



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

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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 $\mu\text{mol DBT min}^{-1} \text{g DCW}^{-1}$ and 0.126 $\mu\text{mol 2-HBP min}^{-1} \text{g DCW}^{-1}$ 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 results in sulfur oxides, which in turn imposes 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 4S¹ 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

1- Four step

cells [31]. Therefore, we could make use of the surfactants and or 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 Na_2HPO_4 5.57 g/l, KH_2PO_4 2.44 g/l, NH_4Cl 2 g/l, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.2 g/l, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 4 mg/l, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 1 mg/l and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 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, 0.5g 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.

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).

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.

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, analogous 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 ($\mu\text{mol DBT min}^{-1}\text{g DCW}^{-1}$) and the production of 2-HBP per minute by one gram of dry cell ($\mu\text{mol 2-HBP min}^{-1}\text{g DCW}^{-1}$).

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, 5 μ m), 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 N₂ isotherms at 77 and 273 K (Micromeritics Gemini 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).

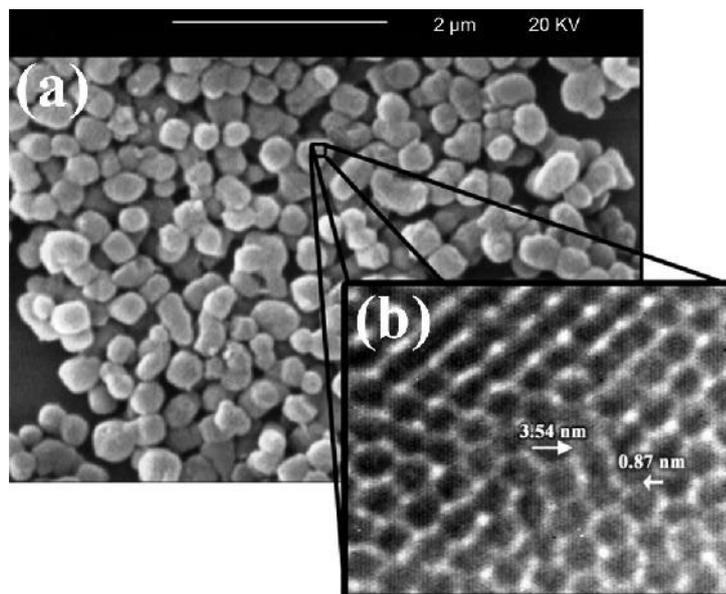


Figure 1. (a) SEM and (b) TEM micrographs of the nanosorbent (MCM-41).

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.

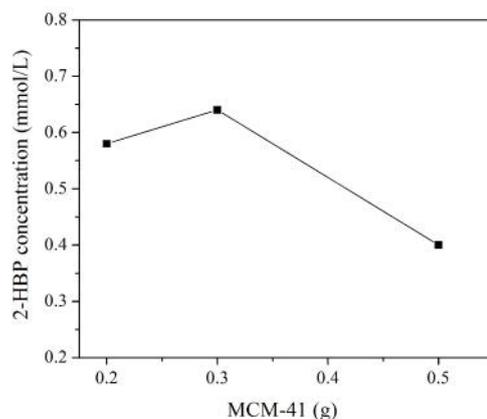


Figure 2. Desulfurization activity of the cells assembled with different amounts of nanosorbent MCM-41 in terms of 2-HBP production. Each data point recorded is an average of three replicate samples. Standard deviation is 5% or less for all data.

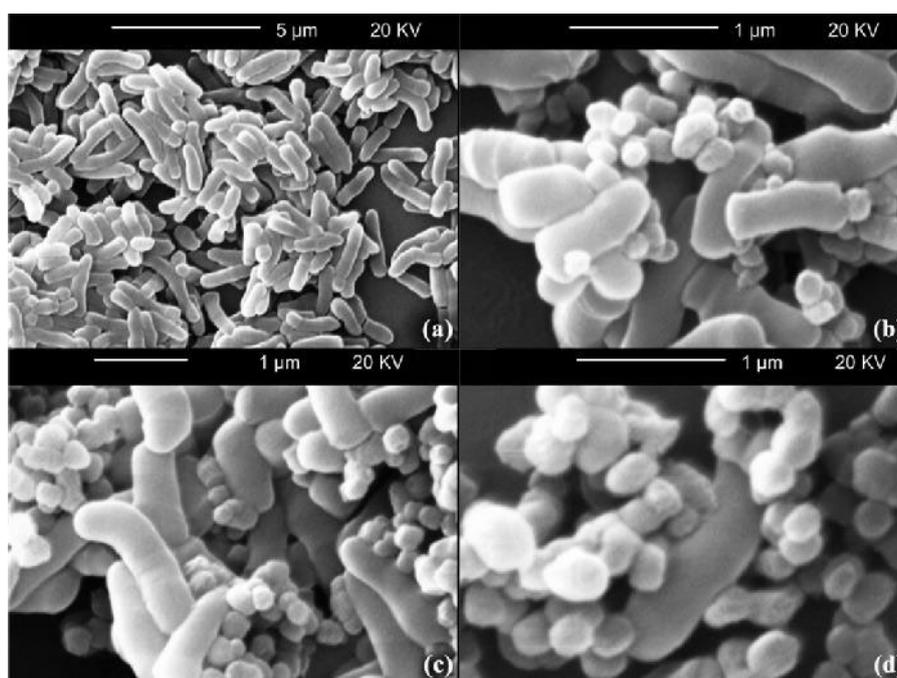


Figure 3. SEM images from the overlapping of MCM-41 particles on the surface of desulfurization cells (a): free cells (b): cellular coating with 0.2 g of the nanosorbent (c): cellular coating with 0.3 g of the nanosorbent (d): cellular coating with 0.5 g of the nanosorbent.

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-HBP 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 ($\mu\text{mol DBT min}^{-1}\text{g}$

DCW^{-1}). Investigations revealed that the peak of desulfurization activity corresponds to the assembled cells during the first hour is $0.34 \mu\text{mol DBT min}^{-1}\text{g DCW}^{-1}$ 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 $0.126 \text{ 2-HBP min}^{-1}\text{g DCW}^{-1}$ 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.

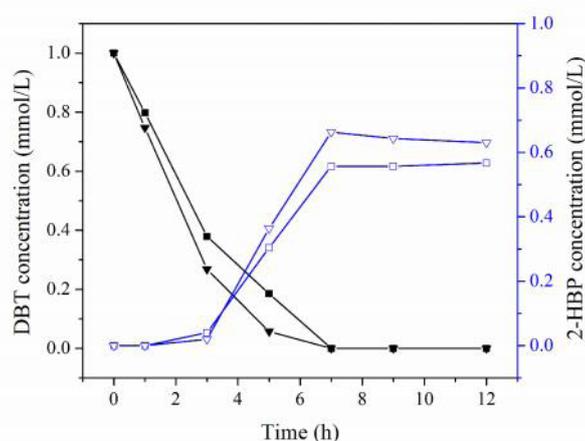


Figure 4. Desulfurization of DBT by free cells and the cells assembled with the nanosorbent: (■) the concentration of DBT consumed by free cells, (▼) The concentration of DBT consumed by the cells assembled with the nanosorbent, (□) The concentration of 2-HBP produced by free cells, (▽). The concentration 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.

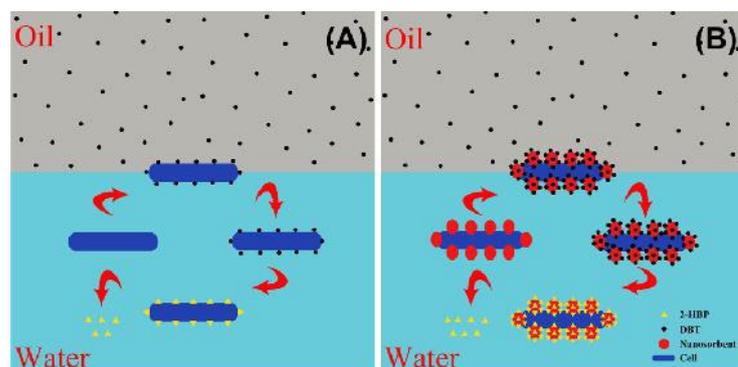


Figure 5. Schematic representation of the desulfurization of DBT performed by (A) the free cells (B) the assembled cells.

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.

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References

- [1] F. Davoodi-Dehaghani, M. Vosoughi, A.A. Ziaee, *Bioresource Technology*, 101, 1102 (2010).
- [2] G. Mohebali, A.S. Ball, B. Rasekh, A. Kaytash, *Enzyme and Microbial Technology*, 40, 578 (2007).
- [3] S. Bhatia, D.K. Sharma, *Biochemical Engineering Journal*, 50, 104 (2010).
- [4] P.S. Kulkarni, C.A.M. Afonso, *Green Chemistry*, 12, 1139 (2010).
- [5] W. Yang, W. Zhou, W. Xu, H. Li, W. Huang, B. Jiang, Z. Zhou, Y. Yan, *Journal of Chemical & Engineering Data*, 57, 1713 (2012).
- [6] A. Alcon, A.B. Martin, V.E. Santos, E. Gomez, F. Garcia Ochoa, *Biochemical Engineering Journal*, 39, 486 (2008).
- [7] X. Cui, D. Yao, H. Li, J. Yang, D. Hu, *J. Hazard. Mater.*, 205-206, 17 (2012).
- [8] Z.A. Irani, M.R. Mehrnia, F. Yazdian, M.

- Soheily, G. Mohebbali, B. Rasekh, *Bioresour. Technol.*, 102, 10585 (2011).
- [9] S. Kumar, V.C. Srivastava, R.P. Badoni, *Fuel*, 90, 3209 (2011).
- [10] W. Li, H. Tang, Q. Liu, J. Xing, Q. Li, D. Wang, M. Yang, X. Li, H. Liu, *Biochemical Engineering Journal*, 44, 297 (2009).
- [11] H.R. Mortaheb, F. Ghaemmaghami, B. Mokhtarani, *Chemical Engineering Research and Design*, 90, 409 (2012).
- [12] M. Seredych, T.J. Bandosz, *Fuel Processing Technology*, 91, 693 (2010).
- [13] J.H. Shan, L. Chen, L.B. Sun, X.Q. Liu, *Energy & Fuels*, 25, 3093 (2011).
- [14] S. Velu, X. Ma, C. Song, *Industrial & Engineering Chemistry Research*, 42, 5293 (2003).
- [15] C.D. Wilfred, C.F. Kiat, Z. Man, M.A. Bustam, M.I.M. Mutalib, C.Z. Phak, *Fuel Processing Technology*, 93, 85 (2012).
- [16] Y. Dong, Y. Nie, Q. Zhou, *Chem. Eng. Technol.*, 36, 435 (2013).
- [17] E. Kaufman, J. Harkins, A. Borole, *Applied Biochemistry and Biotechnology*, 73, 127 (1998).
- [18] V.P. Beškoski, J. Milić, B. Mandić, M. Takić, M.M. Vrvic, *Hydrometallurgy*, 94, 8 (2008).
- [19] J. Calzada, A. Alcon, V.E. Santos, F. Garcia Ochoa, *Biochemical Engineering Journal*, 66, 52 (2012).
- [20] K.J. Kayser, B.A. Bielaga Jones, K. Jackowski, O. Odusan, J.J. Kilbane, *Journal of General Microbiology*, 139, 3123 (1993).
- [21] J. Raheb, S. Ghaffari, M.J. Hajipour, B. Memari, M.E. Kefayati, *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 34, 1318 (2012).
- [22] M. Rashtchi, G.H. Mohebbali, M.M. Akbarnejad, J. Towfighi, B. Rasekh, A. Keytash, *Biochemical Engineering Journal*, 29, 169 (2006).
- [23] V.E. Santos, C. Galdeano, E. Gomez, A. Alcon, F. Garcia Ochoa, *Biochemical Engineering Journal*, 32, 198 (2006).
- [24] M.J. Grossman, M.K. Lee, R.C. Prince, V. Minak-Bernero, G.N. George, I.J. Pickering, *Applied and environmental microbiology*, 67, 1949 (2001).
- [25] K. Boltes, R. Alonso del Aguila, E. Garcia Calvo, *Journal of Chemical Technology & Biotechnology*, 88, 422 (2013).
- [26] S.L. Borgne, R. Quintero, *Fuel Processing Technology*, 81, 155 (2003).
- [27] L. Alves, M. Melo, D. Mendonça, F. Simões, J. Matos, R. Tenreiro, F.M. Gírio, *Enzyme and Microbial Technology*, 40, 1598 (2007).
- [28] W. Chen, F. Bruhlmann, R.D. Richins, A. Mulchandani, *Curr. Opin. Biotechnol.*, 10, 137 (1999).
- [29] X. Jia, J. Wen, Z. Sun, Q. Caiyin, S. Xie, *Chemical Engineering Science*, 61, 1987 (2006).
- [30] B.R. Folsom, D.R. Schieche, P.M. DiGrazia, J. Werner, S. Palmer, *Appl. Environ.*

Microbiol., 65, 4967 (1999).

[31] B. Patel S, J. Kilbane J, A. Webster D, *Journal of chemical technology and biotechnology* (1986), 69, 100 (1997).

[32] H. Zhang, G. Shan, H. Liu, J. Xing, *Surface and Coatings Technology*, 201, 6917 (2007).

[33] M.A. Dinamarca, C. Ibacache Quiroga, P. Baeza, S. Galvez, M. Villarroel, P. Olivero, J. Ojeda, *Bioresource Technology*, 101, 2375 (2010).

[34] A. Mohammadi, J. Badraghi, A.B. Moghaddam, Y. Ganjkhanlou, M. Kazemzad, S. Hosseini, R. Dinarvand, *Chem. Eng. Technol.*, 34, 56 (2011).

[35] H.I. Meléndez Ortiz, L.A. García Cerda, Y. Olivares Maldonado, G. Castruita, J.A. Mercado Silva, Y.A. Perera Mercado, *Ceramics International*, 38, 6353 (2012).

