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KINETIC ANALYSIS FOR BIODESULFURIZATION OF DIBENZOTHIOPHENE USING *R. rhodochrous* ADSORBED ON SILICA

ANALIZA KINETYKI BIODESULFURYZACJI DIBENZOTIOFENU PRZY WYKORZYSTANIU JAKO ADSORBENTU *R. rhodochrous* NA KRZEMIONCE

Abstract: Experimental biodesulfurization (BDS) data for dibenzothiophene (DBT) (1.0-7.0 mM) with *Rhodococcus rhodochorus* immobilized by adsorption on silica, were adjusted with liquid-film kinetic model (Fisher coefficient, $F = 592.74$ and probability value $p << 0.05$ and $r^2 = 0.97$). Simulations predict the presence of considerable amounts of DBT surrounding the particles, which would be available for the cells adsorbed on the surface of silica. The greatest percentage removal (50 %) was obtained for adsorbed cell system over the suspended bacterial cells (30 %), showing that sulfur substrates are more bioavailable when the bacterial cells are adsorbed on silica. The liquid-film modelling with diffusional effects provides proper theoretical basis to explain the BDS performance obtained using adsorbed cells.

Keywords: adsorption, biodesulfurization, immobilization, kinetic analysis, *Rhodoccocus rhodochrous*

Introduction

Biodesulfurization (BDS) emerged as an environmentally friendly technology able to reduce contamination from fossil fuels, under mild pressures and temperatures [1]. Despite numerous studies of this methodology [2-6], the process still presents several unresolved difficulties, among them the high cost of biocatalysis, reactor design, separation of aqueous-organic phases and biocatalytic stability and lifetime [7, 8]. However, the main disadvantage of BDS process is the limited access of the microorganisms to the organic substrates, due to the low bioavailability of sulfur compounds in the bacterial aqueous

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medium [9]. In this context, immobilized bacterial cells [10-14] and the use of surfactants [15-17] constitute an improvement in the field of desulfurization. BDS operation with microbial cells immobilized either, onto the surface of adsorbent material [13, 14] or entrapped into a polymeric matrix [12, 18] has gained special interest during the last decade. In this context, several investigations have been conducted to study different materials and conditions of immobilization [19-25]. To respect we investigated [26] the influence of inorganic supports in the BDS activity of gas oil using a batch systems and a bioreactor packed [27]. However, traditional kinetic studies of BDS reactions have focused on suspended bacterial cells systems [28, 29], and few works have informed about BDS kinetics using immobilized bacterial catalysis entrapped into the polymeric matrix [30, 31]. To our knowledge there are not studies of kinetic analysis of systems involving cells immobilized by adsorption on inorganic supports. This work proposes to apply basic kinetic analysis as theoretical tool in the assessment of biodesulfurization (BDS) of dibenzothiophene (DBT) using bacterial cells adsorbed on silica (Si) and suspended bacterial cells.

Materials and methods

Bacterial strain, sulfur-containing organic molecules and material support

The bacterial strain *Rhodococcus rhodochrous* IGTS8 (ATCC 53968) was grown in sulfur-free Medium A contains 0.5 g KH_2PO_4, 4.0 g K_2HPO_4, 1.0 g NH_4Cl, 0.01 g NaCl, 0.02 g $CaCl_2$, 0.2 g $MgCl_2 \cdot 6H_2O$, $5 \cdot 10^{-3}$ g $ZnCl_2$, $5 \cdot 10^{-3}$ g $MnCl_2 \cdot 4H_2O$, $1 \cdot 10^{-3}$ g $NaMoO_4 \cdot 4H_2O$, $0.5 \cdot 10^{-4}$ g $CuCl_2$, $5 \cdot 10^{-4}$ g Na_2WO_4 and $5 \cdot 10^{-3}$ g $FeCl_2 \cdot 4H_2O$ in 1 dm^3 of deionized water, supplemented with sodium succinate (30 mM) and citrate (0.1 % w/v) as energy and carbon sources, respectively. DBT (0.1 mM) (Merck) dissolved in isooctane (IOA) was used as the only sulfur source. The culture was grown at 30 ºC in a rotary shaker at 250 rpm. Silica (Si) D11-10 BASF, with 80 m^2g^{-1} of specific area, was used as an inorganic support and was sieved through a mesh to obtain particle sizes ranging from 3.4-5.6 mm (3½-6 mesh).

Cell immobilization by adsorption

Cells were collected by centrifugation at 4000 g and 4 ºC for 30 min. The obtained pellets were suspended in $1 \cdot 10^{-2}$ dm^3 of saline solution (0.85 % w/v NaCl). The numbers of immobilized cells were adjusted by measuring the turbidity at 600 nm (OD_{600}) with a Thermo spectrophotometer model Genesys 10S UV-VIS. One unit of optical density was equal to $2.82 \cdot 10^8$ cells [4]. The bacterial suspended were combined with 0.1 g of Si and incubated at 30 ºC in a rotary shaker at 200 rpm for 24 h.

Batch experiments

$1 \cdot 10^{-3}$ dm^3 of various solutions of DBT in IOA (1.0-7.0 mM) was placed in $2.5 \cdot 10^{-2}$ dm^3 flasks containing $1 \cdot 10^{-2}$ dm^3 of Medium A with suspended or adsorbed bacterial cells. The reaction was carried out at 30 ºC in a rotary shaker at 200 rpm. For each initial DBT concentration the bacterial cells and residual DBT concentration were measured. The cultures were centrifuged and turbidity was measured at 600 nm (OD_{600}). The DBT was extracted with ethyl acetate and analyzed by gas chromatography using a Shimadzu GC-2010 equipped with an SPB-5 capillary column (length 30 m, inner diameter 0.25 mm, film thickness 0.25 mm) and a FID detector.

Statistical analysis

Experimental data were analyzed by ANOVA and Fisher LSD tests. Each experiment was repeated at least three times.

Results and discussion

Kinetic analysis of suspended bacterial cells

To analyze the BDS pattern in a suspended bacterial cells system, the initial rate of substrate consumption was determined by computing the slope of the DBT concentration versus time along the linear range of variation. The values for this initial rate were plotted against initial DBT concentration (Fig. 1) and showed a linear dependence with a first order constant of $2.85 \cdot 10^{-11}$ h^{-1} $cells^{-1}$.

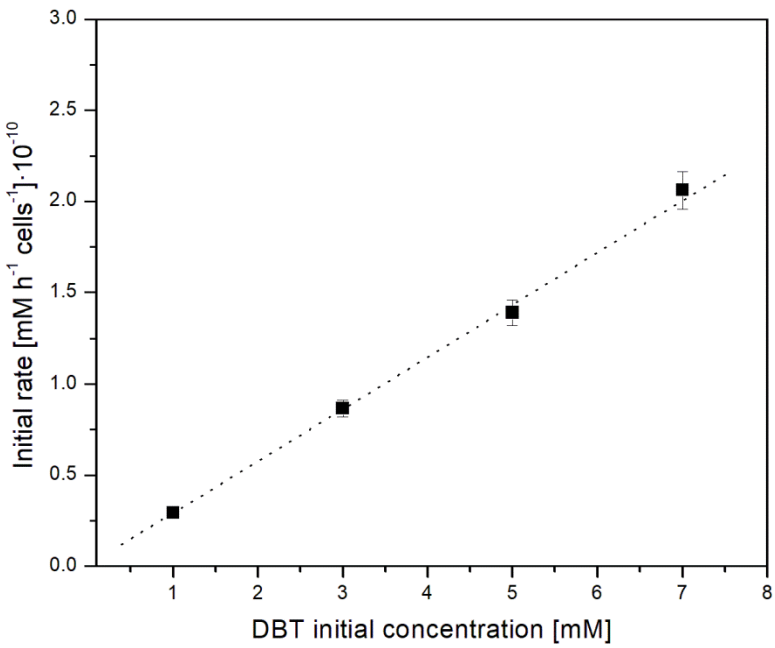

Fig. 1. Initial rate versus initial DBT concentration in suspended bacterial cells system. Data are mean values of three independent assays. Error bars show the standard deviation

The dependence observed is attributed to the proportional increasing of the DBT bioavailability for the cells adhere to the oil-water interface, which may be able to obtain DBT by "drinks from the oil" directly [1]. No growth was observed during the BDS experiments in the suspended bacterial cells system, therefore the removal of DBT was carried out with constant biocatalyst amounts (concentration ranging from $1.1 \cdot 10^{11}$ to $1.5 \cdot 10^{11}$ cells dm^{-3}). Figure 2 show the data for BDS assays with different initial DBT concentrations. Cellular activity proceeded in a manner reasonably predicted by first order kinetics, reaching 30 % sulfur removal up to 9 h. This result was unexpected since authors have reported kinetic data in the context of BDS process, however, previous reports [32, 33]

used Michaelis-Menten kinetics for BDS data fitting purposes, rather than obtaining it experimentally. In contrast, the present work reported the kinetic analysis of *R. rhodochorus* on DBT as the result of the application of a specific methodological procedure.

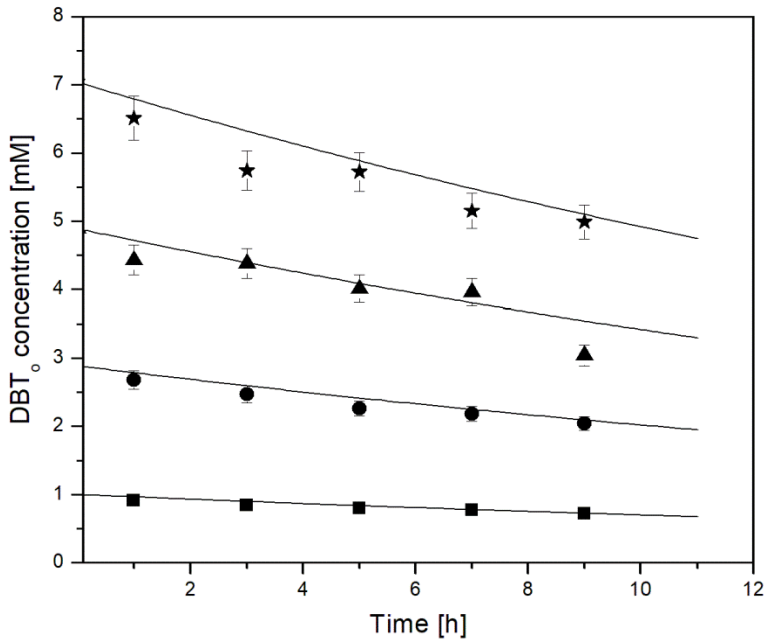

Fig. 2. Comparison of variation in DBT concentration with reaction time for BDS reaction experiments, in suspended bacterial cells system with different initial DBT concentrations in bulk liquid (DBT_o) measured (A = 1 mM (■), B = 3 mM (●), C = 5 mM (▲) and D = 7 mM (★)) and the results of first order modeling (—). Data are mean values of three independent assays. Error bars show the standard deviation

Kinetics of BDS using adsorbed-immobilized cells

In the BDS process using adsorbed cells, solid catalytic particles dispersed in the reaction medium provide the surface on which the biotransformation occurs. The mass transfer of DBT across the static liquid-film surrounding the particles is also carried out at this bioactive liquid-solid interface. To take the DBT mass transfer into account, the kinetics were analyzed using a simple general model of biocatalytic-particles under external diffusion effects [34]. Under these conditions, the BDS process was carried out according to:

$$-\frac{DBT_o}{dt} = h \cdot (DBT_o - DBT) \tag{1}$$

where h - mass transfer coefficient of DBT through the liquid-solid interface [$dm^3 \cdot h^{-1}$]. The subscripts are references to the presence of DBT in bulk liquid (o) or adsorbed on the particle surface (s). Assuming that substrate flux is balanced with its reactive conversion, DBT mass balance at the reactive solid surface is:

$$r = J = v \tag{2}$$

where: r - velocity of DBT transformation; J - mass flux of DBT; v - microbial kinetics. If it is assumed that immobilization on the particle surface does not modify the first-order kinetic behavior of the adsorbed bacterial cells, but any change in reactivity could affect the first order constant, then DBT mass balance can be used to predict DBT_S according to:

$$DBT_S = \frac{h \cdot DBT_o}{h + k_{im}} \tag{3}$$

where k_{im} - the first order kinetic constant of adsorbed cells. Equations (1) and (3) were solved simultaneously for DBT_o and DBT_S, leaving h and k_{im} as parameters of the system estimated by least squares data fitting. BDS assays using adsorbed *R. rhodochorus* and different initial DBT concentrations, and the corresponding modelling predictions are presented in Figure 3.

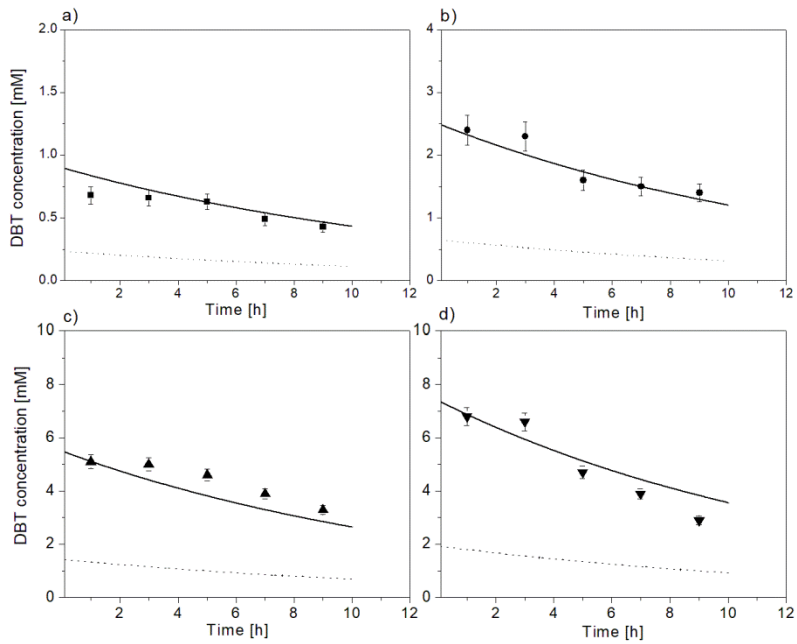

Fig. 3. Comparison of variation in DBT concentration with reaction time for BDS reaction experiments, in adsorbed bacterial cells system with different initial DBT concentrations in bulk liquid (DBT_o) (1 mM (■), 3 mM (●), 5 mM (▲) and 7 mM (★) and modelling predictions (—). The dashed lines (····) correspond to DBT concentration in the film surrounding the particle surface (DBT_S). Data are mean values of three independent assays. Error bars show the standard deviation

The adjustment of the model to the experimental data is shown in Figure 4 (r^2 = 0.97), resulting in significant statistical results: Fisher coefficient, F = 592.74 and probability value $p << 0.05$. Simulations predict the presence of considerable amounts of DBT in the static-fluid film surrounding of Si particles, which would be available for the adsorbed cells on the surface of Si. DBT mass transfer would therefore not be the rate-limiting step in the

BDS process, confirming the general assumption that has been used in modeling the BDS process with the bed-bioreactor [35].

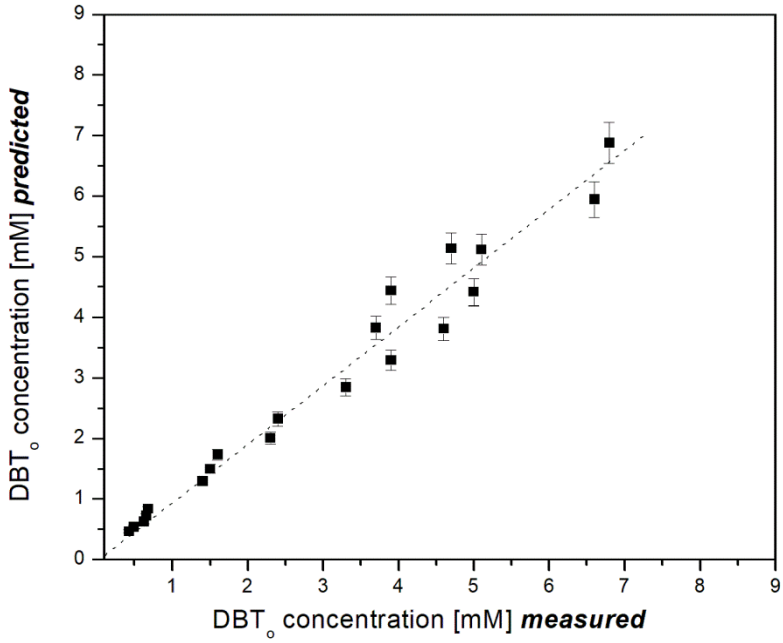

Fig. 4. Adjustment of DBT_o measured concentration with predicted data for the adsorbed bacterial cell system. Error bars correspond to the standard deviation

Calculations based on the amount of cells in contact with Si particles, indicate that $1.11 \cdot 10^9$ cells were retained on the Si surface, which is 15 % lower than the bacterial cells present in the BDS assay with suspended bacterial cells. This level of cell load was chosen because it is close to the limit that can be inmmobilized on Si through the electrostatic and non-electrostatic interactions involved in adsorptive bacterial attachment [26].

The DBT removal was ~50 % at 9 h, showing that the biocatalyst with adsorbed *R. rhodochorus* increased the BDS activity in comparison with the suspended bacterial cells system (Fig. 2). Likewise, the value of the first order kinetic constant was $3.90 \cdot 10^{-10}$ h^{-1} $cells^{-1}$, which is one order of magnitude greater than that obtained with suspended bacterial cells, and DBT mass transfer coefficient was 0.075 dm^3 h^{-1}. This behavior can be explained by the increased interaction of the biomodified support with the organic phase, improving the bioavailability of the sulfur substrates and therefore increasing the BDS activity of DBT, as it was observed in our previous reports [26, 36].

Conclusions

R. rhodochrous adsorbed on silica particles improves the BDS of DBT compared with suspended bacterial cells performance. Liquid film model showed satisfactory fit to the experimental data. Also, the modeling revealed the factors that can explain the better BDS results obtained with adsorbed cells such as the significant DBT amounts surrounding the

catalytic particles that increase the bioavailability of sulfur compounds.

Acknowledgments

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