Improvement of Biodesulfurization Rate by Assembling Nanosorbents on the Surfaces of Microbial Cells

S. Guobin,*[†] Z. Huaiying,*[†] C. Weiquan,*[†] X. Jianmin,* and L. Huizhou*

*Laboratory of Separation Science and Engineering, State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, 100080, China; and [†]Graduate School of the Chinese Academy of Sciences, Beijing, China

ABSTRACT   To improve biodesulfurization rate is a key to industrialize biodesulfurization technology. The biodesulfurization rate is partially affected by transfer rate of substrates from organic phase to microbial cell. In this study, γ-Al$_2$O$_3$ nanosorbents, which had the ability to selectively adsorb dibenzothiophene (DBT) from organic phase, were assembled on the surfaces of Pseudomonas delafieldii R-8 cell, a desulfurization strain. γ-Al$_2$O$_3$ nanosorbents have the ability to adsorb DBT from oil phase, and the rate of adsorption was far higher than that of biodesulfurization. Thus, DBT can be quickly transferred to the biocatalyst surface where nanosorbents were located, which quickened DBT transfer from organic phase to biocatalyst surface and resulted in the increase of biodesulfurization rate. The desulfurization rate of the cells assembled with nanosorbents was approximately twofold higher than that of original cells. The cells assembled with nanosorbents were observed by a transmission electron microscope.

Recently, biodesulfurization of petroleum products has received growing attention as the production of ultra-low-sulfur products (1). However, it is still not a commercial technology because of some problems, such as desulfurization rate, bioreactor design, and the volumetric ratio between the oil and aqueous phases. Among these, desulfurization rate represents the main limiting factor for an industrial application of the biotechnological process. Many progresses have been done in improvement of biodesulfurization rate in the last years by increasing the activity of biocatalysts. Despite considerable progress in improving the expression and copies of the key enzymes (2,3), the flux through the system is still too low for widespread commercial applications (1). Setti et al. (4) and Mehrnia et al. (5) reported that transfer of polycyclic aromatic sulfur heterocycle (such as DBT) from the oil to the water (and then from the water to the cells) can limit the rate of its metabolism.

In this study, a novel biodesulfurization technology was developed by assembling γ-Al$_2$O$_3$ nanosorbent, which can selectively adsorb DBT (a model polycyclic aromatic sulfur heterocycle) from the organic phase, on the surfaces of microbial cell. The approach to increase the rate of biodesulfurization is based on the improvement of transfer rate of DBT.

Pseudomonas delafieldii R-8 (6), was isolated from the sewage pool of Shengli oil field in China, and has the ability to convert DBT to 2-hydroxy-biphenyl (2-HBP) and sulfate.

We synthesized γ-Al$_2$O$_3$ nanosorbent by the following method. We dissolved 25 g Al(NO$_3$)$_3$·9H$_2$O in 100 ml distilled water including 0.1 g of cetyltrimethylammonium bromide (CTAB), and then added dropwise a 10% of NH$_4$HCO$_3$ aqueous solution including 0.1 g of CTAB. The addition was stopped until the sol was formed. The addi-tional amount of NH$_4$HCO$_3$ aqueous solution was ∼50 ml. To continue to stir for 1 h, and then age for 48 h, the products were dried in vacuum for 5 h at 80°C. Finally, γ-Al$_2$O$_3$ nanosorbents were obtained by sintering at 600°C.

Gamma-Al$_2$O$_3$ nanosorbents (0.1 g) and 0.5 g of dry cells were added into 50 ml of saline water (8.5% NaCl). The cells assembled with γ-Al$_2$O$_3$ nanosorbents were harvested and vacuum-dried at −4°C. Each cell preparation (the aforementioned γ-Al$_2$O$_3$-cells or 0.5 of dry cells) was suspended in 15 ml phosphate buffer (0.1 M, pH = 7.0), and the suspension was mixed with 15 ml of model oil (5.0 mM DBT in n-dodecane). The reaction was carried out in 100-ml flasks at 30°C on a rotary shaker at 180 rpm.

Fig. 1, a and b, are two transmission electron microscope (TEM) images of γ-Al$_2$O$_3$ nanosorbent with different magnifications (×10,000 and ×200,000). It is clearly shown that γ-Al$_2$O$_3$ sorbents prepared are very thin long-fiber shape. Its length is ∼100 nm, and its width is only a few nanometers. Thus, the size of the sorbent is much smaller than that of microbial cell, which is about a few micrometers.

Fig. 2, a and b, are TEM images of free cell and microbial cell assembled with γ-Al$_2$O$_3$ nanosorbents, respectively. The γ-Al$_2$O$_3$ nanosorbents were efficiently assembled on the surfaces of microbial cell as shown in Fig. 2.

To investigate the adsorptive ability of γ-Al$_2$O$_3$ nanosorbents, an adsorptive process was performed in normal pressure, which 0.1 g sorbents were added into 5 mL model oil containing 5.0 mM of DBT and 5.0 mM of 2-HBP. Fig. 3 shows the adsorptive curve of γ-Al$_2$O$_3$ nanosorbents at
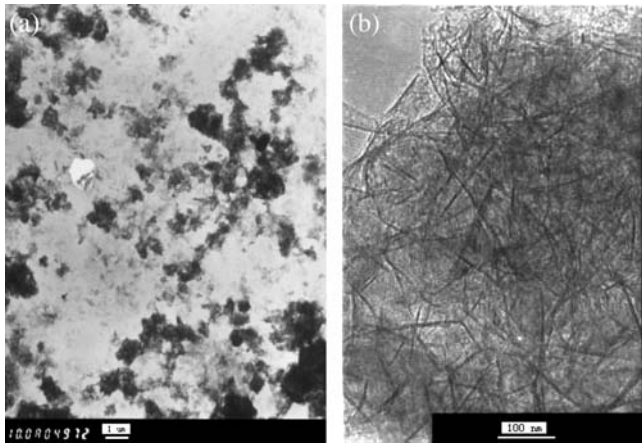
FIGURE 1 TEM images of γ-Al$_2$O$_3$ nanosorbent (obtained by Hitachi 8100, 200 kV). (*a*) ×10,000; (*b*) ×200,000.

FIGURE 3 Adsorption of DBT and 2-HBP using γ-Al$_2$O$_3$ nanosorbents.

30°C. During the first 0.5 h, DBT and 2-HBP concentrations were, respectively, reduced to 2.69 and 2.85 mM from 5.00 mM. According to Fig. 3, the sorbents have been saturated with DBT and 2-HBP for <0.5 h. So, the adsorptive rate of γ-Al$_2$O$_3$ nanosorbents was >446 mmol·kg^{-1}·h^{-1}.

With the purpose of understanding the desulfurizing activity of the γ-Al$_2$O$_3$-cells, we tested the desulfurization rates of free cells and the γ-Al$_2$O$_3$-cells in model oil containing 5.0 mM of DBT, respectively. Fig. 4 shows that the consumption of DBT and the production of 2-HBP using free cells and γ-Al$_2$O$_3$-cells in the model oil. During the first 0.5 h, the rate of DBT consumption by the γ-Al$_2$O$_3$-cells is ~4.93-fold higher than that by free cells. However, during the same time, the rate of 2-HBP produced by the γ-Al$_2$O$_3$-cells is only 1.02-fold higher than that by free cells. The rate of DBT consumption is not consistent with that of 2-HBP produced in model oil. However, during the subsequent 1.0 h, both the rates of DBT consumption and 2-HBP produced by the γ-Al$_2$O$_3$-cells are the same, which are ~1.73-fold higher than those by free cells. It is because that the γ-Al$_2$O$_3$

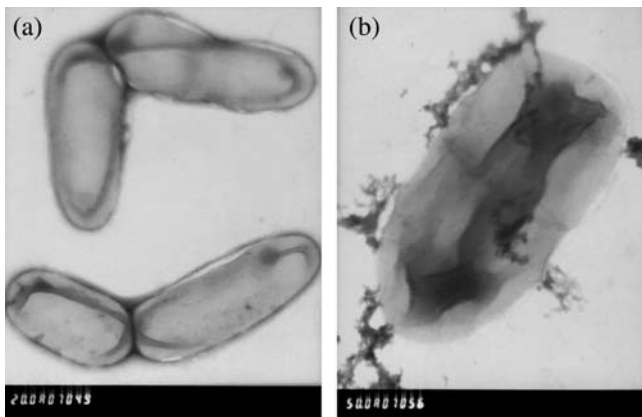nanosorbents can adsorb DBT from model oil, and the rate of adsorption is far higher than that of BDS (Fig. 2). The adsorption process can increase the rate of DBT transfer. During the first 0.5 h, a part amount of DBT molecules, ~0.117 mmol (DBT consumed + 2-HBP produced)/g(sorbent), was adsorbed into the pores of γ-Al$_2$O$_3$ nanosorbent, which resulted in the reduction of DBT and 2-HBP concentrations in oil phase. However, DBT molecules of these cannot be reacted and converted to 2-HBP by microbial cells. Besides, 2-HBP molecules of these produced were adsorbed into the γ-Al$_2$O$_3$ nanosorbents and could not be detected in oil phase. Therefore, the rate of DBT consumption in oil phase was higher than that of 2-HBP produced, detected in oil phase, during the first time. On the other hand, once the

FIGURE 4 Time course of desulfurization of DBT by free cells and γ-Al$_2$O$_3$-cells. Residual DBT concentration after being consumed by free cells (△); 2-HBP concentration produced by free cells (▲); residual DBT concentration after being consumed by γ-Al$_2$O$_3$-cells (□); 2-HBP concentration produced by γ-Al$_2$O$_3$-cells (■).

FIGURE 2 TEM images of free cell and cell assembled with γ-Al$_2$O$_3$ nanosorbent. (*a*) Free cell. (*b*) γ-Al$_2$O$_3$-cell.
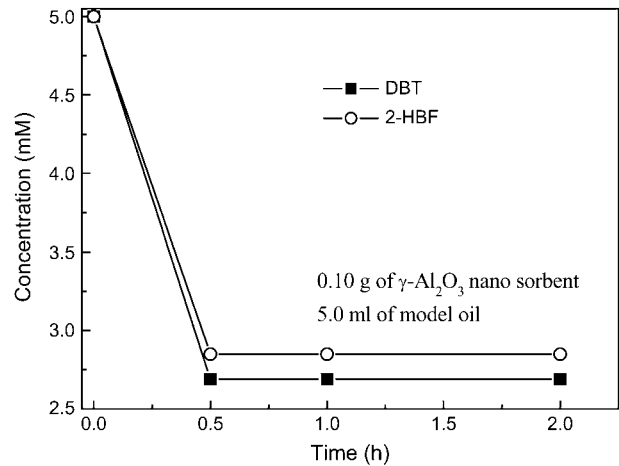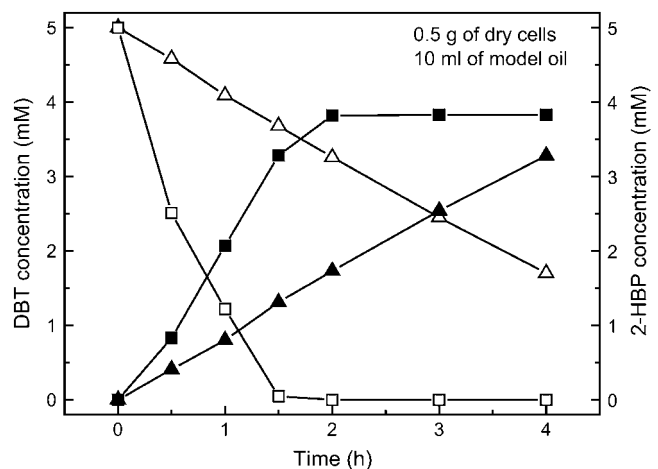
pores of γ-Al$_2$O$_3$ nanosorbents were saturated with DBT and 2-HBP molecules, the consumption of DBT in oil phase can completely be used to convert to 2-HBP, which also was completely transferred to oil phase. Thus, the increase of DBT consumption rate was equivalent to that of 2-HBP production rate after the saturation of sorbents with DBT. The final concentration of 2-HBP produced by γ-Al$_2$O$_3$-cells was $\sim$3.83 mM, less than the initial concentration of DBT. It may deduce that $\sim$0.012 mmol of DBT and 2-HBP were adsorbed into the pores of γ-Al$_2$O$_3$ nanosorbent.

The biodesulfurization rate of R-8 cells has been improved by γ-Al$_2$O$_3$-cell (Fig. 4). It is because that the desulfurization rate was mainly limited by the following two factors, i.e., catalytic activity of *dsz* enzymes in cells and the rate of mass transfer from oil phase to the cell body through aqueous phase. The adsorption of γ-Al$_2$O$_3$ nanosorbents to DBT can accelerate the DBT transfer from aqueous phase to the cell surfaces, which results in the increase of desulfurization rate. According to Fig. 3, the adsorptive rate of the γ-Al$_2$O$_3$ nanosorbent is $>$446 mmol$\cdot$kg$^{-1}\cdot$h^{-1}, which is far higher than the desulfurization rate of microbial cells. Thus, DBT molecules can be quickly congregated on the cell surface, where nanosorbents were located. Consequently, the DBT molecules can be transported into cell for biodesulfurization reaction. Thus, transfer limitation of the DBT molecules can be eliminated to some extent, which results in the improvement of biodesulfurization rate.

REFERENCES and FOOTNOTES

1. Gray, K. A., G. T. Mrachko, and C. H. Squires. 2003. Biodesulfurization of fossil fuels. *Curr. Opin. Microbiol.* 6:229–235.

2. Erwin, K. N., S. Nakano, and P. Zuber. 2005. Sulfate-dependent repression of genes that function in organosulfur metabolism in *Bacillus subtilis* requires Spx. *J. Bacteriol.* 187:4042–4049.

3. Tanaka, Y., O. Yoshikawa, and K. Maruhashi. 2002. The *cbs* mutant strain of *Rhodococcus erythropolis* KA2–5-1 expresses high levels of *Dsz* enzymes in the presence of sulfate. *Arch. Microbiol.* 178:351–357.

4. Setti, L., P. Farinelli, D. S. Martino, S. Frassinetti, G. Lanzarini, and P. Pifferi. 1999. Developments in destructive and non-destructive pathways for selective desulfurizations in oil biorefining processes. *Appl. Microbiol. Biotechnol.* 52:111–117.

5. Mehrnia, M. R., B. Bonakdarpour, J. Towfighi, and M. M. Akbarnejad. 2004. Design and operational aspects of airlift bioreactors for petroleum biodesulfurization. *Environ. Prog.* 23:206–214.

6. Luo, M. F., J. M. Xing, Z. X. Gou, S. Li, H. Z. Liu, and J. Y. Chen. 2003. Desulfurization of dibenzothiophene by lyophilized cells of *Pseudomonas delafieldii* R-8 in the presence of dodecane. *Biochem. Eng. J.* 13:1–6.