Elham Karimi¹
Clayton Jeffries²
Fatemeh Yazdian¹
Abbas Akhavan Sepahi³
Ash Hatamian¹
Behnam Rasekh⁴
Hamid Rashedi⁵
Meisam Omid⁶
Mohammad-Bagher
Ebrahim-Habibi⁴
Seyed Jamal Ashrafi¹

Research Article

DBT desulfurization by decorating *Rhodococcus erythropolis* IGTS8 using magnetic Fe₃O₄ nanoparticles in a bioreactor

Today, crude oil is an important source of energy and environmental contamination due to the continued use of petroleum products is a matter of urgent concern. In this work, two technological platforms, namely, the use of a robust desulfurizing bacteria and the use of nanotechnology to decorate the surface of the bacteria with nanoparticles (NP), were combined to enhance biodesulfurization (BDS). BDS is an ecologically friendly method for desulfurizing petroleum products while avoiding damage to the hydrocarbons due to the high temperatures normally associated with physical desulfurization methods. First, a bacterium known to be a good organism for desulfurization (*Rhodococcus erythropolis* IGTS8) was employed in cell culture to remove a recalcitrant sulfur molecule from a common sulfur-containing compound found in crude petroleum products (dibenzothiophene). 2-Hydroxybiphenyl (2-HBP) produced as a consequence of the BDS of dibenzothiophene was determined using Gibbs' assay. The synthesized NP were characterized by field emission scanning electron microscope, transmission electron microscopy, Fourier transform infrared spectroscopy, X-ray diffraction spectroscopy, and vibrating sample magnetometer. The field emission scanning electron microscope and transmission electron microscopy images showed the size of the NP is 7–8 nm. The decorated cells had a long lag phase, but the growth continued until 148 h (at OD₆₀₀ = 3.408) while the noncoated bacteria grow until 96 h before entering the stationary phase at OD₆₀₀ = 2.547. Gibbs' assay results showed that production of 2-HBP by decorated cells was 0.210 mM at $t = 148$ h, while 2-HBP production by nondecorated cells was 0.182 mM at $t = 96$ h. Finally, the experiments were repeated in a fermenter.

Keywords: Batch processing / Biodesulfurization / Bioreactor / Bioremediation / Fe₃O₄ nanoparticles / Microbial growth

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¹Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran

²Dan F. Smith Department of Chemical Engineering, Lamar University, Beaumont, TX, USA

³Microbiology and Biotechnology Research Group, Islamic Azad University, Tehran, Iran

⁴Microbiology and Biotechnology Research Group, Research Institute of Petroleum Industry, Tehran, Iran

⁵School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran

⁶Protein Research Centre, Shahid Beheshti University, GC, Velenjak, Tehran, Iran

1 Introduction

Fossil fuels are humanity's most important source of energy; about 85% of our energy comes from fossil fuels, another 8%

comes from nuclear power, and 7% from all other sources [1, 2]. After carbon and hydrogen, sulfur is considered as the most plentiful element in petroleum [2, 3]. As a result, the combustion of fossil fuels also combusts the sulfur-containing species within the bulk phase, which have adverse effects on health, environment, and economy [2, 4]. There are various desulfurization methods to eliminate sulfur from fossil fuels. Among them, hydrodesulfurization (HDS) is currently the most commonly used [5, 6]. Although HDS can easily eliminate the inorganic sulfur compounds, HDS is not effective for removing complex polycyclic sulfur compounds. Molecules with alkyl groups adjoined to the sulfur atom are often used as model molecules in deep HDS studies because these molecules are very difficult to desulfurize. However, HDS is problematic for the treatment of fossil

Correspondence: Dr. Fatemeh Yazdian (yazdian@ut.ac.ir), Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran 1439957131, Iran

Abbreviations: 2-HBP, 2-hydroxybiphenyl; BDS, biodesulfurization; BSM, basal salt medium; DBT, dibenzothiophene; FESEM, field emission scanning electron microscope; FTIR, Fourier transform infrared spectroscopy; HDS, hydrodesulfurization; MNPs, magnetic nanoparticles; NP, nanoparticles; TEM, transmission electron microscopy; VSM, vibrating sample magnetometer; XRD, X-ray diffraction spectroscopy

fuels because it is carried out at temperatures and pressures that are so extreme that it will also damage the valuable hydrocarbons and because HDS units are expensive to build and control [7].

Biodesulfurization (BDS) is often considered as a potential alternative to the conventional deep HDS processes used in refineries. In this process, bacteria remove organic sulfur from petroleum fractions without degrading the carbon skeleton of the organic sulfur compounds [5, 8, 9]. Microorganisms, thanks to their enzymatic and metabolic pathways, have the ability to acquire sulfur from different sources. Some microorganisms can concomitantly remove the recalcitrant thiophenic sulfur compounds, such as dibenzothiophene (DBT), from fossil fuels while simultaneously metabolizing this sulfur to meet cellular growth demands [2, 4]. Indeed, microorganisms that can remove sulfur from fossil fuels without changing the structure or heating value of the fuel have already been identified and isolated [10, 11], and many studies have been done to identify the microbial enzymes and mechanisms that are responsible for desulfurization. However, the most BDS studies have been devoted to *Rhodococcus erythropolis* IGTS8 [12–14], which has the 4S pathway specific for the removal of the sulfur atom present in DBT. In this process, the thiophenic group is progressively oxidized without degradation of the carbon structure [13, 14].

In recent years, magnetic nanoparticles (MNPs) have been widely used in the fields of biotechnology and biomedicine, because of their large surface-to-volume ratios, super paramagnetic properties, and low toxicities [15]. MNPs are used for the separation, characterization, and purification and are a well-established alternative to centrifugal separation of biological solutions [15, 16]. At the end of experiments, bacteria coated with MNPs are also easily separated from liquid suspensions by use of a magnetic field [16].

In previous studies, Ansari et al. [17] evaluated the coating of *R. erythropolis* IGTS8 by glycine-modified nanoparticles (NP) with a size range of 40–50 nm. They showed that immobilization of MNPs on the surface of bacterial cells enhances the desulfurization activity [16]. They describe that the decorated cells have a 56% higher desulfurization activity compared to the nondecorated cells. Additionally, Guobin et al. and Li et al. [18, 19] separately immobilized ammonium oleate modified Fe_3O_4 NP (with a size range of 10–15 nm) on two types of bacterial cells, including *Pseudomonas delafieldii* and *R. erythropolis* LSSE8-1. The NP did not have any effect on the desulfurization activity of the bacteria cells. Likewise, Bardania et al. [15] investigated the application of Fe_3O_4 NP to the separation of desulfurizing bacterial cells (*R. erythropolis* FMF and *R. erythropolis* IGTS8) and determined that these MNPs did not affect the desulfurization activity of these strains. The sizes of the NP used by Ansari et al., Li et al., Gubin et al., and Bardania et al. were 40–50, 13, 10–15, and 5–8 nm, respectively. This contradiction with regard to the effects of magnetite NP on the desulfurization activity can be due to the different sizes of NP used in the above-mentioned studies and this study. If the increase in the desulfurization activity is attributed to the immobilization of MNPs and augmentation of membrane permeability to DBT, as described by Ansari et al [16], then the smaller NP prepared and used in this work would have a greater impact on the desulfurization activity when compared to the NP with larger sizes that were prepared

and applied by Ansari et al. Altogether, these studies suggest that there is a metal NP–bacteria interaction that enhances biological activity at the cellular level, but may also contribute to ecotoxicity, challenging the ecofriendly nature of NP. Different classes of bacteria exhibit different susceptibilities, but the mechanism controlling the toxicity is not yet understood. Moreover, different factors such as synthesis, shape, size, composition, addition of stabilizer, and so on, can lead to different conclusions even for very closely related nanosuspensions. So, it is necessary to test each type of functionalization for a given biological application [20].

A surfactant is a substance that affects the surface or interfacial tension of the medium in which it is dissolved. During NP synthesis, surfactants are used in order to reduce interaction between the particles through an increase in repulsive force. Glycine was used as a surfactant to prevent the aggregation of NP [17]. In general, glycine as a surfactant reacts with the positively charged Fe_3O_4 NP through its carboxylic groups, therefore inhibiting aggregation [16, 18]. As was expected, addition of glycine during the process of synthesis led to the confinement of Fe_3O_4 crystals and subsequent synthesis of smaller-sized NP. A more uniform coating of the NP on the surface of bacterial cells can be attributed to larger specific surface area and the higher surface energy of the NP synthesized in the presence of glycine, which leads to less NP aggregation [15].

In the present work, we have made use of a widely studied Gram-positive desulfurization bacterial strain, *R. erythropolis* IGTS8. However, the kinetics of desulfurization by this organism is known to be limited by trans-membrane transport of both the sulfur-based substrates into the cytoplasm and the desulfurized product (2-hydroxybiphenyl (2-HBP)) from the cytoplasm back into the media. Therefore, because NP have been shown to increase the permeability of cellular membranes, the cells were decorated by MNP. By combining these two technologies, it was hypothesized that the utilization of the metabolic machinery of the desulfurizing bacteria would be enhanced by increasing cell membrane permeability induced by the cell-surface decoration by NP. Additionally, NP functionalization by glycine and PEG was investigated in conjunction with these two techniques with an aim to improve NP stabilization and cellular decoration. The effect of MNPs on the desulfurization activity of bacterial cells in basal salt medium (BSM) containing DBT as the sole source of sulfur was investigated. Indeed, we discovered a production enhancement of 15.3% in the NP-decorated cells over the undecorated cells. Transmission electron microscopy (TEM) and SEM, vibrating sample magnetometer (VSM), X-ray diffraction spectroscopy (XRD), and Fourier transform infrared spectroscopy (FTIR) were also employed to investigate the properties of the synthesized MNPs.

2 Materials and methods

2.1 Chemicals

Dibenzothiophene (DBT, 99%) and 2-HBP were obtained from Fisher Scientific (Germany). Ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), glycine, Ringer's solution, and all other chemicals were from

Merck (Germany). The water was purified by ion exchange and reverse osmosis (ELGA-option 3B, ELGA Water, and Marlow, UK).

2.2 Bacterial strain and medium

Rhodococcus erythropolis IGTS8 was purchased from the Research Institute of Petroleum Industry in Iran. The bacteria were cultured in a BSM that had the following composition: 2.44 g KH_2PO_4 , 5.47 g Na_2HPO_4 , 2.00 g NH_4Cl , 0.2 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.001 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.001 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.004 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, and 2 mL glycerol dissolved in 1000 mL. [21]. The 100 ppm DBT stock was the sole sulfur source and was prepared by dissolving 100 mg of DBT in 10 mL of ethanol that was then added to 1 L of BSM. Cell cultivation was carried out at 30°C on a rotary shaker operated at 120 rpm. Mid-exponential phase cultures were collected by centrifugation at $1400 \times g$ for 10 min, washed two to three times with Ringer's solution, and stored at –4°C [15].

2.3 Synthesis and modification of magnetic nanoparticles

The most conventional method for obtaining Fe_3O_4 is by coprecipitation. This method consists of mixing ferric and ferrous ions in a 1:2 molar ratio in highly basic solutions at room temperature or at elevated temperature [22]. To obtain Fe_3O_4 NP, briefly, 25 mL of 0.2 mM ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) was mixed with 100 mL of 0.1 mM ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) solution in a flask and testing began with a temperature of 30°C and after 90 min the temperature reached 80°C in refluxing condition under a nitrogen atmosphere while vigorously stirring the reaction mixture to prevent the development of particles larger than 25 nm to 1 μm [23–25]. Then, 1 g of glycine followed by 140 mL of NH_4OH (25% wt) was added to the reaction mixture under vigorous stirring until the pH was measured to be in the range of pH 10–11 [26]. Then, the black precipitates were collected after cooling the reaction mixture to room temperature. Suspensions of the black precipitates were then washed several times with deionized water [16] and were subsequently lyophilized overnight at –50°C. For complete coverage, MNPs were synthesized by adding PEG in two steps, during (40 mL of solution containing 2 mg/mL PEG 1500 MW) and after synthesis (60 mL of solution containing 2 mg/mL PEG 1500 MW).

2.4 Characterization of magnetic nanoparticles

The synthesized NP were characterized by TEM, field emission scanning electron microscope (FESEM), VSM, XRD, and FTIR. The size and morphology of MNPs were analyzed by a TEM (Philips CM 200, 200 kV TEM, ATM 2×2 k CCD camera) and FESEM (Carl Zeiss, Supra 55) at an accelerating voltage of 15 kV [15]. Before FESEM analysis, the particles were sputter coated with Au/Pd. The saturation magnetization (M_s) and coercive force (H_c) of the samples were measured using a VSM

(Dexing, and Model 250). Saturation magnetization (23 emu/g) was obtained from the hysteresis loop resulting from applying a magnetic field from –10 to +10 kOe [16].

The structure and crystal phase of Fe_3O_4 MNPs were measured by a Bruker D8 advance X-ray diffractometer operating in reflection mode with $\text{Cu-K}\alpha$ radiation. A copper sample holder was used. The scanning range was between 20 and 80° of 2θ [25].

In order to analyze the characteristics of synthesized MNPs, FTIR spectra were used to identify surface groups and determine the chemical bonds. KBr pellets containing 1.5 mg sample and 15 mg KBr were prepared for FTIR measurements (Bruker, Tensor 27, FT-IR spectrometer). Spectra were recorded in the range 450–4000 cm^{-1} [27].

2.5 Statistical analysis

All desulfurization tests were repeated in triplicate, and the results were presented as mean $\pm$ SD. The experimental data were analyzed by the *t*-test and analysis of variance using SPSS version 20 to determine whether the differences are statistically significant.

2.6 Magnetic immobilization of desulfurization cells

The bacteria were grown in BSM until the mid-exponential growth phase and harvested by centrifugation at $1400 \times g$ for 10 min. The cell pellets were washed twice with Ringer's solution and suspended in BSM to $A_{600} = 1.0$. The cells were then decorated with MNPs as follows: 10 mL of an aqueous suspension containing 10 $\mu\text{g/mL}$ Fe_3O_4 NP were added to 100 mL of the cell suspension in BSM that had a final DBT concentration of 0.5 mM [17, 18].

2.7 DBT desulfurization capability

In this study, the desulfurization capability of *R. erythropolis* IGTS8 was tested by using DBT in BSM as the sole sulfur source. Cells were grown until the mid-exponential growth phase and harvested by centrifugation at $1400 \times g$ for 10 min. The cells were then suspended in the same solution to $A_{600} = 1.0$ and used to inoculate the desulfurization studies on the same day. One milliliter of inoculum was added to 250 mL flasks containing 100 mL (1% v/v) of BSM with a DBT concentration of 0.5 mM. Incubation was at 30°C and the flasks were shaken at 100 rpm [16].

2.8 Bacterial growth and desulfurization activity assay in flasks

2-HBP produced as a consequence of the BDS of DBT was determined using Gibbs' reagent (2,6-dichloroquinone-4-chloromide) [21]. The pH was adjusted to 8.0 before the addition 20 μL of Gibbs' reagent to broth samples (2 mL), which reacts with aromatic hydroxyl groups to form a blue-colored complex at this pH. After 30 min of incubation at room temperature, the

Figure 1. X-ray diffraction pattern of PEG-coat Fe_3O_4 NP.

absorbance was measured at 610 nm. The response curve was generated in triplicate using standard solutions of 2-HPB. The response was 6.51 ± 0.5 a.u. per mM 2-HPB. Measurements were prepared in a Nano Drop [16]. The cell growth in flasks was monitored by measuring the optical density of the culture broth samples (2 mL) in triplicate at 600 nm (the samples were diluted in media) using a Jenway 6505 UV/vis spectrophotometer (Paterson Scientific, UK) [19].

2.9 Biodesulfurization activity of immobilization bacteria cells with magnetic nanoparticles in bioreactor

Desulfurization activity of bacteria cells was evaluated in a stirring reactor (a 5 and 2 L jar fermenter) containing 2 L BSM with 0.5 mM DBT and 2 mL/L glycerol. At first, the BSM is prepared and a 20 mL aliquot containing 2 mg/mL of Fe_3O_4 NP was added to the BSM in the fermenter. After 1 day, the fermenters were inoculated with the bacterial cells in the mid-exponential growth phase. Cultivations were conducted in triplicate [28].

3 Results and discussion

3.1 Characterization of magnetic nanoparticles

3.1.1 Determination of crystal phase

In order to understand the chemical composition of synthesized NP, XRD analysis was performed [25]. Output from XRD analysis yields a plot, which shows intensity versus diffraction angle and it can determine the crystallographic phases that are being diffracted [16]. The position and relative intensities of all the characteristic diffraction peaks were observed at 2θ values of the following: 30 (220), 35.4 (311), 43 (400), 53.4 (422), 56.9 (511), and 62.5 (440), as seen in Fig. 1. The MNP synthesized by Wang et al. [29] using the conventional co-precipitation method showed diffraction peaks at 2θ values of 30.4, 35.6, 43.3, 57.3,

Figure 2. (A) SEM and (B) TEM images of PEG-coat Fe_3O_4 NP.

and 62.8, indicative of a cubic spinal structure of the magnet. The crystallite size of the PEG-MNPs synthesized in this study was estimated at 16.3 nm from X-ray line broadening using the Scherer formula [27].

3.1.2 Morphology observation

The morphology of Fe_3O_4 NP was observed by FESEM and TEM [16], as seen in Figs. 2 and 3. TEM micrographs were carried out at an accelerating voltage of 200 kV on carbon-coated copper grids with a mesh of 200–300 (Fig. 2B). It is clearly observed that Fe_3O_4 NP are almost spherical in shape with a size of about 7–8 nm. A morphological characterization of the synthesized Fe_3O_4 NP was obtained using FESEM (Fig. 2A).

3.1.3 Measurement of magnetic properties

The magnetic properties of Fe_3O_4 NP were measured with a VSM [26]. High-field condition was used to assure that saturation magnetization was achieved and measured. Saturation magnetization (23 emu/g) was obtained from the hysteresis loop resulting from applying a magnetic field from -10 to $+10$ kOe at room temperature [16]. Figure 3 shows the magnetization (M_s) versus applied field (H_c) magnetization of Fe_3O_4 NP. The hysteresis loop of synthesized Fe_3O_4 NP indicates super paramagnetic behavior at room temperature as evidenced by zero coactivity and remanence on the magnetization loop [16, 27].

Figure 3. Magnetic hysteresis loop of PEG-coat Fe_3O_4 NP measured at room temperature. (A) Solubility of PEG-coated Fe_3O_4 NP and (B) magnetic separation of PEG-coated Fe_3O_4 NP.

Figure 4. FT-IR spectra (A) glycine- modification Fe_3O_4 NP and (B) PEG-coated Fe_3O_4 NP.

3.1.4 FTIR spectra

Characterization of glycine-modified NPs and PEG-modified NPs was achieved by analyzing the FTIR spectra of the solid samples dispersed in a KBr matrix [30]. FTIR spectra of glycine-modified Fe_3O_4 NP and PEG-coated Fe_3O_4 NP are presented in Fig. 5. The strong FTIR bond observed around 530 cm^{-1} can be attributed to the Fe–O stretching vibration mode of Fe_3O_4 [25]. Figure 4 illustrates the absorption bands for the glycine-modified Fe_3O_4 NP as well resolved, but broad. The bands of the PEG-modified Fe_3O_4 NP are rather broad and few. The 1710

cm^{-1} peak was assigned to C = O vibration [27]. An intense band at 3500 cm^{-1} proposes the presence of nondissociated OH groups [25]. When Mahdavi et al. [30] prepared magnetite NP by a coprecipitation method, their FTIR spectra had absorption peaks at 530 cm^{-1} corresponding to the Fe–O vibration of the magnetite phase. The intense peak at 1710 cm^{-1} was due to the absorption bands of the carboxyl groups. Mukhopadhyay et al. [31] reported a facile and green synthetic approach to prepare magnetite (Fe_3O_4) NPs with a magnetic core and a PEG surface coating. The bare Fe_3O_4 NPs showed characteristic bands related to the Fe–O vibrations near 618 (shoulder) and 569 cm^{-1} . Similar peaks have been observed in the spectra of the PEG-coated NPs (at 633 and 569 cm^{-1}).

3.2 Biodesulfurization activity

Figure 5A compares the growth rate of nondecorated and decorated cells in BSM. Decorated cell growth continues until 148 h while the nondecorated cells decrease in cell number after $t = 96\text{ h}$. The maximum OD_{600} for the decorated cells was 3.4 ± 0.3 at $t = 148\text{ h}$, while the maximum OD_{600} for the undecorated cells was 2.4 ± 0.3 at $t = 96\text{ h}$. The DBT desulfurization was evaluated measuring the 2-HBP production (the end product of DBT desulfurization) [16]. The evaluations of BDS activity of bacterial cells were performed with the spectrophotometric Gibbs' assay and the results were presented in terms of 2-HBP production during 148-h cycles. In Fig. 5B, spectrophotometric Gibbs' assay results showed that the consumption of DBT was observed after the first 24 h of culture, but the accumulation of 2-HBP in the cultivation broth was observed only after 48 h , which might suggest the existence of a concentration-dependent

Figure 5. (A) Comparison of the cell growth of *R. erythropolis* IGTS8 without (solid) and with (dashed) decoration by MNPs and (B) HBP production by *R. erythropolis* IGTS8 without (solid) and with (dashed) decoration by MNPs, in BSM with DBT at the initial ($t = 0$) concentration of 0.5 mM as the sole sulfur source.

efflux system. On the other hand, the maximal extracellular concentration was about 0.182 mM by free cells at $t = 96$ h and 0.210 mM at $t = 148$ h by magnetic-coated cells. Production of 2-HBP by decorated cells was significantly different than by the free cells. It can be seen that whereas the production rate of the nondecorated cells reduced after about 96 h, while the decorated cells continue producing more vigorously until at least $t = 148$ h. Therefore, the decorated cells had a $15.80 \pm 0.01\%$ higher desulfurization capacity compared to the nondecorated cells. The rate-limiting step of the process is transport of DBT into cell, where it undergoes desulfurization [18]. The observation of significantly increased HBP production in the decorated cells suggests that the NP somehow facilitate transport of HBP out of the cells assuming that it is produced in the cytoplasm [4].

3.3 Biodesulfurization activity in bioreactor

The OD₆₀₀ versus time curve for the cells grown in a bioreactor in the presence of iron oxide NPs is presented in Fig. 6A. The cultivation began with a period of linear growth before exponential growth began between 48 and 72 h (Fig. 6A). Fermenter biomass concentration reached a maximum at $t = 120$ h (Fig. 6A), but BDS continued until the cultivation was terminated at $t = 248$ h (Fig. 6B). The reason for an increasing HBP concentration after 120 h could be because the NP affected the cell membrane per-

Figure 6. (A) The growth rate and (B) desulfurization activity of *R. erythropolis* IGTS8 in the presence of magnetic iron oxide NP in bioreactor containing 2 L BSM with 0.5 mM DBT and 2 mL/L glycerol.

meability resulting in intracellularly stored HBP to be excreted into the culture media. The desulfurization activity of bacterial cells in a fermenter in the presence of MNPs is presented in Fig. 6B. According to Fig. 6, BDS is directly related to the amount of biomass, thus increasing the amount of biomass in the bioreactor is associated with the increase of desulfurization rate.

4 Concluding remarks

Rhodococcus erythropolis IGTS8 has shown high activity and selectivity to remove sulfur from organic compounds that commonly exist in crude oil. So, development of bacteria for sulfur removal and design of new BDS processes by improvement of the permeability of the bacteria are required [15, 17]. In this study, we synthesized MNPs using a chemical coprecipitation method [27]. The methods of this study synthesized MNPs of 7–8 nm size. Because if the particles are large, their Brownian energy overwhelms the relatively weak attachment forces. Ansari et al. [16] performed preliminary experiments using iron oxide particles with a diameter of 1 μm (Dynabeads, Dynal Biotech) and showed that they did not attach to the bacteria. Furthermore, the particles should be small enough to be superparamagnetic, that is, smaller than the critical magnetic domain size [17]. The main discovery emerging from these experiments in this study

is that the decorated bacteria achieve a 15.3% higher rate of desulfurization. The cell growth rate in the presence of MNPs was higher than in the absence of MNPs. Because DBT is insoluble in water, its bioavailability to bacteria is low and the resulting sulfur limitation in the cell culture leads to the onset of the stationary phase [4]. In this study, the only sulfur source available to the cell culture was derived from the desulfurization of DBT, so the increase in cell culture density demonstrated the BDS capabilities of the system [17]. If BDS takes place within the cytoplasm the bacterial surface constitutes the rate-limiting barriers to the process (transport of DBT into the cell or HBP out). Ansari et al. [16] proposed the permeabilization of the bacterium is improved through decoration by NP, and some supporting evidence in a model system was obtained. In the presence of MNPs, an increase in the desulfurization yield and the HBP production rates were observed. These experimental results can be related to the favorable production of HBP, as the NP action avoids the accumulation of HBP in the cytoplasm and periplasmic space [32]. On the other hand, the maximal extracellular concentration was about 0.182 mM by free cells at $t = 96$ h and 0.210 mM at $t = 148$ h by magnetic-coated cells in flasks. In conclusion, this study suggests that the addition of NP into a BDS medium does not overcome all of the BDS process limitations, but can avoid the HBP inhibition effects as other authors have reported previously [33].

Practical application

The development of this new method for the biodesulfurization (BDS) could help to maximize the productivity of BDS for industrial and commercial applications. When BDS takes place within the cytoplasm, transport across the bacterial surface constitutes the rate-limiting step to the BDS (transport of DBT into the cell or HBP out). Therefore, the enhanced cellular permeability imparted by nanoparticle decoration of the cell surface, as performed in this study, will improve BDS kinetics. Also, in any industrial process using freely dispersed microorganisms as catalysts, they need to be separated from the products at the end of the process. Therefore, the cellular decoration by magnetic nanoparticles offers the additional benefit of enabling the economical, magnetic separation of the organisms from the product stream.

The authors have declared no conflict of interest.

5 References

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