Modeling of DBT Biodesulfurization by Resting Cells of *Gordonia* sp. WQ-01A Immobilized in Alginate Gel Beads in an Oil-water-immobilization System

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In this study, the resting cells of *Gordonia* sp. WQ-01A, a DBT-desulfurizing strain, were immobilized by calcium alginate. Batch DBT biodesulfurization experiments using immobilized cells and *n*-dodecane as the oil phase were conducted in fermenter under varying operating conditions such as initial DBT concentration, bead loading and the oil phase volume fraction. When the initial DBT concentration is 0.5, 1 and 5 mmol L^{-1}, the DBT concentration dropped almost to zero after $t = 40$, 60 and 100 hours, respectively. The influence of bead loading and the oil-phase volume fraction was small to the DBT biodesulfurization. Furthermore, a mathematical model was proposed to simulate the batch DBT biodesulfurization process in an oil-water-immobilization system, which took into account the internal and external mass transfer resistances of DBT and oxygen, and the intrinsic kinetics of bacteria. To validate this model, the comparison between the model simulations and the experimental measurements of DBT concentration profiles in the oil phase was carried out and the agreement is very good. In addition, the time and radius courses of DBT and oxygen concentrations within the alginate gel beads were reasonably predicted by the proposed model.

Key words:
Dibenzothiophene (DBT), DBT biodesulfurization, alginate, oil-water-immobilization, modeling

Introduction

Biodesulfurization (BDS), which removes sulfur from fossil fuels using a series of enzyme-catalyzed reactions, is expected to be a complement or a promising alternative to the conventional hydrodesulfuration (HDS) for deep desulfurization. Operated under ambient temperature and pressure and removing heterocyclic sulfur compounds such as DBT and DBT derivatives, BDS leads to the development of environment-friendly technology. There have been reported many microorganisms that can metabolize DBT via sulfur-specific pathway (4S pathway), and they are considered to be preferable for DBT biodesulfurization.[1,2,3] Efforts were made to study the desulfurization ability[4,5] or enhance the desulfurization activity based on genetic engineering.[6,7,8,9] However, despite improvements in biocatalysts, some serious problems have to be overcome in order to realize the BDS process being a commercial technology, such as the separation of the oil and the longevity of the catalytic activity.

Cell immobilization, which can solve these problems, has been widely studied and applied recently and is becoming a focus of BDS research. The immobilized *Pseudomonas stutzeri* UP-1 cells entrapped in sodium alginate beads by Hou *et al.*[10] can be used to desulfurize DBT efficiently in the model oil and in the aqueous system. The results showed that sodium alginate was an appropriate material of immobilization and the stability and lifetime of immobilized cells (reach 600 h by reactivation) were much better than those of the non-immobilized cells. Manabu *et al.*[11] selected ENT-4000 as a suitable gel material and succeeded in constructing a biphasic BDS system (immobilized cells and oil) making it easy to recover desulfurized oil and to use the biocatalyst repeatedly for long periods with reactivation. Shan *et al.*[12] used the magnetic polyvinyl alcohol to immobilize the DBT desulfurizing bacterium *P. delafieldii* R-8 and the magnetic immobilized cells can be used to desulfurize DBT efficiently in the model oil. Compared with non-magnetic immobilized cells, the beads of magnetic immobilized cells showed higher

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reaction activity of desulfurization. Lee *et al.*[13] designed a new type of air-lift reactor with immobilized *Gordonia nitida* CYKS1 cells on a fibrous support and used it for the biocatalytic desulfurization (BDS) of diesel oil. It was shown that cells immobilized on nylon fibers well sustained their growth accompanied by desulfurization activity during a series of repeated batch runs over extended period of time. Advantages of easy separation of biocatalyst from the treated fuels, high stability and long lifetime of the biocatalyst using immobilized cells were concluded. At present, themes of BDS using immobilized cells concentrates on selection and optimization of immobilization conditions, the biodesulfurization ability and lifetime of immobilized cells compared to the suspension cells. But, as far as we know, studies on DBT BDS dynamic behaviors by the immobilized cells have not been reported. The BDS reaction using immobilized cells of diesel oil must be accomplished in oil-water-immobilization three-phase system. To know more about the BDS process and enhance the BDS of diesel oil in the three-phase system, knowledge of the reaction mechanism of substrate dissolved in oil and the mass transfer of DBT and oxygen within the alginate gel beads is needed.

The objective of this study is firstly to immobilize the resting cells of *Gordonia* sp. WQ-01A in alginate gel beads, then to use the immobilized cells for batch DBT BDS under different experimental conditions in oil-water-immobilized resting cells system. A mathematical model is proposed for simulating such processes, which should take into account the internal and external mass transfer resistances of DBT and oxygen and the intrinsic kinetics of bacteria. The proposed model is to be validated by the experimental data of DBT concentration profiles in oil phase.

Experiments

Microorganism and culture conditions

Gordonia sp. WQ-01 was isolated and identified to metabolize DBT via sulfur-specific pathway (4S pathway) in our lab.[14] The strain used in this study was the mutant strain of *Gordonia* sp. WQ-01, named *Gordonia* sp. WQ-01A.

Liquid cultures were grown at 30 °C and the pH value of 7.0 in a rotary shaker at 200 rpm. A basic sulfur-free medium (BSM) contains: glucose 5.0 g, NH_4Cl 1.0 g, KH_2PO_4 0.5 g, $Na_2HPO_4 \cdot 12H_2O$ 6.3 g, $MgCl_2$ 0.2 g, $CaCl_2$ 0.04 g, $FeCl_3 \cdot 7H_2O$ 0.04 g, $MnCl_2 \cdot 4H_2O$ 0.008 g, $ZnCl_2$ 0.001 g, $CoCl_2 \cdot 6H_2O$ 0.004 g, $AlCl_3 \cdot 6H_2O$ 0.001 g, $CuCl_2 \cdot 2H_2O$ 0.001 g, H_3BO_3 0.001 g, $NaMoO_4 \cdot 2H_2O$ 0.001 g in 1 L distilled water. The relatively high concentrations of KH_2PO_4 and Na_2HPO_4 in the BSM provided a good pH buffering capacity. DBT was dissolved in ethanol (50 mmol L^{-1}) and supplemented to a sterilized BSM as the sole sulfur source.

Chemical reagents

DBT and 2-HBP were purchased from ACROS ORGANICS (New Jersey, USA). Methanol and ethyl acetate were of HPLC grade. All other chemicals were of analytical grade, commercially available, and used without further purification.

Resting cell suspension preparation

Resting cell suspension of the strain was used in all the studies. Cultivation of the strain was performed in 500 mL Erlenmeyer flask which contained 100 mL BSM supplemented with 0.15 mmol L^{-1} DBT as the sulfur source, at 30 °C with an agitation of 200 rpm. After 36 h cultivation, the culture was centrifuged at 10 000×g for 10 min at 4 °C, washed twice with 0.9 % sodium chloride solution and suspended in 0.9 % sodium chloride solution.

DBT-oxygen intrinsic BDS kinetics

Intrinsic BDS kinetic study for both DBT and oxygen limitations was conducted to regress the Monod constant for oxygen. It was carried out in a 7.5 L fermenter (BioFlo 110, New Brunswick Scientific Co., Inc.) by inoculating 3 L 0.9 % sodium chloride solution with suspension of resting cells of *Gordonia* sp. WQ-01A at the late exponential phase at 30 °C, initial pH of 7.0, agitation speed of 200 rpm and gas volumetric flow rate of 2 L min^{-1}. The initial DBT concentration was 0.005 mmol L^{-1}, which is lower than the saturation concentration of DBT in the water phase and the resting cell density was 0.44 g L^{-1} DCW. Samples were taken every ten minutes for the measurements of DBT concentration. The relative dissolved oxygen (DO) concentration (as a percentage) was measured by the DO electrode, and was calibrated by another absolute oxygen electrode (Mettler Toledo 6050, Switzerland).

Immobilization procedure

Sodium alginate at 4 % (w/v) was used for the immobilization of the resting cells of *Gordonia* sp. WQ-01A. After sterilization of the sodium alginate, resting cell suspension (4.4 g L^{-1} DCW) was added at a ratio of 1:1 (v/v) at room temperature and cooled to 4 °C. The alginate/cell mixture was aseptically extruded by an injector through a needle into a stirred solution of sterile 4 % calcium chloride (4 °C). The height of the needle and rate of stirring of the calcium chloride solution, about

20 cm and 150 rpm respectively, were adjusted to obtain uniform spherical gel beads. Beads were left to harden in the calcium chloride solution for more than 2 h for complete replacement of sodium ions by calcium ions, then were washed with 0.9 % sodium chloride solution and preserved in 0.9 % sodium chloride solution for the next experiment.

Batch DBT biodesulfurization in an oil-water-immobilization system in fermentor

The experimental validation of the proposed model was performed in the 7.5 L fermenter by inoculating 4 L 0.9 % sodium chloride solution with suspension of immobilized cells at 30 °C, initial pH of 7.0, agitation speed of 200 rpm and gas volumetric flow rate of 2 L min^{-1} and under following conditions: initial DBT concentrations of 0.5 mol L^{-1}, 1 mol L^{-1}, 5 mol L^{-1}; bead loadings of 20 %, 30 % and 40 %; the oil phase volume fraction of 10 %, 15 %, 20 %. The oil phase is *n*-dodecane.

Analytical procedures

Culture densities were determined by diluting the suspension with BSM medium and measuring the OD at 600 nm by a Beckman DU 650 spectrophotometer with a 1-cm-path-length cuvette. Dry cell weight (DCW) was determined by drying harvested cell paste to constant weight (DCW) in a CEM 9000 microwave oven with balance (CEM Corp.). A linear relationship between OD and dry cells weight was obtained: dry cell concentration (g L^{-1} DCW) $0.4798 \cdot \mathrm{OD} - 0.0811$ ($R^2 = 0.9939$).

The concentrations of DBT in the aqueous system were analyzed by high-performance liquid chromatography (HPLC) using a LabAlliance (model SeriesIII) system with a C18 column (250 × 4.6 mm, LabAlliance, USA). Elution was performed with 90 % (v/v) methanol/water at 1 mL min^{-1}, and detection was realized with a UV detector (Model 500, LabAlliance, USA) at 280 nm. Aliquots of the culture were acidified to pH 2.0 with 6 mol L^{-1} HCl and extracted with equal volumes of ethyl acetate, but tenfold volumes of ethyl acetate for the intrinsic kinetic study, then vibrated ultrasonically (HS3120 LabAlliance) for 5 minutes. A portion of the ethyl acetate layer was centrifuged at 8 000 × g for 5 min, and the supernatant was analyzed.

The concentrations of DBT in the oil phase were analyzed by gas chromatography (GC) using a DB-17 column (0.25 mm ID×30 m length; J & W Scientific, Folsom, CA, USA) and a flame-ionized detector. The temperature program was 150 °C for 1 min followed by a 10 °C min^{-1} ramp rate to a final temperature of 300 °C, with a final hold for 15 min. Samples were acidified to pH 2.0 with 6 mol L^{-1} HCl, followed by centrifugation at 8 000 × g for 5 min, and the supernatant was analyzed.

All experiments were repeated three times. The data shown in the corresponding figures in the Results and discussion section were the mean values of the experiments.

Model development

In the batch DBT BDS process, a certain volume of *n*-dodecane containing DBT is put into the fermentor, and the oil disperses in the aqueous phase and forms small droplets due to agitation. Cell-immobilized alginate gel beads are suspended in the main liquid phase (oil-water system). The substrate DBT is transferred from the oil droplets to the aqueous phase and then to the surface of the gel beads, whilst oxygen is transferred from the aqueous phase to the surface of the gel beads, then both DBT and oxygen are diffused into the gel beads where they are consumed by the cells, leading to the simultaneous diffusion and reaction of DBT and oxygen.

Based on the analysis of the actual reaction process, the following assumptions are made for the development of the mathematical model simulating the batch DBT BDS process using the resting cells of *Gordonia* sp. WQ-01A immobilized in alginate gel beads:

1) The oil phase disperses in the aqueous phase and exists in small droplets. The diameter of the oil droplets does not change as the DBT concentration decreases.

2) DBT is distributed mainly in the oil phase and the concentration of DBT in the water phase is very low. The water phase in the fermentor is completely mixed and the DBT concentration in the water phase is uniform.

3) The substrate DBT is transferred from within the oil droplets to the oil-water interface and then to the water phase, and transferred from the water phase to the water-solid interface and then to the alginate gel beads, where it is biologically consumed by the bacteria. There is no reaction in the oil phase and water phase. The influence of free cells in the aqueous phase on the batch DBT BDS is negligible.

4) The initial bacteria are distributed uniformly in the alginate gel beads.

5) All alginate gel beads are spherical with the same radius and remain constant during the fermentation.

6) The dissolved oxygen concentration in the aqueous phase is in saturation in the agitating system, and is transferred to the alginate gel beads where it is biologically consumed.

7) Substrate biodesulfurization is limited only by DBT and oxygen in the alginate gel beads while all other nutrients are in excess.

Thus, in view of the assumptions mentioned above, the mathematical model can be developed as follows:

Mass balance of DBT in the oil phase:

$$\frac{\partial c_{DBT,O}}{\partial t} = D_{DBT,O}\left(\frac{\partial^2 c_{DBT,O}}{\partial r^2} + \frac{2}{r}\frac{\partial c_{DBT,O}}{\partial r}\right) \quad (1)$$

where $D_{DBT,O}$ is the diffusion coefficient of DBT in oil phase.

Mass balance of DBT in the water phase, based on the two-film theory (Whitwman, 1923):

$$\frac{\mathrm{d}c_{DBT,W}}{\mathrm{d}t} = k_{DBT,OW}\frac{3}{R_{oil}}\frac{\varepsilon_O}{1-\varepsilon_O-\varepsilon_S}\cdot \cdot (c_{DBT,O}\big|_{r=R_{oil}} - m_{OW}\,c_{DBT,W}) - \quad (2)$$
$$- k_{DBT,WS}\frac{3}{R_S}\frac{\varepsilon_S}{1-\varepsilon_O-\varepsilon_S}(c_{DBT,W} - c_{DBT,S}\big|_{r=R_S})$$

where $k_{DBT,OW}$ is the oil-water mass transfer coefficient of DBT between oil and water phase; ε_O is the volume fraction of oil; $k_{DBT,WS}$ is the liquid-solid mass transfer coefficient of DBT from the aqueous phase to the surface of the cell-immobilized alginate gel beads; ε_S is the volume of cell-immobilized alginate gel beads per unit effective reaction volume (L L^{-1}); m_{OW} is the partition coefficient of DBT in *n*-dodecane and water, its value is 21 158.[14]

Mass balance of DBT in the gel beads:

$$\frac{\partial c_{DBT,S}}{\partial t} = D_{DBT,S}\left(\frac{\partial^2 c_{DBT,S}}{\partial r^2} + \frac{2}{r}\frac{\partial c_{DBT,S}}{\partial r}\right) - \gamma_{DBT} \quad (3)$$

where $D_{DBT,S}$ is the diffusion coefficient of DBT in cell-immobilized alginate gel beads; γ_{DBT} is the intrinsic DBT desulfurization rate in the alginate gel beads.

Mass balance of oxygen in the gel beads:

$$\frac{\partial c_{O_2,S}}{\partial t} = D_{O_2,S}\left(\frac{\partial^2 c_{O_2,S}}{\partial r^2} + \frac{2}{r}\frac{\partial c_{O_2,S}}{\partial r}\right) - \gamma_{O_2,S} \quad (4)$$

where $D_{O_2,S}$ is the diffusion coefficient of oxygen in cell-immobilized alginate gel beads; $\gamma_{O_2,S}$ is the intrinsic oxygen consumption rate in the alginate gel beads.

The initial conditions and boundary conditions are:

$$t = 0, \quad c_{DBT,O} = c^o_{DBT,O}, \quad c_{DBT,W} = 0, \quad c_{DBT,S} = 0, \quad c_{O_2,S} = 0 \quad (5)$$

the oil droplets:

$$r = 0, \quad \left.\frac{\partial c_{DBT,O}}{\partial r}\right|_{r=0} = 0 \quad (6)$$

$$r = R_{oil}, \quad D_{DBT,O}\left.\frac{\partial c_{DBT,O}}{\partial r}\right|_{r=R_{oil}} = k_{DBT,OW}(c_{DBT,W} - c_{DBT,O}\big|_{r=R_{oil}}) \quad (7)$$

the alginate gel beads:

$$r = 0, \quad \frac{\partial c_{DBT,S}}{\partial r} = 0, \quad \frac{\partial c_{O_2,S}}{\partial r} = 0 \quad (8)$$

$$r = R_S, \quad D_{DBT,S}\left.\frac{\partial c_{DBT,S}}{\partial r}\right|_{r=R_S} = k_{DBT,WS}(c_{DBT,W} - c_{DBT,S}\big|_{r=R_S})$$
$$D_{O_2,S}\left.\frac{\partial c_{O_2,S}}{\partial r}\right|_{r=R_S} = k_{O_2,WS}(c^*_{O_2,W} - c_{O_2,S}\big|_{r=R_S}) \quad (9)$$

where $k_{O_2,WS}$ is the liquid-solid mass transfer coefficient of oxygen from the aqueous phase to the surface of the cell-immobilized alginate gel beads; $c^*_{O_2,W}$ is the saturation concentration of oxygen in the water phase, which has a value of 0.236 mmol L^{-1} at 30 °C and normal air pressure.[15] The free cells density in the alginate gel beads was equal to 2.2 g L^{-1} DCW.

The mass balance equations together with their initial and boundary conditions are solved by the finite element analysis method using the software package of Femlab 3.1a and simulated with a 2-D coefficient form using the weak solution with a time step length of 0.01 h and a relative tolerance of $1 \cdot 10^{-4}$ for the calculation of DBT.

Model parameters

Intrinsic kinetic parameters

Based on the experimental data of DBT biodesulfurization by resting cells in pure water phase, the DBT biodesulfurization rate is calculated as:

$$-\frac{\mathrm{d}c_{DBT,S}}{\mathrm{d}t} = \gamma_{DBT} = \mu c_{cell} \quad (10)$$

As the concentration of DBT in the water phase is very low, the effects of the substrate inhibition and product inhibition are negligible. It is assumed that the cells possess a Monod-type response to DBT and the dissolved oxygen concentration. Therefore, the specific growth rate as a function of the concentrations of DBT and oxygen is:

$$\mu = \frac{\mu_{\max} c_{DBT,S}}{K_{DBT} + c_{DBT,S}} \cdot \frac{c_{O_2,S}}{c_{O_2,S} + K_{O_2}} \qquad (11)$$

where $\mu_{\max}$ is the maximum specific growth rate; K_{DBT} is the half-saturation constant for DBT; K_{O_2} is the Monod constant for oxygen.

The kinetic parameters $\mu_{\max}$ and K_{DBT} had been regressed by the experimental data as: $\mu_{\max} = 225$ nm (DBT)/(g min^{-1} DCW), $K_{DBT} = 226.2$ nm L^{-1} (DBT).[14] In our paper, the dissolve oxygen was taken into account in the intrinsic kinetic study, and K_{O_2} was regressed to be 6.961 mmol L^{-1} by the experimental data.

The biodesulfurization reaction in the alginate gel beads consumes DBT and oxygen via the sulfur-specific pathway (4s pathway).[16] Biodesulfurization of one mol of DBT consumes three mols of oxygen. That is to say, the ratio of the reaction rate of oxygen and DBT is 1:3. So the oxygen consumption rate $\gamma_{O_2} = 3\gamma_{DBT}$.

Diffusion coefficients

The diffusion coefficients of DBT in oil phase $D_{DBT,O}$ and in water phase $D_{DBT,W}$ are estimated using the Wilke-Chang equation:[17]

$$D_{DBT,O} = 0.74 \cdot 10^{-14} T \frac{(\varphi_O M_O)^{0.5}}{\eta_O v_O^{0.6}} \qquad (12)$$

$$D_{DBT,W} = 0.74 \cdot 10^{-14} T \frac{(\varphi_W M_W)^{0.5}}{\eta_W v_W^{0.6}} \qquad (13)$$

Diffusion coefficient (D_e) in pure alginate gel decreased to about 90 % of that in water and did not vary with the change of alginate concentration, but D_e decreased considerably with increasing cell concentration.[18] An equation type of Mackie and Meares[19] was proposed to predict the influence of cells on diffusion coefficient in alginate gel beads. It was used in our study to estimate the diffusion coefficients in alginate gel beads of DBT and oxygen:

$$D_{DBT,S} = D_{DBT,W} \cdot \frac{(1-\phi)^3}{(1+\phi)^2} \qquad (14)$$

$$D_{O_2,S} = D_{O_2,W} \cdot \frac{(1-\phi)^3}{(1+\phi)^2} \qquad (15)$$

where $\phi = \frac{\varepsilon_S}{1-\phi_c}$, and ϕ_c is the cell volume fraction. The diffusion coefficient of oxygen in water phase $D_{O_2,W} = 9.14 \cdot 10^{-6}$ m^2 h^{-1} $= 2.539 \cdot 10^{-9}$ m^2 s^{-1}.[20]

Mass transfer coefficients

The liquid-liquid overall mass transfer coefficient of DBT from the oil droplets to the surface of the water phase can be calculated according to the following functions:[14]

$$k_{DBT,OW} = \frac{1}{k_{DBT,O}} + \frac{m_{OW}}{k_{DBT,W}} \qquad (16)$$

where

$$k_{DBT,W} = \frac{2D_{DBT,W}}{d_{p,O}} + 0.31\left(\frac{\eta_{DBT,W}}{\rho_{DBT,W} D_{DBT,W}}\right)^{-\frac{2}{3}}\left(\frac{\Delta\rho\eta_{DBT,W} g}{\rho^2_{DBT,W}}\right)^{\frac{1}{3}} \qquad (17)$$

$$k_{DBT,O} = \frac{Sh_{DBT,O} D_{DBT,O}}{X} \qquad (18)$$

where $Sh_{DBT,O}$ is the Sherwood number, the characteristic length X of the oil droplet is assumed to be equal to $d_{p,O}$. $\Delta\rho$ here is the density difference between the oil phase and the water phase.

The liquid-solid mass transfer coefficients of DBT and oxygen from the water phase to the surface of the cell-immobilized alginate gel beads are calculated using the following correlations:[21]

$$k_{DBT,WS} = \frac{2D_{DBT,W}}{d_{p,S}} + 0.31\left(\frac{\eta_{DBT,W}}{\rho_{DBT,W} D_{DBT,W}}\right)^{-\frac{2}{3}}\left(\frac{\Delta\rho\eta_{DBT,W} g}{\rho^2_{DBT,W}}\right)^{\frac{1}{3}} \qquad (19)$$

$$k_{O_2,WS} = \frac{2D_{O_2,W}}{d_{p,S}} + 0.31\left(\frac{\eta_{O_2,W}}{\rho_{O_2,W} D_{O_2,W}}\right)^{-\frac{2}{3}}\left(\frac{\Delta\rho\eta_W g}{\rho^2_{O_2,W}}\right)^{\frac{1}{3}} \qquad (20)$$

where $\Delta\rho$ here is the density difference between the water phase and the solid phase.

The average oil droplet size

When two immiscible liquids (such as water and *n*-dodecane in our experiment) are placed in a shearing flow such as in an agitated system, a dispersion is formed. The average oil droplet size is the result of an equilibrium being established between drop breakup and coalescence and the function of the physical properties of the immiscible liquids, the degree of agitation and the stirred Bessel geometry.[22] Jia *et al.*[14] considered that coalescence did not dominate over droplet breakup when ε_o was no larger than 25 % (v/v) and used the following correlation to determine the average oil droplet size $d_{p,o}$ in the water-oil system:

$$\frac{d_{p,o}}{D_H} = c_1(1 + c_2\varepsilon_o)(We)^{-\frac{3}{5}} \qquad (21)$$

where $c_1 = 0.047$ and $c_2 = 2.5$ determined by van Heuven and Beek (1971). D_H is the impeller diameter. Moreover, the Weber number expresses the ratio of disruptive forces and the interfacial tension forces.[23]

$$We = \frac{\rho_L N^2 D_H^3}{\sigma_{OW}} \qquad (22)$$

The interfacial tension σ_{OW} in the denominator counteracts the disruptive forces in the numerator. The oil droplet diameter is also affected by the volume fraction of oil ε_o, since higher coalescence rates occur at larger volume fractions of oil.

However, the system studied in this paper is the water-oil system with the presence of alginate gel beads. The beads may have an influence on the oil drop size. However, it was reported that if the oil phase has a low affinity to the solid particles and thus stays predominatly in the water phase, forming droplets that are subjected to hydrodynamic forces which cause distortion and breakup of the droplets.[22] In our research, the density of alginate gel beads is 1048 kg m^{-3}, a little larger than water. It can be observed that the oil phase has hardly affinity for the gel beads, so the effect of the presence of alginate gel beads on the average oil droplet size is negligible in this study. In addition, the oil fraction ε_o in this paper was smaller than 25 % (v/v). So the oil drop size was calculated by the above correlation, and the overall density of the whole system ρ_L depends on the volume fraction of the oil phase ε_o and the bead loading of the immobilized cells ε_S and is a linear combination of the densities of the oil, aqueous phase and alginate gel beads. It can be calculated as:[24]

$$\rho_L = (1 - \varepsilon_O - \varepsilon_S)\rho_W + \varepsilon_O\rho_O + \varepsilon_S\rho_S. \qquad (23)$$

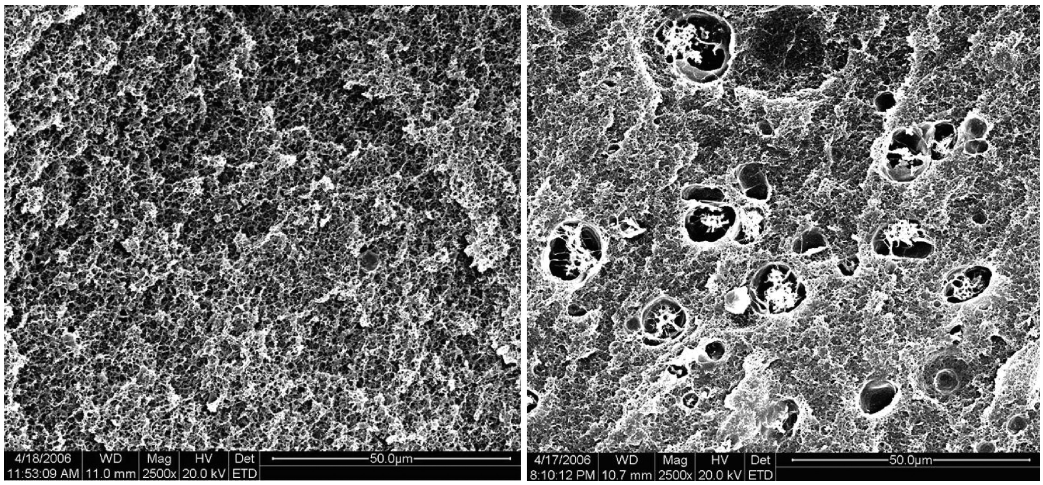
Results and discussion

Microstructure of the alginate gel beads by SEM

Fig. 1 is the SEM photograph of showing that the alginate gel beads were spherical with an average diameter of 2.56 mm. Y. G. Li[25] reported that the desulfurization of DBT by alginate immobilized cells was affected by the size of the immobilized beads. Uniform alginate gel beads were obtained for studying. The SEM photograph also shows that there was a lamella on the surface of beads to prevent cells from leaking out and resist the mass transfer rates of DBT and oxygen. That is to say, that the proposed model should take into account the internal and external mass transfer resistances and the modeling assumption of neglecting the influence of free cells in the aqueous was rational.

Fig. 1 – *SEM photograph of the surface of the alginate gel beads (85×)*

In order to understand cells distribution in the inner alginate gel beads, the alginate gel beads with and without cells entrapped were observed by SEM. The results are shown in Fig. 2. The photographs of the inner of the alginate gel beads with and without cells entrapped are shown in the right side and in the left side respectively. Tiny holes and the netlike configuration could be observed in the beads. From the photograph, it could be observed that agglomerate cells entrapped distribute well in the gel beads. The netlike configuration of the inner alginate gel beads could allow the substrate and oxygen to diffuse in the gel beads and make agglomerate cells immobility as a result of maintaining their distribution to prevent cells from leaking out.



F i g . 2 – *SEM photograph of the inner of the alginate gel beads with and without cells immobilized*

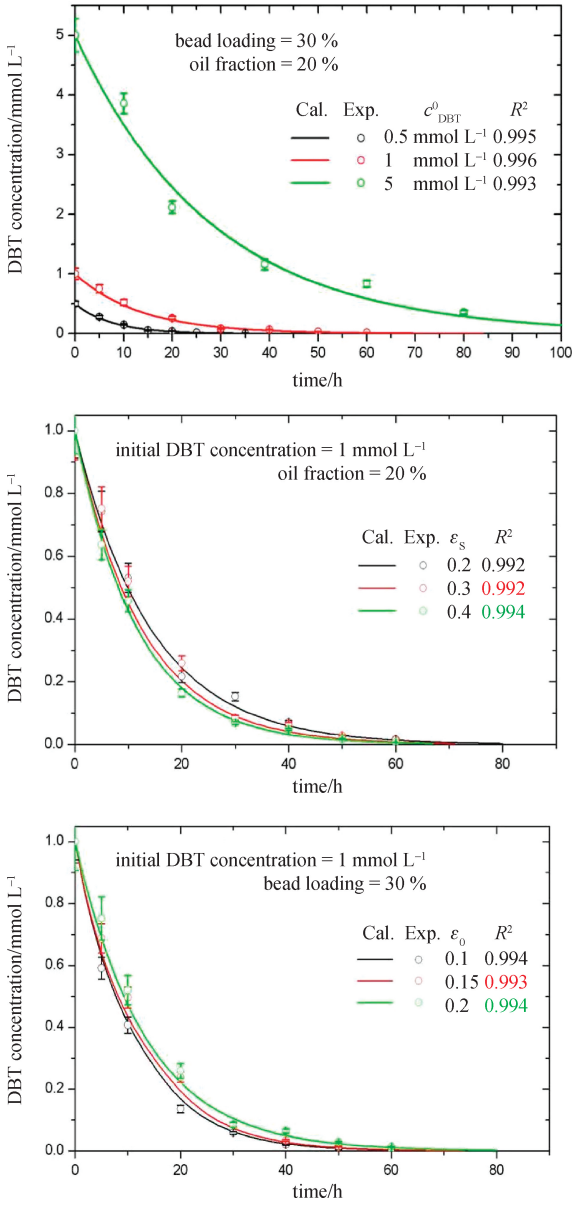
DBT biodesulfurization in oil-water-immobilization system at different operating conditions

N-dodecane dissolving in 0.5, 1 and 5 mmol L^{-1} DBT was prepared and then mixed with the 0.9 % sodium chloride solution suspending immobilized cells. For investigation of the effects of bead loading and the oil phase volume fraction on the biodesulfurization process, the alginate gel bead loadings of 20 %, 30 %, 40 % and the oil phase volume fractions of 10 %, 15 %, 20 % were used. The reactions were carried out in the 7.5 L fermentor at 30 °C with the agitation speed of 200 rpm. Samples were withdrawn at defined time intervals and analyzed for the concentrations of DBT in the oil phase.

The calculated results by the proposed model were the instantaneous distribution of DBT concentrations along the radius in the oil droplets. To compare the simulated results with the experimental data of batch DBT biodesulfurization, the average DBT concentration within the oil phase was calculated as:

$$\overline{c_{DBT,O}} = \frac{\int_0^{R_{oil}} 4\pi r^2 c_{DBT,O}\,\mathrm{d}r}{\frac{4}{3}\pi R_{oil}^3}$$

Then the model simulations of the DBT biodesulfurization behaviors by immobilized cells are provided in Fig. 3, which showed the comparisons of simulated results and experimental data of DBT concentration profiles along time course in the oil phase. From the figure, it is noted that DBT biodesulfurization experiences longer time course at higher initial DBT concentration or lower bead loading (corresponding to lower initial cell concentration) or higher oil fraction (corresponding to



F i g . 3 – *DBT biodesulfurization in oil-water-immobilization system at operating conditions*

larger DBT quantity). For each desulfurization process, the desulfurization rates of the first several hours were much higher than those of the last several hours, which may be the result of the larger substrates concentration differences between the oil phase and the water phase, corresponding to larger driving forces. When the initial DBT concentration is 0.5, 1 and 5 mmol L^{-1}, the DBT concentration dropped almost to zero after 40 hours, 60 hours and 100 hours, respectively. The influence of bead loading and the oil phase volume fraction was small to the DBT biodesulfurization.

The good agreements between the model simulated results and the experimental measured data validated the proposed model ($R^2 \geq 0.992$). And the low relative standard deviations (within ±3 %) ensure the reliability of the experimental data. The DBT concentration decreased incessantly as the simulated curve showed. Klein *et al.*[26] reported that the mass transfer resistance problem normally associated with immobilized cell culture. The mass transfer occurred indeed, but the results confirmed that the rate-limiting step in the oil-water-immobilization system is not mass transfer resistance but bio-conversion.

To understand the dynamic behaviors of the immobilized system, the variations of the concentrations of DBT and oxygen in the alginate gel beads are analyzed by the proposed model.

Fig. 4 illustrates the model predictions of the time course of concentration changes of DBT and oxygen within the alginate gel beads at different initial DBT concentrations of 0.5, 1, 5 mmol L^{-1}, at the fixed bead loading of 30 % and oil fraction of 20 % and radial position of $0.1 \cdot 10^{-3}$ m. The alginate gel beads were assumed free of DBT and oxygen when they were first introduced into the system.

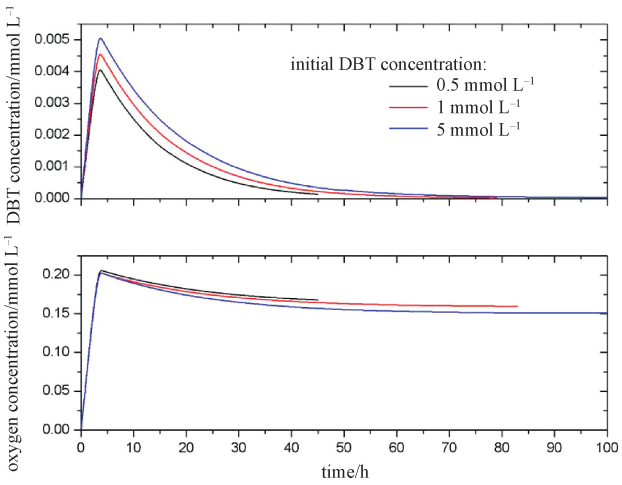


Fig. 4 – *Time course of DBT and oxygen concentrations within the alginate gel bead at different initial DBT concentrations and under fixed bead loading of 30 %, oil fraction of 20 % and radial position of* $1 \cdot 10^{-3}$ *m*

From the Fig. 4, it could be noted that the concentration of DBT and oxygen increase to a plateau and then reduce inside the gel beads. The DBT and oxygen conversion reactions and diffusion of DBT and oxygen inside the gel beads were simultaneous. Initially, the diffusion rate was larger than the conversion rate for higher concentration difference, which resulted in the increasing of the DBT concentration. As the DBT buildup and the process proceeding, the DBT diffusion rate decreased, leading to the reduction of DBT and oxygen concentrations. After about 40 hours, the DBT concentration dropped almost to zero when the initial DBT concentration is 0.5 mmol L^{-1}, while it taked 60 hours and 80 hours for the initial DBT concentration of 1 and 5 mmol L^{-1}, respectively. These simulated results accorded with the experimental results shown in the Fig. 3, which validated the proposed model again.

Differing with DBT, the oxygen concentration dropped very slowly. In order to validate the rationality of the modeling assumption that the dissolved oxygen concentration in the aqueous phase was in saturation in the agitating system, the relative dissolved oxygen (DO) concentration (as a percentage) was measured by the DO electrode and calibrated by another absolute oxygen electrode, so the absolute oxygen concentration profile during the BDS process was got. The results indicated that the dissolved oxygen concentration in the aqueous phase was steady and almost equal to the saturation concentration of 0.236 mmol L^{-1} and the assumption accords with the fact. The saturation concentration of oxygen in the water phase was much higher than that of DBT (0.0057 mmol L^{-1}), so even the reaction speed of oxygen is threefold of that of DBT, the oxygen concentration changed very small with the reduction of DBT concentration. It also could be seen from Fig. 4 that DBT concentrations are higher at any time point for higher initial DBT concentration because higher concentration difference results in quicker diffusion, while the case of oxygen is opposite because of more consumption of oxygen for higher DBT concentration. It could be concluded from this figure that the oxygen concentration is not an important factor affecting the DBT biodesulfurization in the immobilized system.

Conclusion

Hou *et al.*[10] reported that the maximal desulfurization rate of immobilized cells was lower than that of non-immobilized cells. The main advantages of immobilized cells could be repeated and convenient operations. As with most immobilization systems, the diffusion rate of substrates and products

within the bead often limits productivity. The mathematical model validated by the model simulations and the experimental measurements of DBT concentration profiles in the oil phase took into account the internal and external mass transfer resistances of DBT and oxygen and the intrinsic kinetics of bacteria. According to the results, we can conclude: (1) The rate-limiting step in the oil-water-immobilization system is not mass transfer resistance but bio-conversion. (2) Compared to the effect of DBT, oxygen concentration is not an important factor affecting the DBT biodesulfurization in the immobilized system. This work makes us understand the dynamic behaviors of the immobilized system more and may be generally applicable to other area of biocatalytic and biotransformation processes.

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Nomenclature

c – concentration, mmol L^{-1}
c_1, c_2 – constants, dimensionless
D – diffusion coefficient, $m^2\ h^{-1}$
D_H – impeller diameter, m/0.059
d_p – diameter of the alginate gel beads, m/2.56·10^{-4}
$d_{p,O}$ – diameter of the oil droplet, m
g – acceleration of gravity, m s^{-2}/9.81
M – molecular weight, g mol^{-1}
m – partition coefficient, dimensionless
N – stirrer speed, s^{-1}/4.167
k – mass transfer coefficient,m s^{-2}
K_{DBT} – half-saturation constant for DBT, nmol L^{-1} (DBT)
K_{O_2} – Monod constant for oxygen, mmol L^{-1}
R_{oil} – radius of the oil droplet, m
R_S – radius of the alginate gel beads, m
r – radial distance coordinate, m
t – time, h
Sh – Sherwood number, dimensionless
We – Weber number, dimensionless
X – characteristic length, m

Greek letters

γ – rate of reaction, mmol $L^{-1}\ h^{-1}$
η – viscosity, Pa s
η_W – water viscosity, Pa s
ε_S – bead loading, L L^{-1}
μ – specific desulfurization rate, nmol DBT g^{-1} DCW min^{-1}
μ_{max} – maximum specific desulfurization rate, nmol DBT g^{-1} DCW min^{-1}
ρ – density, kg m^{-3}
ρ_L – density of the whole system, kg m^{-3}
φ – associate parameter
ϕ – dimensionless
ϕ_c – cell volume fraction

Superscripts

* – saturation state
0 – initial condition

Abbreviations

DBT – Dibenzothiophene
O_2 – oxygen
O – the oil phase
OW – oil-water system
WS – water-gel beads system
W – the water phase
S – alginate gel beads

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