Improvement of Desulfurization Performance of *Rhodococcus erythropolis* IGTS8 by Assembling Spherical Mesoporous Silica Nanosorbents on the Surface of the Bacterial Cells

Navid Ahmadi Nasab¹², Hassan Hassani Kumleh¹, Mahmood Kazemzad², Farideh Ghavi Panjah², Fatemeh Davoodi-Dehaghami³

¹Agricultural Biotechnology, Faculty of Agricultural Science, University of Guilan, Guilan, Iran. ²Materials and Energy Research Center, Tehran, Iran. ³School of Biology, Faculty of Basic Science, Islamic Azad University, Central Tehran Branch, Tehran, Iran.

(Received 02 Oct. 2014; Final version received 11 Dec. 2014)

Abstract

MCM-41 mesoporous silica is synthesized based on a self assembly method, using a quaternary ammonium template, CTAB for the adsorption of sulfur compounds from model oil (1.0 mmol/l DBT in dodecane solution). Then the adsorption capability of MCM-41 assembled on the surface of bacterium *Rhodococcus erythropolis* IGTS8 is examined regarding the improvement of the biodesulfurization process of the oil compound based on the measurement of DBT consumption rate and 2-HBP production. Study of the model oil desulfurization by the cells assembled with MCM-41 nanosorbent showed a further improvement in DBT reduction rate in comparison with the free cells. The results of the investigations showed that the maximum specific desulfurization activity in terms of DBT consumption rate and 2-HBP production are 0.34 µmol DBT min⁻¹ g DCW⁻¹ and 0.126 µmol 2-HBP min⁻¹ g DCW⁻¹ which indicated an increase of 19% and 16% compared to the highest specific desulfurization activity of free cells, respectively.

**Keywords:** *Rhodococcus Erythropolis IGTS8, Dibenzothiophene, Biodesulfurization, Mesoporous Silica.*
Introduction
By dedicating 0.03 to 7.89 weight percent of crude oil, sulfur is the third abundant element within after carbon and hydrogen [1, 2]. Heterocyclic sulfur compounds result in sulfur oxides, which in turn impose unfavorable effects on health, environment, and economy. Then, presence of sulfur in crude oil has been converted into an important challenge during oil products’ refining, production and consumption [3-5].

There exist various methods for eliminating sulfur from fossil fuels such as solvent extraction, chemical oxidation, adsorption, hydrodesulfurization (HDS) and biodesulfurization (BDS). Currently, hydrodesulfurization is of greater importance compared to the rest, notwithstanding the concerns (no selectivity, need to high temperature and pressure). Nevertheless, the need for alternative technologies to overcome the limitations of the current methods is evident. Amongst, biodesulfurization has exhibited a good potential and could be considered as an alternate or complement to the HDS method [6-17].

Dibenzothiophene (DBT) and its alkylated derivatives are recognized as a main remnant of the organosulfur heterocyclic compounds of fuel showing resistance to the HDS process. In the researches related to BDS mechanism, DBT is of much more use as a model molecule of sulfur compounds of oil. It’s also demonstrated that the bacteria which are able to consume sulfur from DBT have the ability to eliminate this element from its derivatives [18-23].

All recognized microorganisms having a role in desulfurization process do not act the same in decomposing and consuming organosulfur compounds including DBT. The sensible and desirable mechanism within the BDS process is the one which is able to remove sulfur without destroying benzene ring. In other words, it should not damage carbon-carbon bonds presented in DBT structure [24]. In 1990, the specific oxidative desulfurization pathway was introduced for DBT desulfurization. In this pathway bacteria are exclusively able to remove sulfur atoms from DBT by breaking carbon-sulfur bond during the oxidation reactions and produce the final 2-HBP product as a result. The bacteria are also capable of maintaining the heating value of fuel in this pathway without decomposition of the carbon skeleton. As these metabolic reactions occur in four enzymatic stages, this pathway is called $4S^1$ pathway [25, 26].

The kinetics of the desulfurization reaction is affected by the DBT concentration and distribution [30]. The procedure of mass transfer depends on the hydrophobic property of DBT and also the natural hydrophobic essence of the surface of the desulfurization...
cells [31]. Therefore, we could make use of the surfactants and sorbents with the capability of adsorbing heterocyclic aromatic sulfur rings such as DBT [10, 32]. In this manner useful characteristics of the nanoparticles and nanosorbents such as high stability, the possibility of controlling the shape and size of pores, and also having high specific surface area, have brought them into focus compared with surfactants [33, 34]. An appropriate sorbent suitable to this method is the MCM-41 mesoporous silica which based on its unique traits could be effective on the concern of mass transfer. The numbers of pores, ordered porosity and high specific surface area, all have converted mesoporous silica materials into the ideal hosts for receiving a multitude of molecules in different forms, sizes and properties. Furthermore, this nanostructure could provide the adsorbed molecules to cells much better in comparison with zeolites and other micropores due to its larger pores. Finally, MCM-41 is recognized as an eco-friendly sorbent and catalyst because of its silicate structure. This element is present throughout the earth crust as well as the structure of organisms such as the silica skeleton of sponges.

In this experiment, MCM-41 mesoporous silica has been synthesized as an eco-friendly nanosorbent using a quaternary ammonium template, cetyl trimethyl ammonium bromide (CTAB) in basic water-ethanol mixture. Then, MCM-41 is assembled on Rhodococcus Erythropolis IGTS8 bacterium (one of the most common microorganism in the biodesulfurization studies) by means of the physical adsorption. Then, the desulfurization activities of free Rhodococcus Erythropolis IGTS8 bacterium as well as the one assembled with the nanosorbent.

Experimental

Chemicals

DBT (98%), 2-HBP (99%), n-dodecane and ethyl acetate, methanol and acetonitrile solutions of HPLC grade were purchased from Merck Company and utilized without further purifications. Tetraethyl orthosilicate (TEOS 99%) and CTAB were used as the silica source and soft template, respectively. The compounds present in basal salt medium (BSM) which were used for the purpose of growing bacterium IGTS8, consist of Na₂HPO₄ 5.57 g/l, KH₂PO₄ 2.44 g/l, NH₄Cl 2 g/l, MgCl₂.6H₂O 0.2 g/l, MnCl₂.4H₂O 4 mg/l, FeCl₃.6H₂O 1 mg/l and CaCl₂.2H₂O 1 mg/l.

Synthesis of the Spherical Molecules of MCM-41

The MCM-41 mesoporous silica nanosorbent were synthesized at room temperature according to the instructions provided by reported method in literatures [35]. Briefly, 0.5 g CTAB was added to 96 ml of distilled water under mild stirring by magnetic stirrer. After
the solution turned clear, we supplemented
the system with 34 ml of ethanol and then 10
ml of aqueous ammonia solution and it was
allowed to mix for 5 min. Immediately after
that 2.0 ml of TEOS was poured into the
solution under stirring and it continued to be
under mild stirring at room temperature lasted
for 3 hours. The solid product was recovered
by filtration and dried at room temperature
overnight. Finally, the CTAB surfactant was
removed from the composite material by
calcining the sample at 540 °C for 9 h.

Assembling of MCM-41 nanosorbent at the
surface of microorganism
The desulfurization cells having the final
concentration of 10 g/l were added to 50 ml of
the saline (NaCl, %8.5 W/V) of pH 6.8 along
with different amounts of the nanosorbent
including 0.2, 0.3 and 0.5 g and the suspension
resulted from each were gradually mixed in
the stirrer. Finally, the cells and nanosorbent
were separated from the saline by vacuum-
drying and stored at -4°C.

Microorganism
The moderate thermophile (moderate
temperature-loving) bacterium, R. erythropolis
IGTS8 (ATCC 53968) has been utilized in
current work which was obtained from the
Materials and Energy Research Center of Iran.
The *Rhodococcus erythropolis* IGTS8 cell was
cultured in an LB plate containing agar and
the obtained colony were transferred to the
BSM culture medium containing all necessary
elements along with glycerol (5 g/l) as the
carbon source and DBT (0.27 mM) as the sole
source of sulfur. Cell cultivation was carried
out at 30°C on an incubator shaker operated
at 200 rpm. When the cells reached the end of
exponential growth phase, which is occurred
at optical density of around 3 based on the
depicted growth graph, they were collected by
centrifugation in 4°C and at 6000 g for 10 min.
They were stored at -4°C after being washed
three times by phosphate buffer (0.1 molar, pH 7).

Biodesulfurization of Model Oil
The IGTS8 cells assembled with the
nanosorbent were suspended in 30 mL of
phosphate buffer solution (0.1 molar, pH 7)
in order to study the specific desulfurization
activity of the modified biocatalysts. Then,
10 mL of dodecane with the concentration of
1.0 mmol/L DBT were added to the obtained
mixture to prepare an organic-aqueous system,
alogous to oil alkanes. The desulfurization
process was carried out in 200 ml flask, at
30°C and 180 rpm in an incubator shaker.
Finally, the specific desulfurization activity
were calculated during the first 12 hours, in
terms of DBT consumption per minute by one
gram of dry cell (µmol DBT min⁻¹g DCW⁻¹)
and the production of 2-HBP per minute by
one gram of dry cell (µmol 2-HBP min⁻¹g
DCW⁻¹).
**Analytical Methods**

High performance liquid chromatography (HPLC) was utilized for determining the concentration of DBT and 2-HBP in triplicates. HPLC analyses were performed by a KNAUER model equipped with an ODS-3 column (250 mm×4.6 mm, 5μm), and a UV detector operated at the wavelength of 280 nm. The mobile phase was composed of water and acetonitrile with the ratio of 1:4 and with a flow rate of 1 ml/min at 25 °C. For preparation of samples, mixtures separated at various times and their pH was adjusted to 2 by the means of 1-normal Hydrochloric acid to immediately stop the desulfurization activity of the cells. Then each sample was mixed with acetonitrile with ratio of 1:1. Finally, the upper solution was collected through each microtube by centrifugation (14000g) for 15 min at 25°C and then after being passed through the 0.22 micron filter was stored at -4°C up to the analysis time.

The morphology and macro-structure of the nanosorbent MCM-41 and its assembled on the surface of the desulfurization cells were studied using a scanning electron microscope (SEM, S360, Leica/Cambridge) operating at 20 kV. The structure, morphology and size of the pores were investigated with the aid of the transmission electron microscope (TEM), Philips CM 200 model, operating at 200 kV.

Specific surface area measurements and pore size analysis were carried out on samples previously out-gassed for at least 4 h at 300°C, for removal of water and other atmospheric contaminants, by means of N\textsubscript{2} isotherms at 77 and 273 K (Micromeritics Gimini III 2375 instrument). The Brunauer–Emmett–Teller (BET) and the Barrett-Joyner-Halenda (BJH) models were utilized for specific surface area and pore size distribution determination, respectively. UV-Vis spectroscopy (T80+, PG Instrument) were also utilized to study the exponential growth curve of bacteria.

**Results and discussion**

**Characterization of Nanosorbent MCM-41**

The outcome of BET and BJH studies showed that the average diameter of the pores of the synthesized sample is about 3.54 nm and the specific surface area being in the range of 1106 m2/g. The SEM images of MCM-41 sample shows homogeneous distribution of particles with a spherical morphology ranged 200-300 nm. In addition the results derived from the images of HRTEM patterns verify hexagonal pattern of the pores (Figure 1). HPLC analysis has also been utilized to compute the nanosorbent’s ability in adsorbing DBT from the model oil. The results indicate that the nanosorbent of 0.03 g/mL is able to adsorb more than 42% DBT from the model oil in 3 hours (data are not shown here).
**Assembling of MCM-41 particles on the surface of desulfurization cell**

HPLC analysis (Figure 2) and imaging by the means of a scanning electron microscope (SEM) (Figure 3) were utilized to study the assembling, overlapping of the MCM-41 particles on the surface of desulfurization cells and selection of the optimized procedure of assembling for desulfurization. Considering the results derived from the HPLC analysis with different amounts of the nanosorbent MCM-41 and after 8 hours since the start of the reaction, it is exhibited that the maximum desulfurization activity is 0.64 mM 2-HBP which is obtained by 0.3 g of the nanosorbent. Severe reduction of the performance of the cells assembled with 0.5 g of the nanosorbent seems to be due to the agglomeration of particles and enclosure of all cellular surfaces by the particles of the nanosorbent MCM-41 which hinders further biocatalytic activity of the cells. In order to confirm the results of HPLC, microscopic imaging was also performed. According to the images related to the usage of 0.2 g nanosorbent, the consumed particles were not sufficient for homogeneous coating of the surface of the desulfurization cells (final concentration of 10 g/l, equivalent to 0.5 g of cell dry weight) and a considerable part of the cellular surfaces haven’t had any coating. Furthermore by adding the nanosorbent equal to 0.5 g per mentioned dosages, all cell surfaces would be covered by the nanosorbent particles. Finally, the images confirm that the proper coating of the particles onto the cells’ surface could be carried out by the use of 0.3 g of nanosorbent MCM-41 for the mentioned dosages of cells.

---

**Figure 1.** (a) SEM and (b) TEM micrographs of the nanosorbent (MCM-41).
Figure 2. Desulfurization activity of the cells assembled with different amounts of nanosorbent MCM-41 in terms of 2-HBP production. Each data point recorded is an average of three replicate samples. Standard deviation is 5% or less for all data.

Figure 3. SEM images from the overlapping of MCM-41 particles on the surface of desulfurization cells (a): free cells (b): cellular coating with 0.2 g of the nanosorbent (c): cellular coating with 0.3 g of the nanosorbent (d): cellular coating with 0.5 g of the nanosorbent.

The desulfurization of the model oil by the cells assembled with the nanosorbent

The desulfurization activity in model oil was investigated by free cells and cells assembled with the nanosorbent in terms of DBT consumption and 2-HBP production rates (Figure 4). In accordance with the depicted diagrams, the DBT reduction rate by the cells assembled with nanosorbent MCM-41 was enhanced compared with free cells, although
during the first 3 hours of the desulfurization process 2-HBP production by free cells was double of the assembled cells’ On the other hand, in the presence of the assembled cells the DBT is totally consumed after 7 h and the maximum amount of 2-HBP has been produced. Then, the concentration of 2-HBP decreased 5% at 12th h relative to its maximum (at 7th hour) gradually over time while in the presence of free cells 2-BHP was augmented by 2% in the same period. Taking the rapid consumption of DBT being inassembled in the porous nanosorbent’s by the desulfurization cells and the desaturation of these pores by DBT molecules into account, some of the produced 2-HBP could be enclosed by the nanosorbent. The specific desulfurization activity of free cells and the assembled ones were determined in terms of the DBT consumption per minute by a gram of dry cell (µmol DBT min⁻¹g DCW⁻¹). Investigations revealed that the peak of desulfurization activity corresponds to the assembled cells during the first hour is 0.34 µmol DBT min⁻¹g DCW⁻¹ which in comparison with the maximum of desulfurization activity of the free cells (observed at 3rd hour) exhibits a 19% boost. The desulfurization activity of free cells and the assembled ones were also determined based on the production of 2-HBP. The peak of desulfurization activity in terms of 2-HBP production of the free and assembled cells was observed at 7 h after the start of the reaction which is 0.126 2-HBP min⁻¹g DCW⁻¹ for the assembled ones showing 16% rise relative to the maximum desulfurization activity of the free cells. Schematic representation of mass transfer improvement and desulfurization activity by the assembled cells has been depicted in Figure 5.

**Figure 4.** Desulfurization of DBT by free cells and the cells assembled with the nanosorbent: (■) the concentration of DBT consumed by free cells, (▼) The concentration of DBT consumed by the cells assembled with the nanosorbent, (□) The concentration of 2-HBP produced by free cells, (□) The concentration of 2-HBP produced by the cells assembled with the nanosorbent. Each data point recorded is an average of three replicate samples. Standard deviation is 5% or less for all data.
Conclusions

According to the performed experiments and analyses, the synthesized MCM-41 mesoporous silica nanosorbent decreases DBT compositions in model oil by adsorption and entrapment of DBT molecules due to its great specific surface area. As the MCM-41 mesoporous silica nanosorbent does not have any considerable negative effect on the survivability and metabolic activity of cells, these particles could physically be well-assembled onto the surface of desulfurization cells by electrostatic and Van der Waals interactions and provide their DBT molecules to the desulfurization cells which in turn increases the desulfurization activity of the cells by enhancing the mass transfer. Therefore, it is possible to take advantage of the combination of this nanosorbent and cells in biodesulfurization, and probably other similar cases.

Acknowledgements

Authors would like to thank Mr. Ganjkhanlou for his assistance in the preparation of this paper.

References


