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Biodesulfurization of gas oil using inorganic supports biomodified with metabolically active cells immobilized by adsorption

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ABSTRACT

The immobilization of *Pseudomonas stutzeri* using adsorption on different inorganic supports was studied in relation to the number of adsorbed cells, metabolic activity and biodesulfurization (BDS). The electrophoretic migration (EM) measurements and Tetrazolioum (TTC) method were used to evaluate adsorption and metabolic activity. Results indicate that maximal immobilization was obtained with an initial load of 14×10^8 cells mL^{-1} for Al and Sep, whereas Ti requires 20×10^8 cells mL^{-1}. The highest interaction was observed in the *P. stutzeri*/Si and *P. stutzeri*/Sep biocatalysts. The IEP values and metabolic activities indicate that *P. stutzeri* change the surface of supports and maintains metabolic activity. A direct relation between BDS activity and the adsorption capacity of the bacterial cells was observed at the adsorption/desorption equilibrium level. The biomodification of inorganic supports by the adsorption process increases the bioavailability of sulphur substrates for bacterial cells, improving BDS activity.

1. Introduction

Depletion of continental crude oil deposits has forced the exploitation of deeper reservoirs containing petroleum rich in polynuclear aromatic sulphur heterocyclic compounds (Pemex, 2008). The higher sulphur levels in fossil fuels obtained from these sources produce negative environmental impacts when these molecules break down. This makes it necessary to develop new technologies that reduce these pollutants (Fujikawa et al., 2006). The most widely used technology to remove sulphur from crude operates is a chemical process called hydrodesulfurization (HDS) (Gates et al., 1979). In spite the effectiveness of HDS, the operation is a high-energy consumer (Song, 2003). In this context, Biodesulfurization (BDS), a biocatalytic process performed by microorganisms that selectively remove sulphur from hydrocarbon fractions, is an interesting alternative (Kodama et al., 1973). BDS is considered an environmentally friendly process because of the mild operative conditions (pressure and temperature) (Setti et al., 1997) and not require the application of additional technologies to remove toxic molecules generated during the reaction (Le Borgne and Quintero, 2003).

Microbial BDS has been mainly studied in terms of microbiological characterization of bacterial strains, catabolic pathways, and genes involved in sulphur remotion (Castorena et al., 2002; Li W. et al., 2005; Kilbane, 2006; Soleimani et al., 2007; Chen et al., 2008; Li J. et al., 2009; Shavandi et al., 2009). Nevertheless, physical interactions between bacterial cells and sulphured substrates require further studies for to upscale BDS (Yang et al., 2007), in order to resolve problems associated with the limited access of microorganism to organic substrates (Tao et al., 2006). In this context, surfactants and immobilized cells are considered promising solutions to the problem of low solubility.

Biomodification of inorganic supports using cells immobilization increases the interaction between reactants present in two-phase systems, thus avoiding the need to use expensive surfactants (Feng et al., 2006). Bacterial immobilization by adsorption is an improvement over the cell entrapment method that reduces mass transference and the steric effect (Shan et al., 2005; Hou et al., 2005). Bacterial cell adsorption involves the use of inorganic compounds as ideal biosupports. These materials must have controlled porosity and a specific area. They must also be inert to biological attack, insoluble in the growth media and non-toxic to microbial cells. Moreover, adsorbed cells on inorganic supports should be able to maintain the metabolic activity required for the BDS process. Extensive works have been developed about the adsorption of bacteria on mineral surfaces for biolixiviation (Zheng et al., 2001). However, few studies have evaluated the influence of inorganic supports, with different physicochemical properties, on the BDS activity of Dibenzotiophene (DBT) or gas oil (Hwan et al.,

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2000; Zhang et al., 2007), alumina and Celite being the most common supports used in these bioprocess. This work studied the efficiency of adsorption of *Pseudomonas stutzeri* on Silica (Si), Alumina (Al), Sepiolite (Sep) and Titania (Ti) and their influence on the BDS of gas oil, with emphasis on the interaction of inorganic supports on metabolic activity.

2. Experimental

2.1. Materials supports

Silica (Si) D11–10 BASF, (specific surface area of 80 $m^2\ g^{-1}$), Alumina (Al) T-126 Girdler (specific surface area of 200 $m^2\ g^{-1}$), Titania (Ti) P20 Deggussa (specific surface area of 53 $m^2\ g^{-1}$) and Sepiolite (Sep) 120 NF Tolsa (specific surface area of 300 $m^2\ g^{-1}$) were used as inorganic supports. Previous to the adsorption process the supports were pretreated at 200 °C in a furnace for 16 h. All supports were sieved through a mesh in order to obtain a size in a range from 65 to 500 μm. The specific surface area of material supports was calculated using the Brunauer, Emmett and Teller equation (BET) (Brunauer et al., 1938).

2.2. Bacterial strain and culture conditions

P. stutzeri TCE3 (ATCC 53819) was used in this study. To study adsorption, this organism was grown in sulphur-free Medium A (Maghsoudi et al., 2000), supplemented with sodium succinate and citrate as the only energy and carbon sources. DBT (Merck, USA) 0.1 mM was used as the only sulphur source. The culture was grown at 30 °C and 250 rpm in a rotary shaker.

2.3. Cell immobilization

Cells were collected by centrifugation at 4000*g* and 4 °C for 30 min. The obtained pellets were suspended in 10 mL of saline solution (0.85% NaCl), at pH 5.5. For the immobilization process the bacteria cell numbers were adjusted by measuring the turbidity at 600 nm (OD_{600}) (Shan et al., 2005) with a Thermo spectrophotometer model Genesys 10uv, in a range from 1×10^9 to 25×10^9. Obtained bacterial cells were contacted with 0.1 g of respective support in 25 mL flasks at 30 °C in rotary shaker at 200 rpm during 24 h. The number of immobilized cells was measured by loses of turbidity of solution at 600 nm (OD_{600}). The biocatalysts were designated as bacterial cell/solid systems, where the bacterial cells correspond to *P. stutzeri* and the solid corresponds to the inorganic supports.

2.4. Zeta potential measurements

Electrophoretic migration (EM) measurements were carried out in a Zeta-Meter Inc. Model ZM-77 apparatus, using a 30 mg sample suspended on 300 mL of 10^{-3} M KCl solution. The pH was adjusted with HCl or KOH 0.1 M solutions, according to needs. The isoelectric points (IEP) of the adsorbed bacterial cells, the supports, the non-immobilized bacterial cells and the Zeta Potential Charge (ZPC) of the biocatalyst were calculated from the EM rate (Gil-Llambías and Escudey-Castro, 1982).

2.5. Detection by TTC of metabolically viable adsorbed cells

The metabolic activity of viable adsorbed cells was determined using the TTC method, modified for solids (Dinamarca et al., 2007). *P. stutzeri* bacterial cells were adsorbed on each support (range from 2.0×10^8 to 7.0×10^8). The biocatalysts obtained at 24 h were incubated with 0.01% TTC (2,3,5-triphenyl-2H-tetrazolium chloride) in the dark for 4 h and then diluted with ethanol (1 vol.) to dissolve the insoluble formazan that had formed. The amount of TTC formazan produced was determined by measuring absorbance at 490 nm (A_{490}) with a spectrophotometer Thermo model Genesys 10 uv.

2.6. Biodesulfurization

For BDS reaction, the bacterial cells adsorbed on 1 g of each support or the free cells (range from 1.0×10^9 to 25×10^9) were placed in a 25 mL flask containing 10 mL of sulphur-free Medium A. One millilitre of gas oil (4700 mg/L of sulphur) was added for desulfurization. The reaction was carried out at 30 °C in a rotary shaker at 200 rpm for 24 h. The hydrocarbon phase was separated from the aqueous phase by centrifugation at 13,000*g*. The total sulphur content in gas oil was determined using a LECO S-144 DR analyzer. The BDS activity was expressed as the desulfurization capacity (Zhang et al., 2007), which was determined by the amount of total sulphur (g) consumed by the number of bacterial cells per hour ($g_{Sulphur}\ cell^{-1}\ h^{-1}$).

3. Results and discussion

3.1. Cells adsorption

Slopes of *P. stutzeri* adsorption on Al, Si, Sep and Ti supports (expressed as the log of adsorbed bacterial cells per gram of adsorbent material) are shown in Fig. 1. A linear adsorption of bacterial cells was observed in all supports when an initial load of cells from 0 to 5×10^{10} (cells $\times$ mL^{-1}) was used. For Al and Sep, the maximum of cells adsorbed was established at a value of 9.9 (log adsorbed cells $\times$ g^{-1}supports), which was reached when 14×10^8 cells $\times$ mL^{-1} was used. In the case of Ti, the maximum of adsorbed cells was 9.5 (log adsorbed cells $\times$ g^{-1}supports) when 20×10^8 (cells $\times$ mL^{-1}) of initial cells were used in the adsorption process. When the adsorption process of *P. stutzeri* was conducted on Si, saturation of adsorption was not observed. Immobilization results indicate that the lowest interaction between bacterial cells and supports was given between *P. stutzeri* and Ti, whereas adsorption on Al, Sep and Si was more efficient in relation to the initial number of cells required to reach the saturation. In the BDS process the methods for bacterial cells immobilization considered the cells

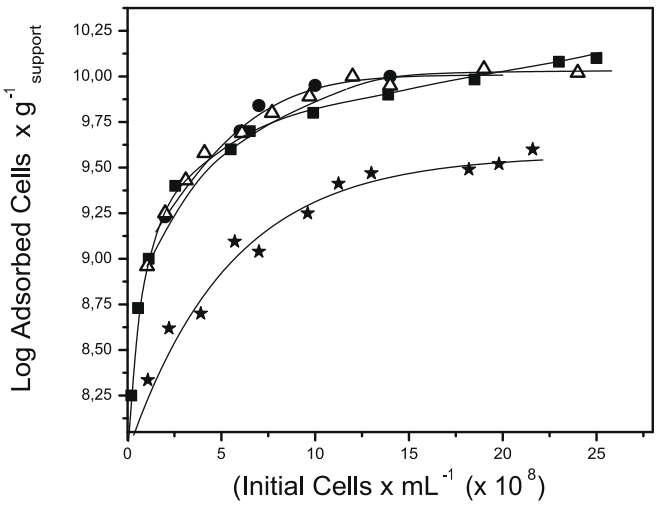

Fig. 1. Effect of initial cell loading on the final number of adsorbed bacterial cells on alumina and silica supports (● – *P. stutzeri*/Al, ■ – *P. stutzeri*/Si, △ – *P. stutzeri*/Sep and ∆ – *P. stutzeri*/T).