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Aerobic biodegradation of odorous dimethyl disulfide in aqueous medium by isolated *Bacillus cereus* GIGAN2 and identification of transformation intermediates

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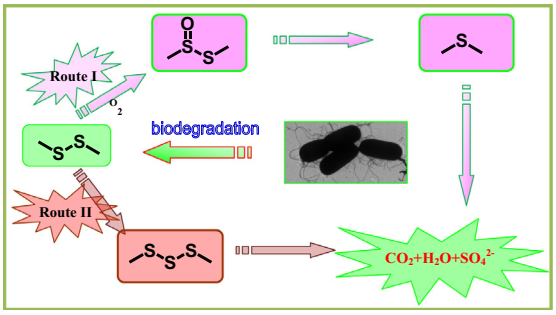
HIGHLIGHTS

- A strain capable of removal DMDS was successfully isolated and identified.
- Optimum conditions with the maximum DMDS biodegradation were obtained.
- Biodegradation kinetics of DMDS was found to follow first-order kinetics model.
- DMDS biodegradation mechanism was proposed based on three identified intermediates.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel, flagellated, rod-shape, Gram-positive facultative aerobe, was isolated and identified as *Bacillus cereus* GIGAN2. It can effectively remove model odorous organics dimethyl disulfide (DMDS) in aqueous solution under aerobic conditions. Initial concentration, pH value and temperature played important role in DMDS biodegradation, and up to 100% of 10 mg L^{-1} of DMDS could be removed within 96 h under the optimum conditions (30 °C, pH 7.0 and 200 rpm) with a maximum biodegradation rate constant of 0.0330 h^{-1} and minimum half-life of 21.0 h, respectively. Three main intermediates were identified using gas chromatography–mass spectrometry during this biodegradation process. Further, a reaction scheme is also proposed to explain the possible DMDS biodegradation mechanism by GIGAN2 based on the above-identified intermediates. Overall, this is the first report to demonstrate a newly isolated strain using high concentrated DMDS as the sole carbon and energy source with high efficiency.

1. Introduction

The odor emission of volatile organic sulfur compounds (VOSCs) like methyl mercaptan, ethanethiol, dimethyl sulfide (DMS), dimethyl disulfide (DMDS), methyl phenyl sulfide, carbon disulfide, carbonyl sulfide as well as hydrogen sulfide (H_2S) has kept on growing in the significance and posed a great threat to the public health over recent decades (Gutarowska et al., 2014; Wan et al., 2010). This kind of sulfur-containing odorant frequently occurred during the manufacture, usage and disposal of a variety of materials from the waste and sewage treatment plants, agricultural and food industries, rendering plants, paper mills, oil petroleum refinery industry (Caceres et al., 2012), and so on. Due to extremely low

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nuisance odor threshold, high toxicity and potential corrosive effect, these VOSCs can injure eyes, respiratory tract, skin, liver, and kidney resulting in high psychological stress, insomnia and loss of appetite (Calderon et al., 2012), and even death after long-term exposure. For example, DMDS has the lowest threshold of 0.1 μg/m^3 (Rosenfeld et al., 2001) among all odorous compounds. Research showed that DMDS concentration higher than 25 and 125 ppm would result in a decrease in the body weight gain, food intake, aspartate aminotransferase, alanine aminotransferase, and blood urea nitrogen in the male and female rats, respectively (Kim et al., 2006). Therefore, considering the unpleasant odor and toxic nature of sulfur-containing compounds, the abatement of them from water and air has drawn increasing attention and attracted considerable interests (He et al., 2009).

Until now, various effective technologies for eliminating odorous waste gas in aqueous medium have been proposed. In most cases, however, some conventional methods like physical/chemical processes (scrubbing, incineration, adsorption and oxidation) are often found to be unsatisfied with the elimination capability to VOSCs, as well as have often been criticized for their high investment and operating costs, possible generation of hazardous secondary wastes and consumption of high level of reagents and energy (Aroca et al., 2007). Comparatively, biological technology is proved as one of the most effective, sustainable, as well as economical interesting methods for the odorous compounds abatement from the contaminant environment (Li et al., 2013) if proper operational conditions are maintained. So far, biological degradation of a number of sulfur-containing compounds in the water and atmosphere by a newly isolated strain or the microorganisms in active sludge or sediments have been widely reported. For instance, methanethiol in wastewater can be removed by methanogenic bacteria (Sipma et al., 2002). What's more, various microbial species, such as strain Au7 (Wang et al., 2011), *Hyphomicrobium* VS (De Bo et al., 2002) as well as *Bacillus sphaericus* (Giri and Pandey, 2013) were isolated to eliminate DMS from waste air environment. The isolated bacteria with the capability to degrade H_2S were also found to be very popularly investigated, for instance, *Bacillus* sp. TSO3 (Ryu et al., 2009) and *Pseudomonas putida* CH11 (Chung et al., 2005).

However, relatively little information is known about the bacteria which can eliminate DMDS in aqueous solution. Most of available works were mainly focused on the co-treatment of DMDS-containing mixture gas by microorganisms in bioreactors, such as *Lysinibacillus sphaericus* RG-1 (Wang et al., 2011), *Thiobacillus thioparus* (Caceres et al., 2012) and B350 microorganisms (Wang et al., 2011). Few studies concentrated on the isolation of a single strain which can use DMDS as the sole energy, except only one paper about the DMDS degradation by isolated *Pseudomonas fluorescens* strain 76 (Ito et al., 2007). Furthermore, very limited research was carried out about the reaction kinetics and biodegradation mechanisms of sulfur-containing exhaust gas by isolated bacterial strains (Caceres et al., 2012; Gadekar et al., 2006) to evaluate the feasibility and effectiveness of biological degradation technology. Up to now, no similar studies have focused on the biodegradation kinetics and mechanisms of DMDS in aqueous by an individual bacterial strain.

The aim of this study is to isolate and identify a new microorganism, which has the ability to decompose DMDS in aqueous medium, and to investigate its biodegradation behavior and mechanism in aqueous solution. The optimal parameters affecting its removal efficiency were disclosed to obtain the best degradation efficiency by this bacterium. To probe the biodegradation kinetics, the maximum removal rate (V_{max}) and the half saturation concentration (K_m) were also studied using a modified Michealis–Menten type kinetics equation. In addition, a tentative degradation pathway of DMDS by the isolated strain, was also proposed based on the identified intermediates using GC–MS.

2. Methods

2.1. Chemicals and basal medium

Dimethyl disulfide (DMDS, 99.5%, Tianjin, China) was used as the sole carbon source and energy source for the microorganism. Dimethyldisulfide, S-oxide (99%), dimethyl sulfide (99%) were obtained from J&K chemical Ltd. All other chemicals were of analytical grade and purchased from Guangzhou Chemical Reagent Co., Inc., China. In all experiments, the mineral medium (MM) for the isolation of DMDS-degrading microbe was autoclaved at 121 °C for 15 min containing (100 mL): $K_2HPO_4{\cdot}3H_2O$ 0.12 g, KH_2PO_4 0.12 g, NH_4Cl 0.04 g, $MgSO_4{\cdot}7H_2O$ 0.02 g and $FeSO_4{\cdot}7H_2O$ 0.02 g, and 100 μL trace element medium stock solution, which was prepared according to our previous work (Wan et al., 2010).

2.2. Bacterial isolation and identification

The DMDS-degrading microorganism was screened from the sludge of a river collected from Guangzhou city, Guangdong province, China, following the modified isolation procedure (Smith and Kelly, 1988). In general, one gram of wet sludge was added to 100 mL sterilized MM containing 1 mg L^{-1} DMDS in 300 mL flask, and incubated at 37 °C with 200 rpm. To obtain DMDS-acclimating bacteria, 10% (v/v) of the above culture was transferred into the MM with the addition of DMDS ranged from 5 to 20 mg L^{-1} every five day for a month. Then, pure strain was achieved on the solid agar medium with 1 mg L^{-1} DMDS by using spread-plate method with 200 μL of the resultant culture. Finally, a newly isolated strain was characterized by morphological and physiological observation as well as the biochemical identification. In addition, 16s rRNA nucleotide sequence was used to confirm the evolutionary relationship of the isolated bacterium with the help of the BLAST software. The universal primer pair 7 F (5′-CAGAGTTTGATCCTGGCT-3′) and 1540 R (5′-AGGAGGTGATCCAGCCGCA-3′) (Tan et al., 2012) were designed to amplify the 16S rRNA gene with the extracted genomic DNA of the strain as the template. The PCR products were subsequently separated and visualized in 1.0% agarose gel. The detailed reaction system and the conditions of PCR amplification experiments were provided in the Supporting information.

2.3. DMDS biodegradation

In the biodegradation experiments, the newly isolated strain was pre-cultured to late logarithmic growth phase in Luria–Bertani at 37 °C and 200 rpm of shaking for 18 h and collected by centrifuged at 8000*g* for 15 min if not specified. Also, there is no replication in cultures. After washing with MM twice, 20 mL of harvested cultures were added into a 300 mL of serum bottle with 100 mL of MM containing 5 mg L^{-1} DMDS to perform the DMDS biodegradation experiments under the conditions of 37 °C, pH 7.4 and 200 rpm. The serum bottles were sealed with a rubber stopper to avoid the volatilization of DMDS. To further investigate the DMDS biodegradation capability of this strain, the effect of three operating parameters, such as initial DMDS concentration, pH value and the temperature on the removal of DMDS was analyzed. For each batch experiment, one of the parameters was changed while the others keeping constant. At biodegradation intervals of 24, 36, 48, 72 and 96 h, 300 μL of the upper gas samples were collected from the bottles using a 500 μL gas-tight locking syringe (Agilent, Australia) for parameter analysis. All samples were measured in triplicate. Moreover, the removal efficiency (RE) of DMDS was calculated according to the following kinetic equation: $RE(\%) = (C_0 - C_t)/C_0 \times 100\%$, where C_0 and C_t are the initial and residual concentration of DMDS at the time indicated, respectively. In addition,