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EXPERIMENTAL ARTICLES

RSM Optimization of Dibenzothiophene Biodesulfurization by Newly Isolated Strain of *Rhodococcus erythropolis* PD1 in Aqueous and Biphasic Systems[1]

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Abstract—Dibenzothiophene (DBT) is a recalcitrant organic sulfur compound which remains in the crude oil after hydrodesulfurization (HDS) process and can be removed by biodesulfurization (BDS). The objective of this study was the isolation of novel strain capable more BDS rate and optimization of DBT removal by both growing and resting cells. Response surface Methodology (RSM) was applied for evaluating the interactive effects of three independent factors including DBT concentration, temperature and pH. The three factors Box-Benken design with three center points was performed to generate the optimum condition for DBT removal by growing cells in aqueous medium and resting cells in biphasic medium. Among the isolated bacteria from oil-contaminated soil, a gram-positive, non-spore forming isolate designated PD1 showed the high BDS rate and capable to convert the DBT to 2-hydroxybiphenyl (2-HBP) as the final product. Analysis of variance (ANOVA) demonstrated that all of the studied parameters in the growing cells system showed significant effect on BDS rate, while in the resting cells effect of pH was not significant ($P > 0.05$). Maximum 2-HBP production (0.21 mM) by growing cells of PD1 strain was obtained at 0.38 mM initial DBT concentration, pH 6.88 and temperature of 27.57°C. For resting cells, maximum BDS activity of PD1 strain was determined as 0.46 μM 2-HBP/min g DCW at optimum pH 6.29, temperature of 26.13°C and DBT concentration of 7.73 mM. The BDS efficiency of *Rhodococcus erythropolis* PD1 (NCBI Gene Bank Accession no. JX625154) was increased by setting each factor at the optimum level.

Keywords: *Rhodococcus erythropolis* PD1, dibenzothiophene (DBT), 2-hydroxybiphenyl (2-HBP), Response Surface Methodology (RSM)

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Sulfur is the third most common element in the crude oil composition after carbon and hydrogen which causes severe environmental pollutions [1]. For example, burning the petroleum based fuels containing sulfur components will release SOx gases to the atmosphere leading to acid rain and corrosion problems. Consequently, strict standards have been stipulated by environmental agencies to prevent the production of such pollutants. As a case in point, US Environmental Protection Agency (EPA) mentioned that the amount of sulfur in mobile and non-mobile sources should not be above 15 mg/L [2]. Therefore, deep desulfurization of the crude oil is compulsory to meet the environmental standards [3].

Hydrodesulfurization (HDS) process which has been usually employed in oil refineries, works at high temperatures and pressures (more than 300°C and 100 atm) [4] and is effective in removing a large amount of inorganic and a part of organic sulfur from the crude oil. However, the major part of organic sulfur content is resistant to HDS and remains in the oil composition after this treatment [5]. As a remedy, Biodesulfurization (BDS) process has been suggested to remove the recalcitrant organic sulfur compounds like dibenzothiophene (DBT) which is often used as a model of heterocyclic sulfuric compounds in biodesulfurization studies. In fact, BDS has several advantages which make it a potentially alternative or at least a complementary process to the HDS. For instance, BDS is more specific and cost effective and takes place at ambient temperature and pressure [6, 7]. In addition, the combustion value of the oil would not be affected in the BDS process because the effective bacterial strains (such as *Rhodococcus* and *Gordonia*) can remove the sulfur through 4S-pathway which attacks to the C–S bounds selectively [6].

Generally, there are four enzymes involve in the 4S-pathway: first, DBT monooxygenase (Dsz C) oxidizes DBT to DBT-sulfone ($DBTO_2$). In the second step, flavomonooxygenase (Dsz A) catalyzes transfor-

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Table 1. Coded values of experimental variables in growing and resting cells

Independent variables	Growing cells			Resting cells		
	−1	0	+1	−1	0	+1
X_1: DBT concentration, mM	0.2	0.5	0.8	1	5.5	10
X_2: Temperature, °C	26	29	30	24	28	32
X_3: pH	6	7	8	5	6.5	8

mation of $DBTO_2$ to 2,2'-hydroxyphenyl benzene sulfinate (HPBS) and in the final step, HPBS is desulfinated by Dsz B to produce 2-hydroxybiphenyl (2-HBP) as the final product [8]. Three catabolic genes, *dsz* A, B, C, are clustered in the *dsz* operon. The fourth enzyme (Dsz D) prepares $FMNH_2$ required for oxygenase reactions and is a chromosomal gene [9]. Clearly, performance and activity of the enzymes in the 4S-pathway is depended on their operating conditions and a high BDS efficiency could be achieved at the optimum level of the effective parameters. Although BDS process has been studied for physiology of microorganisms and their gene modifications [10] as well as the metabolic pathways and kinetics of desulfurization in the model and diesel oil [11], research on the significance of operational factors and their optimization through statistical methods are rare. Response Surface Methodology (RSM) is one of the well known statistical methods which has been utilized to find a relationship between a few effective variables and one or more responses in a system. In this approach, analysis of variance (ANOVA) and regression techniques have been employed to estimate a low degree polynomial model for optimization of the levels of significant explanatory variables in a limited number of experiments [12].

In the present study, *Rhodococcus erythropolis* PD1 which has been isolated from an oil contaminated soil sample was employed to investigate the effects of important operational conditions such as initial DBT concentration, temperature and pH on the BDS efficiency. Box-Behnken RSM was used to determine the optimum value of these factors in BDS process for both resting cells and growing conditions in aqueous and biphasic (aqueous/model-oil) systems. Finally, the BDS efficiency was determined in the obtained optimum conditions.

MATERIALS AND METHODS

Chemicals. Methanol (HPLC grade) was purchased from Sigma-Aldrich (USA). DBT and *n*-tetradecane were purchased from Merck (Germany). Meanwhile 2-HBP was prepared from Fluka Chemika Co. (USA). All other chemicals were analytical grade and commercially available.

Enrichment, isolation and identification of DBT desulfurizing bacteria. Double deionized water was used to prepare sulfur free basal salt medium (BSM). The BSM contains the following composition: $Na_2HPO_4 \cdot 7H_2O$ 8 g/L, KH_2PO_4 4 g/L, NH_4Cl 2 g/L, $MgCl_2$ 0.2 g/L, $FeCl_3$ 0.001 g/L and $CaCl_2$ 0.001 g/L. To isolate effective microorganisms for BDS, more than 100 oil contaminated soil samples were collected from various areas in Iran. One gram of each soil sample was suspended in 50 mL of BSM supplemented with 10 g/L glucose and 0.3 mM DBT as the sulfur source in a 250 mL flask. After incubation for 3 days at 30°C and 180 rpm on a rotary shaker, 5 mL of supernatant was inoculated in 45 mL of fresh BSM supplemented with 0.3 mM DBT and 10 g/L glucose. Subculturing was repeated with 1% v/v inoculums five times. Afterwards, streak culturing was done onto the same medium with 9 g/L agarose. Finally, a single colony was isolated and 2-HBP production was detected by Gibb's assay. One of the Gibb's positive isolated strains designated PD1, was genetically identified by partial 16S rDNA sequencing using DG74 and RW0l general primers.

Characterization of growth and biodesidfurization. One mL of isolated *R. erythropolis* PD1 cells suspension ($OD_{600} = 1$) was inoculated in 100 mL BSM supplemented with 0.3 mM DBT and 15 g/L glucose in a 500 mL flask and incubated at 30°C and 180 rpm shaking for 4 days. During the course of cultivation, aliquots of culture were collected every 6 hours and analyzed for measuring the growth of bacteria, DBT consumption and 2-HBP production by turbidimetry (at 600 nm), UV spectrophotometry (323.8 nm) and Gibb's assay (610 nm) respectively.

Optimization of biodesulfurization in the aqueous system. The optimization of growing cells was conducted in aqueous medium. The aqueous medium was BSM supplemented with different amounts of DBT and 10 g/L glucose. 0.5 mL of cell suspension with OD_{600} of 1 was inoculated to 50 mL of medium and 2-HBP production was measured after 48 h.

Experimental design for growing cells. Initial DBT concentration (X_1), temperature (X_2) and pH (X_3) were considered as the important factors in BDS activity of growing and resting cells. A 3-factor and 3-level Box-Behnken design (BBD) was used to determine the optimum level of the important factors and to study their relationship to the BDS efficiency. Factors and their levels are shown in Table 1. All of the main and interactive effects were investigated by response

surface model. The model of coded units is calculated using equation (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3, \quad (1)$$

where Y is the predicted response, β_0 is the ground average, X_i is the variable, β_i is the linear effect, β_{ii} is the quadratic effect, and β_{ij} is the interaction effect.

A 0.05 significance level was assumed to perform ANOVA for the model coefficients. A total of 15 experimental runs of three factors in different combinations were carried out in duplicates (Table 2). All experimental design and data analysis were performed using Design Expert software package version 8.0.1.

Analytical methods. Cell density was measured by spectrophotometry at 600 nm (OD_{600}) (spectronic 21D Milton Roy). In the growing cells system, BDS activity was monitored using the Gibb's reagent (2,6-dichloroquinone-4-chloroimide) because the levels of 2-HBP and DBT were lower than the detection limit of high-performance liquid chromatography (HPLC). Gibb's reagent reacts with aromatic hydroxyl groups such as 2-HBP at pH 8.0 and forms a blue-colored complex that can be assayed spectrophotometrically at 610 nm. To measure the DBT consumption in the growing cells systems, their pH was adjusted to 2 before mixing with equal volume of ethyl acetate. After centrifuging at 3000 rpm for 5 min, extracted DBT in the organic phase was measured by UV spectrophotometry at 328 nm.

Optimization of biodesulfurization in the biphasic system. The optimization of resting cells was conducted in biphasic system. The biphasic medium consist of BSM (aqueous phase), *n*-tetradecane (organic phase) and different amount of DBT as a sulfur source, were prepared to monitor the BDS activity by the resting cells. The ratio of aqueous to oil phase volume was 2 : 1. For preparation of resting cells, bacterial cells grown on BSM at the late exponential phase were centrifuged at 7000 *g* for 10 min. The harvested cells washed twice, resuspended in a saline serum and inoculated in biphasic medium. Biomass concentration of 15 g/L of dry cell was used in all assays. 2-HBP production was determined after 20 h.

Experimental design for resting cells. For resting cells design, we increased the range between high and low levels of factors (Table 1) to better illustrate the impact of variables.

Analytical method. In the resting cells system HPLC analysis was used to determine the amounts of DBT (retention time = 5.29 min) and 2-HBP (retention time = 3.16 min) in *n*-tetradecane phase. HPLC was performed on a KNAUER advanced scientific instruments (Germany) equipped with an MZ-analysentechnic C18 column (5μ–250 mm) and a UV detector (Smartline 2600) set at 254 nm. The mobile phase was methanol–water (90 : 10, v/v) with a flow rate of 1.5 mL/min.

Table 2. Response surface Box-Behnken design (BBD) and 2-HBP produced by *Rhodococcus erythropolis* PD1 growing cells

Run	A			2-HBP, mM
	X_1, mM	X_2, °C	X_3	
1	0.5	26	8	0.14
2	0.5	29	7	0.20
3	0.2	29	6	0.17
4	0.2	29	8	0.15
5	0.5	30	6	0.14
6	0.8	30	7	0.12
7	0.5	29	7	0.21
8	0.2	26	7	0.18
9	0.2	30	7	0.16
10	0.8	29	8	0.11
11	0.5	30	8	0.10
12	0.5	29	7	0.21
13	0.5	26	6	0.16
14	0.8	26	7	0.15
15	0.8	29	6	0.14

A: growing cell.

RESULTS

Identification of the isolated PD1 strain. The numerous bacterial colonles isolated from diesel contaminated soils were checked for 2-HBP production from DBT as the sole sulfur source. The Gibb's analysis showed that among all of the isolates, PD1 strain had the highest capability in DBT desulfurization and converting it to 2-HBP as the final product. Since 2-HBP production indicates selective cleavage of carbon-sulfur bond, further characterization and optimization was performed on this strain. Gram and acid-fast staining, colony morphology and biochemical activities revealed the PD1 strain as a gram-positive coccoid and short rod, non-spore forming, non-motile, catalase positive and acid-fast bacterium. Partial nucleotide sequence analysis of 16S rRNA gene implied 99% homology with *Rhodococcus erythropolis* HS19 (NCBI GenBank accession no. AY168597). Therefore, PD1 was identified as a novel strain, *Rhodococcus erythropolis* PD1 (NCBI GenBank Accession no. JX625154).

Growth characterization. The growth curve of *R. erythropolis* PD1 has been shown in Fig. 1. The growth was continued for 96 h, simultaneously with the consumption of DBT and the production of 2-HBP. As shown in Fig. 1, the production of 2-HBP is less than DBT consumption.

RSM analysis. The fitted surface and contour plots between DBT concentration and temperature, DBT concentration and pH, temperature and pH have been

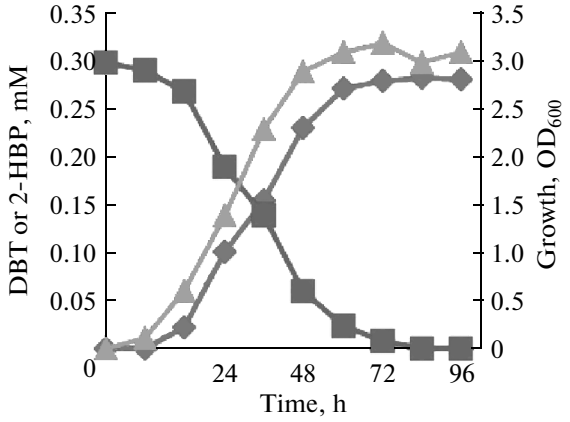

Fig. 1. Growth of *R. erythropolis* PD1, DBT utilization and 2-HBP production during cultivation in BSM medium containing 0.3 mM DBT as sole sulfur source and glucose as sole carbon source incubated at 30°C. The symbols: ■, DBT concentration (mM); ◆, 2-HBP concentration (mM); ▲, growth (OD_{600}).

presented in Fig. 2 and Fig. 3 for growing and resting cells systems, respectively.

Growing cells. A quadratic polynomial equation was estimated to describe the relationship between 2-HBP production of growing cells and variables based on the experimental results of BBD (Table 2). In the growing cells system, the empirical model of coded variables can be expressed as the equation (2) after removing non significant parameters:

$$Y = 2.06 - 0.19X_1 - 0.13X_2 - 0.13X_3 + 0.037X_1X_2 + 0.047X_2X_3 - 0.24X_1^2 - 0.30X_2^2 - 0.042X_3^2. \quad (2)$$

Where Y is the response value (mM), X_1 is the initial DBT concentration (mM), X_2 is temperature (°C) and X_3 is pH. Positive and negative signs before each term indicate synergistic and antagonistic effects respectively [13]. The ANOVA results for 2-HBP production by growing cells have been shown in Table 4. In this case X_1, X_2, X_3, X_1X_2, X_2X_3, X_1^2, X_2^2 and X_3^2 were significant model terms as their calculated P values were less than the 0.05 significance level. The proposed model was proved to be adequate through calculation of the "Lack of Fit" equal to 0.3 and adjusted determination factor (AdjR^2) equal to 0.9927. This means that the Lack of Fit is not significant relative to the pure error and the model predictions were well fitted the experimental results. According to the present model, all of the main factors were significant in the interval of variation and temperature has significant interactions with initial DBT concentration and pH.

Figure 2a shows that the optimum concentration of DBT in growing medium for PD1 strain was 0.38 mM and BDS activity was reduced by both the increasing initial DBT concentration up to 0.8 mM or decreasing it to 0.2 mM due to the limit in the growth rate.

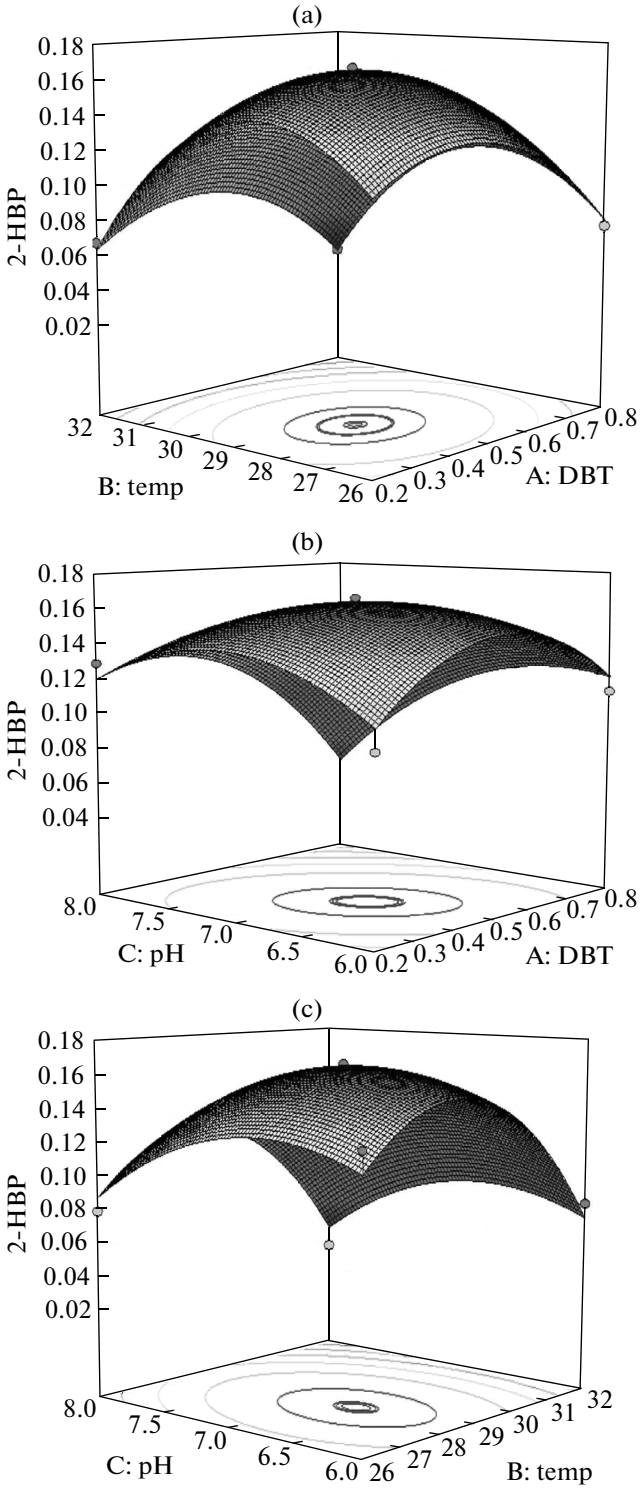

Fig. 2. The response surface and contour plot of 2-HBP production by growing cells of *R. erythropolis* PD1 in aquatic system, (a): interaction of DBT concentration (mM) and temperature (°C), (b): interaction of DBT concentration (mM) and pH, and (c): interaction of temperature (°C) and pH on 2-HBP production.

R. erythropolis PD1 is a mesophilic bacteria and its optimum temperature for BDS of DBT in growing medium was determined at 27.57°C. The surface and contour plot in Fig. 2b indicates that in high and low temperatures, 2-HBP production was low because of the limiting influence of temperature on the growth rate and enzyme activity.

Figure 2c shows the surface and contour plots of pH for 2-HBP production in growing cells system. The optimum pH in growing condition was 6.88 and as shown in Fig. 2c, by a change in pH, 2-HBP production was reduced. The optimum of DBT concentration, temperature and pH for converting the DBT to 2-HBP in the aqueous medium by growing cells were at 0.38 mM, 27.57°C and 6.88, respectively.

Resting cells. The result of 2-HBP production by resting cells was shown in Table 3. The relationship between the 2-HBP production of resting cells and the studied variables after removing non significant parameters has been stated as equation (3);

$$Y = 0.78 + 0.053X_1 - 0.061X_2 - 0.055X_1X_2 - 0.1X_1X_3 - 0.093X_1^2 - 0.095X_2^2. \quad (3)$$

In this equation, all of the coded variables are defined similar to equation (2) in the previous section. The ANOVA results for 2-HBP production by resting cells have been shown in Table 4. P-value of the model was obtained equal to 0.0081 which approved the model significance. In this case, X_1, X_2, X_1X_3, X_1^2, X_2^2 and X_3^2 were significant model terms. The Lack of Fit value of 4.78 indicated the Lack of Fit was not significant relative to the pure error and the adjusted coefficient of determination ($AdjR^2 = 0.8672$) confirmed the significance of the model. In the resting cells BDS, DBT initial concentration, temperature and also the interactions between these factors were significant.

Also in biphasic medium and resting cells system, the optimum concentration was determined at 7.73 mM and as can be seen in Fig. 3a, the BDS activity was reduced by going away from the optimal point. For the resting cells, the optimum point for the initial DBT concentration was far more than the growing cells.

In resting cells, the optimum temperature for the 2-HBP production was 26.13°C (Fig. 3b). Although the growth rate limiting effect was absent in this case, the activity of enzymes can be reduced at the higher or lower temperatures.

Figure 3c shows the surface and contour plots of pH for 2-HBP production in resting cells system. In resting cells BDS of model oil, the optimum pH was obtained equal to 6.29. In contrast with the growing cells, the pH factor in resting cells was not statistically significant (P value = 0.24). In addition, interactions between pH and temperature was not significant (P value = 0.75) (Fig. 3c) in this system.

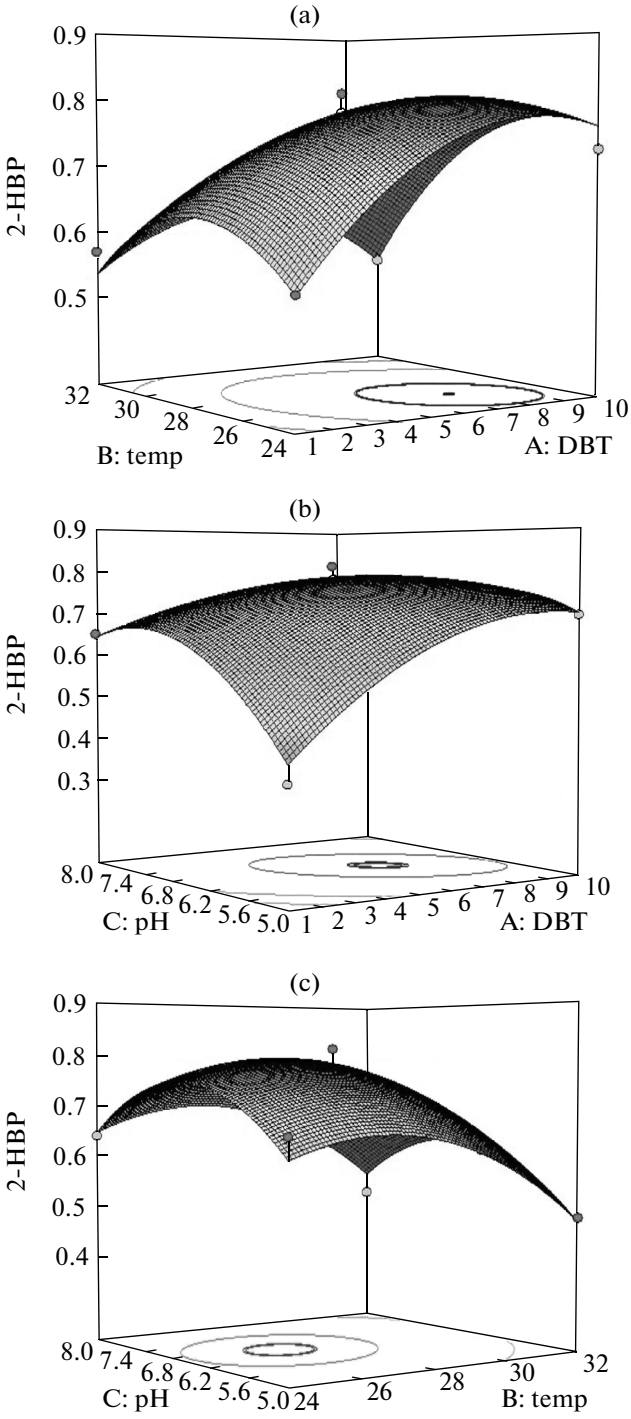

Fig. 3. The response surface and contour plot of 2-HBP production by resting cells of *R. erythropolis* PD1 in biphasic system. Interaction between (a): DBT concentration (mM) and temperature (°C), (b): DBT concentration (mM) and pH, (c): temperature (°C) and pH on 2-HBP production.

The optimum levels of DBT concentration, temperature and pH for maximum biodesulftirization rate by resting cells in the biphasic system were obtained at

Table 3. Response surface Box-Behnken design (BBD) and 2-HBP produsd by *Rhodococcus erythropolis* PD1 resting cells. Biodesulfurization (BDS) activity is calculated in the base of measuring micromol 2-HBP per min g dry CW

Run	B			2-HBP, mM	BDS activity, μM 2-HBP/min g DCW
	X_1, mM	X_2, °C	X_3		
1	1	24	6.5	0.36	0.20
2	1	28	5	0.55	0.31
3	1	28	8	0.59	0.33
4	5.5	28	6.5	0.49	0.28
5	10	28	5	0.50	0.28
6	5.5	28	6.5	0.66	0.37
7	10	28	8	0.70	0.39
8	5.5	32	5	0.53	0.30
9	5.5	24	8	0.76	0.43
10	1	32	6.5	0.73	0.41
11	10	28	6.5	0.64	0.36
12	10	32	6.5	0.78	0.44
13	5.5	24	5	0.81	0.46
14	5.5	28	6.5	0.57	0.32
15	5.5	32	8	0.65	0.37

B: resting cell.

7.73 mM, 26.13°C and 6.29 respectively. In these optimal conditions the maximum BDS activity of *R. erythropolis* PD1 resting cells was determined as 0.46 μM 2-HBP/min g DCW.

DISCUSSION

The production of 2-HBP by *R. erythropolis* PD1 is less than DBT consumption during growth of the biocatalyst, which previously suggested by Caro et al. [14]. This is due to the accumulation of 2-HBP and other 4S-pathway compounds inside and on the surface of cells.

The response surface and its contour plots at the base can represent the regression model developed to investigate the interaction between factors and specify the optimum level of each factor. The interaction of two independent factors can be shown by each response surface with a contour plot while another factor is fixed at the level of zero.

Results indicated that an increase in the initial DBT concentration would make DBT more available to the cells and led to an enhancement in BDS. However, at higher initial concentrations of DBT beyond a critical value, bacterial growth and BDS activity would be inhibited, presumably because of the toxicity of DBT at high concentrations more than the tolerating level of bacteria [15]. Such inhibitory effect of DBT

Table 4. Analysis of variance (ANOVA) for 2-HBP production by growing or resting cells

Source of variance	*d.f.*	Growing cells				Resting cells			
		model coefficients	mean square	F value	P value	model coefficients	mean square	F value	P value
Model	9		0.18	211.57	<0.0001		0.023	11.15	0.0081
Constant		2.06				0.78			
X_1	1	−0.19	0.27	328.56	0.0001	0.053	0.022	10.66	0.0223
X_2	1	−0.13	0.13	150.00	<0.0001	−0.061	0.030	14.51	0.0125
X_3	1	−0.13	0.14	168.54	<0.0001	N.S.	3.613E-003	1.75	0.2435
X_1X_2	1	0.037	5.625E-003	6.75	0.0484	−0.055	0.012	5.85	0.0602
X_1X_3	1	N.S.[1]	1.225E-003	1.47	0.2795	−0.1	0.040	19.34	0.0070
X_2X_3	1	0.047	9.025E-003	10.83	0.0217	N.S.	2.250E-004	0.11	0.7549
X_1^2	1	−0.24	0.20	245.56	<0.0001	−0.093	0.032	15.41	0.0111
X_2^2	1	−0.30	0.33	399.88	<0.0001	−0.095	0.034	16.25	0.0100
X_3^2	1	−0.042	0.64	764.62	<0.0001	N.S.	0.049	23.78	0.0046
Residual	5		4.167E-003				2.068E-003		
Lack of fit	3		1.300E-003	0.30	0.8257		3.025E-003	4.78	0.1780
Pure error	2		2.867E-003				6.333E-004		

[1] N.S.: Non Significant.
Growing cells: R2 = 0.9974 R, adjusted = 0.9927.
Resting cells: R2 = 0.9526 R, adjusted = 0.8672.

has been reported formerly by Ohshiro et al. [16]. Clearly, only water soluble compounds can play an inhibitory role on the cell activity. DBT has an extremely low solubility in water and expected to precipitate in aqueous medium or fully dissolved in the organic phase of the biphasic medium and thus, the initial concentration of DBT expected not to affect the cells or enzymes activity. However, most of the bacterial species with DBT biodesulfurization activity can secrete biosurfactants to enhance the aqueous availability of DBT [17]. For instance, a recombinant *Pseudomonas* strain that have been developed for effective BDS can produce a large amount of biosurfactants which enhanced the biodesulfurization activity [18]. In fact, secretion of biosurfactants would increase the bioavailability of DBT to the cells in the aqueous phase even higher than the tolerating level of bacteria which in turn hinders the BDS activity of the cells. For the resting cells, the optimum point for the initial DBT concentration was far more than the growing cells. In fact, in the biphasic medium, DBT can be dissolved in *n*-tetradecane (organic phase) which reduces its toxic effect on bacteria. Moreover, the produced 2-HBP accumulation in the cells would be lower because of its high solubility in the solvent phase [19].

Another important and potentially limiting factor in biodesulfurization activity is temperature. In particular, temperature should be studied as an interactive factor, because it affects all chemicals and biochemical processes [20]. It has been indicated that in biodesulfurization by *Mycobacterium phlei* WU-F1, conversion of DBT to $DBTO_2$ would be stopped at high temperatures while the conversion of $DBTO_2$ and other intermediate compounds would continue at the same conditions. It can be concluded that the activity of the first enzyme in the 4S-pathway which oxidizing DBT to $DBTO_2$ is more sensitive to temperature variations and is considered as the BDS rate-limiting enzyme [21].

The pH like temperature, carbon source and other factors in culture medium is an effective parameter that controls the bacterial growth rate [22]. In addition, enzymes are affected by the pH variations in the system because of the dependence of the 3D shape of enzymes on the pH. The impact of pH variation is not devoted to only the shape of enzymes. However, it may also affect the shape or electrical charge properties of the substrate in such a way that the substrate cannot bind to the enzyme active site or it cannot undergo catalysis [23]. Previous studies showed that the highest DBT biodesulfurization by the bacterial strains were obtained at pH near to neutral conditions [3, 24]. Also, it has been indicated that the purified 4S-pathway enzymes such as flavin reductase have higher activities at the pH interval of 6 to 8 [25]. The optimum pH in growing condition was 6.88 and 2-HBP production was reduced by decreasing or increasing of pH. Optimum pH was obtained as 6.29 for BDS of DBT in model oil system. In contrast with the growing cells, the pH factor and interactions between pH and temperature were not significant for BDS by resting cells in biphasic system.

Maximum BDS activity of PD1 resting cells was determined as 0.46 μM 2-HBP/min g DCW at optimal conditions. Nassar and colleagues [26] reported that newly isolated *Brevibacillus invocatus* C19 relative to *R. erythropolis* IGTS8 removed 66.85 and 50% of the initial 1000 ppm DBT and produced 31.98 and 31.34 ppm 2-HBP, respectively. Isolated PD1 strain in this study showed higher ability to removal of DBT mainly from oil-model thus could be used for petroleum DBT biodesulfurization.

Finally, it can be concluded that at the optimum pH, temperature and DBT concentration, cell efficiency increases and the highest rates of biodesulfurization activity can be obtained by setting all these effective factors at the optimum levels.

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