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Microbial Desulphurization Study of Dibenzothiophene and Crude Oil by a Soil Isolate

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ABSTRACT

The desulphurization of crude oil is mandatory to conquer the problem transpiring from burning of high sulfur containing fuels. Acid rain, respiratory and cardiopulmonary problems are the major consequences of sulfur dioxide emission. In current scenario, biodesulphurization is an attractive approach capable of removing complex sulfur compounds under mild conditions as compared to other physiochemical techniques. In the present study, biodesulphurization of dibenzothiophene (DBT) and crude oil was studied by a specific desulphurizing microbe isolated from oil contaminated soil sample. The isolate E1 found closely related to *Bacillus* sp. was sufficient to desulphurize 88% of DBT (2 mM) in synthetic media in 72 h using 4S pathway. The DBT degradation was also validated by GC-MS analysis. The resting cells of isolate E1 presented better desulphurization efficiency with immobilization (57.9 ± 0.1%) and biphasic system (63.6 ± 1.93 %) as compared to free resting cells in aqueous phase system (53.9 ± 5.1%) in just 24 h. The resting cells of isolate exhibited the complete degradation of DBT **(~**0.1 mM) in crude oil and appeared as a potent candidate for biodesulphurization of crude oil.

INTRODUCTION

The release of sulfur dioxide from the combustion of sulfur containing fuel is imposing threat to the environment and health [1]. The discharge of sulfur dioxide (SO_2) leads to acid rain, aerosol formation and low pH fog. Being small in size (2.5µm), the aerosol may enter the lungs and cause the respiratory and cardiopulmonary problems [2]. Acid rain damages the soil and foliage; disturbs the aquatic life, food chains, forests and agricultural ecosystems. Therefore, the removal of sulfur from fossil fuels is mandatory and beneficial process [3]. Being inevitable part of the fuel, it is impractical to have zero sulfur level. Although the environmental regulations are becoming stringent day by day taking into account the need of ultra-low sulfur level. As the detrimental effects show their influence globally, the nations of whole world are actively contributing in controlling SO_2 emission. Most of the developed countries have adopted the sulfur limit of 10-15 ppm in crude oil while other developing countries are still targeting to reduce the limit up to 500 ppm. In India, a sulfur limit of 50 ppm is acceptable in the metropolitan areas since 2010 [4].

To reduce the sulfur content from the fuel, refineries are presently employing the hydrodesulphurization process which employs the use of $CoMo/Al_2O_3$ and $NiMo/Al_2O_3$ catalysts at high temperature (200-425˚C) and pressure (150-250 psi) [5-7]. Biodesulphurization is an excellent 'green remedy' to remove sulfur from the fuels using metabolic potential of the microbes which operates at mild temperature-pressure with low capital and operational cost. It possesses the ability to degrade the complex aromatic sulfur compounds of crude oil including alkyl derivatives of benzothiophene and dibenzothiophene which are otherwise challenge for conventional hydrodesulfurization and thermal conversion [6].

Two pathways are involved in the desulphurization of organosulphur compounds by microorganisms *i.e.* Kodama Pathway (Exhaustive Pathway) and 4S Pathway (Specific Pathway). Kodama pathway includes destruction of C-C bond of benzene ring [8] while 4S pathway which is highly selective pathway includes the oxidation followed by cleavage of C-S bond to form 2-Hydroxybiphenyl (2-HBP) [9]. Microbes able to desulphurize via 4S pathway retaining the calorific value of fuel are preferred for biodesulphurization. Many species were found to involve in desulphurization including *Rhodococcus erythropolis* [6,10], *Klebsiella* [11], *Corynebacterium, Arthrobacter* and *Xanthomonas* [12], *Paenibacillus* [13], *Nocardia* CYK2 [14], *Gordonia amicalis* IEGM [15], *Microbacterium* strain ZD-M2

[16], *Mycobacterium* sp. [17], Yeast *Trichosporon* sp. [18], *Shewanella putrefaciens* NCIMB8768 [19], *Rhizobium* sp. MS4 [9], *Stachybotrys* sp. WS4 [20], *Lysinibacillus sphaericus* DMT-7 [21], *Pantoea agglomerans* [22], *Bacillus* sp. KS1 [23], *Corynebacterium mycetoides* EMB113 [24] and *Chelatococcus* sp. [25]. The research has been carried out throughout the world to find out the capability of microbes to degrade the DBT and its alkyl derivatives efficiently.

Resting cells are the preferred biocatalyst used in desulphurization than growing cells [26]. Further, the immobilization of biocatalyst helps in easy separation and reusability of the biocatalyst [19]. The low water content enhances the solubility and availability of hydrophobic substrates to the biocatalyst but the viability of bacteria decreases due to toxicity of solvent. Therefore, the biphasic reaction systems are considered efficient for biodesulfurization which also avoid the biocatalyst inhibition by hampering the accumulation of 2-HBP. The high water-to-oil ratio was found suitable for biphasic system [27-28].

The present study involves the isolation of specific desulphurizing microbe and its use in the desulphurization of DBT in the synthetic media as well as in the crude oil. The immobilized resting cells and biphasic system presented the better desulphurization efficiency than free resting cells in the aqueous system.

MATERIALS AND METHODS

2.1 Chemicals

The analytical grade chemicals were used throughout the study. Dibenzothiophene (DBT), 2-Hydroxybiphenyl (2-HBP) and 2,6-dichloroquinone-4-chloramide (Gibb's reagent) were purchased from Hi-Media, Mumbai. Soil samples were collected from oil contaminated sites like bus stands, petrol pumps and automobiles shops of Patiala, Punjab (India). Crude oil was provided kind courtesy by Guru Gobind Singh Refinery, Bathinda, Punjab (India).

2.2 Media

The sulphur free minimal salt medium (MSM) was used for the screening and desulphurization study which comprised of 2.0 gL^{-1} Na_2HPO_4, 1.0 gL^{-1} KH_2PO_4, 0.4 gL^{-1} $MgCl_2.6H_2O$ and 0.4 gL^{-1} NH_4Cl and 2 mL (per liter) of trace element solution constituting 0.5 gL^{-1} $SnCl_2.2H_2O$, 0.1 gL^{-1} $Al(OH)_3$, 0.05 gL^{-1} KI, 0.05 gL^{-1} LiCl, 0.8 gL^{-1} $MnCl_2.4H_2O$, 0.5 gL^{-1} H_3BO_3, 0.1 gL^{-1} $ZnCl_2$, 0.1 gL^{-1} $CoCl_2.6H_2O$, 0.1 gL^{-1} $NiCl_2.6H_2O$, 0.05 gL^{-1} $BaCl_2$

and 0.05 gL^{-1} $(NH_4)_6Mo_7O_{24}.4H_2O$. Sodium succinate was supplemented as a carbon source to a final concentration of 10 mM. The media was sterilized by autoclaving at 121°C at 15 psi for 20 min. Filter sterilized DBT (dissolved in ethanol) was supplemented as a sulfur source in sterilized media to final concentration of 2 mM [29].

2.3 Isolation, Screening and Characterization of specific desulphurizing strain

The microbes able to remove sulfur from organosulphur compounds were isolated from the oil contaminated soil samples by providing DBT as sole sulfur source in the medium. One gram of each soil sample was suspended in 100 mL of MSM with DBT (2mM) and sodium succinate (10 mM) as sulfur and carbon source and incubated at 30^0C for 72 h at 150 rpm on orbital shaker [6,29]. Following that, an aliquot of the each sample was transferred aseptically to the same media for enrichment of the desulphurizers. After three subculturing, 0.1 mL of 10^{-5} dilutions were streaked on the nutrient agar plates and incubated at 30^0C for 36-48 h. The morphologically distinct colonies were obtained after repeated streaking [30]. The pure isolated cultures were maintained at 4^0C. The screening of the specific desulphurizers was performed with Gibb's assay based on the appearance of bluish purple color [22]. The selected strain was identified by microscopic, morphological and biochemical characterization [23].

2.4 Desulphurization activity of selected bacteria

2% of the selected culture (E1 isolate) grown in nutrient agar was inoculated in the 100 mL of MSM with sodium succinate (10 mM) and DBT (2 mM) and incubated at 30^0C in an orbital shaker at 150 rpm for 96 h. A small aliquot was withdrawn at the interval of 24 h to estimate the amount of DBT degradation and 2-HBP production by spectrophotometrically and Gibb's assay respectively as explained in analytical techniques. The desulphurization products were also analyzed by GC-MS [21].

2.5 Desulphurization with immobilized resting cells

The resting cells were found more efficient to remove sulfur as compared to growing cells [9]. The microbial cells of E1 isolate were harvested from 24 h grown culture in MSM with sodium succinate and DBT by centrifugation at 6,000 rpm for 15 min. The harvested cells were washed twice thoroughly with 0.1 mM potassium phosphate buffer (pH 7.2) and resuspended (10 g dry cells/liter) in the same buffer. The resting cells were used for immobilization by calcium alginate method [31]. The slurry of sodium alginate (3%) was

prepared in potassium phosphate buffer containing 2.5 mL suspension of resting cells. The calcium alginate beads were prepared by adding sodium alginate slurry dropwise in the chilled solution of 0.1 M $CaCl_2$ solution while stirring on magnetic stirrer. The beads were stored in potassium phosphate buffer. The immobilized resting cells and free resting cells suspension (2.5 mL) of E1 isolate were used for desulphurization study. Both free and immobilized resting cells were added in 250 mL flasks containing 100 mL of 2 mM DBT solution and incubated on orbital shaker at 30^0C. 2 mL sample was withdrawn at short interval of 6-6 h for 24 h to check 2-HBP production and DBT degradation [19].

2.6 Desulphurization in biphasic system and crude oil

The desulphurization ability of resting cells was compared in the aqueous and biphasic system. 25 mL of resting cells suspension (10 g dry cells/liter) in potassium phosphate buffer (0.1 M, pH = 7.2) with 2 mM DBT was used for the aqueous desulphurization process. On the other hand, in the biphasic system 12.5 mL of resting cells suspension (20 g dry cells/liter) in potassium phosphate buffer along with 12.5 mL of hexadecane was employed. The desulphurization experiment was carried out at 30°C with 150 rpm. Samples were collected at each 2 h interval for the estimation of DBT and 2-HBP [13]. The resting cells of E1 isolate were also used to desulphurize the crude oil. 15 mL of resting cells suspension (10 g dry cells/liter) were added in 5 mL of crude oil and incubated at 30°C/200 rpm. Following 3 days incubation, the cells were separated by centrifugation at 10,000 rpm for 10 min from oil and aqueous phases. The oil phase was acidified to pH 2 and analyzed by GC-MS. Un-inoculated diesel oil sample served as control [29].

Analytical Techniques

UV-Vis spectrophotometer was used to estimate the amount of DBT degradation. λ_{max} of DBT (dissolved in acetone as well as in aqueous media) was found to be 325 nm after scanning in the range of 200-800 nm. Derikvand and others [32] found the λ_{max} of DBT at 323.8 nm. The absorbance of the treated (biodesulphurized) sample and uninoculated media was measured. The degradation of DBT was estimated by taking absorbance of media at 325 nm after and before desulphurization treatment. The percent degradation was calculated by using following formula where control is uninoculated media and sample is biodesulphurized media.

$$\% \text{ DBT Degradation } = \frac{\text{O. D. (325 nm) of control} - \text{O. D. (325 nm) of sample} * 100\%}{\text{O. D. (325 nm) of control}}$$

Gibb's assay [33] is an analytical method used to detect the presence of 2-HBP, end product of 4S pathway which is indicated by color development. To estimate the 2-HBP production, 20 µL of Gibb's reagent (1 mg/mL ethanol) was added in the 2 mL of cell-free culture (pH=8 adjusted using 10% Na_2CO_3 solution) and incubated at 30^0C for 30 min for the color development. The absorbance was noted down at 610 nm. For Gas chromatography- Mass spectrometry (GC-MS) analysis of metabolites of desulphurization, the cell-free supernatant of 72 h grown culture was acidified to pH = 2 and was extracted with equal volume of ethyl acetate (Bhatia and Sharma 2010). The fraction of ethyl acetate layer was characterized by GC-MS equipped with RTX5-MS GC column (0.25 mm i.d. x 30m length) [34].

RESULTS AND DISCUSSION

3.1 Identification of bacterial strain

Thirty two bacterial cultures were isolated from six different soil samples following enrichment with DBT as a sole sulfur source in the minimal salt media (MSM) which were further screened for specific desulphurization by Gibb's assay. Five isolates have shown bluish-purple color development with Gibb's reagent. Among these, the isolate E1 was selected for further study based on the highest 2-HBP production by measuring color intensity at 610 nm. The gram-positive isolate E1 observed as creamy and round colonies on the nutrient agar. Based on morphological and biochemical characterization (Table 1) the isolate was observed to be closely related to *Bacillus* sp. according to Bergey's manual of systematic bacteriology [23].

Table 1: Biochemical Characterization of Isolate E1

Biochemical Tests	Results
Dextrose Fermentation	-ve
Simmons's Citrate	+ve
TSI Agar	+ve
Catalase	+ve
Gelatin	+ve
Starch Agar	+ve
Motility	+ve
Methyl Red	+ve
Indole	-ve
Urease	+ve

3.2 Study of desulphurization activity of selected bacteria with time

The desulphurization of DBT (2 mM) in minimal salt media was studied with growing cells of isolate E1 for 96 h. The DBT degradation and 2-HBP production were estimated after every 24 h (Figure 1). The maximum % DBT degradation was observed at 72 h (88.0 ± 1.04 %) after that it became almost stable (87.5 ± 2.07 %). On the other hand, maximum 2-HBP production was found at 48 h (1.103 ± 0.051 mM) after that started decreasing. It can be explained by the conversion of 2-HBP into another metabolite like 2-methoxybiphenyl (2-MBP) or biphenyl [16].

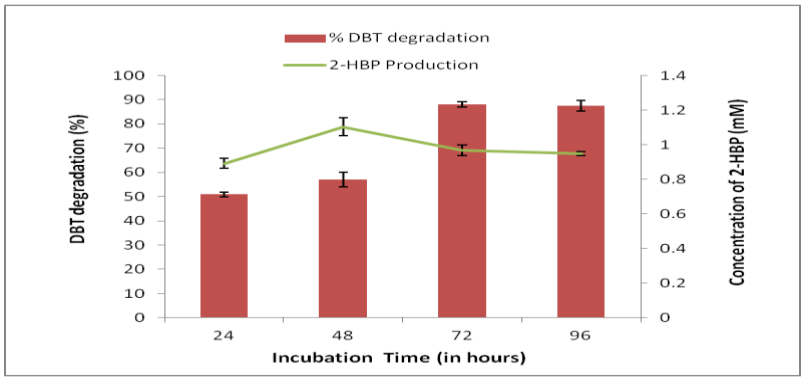

Figure 1: Study of DBT degradation (%) and 2-HBP production with time

3.3 Desulphurization with immobilized resting cells

The resting cells were found more efficient to remove sulfur as compared to growing cells [9, 35]. Immobilization of resting cells further enhanced the efficiency for desulphurization as compared to free resting cells [35, 36]. A comparative study of sodium alginate immobilized cells and free resting cells exhibited higher DBT degradation (57.9 ± 1.1%) and 2-HBP production (0.846 ± 0.037 mM) with immobilized cells as compared to the free resting cells in 24 h. Only 53.9 ± 2.2% DBT degradation and 0.493 ± 0.084 mM 2-HBP production was observed with resting cells (Figure 2). Initially, the performance of free resting cells was found better than immobilized cells up to 12 h which might be due to low distribution of substrate and products across the matrix [37]. The cells of *S. subarctica* T7b immobilized with sodium alginate completely degraded the DBT (0.54 mM) after 72 h [38]. This may be due to increased volumetric reaction rates, utilization of high cell concentrations of the biocatalyst and increased transport rate of sulphur-containing substances into biocatalytic cells as compared to free cells [39].

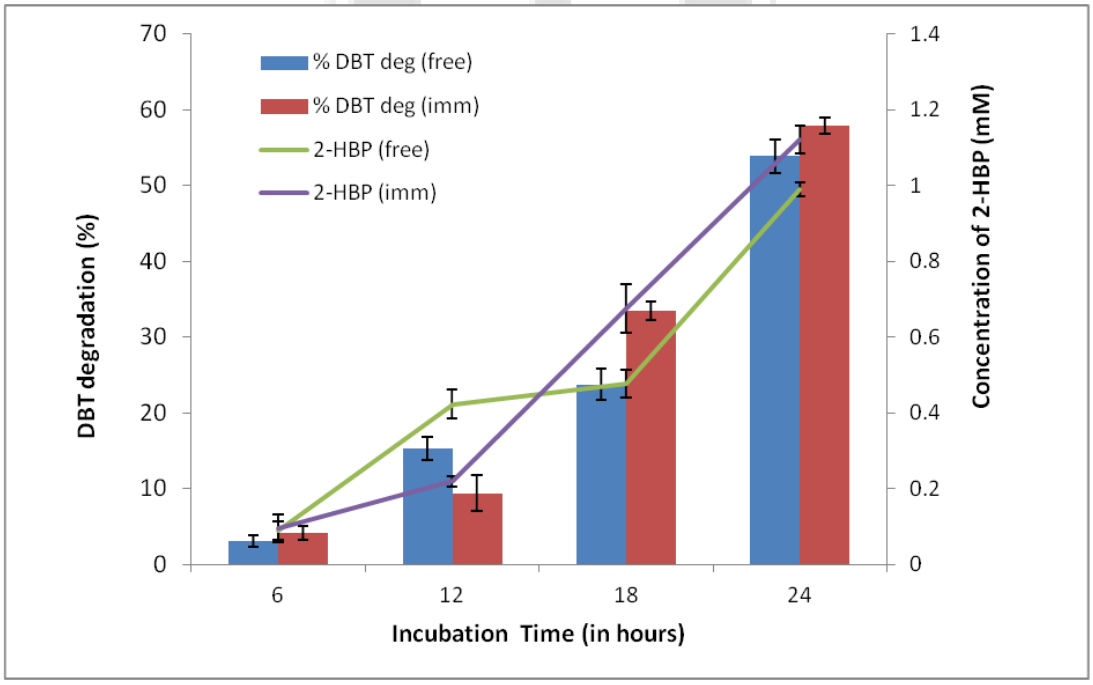

Figure 2: Comparative study of DBT degradation (%) and 2-HBP production with free and immobilized resting cells

3.4 Desulphurization in biphasic system

The enhanced desulphurization of DBT (2 mM) was observed in biphasic system using resting cells of isolate E1 as compared with aqueous system (Figure 3). At 24 h, the amount

of HBP production was higher in biphasic system (1.291 ± 0.095 mM) than in the aqueous phase (0.913 ± 0.014 mM). DBT degradation was found to be more in biphasic system (63.6 ± 1.93 %) than in aqueous phase (54.1 ± 2.19 %). The resting cells of *S. subarctica* T7b in biphasic system were studied to exhibit complete degradation of DBT (1.36 mM) in 24 h [38]. *Bacillus* strain KS1 was also found to show higher desulphurization activity in biphasic system than in aqueous phase [23]. This might be due to more else for substrate availability and lesser end-product inhibition. The inhibitory effect of HBP was avoided by channeling the hydrophobic compound (DBT) to the organic phase, allowing the desulphurization process to continue unhindered in the aqueous phase [40-41].

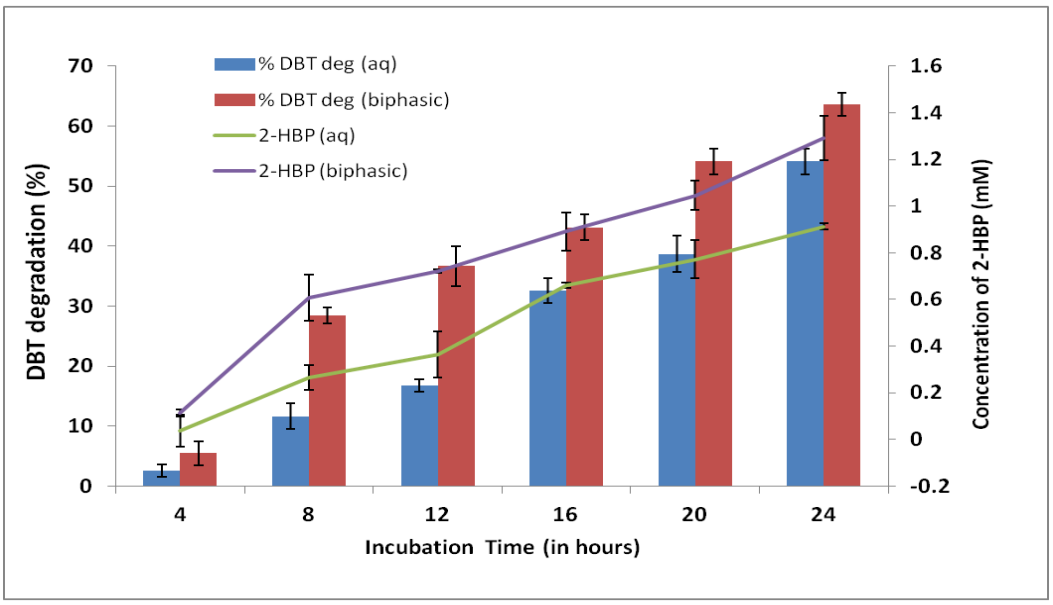

Figure 3: Comparative study of DBT degradation (%) and 2-HBP production with resting cells in aqueous and biphasic media

3.5 GC-MS analysis of desulphurization

The metabolites of DBT desulphurization within synthetic media as well as in the crude oil were studied with GC-MS. The GC chromatograms of the ethyl acetate extracts of uninoculated media containing DBT (A) and 72 h cultured media of E1 isolate containing degraded product of DBT (B) have shown five major peaks at the retention times of 9.40, 11.47, 14.37, 16.95 and 24.90 min (Figure 4). The peaks were further studied by mass spectrometry. Out of five peaks, peak (II) at the retention time of 11.47 min corresponded to DBT as analyzed from its MS spectrum with m/z 184 (Figure 5). The peak area of peak II (DBT) found to reduce from 14,000,000 to 3,500,000 indicating the degradation of DBTs.

Figure 6 showed the GC chromatogram overlap of crude oil before and after treatment with the resting cells of isolate E1. The overlapping chromatograms showed the degradation of crude oil components especially organosulphur compound DBT (Peak I) as indicated from its retention time 11.47. The concentration of DBT in crude oil was found approximately 0.1 mM as indicated from the peak area. The peak corresponding to DBT completely disappeared presenting the complete removal of DBT with resting cells of *Bacillus* sp. (Isolate E1). Therefore, it can be considered as a suitable candidate for biodesulphurization. The degradation of diesel oil compounds was also studied with *Trichosporon asahii* [42].

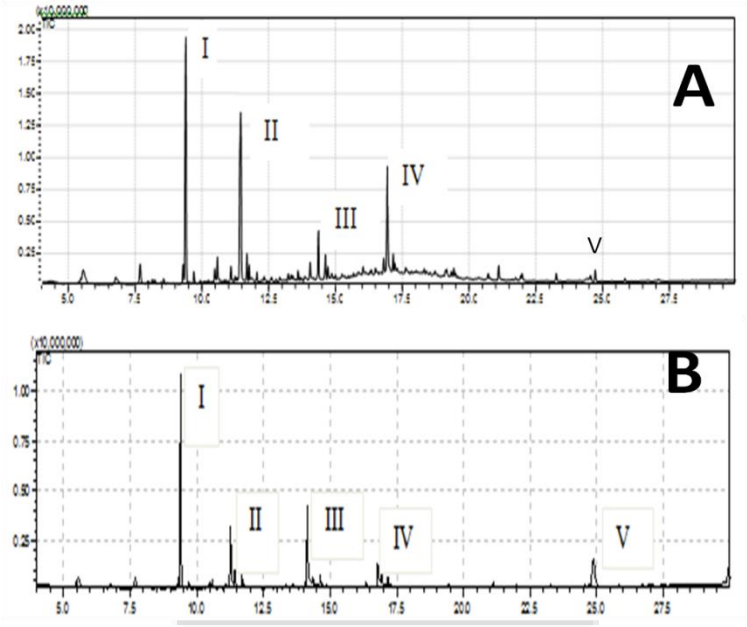

Figure 4: GC chromatograms (A) Uninoculated media containing DBT (B) 72h cultured media (isolate E1) containing degraded product of DBT

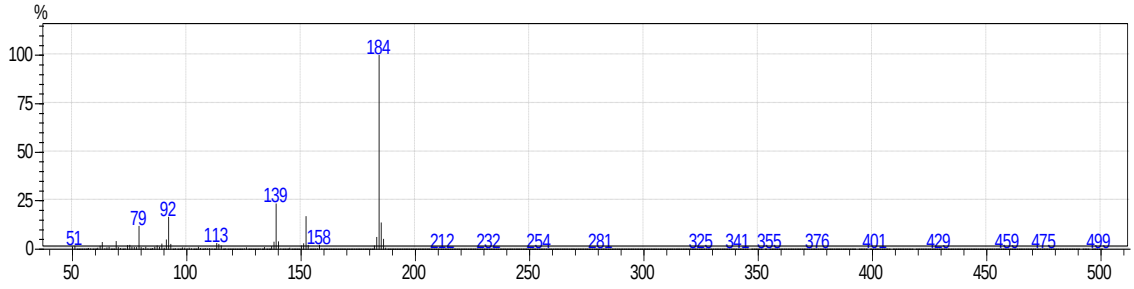

Figure 5: MS spectrum of DBT with m/z 184 (corresponded to peak II, 11.47 min)

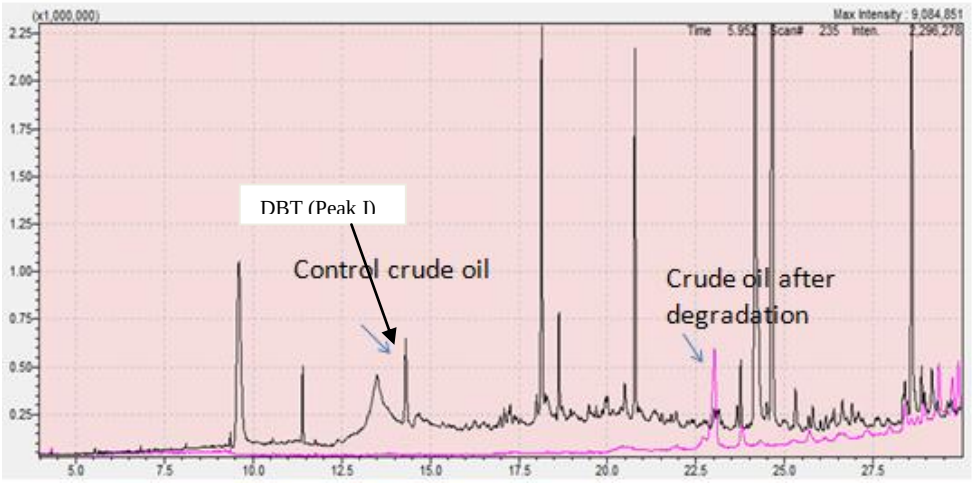

Figure 6: GC chromatograms of crude oil before and after degradation

CONCLUSION

Out of 32 bacterial isolates enriched from six oil contaminated soil samples, five have shown positive results with Gibb's assay indicating the 4S pathway for sulfur removal. Among five isolates, isolate E1 was selected based on maximum color intensity at 610 nm giving indication of maximum 2-HBP production. Based on morphological and biochemical analysis, the isolate E1 was found to be closely related to *Bacillus* sp. Isolate E1 has shown 88.0 ± 1.04 % desulphurization of DBT (2 mM) at 72 h which was also validated by GC-MS analysis. The maximum 2-HBP production (1.103 ± 0.051 mM) was measured at 48 h after which it might have started conversion into the other metabolites like biphenyl or 2-MBP [16]. The immobilized resting cells and biphasic system exhibited better desulphurization efficiency than free resting cells in aqueous phase. The resting cells of isolate E1 exhibited complete degradation of DBT in crude oil besides other components. The isolated *Bacillus* sp. appeared as a potential candidate for biodesulphurization of crude oil.

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REFERENCES

1. Yu B, Xu P, Shi Q, Ma C. Deep desulfurization of diesel oil and crude oil by a newly isolated *Rhodococcus erythropolis* strain. Appl Environ Microbiol. 2006;72:54–58.
2. Mohebali G, Ball AS. Biocatalytic desulfurization (BDS) of petrodiesel fuels. Microbiol. 2008;154:2169–2183.

3. Sohrabi M, Kamyab H, Janalizadeh N, Huyop FZ. Bacterial desulfurization of organic sulfur compounds exist in fossil fuels. J Pure Appl Microbiol. 2012;6:717-729.
4. USEPA, United States Environmental Protection Agency 2016.
5. Bandyopadhyay S, Chowdhury R, Bhattacharjee C, Pan S. Simultaneous production of biosurfactant and ULSD (ultra low sulfur diesel) using *Rhodococcus* sp. in a chemostat. Fuel. 2013;113:107–112.
6. Mohamed ME, Al-Yacoub ZH, Vedakumar JV. Biocatalytic desulfurization of thiophenic compounds and crude oil by newly isolated bacteria. Frontiers Microbiol. 2015;6:1-12.
7. Siddiqui SU, Ahmed K. Methods for desulfurization of crude oil: a review. Sci Int. 2016;28:1169-1173.
8. Gregorio DS, Zocca C, Sidler S, Toffanin A, Lizzari D, Vallini G. Identification of two new sets of genes for dibenzothiophene transformation in *Burkholderia* sp. DBT1. Biodegradation 2004;15:111–123.
9. Pokethitiyook P, Tangaromsuk J, Kruatrachue M, Kalambaheti C, Borole AP. Biological removal of organic sulfur by bacterial strains isolated in Thailand. Sci Asia 2008;34:361–366.
10. Davoodi-Dehaghani F, Vosoughi M, Ziaee AA. Biodesulfurization of dibenzothiophene by a newly isolated *Rhodococcus erythropolis* strain. Bioresour Technol. 2010;101:1102–1105.
11. Dudley MW, Frost JW. Biocatalytic desulurization of aryl sulfonates. Bioorg Med Chem. 1994;2:681-690.
12. Constanti M, Glralt J, Bordons A. Desulfurization of dibenzothiphene by bacteria. World J Microbiol Biotechnol. 1994;10:510-516.
13. Konishi J, Ishii Y, Onaka T, Okumura K, Suzuki M. Thermophilic carbon–sulfur-targeted biodesulfurization. Appl Environ Microbiol. 1997;63:3164–3169.
14. Chang JH, Rhee SK, Yong KC, Chang HN. Desulfurization of diesel oils by newly isolated dibenzothiophene degrading *Nocardia* sp. strain CYKS2. Biotech Progress. 1998;14:851-855.
15. Kim SB, Brown R, Oldfield C, Gilbert SC, Iliarionov S, Goodfellow M. *Gordonia amicalis* sp. nov., a novel dibenzothiophene-desulfurizing actinomycete. Int J Systematic Evolutionary Microbiol. 2000;50:2031–2036.
16. Li W, Zhang Y, Wang MD, Shi Y. Biodesulfurization of dibenzothiophene and other organic sulfur compounds by a newly isolated *Microbacterium* strain ZD-M2. FEMS Microbiol Lett. 2005;247:45–50.
17. Li F, Xu P, Feng J, Meng L, Zheng Y, Luo L, Ma C. Microbial desulfurization of gasoline in a *Mycobacterium goodii* X7B immobilized-Cell System. Appl Environ Microbiol. 2005;71:276-281.
18. Zahra E, Giti E, Sharareh P. Removal of dibenzothiophene, biphenyl and phenol from waste by *Trichosporon* sp. Scientific Res Essay. 2006;1;72-76.
19. Ansari F, Prayuenyong P, Tothill I. Biodesulfurization of dibenzothiophene by *Shewanella putrefaciens* NCIMB 8768. J Biol Phys Chem. 2007;7:75-78.
20. Torkamani S, Shayegan J, Yaghmaei S, Alemzadeh I. Study of the first isolated fungus capable of heavy crude oil biodesulfurization. Ind Eng Chem Res. 2008;47:7476–82.
21. Bahuguna A, Lily MK, Munjal A, Singh RN, Dangwal K. Desulfurization of dibenzothiophene (DBT) by a novel strain *Lysinibacillus sphaericus* DMT-7 isolated from diesel contaminated soil. J Environ Sci. 2011;23:975–982.
22. Bhatia S, Sharma DK. Biodesulfurization of dibenzothiophene, its alkylated derivatives and crude oil by a newly isolated strain *Pantoea agglomerans* D23W3. Biochem Eng J. 2010;50:104–109.
23. Rath K, Mishra B, Vuppu S. Biodegrading ability of organosulfur compound of a newly isolated microbe *Bacillus* sp. KS1 from the oil contaminated soil. Biosci Technol. 2012;4:465-471.
24. Olabemiwo OM, Adelowo OO, Tella AC, Bello IA. Preliminary study on biodesulfurization of Nigerian natural bitumen. Int J Basic Appl Sci. 2013;13:79-86.
25. Bordoloi NK, Rai SK, Chaudhuri MK, Mukherjee AK. Proteomics and metabolomics analyses to elucidate the desulfurization pathway of *Chelatococcus* sp. PLoS ONE 2016;11:e0153547
26. Chang JH, Chang YK, Cho KS, Chang HN. Desulfurization of model and diesel oils by resting cells of *Gordona* sp. Biotechnol Lett. 2000;22:193–196.
27. Marcelis CLM, Leeuwen MV, Polderman HG, Janssen AJH, Lettinga G. Model description of dibenzothiophene mass transfer in oil/water dispersions with respect to biodesulfurization. Biochem Eng J. 2003;16:253-264.
28. Kawaguchi H, Kobayashi H, Sato K. Metabolic engineering of hydrophobic *Rhodococcus opacus* for biodesulfurization in oil–water biphasic reaction mixtures. J Biosci Bioeng. 2011;113:360-366.

29. Labana S, Pandey G, Jain RK. Desulfurization of dibenzothiophene and diesel oils by bacteria. Lett Appl Microbiol. 2005;40:159–163.
30. Saha A, Santra SC. Isolation and characterization of bacteria isolated from municipal waste for production of industrial enzymes and waste degradation. J Microbiol Exp. 2014;1:00003.
31. Derikvand P, Etemadifar Z. Improvement of biodesulfurization rate of alginate immobilized *Rhodococcus erythropolis* R1. Jundishapur J Microbiol 2014;7:1-7.
32. Derikvand P, Etemadifari Z, Saber H. Sulfur removal from dibenzothiophene by newly isolated *Paenibacillus validus* strain pd2 and process optimization in aqueous and biphasic (model-oil) systems. Polish J Microbiol. 2015;64:47-54.
33. Raheb J, Memari B, Hajipour M J. Gene-manipulated desulfurizing strain *Pseudomonas putida* reduced energy consuming in the biodesulfurization process. Energy sourc Part A 2011;33;2018-2026.
34. Chen H, Zhang WJ, Cai YB, Zhang Y, Li W. Elucidation of 2-hydroxybiphenyl effect on dibenzothiophene desulfurization by *Microbacterium* sp. strain ZD-M2. Bioresour Technol. 2008;99:6928–6933.
35. Yang J, Hu Y, Zhao D, Wang S, Lau PCK, Marison IW. Two-layer continuous-process design for the biodesulfurization of diesel oils under bacterial growth conditions. Biochem Eng. 2007;37:212–218.
36. Li G, Li S, Zhang M, Wang J, Zhu L, Liang F, Liu R, Ma T. Genetic rearrangement strategy for optimizing the dibenzothiophene biodesulfurization pathway in *Rhodococcus erythropolis*. Appl Environ Microbiol. 2008;76;971–976.
37. Naito M, Kawamoto T, Fujino K, Kobayashi M, Maruhashi K, Tanaka A. Long-term repeated desulfurization by immobilized *Rhodococcus erythropolis* KA2-5-1 cells. Appl Microbiol Biotechnol. 2001;55:374–378.
38. Gunam IBW, Kenta Y, Nengah IS, Nyoman SA, Wayan RA, Michiko T, Fusao T, Teruo S, Kozo A. Biodesulfurization of dibenzothiophene and its derivatives using resting and immobilized cells of *Sphingomonas subarctica* T7b. J Microbiol Biotechnol. 2013;23:473–482.
39. Amin GA, Bazaid SA, Halim AM. A two-stage immobilized cell bioreactor with *Bacillus subtilis* and *Rhodococcus Erythropolis* for the simultaneous production of biosurfactant and biodesulfurization of model oil. Pet Sci Techno. 2011;31:2250-2257.
40. Caro A, Boltes K, Leton P, Garcia-Calvo E. Dibenzothiophene desulfurization in resting cell conditions by aerobic bacteria. BiochemEng J. 2007;35:191-197.
41. Nuhu AA. Bio-catalytic desulfurization of fossil fuels: a mini review. Rev Environ Sci Biotechnol. 2013;12:9-23.
42. Chandran P, Das N. Biosurfactant production and diesel oil degradation by yeast species *Trichosporon asahii* isolated from petroleum hydrocarbon contaminated soil. Int J Eng Sci Techno. 2010;**2**:6942-6953.