Selection of adsorbents for *in-situ* coupling technology of adsorptive desulfurization and biodesulfurization

ZHANG HuaiYing[1,2], LIU QingFen[1], LI YuGuang[1,2], LI WangLiang[1,2], XIONG XiaoChao[1,2], XING JianMin[1†] & LIU HuiZhou[1†]

[1] State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, China;
[2] Graduate University of Chinese Academy of Sciences, Beijing 100049, China

***In-situ* coupling of adsorptive desulfurization and biodesulfurization is a new desulfurization technology for fossil oil. It has the merits of high-selectivity of biodesulfurization and high-rate of adsorptive desulfurization. It is carried out by assembling nano-adsorbents onto surfaces of microbial cells. In this work, *In-situ* coupling desulfurization technology of widely used desulfurization adsorbents of γ-Al$_2$O$_3$, Na-Y molecular sieves, and active carbon with *Pseudomonas delafieldii* R-8 were studied. Results show that Na-Y molecular sieves restrain the activity of R-8 cells and active carbon cannot desorb the substrate dibenzothiophene (DBT). Thus, they are not applicable to *in-situ* coupling desulfurization technology. Gamma-Al$_2$O$_3$ can adsorb DBT from oil phase quickly, and then desorb it and transfer it to R-8 cells for biodegradation, thus increasing desulfurization rate. It is also found that nano-sized γ-Al$_2$O$_3$ increases desulfurization rate more than regular-sized γ-Al$_2$O$_3$. Therefore, nano-γ-Al$_2$O$_3$ is regarded as the better adsorbent for this *in-situ* coupling desulfurization technology.**

adsorptive desulfurization, biodesulfurization, *in-situ* coupling, adsorbents

Organic sulfur compounds in fossil oil and its products have caused many problems. For example, when fossil fuels are combusted, SO$_x$ are released, resulting in serious environmental pollutions[1]. In order to protect the environment, sulfur levels of diesel oil and gas oil have been required to be very low (sulfur content less than 10 −15 μg·g^{-1}). The aim is to obtain clean fuels[2].

Due to its mild reaction conditions, high selectivity, and little impact on fuel quality, biodesulfurization is considered as a promising technology in deep desulfurization for fuels[3]. However, there are still many issues in the way of its industrialization, e.g., low reaction rate, difficulty in products separation and so on. At present, biological techniques, such as metabolic engineering, have been employed to increase desulfurization rate[4]. Meanwhile, Kobayashi[5] has discovered that mass transfer of substrate is one of key factors which impact biodesulfurization rate. Setti[6] proved it by a *Pseudomonas* system with loop lyase similar to dioxygenases,

discovering that the transfer rate of dibenzothiophene (DBT) from oil phase to water and then to cells does impact biodesulfurization rate.

Compared with biodesulfurization, adsorptive desulfurization has a much faster reaction rate[7]. Adsorbent preparation is the key of adsorptive desulfurization. Recently, most adsorbents for desulfurization were based on π-complexation[8,9] or formations of metal-sulfur bonds (such as Ni-S, La-S)[10]. Adsorbents based on π-complexation are easy to regenerate, but their selectivity is very low, resulting in a loss of fuel quality. Meanwhile, adsorbents that format metal-sulfur bonds with sulfur have high selectivity but are difficult to be regenerated. Hence, adsorptive desulfurization technology also has a long way to go before being industrialized. If a desulfu-

rization technology has both the high reaction rate of adsorptive desulfurization and the high selectivity of biodesulfurization, it can increase the desulfurization rate without damaging fuel quality.

Dr. Shan Guobin in our lab has started to study the combination of adsorptive desulfurization and biodesulfurization, and he proposed an *in-situ* coupling technology of them[11]. It is carried out by assembling nano-adsorbents on the surface of microbial cells. The nano-adsorbents on the surface of cells adsorb DBT quickly from the oil phase and transfer it to cells for biodegradation, thus improving the transfer rate of substrate and increasing desulfurization rate about 2.5 times. Adsorbent is an important factor of this coupling technology because different adsorbents have different interaction to organic sulfur compounds and cells, which would affect their assembly onto the cell surfaces and desorption behaviour of organic sulfur from them. Because the adsorbents are assembled on the cells' surfaces, the property of cell surface is another factor which impacts the coupling technology. Besides, desulfurization conditions, such as temperature and volume ratio of oil to water phase, would also affect *in-situ* coupling technology.

In this work, different kinds of adsorbents (alumina, molecular sieves, and active carbon) were used in *in-situ* coupling technology of adsorptive desulfurization and biodesulfurization. Effects of category and property of adsorbent on the coupling technology were analyzed and discussed.

1 Materials and methods

1.1 Materials

Dibenzothiophene (DBT, 99%) was purchased from Acros Organics, USA; 2-Hydroxybiphenyl (2-HBP, 99%) was purchased from TCI, Japan. n-Octane was purchased from Shanghai Chemical Reagent Corporation, China Medicine Group; Methanol for HPLC was purchased from Tianjin Siyou Chemical Reagent Corporation in China. All other chemicals were of reagent grade.

Na-Y molecule sieves were provided by Dr. Li Wangliang in our lab; nano-γ-Al$_2$O$_3$ was provided by Dr. Cai Weiquan in our institute, recorded as γ-Al$_2$O$_3$-1; trefoil-shaped γ-Al$_2$O$_3$ was provided by Dr. Shan Guobin in our lab, recorded as γ-Al$_2$O$_3$-2 and pulverized for desulfurization. Active carbon was purchased from Beijing

Guanghua Mucai Factory.

1.2 Strain and cultivation

Strain: *Pseudomonas delafieldii* R-8 (No. CGMCC 0570) was isolated by our laboratory.

Basic salt medium (BSM) for cultivation : KH$_2$PO$_4$, 2.44 g; Na$_2$HPO$_4$·12H$_2$O, 12.03 g; MgCl$_2$·6H$_2$O, 0.4 g; NH$_4$Cl, 2.0 g; CaCl$_2$, 0.75 mg; FeCl$_3$·6H$_2$O, 1 mg; MnCl$_2$·4H$_2$O, 4 mg; Glycerol, 10 g; deionized water, 1000 mL. Sulfur source is 0.1 mmol·L^{-1} DBT.

Cultivation conditions: 25 mL culture medium in 100 mL flasks; (30±1)℃, 170 r/mim in a reciprocal shaking incubator; harvested by centrifugation after 50 h cultivation, washed 2－3 times by NaCl solution (0.85%), frozen, dried in a condenser vacuum drier at 30 mtorr (1 mtorr = 0.133 Pa), −64℃ for 24 h, and saved at −4℃.

1.3 Desulfurization methods

The model diesel oil for desulfurization was *n*-octane, containing 1－2 mmol/L of DBT.

Biodesulfurization method: 0.5 g of dry cells were added into 10 mL of saline water (0.85% NaCl), and the suspension was mixed with 5 mL of model oil. The reaction was carried out in 100 mL flasks at 30℃ on a rotary shaker at 170 r/min.

In-situ coupling desulfurization method: 0.5 g of dry cells were added into 10 mL of saline water, and then adsorbent was added into the suspension and mixed with cells. Then, 5 mL of model oil was added into the mixture for desulfurization. The reaction was carried out in 100 mL flasks at 30℃ on a rotary shaker at 170 r/min.

Adsorptive desulfurization of adsorbents in saline water: 100 mg of adsorbents were suspended in 5 mL of saline water, and 2 mL of model oil was added (containing the same concentration of DBT and 2-HBP) for desulfurization. The reaction was also carried out in 100 mL flasks at 30℃ on a rotary shaker at 170 r/min.

Generally, the qualities of cells used are the same in each section. DBT and 2-HBP tested are in oil phase, and that has not been tested on adsorbents.

1.4 Analysis methods

The concentrations of DBT and 2-HBP were tested using High Performance Liquid Chromatography (HPLC) (1100 series, Agilent Technologies, USA). The morphology of adsorbents was observed with a scanning electron microscope (SEM) (JSM-5800, Hitachi, Japan).

The specific surface area and porous ratio of adsorbents were determined using a Nova 1200 Quantachrome, USA. The assembly of adsorbents on the surface of cells was observed by a transmission electron microscope (TEM) (Techai 20, Philips, the Netherlands).

2 Results and discussions

2.1 Characterization of desulfurization adsorbents

Figure 1 shows that γ-Al_2O_3-1 particle is less than 10 nm in width and $100-200$ nm in length. Its structure is loose with little congregation. Its specific surface area is 339 m^2/g, density is 3.65 g/cm^3, and porous ratio is 81%. Particle γ-Al_2O_3-2 is a heavy congregation of blocks of $100-200$ nm in size. Its specific surface area is 99 m^2/g, density is 3.65 g/cm^3, and porous ratio is 43%. Na-Y molecule sieves are compacted with bigger-sized blocks. Its specific surface area is 645 m^2/g, density is 2.91 g/cm^3, and porous ratio is 69%. Active carbon is loose and porous. Its specific surface area is 1843 m^2/g, den-

sity is 2.15 g/cm^3, and porous ratio is 87%.

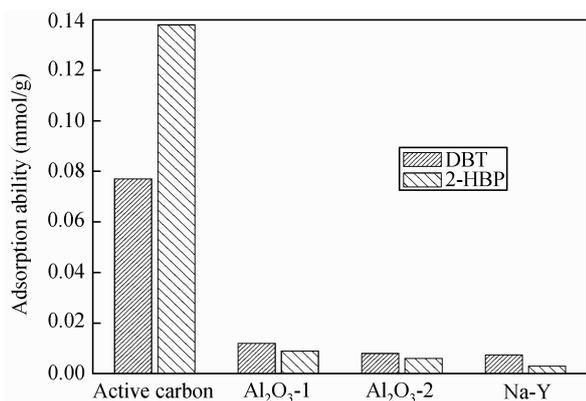### 2.2 Adsorptive desulfurization of adsorbents in aqueous phase

Adsorptive desulfurization of adsorbents was tested in saline water because the *in-situ* coupling technology was conducted in saline water. Figure 2 shows that active carbon has high adsorption capacity of 0.077 mmol/g to DBT and even higher capacity of 0.138 mmol/g to 2-HBP. Meanwhile, other adsorbents have low adsorption capacity to DBT and 2-HBP. The adsorption capacity of γ-Al_2O_3-1 to DBT is 0.012 mmol/g, and to 2-HBP is 0.009 mmol/g. The adsorption capacity of γ-Al_2O_3-2 to DBT is 0.008 mmol/g, to 2-HBP is 0.006 mmol/g, and that of Na-Y molecule sieves to them is only 0.007 mmol/g and 0.003 mmol/g, respectively.

2.3 *In-situ* coupling desulfurization of different kinds of adsorbent

Figure 3 shows that coupling systems of different kinds of adsorbent have different DBT consumption rates and
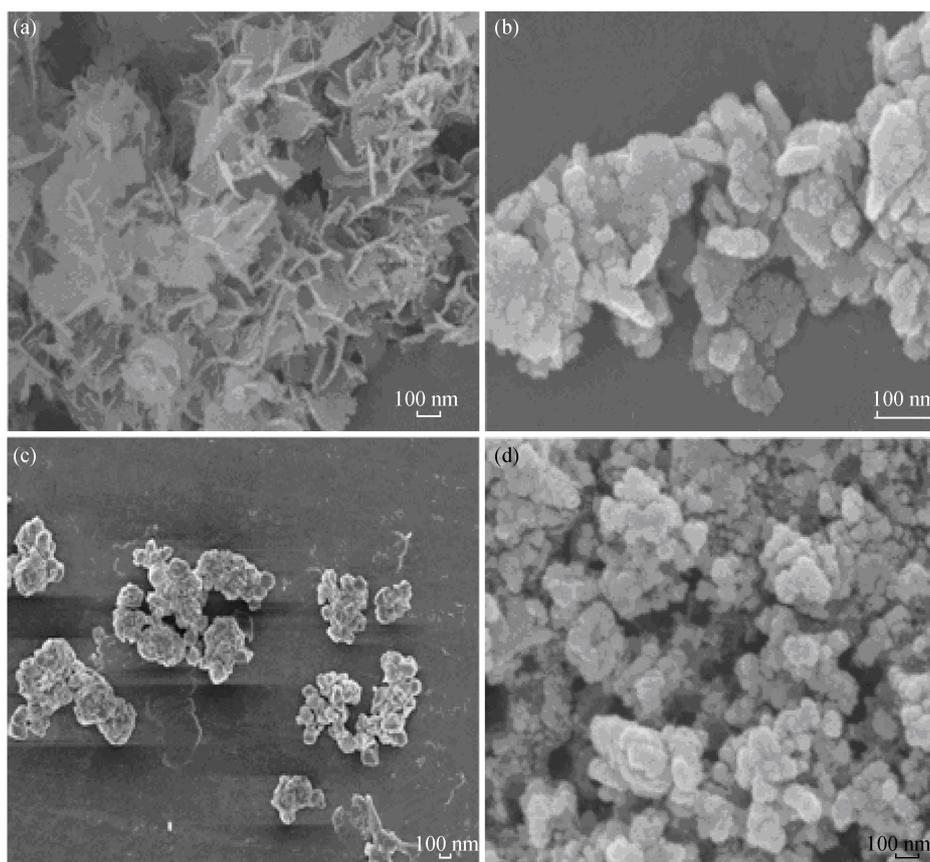

Figure 1 SEM images of adsorbents. (a) γ-Al_2O_3-1; (b) γ-Al_2O_3-1; (c) Na-Y molecule sieves; (d) active carbon.

Figure 2 Adsorptive desulfurization capability of adsorbents in aqueous phase.

2-HBP production rates when coupled with the same amount of R-8 cells. The order of DBT consumption rates is listed as follows: Active carbon-R-8 system> γ-Al$_2$O$_3$-1-R-8 system > γ-Al$_2$O$_3$-2-R-8 system >R-8 system >Na-Y molecule sieves-R-8 system, while the order of 2-HBP production rates is listed as follows:

γ-Al$_2$O$_3$-1-R-8 system > γ-Al$_2$O$_3$-2-R-8 system >R-8 system >Na-Y molecule sieves-R-8 system >active carbon-R-8 system. It also shows that both DBT consumption rates and 2-HBP production rates in the coupling systems of γ-Al$_2$O$_3$ are higher than that in free R-8 cell system. At the same time, DBT consumption rate and 2-HBP production rate in coupling system of γ-Al$_2$O$_3$-1 are higher than that in coupling system of γ-Al$_2$O$_3$-2. In the coupling system of Na-Y molecule sieves, both DBT consumption rate and 2-HBP production rate are lower than that in free R-8 cell system, which means that molecule sieves do not increase desulfurization rate but decrease it. In the coupling system of active carbon, DBT consumption rate is much higher than that in free R-8 cell system. However, 2-HBP production rate is lower than that in free R-8 cell system, and even lower than that in the coupling system of Na-Y molecule sieves. Figure 3(c) is the curves of total concentrations of DBT and 2-HBP in each system over time. It shows that total concentration decreases over time in active carbon-R-8
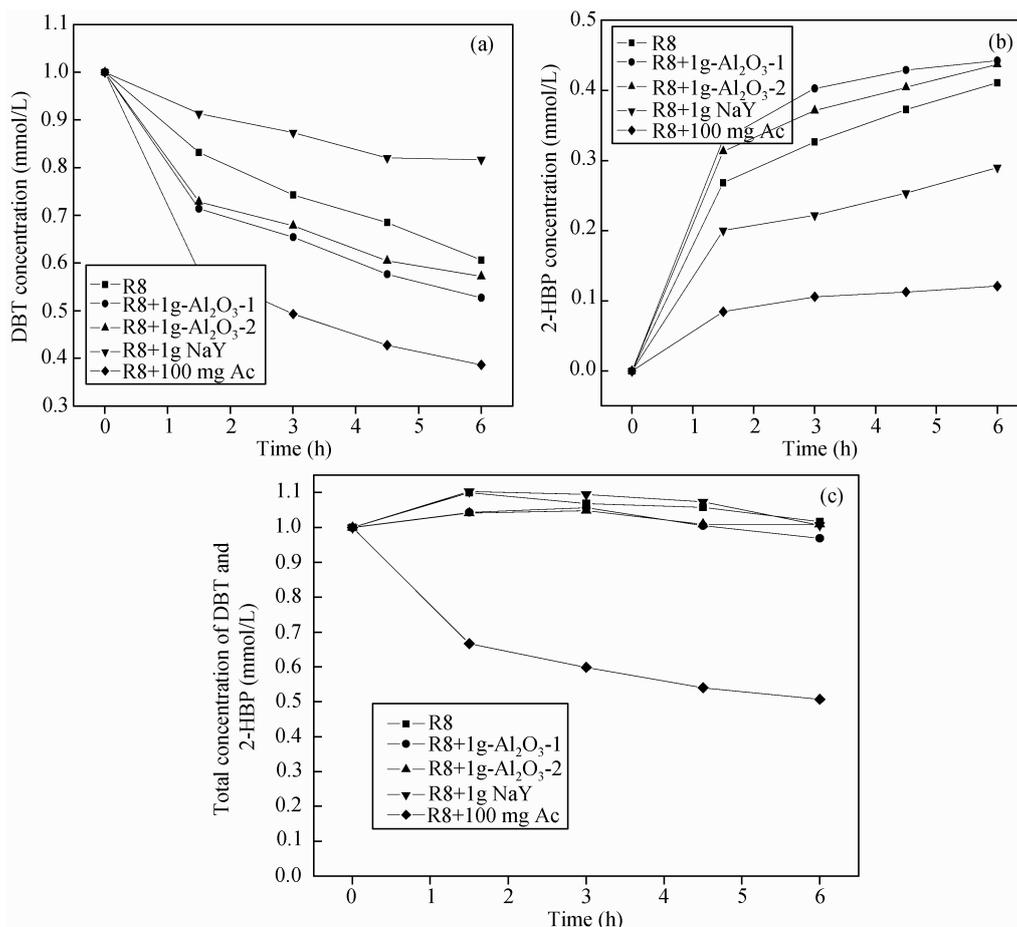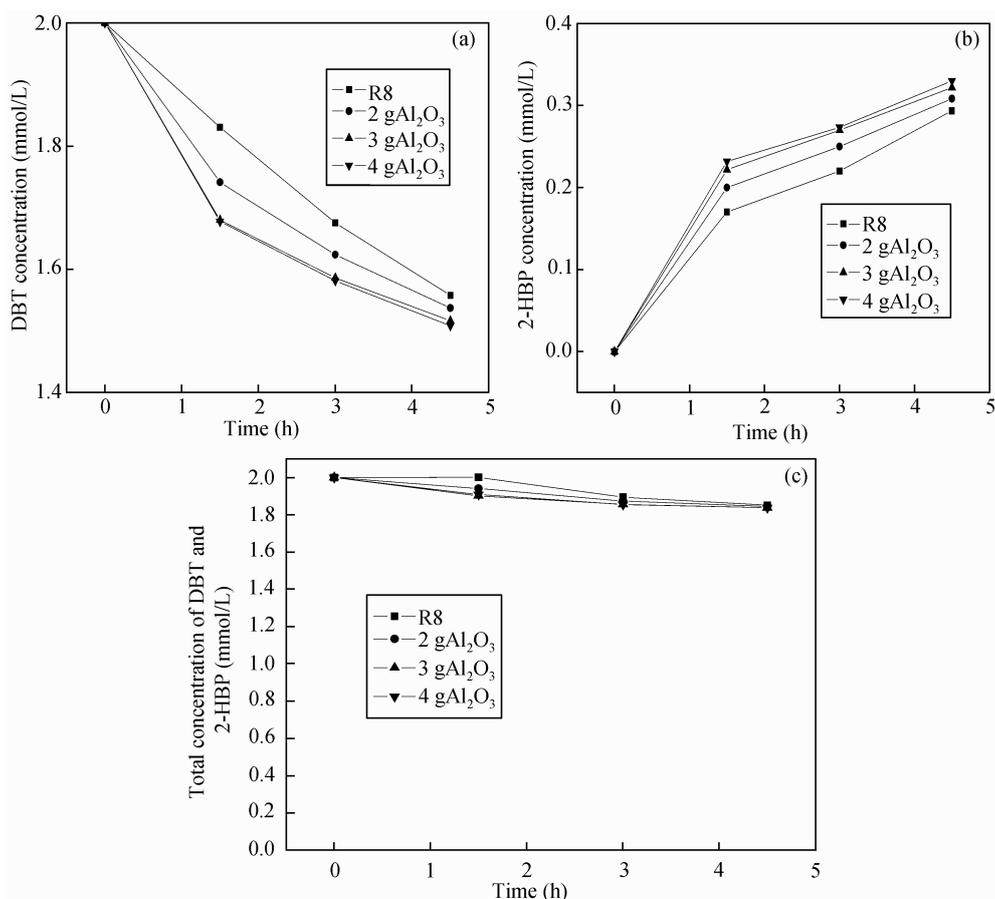

Figure 3 DBT concentration-time (a), 2-HBP concentration-time (b), total concentration of DBT and 2-HBP-time (c) curves of different adsorbents in the coupling desulfurization.

coupling system. It has decreased to half of its original at the 6th hour, which means that a lot of DBT or 2-HBP was left on active carbon. There is little change in total concentration of DBT and 2-HBP in other systems, which is to say that little DBT and 2-HBP was left on γ-Al$_2$O$_3$ or Na-Y molecule sieves.

2.4 Effects of γ-Al$_2$O$_3$-2 amounts on coupling desulfurization

According to Figure 3, the coupling of γ-Al$_2$O$_3$ with R-8 can increase desulfurization rate. In order to further understand this result, effects of γ-Al$_2$O$_3$-2 amounts on coupling desulfurization were studied, as shown in Figure 4. It can be seen that as γ-Al$_2$O$_3$ amount increases, DBT consumption rate and 2-HBP production rate also increase. It means that desulfurization rate increases with γ-Al$_2$O$_3$ amount. However, when the amount of γ-Al$_2$O$_3$-2 used is 3 g and 4 g, their DBT concentration-time curves are almost the same, and so are their 2-HBP concentration curves. That is to say, when the amount of γ-Al$_2$O$_3$-2 is more than 3 g, γ-Al$_2$O$_3$-2 does

not increase desulfurization rate anymore. It has two possible reasons. On one hand, the transfer rate of substrate is one of limitation rates but not the only one in biodesulfurization. On the other hand, it is reported[12] that if particles on a cell occupy more than 2/3 of its surface area, cell activity will be affected. Here in the coupling system of 4 g γ-Al$_2$O$_3$-2, so much γ-Al$_2$O$_3$ was assembled onto the cell surface that the biodegradation activity of cells was damaged. As a result, total desulfurization rate is similar to that in the coupling system of 3 g γ-Al$_2$O$_3$-2. Thus, we can conclude that the optimal amount of γ-Al$_2$O$_3$-2 in γ-Al$_2$O$_3$-2-R-8 coupling system is between 2−3 g. Considering that dry cells we used are 0.5 g, the optimal amount of γ-Al$_2$O$_3$-2 is between 4−6 g Al$_2$O$_3$/ g cell. Figure 4(c) is curves of total concentrations of DBT and 2-HBP in each system overtime. It can be seen that less than 5% of its total concentration of DBT and 2-HBP has decreased. It means that little DBT and 2-HBP was left on γ-Al$_2$O$_3$-2, even when its weight was 4 g.

Figure 4 DBT concentration-time (a), 2-HBP concentration-time (b), total concentration of DBT and 2-HBP-time (c) curves of different amounts of Al$_2$O$_3$-2 in the coupling desulfurization.

2.5 Effects of Na-Y molecule sieves amounts on coupling desulfurization

According to Figure 3, Na-Y molecule sieves do not increase desulfurization rate when coupled with R-8. In order to verify this result, effects of Na-Y molecule sieves amounts on coupling desulfurization were studied, as shown in Figure 5. It can be seen that in coupling systems of Na-Y molecule sieves with R-8, DBT consumption rates and 2-HBP production rates are lower than that in free R-8 system, and with the increase of Na-Y molecule sieves amounts, both DBT consumption rates and 2-HBP production rates decrease. That is to say, Na-Y molecule sieves restrain the desulfurization activity of cells, and the restraint increases with Na-Y molecule sieves amounts.

2.6 Effects of active carbon amounts on coupling desulfurization

According to Figure 3, DBT consumption rate in the coupling system of active carbon is much higher than that in free R-8 cell system, but 2-HBP production rate is much lower than that in free R-8 system. In order to verify this result, effects of active carbon amounts on coupling desulfurization were studied in this section. It can be seen from Figure 6(a) that with the increase of active carbon amount, DBT consumption rate increases. What Figure 6(b) indicates is that with the increase of active carbon amount, 2-HBP production rate does not increase as well, but decreases. This must be due to the high adsorption capacity of active carbon to DBT and 2-HBP shown in Figure 2. However, it does not tell whether active carbon increases coupling desulfurization rate or not.

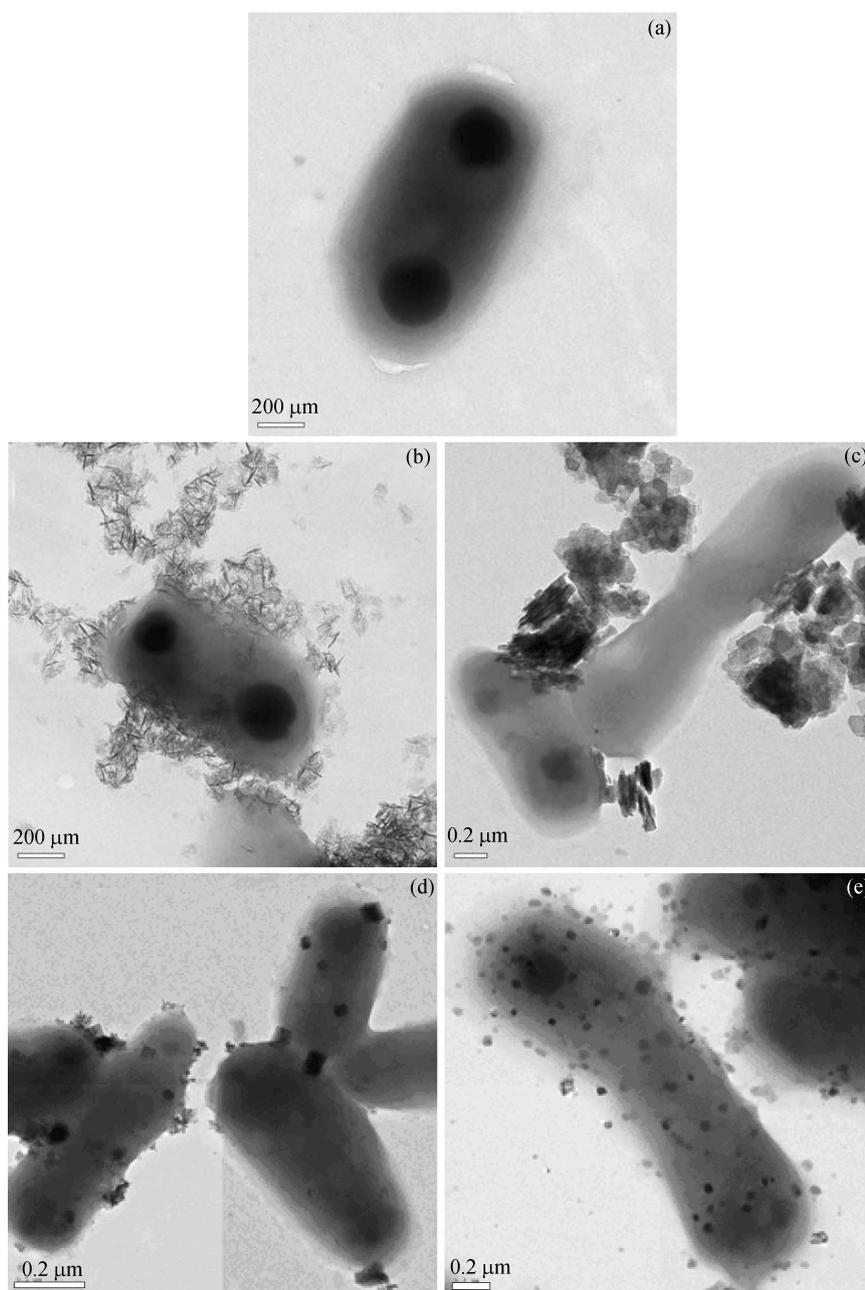## 2.7 Assembly of adsorbents on cell surfaces

In order to study the *in-situ* coupling of R-8 cell with different adsorbents, TEM was used to investigate the assembly of adsorbents on cell surfaces, as shown in Figure 7.
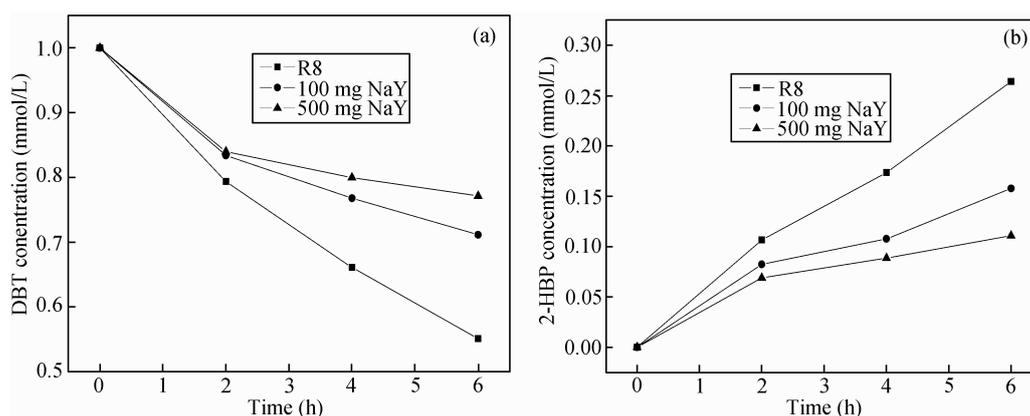
Figure 5 DBT Concentration-time (a) and 2-HBP concentration-time (b) curves of different amounts of Na-Y molecule sieves in the coupling desulfurization.
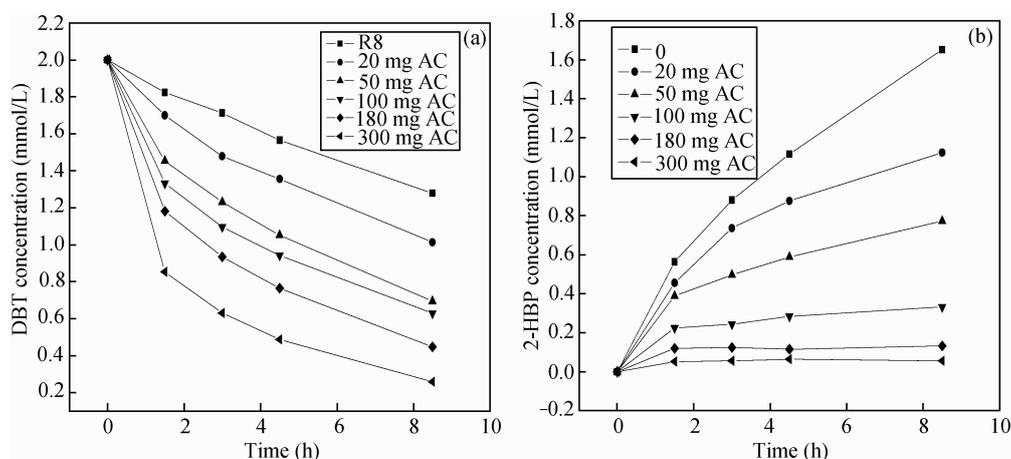
Figure 6 DBT concentration-time (a) and 2-HBP concentration-time (b) curves of different amounts of active carbon in the coupling desulfurization.

Figure 7 Adsorbents assembled on cell surfaces. (a) Free cell, 10500 times; (b) Al$_2$O$_3$-1, 10500 times; (c) Al$_2$O$_3$-2, 7800 times; (d) Na-Y, 6500 times; (e) Active carbon, 9600 times.

From Figure 7(a), Figure 7(b), and Figure 7(c), we can see that R-8 cell adsorbs γ-Al$_2$O$_3$-1 onto its surface very well, and it can also adsorb γ-Al$_2$O$_3$-2 onto its surface. In *in-situ* coupling system, the consumption of substrate DBT includes two parts. One is that adsorbed onto adsorbents; the other is that transferred into cells and biodegraded. Only the consumption in the second part results in 2-HBP production. As well, one part of the 2-HBP produced would be adsorbed by adsorbents,

and another part would be transferred to oil phase and tested. It can be seen from Figure 3 that DBT consumption rates and 2-HBP production rates in coupling systems of γ-Al$_2$O$_3$ with R-8 are higher than that in free R-8 system. At the same time, according to Figure 2, γ-Al$_2$O$_3$ adsorbs little DBT and 2-HBP in water. It means that in the coupling systems of γ-Al$_2$O$_3$ with R-8, the decrease of DBT concentration is not only caused by the adsorption of γ-Al$_2$O$_3$ to DBT, but also partly by bio-

degradation. Because of the low adsorption capacity of alumina to DBT, the adsorption between them can by saturated quickly. It is reported that the adsorption between γ-Al$_2$O$_3$ and DBT is electrostatics, and the weak force based on acid ports on γ-Al$_2$O$_3$ surfaces; the adsorption between them is reversible and DBT can be desorbed easily[13]. The desorbed DBT then transfer to cells for biodegradation. It is faster for cells to obtain DBT from oil phase by this way, thus 2-HBP production rate is increased. That is to say, the coupling of γ-Al$_2$O$_3$ with R-8 can efficiently increase desulfurization rate.

By comparing Figure 7(b) and Figure 7(c), it can be seen that γ-Al$_2$O$_3$-2 is not assembled onto cells as much as γ-Al$_2$O$_3$-1 because of its big size. This could explain the result in Figure 3 that desulfurization rate in γ-Al$_2$O$_3$-1-R-8 system is faster than that in γ-Al$_2$O$_3$-2-R-8 system. With its smaller size, bigger surface area, γ-Al$_2$O$_3$-1 is the better adsorbent for coupling desulfurization.

Figure 7(d) indicates that little Na-Y molecule sieves are assembled onto cell surface, which could contribute to the result that Na-Y molecule sieves do not increase desulfurization rate when coupled with R-8. However, it cannot explain the results that the desulfurization rate in Na-Y molecule sieves-R-8 coupling system is lower than that in free R-8 system, and the coupling desulfurization rate decreases with the Na-Y molecule sieves amounts. It may be caused by the restraint of Na-Y molecule sieves to desulfurization activity of R-8 cells, and the restraint has nothing to do with the size and morphology of Na-Y molecule sieves.

Figure 7(e) shows that active carbon can be assembled to cell surface very well. However, according to Figure 3, active carbon-R-8 coupling system has the highest DBT consumption rate but the lowest 2-HBP production rate. As discussed before, the consumption of substrate DBT includes two parts. One part is that adsorbed onto adsorbents; another part is that transferred into cells and biodegraded. Only the consumption in the second part can result in 2-HBP production. As well, one part of the production 2-HBP would be adsorbed by adsorbents, and the second part would be transferred to oil phase and tested. Thus, there might be two possible explanations for DBT consumption and 2-HBP production in active carbon-R-8 coupling system. One is that active carbon does not increase desulfurization rate. In this

situation, the strong adsorption of active carbon to DBT causes the quick consumption of DBT in the coupling system. The rest DBT can be biodegraded only after the adsorption is saturated. 2-HBP produced from biodegradation is adsorbed by active carbon, and it can be tested only after the adsorption is also saturated. Another one is that active carbon increases the substrate transfer rate. In this situation, after DBT is adsorbed to active carbon, it is desorbed and transferred to cells for biodegradation, resulting in the increase of 2-HBP concentration. However, because of the high capacity of active carbon to 2-HBP, 2-HBP concentration tested is not more but less than that in free cell system. It is reported that the adsorption force between active carbon and DBT is hydrogen bond[13]. It is very strong and thus hard to desorb. Therefore, the first explanation seems to have more possibility. However, no matter what situation is, the fact is that active carbon is not applicable to *in-situ* coupling desulfurization technology because of its high adsorption capacity of DBT and 2-HBP.

3 Conclusion

Applications of widely used desulfurization adsorbents of γ-Al$_2$O$_3$, Na-Y molecular sieves and active carbon in the *in-situ* coupling of adsorptive desulfurization and biodesulfurization technology were studied in this paper. Results show that γ-Al$_2$O$_3$ can adsorb DBT from the oil phase quickly and transfer it to microbial cells for biodegradation, and thus increase the desulfurization rate. Results also show that nano-sized γ-Al$_2$O$_3$ performed better in the coupling technology than regular-sized γ-Al$_2$O$_3$. However, too much γ-Al$_2$O$_3$ cannot increase desulfurization rate further; in γ-Al$_2$O$_3$-2-R-8 coupling system, the optimal amount of γ-Al$_2$O$_3$-2 is between 4－6 g Al$_2$O$_3$/ g cell. It was also found that Na-Y molecular sieves cannot increase desulfurization rate in the coupling technology but restrain the biodesulfurization activity of R-8 cells. Active carbon is not applicable into *in-situ* coupling desulfurization technology because of its high adsorption capacity to DBT and 2-HBP. Therefore, we concluded that nano-γ-Al$_2$O$_3$ is a better adsorbent for *in-situ* coupling technology.

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