown in Fig. 8A (column 1) and Fig. 8B (column 2) after 415 days. Morphological diversity in column 1 was more complex than in column 2, and they had been shown by FISH technique. Good structure of biofilm was effectively retained, and integrity of the biofilm was intact. In addition, some precipitation was observed in micrographs, and the precipitation had been demonstrated to be predominantly elemental sulfur by the analysis of SEM equipped with an energy-dispersive X-ray analyzer (EDX), and the result corresponded with metabolite analysis (data not shown). Whether other microorganisms are capable of causing or assisting the biodegradation of VSC is still unclear.

4. Conclusions

In this study, GAC has been successfully applied to reduce VSC emission from solid–liquid separation tank in swine wastewater treatment system. The biofilter inoculated with *Pseudomonas* sp. is confirmed to effectively reduce most mixed sulfur compounds (100%) and odor (86%) at short residence time (13 s). Among the emission of these VSC at an EBRT of 30 s, DMS was more difficult to degrade (∼99%). No acidification occurred in this system since a stable buffer capacity was maintained, and the primary oxidation metabolite was elemental sulfur. Although other bacteria containing non-inoculated *Pseudomonas* sp. were found in the biofilter, and their ratios increased with increasing time, high immobilization efficiency can lead to predominant population of inoculated *Pseudomonas* sp. after 415 days operation and result in the stable long-term high removal efficiencies.

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