Research Article

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Removal of H₂S by vermicompost biofilter and analysis on bacterial community

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Abstract: The vermicompost collected from dewatered domestic sludge as packing material in biofilter was investigated for hydrogen sulfide (H₂S) removal. No nutrients or microbial inoculation was added throughout the experiment. The corresponding bacterial community characteristics in the vermicompost biofilter of different spatial levels were evaluated by Miseq high-throughput sequencing technique. The results showed that the vermicompost biofilter performed well during operation. The H₂S removal efficiency reached nearly 100% under condition of the inlet concentration <350 mg m⁻³ and 0.25–0.35 m³ h⁻¹ gas flow rate. The maximum elimination capacity of 20.2 g m⁻³ h⁻¹ was observed at a flow rate of 0.35 m³ h⁻¹. Furthermore, the amounts of biodegraded products and pH varied accordingly. In addition, the results from high-throughput sequencing revealed pronouncedly spatial variation of the vermicompost, and the *Rhodanobacter*, *Halothiobacillus*, *Mizugakiibacter* as well as *Thiobacillus*, which can play an important role in removing H₂S, were predominant in the final vermicompost. These results imply that the vermicompost with diverse microbial communities has a good potential for eliminating H₂S.

Keywords: Bacterial community, high-throughput sequencing, hydrogen sulfide, vermicompost

1 Introduction

The odor is one of the world's seven typical public pollutions. It is harmful to people's living environment and health, and it can affect the growth of plants and animals. Furthermore, it is a potential safety concern. The hydrogen sulfide (H₂S) which smells like rotten eggs is the most common, highly toxic, and malodorous gas. It is a colorless, corrosive, and flammable gas that is an environmental pollutant [1]. The combustion of H₂S leads to a formation of SO_x, such as SO₂, which is an acid rain precursor and causes serious damages to ecological systems and human health [2]. H₂S is emitted from natural and anthropogenic sources. For instance, the natural emission sources are volcanoes, sulfur springs, and undersea vents. The major anthropogenic H₂S emission sources are petrochemical processes, paper manufacturing, food waste treatment, biogas production, composting, wastewater treatment, landfills, and livestock manure [1,3]. The olfactory threshold of H₂S is approximately 0.00047 ppm. The American Conference of Governmental Industrial Hygienists (ACGIH) points out that people's olfactory neurons will become fatigued when exposed to H₂S at 150–200 ppm. At approximately 1,000 ppm, people will lose consciousness and could even die in seconds owing to respiratory paralysis and cellular anoxia [4]. Therefore, H₂S must be purified from waste gas for the sake of public health and safety as well as environmental protection.

The common methods for H₂S removal are physical, chemical, and biological treatments. The first two methods are based on adsorption, chemical oxidation, and precipitation adsorption technologies [5,6]. However, these processes have high operating and processing costs related to energy and chemicals, and also produce hazardous byproducts that must be treated before discharge [7]. In contrast, the biological treatments (biofilters, biotrickling filters, and bioscrubbers) have advantages of low operating cost and energy consumption; high removal efficiency; less secondary pollution; and public acceptance as an environmentally friendly technology [8,9]. A biofilter has been proposed as a feasible and effective option for abatement of H₂S in recent

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years [10]. The removal capability and steady operation of the biofilter depend on the synergistic effects of the microbial activity, diversity, and benign environment. The packing material acts as the carrier for the microorganisms. Its performance directly influences the environment for microbial growth and activities, mass transfer, absorption, and adsorption [11,12] and it is considered as the backbone of the biofiltration. Therefore, selection of an appropriate filter media helps to improve the efficiency and capacity of the biofilter. Many studies have shown that the packing materials, such as soil, compost, peat, volcanic rock, polyethylene pellet, compost and biochar, expanded schist polyurethane foam and activated carbon are commonly be used for H₂S treatment [7,13–15]. Morgan-Sagastume and Noyola [16] showed that mixing filter media of a compost biofilter affected the H₂S removal efficiency. Moreover, the removal capacity has remained constant and close to 100% under the mixed compost conditions. As the treatment progressed, internal changes within the biofilter were correlated with its moisture content. However, the investment and operational costs must be considered before implementing this technology. As mentioned by Kante et al. [17] and Nor et al. [18], activated carbon had a good H₂S removal performance; however, it alone does not provide the characteristics for microorganism survival, thus it is mostly used in combination with other packing materials that have beneficial properties.

Vermicomposting involves interaction between microorganisms and earthworms. Biological degradation and stabilization of organic solid wastes occur via digestion by earthworms that transform the waste into useful vermicompost. Vermicompost has high porosity and a large specific surface area; it is a homogenized and humic substances; it has an abundant organic carbon and bacterial community [19,20]. Just like the compost with substantial fertilizer value, the vermicomposting product is an efficient growth promoter for plants as it contains higher available nutrients and much more diverse agricultural probiotics [21,22]. Several investigations have been conducted about the use of vermicompost in abatement of waste gases such as ethylene, methane, formaldehyde, ammonia, and hydrogen, as well as odor from human feces and livestock farming [23–26]. Up to now, there is little research available on vermicompost as packing material to remove H₂S and the microbial analysis in vermicompost.

The main objectives of this study were to assess the feasibility of removing H₂S using vermicompost and to investigate the associated microbial community. The vermicompost obtained from dewatered sludge was chosen as a media of bioreactor, and the changes of

microbial community were analyzed by Miseq high-throughput sequencing technique in this study. The results of this research may provide a basis for vermicompost as a biomedica to remove hydrogen sulfide and could be used as a guide for further design and operation of industrial-scale systems.

2 Materials and methods

2.1 Vermicompost and hydrogen sulfide

The initial physicochemical characteristics of vermicompost are given in Table 1 [27]. The vermicompost was collected from a vermicomposting reactor of dewatered domestic sewage sludge. In detail, the vermicomposting experiment was performed at a 1/5 weight ratio of earthworms to sludge in the laboratory at $25 \pm 1^\circ\text{C}$ for 60 days based on the method of Fu et al. [28]. After vermicomposting, all earthworms were picked out and the obtained vermicompost was sieved using a standard sieve with a mesh range of 2.36–4.75 mm. The vermicompost was loaded in the bioreactor by hand.

Hydrogen sulfide was supplied from a concentrated standard gas cylinder (99.99% purity; 8,000 mg m⁻³ concentration; balanced gas N₂; Chengdu Keyuan Gas Co., Ltd, China) and H₂S was diluted to different concentrations using air from a compressor.

2.2 Bioreactor set-up and operation

The bench-scale horizontal biofilter system was composed of a prehumidification system, a bioreactor, and a

Table 1: Physical and chemical properties of the vermicompost values are the means $\pm$ standard errors ($n = 3$)

Parameter	Value
Moisture content (%)	58.65 ± 0.01
pH	7.15 ± 0.00
Packing density (g L ⁻¹)	717.59 ± 8.02
Specific surface area (m ² g ⁻¹)	7.65 ± 0.26
Pore size (nm)	16.40 ± 0.42
Organic matter (%)	24.04 ± 0.01
Total nitrogen (g kg ⁻¹)	23.59 ± 0.37
C/N ratio	10.35 ± 0.03
Total phosphorus (g kg ⁻¹)	5.95 ± 0.42
Total kalium (g kg ⁻¹)	2.12 ± 0.30
Total sulfur (g kg ⁻¹)	71.66 ± 0.10

tail gas absorber (Figure 1b). The biofilter was a cuboid of Perspex packed with vermicompost. Its length, width, and height were 400 mm, 130 mm, and 210 mm, respectively. The vermicompost was placed in four Teflon mesh segments ($80 \times 12 \times 200$ mm in height, each), which provided an overall thickness of 320 mm and an effective packing volume of 7.37 L. Five H_2S sampling ports were distributed within bioreactor: one each at the inlet and outlet points and three along the length of the bioreactor. Compressed air was introduced into the bioreactor system after passing through an activated carbon filter to remove impurities. The airstream was prehumidified in a distilled water container before mixing the streams. Hydrogen sulfide was safely discharged from the cylinder using a pressure-reducing regulator and a flow meter. It was diluted with prehumidified air in a mixing chamber and then entered the system from the left of the biofiltration. The H_2S stream, after traveling throughout the bed, exited the system at the right, passed through the three-way valve, then entered into a bottle containing an absorbing solution of NaOH (10%) and, finally, into the exhaust hood. H_2S passed through the bioreactor for removal by the microbial community in the vermicompost. The flow rates of the streams were controlled with a flow meter. The overhead view of the biofiltration bed packed with vermicompost is shown in Figure 1a.

The system was operated at room temperature ($20\text{--}28^\circ\text{C}$) and no nutrients or microbial inoculation was added during the whole experimental process. The experimental conditions were as follows: gas flow rate was adjusted to 0.25, 0.35, 0.45, and $0.55\text{ m}^3\text{ h}^{-3}$, corresponding to an empty bed gas retention time of 106, 76, 59, and 48 s, respectively. The concentrations of H_2S varied between the range of $100\text{--}500\text{ mg m}^{-3}$ for each stage.

2.3 Sampling

H_2S samples were periodically collected from the bioreactor using 0.5 L Tedlar bags (Cole-Parmer, USA). One fresh vermicompost sample was collected at the start of the experiment and is referred as a CK. Four samples were collected from first to fourth segments of bioreactor at the end of the experiment and are referred as T1–T4. Each sample was collected in duplicate. The collected samples were divided into two parts for different analyses. One part was immediately stored at -20°C to analyze the bacterial populations in the biofilter, while the other was air-dried, ground, and sieved using an 80 mesh to determine the physicochemical properties.

Figure 1: (a) Vertical view of the bioreactor packed with vermicompost; “ $\curvearrowright$ ”: Gas flow direction. (b) Schematic diagram of the experimental system: 1. H_2S cylinder; 2. flow meter; 3. air compressor; 4. air filter; 5. humidifier; 6. expansion tank; 7. bioreactor packed with vermicompost; 8. H_2S sampling; 9. NaOH container.

2.4 Analysis of physicochemical properties

All measurements were determined in triplicate. The amount of H₂S concentration was periodically determined by gas chromatography (GC-2014, Shimadzu, Japan) with a flame photometric detector (FPD) [29] and DB-5 capillary column (15 m × Ø 0.53 mm × 1.0 µm; Hewlett Packard, USA). The temperatures of the oven, injector, and detector were fixed at 100, 50, 200°C, respectively. The flow rate was 50 mL min⁻¹. A six-port sampling needle valve automatically regulated the injection of product gases into the GC. Nitrogen was used as the carrier gas.

The surface area of the vermicompost was detected by a surface area and porosimetry analyzer (JW-BK200, Germany). The pH was measured with a pH meter (pH-3C, Shanghai, China), after mixing 1 g of vermicompost with 10 mL of distilled water. The moisture content of the vermicompost-based bioreactor was determined by taking the samples, then weighing and drying it for 12 h at 105°C in an oven to a constant weight. The organic matter content was measured by oven for 6 h at 550°C. The total nitrogen and phosphorus content were determined by spectrophotometry after separating alkaline potassium persulfate and sodium hydroxide. Total sulfur was analyzed by alkali fusion ion chromatography (Shimadzu, Japan) [30]. Elemental sulfur was measured with spectrophotometry. The forms of inorganic sulfur were determined by sequential extraction, based on the method described by Hu et al. [31]. Modifications to the methods were as follows:

(1) Water-soluble sulfate (H₂O-S): 1 g sieved dry sample was placed into a centrifuge tube; the sample was mixed with 10 mL ultrapure water (ratio 1:10); the mixture was shaken for 1 h at room temperature (20 ± 2°C); the mixture was then centrifuged at 5,000 × g for 10 min; the supernatant was filtered through a 0.45 µm membrane; and H₂O-S was determined by ion chromatography.

(2) Adsorbed sulfate (Adsorbed-S): after centrifugation, the supernatant was discarded and then 10 mL of 0.032 mol L⁻¹ KH₂PO₄ was added (this was also from a 1 g sample); and determine adsorbed-S by ion chromatography.

(3) Hydrochloric acid-soluble sulfur (HCl-Soluble-S): the sample without supernatant was extracted with 10 mL of 2 M HCl. S-free activated charcoal (100 mg) was added to 10 mL of the HCl extracts to eliminate soluble organic sulfur and the associated organic S from the 2 M HCl extracts. The amount of the HCl-Soluble-S for 1 mL extracting solution was then analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900, USA) with hexapole collision cell technology. Extraction process of adsorbed-S and HCl-Soluble-S was the same as H₂O-S.

2.5 Microbiological analysis

The microbial community structure in the media influences the performance of the bioreactor and the operational costs. Hence studying the microbial community of odorants can help to better understand the functions of different bacterial species and is significant in optimizing the H₂S removal process. Changes in the microbial community of different layers of vermicompost-packed bioreactor were analyzed by high-throughput sequencing.

2.5.1 DNA extraction and purification

Genomic DNA was extracted from vermicompost using the Power Soil® DNA Isolation kit (MOBIO QIAGEN, USA) according to the manufacturer's instructions. The purity and concentration of the total gDNA were detected by 1% agarose gels electrophoresis. The qualified DNA samples were stored at -20°C prior to use. DNA was diluted to 1 ng µL⁻¹ using double-distilled water prior to use as a PCR template.

2.5.2 PCR amplification and high-throughput 16S rRNA gene sequencing

The V3-V4 hypervariable region of the 16S rRNA gene was targeted for amplification using the primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTAC-HVGGGTWCTAAT-3') with barcodes (Huang et al., 2018). All PCRs were carried out in 30 µL reactions containing 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 0.2 µM of forward and reverse primers and about 10 ng of template DNA. Amplification cycling consisted of an initial denaturation at 98°C for 1 min; followed by 30 cycles at 98°C for 10 s, 50°C for 30 s, and 72°C for 30 s; and finally 72°C for 5 min. The PCR products were mixed with the same volume of 1× loading buffer (contained SYB green) and then detected by 2% agarose gel electrophoresis. PCR products were mixed in equidensity ratios. The mixed PCR products were purified using the GeneJET™ Gel Extraction Kit (Thermo Scientific, USA), as specified by the manufacturer. Sequencing libraries were prepared with the Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific, USA) and quantified by using a Qubit®2.0 Fluorometer (Thermo Scientific, USA). Amplicon sequencing was performed by Novogene Bioinformatics Technology Co., Ltd (Beijing, China) on the Ion Torrent S5™ platform.

2.5.3 Sequence analyses

The raw reads were optimized by length, quality, primer, and barcode mismatches. Subsequently, high-quality tagged sequences were gained after chimera detection and removal using the QIIME (Version 1.7.0). The sequences were then clustered into an operational taxonomic unit (OTUs) at 97% similarity using UPARSE package. The representative sequence for each OUT, which was the most abundant sequence, was used for taxonomic classification. Using the RDP classifier (version 2.2), the taxonomic information of the sequences was annotated separately. To indicate the microbial diversity in vermicompost, the α -diversity indices were quantified in the light of OUT richness.

2.5.4 Statistical analysis

Significant differences were analyzed by one-way analysis of variance (ANOVA) at 95% confidence level using the SPSS 22.0 statistical software. The Shannon index, Chao1 species richness estimators, and beta diversities of the bacterial communities were calculated using QIIME (Version 1.9.1, <http://qiime.org/index.html/>) [32]. Cluster analysis of the unweighted and weighted UniFrac distance metrics of the OTUs was performed by principal coordinate analysis (PCoA).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Performance of the vermicompost-packed bioreactor

The performance of the vermicompost-packed biofilter was monitored over 106 days under the gas flow rates of 0.25, 0.35, 0.45, and 0.55 m³ h⁻¹. The H₂S concentration and removal efficiencies of the bioreactor during the different phases are shown in Figure 2a. After acclimation to the low concentration of H₂S, the bioreactor took 6 days to accommodate the concentration. H₂S removal then increased quickly reaching 98%, thereby revealing adaptation of the microorganisms with the environment. The H₂S removal efficiency was close to 100% when the inlet concentration was less than 350 mg m⁻³ and the gas flow rate was between 0.25 and 0.35 m³ h⁻¹. However, further experiments demonstrated that removal efficiency declines

with increasing gas flow. At the end of each phase, the removal efficiencies decreased to 91%, 87%, 73%, and 60% at the gas flow rates of 0.25, 0.35, 0.45, and 0.55 m³ h⁻¹, respectively. The standard deviation for lower gas flow rates verifies the stability in the biofilter operation [16].

An increase in elimination capacity of the biofilter is due to the elevated inlet concentration or flow rate and plays a role in the treatment efficiency. The loading rate and the elimination capacity are used to reflect the performance of bioreactor. Researches have shown that the removal efficiency decreases in high loading rates, because H₂S molecules do not have enough time to diffuse inside the biofilm, and the biofilm cannot fully degrade the pollutant [13,33]. The H₂S elimination capacity and removal efficiency as a function of the inlet H₂S loading was investigated and is shown in Figure 2b. The H₂S removal efficiency abated gradually which was affected by the inlet H₂S loading rate of 5.6–23.3 g m⁻³ h⁻¹ at a constant flow rate of 0.35 m³ h⁻¹. Moreover, there existed a near-linear relation between the elimination capacity and the inlet H₂S loading rate. In addition, the maximum observed elimination capacity was 20.2 g m⁻³ h⁻¹, and bioreactors reached above 92.4% when the inlet H₂S loading was less than 15.1 g m⁻³ h⁻¹. However, the elimination capacity of the vermicompost-mediated bioreactor slowly improved and lower efficiencies were obtained when a higher concentration of H₂S and an increased flow rate were used. The decreased removal efficiency at the high loading rate was probably due to effective biomass and gas–liquid mass transfer limitations in the bioreactor since biological activity could be inhibited when the substrate conversion ability of the biomass was near the maximum [33]. In addition, exposure to high concentrations of H₂S could result in toxicity for the vermicompost microbial community. Hence, the vermicompost-mediated bioreactor has an increased ability to remove H₂S from contaminated air and may be more suitable for practical application.

The removal rate of contaminants from waste gas in a biofilter was correlated with concentration [34]. Hydrogen sulfide samples from different bioreactor thickness (1[#] – 80 mm, 2[#] – 160 mm, 3[#] – 240 mm, and 4[#] – 320 mm) were periodically investigated to determine the distribution of H₂S. The influence of thickness on the removal efficiency of H₂S and changes in concentration are demonstrated in Figure 3. The results showed that the removal efficiency of H₂S could be notably enhanced with increasing vermicompost thickness but the concentration of hydrogen sulfide reduced gradually. The removal efficiency increased because the path length and the contact area that hydrogen sulfide passed through in the vermicompost increased with the greater thickness. Thus, H₂S molecules had enough time to diffuse inside the biofilm and

Figure 2: (a) Removal performance for hydrogen sulfide in the vermicompost-packed bioreactor at different gas flow rates and inlet H_2S concentration and (b) influence of inlet loading on the elimination capacity and removal efficiency.

accelerate biodegradation. Different packing material thicknesses contributed differently to the H_2S removal efficiency. For instance, the removal efficiency at 80 mm made up over 50% and was the main area of deodorization. Furthermore, the removal efficiency of H_2S decreased at 80 mm and increased for the rest of the thickness. The H_2S contaminated air stream first entered the bioreactor segment #1 (left side of the bioreactor) through the inlet and then successively passed through segments 2 to 4 (right side). When the gas flow rate remained constant, the H_2S loading rate relied on the concentration. Consequently, the bioreactor achieved the highest H_2S load in segment #1 when compared to the other segments. The different loads along the bioreactor resulted in differences in the elimination capacity for different segments.

Similar observations have been achieved in other studies [29,35].

3.2 Effect of pH changes

The removal of H_2S using biological methods mainly depends on the metabolic activity of the sulfur-oxidizing microorganisms that are present in the packing material and biological metabolism is inseparable from the enzymatic catalysis [36]. Furthermore, enzymes can only work at certain pH values. Hence, pH also plays an important role in biofilter performance. Results showed that the pH decreased from 7.15 to 5.18 with increasing feed gas flux during the

Figure 3: The concentrations and removal efficiencies of H_2S along the bioreactor packed with vermicompost.

entire experimental period without additional treatment and there was a significant drop in the pH of T1 (Figure 4). However, no significant difference was obtained from T4. The decrease in pH might have been caused by sulfide oxidizing bacteria in the vermicompost that adsorbed and degraded the hydrogen sulfide, which was biologically oxidized to H^+ , sulfate, and sulfuric acid as presented in equations (1)–(4) [37]. Furthermore, this indicated that the pH change resulted in decreased biological activity and a change of transformation in metabolism. Omri *et al.* [33] showed that a pH drop resulted from oxidation by SOB and

the biodegradation of H_2S gas was achieved in the bottom and middle layers. Neisi *et al.* [38], reported that H_2S was efficiently removed from a septic tank with a vermicompost biofilter in the pH range of 4.7–7.8 when the H_2S concentration varied between 33 and 54 ppm.

Figure 4: Variation of pH in the four layers of bioreactor packed with vermicompost.

3.3 Analysis of metabolic products

Sulfur fertilizer has become the fourth fertilizer following N, P, and K fertilizers applied in agriculture. Sulfur exists in soils in varieties of inorganic and organic species. Inorganic sulfur is more mobile, it can be divided into SO_4^{2-} in the soil solution ($\text{H}_2\text{O-S}$), adsorbed SO_4^{2-} (Adsorbed-S), and hydrochloric acid-soluble sulfur (HCl-Soluble-S) [31,39]. Inorganic sulfur plays a critical role in the regulation of plant growth and development. The major end-products of H_2S oxidation are elemental sulfur (S_0) and sulfate (SO_4^{2-}) [40]. Hence, the determination of inorganic sulfur in different vermicompost samples was performed before and after the experiment. Compared with CK, the $\text{H}_2\text{O-S}$ and adsorbed-S concentration in vermicompost samples increased significantly and accumulated the most in T1, whereas HCl-Soluble-S decreased slowly (Figure 5). In addition, adsorbed-S is inorganic sulfur that is kept on the surface of soil colloids by anion exchange and coordination adsorption, which has strong stability. The order of adsorbed-S content in the different vermicompost samples was $\text{T1} > \text{T2} > \text{T3} > \text{T4}$. This was mainly because the pH of T1 was lower than the others and this less-acidic environment was attributed to SO_4^{2-} accumulation; therefore, T1 was the main area of deodorization. The increase of sulfate indicated that H_2S was degraded by the vermicompost and that H_2S removal by the vermicompost in the bioreactor was due to a combination of adsorption and biodegradation mechanisms. Vermicompost is rich in organic carbon, mineral nutrients, and inorganic impurity catalysts (iron oxide, calcium oxide, and magnesia) (Table 1), which could facilitate H_2S oxidation and convert it to S_0 in the presence of oxygen [37]. Meanwhile, S_0 would be oxidized slowly to sulfate as shown in equation (4). Hence, a yellow

Figure 5: Inorganic S in vermicompost and control during hydrogen sulfide treatment.

substance, which was deposited on the vermicompost after certain running time, was determined to be elemental sulfur ($10.85\text{--}16.58 \text{ mg g}^{-1}$) through analysis. These results are in correspondence with the literature reports [29,41]. Thus, the vermicompost with H_2S removed can become a sulfur fertilizer resource, offering nutrients to the growth of plants and enriching and improving the soils as well.

3.4 Bacterial communities developed in vermicompost for removal hydrogen sulfide

3.4.1 α -diversity and β -diversity of the bacterial communities

Diversity indices of vermicompost bacterial communities and the control during hydrogen sulfide treatment are presented in Table 2 [27]. Five samples were sequenced and subjected to quality filtering. Each sample had an average of 847 high-quality OTUs with coverage rates above 99% (Table 2). This indicated that the sequencing depth was reasonable and truly reflected the diversity of the bacterial community. Chao 1 indices simply indicate the number of species in a community, and the higher values reflect the more rich species in an environmental system. Shannon diversity index indicates some relation between the number of species and number of individuals, and truly reflects the diversity of bacterial community [42]. As shown in Table 2, in contrast to the control treatment, vermicompost showed relatively low Shannon and Chao 1 indices after treating hydrogen sulfide and suggested that these samples had lower diversity and richness than the control. Furthermore, this indicated that the bacterial community in different segments significantly varied. These results were closely related to gas admission. The contaminated air stream was

Table 2: Diversity indices of vermicompost bacterial communities and the control during hydrogen sulfide treatment

Sample	OTU	Shannon	Chao1	Goods coverage/%
CK	1,156	7.51	1161.78	99
T1	734	3.27	734.67	99
T2	994	6.62	984.71	99
T3	1,103	6.08	1093.28	99
T4	1,042	7.24	1052.46	99

directed and the H₂S concentration decreased horizontally in the bioreactor from left to right; therefore, microorganisms

of segment #1 were first exposed to higher concentrations of H₂S, while the higher H₂S concentration and sulfuric acid produced by the bacteria had a great toxic effect on the microorganisms. Thus, species richness and community diversity were the lowest in T1.

The similarity/dissimilarity of the 16S rRNA gene sequences among the different samples was determined by the weighted UniFrac clustering method. PCoA results based on fast UniFrac analysis showed that the bacterial communities in different vermicompost samples were significantly different (Figure 6a). However, T2 and CK had similar microbial communities.

Figure 6: (a) Bacterial community clustering based on weighted UniFrac PCoA in vermicompost samples and (b) bacterial community clustering based on weighted UniFrac distances in different sampling vermicompost. The tree was calculated by weighted UniFrac distance based on relative abundances at the phylum level.

3.4.2 Bacterial community composition

As illustrated in Figure 6b, the bacterial community compositions differed significantly in each sample. A total of 41 phyla were identified in the samples. The five most dominant phyla were Proteobacteria, Gemmatimonadetes, Bacteroidetes, Actinobacteria, and Firmicutes, accounting for 82.1–96.6% of the bacterial rRNA gene sequence abundance. Proteobacteria was identified as a prominent member representing sulfur-oxidizing bacteria, which were mainly responsible for the degradation of hydrogen sulfide [43]. This phylum was the single largest group in the vermicompost, accounting for 85.0%, 46.1%, 62.0%, and 44.0% in samples CK, T1, T2, T3, and T4, respectively, after 106 days of operation. In contrast, compared to the control treatment, Gemmatimonadetes decreased in different vermicompost samples and only accounted for 2% in T1.

The metabolic capacity of phylogenetically related microbial species is similar [8]. Thus, community structures at the genus level were analyzed to determine the main reasons for the differences in vermicompost from different spatial distributions. The results showed that the genus *Rhodanobacter* had the highest abundance in the four samples used for hydrogen sulfide treatment, accounting for an average of 28.7% of the bacterial 16S rRNA gene abundance (Figure 7). The genus *Rhodanobacter* is a Gram-negative, aerobic, yellow-colored bacteria, belonging to the family Xanthomonadaceae of the phylum Proteobacteria [44], and has been shown to remove hydrogen sulfide [45]. Additionally, the abundance of *Halothiobacillus* (mean 5.0%), *Thiobacillus* (mean 3.5%), and *Mizugakiibacter* (mean 2.7%) was significantly higher to those in the control group. However, the abundance of *Bacillus* reached its maximum value in the control treatment and then decreased distinctly in the vermicompost. The genus

Figure 7: Predominant bacterial genera in different vermicompost samples.

Halothiobacillus belongs to the γ -subclass of the Proteobacteria (family Halothiobacillaceae) and it is a mesophilic, obligately chemolithoautotrophic, sulfur-oxidizing bacterium. *Halothiobacillus* cells grow chemolithoautotrophically using sulfur, sulfide, sulfite, thiosulfate, and tetrathionate as electron donors and carbon dioxide as a carbon source [46]. Elemental sulfur production obtained from the bioreactor packed with vermicompost was higher, which indicated that vermicompost contributed to the growth of *Halothiobacillus* sp. Previous reports have found that the genus *Halothiobacillus* was the dominant bacterial community member in high salinity wastewater containing sulfide and a biotechnology-based deodorizing system containing high concentrations of hydrogen sulfide [46,47]. *Thiobacillus* is a colorless, strictly aerobic, chemolithoautotrophic sulfur-oxidizing bacteria that being able to oxidize sulfide to elemental sulfur, sulfite, and thiosulfate. Furthermore, it has been identified as the dominant bacterial community member in desulfurization systems [48,49]. The abundance of *Thiobacillus* increased with increasing vermicompost thickness. The results also indicated that segment 4# was more suitable for the growth of this genus during the late period. This could be connected with the fact that *Thiobacillus* species was able to effectively degrade H₂S [50]. In addition, the different H₂S concentrations resulted in variations in the bacterial community and abundance as the thickness of the bioreactor increased. The relatively high bacterial diversity that was observed in the vermicompost contributed to a good H₂S removal performance.

4 Conclusions

A vermicompost-packed biofilter effectively removed hydrogen sulfide in contaminated air. The H₂S removal performance varied with the gas flow rate, concentration, thickness, and microbial community composition. Accordingly, different forms of sulfate and sulfur were identified as biodegradation products. It is conceivable that the vermicompost with H₂S removed contained relatively high sulfur, which can improve its fertilizer value. Moreover, high-throughput sequencing results indicated that the different spatial levels of vermicompost exhibited differences in bacterial diversity. *Rhodanobacter*, *Halothiobacillus*, *Thiobacillus*, and *Mizugakiibacter* were identified as predominant bacterial genera in the final vermicompost. Studying the microbial communities associated with different biofilter layers can help to better understand the functions of different bacterial species and is significant in optimizing the H₂S removal process.

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