J. Microbiol. Biotechnol. (2003), **13**(6), 866–871

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

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Removal of Dimethyl Sulfide in Ceramic Biofilters Immobilized with *Thiobacillus thioparus* TK-m

KIM, JONG-YEON AND BYUNG-WOO KIM*

Department of Chemical Engineering, Sungkyunkwan University, Suwon 440-746, Korea

Received: April 10, 2003
Accepted: October 15, 2003

Abstract Malodorous gas of dimethyl sulfide (DMS) was biologically oxidized to sulfate by *Thiobacillus thioparus* TK-m (DSM5368) immobilized in/on ceramic beads. More than 99.99% of DMS removal efficiency was obtained in a ceramic-biofilter reactor of 3.9 l when the feed concentrations were about 27.5 and 55.0 mg DMS/l at 30°C. However, the removal efficiency of the biofilter at above 40°C decreased to 4.5 mg DMS/(l·min) which was 85% of that at 30°C.

Key words: Dimethyl sulfide, immobilization, *Thiobacillus thioparus* TK-m, ceramic biofilter

Biofiltration has been frequently applied in the treatment of odor, VOC, and toxic chemicals because of low capital and operating costs, low energy requirements, and an absence of residual products requiring further treatment or disposal. As is well known, biofiltration units are microbial systems incorporating microorganisms grown on a porous solid media like compost, peat, soil, bark, and synthetic substances, or a mixture of these materials. The substrate provides the microorganisms with a favorable environment in terms of oxygen, temperature, moisture, nutrients, pH, and a carbon source of energy for their growth and development. The microorganisms utilize these favorable conditions to metabolize carbon-based compounds to their primary components, carbon dioxide and water, with additional biomass and metabolic byproducts.

Filter media are the key components of a biofilter. Filter media not only support the absorption effects, thereby ensuring adequate residence time for metabolic destruction, but also reserve substrate and humidity for the microorganisms and serve as the mechanical support for the maintenance of the internal structure of the filter bed [4, 12, 16, 27]. The intrinsic biological property of ideal filter media is not much different from the other common media described above. The key property of an intrinsically active filter media for biological oxidation is its structure. Nonhomogeneous media cause channeling, in which air passes only through the most permeable sections of the filter, which enhances drying of the more permeable zones and reduces the retention time.

Filter media include peat, compost, wood bark, soil, carbon particles, inert synthetic packing materials, or a combination of these. Soil beds both adsorb and oxidize the odorous compounds with a removal efficiency of 99% [23]. However, they are limited in effectiveness, because they are prone to clogging and short circuits. Compost has been more widely used than soil. The useful properties of compost are its high surface area, air permeability, water permeability, holding capacity, and microbial density, as well as low cost. However, compost does suffer from aging effects, because of microbial mineralization. Peat has both absorption and adsorption properties, high cellulose content, large moisture retention capacity, buffering capacity, and easy availability [3, 22]. Wood bark has also been used as packing material, because of its excellent air permeability, easy availability, and low cost. Granulated activated carbon (GAC) as a sole support medium has performance superior to soil and diatomaceous earth, because of its higher adsorptive capacity, however, the improvement over compost is not so much as to justify the price difference. Besides, some microorganisms cannot easily acclimatize with the GAC [10]. GAC has been reported to be useful in the biofiltration of hydrophobic contaminants [19]. Inorganic inert materials like polystyrene spheres, perlite, and ground scrap-tires can be added to the organic media in order to maintain bed porosity, and to prevent compaction and the development of lumps.

Ceramics have been shown to be effective filter materials with good adsorptive characteristics and porosity. Moreover, advantageous properties of ceramics are chemically inert, incompressible, reusable, and resistant to microbial attacks [20, 26]. They provide an additional buffering capacity for

*Corresponding author
Phone: 82-31-290-7246; Fax: 82-31-290-7272;
E-mail: bwkim@skku.ac.kr

spikes or shock loads of contaminants, however, the initial capital costs are higher than those for organic materials [5].

Some packing materials of natural origin like compost contain a sufficient number of different microorganisms such as bacteria, actinomycetes, and fungi. The efficiency of the purification process is enhanced, following the growth of active strains during the adaptation time after the startup of the biofilter. For easily biodegradable organic compounds, acclimation can typically take about ten days [21].

Following the use of natural materials, biological gas cleaning has made considerable progress, but is still in its developing stages for application to the control of VOCs and toxic chemicals in industrial use. DMS is a malodorous compound, which has an odor threshold of 120 ppb and an emission limit of 0.2 ppm in Korea. It is produced in the vulcanization process of rubber, and in the wood-pulping industry, oil refineries, and sewer systems, as well as from the methylation of sulfide in fresh water sediment, and it causes malodorous air pollution [18]. Various methods have been used for the removal of malodorous compounds from contaminated air. However, the removal efficiency for sulfur-containing malodorous compounds is reported to be poor. Fukuyama *et al.* [9] reported that the removal rate of DMS was 9 g/(kg-MLSS·d), when a culture of acclimated activated sludge was used for DMS. Tanj *et al.* [25], using *Thiobacillus thioparus* TK-m to remove DMS, reported that the outlet concentration of DMS was 0.72 mg/l when the inlet concentration was 19.0 mg/l.

In this study, light ceramic beads immobilized with *Thiobacillus thioparus* TK-m were applied to increase the reactor performance as well as the bed height in the field. In order to obtain the information for a real field application, long runs were tried by varying reactor temperatures and concentrations of DMS.

Materials and Methods

Microorganism and Immobilization

Thiobacillus thioparus TK-m (DSM 5368, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) was used in these experiments. Optimal pH and temperature were estimated as 6 and 30°C, respectively, in shaking-flask experiments, and the specific growth rate was 0.095 h^{-1} [1]. Cells were inhibited at above the DMS concentration of 0.17 g/l. The addition of nutrients such as nitrogen, phosphorus, sulfur, and trace elements to synthetic biofilters, showed, unlike the effect of natural supporters, significant improvement in the degradation rates of toluene [1] and other chemicals [11]. Therefore, the basal medium in 1 l of distilled water consisted of 2 g KH_2PO_4, 2 g KH_2PO_4, 0.4 g Na_2CO_3, 3.8 g KNO_3, 0.2 g $MgCl_2 \cdot 6H_2O$, with a 5 ml trace metal solution and 5 ml vitamin mixture. The trace metal solution in distilled water with a final volume of 1 l was composed of 50 g disodium EDTA, 11 g $ZnSO_4 \cdot 7H_2O$, 5 g $CaCl_2$, 2.5 g $MnCl_2 \cdot 4H_2O$, 0.5 g $CoCl_2 \cdot 6H_2O$, 0.5 g $(NH_4)_6 \cdot Mo_7O_2 \cdot 7H_2O$, 5 g $FeSO_4 \cdot 7H_2O$, and 0.2 g $CuSO_4 \cdot 5H_2O$. The vitamin mixture in distilled water with a final volume of 1 l was composed of 10 mg thiamin HCl, 20 mg riboflavin, 10 mg pyridoxin, 20 mg nicotinic acid, 10 mg p-aminobenzoic acid, 20 mg calcium pantothenate, 1 mg biotin, and 1 mg cyanocobalamin.

Strain TK-m was precultured at pH 6 and 30°C for two weeks in 150 ml medium containing 3.4 mg DMS/l in 500-ml shaking flasks sealed by a silicone stopper. DMS was added intermittently according to the growth of the bacteria. The biofilter reactor was packed with porous ceramic beads which were immobilized by physical adsorption in the culture solution of 46 mg cells/l. Initial cell loading in the immobilized beads was done with the concentration of about 90 mg cells/l ceramic.

Analytical Methods

Cell concentrations in a batch reactor were determined at absorbance 660 nm with a UV/Vis spectrophotometer (UV-160A, Hitachi, Japan) [14]. Absorbance of 0.1 corresponded with 65.0 mg dry cell/l. Sulfate was measured turbidimetrically at 420 nm as barium sulfate [8]. Dimethyl sulfide concentration was determined at 300 nm with a spectrophotometer after extracting with 5 ml of 2,2,4-trimethylpentane and then mixing 2 ml of this solution with 2 ml of 0.2% (w/v) iodine in trimethylpentane [13].

The segments of the sectioned cell-immobilized beads were fixed for 1 day using 2% glutaraldehyde in 0.1 M Tris-HCl buffer (pH 6.8) and postfixed for 1 h in aqueous solution of 1% uranyl acetate. The stained sections were freeze-dried for 1 day, and remaining water was dehydrated further in a vacuum drier for 1 day. Following the dehydration, the gold-coated specimen was examined in a scanning microscope (SEM S-2400, Hitachi, Kathuda, Japan).

Biofilter Reactor

A cylindrical biofilter reactor with an inside diameter of 10 cm and height of 50 cm packed to a bed height of 33 cm was used (Fig. 1). The average mechanical strength and porosity of a ceramic bead was 50 kg_f/cm^2 and 80%. A bead diameter ranged between 10 and 15 mm and the specific surface area was 6.3 m^2/g. Moisture content of 50–60% was maintained by adding the buffer solution with a spray nozzle in a humidifier (Model SKU, Suhkwang Instrument Co., LTD, Korea).

Two sampling ports were located at both the inlet and outlet of the biofilter. DMS was supplied at a constant flow rate of 250 ml/min to the lower part of the biofilter after dilution with air. DMS concentration fed into the biofilter reactor was controlled by mixing 0.1% DMS standard gas with air, using a mass flow controller (UFC-8100, UNIT Instruments Inc., U.S.A.).

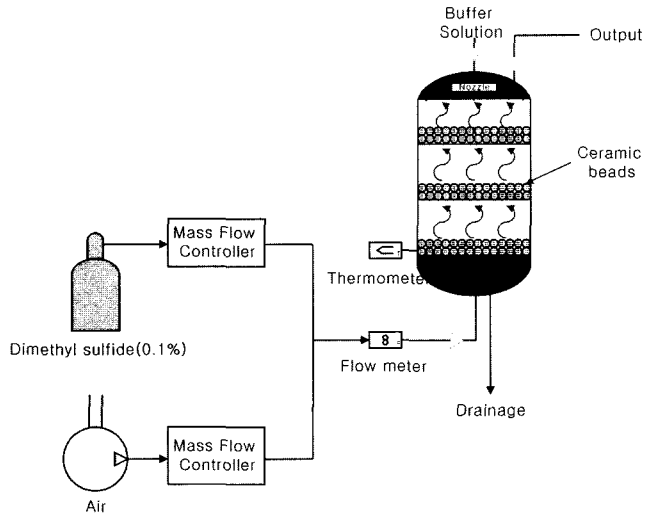

Fig. 1. Schematic diagram of a biofilter reactor immobilized with *Thiobacillus thioparus* TK-m so as to remove DMS.

RESULTS AND DISCUSSION

A Typical Time Course in a Batch Reactor

Kanagawa and Kelly [13] suggested that *T. thioparus* TK-m grows autotrophically while oxidizing DMS and does not appear to assimilate DMS-carbon by methylotrophic mechanisms such as the serine pathway. In contrast to the oxidation stoichiometry in the oxygen electrode, form aldehyde and sulfate are produced by the following equation:

$$(CH_3)_2S+3O_2=2\ HCHO+H_2SO_4$$

In order to investigate this tendency toward sulfate production, *T. thioparus* TK-m was first grown in shaking flasks. Sulfate concentration increased up to about 10 mg SO_4^-/l, as the cell concentration increased to an absorbance of 0.05 at 660 nm measured by a spectrophotometer.

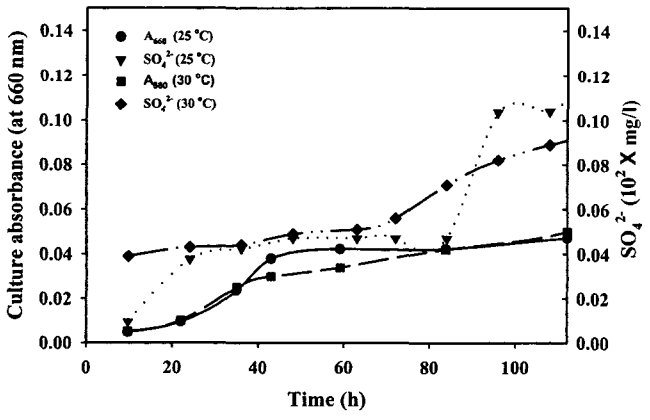

Fig. 2. Batch culture of *T. thioparus* TK-m in a 500-ml shaken flask containing 200 ml medium of 2.48 g/l sodium thiosulfate at 25 and 30°C.

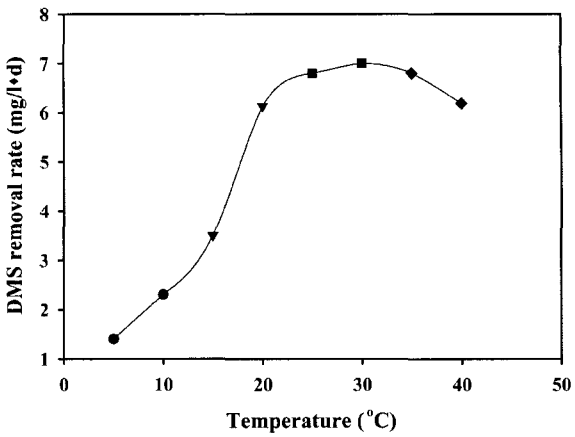

Fig. 3. Effect of temperature on the biological degradation of DMS by *T. thioparus* TK-m.

Optimal Temperature and pH in a Batch Reactor

The influences of temperature and pH on the biological removal of DMS were examined in batch cultures. Temperature variation should be considered, since the actual temperature increase depends on the nature and concentration of the contaminant to be oxidized, and ranges between 2-4°C with occasional hikes up to 10°C and more. Metabolic heat production dries the packing material and promotes the development of heterogeneous zones, ultimately resulting in nonuniform gas distribution and reduction in microorganisms.

The optimum temperature was 30°C, at which the removal rate of DMS was 0.43 mg/(l·min) (Fig. 3). The removal rate did not vary much in the temperature range of 25-35°C, however, it decreased sharply at temperatures below 20°C. Removal rates at 15°C, 10°C, and 5°C were shown as 1/2, 1/3, and 1/5 of the rate at 30°C, respectively.

The optimum pH was 6, while the removal rate of DMS was 0.40 mg/(l·min) (Fig. 4). The removal rate decreased

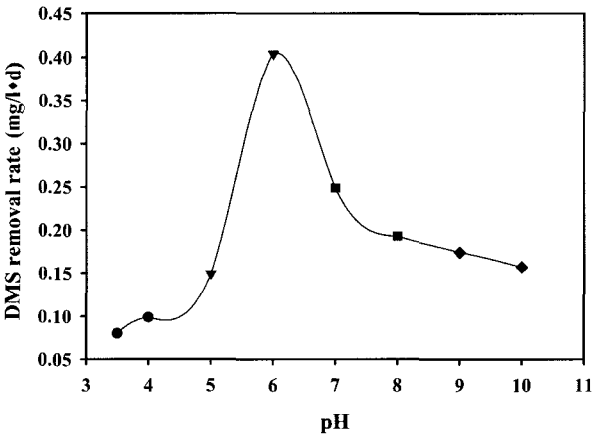

Fig. 4. Effect of pH on the biological degradation of DMS by *T. thioparus* TK-m.

sharply at pH values below 5. Removal rates at pH 4 and pH 3.5 were shown as 1/4 and 1/5 of that at pH 6.

Initial Acclimation of Bacteria

Biofilters operate essentially as plug flow devices. Concentrations of contaminant decline as air passes through the biofilter, so that concentrations are much higher near the influent end than at the effluent end. The characteristics of the biological community change accordingly. The influent end supports a dense biomass, possibly because the substate was not limited. In this study, DMS concentration was determined at both inlet and outlet ports.

After packing ceramic beads, a decomposition experiment was carried out for 60 days. Biological degradation at 30°C is shown at the initial stage in Fig. 5, where DMS with a concentration of 27.5 mg/l was supplied at 250 ml/min. The inlet and outlet DMS concentrations and the removal percentage of the biofilter are described in Fig. 5.

Inoculation of a biofilter with a laboratory grown culture of microorganisms could drastically reduce the acclimation period for the biodegradation of dichloromethane from 10 weeks to 10 days [7]. Microorganisms can survive for fairly long periods with hardly any loss of microbial activity, when the biofilter is not loaded. This activity can go on up to two months if sufficient nutrients are available from the filter material [17].

In this study, the acclimation period required more than 15 days for the biofilter to become effective. After the initial acclimation phase of two weeks, the removal capacity increased from 20% to 99.99%.

Effect of Variations in DMS Feed Concentration and Reactor Temperature

On the 17th day, two days after a fully acclimated situation, DMS concentration increased from 27.5 to 55.0 mg/l with the same flow rate of 250 ml/min. It took about 24 h for the biofilter to adapt to the higher concentration, with a

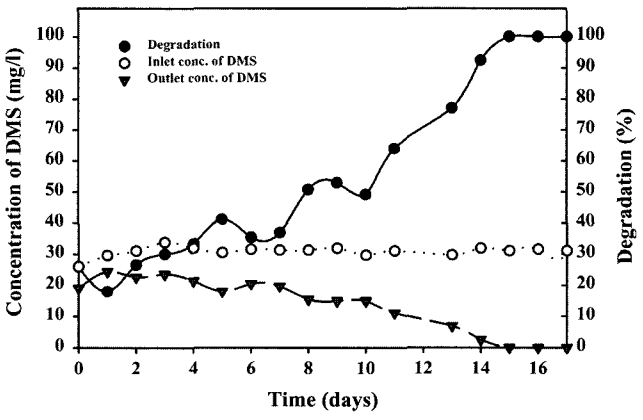

Fig. 5. Biological degradation at 30°C, where DMS was supplied at 250 ml/min with a concentration of 27.5 mg/l.

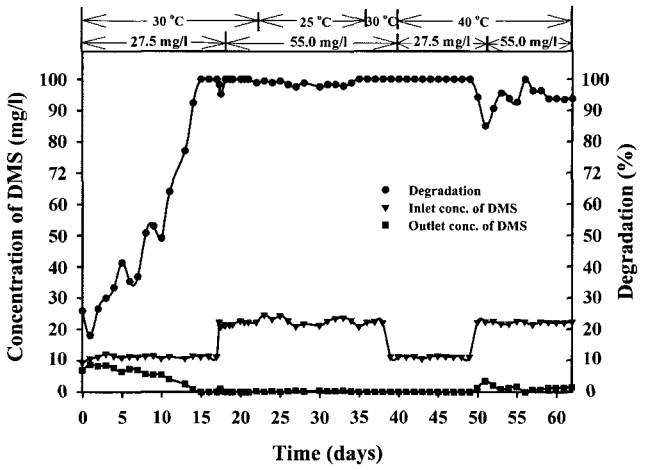

Fig. 6. Effect of reactor temperature and feed concentration on the removal rate of DMS. DMS gas was supplied at a flow rate of 250 ml/min.

DMS removal efficiency of 99.99%. In order to investigate the effect of temperature on the removal efficiency, the temperature of the biofilter was decreased on the 21st day from 30 to 25°C at the same DMS concentration and flow rate. Little variation in removal efficiency is shown in Fig 6. The temperature was increased from 25 to 30°C on the 33th day after twelve-day operation. Then, the temperature was increased again from 30 to 40°C and DMS concentration was raised from 27.5 to 55.0 mg/l on the 47th day, after having decreased to 27.5 mg/l. The removal rate of DMS at 40°C decreased to 4.5 mg/l·min, which was 85% of that at 30°C.

Too little moisture content causes drying of the filter bed, depriving microorganisms of water, and consequently biological activity is significantly reduced and the bed material contracts, creating fissures that cause channeling and short circuiting and a decrease in the retention time. Scanning electron micrographs of a ceramic bead with a biofilm are shown in Fig 7. A whole ceramic bead immobilized with the cells (Fig. 7a) has an average diameter of 1 cm with a pore size of 0.1- 1 mm. To prevent excessive head-loss, it has been recommended that 60% (by weight) of biofilter media be composed of particles greater than 4 mm in diameter [24]. In an optimal growth condition of 30°C, the biofilm (Fig. 7b) shows abundant cell aggregates. However, the size of cell aggregates tends to decrease and the cells tend to detach from the ceramic bead surface (Fig. 7c) due to the dry conditions at 40°C.

On the other hand, too much water decreases the transfer of oxygen to the biofilm, limiting the reaction rate. This is typically referred to as "blinding" of the biofilm, and promotes development of anaerobic zones within the filter bed, resulting in foul smelling emissions, and increasing back-pressure. For a compost bed, a moisture level of 40- 50% is recommended [2], and for peat moss it should

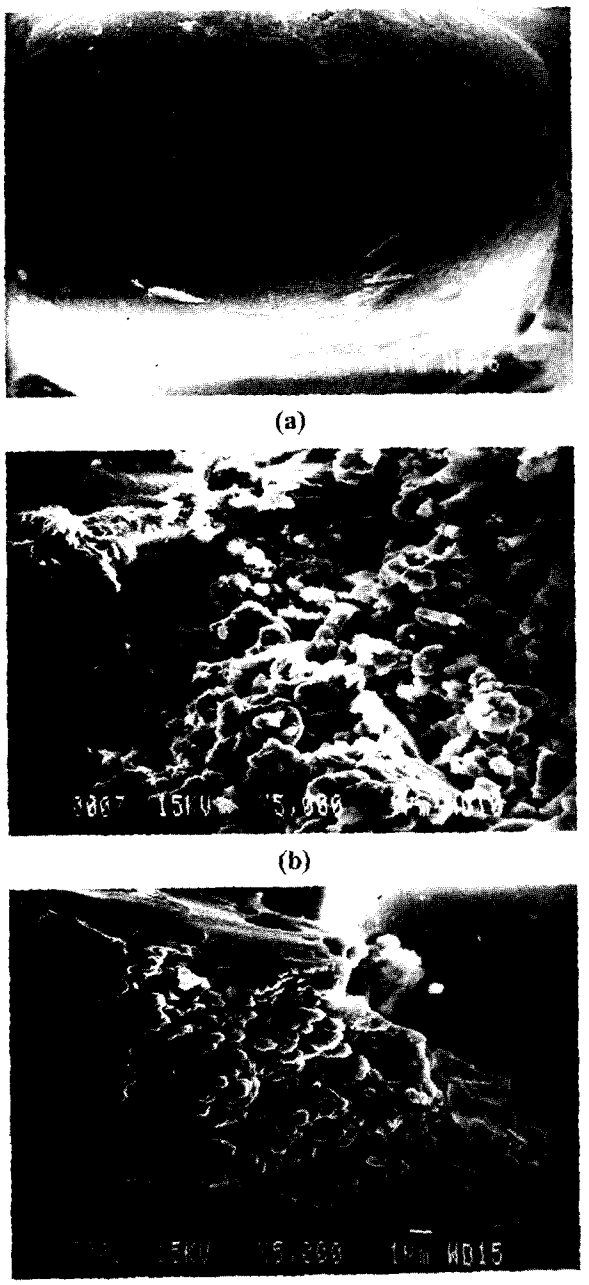

Fig. 7. Scanning electron micrographs.
(a) A whole ceramic bead immobilized with *T. thioparus* TK-m, (b) the biofilm surface grown at an optimal temperature of 30°C, and (c) the biofilm surface dried at 40°C.

be 40-60% [23]. For direct moisturization, water droplet diameters should not be greater than 1 mm because the impact of the falling droplet increases by the fifth power of the diameter. In this study, moisture content was controlled by adding the buffer solution through a spray nozzle from a humidifier. However, the relative humidity tended to decrease as the reactor temperature increased.

CONCLUSION

DMS, a malodorous gas, was efficiently removed by *Thiobacillus thioparus* TK-m immobilized in/on ceramic beads. Removal efficiency was 99.99% with a removal capacity of 5.3 mg DMS/(l·min), which was much higher than 0.67×10^{-3} mg slurry/(l·min) [18]. A maximal removal rate of 2.8 mg DMS/g(dry cell wt)·min was reported [14], however, this result cannot directly be compared with the result in this study. When the temperature was above 40°C, the removal capacity of the biofilter decreased to 4.5 mg/l·min which was 85% of that at 30°C, due to the drying of the filter bed which deprives the microorganisms of water.

Acknowledgment

This work was supported by grant No. R01-2000-00328 from the Basic Research Program of the Korea Science & Engineering Foundation.

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