Construction of a Biofilter Immobilized with *Rhodococcus* sp. B261 for Removal of H₂S Gas Generated by Livestock

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To explore the optimal conditions for the removal of H₂S gas by biofiltration, various conditions, including inlet H₂S concentration, flow rate, moisture, and cell number, were examined. Heterotrophic bacteria were isolated from the compost of the animal excreta. A strain that effectively removed H₂S was selected and identified as *Rhodococcus rhodochrous* B261 by analysis of its 16S rDNA sequence. A cell number of 10⁷ cfu/g compost was sufficient to dominate the microbiota, and an effective removal was observed at H₂S gas concentrations below 220 mg/L. The moisture content of 33-38% was suitable for activation of the microbial activity and delaying the desiccation. Higher flow rates resulted in lower removal rates of the H₂S gas. Under the conditions of 10⁷ cfu/g compost, H₂S gas concentrations of 220 mg/L, and moisture content of 33-38%, the inlet H₂S gas concentrations of 120 and 400 mg/L were completely removed for 34 and 12 days, respectively. The amount of sulfur removed was 2.99×10⁻⁶ H₂S-S/cell, which was suggested as the amount of sulfur removed by a single cell. The biofilter consisting of the compost and *R. rhodochrous* B261 could be suitable for a long-term biofiltration for the removal of H₂S and other malodorous compounds.

**Key words:** biofilter, compost, hydrogen sulfide, PCR, *Rhodococcus rhodochrous* B261

Malodor generated by livestock has become a serious pollution problem in many countries. The main malodorous compounds emitted from the livestock houses are hydrogen sulfide, ammonia, trimethylamine, and volatile fatty acids. High concentrations of these compounds, especially hydrogen sulfide, are harmful to the health of the workers, animals, and local residents [Haggard et al., 1992]. Therefore, elimination or reduction of the sources of malodors is very important from the environmental health standpoint. Several microbial methods for the removal of malodor compounds have been reported in the last two decades [Ottengraf et al., 1986; Leson and Winer, 1991; Wani et al., 1997]. These methods share the common characteristics in that they provide easier operation and lower maintenance costs, and are more environmentally friendly, because no secondary pollution is produced as compared to the chemical and physical methods. There have been a number of studies on the elimination of hydrogen sulfide using biofilters [Furusawa et al., 1984; Cho et al., 1991; Shoda, 1991; Park et al., 1993; Shinabe et al., 1995]. Most of these studies focused on the identification of more effective carriers to prevent the drop in the pressure responsible for reducing the removal efficiency in biofiltration over long periods.

A number of microorganisms with sulfide-oxidizing ability have been reported, including the autotrophic bacteria *Thiobacillus* spp., the photosynthetic bacterium *Chromatium vinosum*, the methylo trophic bacterium *Hyphomicrobium*, and *Xanthomonas* [Fukumori et al., 1979; Zhang et al., 1991; Cho et al., 1992]. Autotrophs use sulfide as an energy source, the mechanism of which has been investigated in a number of studies. However, there have been few studies on the heterotrophs capable of eliminating hydrogen sulfide. *Streptomyces* and *Bacillus* spp. in the livestock waste treatment programs are heterotrophs capable of reducing the level of hydrogen sulfide produced during composting [Nakada

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**Abbreviations:** PCR, polymerase chain reaction; PE, feces extract medium; PEA, 10% pig feces extract agar medium; PEC, 10% pig feces extract centrifuged medium; PFE, pig feces extract medium

and Ohta, 1997; Ohra et al., 1997; Nakada and Ohta, 1999]. Studies have found that rapid composting of the animal excreta by the deodorizing microorganisms removed the main malodorous compounds generated during the composting of the animal excreta, including volatile fatty acids and hydrogen sulfide [Ohta and Ikeda, 1978; Ohta and Kuwada, 1978].

The objective of the present study was to develop biofilters using these microbes for an effective removal of the hydrogen sulfide generated in the livestock houses. Attempts were made to determine the factors required for the effective elimination of the hydrogen sulfide gas.

Materials and Methods

Strains. Twenty strains were isolated from the compost of pig feces using 10% pig PFE. The strain that showed the highest ability to remove H₂S was selected and used for the experiments.

Identification. The strain showing the highest H₂S removal activity was identified by the morphological and the physiological characterizations [Nakamura and Yoda, 1978; Krieg and Holt, 1984] and by the analysis of its 16S rDNA sequence amplified by PCR.

DNA sequence. The strain was cultured in a nutrient broth at 37°C for 24 h with shaking at 250 rpm, and DNA of the strain was extracted (InstaGen Matrix Catalog 732-6030; Bio-Rad, Hercourles CA) and heated using a block incubator (BI-525, Astech, Fukuoka, Japan) at 100°C for 5 min and quenched on ice. The DNA extract was added to the nuclease P₁ solution (Yamasa Shoyu Co., Tokyo, Japan) and incubated at 50°C for 1 h to induce fragmentation of the DNA. The DNA fragments were analyzed on an L-7400 type HPLC apparatus (Hitachi, Tokyo, Japan) equipped with an ODS column (Cosmosil 5C18, Nakalai Tesque, Kyoto, Japan). A gradient of ammonium hydrogen phosphate (0.05%) was used, and the absorbance was determined at 265 nm.

16S rDNA analysis. Identification of the strain capable of H₂S removal was performed by NCIMB (Shizuoka Japan).

Compost used. A mixture of 5 kg of fresh pig feces, 2.5 kg of mixed feed culture, and 1.5 kg of rice bran was composted in a treatment box at room temperature for 5 days. The compost was dried to a moisture content of approximately 35%. The dried compost contained 10⁸ cells g⁻¹ of deodorizing bacteria.

Medium preparation. Ten percent each PE, PEC, and PEA media were used as growth media for R. rhodochrous B261. Ten percent pig feces extract was prepared by passage through a cotton filter. All media were autoclaved at 121°C for 20 min, and the pH was adjusted to 7.0 with 10% Na₂CO₃ or 1 N HCl under aseptic conditions.

Culture of strain for biofilter. R. rhodochrous B261 was inoculated into 500 mL of PE medium and incubated at 37°C with shaking at 240 rpm for 16 h. A volume of 300 mL of the precultured broth was transferred into a 10-L jar fermentor containing 6 L of fresh PE medium and incubated at 37°C at a flow rate of 3.5 L/min and an agitation speed of 300 rpm for 9 h. The cultured cells were harvested by centrifugation at 17,000×g and suspended in distilled saline solution. The suspended cells were mixed with the dried compost and packed into a column.

A deodorization column for H₂S as designed by Furusawa et al. [1984] was used. Compressed air at a pressure of 2 kg cm⁻² was passed through the H₂S-generating column at a specified flow rate controlled by the regulator. Sodium sulfide and HCl solution were passed through the H₂S-generating column at 1 mL/min using a peristaltic pump. Two hundred glass beads (ø 10 mm) were included in the column to ensure effective generation of the H₂S gas. The generated gas was passed through the deodorizing column at the flow rate controlled by a flow meter (Ryutai Kogyo, Tokyo, Japan). The inlet and outlet concentrations of H₂S were determined using an SB type syringe H₂S detector (Kitagawashiki, Tokyo, Japan). The inlet H₂S concentration was controlled at 60-400 mg/L by mixing HCl and sodium sulfate at 1.2-23.6% and 4-10%, respectively.

Samples of 500 g of the mixed and the pure cultures with specified moisture levels were packed into the column. The physical properties of the compost and the operating conditions are shown in Table 1.

Enumeration of viable cells. Viable cells on the PEA medium adjusted to pH 8.0 were counted. In brief, 10 g each sample was diluted in 90 mL of the saline solution. The suspensions were serially diluted to 10⁻¹⁻⁹ and inoculated onto the PE plates. The plates were incubated at 37°C for 3 days, and the colonies formed as the viable cells were counted.

Analysis. Moisture contents of the samples were determined by measuring the difference between the wet and dry weights after drying the samples. To maintain the moisture content, an X-ray moisture detector (Kett, F-18, Tokyo, Japan) was used for measuring the moisture content, and the weight of water required to maintain the moisture content was supplied into the column. The water holding capacity was determined as the difference in wet and dry weights of the compost. Porosity of the samples was measured as the difference in volume between the mixture of water and sample, and that of water alone.

Hydrogen sulfide and sulfide compounds. One
Table 1. Physical properties of compost and operating conditions for H₂S removal

<table>
<thead>
<tr>
<th>Compost</th>
<th>Density</th>
<th>0.33 g/cm³</th>
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<tbody>
<tr>
<td>Porosity</td>
<td></td>
<td>77.8%</td>
</tr>
<tr>
<td>Water-holding capacity</td>
<td></td>
<td>65%</td>
</tr>
<tr>
<td>Packed weight</td>
<td></td>
<td>500 g</td>
</tr>
<tr>
<td>Size (φ×height)</td>
<td></td>
<td>7×50 cm</td>
</tr>
<tr>
<td>Packed volume</td>
<td></td>
<td>1530 mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Operating conditions</th>
<th>Flow rate</th>
<th>0.1-1.2 L/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column initial pH</td>
<td></td>
<td>7.5-8</td>
</tr>
<tr>
<td>Column temperature</td>
<td></td>
<td>37°C</td>
</tr>
<tr>
<td>Inlet concentration</td>
<td></td>
<td>100-400 mg/L</td>
</tr>
<tr>
<td>Initial cell number</td>
<td></td>
<td>10⁶³ CFU/g-ceramic beads</td>
</tr>
<tr>
<td>Moisture content</td>
<td></td>
<td>33-38, 40-45, 55-60, 50-55%</td>
</tr>
</tbody>
</table>

hundred grams each of the samples were transferred into 500-mL flasks, made air-tight with rubber tubings, and incubated at 40°C for 1 h. The headspace gas in the flask was sampled by an air-tight microsyringe (Hamilton, Reno, NV) and injected into a gas chromatograph (GC-14B, Shimadzu, Tokyo, Japan) equipped with an Flame Photometric Detector (Shimadzu, Tokyo, Japan). A column packed with polyphenyl ether (5-rings) OS-124 (10%, 60-80 mesh, Shimadzu) was used. Nitrogen was used as a carrier gas, and hydrogen was used as a fuel at a flow rate of 40 mL/min. The levels of sulfur, sodium thiosulfate, sodium sulfate, and sulfate in the compost were determined by the method of Tachihara [1985]. Ten grams each of the chemicals were diluted with 100 mL of distilled water and extracted using a Soxhlet apparatus. The extracts were used in the sulfate barium precipitation and the ureic acid titration.

**Results and Discussion**

**Identification of strain.** Strain B261 was identified as *Rhodococcus rhodochrous* B261 based on its morphological, biological, and chemical properties, as well as through the analysis of its 16S rDNA sequence amplified by PCR (Fig. 1). Moreover, in the neighbor joining phylogenetic tree, a significantly high similarity (98.19%) was observed with the 16S rDNA sequence of *R. B261* in the database using MicroSeq™ Identification System Software V.14.1 and BLAST (National Center for Biotechnology Information, Bethesda, MD).

**Comparison of pure and mixed cultures.** At the flow rate of 250 mg/L, H₂S gas was removed completely for 12 days in the column packed with the pure culture (Fig. 2). From day 13, only 3 mg/L was detected from the outlet port of the column and resulted in a decrease in H₂S removal rate. In the case of mixed cultures, H₂S was removed completely for 8 days. The removal rate of H₂S in the columns of the autoclaved pure and mixed cultures decreased after 4 and 2 days, respectively. This result clearly showed that the pure culture had higher removal capacity than the mixed culture. In addition, the degrees of removal of H₂S were significantly different between the autoclaved and non-autoclaved cultures, indicating that the living deodorant microorganisms are responsible for the removal of H₂S. The removal of H₂S by the autoclaved cultures was suggested to be due to the abiotic adsorption or the remaining viable cells (about 10⁷ cells/g).

![Fig. 1. Neighbor-joining phylogenetic tree of *Rhodococcus sp.*B261.](image)

The values in parentheses show similarity with strain B261 as determined by 16S rDNA sequence analysis.
Fig. 2. Comparison of H₂S removal by biofilters packed with pure, mixed, autoclaved pure, and autoclaved mixed cultures (a) and removal rates (b). Symbols: (■), Inlet H₂S concentration; (▲), Pure culture; (○), Mixed culture (△), Autoclaved pure culture; (○), Autoclaved mixed culture.

The initial pH values of pure and mixed cultures were almost the same, i.e., pH 9.6, as composting progressed for 4 days. However, after autoclaving, both the pure and the mixed cultures showed decreases in pH to about 8.3 and 7.6, respectively. The pH decreased with the increasing rate of H₂S removal. Finally, the pH values of pure cultures, mixed cultures, autoclaved pure cultures, and autoclaved mixed cultures on day 14 were 8.1, 7.7, 7.3, and 7.1, respectively, indicating that alkaline pH is associated with the removal of H₂S. Moisture contents adjusted to 35% respectively decreased to 18.3, 17.1, 16.4, and 13.0% in the pure cultures mixed cultures, autoclaved pure cultures, and autoclaved mixed cultures on day 14.

Effect of flow rate on H₂S removal. At the flow rate of 4.8 m³/kg/d, H₂S gas was removed completely for 28 days (Fig. 3). At flow rates of 9.6 and 14.4 m³/kg/d, complete removal of H₂S gas was maintained for 14 and 11 days, respectively. The pH decreased gradually from the initial value of 9.4 to 7.9 at the flow rate of 4.8 m³/kg/d, to 8.0 at 9.6 m³/kg/d, and to 7.8 at 14.4 m³/kg/d. At higher flow rates, the moisture content decreased rapidly reaching a final stable value of 10-13%. At the flow rate of 4.8 m³/kg/d, removal of H₂S gas remained at 100% for 7 days even after the moisture content decreased to about 12%. In addition, it was clear that lower moisture content, below 12%, at the higher flow rate of 14.4 m³/kg/day caused a reduction in the H₂S removal rate.

Effect of moisture content on H₂S removal. At 60 mg/L of the inlet H₂S concentration, H₂S gas was completely removed for 23 days (Fig. 4), and then the inlet H₂S concentration increased to 120 mg/L. When the moisture content was maintained at 40-45%, 3 mg/L of

Table 2. Characteristics of No.B261 strain by NCIMB*  
<table>
<thead>
<tr>
<th>Items</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td>Gram</td>
<td>+</td>
</tr>
<tr>
<td>Spores</td>
<td>-</td>
</tr>
<tr>
<td>Mobility</td>
<td>-</td>
</tr>
<tr>
<td>Colonial morphology</td>
<td>Round</td>
</tr>
<tr>
<td></td>
<td>Smooth</td>
</tr>
<tr>
<td></td>
<td>Low convex</td>
</tr>
<tr>
<td></td>
<td>Orange pink</td>
</tr>
<tr>
<td></td>
<td>1-1.5 mm in diameter</td>
</tr>
<tr>
<td>Temperature growth</td>
<td>- 37°C</td>
</tr>
<tr>
<td></td>
<td>- 10°C</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase, Kovacs</td>
<td>-</td>
</tr>
<tr>
<td>O-F glucose</td>
<td>- (alkaline)</td>
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<tr>
<td>First, stage identification</td>
<td>Rhodococcus sp.</td>
</tr>
</tbody>
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*National Collection of Industrial Bacteria Torry Research Station.
H₂S was emitted from the column on day 24. At the moisture contents of 55-60 and 50-55%, 14 and 1 mg/L of H₂S were emitted from days 26 and 28, respectively. However, in the column maintained at a moisture content of 33-38%, H₂S was removed completely until the end of the experimental period. In particular, the color of the culture in the column with a moisture content of 40-45% changed to white after 1 week. Based on these results, the optimum initial moisture content for H₂S removal was determined to be 33-38%.

The initial cell number of 1.85×10⁸ cells·g⁻¹ was not significantly changed in any of the columns with moisture contents of 33-38, 40-45 or 50-55% on day 7 (Table 3). However, at the end of the experimental period, the viable cell number was reduced to about 10⁶ cells·g⁻¹ in all columns. The columns showed contamination rates of 21.9, 51.4, 47.8, and 30.4% and the contamination rate in the column decreased in the order of moisture contents of 40-45>50-55>55-60>33-38%. The extent of contamination was related to the emission of H₂S from the column (Fig. 4). Based on the results of inhibition of the H₂S removal activity by R. rhodochrous B261, the reduction of removal rate in the columns with moisture contents maintained above 33-38% may have been due to contamination. However, when the moisture content was maintained below 30%, H₂S removal was also reduced because of the significant decrease in the viable cell number (Table 3). Therefore, a moisture content of 33-38% is needed for a long-term removal of H₂S gas.

**Effect of cell number on H₂S removal.** In the columns containing 10⁷ and 10⁶ cells/g, H₂S gas was completely removed after 3 h and 1 day, respectively (Fig. 5). However, in the column containing 10⁵ cells/g, complete removal of H₂S gas was maintained until the end of the experimental period. The period of complete removal of H₂S was clearly extended in proportion to the R. rhodochrous B261 cell number. The initial pH values in the columns with cell numbers lower than 10⁷ were not

### Table 3. Cell numbers of R. rhodochrous B261 in pure cultures under controlled moisture content conditions during H₂S removal

<table>
<thead>
<tr>
<th>Moisture content of pure culture (%)</th>
<th>Microbial number (cells/g-dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>33-38</td>
<td>1.85×10⁸</td>
</tr>
<tr>
<td>40-45</td>
<td>1.85×10⁸</td>
</tr>
<tr>
<td>50-55</td>
<td>1.85×10⁸</td>
</tr>
<tr>
<td>55-60</td>
<td>1.85×10⁸</td>
</tr>
</tbody>
</table>

The numbers in parentheses show contamination ratios. (Total microbial cell number-R. rhodochrous B261 cell number/total microbial cell number)×100.
Fig. 6. Effect of inlet concentration of H\textsubscript{2}S on removal in biofiltration at different flow rates (a) and removal rates (b) at a moisture content of 35%. Symbols: (a), Inlet H\textsubscript{2}S concentration; (Δ) 4.8 m\textsuperscript{3}/kg/day; (○) 9.6 m\textsuperscript{3}/kg/day; (●) 14.4 m\textsuperscript{3}/kg/day; (b) (―), 100% removal.

significantly reduced. However, in the columns with cell numbers of 10\textsuperscript{7} or higher, the pH of 9.5 did not change for 4 days and declined rapidly to pH 7.7 for 10 days.

**Effect of inlet concentration on H\textsubscript{2}S removal.** Figure 6 shows the removal of H\textsubscript{2}S in the columns with the moisture content maintained at 33-38%, inlet H\textsubscript{2}S concentration of 100, 200 or 400 mg/L, and at different flow rates of 4.8, 9.6, and 14.4 m\textsuperscript{3}·kg\textsuperscript{-1}·d\textsuperscript{-1} for 48 days. In all columns, H\textsubscript{2}S gas, at an inlet concentration of 100 mg/L, was removed completely for 18 days.

The columns were autoclaved and used to determine the abiotic removal under the same conditions as those of the controls (data not shown). At the flow rates of 4.8, 9.6, and 14.4 m\textsuperscript{3}/kg/d, no H\textsubscript{2}S gas was emitted from the columns from days 1, 3, and 6, respectively, after autoclaving. The results at 4.8 m\textsuperscript{3}/kg/d showed that the surviving cells remaining in the column after autoclaving were responsible for the maintenance of complete removal of H\textsubscript{2}S gas for 6 days.

With the increasing inlet concentration from 100 to 200 mg/L, H\textsubscript{2}S gases from the columns were detected at 4.8, 9.6, and 14.4 m\textsuperscript{3}/kg/d at days 21, and 26, and 34, respectively. Moisture contents of all columns decreased to below 15%, and this decrease was responsible for the observed decrease in the H\textsubscript{2}S removal. Therefore, to reanimate the microorganisms, water was added to bring up the moisture content of all columns to 35%. Inlet concentration decreased to 100 mg/L from days 36 to 37. In the columns with the flow rates of 9.6 and 14.4 m\textsuperscript{3}/kg/d, the 99% removal rate of H\textsubscript{2}S was maintained after the addition of water. Increasing the inlet concentration of H\textsubscript{2}S from 100 to 400 mg/L decreased the H\textsubscript{2}S removal at days 2 and 6 at the flow rates of 9.6 and 14.4 m\textsuperscript{3}/kg/d, respectively. However, the removal in the column supplied with 400 mg/L H\textsubscript{2}S at the flow rate of 4.8 m\textsuperscript{3}/kg/d was maintained for almost twice as long as that at the flow rate of 9.6 m\textsuperscript{3}/kg/d with the addition of water. However, the moisture content showed almost no effect on the physicochemical H\textsubscript{2}S removal activity. H\textsubscript{2}S gas dissolved in the water was suggested to be degraded by *R. rhodochrous* B261, resulting in the increased removal rate. In addition, the decrease in pH was delayed with the addition of water. These results indicated that the higher removal activity was due to not only increases in the level of the dissolved H\textsubscript{2}S in the water phase, but also the physiological activation of *R. rhodochrous* B261 in the pure culture columns.

**Changes in forms of sulfur compounds.** The concentrations of single-sulfur, thiosulfate-S, sodium sulfite-S, and sulfate-S increased with the progressive removal of H\textsubscript{2}S (Fig. 7), indicating that hydrogen sulfide is oxidized first into a single-sulfur, then into thiosulfate-S, and finally into sulfite-S and sulfate-S. The decrease in pH was also suggested to be caused by the accompanying increase in the sulfur level.

Use of the compost biofilters mixed with microorganisms capable of degrading the malodorous compounds is a promising and economical method for the removal of these compounds, which are produced not only by livestock and fisheries, but also by various kinds of industrial processing plants, such as those used for the sewage treatment, and chemical and fertilizer industries. This is because compost provides well-acclimatized microorganism communities, high bulk density, high specific surface area, and good water-holding capacity and moisture content [Van Groenestijn and Hesselink, 1993]. Smet et al. [1996] also reported that compost is a better carrier material for biofiltration.

Flow rate and pH are the major factors regulating the effective removal of hydrogen sulfide [Cho et al., 2000;
Park et al., 2002]. Neutral or weak alkaline pH (pH 7-9) has been shown to be suitable for achieving the maximum removal of hydrogen sulfide [Yun and Ohita, 1998; Cho et al., 2000; Park et al., 2002]. Cho et al. [2000] obtained the maximum removal capacity of 428 g-S/m²/h at SV 300 h⁻¹; the removal capacity was reduced at higher space velocity. However, they found the inlet H₂S concentrations from 300 to 500 mg/L have no effect on the removal capacity at SV 300 h⁻¹. In addition, Park et al. [2002] obtained the maximum inlet H₂S concentration necessary for maintaining the maximum removal capacity in biofilters containing the immobilized Thiothrix sp. IFW using the Ca-alginate beads. However, in the present study removal efficiency of H₂S was affected by both the flow rate and the inlet H₂S concentration above 200 mg/L. To maintain a constant H₂S removal capacity, the inlet H₂S concentration should be ≤100 mg/L at flow rates below 8 m³/kg/d.

In general, removal of malodorous compounds, such as H₂S and ammonia, is affected by the moisture content and the water-holding capacity due to the solubility of these compounds in water and the requirement of microbial activity [Wani et al., 1997; Atlas and Bartha, 1997]. Cho et al. [2000] reported the removal of H₂S using biofilters consisting of porous lava packed with the autotrophic bacterium, Thiobacillus thiooxidans. They suggested that increasing the water-holding capacity increases the deodorization efficiency. However, in the present study, a lower moisture content of 33-38% resulted in the maximum removal rate. The low removal rate of hydrogen sulfide was suggested to be due to the drop in the pressure caused by the high moisture content, which reduces the solubility of hydrogen sulfide in water. However, the pressure drop was not detected in the present study. Therefore, 33-38% is suggested to be a suitable initial moisture content to facilitate the microbial activity, especially that of *R. rhodochrous* B261, and the column pass-through.

The amount of sulfur removed by the *R. rhodochrous* B261 cells in the biofilter was calculated by the following equation:

\[
C = 2.99 \times 10^{-6} \times N \times 1.437 \times 10^{-3} \times FR \times HC \times T
\]

Where C=removal capacity (g-S/kg), N=R. rhodochrous B261 cells number (cells/kg), FR=flow rate (m³/kg/d), HC=H₂S concentration (mg/L), and T=time required for H₂S removal.

The amount of sulfur removed was 2.99×10⁻⁶ H₂S-S/cell, which corresponded to the value reported previously for the amount of sulfur removed by a single cell [Zhang et al., 1991]. The removal capacity of H₂S was proportional to the cell number, whereas the removal time was inversely proportional to the flow rate and the concentration of the inlet H₂S.

The removal capacity of H₂S in the box sealed hermetically with the immobilized *R. rhodochrous* B261 was not significantly different from the results obtained with the active carbon (data not shown). However, active carbon is more expensive than the immobilized *R. rhodochrous* B261 biofilter. In addition, the heterotrophic bacterium *R. rhodochrous* B261 is capable of degrading volatile fatty acids, which are one of the major malodorous compounds generated by the livestock, especially swine [Yun and Ohita, 1998]. As the compost is already at alkaline pH during composting, *R. rhodochrous* B261 can dominate the microbiota, resulting in the increased removal capacity. Therefore, compost with *R. rhodochrous* B261 might be suitable for the long-term biofiltration in the removal of H₂S and other malodorous compounds.

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**References**


