The influence of moisture content on removal of H$_2$S using the vermicompost bi filter and analysis on microbial community

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**Abstract.** The utilization of bio filters provides a promising option, serving as an environment-friendly and economically beneficial strategy for waste gas abatement. In this study, the different moisture content of vermicompost with indigenous microorganisms as a filter bed material was evaluated for the performance of a bio filter in terms of hydrogen sulfide removal efficiencies and the bacterial dynamics. Maximum removal efficiency of hydrogen sulfide was confirmed when the moisture content of the packing material was from 50% to 60%. By 16S rDNA gene-Illumina Miseq high-throughput sequencing technology, The Shannon and Simpson index of the vermicompost microbial community had significantly decreased after treating hydrogen sulfide. The predominant bacteria in vermicompost samples were Proteobacteria, Gemmatimonadetes, Bacteroidetes and Actinobacteria. Rhodanobacter and Mesorhizobium were isogenous with sulfur-oxidizing bacteria, which play a critical role in vermicompost biodegrading hydrogen sulfide.

1. Introduction

Hydrogen sulfide (H$_2$S) is a toxic gas with acute neurotoxicity, which is the most commonly occurring and hazardous environmental pollutant found in sulfur-containing malodor. H$_2$S is primarily emitted or generated by natural sources, including volcanoes, sulfide hot springs, submarine sediments. Additionally, anthropogenic sources such as petroleum refining, paper making, sewage treatment, waste disposal, textile industry, and livestock farming also contribute to H$_2$S emissions[1-3]. H$_2$S exhaust is a broad-spectrum poison. Short-term exposure to concentrations above 10 ppm can have adverse effects. Exposure to above 600 ppm H$_2$S can cause unconsciousness and even death within seconds[4]. This is due to H$_2$S binds to Fe$^{3+}$ in oxidized cytochrome oxidase in the blood, inhibiting cellular respiration or causing respiratory paralysis[5]. Additionally, the presence of H$_2$S can corrode power equipment and metal piping, and also formation of "hydrogen embrittlement" leads to safety accidents[5]. Air quality standards for H$_2$S emissions have increasingly become stringent in recent years. The Scientific Advisory Board on Toxic Air Pollutants (USA) has specified the acceptable range of 20 to 100 ppm in environment[3], and the instantaneous threshold of H$_2$S is 50 ppm. Therefore, in order to mitigate the toxic effects and other negative impacts of H$_2$S, it is essential to effectively manage malodorous substances such as H$_2$S. This holds paramount significance in enhancing environmental air quality and minimizing risks to human health.

Compared to physical-chemical methods, the common bioreactors include bio filters, bio-scrubbers and bio-trickling filters, which utilize microorganisms for removing gaseous pollutants and show more effective and economically viable treatment ability. Owing to the low cost and high efficiency, bio-filtration has recently gained more and more attention for the removal of H$_2$S. The main factors influencing the removal of H$_2$S using bio-filtration include microorganisms, the type and moisture content of the filler, ambient temperature, pH, and the reactor's structure. The efficiency of bio-filtration relies on the moisture content of the packing material, which influences gas-liquid mass transfer and the growth of microorganisms. The moisture content of the biofilter packing material is a critical parameter that must be controlled to optimize the performance of the biofilter. Omri et al. reported that the peat biofilter has a great microbial growth environment and strong adsorption and degradation of H$_2$S when the moisture content kept in 50%[6]. Plaza et al. found that different moisture content caused changes in filler properties, which in turn affected the biofiltration system's ability to remove H$_2$S[7]. It has been shown that the moisture content of 40% was suitable to remove the BTEX mixture by using compost and perlite as biofiltration carriers[8]. Pinnette et al. recommended to use a compost biological filter, which achieved the satisfactory operation effect when the moisture content of the filler was at most 60%[9].

Vermicompost, also known as "land treatment pill", has a specific surface area, properties of the porous structure, including a variety of probiotics and protease, urease, esterase, and other biological properties. These biological characteristics determine that it can be used as...
a gas adsorbent. Previous studies were demonstrated the effectiveness of vermicompost in removing pollutants such as mercaptans, ethanol, hydrogen sulfide, ammonia, and low fatty acids\cite{1,4,10}. However, there is a lack of research on the impact of vermicompost moisture content on the treatment of H$_2$S and changes in microbial community in recent years. Therefore, this study aims to investigate the removal of H$_2$S by using vermicompost bioreactor, analyzing the deodorization performance at different moisture content and using 16S rDNA gene-Illumina Miseq high-throughput sequencing technology assess the changes in microbial population of vermicompost under different conditions. The findings of this study will contribute to a better understanding of the efficiency of vermicompost in removing H$_2$S and provide a valuable theoretical basis and reference for future applications.

2. Materials and methods

2.1. Experimental materials

The vermicompost which earthworm treated urban domestic sewage sludge was used as filler. After sifting through a screen with a diameter of 2.36mm ~ 4.75mm, it was distributed into the Erlenmeyer flask bioreactor. The H$_2$S was from a concentrated standard gas cylinder (Chengdu Keyuan Gas Co., LTD., purity 99.99%). The fundamental properties of vermicompost was described in the literature\cite{10}.

2.2. Design of Experiments

150 g of sieved vermicompost was naturally dispensed into five 1000 ml triangular flasks with butyl rubber stoppers. Moisture content of vermicompost samples was maintained in 30%, 40%, 50%, 60%, and 70% respectively. 200 ppm H$_2$S was injected into each bioreactor by an airtight syringe. Each container was labeled and incubated in a constant temperature chamber without shaking. A series of five experiments were set up and was replicated three times for each treatment. H$_2$S from the head-space of bioreactor was extracted regularly by an airtight syringe and detected. When the H$_2$S concentration decreased to less than 0 ppm, the stopper of the triangular flask was opened for 1 h to replace the H$_2$S inside the flask with air. H$_2$S was re-injected to a same concentration, and the test was repeated multiple times.

2.3. Sample collection and analysis

Initial sample and vermicompost after the experiment at different moisture content of filler were collected and stored in a refrigerator at -80°C for DNA extraction and high-throughput sequencing analysis. The samples by air-drying and grinding were placed in a sealed bag and stored in a cool and dry place before use. H$_2$S samples were periodically collected from the bioreactor using 0.5 L Tedlar bags (Cole-Parmer, USA) and the concentration of H$_2$S was detected by gas chromatography (GC-2014, Shimadzu, Japan) with a flame photometric detector (FPD)\cite{10}.

2.4. DNA extraction and high-throughput sequencing

At the end of the experiment, vermicompost samples from different moisture content conditions were collected and labeled as MC3, MC4, MC5, MC6, and MC7. The initial vermicompost sample was marked as CK, and the parallel samples were mixed as one DNA sample. DNA was extracted using the Power Soil® DNA Isolation Kit (Mo Bio, USA), and the extracted DNA was tested for integrity, purity and concentration using 1% agarose gel electrophoresis. The V3–V4 region of 16S rRNA genes bacterial amplification by PCR was performed using the high-fidelity enzyme Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs, USA). Bacterial primers 341F (5’-CCTAYGGGRBGCASCAG-3’) combined with bar code and 806R (5’-GGACTACHVGGGTWTCTAAT-3’), were used to amplify bacteria. The PCR products were detected and recovered using 2% agarose gel electrophoresis, purified finally using the Gene JET Gel Extraction Kit (Thermo Scientific, USA). The library was constructed using Ion Plus Fragment Library Kit 48 rxns (Thermofisher, USA), and then the high-throughput sequencing was performed by Novogene Bioinformatics Technology Co., Ltd (Beijing, China) after quantificating Qubit and qualifying the library.

2.5. Data analysis

The α-diversity and β-diversity of samples were analyzed using different bio-informatics algorithms and MUSCLE software according to the relationship between the number of OTUs and the sequencing depth. The relativity of data was analyzed with IBMSPASS22.0 software. Species number, Chao1 index, Shannon index, coverage and others were calculated by Qiime software. All data were plotted with Sigmaplot 16.2 software.

3. Results and discussion

3.1. Influence of filler moisture content on H$_2$S removal efficiency

The moisture content of filler is one of the important factors affecting the efficiency of H$_2$S removal. The H$_2$S removal efficiency of vermicompost under different moisture content conditions was investigated periodically during the experiment (Figure 1.). The results indicated that the H$_2$S removal varied significantly at different moisture content. A lag period of 2 days was demonstrated before H$_2$S degradation in the vermicompost of bioreactor with the 1st injection of H$_2$S at moisture content of 30%. The H$_2$S removal rate was only 22.5% after 6 days of operation. Meanwhile the concentration of H$_2$S remained almost unchangable from 7 to 10 days and these results
indicated that the removal of H$_2$S by the vermicompost was not significant. Furthermore, it was observed that the vermicompost had a lighter color and took on a dry granular form. This showed that the microbial community of filler was in a poor physiological condition and low microbial activity. Consequently, effects of earthworm cast on H$_2$S removal declined at moisture content of 30%. On the other hand, when the vermicompost moisture content ranged from 50% to 60%, a lag period of 1 day was observed after the initial injection of 200 ppm H$_2$S, but it disappeared during subsequent re-injections of the exhaust gases, and the domestication period was completed by 7 days. When the H$_2$S was injected subsequently, efficient removal of H$_2$S was achieved within a short time. It indicated that the microorganisms of the vermicompost adapted quickly and led to grow rapidly with higher activity. Sulfur-oxidizing bacteria can produce H$_2$S-degrading enzyme, such as H$_2$S: quinone oxidoreductase and flavocytochrome c hydrogen sulfide dehydrogenase, which stimulate enzyme activity and increase the ability to biodegrade H$_2$S$^1$. However, the bioreactor operated for 12 days, H$_2$S concentration was nearly 160 ppm and the removal ratio only about 20% at the moisture content of 70%.

Furthermore, it was observed that a small amount of liquid had leaked from the system, which possibly due to higher moisture content resulting in an expansion of the surface area of vermicompost. Consequently, there was an augmented thickness in the surface liquid film, accompanied by reduced porosity, impeding the transmission of airflow. It led to an elevation in the resistance coefficient of earthworm castings, influencing the efficiency of mass transfer between the water film and the biofilm. As a result, it decreased the biological oxidation capacity of H$_2$S. Besides, the excessive moisture content in the filler may lead to the formation of anaerobic zones within the earthworm castings and result in the compaction of the filler layer. In summary, it was elucidated that vermicompost demonstrated effective H$_2$S removal within the moisture content range of 50% to 60%.

![Figure 1. H$_2$S removal effect at different moisture content (point is the concentration of H$_2$S, inverted triangle is H$_2$S reinjection).](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Shannon</th>
<th>Simpson</th>
<th>Chao1</th>
<th>ACE</th>
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<tr>
<td>CK</td>
<td>6.539</td>
<td>0.964</td>
<td>932.547</td>
<td>924.123</td>
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<tr>
<td>MC3</td>
<td>6.486</td>
<td>0.961</td>
<td>863.000</td>
<td>845.006</td>
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<tr>
<td>MC4</td>
<td>6.431</td>
<td>0.956</td>
<td>859.099</td>
<td>849.151</td>
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<tr>
<td>MC5</td>
<td>6.127</td>
<td>0.918</td>
<td>915.667</td>
<td>899.283</td>
</tr>
<tr>
<td>MC6</td>
<td>6.045</td>
<td>0.900</td>
<td>905.443</td>
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<tr>
<td>MC7</td>
<td>6.329</td>
<td>0.919</td>
<td>967.863</td>
<td>953.976</td>
</tr>
</tbody>
</table>

3.2. Analysis of the microbial composition of vermicompost

3.2.1 $\alpha$-diversity of vermicompost bacterial communities.

The $\alpha$-diversity index analysis was conducted on valid sequence data obtained from vermicompost samples under different conditions. This analysis aimed to explore the richness and homogeneity of microbial communities in individual samples from different moisture content of vermicompost during the treatment of H$_2$S (Table 1.). The Shannon and Chao1 indices were used to represent the diversity index and the number of operational
taxonomic units (OUTs) in bacterial communities, respectively. And the ACE index was used to represent the richness and homogeneity of species composition in a sample. Table 1. shows that the Chao1 index and ACE index of samples MC3, MC4, MC5 and MC6 were significantly lower than CK after the treatment of H2S with vermicompost at different moisture content. However, the Chao1 index and ACE index were higher than CK in sample MC7. The Shannon index of vermicompost was lower than that of CK for all the H2S treatments, with the lowest value observed in sample MC6. The highest and lowest Simpson index were observed in the CK and MC6 group, respectively. These results indicate that the composition and structure of the vermicompost bacterial community had significant changes after treating H2S with different moisture content of vermicompost.

3.2.2 Microbial composition and structure analysis.

(1) Microorganisms of vermicompost Comparative at the phylum level. Microbial community composition of vermicompost samples at the phylum level under different moisture content conditions was investigated by high-throughput sequencing of the 16S rDNA. Subsequently a total of 37 Microbial bacteria phyla were identified in the samples. The results of the analysis of the first 15 major bacterial phyla were shown in Figure 2.

The main bacteria community at the phylum level in vermicompost under different moisture content in this experiment were consisted of Proteobacteria, Gemmatimonadetes, Bacteroidetes, Actinobacteria, Firmicutes, Verrucomicrobia, Chloroflexi, Latescibacteria, Thermomicrobia, Thaumarchaeota, Chlorobi, Acidobacteria, Saccharibacteria, Ignavibacteriae, unassorted and small microflora. The dominant bacteria in vermicompost samples were Proteobacteria, Gemmatimonadetes, Bacteroidetes and Actinobacteria. Proteobacteria accounted for 36%, 40.3%, 34%, 29% and 31.5% of the total bacterial abundance in the MC3, MC4, MC5, MC6 and MC7, respectively. Gemmatimonadetes made up 12.7%, 18.9%, 31.3%, 35.6% and 32.2% of the total bacterial abundance in the MC3, MC4, MC5, MC6 and MC7, respectively. Compared to CK, a significant increase was observed in Proteobacteria and Gemmatimonadetes after treating H2S with vermicompost. These findings indicate that sulfur-oxidizing bacteria, which was isogenous with Proteobacteria and Gemmatimonadetes, was involved in the biological oxidation of H2S by vermicompost. Additionally, Proteobacteria and Gemmatimonadetes of the MC6 accounted for 66.9%. Combined with the information presented in Figure 1, it further showed sulfur-oxidizing bacteria play a dominant role in treating H2S by vermicompost. Furthermore, Actinobacteria of the total bacterial abundance in the CK, MC3, MC4, MC5, MC6 and MC7 were found to be 31.9%, 5.8%, 8.2%, 6.5%, 7.6% and 8.2%, respectively. The abundance of Actinobacteria showed a significant decrease in all samples.

Figure 2. The relative abundance of bacteria in different vermicompost samples at the phylum level.
(2) Compare and classification of microorganisms in vermicompost at the genus level. Based on the species annotations and abundance information of the samples at the genus level, the top 35 genera in terms of abundance information of the genera in samples were selected using the maximum value ranking method, which was clustered at the species level, and plotted as a heat map (Fig. 3.). The results of heat map analysis on R language showed that the microbial community was significantly changed after the treatment of H$_2$S with vermicompost, as well as Rhodanobacter and Mesorhizobium dominated the vermicompost. Rhodanobacter had the highest relative abundance, which accounted for 11.39%, 15.30%, 10.22%, 6.31% and 5.82% in the MC3, MC4, MC5, MC6 and MC7, respectively. Rhodanobacter, Gram-negative, strictly aerobic, rod-shaped, and yellow, use carboxylic and amino acids as carbon sources and oxidizes thiosulphate to sulfate under aerobic conditions[11]. The relative abundance of Mesorhizobium in the samples after treatment of H$_2$S was higher than that of the control product. Mesorhizobium belongs to the Phyllobacteriaceae and is usually found in symbiosis with leguminous plants. In addition it is a genus of nitrogen-fixing bacteria and promotes plant growth. However, the role of Mesorhizobium on treatment of H$_2$S with vermicompost has not been reported. The microbial community structure after the treatment of H$_2$S with vermicompost at different moisture content was significantly different from the initial vermicompost. This is mainly because H$_2$S is the sole energy source of microorganisms and subsequent sulfur-oxidizing bacteria are the most dominant species, and also plays a critical role in biodegrading H$_2$S. Yet now the analysis mechanism of bio-degradation H$_2$S is not clear and needs to be further analyzed and studied.

4. Conclusions

(1) Vermicompost demonstrated efficient removal of H$_2$S within the moisture content range of 50% to 60%. The microbial community of the bed material was
physiologically sound and sulfur-oxidizing bacteria were the most active with a high capacity for the adsorption and degradation of H$_2$S.

(2) Compared to CK, Shannon, Simpson, and chao1 index showed a decreasing trend in the five samples of vermicompost. The results indicated that the composition and structure of the vermicompost bacterial community had significant changes after treating H$_2$S with different moisture content of vermicompost.

(3) Analysis of the diversity and composition of the bacteria microbial community structure of vermicompost from different samples, which revealed the most dominant phylum at the phylum level was Ascomycetes, followed by Bacillariophyta, with their abundance percentages ranging from 29 to 40.3% and 12.7 to 35.6%, respectively. These findings suggested that the phylum Proteobacteria was the dominant population in the microecological bacterial community of vermicompost. Rhodanobacter and Mesorhizobium, with the dominant genera of five vermicompost samples, took up a higher abundance and played a crucial role in the adsorption degradation of H$_2$S.

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References