

Microbial Desulfurization of Dibenzothiophene and 4,6-Dimethyldibenzothiophene in Dodecane and Straight-Run Diesel Oil

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Abstract—The desulfurization of dibenzothiophene (DBT), 4,6-dimethyldibenzothiophene (4,6-DMDBT) and their mixture by lyophilized cells of *Pseudomonas delafieldii* R-8 was studied in the presence of dodecane. The desulfurization rate for 4,6-DMDBT was found to be about 40% in comparison with that for DBT. The desulfurization process for DBT and 4,6-DMDBT proceeded simultaneously without preference for either one. The desulfurization rate for each compound was decreased when they were mixed together. The extent of desulfurization of 4,6-DMDBT was increased with the increase of cell concentration and the decrease of the volume ratio of oil-to-water used. The specific desulfurization rate for 4,6-DMDBT could be reached to 10.4 mmol sulfur kg⁻¹ (cell) h⁻¹ [approximately 0.33 mg sulfur g⁻¹ (cell) h⁻¹]. *Pseudomonas delafieldii* R-8 showed high desulfurization capability for straight-run diesel oil (containing 1,807 mg/L of sulfur). About 1,000 mg/L of sulfur in diesel oil was removed by resting cells of this strain in 24 h of reaction. The specific desulfurization rate was 8.75 mmol sulfur kg⁻¹ (cell) h⁻¹.

Key words: Biodesulfurization, Dibenzothiophene, Diesel Oil, *Pseudomonas delafieldii*, Two-phase System

INTRODUCTION

Many forms of sulfur-containing compounds exist in fossil fuels, the combustion of which produces sulfur oxides. The emission of sulfur oxides to the air has resulted in serious environmental problems. Hydrodesulfurization (HDS) is the commonly used method at present for fuel desulfurization. However, it does not work well on the polyaromatic sulfur heterocyclics (PASHs) such as dibenzothiophene (DBT) and its derivatives, especially 4,6-dimethyldibenzothiophene (4,6-DMDBT), found in heavier fractions of oil. Biodesulfurization (BDS) is regarded as a promising method for the production of ultra-low-sulfur fuels for its mild operating conditions and its ability to remove the sulfur from PASHs [Monticello and Finnerty 1985; Ohshiro and Izumi, 1999]. *R. erythropolis* IGTS8 was the first reported bacterium that could metabolize DBT to 2-hydroxybiphenyl (2-HBP) and sulfite [Kilbane and Jackowski 1992; Gray et al., 1996]. This metabolic pathway was considered as the most promising one for fuel desulfurization. Since then, many microorganisms with such a metabolic pathway for DBT have been isolated such as *R. erythropolis* N1-36 and D-1, *Gordona* sp. CYKS1 and *Paenibacillus* sp. A11-2. Most of them are gram-positive ones. To our knowledge, few gram-negative bacteria with such a metabolic pathway were reported [Gallagher et al., 1993; Darzins and Mrachko 2000]. *Pseudomonas delafieldii* R-8, isolated by our research group, was capable of desulfurizing DBT to 2-hydroxybiphenyl (2-HBP) through the formation of DBT sulfone [Jiang et al., 2002]. In previous work we studied the desulfurization of DBT by this strain in a model oil (dodecane) system [Luo et al., 2003]. As we know, alkylated DBTs are more recalcitrant to HDS. 4,6-DMDBT is one of the representatives. Here, the desulfurization of

4,6-DMDBT was studied by using lyophilized cells of *Pseudomonas delafieldii* R-8 in the same model oil system. The rate of desulfurization of 4,6-DMDBT was compared with that for DBT. The capability of desulfurizing straight-run diesel oil with high sulfur content was evaluated.

MATERIALS AND METHODS

1. Bacterial Strain and Cultivation

Pseudomonas delafieldii R-8 (CGMCC 0570) was isolated by our research group [Jiang et al., 2002]. Cells were cultivated in 500 ml flasks containing 150 ml sulfur-free medium (SFM) [Luo et al., 2003]. The sulfur source for cell growth was about 0.1 mmol/L of DBT.

2. Desulfurization in the Presence of Dodecane

The reaction solutions contained 0.1 M phosphate buffer (pH 7.0), dodecane (containing either of DBT, 4,6-DMDBT or their mixture) and lyophilized cells of *Pseudomonas delafieldii* R-8 as previously described [Luo et al., 2003]. The cell concentration was 20 g/L based on the aqueous phase. The volume ratio of oil-to-water was 1 : 1 (unless otherwise stated).

Cell cultivation and desulfurization reaction were carried out at 30 °C on a rotary shaker operated at 170 rpm. The specific desulfurization rate was expressed as micromoles of DBT or sulfur transformed per kilogram of lyophilized cells per hour, mmol sulfur kg⁻¹ (cell) h⁻¹.

3. Analytical Methods

DBT, 4,6-DMDBT and 2-HBP in the dodecane phase were assayed by high performance liquid chromatography (HPLC) as previously described [Luo et al., 2003]. The sulfur content of diesel oil was analyzed with WK-2B Microcoulomb Analyzer (Jiangsu Electroanalysis Instruments, China).

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RESULTS AND DISCUSSION

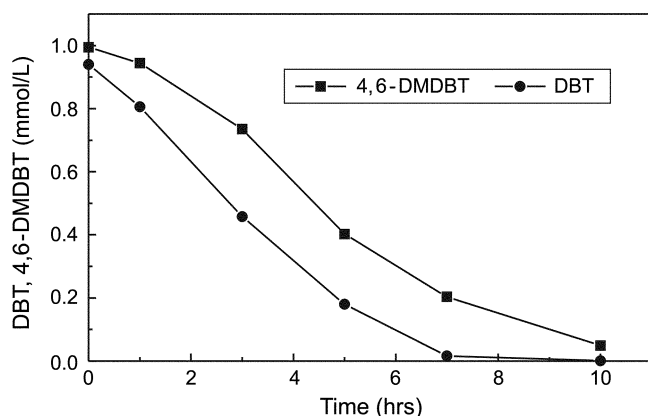


Fig. 1. Time courses of desulfurization of DBT and 4,6-DMDBT by lyophilized cells of R-8 in the presence of dodecane. The cell concentration was 20 g/L of aqueous phase. The phase ratio of oil-to-water was 1 : 1.

1. Biodesulfurization of DBT, 4,6-DMDBT and Their Mixture

The desulfurization rate of *Pseudomonas delafieldii* R-8 for DBT, 4,6-DMDBT and their mixture was examined. Fig. 1 shows the time course of desulfurization of DBT and 4,6-DMDBT, respectively. The initial concentration of DBT and 4,6-DMDBT was 0.94 and 1.0 mmol/L, respectively. The results showed that about 98% of DBT was desulfurized in 7 h. The initial rate of desulfurization of DBT was 6.5 mmol kg⁻¹ (cell) h⁻¹. About 75% of 4,6-DMDBT was removed in 7 h. The initial rate of desulfurization of 4,6-DMDBT was 2.5 mmol kg⁻¹ (cell) h⁻¹, which was only about 40% of that for DBT. This result is very similar to that given by Ohshiro et al. [1996]. In their studies, the initial reaction rate of *Rhodococcus erythropolis* H-2 for 4,6-DMDBT was about 60% of that for DBT.

Fig. 2 shows the time course of desulfurization of the mixture of DBT and 4,6-DMDBT. The concentration of each in the dodecane phase was about 0.5 mmol/L. It indicates that the desulfurization process of DBT and 4,6-DMDBT proceeded simultaneously without showing any preference to either one. The initial rate of desulfurization of DBT and 4,6-DMDBT was 1.7 and 0.75 mmol sulfur

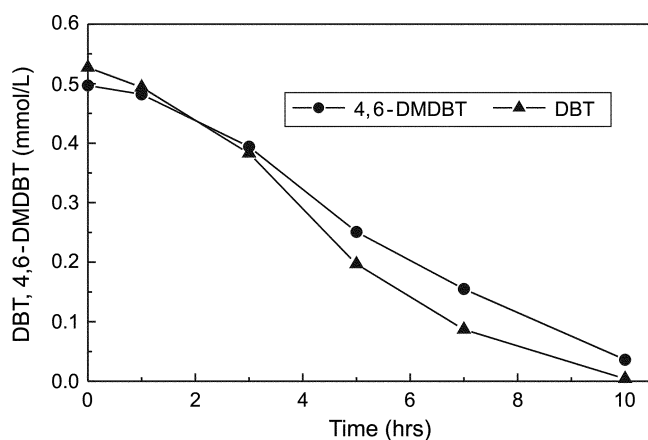


Fig. 2. Time course of desulfurization of the mixture of DBT and 4,6-DMDBT by lyophilized cells of R-8 in the presence of dodecane. The cell concentration was 20 g/L based on the aqueous phase. The phase ratio of oil-to-water was 1 : 1.

kg⁻¹ (cell) h⁻¹, respectively. The desulfurization pattern of DBT of *Pseudomonas delafieldii* R-8 could be represented by the Michaelis-Menten Equation as Eq. (1) [Luo et al., 2003], where V_{max} and K_m represent as the limiting maximal velocity and Michaelis constant, respectively.

$$v = \frac{V_{max}S}{K_m + S} \quad (1)$$

So, if $S_1 = 2 S_2$, then,

the calculated desulfurization rate for S_1 and S_2 can be expressed as:

$$\frac{v_1}{v_2} = \frac{V_{max}S_1(K_m + S_2)}{(K_m + S_1)V_{max}S_2} = 1 + \frac{K_m}{K_m + 2S_2} < 2 \quad (2)$$

From the above result, we know that when S_1 was 1.0 mmol/L, v_1 was 6.5 mmol sulfur kg⁻¹ (cell) h⁻¹. Then, when S_2 was 0.5 mmol/L, the corresponding desulfurization rate (v_2) should be larger than 3.2 mmol sulfur kg⁻¹ (cell) h⁻¹ according to Eq. (2). In fact, when the mixture of DBT and 4,6-DMDBT was desulfurized, the desulfurization rate for DBT was 1.7 mmol sulfur kg⁻¹ (cell) h⁻¹, lower than the v_2 calculated. In other words, the desulfurization rate for DBT, when mixed with 4,6-DMDBT, was reduced as found also by Morio et al. [2001]. Similarly, it might be concluded that the desulfurization rate for 4,6-DMDBT, when mixed with DBT, was reduced. Morio et al. [2001] explained this phenomenon as apparent competitive inhibition of substrates. The above results showed that such phenomena in desulfurization by gram-positive desulfurizing bacteria also exist in gram-negative ones.

2. Desulfurization of 4,6-DMDBT at Various Cell Concentrations

The cell concentration was changed from 10 to 50 g/L based on the aqueous phase. The initial concentration of 4,6-DMDBT in the dodecane was about 10 mmol/L (corresponding to 320 mg/L of sulfur). The phase ratio of oil-to-water was 1 : 1. As shown in Fig. 3, the DBT degradation rate was increased with increasing of cell concentration. When the cell concentration was 50 and 10 g/L, the percent of degradation of 4,6-DMDBT was about 94 and 15%, respectively. However, for the specific desulfurization rate, the optimal cell concentration was about 20 g/L. At this cell concentration, the specific desulfurization rate could reach to 10.3 mmol sulfur kg⁻¹ (cell) h⁻¹. The specific desulfurization rate did not increase with the

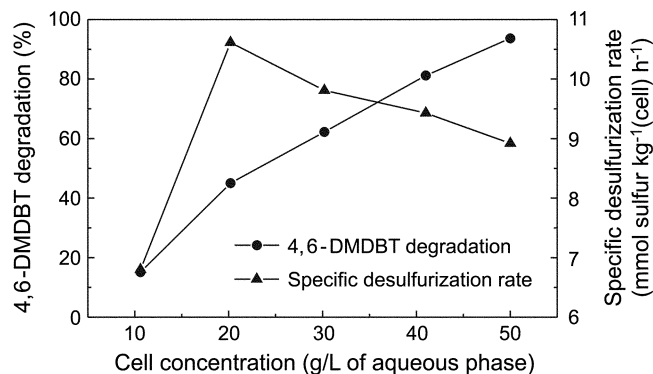


Fig. 3. The effect of cell concentration on the desulfurization of 4,6-DMDBT. The initial concentration of 4,6-DMDBT in the dodecane is about 10 mmol/L (corresponding to 320 ppm of sulfur). The phase ratio of oil-to-water was 1 : 1.

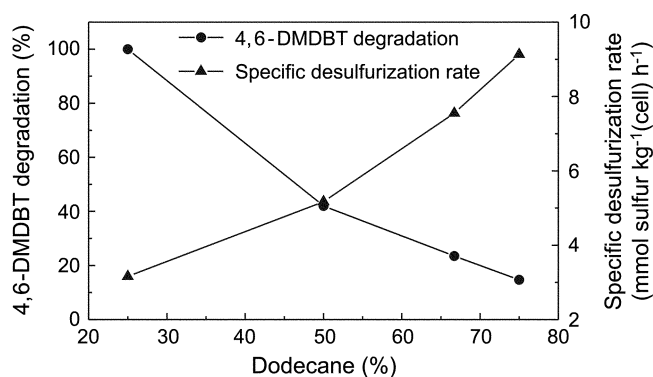


Fig. 4. The effect of phase ratio on the desulfurization of 4,6-DMDBT. The initial concentration of 4,6-DMDBT in the dodecane is about 10 mmol/L (corresponding to 320 ppm of sulfur). The cell concentration was 50 g/L of aqueous phase.

further increase of the cell concentration, which might be due to the limitation of oxygen supply as found in *R. erythropolis* D-1 and H-2 [Ohshiro et al., 1995; Izumi et al., 1994] or the rate of the renewal of surface as new cells at the organic/aqueous interface to acquire the substrate [Eric et al., 1998].

3. Desulfurization of 4,6-DMDBT at Various Phase Ratios

The phase ratio is one of the main factors to influence the size of the bioreactor to be used. In this work, the percent of dodecane volume with respect to aqueous phase was changed from 25 to 75%, which corresponds to the phase ratio of oil-to-water changed from 1 : 3 to 3 : 1. The cell concentration in the aqueous phase was 50 g/L. Fig. 4 shows the specific desulfurization rate and the rate of degradation in 21 h of reaction. It indicates that the percent of degradation was decreased with the increase of dodecane in the tested range of volume of dodecane. Ten mmol/L of 4,6-DMDBT could be completely desulfurized in 21 h as the volume of dodecane was 25%. However, the percent of 4,6-DMDBT degradation was lower than 20% when the volume of dodecane was 75%. The specific desulfurization rate was increased with the increase of the amount of dodecane within the range tested.

4. Desulfurization of Diesel Oil

The straight-run diesel oil, kindly provided by SINOPEC Dushanzi Institute of Petrochemicals Corporation Ltd, was treated by lyophilized cells of R-8 with the phase ratio of oil-to-water as 1 : 3. The sulfur content of the diesel oil was decreased from 1,807 to 808.9 mg/L in 24 h. About 1,000 mg/L or 55.2% of sulfur was removed. The specific desulfurization rate was 8.75 mmol sulfur kg⁻¹ (cell) h⁻¹.

Rhodococcus sp. P32C1 was reported to remove 48.5 and 23.7% of sulfur from two types of diesel oils with the initial sulfur contents as 303 and 1,000 mg/L, respectively [Maghsoudi et al., 2001]. The resting cells of *Gordona* strain CYKS1 could be used to decrease the total sulfur in a middle distillate unit feed from 1.50 to 0.61 g/L in 10 h with the desulfurization rate as 9.4 mmol sulfur kg⁻¹ (cell) h⁻¹ [Chang et al., 2000]. It showed that the capability of *Pseudomonas delafeldii* R-8 for diesel desulfurization was similar to that of the above strains. This indicates that *Pseudomonas delafeldii* R-8

should be useful for the desulfurization of diesel oil.

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