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DBT desulfurization by decorating bacteria using modified carbon nanotube

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ABSTRACT

After food, fossil fuel is humanity's most important source of energy. Sulfur oxides are produced by the oxidation of the available sulfur in the fuel. Biodesulfurization (BDS) could be an alternative technology to hydrodesulfurization (HDS) to remove sulfur from the recalcitrant organic compounds dissolved in crude oil fractions. It can be seen that all carbon nanotubes (CNTs) exhibited excellent catalytic performance for dibenzothiophene (DBT) oxidation when using molecular oxygen. In this study, chemical modification of Multiple Wall Carbon Nanotubes (MWCNTs) via oxidation followed by side wall functionalization using polyethylene glycol (PEG) was performed to improve the solubility of MWCNTs in aqueous solution. TEM, SEM, FT-IR spectra and Raman spectra were done for characterization of modified MWCNTs. Solubility of the modified MWCNTs studied using different solvents; deionized water, ethanol, and dimethyl sulfoxide (DMSO). Biodesulfurization capability investigated for *Rodococcus erythropolis* IGTS8 and *Gordona rubropertinctus* PTCC 1604 of DBT as sole sulfur source in basal salts medium. The results indicated in the presence of carbon nanotubes growth rate of *R. erythropolis* IGTS8 around 8% increases and *G. rubropertinctus* PTCC 1604 5% reduction was observed after 96 h. Gibb's assay results in the presence of carbon nanotubes showed desulfurization activity of *R. erythropolis* IGTS8 an increase of about 12% and for *G. rubropertinctus* PTCC 1604 was estimated about 15%.

1. Introduction

The most important sources of energy for people, are fossil fuels after food [1]. Since there are several impurities such as particulates and various gases like sulfur dioxide, nitrogen oxides and volatile organic compounds in the fuels, combustion of fossil fuels make waste products. The oxidation of the available sulfur in the fuels leads to production of sulfur oxides. The combination of water vapor, nitrogen oxides and sulfur oxides in clouds to form nitric and sulfuric acids, caused to make acid rain with serious air pollution problems [1,2]. In order to remove the sulfur from fuels, hydrodesulfurization (HDS) is applied in petroleum industry which treats the crude oil [3,4]. This treatment is a catalytic process converting organic sulfur to hydrogen sulfide gas which requires high temperature and high pressure [4,5]. The big part (up to 70%) of sulfur in fuels is found as refractory molecules like dibenzothiophene (DBT) and substituted DBTs [6]. As a disadvantage of HDS, it is unable to remove heterocyclic sulfur compounds such as DBT and DBT derivatives [4]. To overcome this drawback, biological desulfurization (BDS) of fossil fuels may be an alternative process to remove the recalcitrant sulfur. The advantage of BDS, which takes place in oil–water system, as a biological process is that it needs relatively mild conditions (low pressures and low temperatures) [6]. In 1990, Kilbane suggested a sulfur specific pathway (4S pathway) for the purpose of BDS of petroleum products, where the bacteria oxidize the sulfur atom in DBT selectively without cleavage of C—C bonds [6,7]. This pathway metabolizes DBT model molecule via the sequential formation of sulfoxide, sulfone, sulfonate and finally 2-hydroxyiphenyl (2-HBP) [8]. While, some parameters such as desulfurization rate, bioreactor design and the volumetric ratio of oil: aqueous phases are the largest obstacles in BDS industrial application [9]. A study showed

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that the microbial desulfurization activity is affected by mass transfer rate of reactants as an important factor [10]. Setti et al. investigated that the metabolic rate of DBT has an effect on transfer rate of DBT from oil to water and then to the surface of cells [11]. The biosurfactants are effective in the solubility enhancement of hydrocarbon substrates; Certain species of *Rhodococcus,* such as *Rhodococcus erythropolis, Rhodococcus aurantiacus, Rhodococcus ruber, Rhodococcus rubropertinctus, Rhodococcus terrae*, *Rhodococcus percolates* and *Gordona* sp. are also important biosurfactant producers [12]. Although synthetic surfactants have been shown to increase DBT solubility in the aqueous phase, they have been associated with negative effects on biodesulfurization activity due to their toxicity. Biological surfactants (biosurfactants) present several advantages over synthetic surfactants. They are biodegradable, non-toxic, and function even at high temperatures, extreme pH or salinity. As well, they can be produced from renewable raw materials [13].

Using BDS process helping for biocatalyst characterization and improvement by classic microbiological methods and genetic engineering. There are very few reports on BDS process designs and cost analysis [1]. The magnetic immobilization of desulfurization cells was studied by Shan et al. In their study, Fe_3O_4 magnetic nanoparticles were coated on the cells in order to facilitate the reuse of the cells [14]. In another study, BDS activity was enhanced by assembling Nano-γ-Al_2O_3 particles on the magnetic immobilized *Rhodococcus erythropolis LSSE8-1-vgb*. The reaction rates were raised nearly 20% in the first 9 h [15]. Li et al. showed almost 50.8 emu/g saturation magnetization of *R. erythropolis LSSE8-1* cells by using super magnetic Fe_3O_4 nanoparticles modified with oleate. To improve BDS efficiency, these magnetized cells could be separated from the fermentation broth with the aid of an external magnet. The probable reason of enhancing performance of the biocatalytic process, is increment of permeability of the organism cells to DBT [16]. In other study, adsorption of Fe_3O_4 nanoparticles with average size of 45–50 nm on the surface of *R. erythropolis* IGTS8 leads to increase in DBT desulfurization up to 56% [17]. The results of a study showed that harnessing the potential of super-paramagnetic polyvinyl alcohol (PVA) beads for immobilizing the cells of *P. delafieldii* R-8*,* leads to lower cost of desulfurization of DBT in model oil and higher efficiency [14].

Carbon nanotubes (CNTs) are the materials with unique physical and chemical properties. Nowadays, CNTs have more paid attention in biological and biomedical applications, due to their modification which are caused to serve them as protein transporters or drug carriers. While, the low solubility of CNTs in aqueous media is a big problem [18]. Functionalization of CNTs by polymers is a usual method to solve this difficulty. The polymer chains can help the nanotubes dissolve in solvents and improve dispersion of the nanotube bundles [19]. Therefor all CNTs exhibited excellent catalytic performance for DBT oxidation when using molecular oxygen. In study of Zhang et al., CNTs as novel catalysts and molecular oxygen as oxidant for the oxidative desulfurization (ODS) of a model fuel were used. This model contains benzothiophene (BT), dibenzothiophene (DBT) and 4,6-dimethyldibenzothiophene (4,6-DMDBT) at atmospheric pressure and low temperature. It can be seen that all CNTs exhibited excellent catalytic performance for DBT oxidation when using molecular oxygen. It meant that the selectivity of the CNT catalyst for the conversion of the sulfur compound into its corresponding sulfone was 100%. Thus, the yield of oxidation reaction products of these sulfur compounds should be equal to the amount converted of these sulfur compounds. Therefore, the catalytic activity of CNTs requires contact with DBT [20]. Immobilization often mimics what occurs in nature when cells grow on surfaces or within natural structures. Many microorganisms have the ability to adhere to and form a biofilm on different kinds of surfaces in nature. Multiwall CNTs are relatively affordable materials, making them an attractive option as artificial flocculation agents [21]. However, a drawback to their applications is low solubility in water or organic solvents [22]. The CNTs will not disperse in the culture media and will therefore not be accessible to bind to the cells unless they are modified to be soluble. In this study, we suggest the CNTs as nano-sorbents. For increment of the CNT solubility, the polymer polyethylene glycol (PEG) was used. Then it was added to the culture medium microorganisms.

2. Materials and methods

2.1. Chemicals

2-Hydroxybiphenyl (HBP) and dibenzothiophene (99%) were purchased from Fisher scientific (Germany). Gibbs reagent, 2,6-dicholoroquinone-4-chloromide were obtained from Sigma (USA). Acetyl chloride (CH_3COCl), polyethylene glycol (PEG, weight average molecular weight ~1500 g/mol) were purchased from Sigma–Aldrich. DMF from Riedel de Haën (Seelze, Germany) and Multi-Walled Carbon Nanotubes (MWNTs) (outer diameter 15 ± 5 nm, lengths 50 μm by TEM, 95% purity) were purchased from Nanosuny Corporation in Iran and their production method is CVD [23]. All other reagents were of the highest purity commercially available and were used without further purification.

2.2. Bacterial strain and medium

The microorganisms used in this study were *Rodococcus erythropolis* IGTS8 and *Gordona rubropertinctus* PTCC 1604 that obtained from the Research Institute of Petroleum Industry and Research Organization for Science and Technology (ROST). These strains were capable of desulfurizing DBT to 2-hydroxybiphenyl (2-HBP) and sulfite as end-products via a sulfur-specific pathway [16]. The strains were maintained at 4 °C on LB-Agar plates and it was transferred every 14 days. The inoculum was obtained by incubating a loop of bacteria from LB-agar plates into 50 mL of Luria Broth in 250 mL Erlenmeyer flasks agitated at 120 rpm and 30 °C, during 24 h on a rotary shake [5]. The basal salts medium (BSM) used for the cultivation/maintenance of this microorganisms and further for the desulfurization tests, was containing 2.00*g* NH_4Cl, 0.2*g* $MgCl_2$. 6 H_2O, 0.001*g* $CaCl_2$. 2 H_2O, 2.44*g* KH_2PO_4, 5.47*g* Na_2HPO_4, 0.001*g* $FeCl_3{\cdot}6H_2O$, 0.004*g* $MnCl_2{\cdot}4H_2O$ and 2 mL glycerol in 1000 mL deionized water and its final pH was adjusted to 7.5 before autoclaved at 121 °C, 1 atm. for 15 min [22]. DBT dissolved in ethanol was added to give a final concentration of 0.5 mM (100 ppm) as the sole sulfur source. Cells cultivation were carried out at 30 °C on a rotary shaker operated at 120 rpm, when cultured to the mid-exponential growth phase collected by centrifuged at 1400*g* for 10 min, washed 2–3 time with Ringer's solution and stored in – 4 °C [17] The bacterial inoculum was grown (2% from a bacterial frozen stock (glycerol 0.99%) [22].

2.3. Multiple Wall Carbon Nanotube (MWCNTs) modification procedure

Chemical modification of MWCNTs via oxidation followed by side wall functionalization using polyethylene glycol (PEG) was performed to improve the solubility of MWCNTs in aqueous solution [23].

2.3.1. Oxidation of MWCNTs

500 mg of carbon nanotubes were added to 250 mL of a mixture of concentrated sulfuric acid (95%), and nitric acid (52%) (With a volume ratio of 3:1). The mixture was sonicated in a bath for 5 h at 60°C. After cooling to room temperature, the reaction mixture was diluted with 250 mL of deionized water. Then this mixture harvested by centrifugation at 3000 rpm for 10 min. and washed with deionized water for several times until pH 7 was obtained. The product was dried in an oven at 100 °C [24].

2.3.2. Formation of carbonyl chloride groups on MWCNTs

After oxidation of the MWCNTs with the nitric acid and the introduction of carboxylic groups (MWCNTs–COOH), the MWCNTs with