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Hydrogen Sulfide Removal by Immobilized *Thiobacillus novellas* on SiO_2 in a Fluidized Bed Reactor

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Abstract The removal of hydrogen sulfide (H_2S) from aqueous media was investigated using *Thiobacillus novellas* cells immobilized on a SiO_2 carrier (biosand). The optimal growth conditions for the bacterial strain were 30°C and initial pH of 7.0. The main product of hydrogen sulfide oxidation by *T. novellus* was identified as the sulfate ion. A removal efficiency of 98% was maintained in the three-phase fluidized-bed reactor, whereas the efficiency was reduced to 90% for the two-phase fluidized-bed reactor and 68% for the two-phase reactor without cells. The maximum gas removal capacity for the system was 254 g $H_2S/m^3/h$ when the inlet H_2S loading was 300 $g/m^3/h$ (1,500 ppm). Stable operation of the immobilized reactor was possible for 20 days with the inlet H_2S concentration held to 1,100 ppm. The fluidized bed bioreactor appeared to be an effective means for controlling hydrogen sulfide emissions.

Key words: *Thiobacillus novellas*, hydrogen sulfide, immobilized cells, SiO_2, biosand, fluidized-bed bioreactor

Recent years have seen a considerable amount of research activity into the reduction of offensive hydrogen sulfide (H_2S), pollutants from our environment [6]. A large variety of microorganisms are known to grow chemolithotropically on sulfide compounds, including hydrogen sulfide, and a common characteristic is the manner in which they obtain energy for growth; *i.e.*, by oxidizing sulfide components. In general, the following genera have been evaluated for their sulfide properties: *Thiobacillus* sp., *Thiosphaera* sp., *Thiomicrospira* sp., *Thermothrix* sp., *Beggiatoa* sp., *Pseudomonas* sp., and *Sulfolobus* sp. [1, 5]. Among these bacteria, *Thiobacillus* sp. has been studied extensively for its ability to remove hydrogen sulfide from gases. The *Thiobacillus* sp. is more effective than the other sulfide-oxidizing microorganisms in terms of their required growth conditions and the utilization of various types of substrates. The sulfide compounds utilized by *Thiobacillus* sp. as energy sources include H_2S, S, and $S_2O_3^{2-}$ [8–10, 12, 15]. In its liquid phase, hydrogen sulfide exists in the forms of H_2S, HS^-, and S^{2-}, depending on the pH, temperature, and ion intensity. Below a pH of 7.0, H_2S is the main dissolved component, whereas in the pH range of 7.0 to 14.0, HS^- is the main component. The concentration of S^{2-} in wastewater is generally ignored [8].

Biological treatment techniques for the removal of noxious hydrogen sulfide have included biofilters, biological trickling filters, bioscrubbers, and fluidized-bed reactors [4, 11, 13]. To effectively remove hydrogen sulfide by a biological method, we have identified and isolated a new sulfide-oxidizing strain of bacteria that satisfies growth and reactor operation conditions [3]. We report here on hydrogen sulfide removal using sulfur-oxidizing strain *T. novellas* SRM immobilized on a novel SiO_2 (biosand) carrier in a 3-phase fluidized-bed reactor.

Materials and Methods

Bacterial Strain, Media, and Culture Conditions

The strain selected for use in this study was *Thiobacillus novellas* SRM, as reported previously [3]. The basal medium chosen for daily culture was based on thiosulfate. The medium for the growth and degradation of hydrogen sulfide was prepared by dissolving 4.0 g of K_2HPO_4, 4.0 g of KH_2PO_4, 0.5 g of NH_4Cl, 0.05 g of $FeSO_4 \cdot 7H_2O$, 8.0 g of $Na_2S_2O_3 \cdot 5H_2O$, and 2.0 g of yeast extract in 1 l of distilled water and the pH adjusted to 7.0 using 2 N NaOH and 2 N HCl. A sulfide-limiting condition in the actual fluidized-bed bioreactor was created by using a yeast extract only in basal medium as the reaction medium. The SiO_2 crystal

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biosand used as an immobilization carrier for this study was purchased from Jeonwoo Art System (Seoul, Korea) and stored in a vacuum desiccator for more than 24 h before use. The physical properties of the biosand were as follows: diameter 1–2 mm, density 1.27 g/cm^3, specific surface area 540 m^2/g, total pore area 589 m^2/g, and pore volume 740 cm^3/g.

Cell Concentration Assay

The optical density (600 nm) of cells was measured using a spectrophotometer (UV-150A, Shimadzu Corp., Kyoto, Japan) and dry cell concentrations calculated using a calibration curve. In order to measure the concentration of the cells in the immobilized biosand, the biosand was mixed with sterile saline solution for 3 h and then centrifuged. The amount of viable cells was measured in the supernatant after mixing the biosand and fresh medium under an optical microscope using a hemocytometer.

Measurement of Hydrogen Sulfide

Hydrogen sulfide concentrations of more than 50 ppm were detected and measured by gas chromatography (DS 6200, Donam Instruments, Inc., Sungnam, Korea). GC was conducted using a separation column (SPB-1 sulfur fused silica capillary column, 30 m×0.32 mm ID, 4 μm film, Sigma-Aldrich Co., St. Louis, U.S.A.) at 200°C, an FPD detector at 250°C, and an isothermal inlet temperature of 60°C. N_2 was used as a carrier gas. H_2S concentrations of less than 50 ppm were measured with a H_2S detector (Komyo Mb-500, Tokyo, Japan).

Measurement of Ionic Sulfur Compounds

The concentrations of sulfur ion were measured by ion chromatography (IC500, Yokokawa-Hokusin Electric, Japan). Concentrations of thiosulfate were measured by the method of Kelly *et al.* [7]. Concentrations of sulfate were measured by mixing 2 ml of 10% $BaCl_2$ $2H_2O$ and 2 ml of culture broth and subsequently measuring the absorbance at 450 nm with a UV-VIS spectrophotometer (UV150A, Shimadzu Corp., Kyoto, Japan).

Cell Immobilization and Reactor Operations

To compare efficiencies of the H_2S removal, the same reactor was operated following the three different methods. First, the efficiency of H_2S treatment by chemical oxidation without cells (*i.e.*, only by oxygen gas) was investigated in a 2-phase fluidized-bed reactor (Reactor A). Second, the effects of free cells in their exponential growth phase were investigated in a 2-phase condition (Reactor B). Third, immobilized cells were loaded into the fluidized-bed reactor and the effect then was investigated in a 3-phase condition (Reactor C). Three-hundred ml of growth medium and biosand (immobilized with 2 g/l of the bacterial strain) was placed in a flask and inoculated with

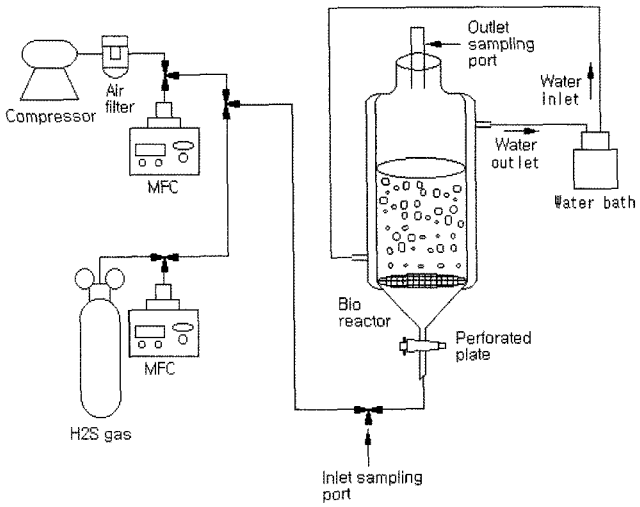

Fig. 1. Schematic diagram of the H_2S removal bioreactor.
MFC stands for mechanical flow counter, measuring gas flow.

Thiobacillus novellus SRM and placed on a shaker incubator (30°C at 120 rpm). The cells in logarithmic growth phase were immobilized on the biosand carrier and added to the fluidized-bed bioreactor. The schematic diagram of these reactors is depicted in Fig. 1. The glass reactor measured 90 mm diameter by 1,000 mm height. H_2S gas was supplied by mixing 30,000 ppm H_2S with dry air. A filter (0.1 μm) was installed at the base of the reactor increasing the contact area between H_2S gas and immobilized cells and decreasing potential shear stresses. Before operating the reactor, the bacterial strain was adapted to a medium containing a relatively low concentration of H_2S (30 ppm, 1 l/min gas flow).

Sulfide removal efficiency was defined as follows: removal efficiency (%)=(1−outlet gas concentration/inlet gas concentration)×100 under the condition of a continuous feed of hydrogen sulfide into the bioreactor. A steady state reactor operation condition was assumed for sulfide removal, and the feed concentration of the hydrogen sulfide was also assumed to be constant.

Effects of Temperature and pH

A constant reaction temperature of 30°C was maintained by using a temperature-controlled circulating water bath. The load factor for hydrogen sulfide was controlled by the sulfide concentration of the gas feed. The pH was adjusted using sterilized 2 N NaOH and 12 N HCl [14]. The effects of temperature and pH on the microorganisms were investigated by conducting experiments at an aspect ratio (the ratio of the reactor width to height) of 1.0, a generally accepted ration in fluidized-bed operation. To adjust the load factor, the hydrogen sulfide concentration was varied within the range of 200–400 ppm, which is the typical concentration range of hydrogen sulfide found in odorous

discharges in the environment. The gas flow was varied within a range of 0.5 to 2.0 l/min around a base level of 1.0 l/min, at which the bubble flow was maintained in the 3-phase fluidized-bed conditions.

RESULTS AND DISCUSSION

Hydrogen Sulfide Removal by Free and Immobilized Cells

Thiobacillus novellas SRM cells grown by a flask culture were fed into the fluidized-bed reactor without carrier, and the hydrogen sulfide removal efficiency by the free cells was investigated. The reactor operations were carried out at pH 7, 30°C, an aspect ratio of 1, gas flow of 1.0 l/min, and a feed concentration of 200 ppm of H_2S for 6 h. Since no biological oxidation was expected in Reactor A, any removal of hydrogen sulfide for these conditions was considered to be due to chemical oxidation by O_2 gas. The extent of biological oxidation by suspended cells was recognized in Reactor B, the 2-phase fluidized-bed reactor. In Reactor C, the 3-phase fluidized-bed reactor, the extent of oxidation by the attached cells was readily identified. The removal efficiency stabilized within 1 to 2 h of reaction initiation, and had plateaued by 12 h (data not shown). In addition it was possible to maintain high efficiencies (above 98%) with the 3-phase fluidized-bed bioreactor and the 2-phase fluidized-bed bioreactor (90%) compared with approximately 68% in the absence of the bacterial cells. The effects of adsorption onto biosand and the removal of H_2S were negligible (data not shown). These results indicated that oxidation by suspended cells approximated 22%, and that by immobilized cells 30% (Fig. 2). Thus, the biological oxidation by *T. novellus* SRM played a significant role in the removal of H_2S gas. The morphological characteristics of the cells before their immobilization onto the biosand were studied with a scanning electronic microscope (SEM), and representative scans are shown in Fig. 3. These results led us to conclude that the cells immobilized onto biosand directly participated in sulfur oxidation.

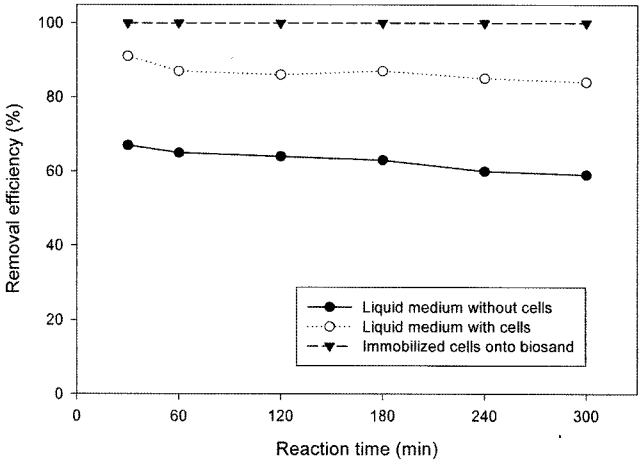

Fig. 2. Temporal profiles of H_2S removal efficiencies from aqueous media with and without cells.
Cells were immobilized on 10 g of biosand (feed 200 ppm of H_2S and aspect ratio of 1).

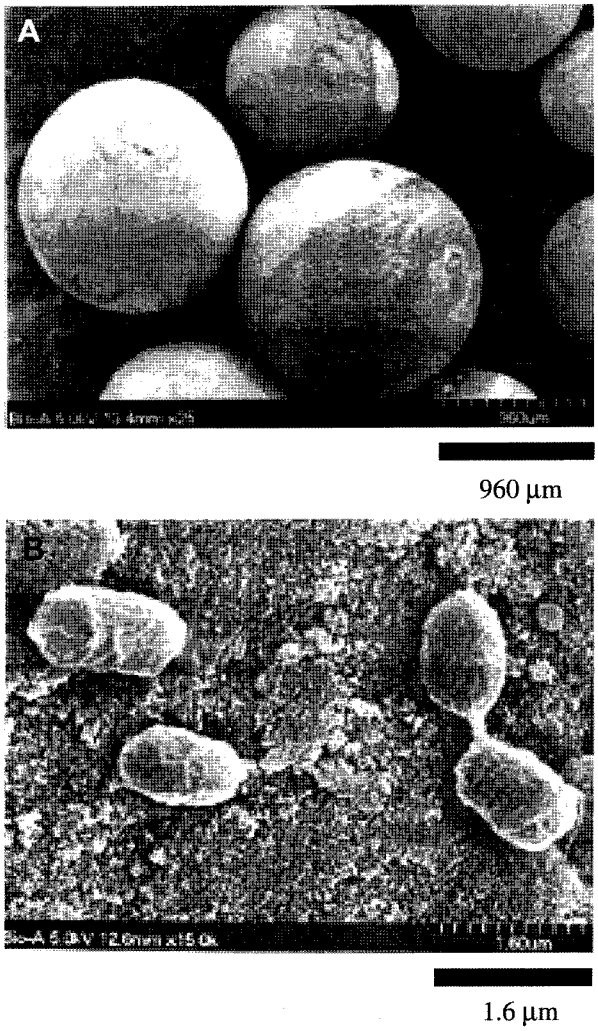

Fig. 3. Scanning electron microscope pictures of (**A**) biosand carrier and (**B**) immobilized cells on biosand.
The particle sizes are indicated on the pictures as a bar.

Effects of Initial pH and Temperature

The reactor received 300 ml each of the carrier immobilized with 2 g/l of the bacterial strain, or slurry prepared by adding the bacterial strain and the carrier to 1 l of the reaction medium. A gas feed containing 200 to 1,500 ppm of hydrogen sulfide was introduced into the reactor at 25–35°C and an initial pH of 5–9. This study investigated the optimal pH for hydrogen sulfide removal by immobilized *T. novellas* SRM and demonstrated the effects of initial pH on hydrogen sulfide removal by the immobilized cells. The

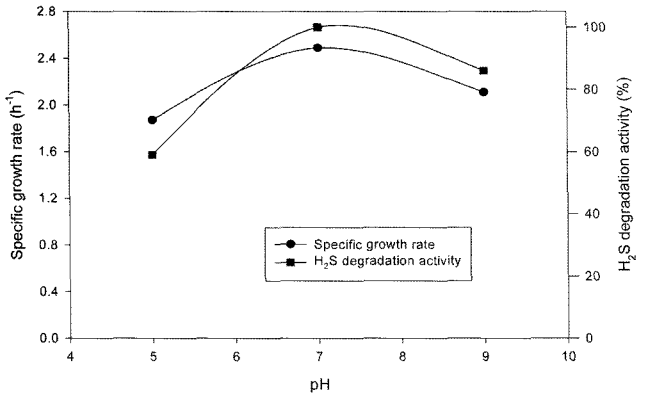

Fig. 4. Effects of initial pH on specific growth rates and H_2S degradation activity.

relative activity (%) was defined as the hydrogen sulfide removal rate at a specific pH relative to the removal rate at the optimal pH of 7.0 (Fig. 4). The relative activity was 100% at pH 7.0 up to a level of 300 ppm (data not shown). At pH 9.0, the relative activity was 84% and at pH 5.0 was 59%, indicating that immobilized *T. novellas* SRM shows a broader pH optimum than that of free cells [3]. Park *et al.* [14] have also reported that when enzymes or cells were immobilized using a carrier, their activity was observed within a broader pH range as compared with free enzymes or free cells.

These results indicated that the H_2S removal was temperature dependent: 57% at 20°C, 81% at 25°C, and 100% at 30°C (Fig. 5). We speculated that this pattern representing the chemical oxidation rate of hydrogen sulfide by submerged oxidation was much higher than the biological removal efficiency. The results of this study were in agreement with a previous report that the optimal temperature for hydrogen sulfide removal by immobilized *T. novellas* SRM was 30°C, and that removal was possible at up to 35°C [3].

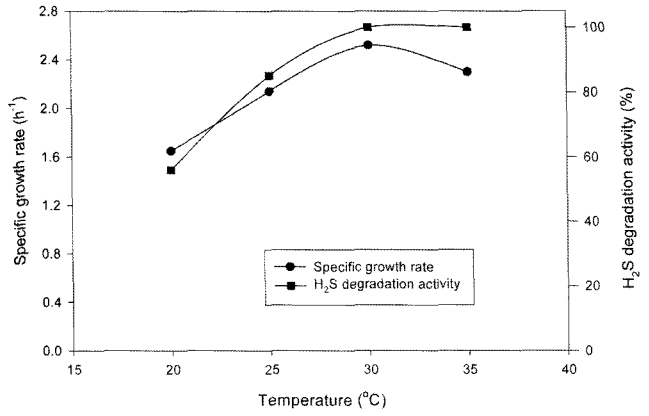

Fig. 5. Effects of temperature on specific growth rates and H_2S degradation activity.

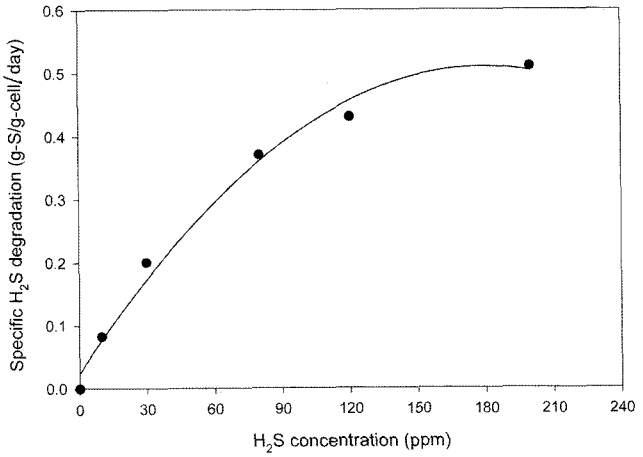

Fig. 6. Effect of inlet H_2S concentrations on specific H_2S degradation.

Effects of H_2S Concentrations

The effect of the concentration of hydrogen sulfide on its removal by immobilized *T. novellas* SRM was investigated by feeding various concentrations of hydrogen sulfide (10, 30, 80, 120, and 200 ppm) into the lower end of the reactor under the following conditions: 30°C, pH 7, 2 g/l cells, and 300 ml of biosand. The oxidation of hydrogen sulfide by the *T. novellas* SRM used in this study approximated the Monod-type kinetics, and almost no inhibition was observed up to 200 ppm (Fig. 6). With a feed concentration in the range of 15 ppm to 120 ppm, no hydrogen sulfide was detected in the effluent during a 1 h to 9 h period of operation. The specific growth rate of the bacteria was 20–30 times as high as the other hydrogen sulfide-oxidizing *Thiobacillus* sp. [2].

Operational Stability of the Fluidized-Bed Bioreactor

The H_2S gas was fed into the fluidized-bed reactor to check the stability of immobilized cells within the reactor. The

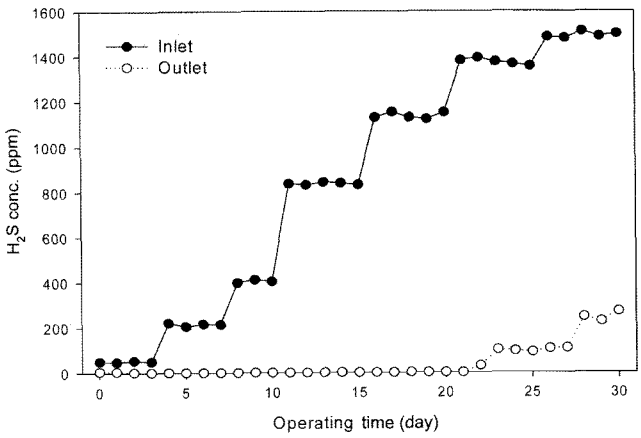

Fig. 7. Degradation of H_2S for a continuous supply of different concentrations of the gas by the immobilized *T. novellas* SRM at 30°C and pH 7.0.

space velocity was controlled at 50 l/h and the results obtained were shown in Fig. 7. The H_2S removal efficiency was over 99% at a H_2S concentration of up to 1,100 ppm. As the H_2S concentration increased, the removal efficiency was decreased to 93% and 85% at the inlet H_2S concentration of 1,300 and 1,500 ppm, respectively. The optimal concentration for the removal of H_2S was determined to be 1,100 ppm. The maximum removal capacity was 254 g $H_2S/m^3/h$ when the inlet H_2S loading was 300 $g/m^3/h$ (1,500 ppm). Stable operation of the immobilized reactor was possible for 20 days when the inlet H_2S concentration was maintained at 1,100 ppm. In conclusion, the bioreactor fluidized with immobilized *T. novellas* SRM cells appeared to be an effective means for controlling hydrogen sulfide emissions.

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