DBT desulfurization by decorating bacteria using modified carbon nanotube

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A R T I C L E    I N F O

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Gordona rubropertinctus PTCC 1604

A B S T R A C T

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. Gibbs 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–S bonds [6,7]. This pathway metabolizes DBT model molecule via the sequential formation of sulfoxide, sulfone, sulfonate and finally 2-hydroxyphenyl (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
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 auranticus, Rhodococcus ruber, Rhodococcus ruberputinus, Rhodococcus terreus, Rhodococcus percolates and Gordana 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, Fe3O4 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-Al2O3 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 Fe3O4 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 Fe3O4 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. delafeldii 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-dimethylbenzothiophene (4,6-DMDT) 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 dibenzo thiophene (99%) were purchased from Fisher scientific (Germany). Gibb’s reagent, 2,6-dichloroquinone-4-chloromide were obtained from Sigma (USA). Acetyl chloride (CH3COCl), polyethylene glycol (PEG, weight average molecular weight ~ 1500 g/mol) were purchased from Sigma–Aldrich. DMF from Riedel de Haën (Seele, Germany) and Multi-Walled Carbon Nanotubes (MWNs) (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 ruberputinus 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 sul fate 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 microorganism and further for the desulfurization tests, was containing 2.00 g NH4Cl, 0.2g MgCl2 6 H2O, 0.001g CaCl2 2 H2O, 2.44g KH2PO4, 5.47g Na2HPO4, 0.001g FeCl3 6H2O, 0.004g MnCl2 4H2O 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 1400g 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
Carboxyl chloride groups (MWCNTs–COCl) were prepared as follows: Oxidized MWCNTs (MWCNTs–COOH), 20 mg, were stirred in 10 mL of dimethylformamide (DMF) and in the presence of 3 mL Acetyl chloride (CH3COCl) of at 50 °C for 24 h. After centrifugation of the reaction solution, the brown–black supernatant was decanted and the remaining solid was washed with water, the remaining solid was dried in an oven at 100°C for overnight [25].

2.3.3. Formation of MWCNTs–PEG
After MWCNTs–COCl had been prepared, the dried solid was reacted with polyethylene glycol (PEG) as follows: MWCNTs–COCl, 5 g, was mixed with 1.2 g of polyethylene glycol (PEG) in 40 mL DMF solvent mixture and stirred for 40 h at 80°C. Hence, 24 mg of PEG is present in per gram of carbon nanotubes. After centrifugation, the black solid was washed with deionized water until the wash-water was clear. The remaining black solid was retained after drying at vacuum [22,25].

2.4. Sample dispersion in solvents
The untreated and treated MWCNTs were tested for dispersion in three solvents with different polarity indexes: water, ethanol, and Dimethyl sulfoxide (DMSO). 5 mg each of the samples was immersed into 10 mL of solvent and sonicated. After standing for 72 h, photographs were taken to evaluate the degree of dispersion.

2.5. Capability of DBT biosulfurization
In this study, DBT as sulfur source in BSM was used for desulfurization capability of R. erythropolis IGTS8 and G. rubropertinctus PTCC 1604. Cells in mid-exponential growth phase were grown. Then, cells were centrifuged at 1400 × g for 10 min and suspended in the same solution to A600=1. Next, 2 mL of inoculum was added to 250 mL were centrifuged at 1400 × g for 10 min and suspended in the same solution to A600=1. Next, 2 mL of inoculum was added to 250 mL of reaction mixture and stirred for 40 h at 80 °C. Hence, 24 mg of PEG is present in per gram of carbon nanotubes. After centrifugation, the black solid was washed with deionized water until the wash-water was clear. The remaining black solid was retained after drying at vacuum [22,25].

2.6. Immobilization of desulfurization cells
The bacteria were grown in BSM until the mid-exponential growth phase and harvested by centrifugation at 1400g for 10 min. The cell pellets were washed twice with Ringer’s solution and re-suspended back in BSM to A600 = 1.0. The cells were then immobilized with PEG-MWCNTs as follows: 10 mL of a suspension containing 10 µg/mL PEG-MWCNTs per mL of water with 100 mL of the cell suspension in BSM with DBT at a final concentration of 0.5 Mm [3,18].

2.7. Experimental design
Optimization of BDS process of DBT at a final concentration of 0.5 mM by growing cells of R. erythropolis IGTS8 and G. rubropertinctus PTCC 1604, in Presence of modified carbon nanotubes by polyethylene glycol (PEG) was carried out in 100 mL scale. Carbon nanotubes concentration (A), initial sulfur concentration (B) and cell concentration (C) that were selected due to several preliminary tests, were regarded as effective factors for this process and the influence of these parameters on sulfur reduction was studied. CCD and RSM were used in order to investigate the relationship between these variables and the optimum levels of them. For this purpose, 16 experimental runs were required as per three-level three-factor fractional factorial CCD. The coded and non-coded values of the variables are shown in Table 1.

2.8. Analytical methods

2.8.1. Bacterial growth and desulfurization activity assay
Cell growth was monitored as turbidity increase in culture medium by reading optical density (OD) at 600 nm with a spectrophotometer [5]. Gibb’s assay was used to screen the conversion of DBT to phenolic compounds [2]. 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-colored complex which can then be monitored spectrophotometrically (Jenway-6505 UV/visible) at 610 nm after 30 min incubation at room temperature [26]. Spectrophotometer and absorbance changes were converted to HBP concentration with the aid of a 2-HBP-generated Standard curve. All desulfurization tests were repeated in triplicate, and the results were presented as mean ± SD and shown with error bars.

2.8.2. Characterization of the chemically modified MWCNTs
Fourier transform infrared (FTIR) measurements of the functionalized MWCNTs were performed on an FTIR Spectrometer (Bruker, Tensor 27, FT-IR spectrometer) using KBr pellets [23]. Raman spectroscopy with a 785-nm laser and diffusive reflectance method (SIN-TERRA (2009) Model, BRUKER (Germany) was applied to characterize the structure of PEG–MWCNTs [25]. Morphological characteristics of PEG–MWCNTs after functionalization with PEGs were examined by Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (JEOL JSM-6400 Digital) [23,25].

3. Results and discussions

3.1. Characterization of Multiple Wall Carbon Nanotube (MWCNTs)

3.1.1. Morphology observation
Morphological characteristics of MWCNTs–PEG after functionalization with PEGs were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (Fig. 1). TEM and SEM images of modified carbon nanotubes by PEG confirm the attachment of shortened MWCNTs with PEGs in comparison with pure MWCNTs, which appear as interconnected networks of tubes [23]. The MWCNTs were wrapped by a PEG shell with a thickness of about 2–6 nm. TEM images of MWCNTs–PEG show that the morphology and length of MWCNTs are well retained; indicating that modified carbon nanotubes by PEG does not affect the tubular structure of MWCNTs [18,25]. Although, side-walled functionalization with PEG did not alter the morphological structure of MWCNTs surfaces significantly, as observed from their SEM images, the diameter of the CNTs upon modification increased slightly.

3.1.2. FTIR spectra
Fig. 1 shows the FT-IR spectra of carboxylate carbon nanotubes (MWNT-COOH) and modified MWNTs–PEG. Spectrum of carboxylate MWNTs, peaks at 1704 cm⁻¹ and 1206 cm⁻¹ correspond to C=O and C–O–C asymmetric stretches, respectively [24]. These spectra confirm that the MWNTs have been functionalized by carboxyl group. The peak at approximately 3500 cm⁻¹ corresponds to an O–H stretch. The peak at approximately 3500 cm⁻¹, characteristic of an H bonded O–H stretch, became more pronounced in MWCNTs–PEG than the oxidized MWCNTs. This is may be due to the introduction of more O–H groups with the PEG on the surface of MWCNTs. A sharp peak at 1730 cm⁻¹ corresponding to C=O stretching vibration of the carboxylic acid became less intense with the MWCNTs–PEG. The MWCNTs–PEG spectrum shows a peak at 1100 cm⁻¹ corresponding to the C–O stretch vibration of the ether group of PEG (same peak appears in pure PEG) [25].

### Table 1
Coded and non-coded values of the variables.

<table>
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<th>Number</th>
<th>Term</th>
<th>Values</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>A Concentration of multi-walled carbon nanotubes (g/mL)</td>
<td>0.0005 0.001 0.0015</td>
</tr>
<tr>
<td>2</td>
<td>B Concentration of sulfur (mM)</td>
<td>0.15 0.3 0.45</td>
</tr>
<tr>
<td>3</td>
<td>C Cell concentration (v/v)</td>
<td>1% 2% 3%</td>
</tr>
</tbody>
</table>
3.1.3. Raman spectroscopy

Raman spectroscopy is a valid method to track the carbon nanotube surface modification, which have been used by researchers [27]. Fig. 2 shows the Raman spectra of the MWCNTs-COOH and MWCNTs-PEG samples excited by a 532-nm laser. We observe an intense peak at $\sim 1342 \text{ cm}^{-1}$ (D band), a weaker peak at $\sim 1595 \text{ cm}^{-1}$ (G band) [19]. D band is attributed to disordered carbon atoms of MWCNTs corresponding to sp3-hybridized and G band is related to the sp2-hybridized carbon atoms. Raman spectroscopy is a suitable method for evaluating solubility and dispersion of carbon nanotubes in soluble form. The ratios of the intensity of the G and D bands (I_G/I_D) are often used to estimate carbon impurities and the density of functional groups on carbon nanotubes. This ratios is reduced for the PEG modified carbon nanotubes [27]. The I_G/I_D ratios decrease from 0.81 to 0.71 for the PEG modified MWCNTs.

3.2. Modified Multiple Wall Carbon Nanotube Solubility

Solubility of the modified MWCNTs were studied using different solvents; deionized water, ethanol, and Dimethyl sulfoxide (DMSO). Fig. 3 shows the photos of pristine MWCNTs, and MWCNTs–PEG, suspensions in different solvents. It is clear from the Figure that pristine MWCNTs were completely insoluble (un-suspendable) in water. This is due to the hydrophobicity of the MWCNTs, which allows them to be suspended in non-polar solvents. The MWCNTs–PEG was completely soluble in all three solvents, however MWCNTs–PEG solubility in ethanol is low, as the results indicated PEG–MWCNTs have fairly good solubility in water and is stable for up to seven days.

3.3. Bacterial growth and desulfurization activity assay

The cell growth and production of 2-HBP from DBT degradation at the concentration of 0.5 mM by growing R. erythropolis IGT8 and G.
rubropertinctus PTCC 1604 were monitored by measuring optical density (OD) at 600 nm and Gibbs assay, respectively [28]. Fig. 4 compared the growth rate of bacterial cells in basal salt medium (BSM), in A) Absence or B) Presence of modified carbon nanotubes by polyethylene glycol (PEG) C) Unmodified carbon nanotubes. The results showed the highest growth rate for the microorganisms can be obtained in 96 h and a phase delay for any two microorganisms were detected in the first stage of growth. This phase is short for microorganism R. erythropolis IGTS8 and faster than G. rubropertinctus PTCC 1604 to enter the log phase. We also showed that the cellular growth rate was reduced in the presence of unmodified carbon nanotubes, so unmodified CNTs may have a toxic effect on the cultures.

The DBT desulfurization was evaluated measuring the 2-HBP production (the final of DBT desulfurization) [14]. The evolution of
The biodesulfurization activity of bacterial cells was performed with the spectrophotometric Gibb's assay and the results were presented in terms of 2-HBP production during 148 h cycles. In Fig. 5, 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 cultivation broth was observed only after 48 h which might suggest the existence of a concentration-dependent efflux system [26]. In Fig. 5 spectrophotometric Gibbs assay results showed that the accumulation of 2-HBP in cultivation broth was observed only after 24 h. In hand other, the maximal extracellular concentration for *R. erythropolis* IGTS8 was about 0.357 mM in 96 h and 0.399 mM in 148 h in absence and presence of modified carbon nanotubes by polyethylene glycol (PEG). Production of 2-HBP in presence of MWCNTs-PEG were significantly different from their absence. It can be seen that whereas desulfurization activity rate of *R. erythropolis* IGTS8 in absence MWCNTs-PEG decreasing after about 96 h, while in presence of MWCNTs-PEG continue producing more vigorously until at least 148 h. In order, the results shown that in presence *R. erythropolis* IGTS8 had a 12% and *G. rubropertinctus* PTCC 1604 16% higher desulfurization activity compared to the absence of modified multiple carbon nanotubes. We also showed that the BDS activity rate was reduced in the presence of unmodified carbon nanotubes. All desulfurization tests were repeated in triplicate, and the results were presented in Figs. 4 and 5 as mean ± SD and shown with error bars.

![Table 2](image)

<table>
<thead>
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<th>A</th>
<th>B</th>
<th>C</th>
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<tbody>
<tr>
<td>0.55</td>
<td>-0.94</td>
<td>-0.96</td>
<td>3.2684</td>
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R-Squared: 0.9202.

\[ R1 = 3.16 + 0.022A - 0.041B - 0.11C - 0.14 AB + 0.040 AC - 0.26 A^2. \]

![Table 3](image)

<table>
<thead>
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<th>A</th>
<th>B</th>
<th>C</th>
<th>R1</th>
</tr>
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<tr>
<td>-0.92</td>
<td>-0.92</td>
<td>-0.92</td>
<td>3.539</td>
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R-Squared :0.9735.

\[ R1 = 3.36 - 0.045 A + 7.000E - 003B + 0.053 C + 0.12 AB - 0.040 AC + 0.068. \]
3.4. Regression analysis

The growth rate of *R. erythropolis* IGTS8 and *G. rubropertinctus* PTCC 1604 in optimal conditions shown in Fig. 6. The Figure (I) is understood that by increasing the parameter A (concentration of multi-walled carbon nanotubes) to +1 (0.0015 g/ml) and increase parameter B (concentration of sulfur) to +1 (0.45 mM) the value of R1 (growth rate) Response is increased that equivalent to an increase absorbance at nm 600. As regards, the mass transfer rate of Sulfur compounds is very low and carbon nanotubes as a new generation of adsorbents, Availability of organic sulfur compounds by adsorption are increasing to bacterial cells. So by increasing the concentration of carbon nanotubes, greater concentration of sulfur compounds is used by microorganisms, as well as grow and desulfurization rate increased. Fig. 6 shows three-dimensional diagram of variables interaction in the growth rate of (I) *R. erythropolis* IGTS8 and (II) *G. rubropertinctus* PTCC 1604.

Table 4
The optimum conditions to R2 (2-HBP concentration) respond for the microorganisms *R. erythropolis* IGTS8.

<table>
<thead>
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<th>A</th>
<th>B</th>
<th>C</th>
<th>R2</th>
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<tbody>
<tr>
<td>0.84</td>
<td>-0.95</td>
<td>-0.93</td>
<td>0.479066</td>
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</table>

R-Squared: 0.9197.

\[ R^2 = 0.47 + 4.200E^{-0.020} A - 0.025 AB + 7.375E^{-0.020} AC - 0.048 A^2. \]

Table 5
The optimum conditions to R2 (2-HBP concentration) respond for the microorganisms *Gordona rubropertinctus* PTCC 1604.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.98</td>
<td>-0.12</td>
<td>0.96</td>
<td>0.441055</td>
</tr>
</tbody>
</table>

R-Squared: 0.8822.

\[ R^2 = 0.36 + 4.000E^{-0.003} A + 4.000E^{-0.003} C - 1.375E^{-0.003} BC - 0.016 B^2 + 0.042 C^2. \]
compounds to the microorganisms can be done in the presence of carbon nanotubes. Therefore, High growth rate of bacterial cell can be seen even at low levels of sulfur compounds. Table 3 shows The optimum conditions to R1 respond for the microorganism’s G. rubropertinctus PTCC 1604.

Fig. 7D shows, the desulfurization activity rate of R. erythropolis IGTS8 and increased in high concentration of carbon and sulfur compounds. It represents the effective transformation of sulfur-containing compounds. Table 4 shows the optimum conditions to R2 (2-HBP concentration) respond for the microorganisms R. erythropolis IGTS8.

Fig. 7D shows, the desulfurization activity rate of G. rubropertinctus PTCC 1604 increases in a low concentration of carbon and sulfur compounds. Because, as the results of cell growth rate was observed, the high concentration of carbon nanotubes reduces growth rate and also long leg phase, on the other hand, of surfactant production by microorganisms enough of sulfur compounds even at low levels is available as well. Table 5 shows the optimum conditions to R2 (2-HBP concentration) respond for the microorganisms G. rubropertinctus PTCC 1604.

4. Conclusion

Biological desulfurization is carried out at ambient temperature and pressure and reduces the costs of setting up and operating. It also removes sulfur from organic compounds selectively and without destroying the carbon skeleton and maintaining the heating value of the fuel. However, because low rate of mass transfer, multi-phase system and biocatalyst separation at the end of the process, for industrial use biological desulfurization, increase the activity of microorganisms and their separation is required [14,29,30]. Zhang et al. in 2014, the carbon nanotubes used for catalytic activity in oxidative desulfurization process [28]. The low solubility of carbon nanotubes is their main disadvantage, so to use them as Nano-absorbent in multi-phase systems requiring functionalization and modification of their surface [19,25]. In this study, the carbon nanotubes were carbonylated then were coated with the polymer polyethylene glycol. The average diameter of the outside wall of pure multiwall carbon nanotubes is 15–5 nm, compare TEM images of pure multiwall carbon nanotubes and coated with polyethylene glycol and show that the polymer is connected successfully. In this study, Raman spectroscopy study of multiwall carbon nanotubes coated with polyethylene glycol showed two Spectrum D and G in the range of 1344 and 1595 cm$^{-1}$ respectively. Fourier transform infrared spectroscopy (FTIR) study shows the presence of carboxyl, hydroxyl and carbonyl groups that indicating carbon nanotubes are carbonylate correctly. The presence related spectrum to –CH groups represents multiwall carbon nanotubes is cover correct with polyethylene glycol polymer. In this study, solubility and stability of multiwalled carbon nanotubes in solvents, deionized water, ethanol and dimethyl sulfoxide (DMSO) showed that the carbon nanotubes coated in deionized water was solubility more than other solvents and their stability was maintained for up to a week. Growth and desulfurization activity of both microorganisms showed 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. In fact, the most important effect of carbon nanotubes on the growth of G. rubropertinctus PTCC 1604 is to increase the lag phase. Gibbs assay results showed in the presence of carbon nanotubes, the production rate of 2-hydroxy biphenyls by R. erythropolis IGTS8 increase up to 148 h, while in the absence of carbon nanotubes highest desulfurization activity and the production of 2-hydroxy biphenyls was in 96 h. So, de-sulfurization activity of the R. erythropolis IGTS8 in the presence of carbon nanotubes showed an increase of about 12%. As well as, the highest desulfurization activity and the production of 2-hydroxy biphenyls for G. rubropertinctus PTCC 1604 in the absence and presence of carbon nanotubes were in 96 h. So, their desulfurization activity in the presence of carbon nanotubes was estimated at about 15%. If BDS takes place in the cytoplasm the bacterial surface comprises the rate limiting barriers to the process of DBT transportation into the cell or HBP out [26]. The observation of significantly increased of HBP production in bacteria cells in the presence of CNTs suggests that the CNTs somehow facilitate transportation of DBT inside and facilitate transportation of HBP out of the cells assuming that it is produced in the cytoplasm by increasing the permeability of the membrane and increased availability of DBT [17]. The only reason of CNTs functionalization, is to increase their solubility and stability in aquatic medium and reduce toxicity, in order to increase their ability to increase the availability of DBT for bacterial cells. The effect of carbon nanotubes as Nano-sorbent on desulfurization is increasing the mass transfer rate. In this study, the effective variable on biodesulfurization processes was optimized in the presence of carbon nanotubes.

References


