

Effects of Biodesulfurization by bacterial cell of crude oil toreduce air and the environment pollutions

Elham Karimi¹, Fatemeh Yazdian^{1*}, Behnam Rasekh², Ashrafolsadat Hatamian¹, Abbas Akhavan Sepahy³

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

yazdian@ut.ac.ir

²Department of Petroleum Biotechnology, Biotechnology Research Center, Research Institute of Petroleum Industry, Tehran, Iran

³ Islamic Azad University, North Tehran Branch

Abstract

Combustion of sulfur-containing fossilfuels emits sulfur oxides, which can cause adverse effects on health, environment and economy. Among sulfur oxides, SO₂ is abundant is produced in the lower atmosphere. Furthermore, SO₂ can be the cause of sulfate aerosol formation. Hydrodesulfurization (HDS) has been used to reduce the sulfur composition. Hwever increasingly higher and higher temperatures and pressure are required to improve sulfur removal using HDS, leading to increased costs and atmosphere emissions. To overcome the problem, Biodesulfurization (BDS) has been considered as a potential alternative to the conventional deep HDS processes. Biodesulfurization is a non-invasive approach that can specifically remove sulfur from refractory hydrocarbons under mild condition and it can be potentially used in industrial desulfurization. The Basal Salts Medium(BSM) used for the cultivation/maintenance of this microorganism and further for the desulfurization tests. The strain grew well in the media containing glycerol as the sole carbon and energy source and DBT as the sole sulfur source. The Gibb's assay was used to detect and quantify 2-HBP produced by the strain after incubation with DBT and DBT sulfone. The strain grew well in the media containing glycerol as the sole carbon and energy source and DBT as the sole sulfur source. concomitant with growth, the concentration of 2hydroxybiphenyl increased.

Keywords: Sulfur Oxides, Hydrodesulfurization, Biodesulfurization, Bacterial cell.

Introduction

Petroleum is a naturally occurring and mixture of solids, liquids, gases, mainly hydrocarbons. also, Crude oil has been an important source of energy, particularly in transport and electrical energy[1]. after The carbon and hydrogen, sulfur is the third most abundant element in crude oil, And its amount in crude oil from 0.03 to 7.89 wt% with respect to the source is variable[1,2]. Increased consumption of fossil fuels rich in sulfur, resulting in the release of harmful chemicals such as sulfur oxids. Among sulfur oxides, SO2 is abundant and is produced in the lower atmosphere[3]. The aerosol particles have anaverage diameter of 2.5

µm that can be transported into the lungs and cause respiratory illnesses [2,3]. The SO₂ emissions cause environmental problems such as acid rain, the destruction of buildings and damage to aquaticand terrestrial organisms and agricultural land, and air pollution leads [3,4,5,6]. Since the quality of fossil fuels has direct effect on the environment, decreasing of the sulfur content reduce pollution from burning fossil fuels is essential [1]. Sulfur-containing compounds in crude oil and coalare generally divided into two major groups: inorganic sulfur and organic sulfur. Sulfur compounds in crude oil include thiols, sulfides, polysulfides, thiophenic and alkyl-substituted isomers of thiophenic compounds containing a variety of aromatic rings (i. e. polycyclic aromatic sulfur heterocycles such as thiophene, benzothiophene, dibenzothiophene, and benzon phthothiophene) which are carcinogenic [6,7,8,9,10]. (Figure 1).



Figure(1)- Chemical structure of typical organic sulfur compounds in fossil fuel[10].

There are various desulfurization methods to remove sulfur from fossil fuels. Among these, hydrodesulfurization (HDS) is currently considered as the most important one[1,2,3]. HDSprocessasanefficient technology, not onlyremoval sulfur, but alsofor these paration nitrogen and metals fromthedistillation of variousknown. Conventional HDS is a highpressure (150-200 psig) and high-temperature (200-450°C) catalytic process that converts organic sulfur to hydrogen sulfide gas by reacting crude oil fractions with hydrogen in the presence of an efficient inorganic catalyst [2,3,4,5]. But in the processmany complex moleculesisoftendibenzothiopheneand its derivativescompriseabout70% of thesulfur contentof crude oilremains[8,9,10]. One of the alternative options to remove sulfur from fossil fuel is by biological methods [13]. Biological processes require relatively mild conditions (low pressures and low temperatures), which could be a major advantage of biodesulfurization [14]. It can be noticed that biocatalytic desulfurization offers the petroleum industry several benefits over hydrodesulfurization (HDS) processes: capital cost savings, operating cost saving, flexibility to handle a wide range of petroleum streams, more rapid engineering and construction time, safer and milder conditions[8,9,10].

Method and material

Chemicals

Dibenzothiophene(DBT)were purchased from Merck. 2_Hydroxybiphenyl(2-HBP) was purchased from Sigma.anddimethylformamide (DMF) was from Riedel de Haën.

Bacterial strain

The microorganism used in this study was Gordonarubropertinctus PTCC 1604, that this strain purchased from Iranian Research Organization for Science and Technology (IROST).

Media

Nutrient agar and Nutrient broth culture mediums used for the maitanence of microorganisms. The bacteria were maintained by sub-culturing into a liquid medium or plating on a solid medium (nutrient agar) weekly. The Basal Salts Medium(BSM) used for the cultivation/maintenance of this microorganism and further for the desulfurization tests. This medium contented (gram per liter of deionized water) NH4 Cl (1.2), KH2PO4 (6.0), Na2HPO4 (4), FeCl3 (0.001), MnCl2·4H2O (0.004), MgCl2·6H2O (0.75), CaCl2·2H2O (0.001). All media sterilised by autoclaving at 121 °C for 15 min. for desulfurization experiments by growing cells, cultivations were carried out in 100 ml erlenmeyer flasks containing 50 ml of BSM medium at 30 °Cand under rotary shaking at 120 rpm.

Bacterial growth

Growth of R. erythropolis IGTS8 under their optimal conditions were measured from their optical densities at 600 nm (A600) using a UV/Visible spectrophotometer. Bacterial strain were cultured by using 1 ml inoculum added to a 100 mL of nutrient broth in a 250-mL flask. Flasks were then incubated at 30 °Cand under rotary shaking at 120 rpm. Samples were taken every three hours and monitored for bacterial growth by a spectrophotometer at 600 nm until a stable optical density was reached.

DBT desulfurization ability

Cells were grown until the mid-exponential growth phase and harvested by centrifugation at 6000 rpm for 15 min. The cells were then re-suspended in the same solution to A600 = 1.0 and used on the day of harvesting. One mL of inoculum was added to 250 mL flasks containing 100 mL of BSM with 0.3 mM of DBT-ethanol solution and incubated at 30 °C(100 rpm).

Gibbs assay

The 2-hydroxybiphenyl (2HBP) produced as a consequence of the BDS of DBT was determined using Gibbs reagent (2, 6-dichloroquinone-4-chloroimide)(Figure2).



Figure (2)-conversion of DBT to 2-HBP[9].

The Gibb's assay was used to detect and quantify 2-HBP produced by the strain after incubation with DBT and DBT sulfone. The media must be adjusted to pH 8.0 before the Gibb's reagent is added. Gibb's reagent, the principle reagent of this assay, can react with the aromatic hydroxyl groups at pH of 8.0 to form a blue-coloured complex which can then be monitored spectrophotometerically at 610 nm after 30 min incubation at room temperature. The absorbance of the supernatant determined at 610 nm was converted to concentration (mg/L) with the aid of 2-HBP generated standard curve.

Results

In this study, aerobic bacterial cell were investigate: Rhodococcuserythropolis IGTS8. Growth curves of R. erythropolis in nutrient medium at their optimal temperature are shown in figure 3. Growth patterns of bacteria strain under their respective optimal condition were typical for bacterial growth.



Figure (3)-Growth patterns of bacteria strain

Dibenzothiophene (DBT) as a sole sulfur source in BSM in three concentrations. Production of 2-HBP from DBT degradation at the concentration of 0.3 mM of DBT by growing R. erythropolis was monitored by Gibb's assay and are presented in (Figures4).



Figure (4)- Production of 2-HBP from DBT degradation at the concentration of 0.3 mM of DBT

Discussion and Conclusions

The strain grew well in the media containing glycerol as the sole carbon and energy source and DBT as the sole sulfur source. concomitant with growth, the concentration of 2-hydroxybiphenyl increased. The yield of 2-HBP was maximum at the time of the transition from late exponential phase to stationary phase. It has been reported that 2-HBP is toxic to bacterial cells, hence biodesulfurization is inhibited by accumulation of 2-HBP [10].

Reference

- 1. Gupta, N., Roychoudhury, P.K., Deb, J.K. Biotechnology of desulfurization of diesel: prospects and challenges. Appl Microbiol Biotechnol 66 (2005) 356–366.
- Mohebali, G., Ball, A. S. Biocatalytic desulfurization (BDS) of petrodiesel Fuels. Microbiology 154 (2008) 2169–2183.
- Soleimani, M., Bassi, A., Margaritis, A .Biodesulfurization of refractory organic sulfur compounds in fossil fuels. Biotechnology Advances 25 (2007) 570–596.
- Kilbane, J.J. Microbial biocatalyst developments to upgrade fossil fuels. Current Opinion in Biotechnology 17 (2006) 305–314.
- Raheb, J., Rasekh, B., Irani, Sh., Hajipour, M.J., MozaffariTabatabaei, M., Kefayati, M.E., Memari,B. The study of Biodesulfurization activity in recombinant E. coli Strain by cloning the dsz Genes involve in 4S pathway. Journal of Sciences, Islamic Republic of Iran 22(3) (2011) 213-219.
- Raheb, J., Hajipour, M.J., Saadati, M., Rasekh, B., Memari, B. The Enhancement of Biodesulfurization Activity in a Novel Indigenous Engineered Pseudomonas putida. Iranian Biomedical Journal 13 (2009) 141-147.
- Kilban, J.J.Biodesulfurization of water- soluble coal-derived material by Rhodococcus rhodochrous IGTS8., Institute Gas Technology 40 (1992) 1107-1114.
- 8. Ansari, F., Prayuenyong, P., Tothill, I. Biodesulfurization of dibenzothiophene by Shewanellaputrefaciens NCIMB 8768. Journal of Biological Physics and Chemistry 7 (2007) 75-78.
- Gonçalves Alves, Luís Manuel., Dibenzothiophene desulfurization by Gordoniaalkanivorans strain 1B. PhD thesis, Universidade de Lisboa, (2007).
- 10. Ansari, F. The using of magnetic nanoparticles to enhance biodesulfurization. PhD Thesis, University of cranfield (2008).
- 11. Kilbane, J.J. Desulfurization of coal: the microbial solution. Tibtech 7 (1989) 97-101.
- 12. Kilbane, J.J. Sulfur-specific microbial metabolism of organic compounds. Resource Conservation Recycling, 3 (1990) 69-79.
- Chen, H., Zhang, W-J., Chen, J-M., Cai, Y-B., Li, W. Desulfurization of various organic sulfur compounds and the mixture of DBT + 4,6- DMDBT by Mycobacterium sp. ZD-19. Bioresource Technology 99 (2008) 3630–3634.
- 14. Mohebali, G., Ball, A., Kaytash, A., Behnam, R. Stabilization of water/gas oil emulsions by desulfurizing cells of Gordoniaalkanivorans RIPI90A. Microbiology 153 (2007) 1573–1581.