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Characteristics of dibenzothiophene desulfurization by *Rhodococcus erythropolis* R1 and its *Dsz*-negative mutant

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Abstract

Introduction: Biodesulfurization is used as a selective method for lowering the sulfur content of petroleum products.

Materials and methods: A sulfur-oxidation bacterial strain named *Rhodococcus erythropolis* R1 (NCBI GenBank Accession No. GU570564) was used in this study for desulfurization of dibenzothiophene (DBT).

Results: The induced culture of strain R1 was able to produce 2-hydroxybiphenyl (2-HBP) from DBT followed 4S pathway without further degrading carbon backbone. This process confirmed by gas chromatography (GC) analysis. The specific activity of DBT desulfurization by R1 was 45 µM (g dry wt)$^{-1}$ h^{-1}. The addition of Tween 80 as surfactant and glycerol as carbon source determines a 100% rate of DBT-desulfurization during 3 days. The heavy plasmid detected in R1 strain carries *dsz* genes responsible for biodesulfurization of DBT that was shown by PCR reaction. The mutant strains which had lost this plasmid also had lost desulfurization phenotype. Both mutant and wild strain were sensitive to high concentration of 2-HBP and some antibiotics.

Discussion and conclusion: Strain R1 desulfurize DBT through the sulfur-specific degradation pathway or 4S pathway with the selective cleavage of carbon-sulfur (C-S) bonds without reducing the energy content. Addition of surfactant enhanced the desulfurization of DBT by increasing its bioavailability and also could improve the growth and desulfurization rate. The location of desulfurization genes was on a heavy plasmid in strain R1. Based on the results of this study, *R. erythropolis* R1 could serve as a model system for efficient biodesulfurization of petroleum oil without reducing the energy value.

Key words: Biodesulfurization, 2-Hydroxybiphenyl (2-HBP), *Rhodococcus erythropolis* R1, Mutant

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Introduction

Organic sulfur in fossil fuels has been a major cause of environmental pollution (1). Combustion of fossil fuels leads to the release of toxic sulfur dioxides into the environment, contributing significantly to air pollution and being the principal cause of acid rain (2- 4). Therefore petroleum is treated by hydrodesulfurization (HDS) using metallic catalysts in the presence of hydrogen gas under extremely high temperature and pressure. Although HDS can remove various types of sulfur compounds, some types of heterocyclic sulfur compounds such as dibenzothiophene (DBT) and its derivatives can not be removed (5, 6). Moreover, because some of these heterocyclic compounds are mutagenic and carcinogenic, ecosystems contaminated with these compounds may elicit serious health risks (7, 8).

To achieve more complete desulfurization of petroleum, it is necessary to establish other methods that can remove these sulfur compounds. Biodesulfurization using microbial catalysts capable of desulfurizing DBT and their alkylated compounds is suitable for this purpose (5). Substantial efforts by industrial and academic research communities have been directed toward the development of biotechnology for desulfurization of petroleum and coal (1, 4). DBT has been widely used as a model compound to screen microorganisms which might be used in desulfurization of fossil fuels (3-5, 8, 9).

Rhodococcus erythropolis strain IGTS8 is a prototype sulfur specific desulfurization bacterium (10). Some mesophilic and thermophilic DBT-desulfurizing microorganisms have been isolated, for example, *R. erythropolis* D-1 (11), *R. erythropolis* H-2 (12), *R. erythropolis* XP (4) and *Lysinibacillus sphaericus* DMT-7 (13). Most of these bacteria desulfurize DBT through the sulfur-specific degradation pathway or 4S pathway with the selective cleavage of carbon-sulfur (C-S) bonds without reducing the energy content (14, 15). The 4S pathway does not rely on aromatic ring cleavage and therefore there is no possibility of side reactions leading to degradation of the aromatic fraction of fuel and hence to a reduction in octane rating. The product of this pathway is 2-hydroxybiphenyl (2-HBP) and sulfate (16).

The desulfurization genes *dsz*ABC, coding for the enzymes of the 4S metabolic pathway in *R. erythropolis* IGTS8, have been found in large plasmids (17). Loss of the DBT-desulfurizing phenotype has been clearly related to the loss of these plasmids (18). The genes *dsz*ABC are grouped in a 3.7 kb desulfurization operon called the *dsz*ABC operon. This system is repressed in the presence of easily metabolizable sources of sulfur such as sulfate or sulfur containing aminoacids (19).

In this study we analyzed the DBT desulfurization characteristic of *R.erythropolis* strain R1 formerly isolated from gasoline-contaminated-soil (20) when is cultivated with glucose or glycerol as carbon source and different concentrations of surfactant for further application in removal of DBT from contaminated environments. Also the induced Gibbs-negative mutant of the isolate by plasmid curing was compared with wild type (Gibbs- positive).

Materials and methods

Bacteria

Bacteria used in this research were *R.erythropolis* strain R1, isolated formerly by us (20), and its Gibbs negative mutant, isolated in this research.

Preparation of media

DBT-desulfurizing bacterium was cultivated on basal salt medium containing: 4 g KH_2PO_4, 4 g Na_2HPO_4, 2 g NH_4Cl, 0.2 g $MgCl_2$, 0.001 g $CaCl_2$ and 0.001 g $FeCl_3$ per liter, added with glucose (5 g per liter) or glycerol (2 g per liter) and DBT (0.3 mM) as sole carbon and sulfur sources respectively, incubated at 30 °C with rotation at 140 rpm for 72-100 hours (Shaking Incubator, n-Biotek) (10).

BSM agar was made by addition of 0.8% agarose (Merck) for colony formation.

Nutrient agar (Merck) and nutrient broth (Merck) were used for plasmid curing in *R. erythropolis* strain R1.

Antibiotic and cadmium sensitivity test were performed on Luria bertani agar (LA, 10 g peptone, 10 g NaCl, 5 g yeast extract and 15 g agar per liter) added with appropriate antibiotic or cadmium nitrate.

Effect of surfactant was assayed in BSM broth supplemented with glucose and DBT as carbon and sulphur source respectively and tween80 as surfactant.

Characterization of cell growth and DBT-desulfurizing ability of R1

Cell growth was measured turbidimeterically at 600nm using a spectrophotometer (Specord S 10 Carl Zeiss Technology) (21).

Removal of DBT was assayed with UV spectrum at 200-600nm after acidifying the samples to pH 2.0 and extraction with ethyl acetate (6). The concentration of DBT was determined by spectrophotometeric analysis in a UV spectrophotometer (Specord S 10 Carl Zeiss Technology), using absorption maxima at 323.8nm wavelength. The standard curve of DBT concentration was prepared with DBT in the range 0-0.3 mM in triplicate experiments. The concentration of DBT removal was estimated by mM per liter (22).

Gibbs assay was used for DBT desulfurization and 2-HBP production as follows: An aliquot (1.5 ml) of bacterial culture broth transferred to Eppendorf tube and centrifuged (12000 rpm in microcentrifuge, Sigma-110, 2 min) to remove cells. A volume of 200 µL of sodium bicarbonate ($NaHCO_3$, pH 8.0) and 20 µL Gibbs reagent solution (1 µg μL^{-1}) in ethanol were added to supernatant (1 ml). The reaction of mixture was agitated at 30 °C for 30 min to allow the formation of a blue complex by the Gibbs reagent and aromatic hydroxyl groups such as 2-HBP. The absorbance was measured at 610_{nm} (4, 23). The standard curve of 2-HBP concentrations was prepared with 2-HBP in the range 0-0.3 mM in triplicate experiments. The content of produced 2-HBP was estimated by mM per liter (24).

GC method: DBT desulfurization was confirmed using a gas chromatograph (model GC-2010; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a Durabond Free Fatty Acid Phase (DB-FFAP) column (60 m × 0.25 mm internal diameter, 0.5 µm film thickness; Agilent Technologies, Palo Alto, CA, USA), using helium as the carrier gas. The injection volume and split ratio were adjusted to 1 µL and 1:50, respectively. The column temperature was programmed to increase from 40 to 170 °C with a linear gradient of 10 °C/minute.

Specific activity of DBT desulfurization

For induction of desulfurization enzymes *R. erythropolis* R1 was grown on BSM containing 2 g L^{-1} glycerol and 0.3 mM DBT, and incubated at 30 °C with 140 rpm shaking (Shaking Incubator, n-Biotek) for 80 hours. Cells were harvested by centrifugation at 3000 rpm (microcentrifuge, Sigma-110) for 10 min, washed twice with phosphate buffer saline (PBS: g/L 45 NaCl, 15.2 Na_2HPO_4, 3.93 NaH_2PO_4, pH 7.4) and resuspended in the

same buffer to adjust the cell mass to an optical density at 600nm (OD 600nm) of 30 after dilution (equal to 17.1 g dry weight cells per liter). This cell suspension was inoculated to BSM containing 0.3 mM of DBT dissolved in ethanol without another carbon source and incubated at 30 °C in shaker (180 rpm, Shaking Incubator, n-Biotek for 60 min). At 10 min intervals Gibbs assay was performed. The linear regression slope of a plot of produced 2-HBP concentration *versus* time gave the reaction rate (µM h^{-1}); the specific activity (µM g^{-1} h^{-1}) was obtained by dividing the rate by the cell concentration (g dry wt L^{-1}) (24).

Effect of surfactant on biodesulfurisation activity in *R. erythropolis*R1

Different percentage of tween 80 as surfactant added to BSM broth supplemented with Glucose (1 gL^{-1}) and DBT (0.3mM). These medium incubated in growth condition for the bacterium *R. erythropolis*. The growth and DBT transformation were assayed as mentioned above.

Plasmid-curing method for wild-type *R. erythropolis*R1

R1 was cultivated on nutrient broth (N.B.) at 30 °C with shaking (140 rpm, Shaking Incubator, n-Biotek). After 24 hours, 1 ml of first culture was transferred to new medium of N.B. and these subcultures were repeated for 8 times (17). In each subculture, media were diluted with sterile phosphate buffer saline (PBS), spread on nutrient agar (N.A.) and incubated at 30 °C for 24 hours. Each individual colony was transferred on replicate plate contained N.A. and DBT agar. After incubation of plates at 30 °C for 3 days, Gibbs reagents including sodium bicarbonate and Gibbs reagent were added. The colonies without blue color around them in DBT agar after a few minutes were selected as Gibbs-negative. These Gibbs-negative mutants were transferred on BSM containing DBT in flasks and were assayed with reagent of Gibbs after 48-72 hours. The Gibbs negative mutant strains after 7-10 days were selected as plasmid-cured strains of wild type soil-isolated *R. erythropolis* strain R1.

Isolation of plasmid DNA

The procedure for isolating plasmids from *R. erythropolis* strain R1, which is useful in the isolation of large and small plasmids, was according to Denis-Larose *et al.* (17). This method is based on alkaline lysis for isolation of plasmid with several modifications.

Polymerase chain reaction (PCR) amplification of desulfurization gene (*dsz*C)

Plasmid DNA isolated from R1 was used as target DNA in PCR analysis. The PCR primers for amplification of the desulfurization gene of *dsz*C were designed using DNA sequences from strain IGTS8 (GenBank accession number U08850). PCR primers for the *dsz*C gene amplification were: *dsz*C Forward (position: 277- 296), 5′-CTGTTCGGATACCACCTCAC-3′ and *dsz*C Reverse (position: 651 - 668), 5′-ACGTTGTGGAAGTCCGTG-3′ (25). PCR amplification was performed in a thermal cycler (EppendorfMastercycler™ Personal Thermal Cycler, Eppendorf AG, Hamburg, Germany). The PCR mixtures were prepared with 2 µl of target plasmid DNA, 5 µl of 10X PCR buffer (10 mM Tris-HCl [pH 8.3], 10 mMKCl), 200 µmol of each desoxyribonucleoside triphosphate, 3.75 mM $MgCl_2$, 20 pmol of each of appropriate primers, 1% (v/v) formamide, and 5 U of Taq DNA polymerase in a 50 µl final volume. Negative control (PCR mixture without added target DNA) was included in PCR. The PCR product size was 392 bp and the PCR conditions were as follow: one cycle of 4 min at 94 °C; 35 cycles consisting of 1 min at 94 °C, 1.5 min

at 55 °C and 2 min at 72 °C; and a final cycle of 10 min at 72 °C.

Antibiotics and cadmium sensitivity assays in R1 and its Gibbs-negative mutant

Plates of LA (20 ml) were made containing the appropriate concentrations of antibiotic. The concentrations of antibiotics used were chosen as the concentrations surrounding the commonly used doses described in (26); those not listed were used at a range of concentrations between 10-100 µg per ml. R1 and its Gibbs-negative mutant named 49mut were examined for susceptibility test. Cells were streaked onto each concentration of antibiotic and growth assessed after 5 days at 30 °C.

Chemicals

Dibenzothiophene (DBT, $C_{12}H_8S$) was purchased in Iran which was made by Merck-Schuchardt Hohenbrunn, Germany. 2-Hydroxybiphenyl (2-HBP, $C_{12}H_{10}O$) was purchased in Iran that was made by Fluka (EC No. 2019935). Gibbs reagent (2, 6-Dichloroquinone-4-chloroimide, $C_6H_2Cl_3NO$) made by Merck in Germany. All other chemicals were of analytical grade and used without further purification.

All experiments were performed in triplicate with blank media.

Results

16S rDNA sequencing of strain R1

Gram positive bacilli were isolated from gasoline-contaminated-soil in Isfahan by us that have the ability to convert DBT to 2-HBP. The isolated strain was oxidase negative, catalase positive, no spore forming and partially acid-fast bacilli. This strain identified as *Rhodococcus* sp. by more biochemical tests previously (20).

Partial sequences of 16S rRNA gene showed 97% homology with *R. erythropolis* GenBank accession numbers: EF690428.1, EF204438.1, etc. Thus isolated strain has been assigned *R. erythropolis* strain named R1. Information of bacterial genome registered in NCBI database as *R. erythropolis* strain R1 and Accession No. is GU570564.

Growth and desulfurization

R. erythropolis R1 grew in BSM medium with glucose (or glycerol) and DBT as sole sources of carbon and sulfur respectively. The time course of DBT degradation by growing cells of this strain is shown in Fig. 1. This strain degraded 100% of 0.3 mM DBT within 4 days, produced 2-HBP and reduced pH value of medium during growth and desulfurization. On the other hand, it was confirmed that this strain did not utilize DBT as the sole source of carbon (data not shown), either in BSM containing DBT dissolved in dimethylformamide (as solvent) or only DBT.

The effect of the initial DBT concentration is shown in Fig. 2. The initial DBT concentration was varied in a range from 0.1 to 6 mM. The desulfurization rate was observed not to be affected by DBT in 0.1 to 4 mM, but from 5mM or higher this phenomenon was occurred. The 2-HBP that is the end-product of desulfurization of DBT in this bacterium increased from 1.25 in 0.1 mM DBT to 2.75 in 4 mM DBT initial concentration which is about 2.18 times higher than that at 0.1 mM. But, the increase in the initial DBT concentration above 4 mM resulted in a lower 2- HBP production.

Desulfurization of DBT in 3M concentration by strain R1 confirmed by GC assay that is shown in Fig. 3.

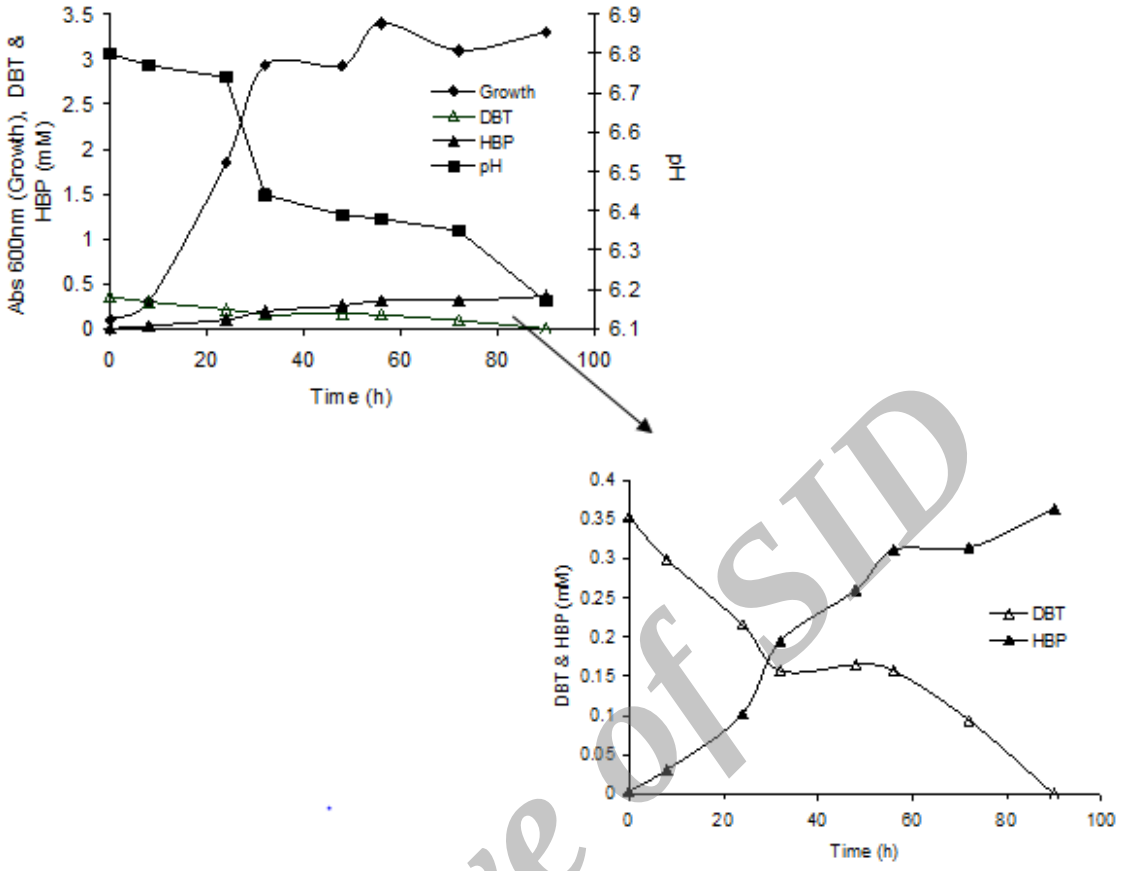

Fig. 1- Growth rate, remained DBT, 2- HBP produced and pH reduction by *Rhodococcus erythropolis* R1 during cultivation on BSM with DBT (0.3 mM) and glucose (2 g l^{-1}).

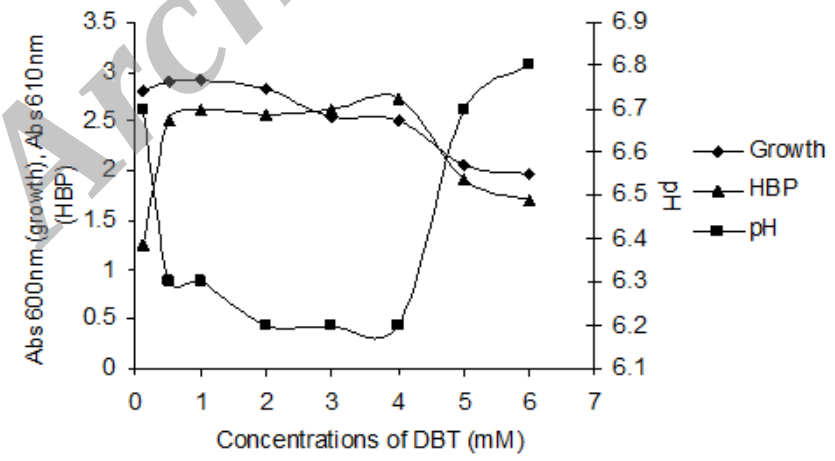

Fig. 2- Effect of initial DBT concentration on growth, hydroxybiphenyl (2-HBP) production and pH variation in *Rhodococcus erythropolis* R1. Reactions were performed at 30°C, 140 rpm shaking (Shaking Incubator, n-Biotek), 10 g l^{-1} glucose, and lasted for 72 hs.

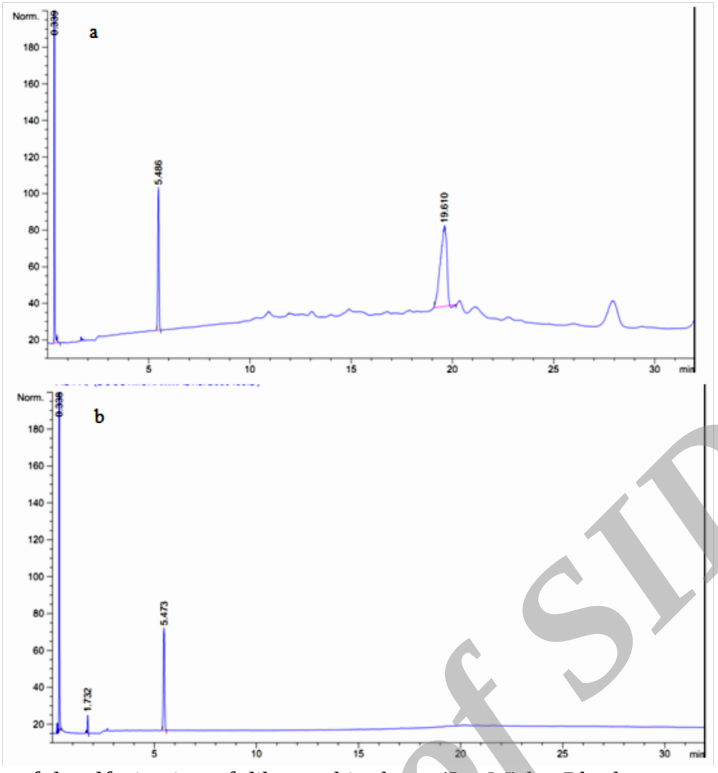

Fig. 3- The GC analysis of desulfurization of dibenzothiophene (3 mM) by *Rhodococcus erythropolis* R1 after 3 days incubation at 30 C and 140 rpm shaking. The blank DBT (a), desulfurized DBT by strain R1 (b).

Effect of different solvents on Growth

Growth in different solvents by DBT can be seen as sole sulfur source is shown in Fig. 4. As it is shown R1 strain grew on ethanol as solvent, and did not grow in other solvents.

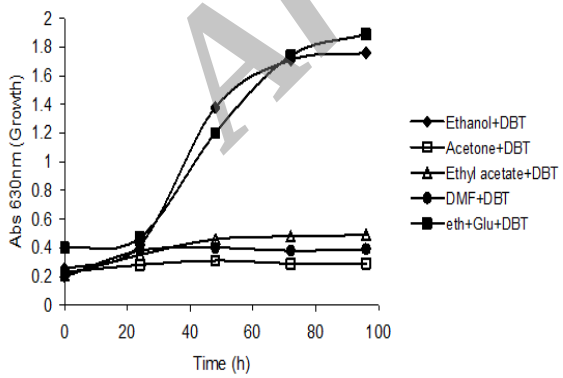

Fig. 4- Growth of *Rhodococcus erythropolis* R1 in different solvents (ethanol, acetone, ethyl acetate, dimethylformamide). Reactions were performed at 30°C, 140 rpm shaking (Shaking Incubator, n-Biotek), 0.3 mM DBT as sole sulfur source, and lasted for 100 hs.

Inhibition of Growth and desulfurization by 2-HBP

Inhibition of cell growth and desulfurization activity or 2-HBP production of the *R. erythropolis* R1 by the end product from DBT desulfurization, 2-HBP, were shown in Fig. 5. As shown in Fig. 4a the growth of *R. erythropolis* R1 is relatively high in the presence of 2-HBP except at 0.25 mM. At this concentration of 2-HBP reduction of DBT and pH (that is equal to sulfate production) have not seen (shown in Fig. 5b and 5c).

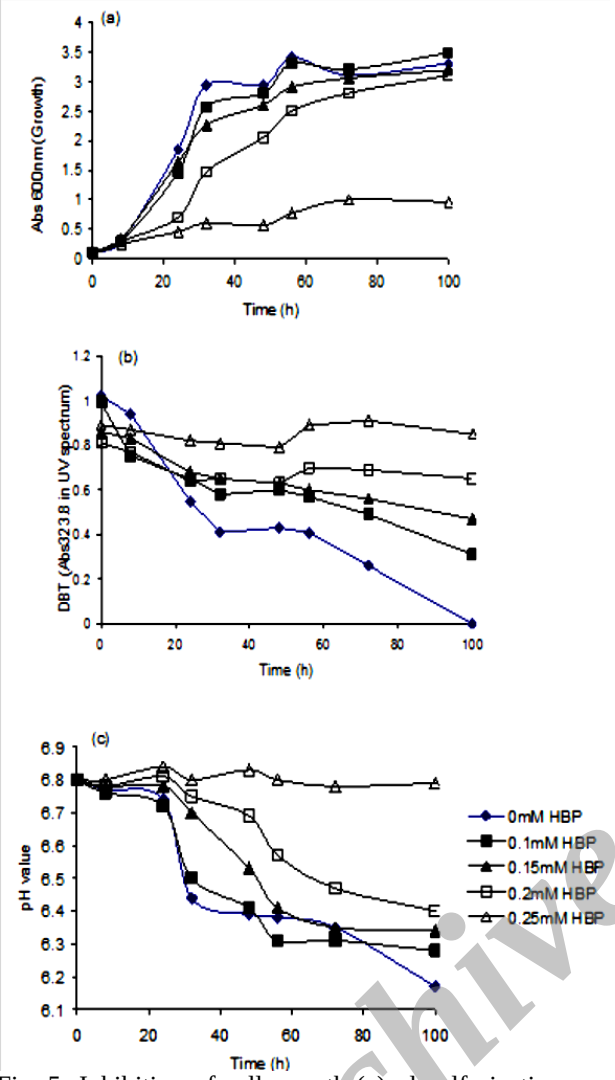

Fig. 5- Inhibition of cell growth (a), desulfurization activity (b) and pH reduction (c) by different concentrations of 2-HBP during cultivation of *R. erythropolis* R1 on BSM with DBT (0.3 mM) as sole sulfur source and glucose (10 g l^{-1}).

Specific activity of DBT desulfurization

The cell suspension of *R. erythropolis* R1 from BSM containing DBT as sole sulfur source inoculated to BSM containing DBT for analysis specific activity of desulfurization. The specific activity of 2-HBP production by the isolate was determined as 45 µM g^{-1} h^{-1}. If the cell suspensions prepared from the $MgSO_4$ instead of DBT as sole sulfur source, no reduction of DBT or production of 2-HBP had been detected.

Effect of surfactant

Fig. 6 shows that Tween 80 in 0.5 - 5 g per liter is suitable for growth and production of 2- HBP by *R. erythropolis* R1. The optimum value of Tween 80 concentration for desulfurization was about 1 g per liter. The amount of 2-HBP formed with this concentration of Tween 80 was about 27% more than formed without surfactant after 24 h of incubation.

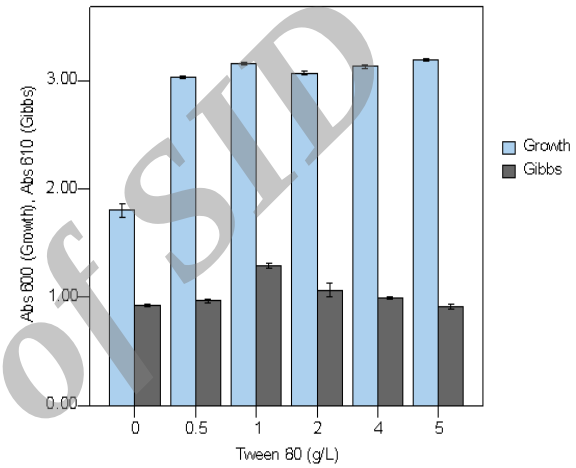

Fig. 6- Effect of Tween 80 on growth and 2- HBP production (Absorbance of Gibbs assay) of *R. erythropolis* R1. Medium contain BSM with DBT (0.3 mM) and glucose (10 g l^{-1}) by different concentrations of Tween 80 as surfactant. (Error bars ± 1 SD)

Induction of 2- HBP negative mutant

Wild type strain of *R. erythropolis* R1 produced 2-HBP from DBT lost its ability after several subcultures into N.B. and N.A. After one subculture the mutation occurred by 10%. Following subculturing of *R. erythropolis* R1, approximately 700 colonies from consecutive subcultures were examined individually to determine whether they had lost the ability to convert DBT to 2-HBP. Among mutant colonies one mutant named 49 mut that could not metabolize DBT even after extended incubation (6 to 10 days) was selected. These data show that genes responsible for desulfurization of DBT in isolated strain are plasmid-coded. The large plasmid

(more than 60 kb) was seen by specific large plasmid isolation (Fig. 7). The *dsz* (sox) plasmid had no detectable plasmid-linked antibiotic resistance or heavy - metal resistance markers by comparison between wild-type *Rhodococcus* and Gibbs-negative mutant strain (49 mut) (Table 1).

PCR analysis showed that *dsz*C gene was in wild R1 strain but did not exist in 2-HBP negative mutant strain or 49 mut (Fig. 8). Therefore the 49 mut strain is *Dsz*C negative mutant with Gibbs negative habit. *Dsz* plasmid was found in R1 with plasmid curing and lost of plasmid.

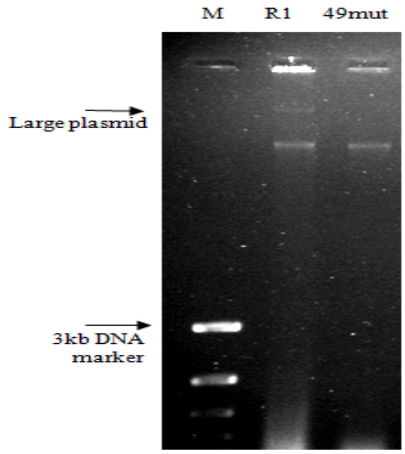

Fig. 7- Large plasmid detection in wild strain and Gibbs-negative mutant of *Rhodococcus erythropolis* R1. The large plasmid was seen in wild strain but not in its Gibbs-negative mutant (49 mut), (M: DNA marker).

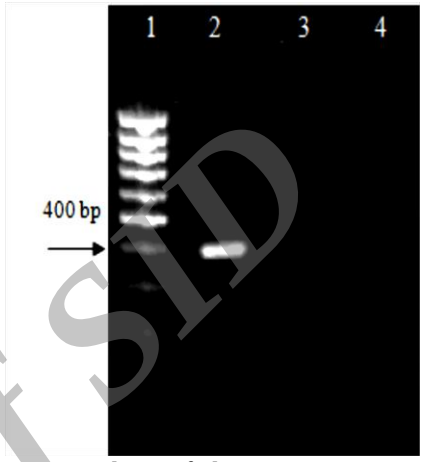

Fig. 8- PCR analysis of *dszC* gene. Lane 1: 50 bp DNA ladder, lane 2: wild *R. erythropolis* strain R1, lane 3: Gibbs-negative mutant strain (49 mut), lane 4: control negative.

Table 1- Antibiotic and cadmium sensitivity assays in *Rhodococcus erythropolis* R1 and its Gibbs-negative mutant (49 mut).

Antimicrobial agents	*R. erythropolis* R1	mutant strain 49 mut
Kanamycin (50 µg ml^{-1})	R	R
Erythromycin (50 µg ml^{-1})	S	S
Tetracycline (50 µg ml^{-1})	S	S
Oxytetracycline (50 µg ml^{-1})	S	S
Chloramphenicol (170 µg ml^{-1})	S	S
Chloramphenicol (25 µg ml^{-1})	R	R
Spectinomycine (50 µg ml^{-1})	R	R
Ampicillin (50 µg ml^{-1})	S	S
Penicillin (10 U ml^{-1})	R	R
Vancomycin (30 µg ml^{-1})	S	S
Cefoxitin (30 µg ml^{-1})	S	S
Erythromycin (15 µg ml^{-1})	S	S
Cephalothin (30 µg ml^{-1})	S	S
Amikacin (30 µg ml^{-1})	S	S
Amoxicillin (30 µg ml^{-1})	S	S
Gentamycin (10 µg ml^{-1})	S	S
Carbenicillin (100 µg ml^{-1})	S	S
Novobiocin (5 µg ml^{-1})	R	R
Cadmium nitrate (27.5µg ml^{-1})	R	R

^{R} Resistance, ^{S} Sensitive

Growth of wild and mutant strains of *R. erythropolis* strain R1 in 2- HBP

As it is shown in Fig. 9 both wild type and mutant strains did not grow in concentrations of 0.2 mM or higher of 2-HBP. However the growth rate of wild strain is higher than mutant strain. These results showed that the inhibition effect of 2-HBP on isolated *Rhodococcus* sp. did not relate to *dsz* genes.

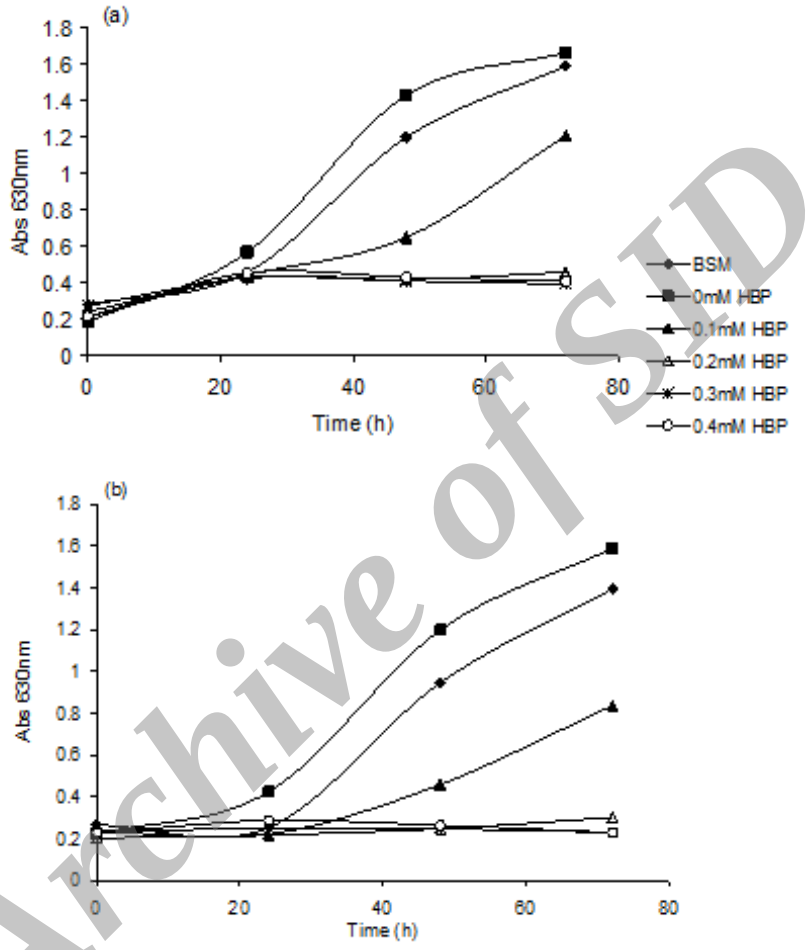

Fig. 9- Growth of *Rhodococcus erythropolis* R1 (a) and Gibbs-negative mutant strain or 49mut (b) in different concentrations of 2-HBP in BSM containing glycerol (2 g l^{-1}) and sulfate (0.2 g l^{-1}).

Discussion and conclusion

There is significant interest in the development of desulfurizing bacteria for commercial fuel desulfurization. Specific interest in desulfurizing bacteria is a consequence of the inability of these strains to degrade the carbon backbone of the original organosulfur compound, following desulfurization. The strain IGTS8, grown in mineral salt medium containing a suger as carbon source and DBT as sole sulfur source, consumed the organosulfur compound and the desulfurized carbon backbone accumulates in the medium (23). *R. erythropolis* R1 isolated formerly by us (20) uses a co-metabolism reaction with DBT and other carbon source for desulfurization of DBT. This strain desulfurize DBT through the sulfur-specific degradation pathway or 4S pathway with

the selective cleavage of carbon-sulfur (C - S) bonds as shown in other strains of *Rhodococcus* (4, 14). In this pathway the aromatic ring cleavage does not occur and the products of this pathway are 2-HBP and sulfate (16).

DBT is intrinsically a xenobiotic compound and there have been several reports that indicate a high concentration of DBT has inhibition effects on cell growth and desulfurization activity (27, 28). The inhibitory effect of DBT on desulfurization of DBT was approved in *R. erythropolis* R1 that is over 4mM of DBT.

Inhibition of cell growth and desulfurization activity or 2-HBP production by the end products from DBT desulfurization, 2-HBP and sulfate, were previously reported (27, 28). The desulfurization activity, cell growth and pH reduction (sulfate production) of the *R. erythropolis* strain R1 were also affected by adding 2-HBP to desulfurization medium. These results indicate that 2-HBP not only inhibited the enzymes involved in desulfurization but also certain enzymes for cell growth.

The specific activity of strain IGTS8 was obtained as 35 μM g^{-1} h^{-1} (24). The specific activity of DBT desulfurization in R1 was comparable with the strain IGTS8 and showed more activity. The induction of enzymes required for desulfurization of DBT in medium containing DBT was necessary for R1, as also shown by Tanaka *et al.* (6) for other strains.

There is some problem in biocatalytic conversions of heterocyclic compounds. In some cases, problems arise from substrates and products that are poorly soluble in water and/or display inhibitory or toxic effects on the biocatalyst. The nonionic chemical surfactant Tween 80 can enhance the biodesulfurization activity in both aqueous and biphasic systems by reducing the concentrations of the products around the cells. Conversely, Tween 80 can also reduce the concentrations of hydrophobic substrates associated with the cells. As long as the concentrations support adequate reaction rates, this reduction will not limit the overall conversion (29). On the other hand addition of surfactant enhanced the desulfurization of DBT by increasing its bioavailability. Tween 80 could improve the growth and desulfurization rate in *R. erythropolis* R1. This effect showed also by Sett*iet al.* (22) on IGTS8 with cyclodextrine and by Wang *et al.* (30) on *Corynebacterium* sp. ZD-1 with Tween 80.

Most microbial strains with DBT desulfurization activity are known to secrete some biosurfactants to solubilize and thus enhance the bioavailability of DBT.

The results in this study show that the genes responsible for desulfurization of DBT in isolated strain are plasmid–coded; and plasmid curing by consecutive sub culturing cause loss of desulfurization phenotype. Denome *et al*. (10) showed the location of desulfurization genes on an endogenous 120 kb linear plasmid in strain IGTS8. Denis - Larose *et al.* (31) showed a large plasmid of 150 kb in IGTS8 and 100 kb in the other strains of *Rhodococcus*. *R. erythropolis* R1 have the large plasmid that is responsible for desulfurization ability in this strain.

Concluding Remarks

In this study we analyze the biodesulfurization characteristics of sulfur-oxidative *R. erythropolis* strain R1. This strain has high activity of DBT desulfurization without breaking the carbon backbone of aromatic ring. The ability of desulfurization may increase by addition of surfactant. *Dsz* plasmid is found with plasmid isolation. The strain R1 can be applied for removal of DBT from different environments and fossil fuels in future researches.

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ویژگی‌های سولفور زدایی از دی‌بنزوتیوفن توسط *Rhodococcus erythropolis* R1 و موتان *Dsz*-negative آن

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چکیده

مقدمه: سولفور زدایی زیستی به عنوان یک روش انتخابی برای کاهش میزان گوگرد محصولات نفتی به کار می‌رود.

مواد و روش‌ها: در این تحقیق، یک سویه باکتریایی اکسید کننده گوگرد بـه نـام رودوکوکـوس اریتروپـولیس R1 (شماره دسترسی GU570564 ژن بانک NCBI) برای سولفورزدایی از دی‌بنزوتیوفن (DBT) استفاده شد.

نتایج: کشت القا شده سویه R1 توانایی تولید ۲-هیدروکسی‌بای‌فنیل (2-HBP) از DBT را طی مسیر 4S بدون تجزیـه اسکلت کربنی آن داشت. این فرایند به وسیله تحلیل کروماتوگرافی گازی (GC) تایید شد. میـزان فعالیـت اختصاصـی سولفورزدایی از DBT به‌وسیله سویه R1 ۴۵ میکرومول در گرم وزن خشک در ساعت به دست آمد. سـولفورزدایی از DBT با افزودن توئین ۸۰ به‌عنوان سورفاکتانت و گلیسرول به‌عنوان منبع کربن طی ۳ روز به میـزان ۱۰۰ درصـد تعیـین شد. پلاسمید سنگین در سویه R1 تشخیص داده شدکه به‌وسیله واکنش PCR وجود ژن‌های *dsz* مسؤول سولفورزدایی زیستی از DBT در آن نشان داده شد. سویه‌های موتانی که این پلاسمید را از دست داده بودنـد فنوتیـپ سـولفورزدایی را هم از دست دادند. هر دو سویه وحشی (R1) و موتان به غلظت‌های بالای 2-HBP و برخی آنتی‌بیوتیک‌هـا حسـاس بودند.

بحث و نتیجه‌گیری: سویه R1 از طریق مسیر تجزیه اختصاصی سولفور یا مسیر 4S با شکست انتخابی باندهای C-S بدون کاهش انرژی از DBT سولفورزدایی می‌کند. افزودن سورفاکتانت سـولفورزدایی از DBT را بـا افـزایش قابلیـت دسترسی زیستی آن افزایش داده، همچنین می‌تواند میزان رشد و سولفورزدایی سویه را اصلاح کنـد. جایگـاه ژن‌هـای سولفورزدایی در سویه R1 بر روی یک پلاسمید سنگین بود. با توجه بـه نتـایج حاصـل از ایـن تحقیـق رودوکوکـوس اریتروپولیس R1 می‌تواند به عنوان یک سیستم مدل برای سولفورزدایی زیستی موثر از نفت خام بـدون کـاهش ارزش سوختی نفت به‌کار رود.

واژه‌های کلیدی: سولفورزدایی زیستی، ۲-هیدروکسی‌بای‌فنیل (2-HBP)، رودوکوکوس اریتروپولیس R1، موتان

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