Comparative elimination of dimethyl disulfide by maifanite and ceramic-packed biotrickling filters and their response to microbial community

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HIGHLIGHTS

- The seeded GIGAN2 became dominant species after 45 days accumulation in twin BTFs.
- Higher ECs and REs were achieved in maifanite-packed BTF than ceramic-packed BTF.
- Max EC in BTF with maifanite (19.0) is higher than ceramic (16.6 g m\textsuperscript{-3} h\textsuperscript{-1}).
- Higher abundance of GIGAN2 on maifanite proved its higher DMDS removal capability.

GRAPHICAL ABSTRACT

Unpleasant odor emissions have traditionally occupied an important role in environmental concern. In this paper, twin biotrickling filters (BTFs) packed with different packing materials, seeded with \textit{Bacillus cereus} GIGAN2, were successfully constructed to purify gaseous dimethyl disulfide (DMDS). The maifanite-packed BTF showed superior biodegradation capability to the ceramic-packed counterpart in terms of removal efficiency and elimination capacity under similar conditions. At an empty bed residence time of 123 s, 100\% of DMDS could be removed by maifanite-packed BTF when DMDS inlet concentration was below 0.41 g m\textsuperscript{-3}. To achieve same effect, the inlet concentration must be lower than 0.25 g m\textsuperscript{-3} for ceramic-packed BTF. The bacterial communities analyses found higher relative abundance of GIGAN2 in the maifanite-packed BTF, suggesting that maifanite is more suitable for GIGAN2 immobilization and for subsequent DMDS removal. This work indicates maifanite is a promising packing material for real odorous gases purification.

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1. Introduction

Volatile organic sulfur compounds (VOSCs) are the primary irritants and one of the most offensive malodorous pollutants. They have attracted much attention in recent decades due to their adverse effects on human health (Wu \textit{et al.}, 2010) and their atmospheric chemistry activity relevant to the formation of sulfate aerosols (Andreia \textit{et al.}, 1997; Rumsey \textit{et al.}, 2014). The VOSCs mainly emitted from pulp and paper facilities, municipal sewage treatment plants, food industries and agricultural production processes can cause serious annoyance to
adjacent residents even in trace-level concentrations due to very low sensory thresholds (Li et al., 2015; Luvsanjamba et al., 2008). As a typical VOSC, dimethyl disulfide (DMDS) possesses the lowest threshold value of 0.1 μg m⁻³ among all odorous compounds and can cause fatigue, dyspnea, and upper respiratory tract irritation experiences at high concentration (Wan et al., 2011). In addition, it is also potentially related to the disturbance of human heme synthesis system (Klingberg et al., 1988). Thus, it is very meaningful and essential to develop safety and effective ways to remove this kind of compounds from waste gas effluents.

To eliminate the VOSCs threat, every relevant discharge should be subject to scrutiny and various treatment, and disposal technologies are also needed to be employed in industrial sites. Conventional control methods such as adsorption, condensation and thermal incineration are mainly based on physicochemical or chemical processes, which just simply transfer pollution sources from gas phase to liquid or solid phase, and need further processing (Ralebitso-Senior et al., 2012). Moreover, these traditional approaches show efficient and economical merits merely for the high-concentration pollutants. However, in the most cases, the VOSCs-contaminated gas was emitted with high-flow gas streams but at low concentrations, which are more preferred for the biological degrading processes (Jin et al., 2007). Biofiltration technologies have been attracting much attention definitely due to their cost-efficient, sustainable and environmentally friendly characters (He et al., 2009). Among them, biofilters and biotrickling filters (BTFs) are the most ubiquitously used and promising biotechnologies, while biofilters are less popular since they require to dissolve gaseous pollutants in the short residence time and is less suitable for less water-soluble compounds. However, most of the target VOCs are volatile and less water-soluble (Shareefdeen and Singh, 2005). Generally, biofilters use organic materials as packing material with nutrient solution added periodically. Whereas, recirculated nutrient solution is trickled continuously in BTFs. More importantly, BTFs normally require inert packing material to avoid bed compaction and subsequent sharply elevated gas pressure drop, which frequently occur in biofilters during the operation processes (Ding et al., 2011). Furthermore, the continuous trickling model of BTFs can also prevent the packing bed from drying and enhance the renewal rate of biofilm. A great majority of BTFs are proved to be more efficient than conventional biofilters because of their higher maneuverability of operating conditions with respect to long-term operation, especially when the pollutants are degraded with acids released such as chlorine-based and sulfur-based volatile organic compounds (Cox et al., 2002).

Packaging material and metabolic population are the key factors to determine the biodegradation performance in BTFs (Ralebitso-Senior et al., 2012). The major evaluation factor of packing material is its cost and mechanical strength. The most commonly used packing materials include activated carbon, lava rock, plastic ring, ceramic particle, polyurethane foam, molecular sieve and perlite (Delhomenie and Heitz, 2005). Maifanite is a kind of granitoid silicate produced from basic or acidic intrusive rocks by weathering and denudation, and is widely used in various fields, including medical care, food additives, antisepsis, and heavy metal decontamination in East Asia countries for its innocuous and neutral characteristics (Fu et al., 2013; Jiang et al., 2013). It also presents a great potential as packing material for BTFs due to its good mechanical properties to avoid the collapsing of fillings and cavernous porosity which is beneficial to microbial immobilization. Furthermore, various micro-nutrient elements such as Ca²⁺, Na⁺, K⁺, Fe³⁺, Cu²⁺, Mg²⁺, Zn²⁺ and Mn⁴⁺ can also be released into the liquid phase from maifanite as mineral nutrition source for bacterial growth (Gao et al., 2013). However, no study has been conducted to investigate the biodegradation promoting behavior of maifanite as the packing material in the BTFs yet.

Generally, the functional bacteria or consortium targeted to the specific pollutant should be inoculated on the inert packing material in BTFs. Previously, various VOSCs degradable strains were isolated and introduced into BTFs for VOSCs removal (An et al., 2010; de Bok et al., 2006; Giri et al., 2010; Hayes et al., 2010). Some reported functional species relative to the DMDS degradation with their main parameters and degradation performances in BTFs were listed in Table S1. After inoculation, the bacterial strain might experience a slow acclimation period and became the dominant species once the acclimation was achieved. Therefore, monitoring the dynamic of bacterial community is essential to understand the acclimation process. Nevertheless, few research was relative to the exploration of bacterial community dynamic in acclimation process, especially for the DMDS biodegradation.

Thus, in this study, twin BTFs were firstly designed and constructed. Considering its various merits and further application, maifanite was chosen as packing material in one column, and the most commonly used ceramic particle was packed in another column for comparison. Bacillus cereus GIGAN2, previously isolated for degradation of odorous DMDS in aqueous medium by our research group (Liang et al., 2015), was inoculated into twin BTFs to purify gaseous DMDS. The effect of empty bed residence time (EBRT) and inlet DMDS concentration on the RE as well as elimination capacity (EC) were extensively compared. Further, the bacterial compositions were comparatively analyzed in the twin BTFs based on PCR-DGGE technology.

2. Methods

2.1. Metabolic strain, packing materials and mineral salts medium

A novel DMDS degrader Bacillus cereus GIGAN2 previously isolated from sludge of a river in Guangzhou city, China by our group was seeded in twin BTFs (Liang et al., 2015). Maifanite and ceramic particles were used as packing material respectively in the twin BTFs, and the physical characteristics of the packing materials are presented in detail in Table S2. The specific surface area of maifanite and ceramic particles were 3.882 and 3.182 m² g⁻¹ and the porosity volume for the pile reached 49% and 44%, respectively. Overall, their physical characteristics presented above didn’t show significant differences. The scanning electron microscope images show that the surface of both packing materials is rather rough (Fig. S1). DMDS (99.5%) purchased from J&K chemical Ltd. was employed as sole carbon source. All other chemicals were of analytical grade and obtained from Guangzhou Chemical Reagent Co., Inc., China. Mineral salts medium was used for trickling the packed materials from top of BTFs, and the recipe was provided in supporting information. After each 30-day operation, half volume of mineral medium was replaced with isovolumetric fresh mineral medium to avoid adverse effect on microbial growth by the produced intermediates.

2.2. Description and operation of biotrickling filter

As Fig. 1 shows, a twin custom laboratory-scale BTF unit was employed in the experiment which comprised a couple of layers with the same configurations to ensure their comparability. The twin BTFs were made of high-transparent plexiglass and fixed to a horizontal steel frame. The external diameter and height of each filtration column was of 60 and 1200 mm, respectively. Maifanite and ceramic particles were divided into six equal layers and were packed into the BTFs respectively with a total volume of 1.37 L in each column. The packing height of each layer was approximately 100 mm. Sampling ports were opened in each layer. All the air tubes were made of polytetrafluoroethylene. Ambient air purified
program was: initial temperature of 80 °C for 2 min, increase to 150 °C with the rate of 10 °C min\(^{-1}\) for 2 min. The detector temperature was set at 250 °C. 250 μL sample gas was collected from each sampling port and was injected into the column by a 500 μL airtight syringe (Agilent) for the concentration determination in splitless mode.

The mass of the biofilm was expressed in mg per gram of dry particles and detected by the weight loss. About 20 g maifanite or ceramic particles were withdrawn from the BTF in triplicates, washed slightly using distilled phosphate buffer to eliminate the unimmobilized microorganism. Then the washed particles were placed in a weighed crucible (W1) and dried in an oven at 100 °C for 24 h. After that, the crucible were reweighed (W2) to calculate the weight of dry particles (W2–W1) and transferred into muffle furnace to burn off the attached biofilm at 560 °C for 1 h. Finally, by weighting the crucible (W3), the mass of volatile attached solids can be obtained by subtract W3 from the weight of dry particles. To exclude the weight loss of packing materials, the blank control groups with uncultured particles were processed in parallel.

2.4. Microbial community diversity analysis

The PCR (polymerase chain reaction)-DGGE (denaturing gradient gel electrophoresis) was employed to analyze microbial community in the twin BTFs. Firstly, DNA was extracted using genomic DNA isolation kit (Sangon Biotech Co., Ltd., Shanghai) from the biomass in twin BTFs after 30-day acclimatization. A pair of universal primers: 357F (5’-CTCTTACGCGGCGCAGCAG-3’) and 519R (5’-GWATTACCGCGGCKGCTG-3’) was used to amplify the target DNA. To enhance the definition of DGGE profile, a 40 bp GC clamp (5’-GGGGC-3’ to 5’-GGGGG-3’) was added in the forward primers. The PCR temperature cycling condition was initially set at 94 °C for 5 min, followed by 20 cycles of the procedure: 94 °C for 1 min, 65 °C for 1 min with a gradual decrease of 0.5 °C per cycle, 72 °C for 1 min, and 15 cycles of: 94 °C for 1 min, 55 °C for 1 min with a gradual decrease of 0.5 °C per cycle, 72 °C for 1 min, and 15 cycles of: 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min. An excess 7 min at 72 °C was undertaken at the last stage. Prior to the DGGE operation, the PCR product fragments were purified by 1% agarose gel electrophoresis. The DGGE was carried out in a Dcode Universal Mutation Detection system (BIO-RAD Laboratories) by 8% acrylamide gel concentration and an increasing linear denaturant gradient from 35% to 65%. The electrophoretic voltage was controlled at 120 V for 9 h with the electrophoretic buffers at 60 °C. The bands were visualized by silver staining.

3. Results and discussion

3.1. Start-up of BTFs

Inlet and outlet DMDS concentrations were detected once every four days, and each DMDS concentration value was the mean value of a triplicate measurement during start-up period. REs were calculated and plotted against the operating time in Fig. 2. During the first 36 days, REs of DMDS in twin BTFs increased gradually with fluctuations. Nevertheless, the REs inclined to maintain at approximately 95% (maifanite particles) and 80% (ceramic particles) within the following 20 days, revealing the gradual acclimation of GiGAN2 to DMDS waste gas. On day 20th and 28th, the sudden raise of inlet concentrations to 0.49 and 0.81 g m\(^{-3}\) respectively led to the significant decline of REs in both BTFs, indicating that the degradation performances of degrading bacterium were relatively susceptible to the DMDS concentration during the start-up period. Similar situation also occurred in our previous work about the biodegradation of ethanethiol in BTF seeded with strains RG-1 or B350 mixed microorganisms (An et al., 2010). This was attributed by activated carbon adsorption column was employed to dilute and blend the generated DMDS vapor in the gas tank. And then the synthetic waste gas was fed into the BTFs with a counter-current flow model to increase the mass transfer rate of DMDS. The purified gas was demisted by a mesh mist eliminator (diameter of 54 mm and height of 30 mm) before discharge.

After 18 h cultivation with nutrient broth, 100 mL of bacterial suspension (1.1 × 10\(^7\) colony-forming units per mL) was centrifuged at 4000 rpm for 10 min to concentrate bacteria. 10 mL was set constantly at 1.5 mL min\(^{-1}\) of 54 mm and height of 30 mm) before discharge.

Meanwhile, the pH value of mineral salts medium was regularly detected and adjusted to 7.0 with 0.1 M NaOH. The gas samples were collected from each sampling port to detect DMDS concentrations. In the EBRT experiments, gas rotameters were used to adjust the gas flow rates from 30 to 80 L h\(^{-1}\) corresponding to EBRTs from 164 to 62 s at fixed inlet DMDS concentration of 0.41 g m\(^{-3}\). After four-day stabilization, the DMDS concentrations in each layer were detected to calculate the RE and EC, and the following batch of experiments were operated by changing the inlet DMDS concentration or EBRT. Gradually increasing and then leveling off of RE is considered as an indicator of successful formation of biofilm onto packing materials. For comparison, the twin BTFs were operated in parallel under similar conditions except the packing materials.

2.3. Analytical methods

A HP 5890 gas chromatography (Hewlett-Packard, USA) equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) with a FID detector was employed to detect DMDS concentrations. The flow rate of carrier gas (ultra-high purity nitrogen) was set constantly at 1.5 mL min\(^{-1}\). The column temperature fluctuations. Nevertheless, the REs inclined to maintain at approximately 95% (maifanite particles) and 80% (ceramic particles) within the following 20 days, revealing the gradual acclimation of GiGAN2 to DMDS waste gas. On day 20th and 28th, the sudden raise of inlet concentrations to 0.49 and 0.81 g m\(^{-3}\) respectively led to the significant decline of REs in both BTFs, indicating that the degradation performances of degrading bacterium were relatively susceptible to the DMDS concentration during the start-up period. Similar situation also occurred in our previous work about the biodegradation of ethanethiol in BTF seeded with strains RG-1 or B350 mixed microorganisms (An et al., 2010). This was attributed

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Inlet

In the maifanite-packed BTF, the maximum EC of 19.0 g m$^{-3}$ was obtained when the inlet concentration of DMDS ranged from 0.41 to 0.61 g m$^{-3}$, which was slightly lower than the BTF reported by Arellano-Garcia et al. (17 g m$^{-3}$) under alkaline condition (Arellano-Garcia et al., 2012). However, the maximum EC (53 g m$^{-3}$) in the BTF reported by Ramirez et al. (2013) is higher than that of our work are well consistent with this phenomenon. In addition, the usage of maifanite as the packing material would not cause heavy metal pollution to the strain.

3.2. Effects of inlet DMDS concentrations

Combined with gas flow rate, inlet concentration of pollutant is an essential operating factor for BTFs (Li et al., 2015). As shown in Table S1, the maximum ECs in the twin BTFs was slightly lower than the BTF reported by Arellano-Garcia et al (17 g m$^{-3}$) under alkaline condition (Arellano-Garcia et al., 2012). However, the maximum EC (53 g m$^{-3}$) in the BTF reported by Ramirez et al. (2013) is much higher than ours (Ramirez et al., 2011). The various maximum EC in different reports may be the different characteristics of the degrading strain, the operating parameters or the reactor configurations.

The EBRT depended on the gas flow rate in specific BTF have great influence on the biodegradation processes (Jiang et al., 2000). The variation of REs and ECs were investigated with EBRTs from 164 to 62 s at fixed inlet DMDS concentration of 0.41 g m$^{-3}$. As shown in Fig. 4a and b, at EBRT of 164 s, DMDS could be completely removed by both BTFs packed with maifanite and ceramic particles after passing through the 4th and 5th layer, respectively. Further, decrease EBRT to 132 s, the RE significantly descended to 85.6% for ceramic-packed BTF, while 100% RE still could achieve for maifanite-packed BTF. The REs in maifanite-packed BTF were much higher than those in the ceramic-packed BTF under the identical conditions as EBRT less than 123 s. As shown in Fig. 4c and d, for the maifanite-packed BTF, when the EBRT ranged from 164 to 82 s, the EC showed an upward tendency from 8.9 g m$^{-3}$ to 13.6 g m$^{-3}$, but further decrease of EBRT to 62 s led to the EC slightly descended to 12.4 g m$^{-3}$. Comparatively, distinct trends can be observed for the EC in the twin BTFs. The maximum EC in ceramic-packed BTF was 10.4 g m$^{-3}$, which was much lower than that in maifanite-packed BTF. Further, the ECs were also plotted against the inlet loading of DMDS depending on EBRT. As shown in Fig. 4e and f, the EC sharply increased to 13.6 g m$^{-3}$ and then gradually decreased to 12.4 g m$^{-3}$ with the inlet loading increased from 8.92 to 24.56 g m$^{-3}$ in maifanite-packed BTF, whereas ECs in ceramic-packed BTF tended to maintain at approximately 10 g m$^{-3}$, which was far below the value in maifanite-packed BTF under the same inlet loading.

In all, the BTF packed with maifanite showed higher removal performance to the DMDS with respect to the REs and ECs under the identical operating conditions. Gao et al. reported that maifanite could act as promoting additive to enhance the polychlorinated biphenyl degradation capacity by two penicillium fungus in aqueous medium (Gao et al., 2013). Further analysis of zeta potential showed higher negative potential in culture medium with maifanite added which could form migration potential and increase the probability of collision between the pollutant molecules and degrading strains. The experimental results of BTF obtained from our work are well consistent with this phenomenon. In addition, it is reported that the released mineral elements from maifanite could promote bacterial growth, and subsequently to the polychlorinated biphenyl biodegradation (Gao et al., 2013). Further, Jiang et al. discovered that if 10 g of maifanite was immersed into 100 mL deionized water, the detected concentrations (µg/mL) of the elements in the water are very low: Fe 0.068, Cu 0.022, Mn 0.049, Zn 0.037, Se 0.015, and Al 0.001 (Jiang et al., 2013). Thus, the usage of maifanite as the packing material would not cause heavy metal pollution to the strain.
3.3. Long-term performance of the BTF

The long-term stability of a BTF was important for the practical application. Fig. S4 shows the removal performance of the twin systems during whole 210 days. Generally, stable removal of DMDS could be achieved by the twin BTFs when the inlet concentration was maintained around 0.41 g m$^{-3}$ with the EBRT of 123 s. However, when comparing the twin BTFs, the maifanite-packed BTF showed higher removal efficiency than the ceramic-packed one. For example, on the day 108–176, REs were over 95% in maifanite-packed BTF, whereas they were approximately 80% under the same condition. In real situation, the interruption in the plant operation, weekend recess, holiday breaks, or equipment malfunctions might constantly occur, which lead to the starvation of polluted air. The fast recovery capability of BTF was well essential for its employment in industrial settings. To further assess the robustness of the systems, the effect of pollutant starvation was investigated on the day 176. After 8-day starvation, the inlet concentration was resumed to 0.41 g m$^{-3}$. It can be seen that, for both BTFs, the REs could be quickly recovered to normal.

The pH values of mineral medium were also detected once a day. As Fig. S5 shows, pH changed from neutrality to acidity, and then we adjusted to 7.0 as dropping to near or below 5.0. This is due to that the conversion of S in DMDS to SO$_4^{2-}$ during the biodegradation processes (Ho et al., 2008). Similar phenomenon was also observed as ethanethiol was treated by *Lysinibacillus sphaericus* RG-1 or B350 mixed microorganisms in BTFs (An et al., 2010).

The pressure drop is one of the important parameters of long-term stability which could be a valuable indicator of the biomass formation, cracks in the packing material, and channeling in the gas flow for a biotrickling filter. As Fig. S6 shows, the pressure drop increased to 69 and 50 Pa and then slowly increase to 118 and 83 Pa at day 176 for maifanite-packed and ceramic-packed BTF, respectively. The 8-day starvation led to a slight decrease of the pressure drop, suggesting that the starvation protocol may be useful for the pressure drop control and long-term stability.

3.4. Microbial community diversity in the twin BTFs

As known, metabolic population is a key factor to determine the biodegradation performance of BTFs (Ralebitso-Senior et al., 2012). Although only *Bacillus cereus* GIGAN2 (DMDS degrader) was inoculated, the ambient microbes in the air could migrate into BTFs and then immobilized on the packing material surface to form the biofilm during the operation of BTFs. To further understand the DMDS
**Fig. 4.** DMDS RE at different EBRT with fixed inlet concentration of 0.41 g m\(^{-3}\) using (a) maifanite and (b) ceramic particle as packing material; EC of BTFs to remove DMDS at different EBRT with fixed inlet concentration using (c) maifanite and (d) ceramic particle as packing material; EC versus the inlet loading of DMDS at various EBRT with fixed inlet concentration of 0.41 g m\(^{-3}\) using (e) maifanite and (f) ceramic particle as packing material.

**Fig. 5.** The patterns of DGGE gel of purified PCR product of DNA extracted from biofilm in the twin BTFs (lane 1: in maifanite-packed BTF; lane 2: in ceramic-packed BTF) with 15–45 d.
biodegradation with the distinct trends in the twin BTFs, PCR-DGGE was used to analyze the bacterial community structures immobilized on the maifanite and ceramic particles after 15, 30 and 45 days acclimatization. The DNA base sequences of 16Sr DNA V3 fragments were employed for the identification of bacteria. Fig. 5 shows the results of DGGE gel purified from the PCR product of DNA extracted from the biofilm in twin BTFs. The main bands and their relative abundance were profiled by Quantity One software. Some clear bands were cut off for the DNA cloning and sequencing. The nucleotide sequence of each target V3 fragments was submitted to the ribosomal database project classifier for the identification of their taxonomic placement and GenBank for comparison to achieve the closest relatives. The detailed information of each band is showed in Tables S3 and S4. The analysis of phylogenetic trees for each band are also presented in Table S5 and Fig. S7.

4. Conclusion

A novel DMDS-degrader, Bacillus cereus GIGAN2, was successfully inoculated to twin BTFs to purify gaseous DMDS. Higher ECs and REs were achieved in maifanite-packed BTF than ceramic-packed BTF. The increasing abundance of GIGAN2 and the decrease of Shannon–Wiener diversity index showed the acclimatization process of GIGAN2. Higher biomass and higher relative abundance of functional strain GIGAN2 on the packing material also suggested that the maifanite was a good packing material for BTF to promote DMDS biodegradation ability. Overall, GIGAN2 and maifanite could be a promising integration to purify DMDS emitted from various real industries.

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A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.11.081.
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