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Growth-phase dependent biodesulfurization of Dibenzothiophene by *Enterobacter* sp. strain NISOC-03

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ABSTRACT: Petroleum-polluted soil samples from Ahvaz oilfield were enriched, using three methods to detect microorganisms with different dibenzothiophene degradation capabilities. Strain NISOC-03, a nitrate-reducing, oxidase negative, catalase, citrate, and urease positive, gram negative rod, showed interesting dibenzothiophene desulfurization behavior, designated as *Entreobacter* sp. strain NISOC-03 based on phenotype and genotype analyses. Gas chromatography, biomass measurement, and Gibb's assay showed that in the presence of benzoate as the carbon source, strain NISOC-03 utilized 64% of 0.8 mM dibenzothiophene, producing 0.27 mM phenyl phenol during the exponential growth phase, though the produced phenyl phenol was degraded in the stationary growth phase. In the presence of glucose as the carbon source, however, strain NISOC-03 metabolized only 19.6% of 0.8 mM dibenzothiophene. Furthermore, replacing glucose with ethanol or glycerol led to the same reduction of the dibenzothiophene utilization. It is thus concluded that the chemistry of the potential carbon source(s) in the culture medium has a significant influence on the quality and the rate of dibenzothiophene metablization, and the enrichment designation has a very vital effect on the biodegradation efficiency of the isolated microorganisms.

Keywords: biodegradation, biodesulfurization, dibenzothiophene, enterobacter, phenyl phenol.

INTRODUCTION

Typically sulfur level in crude oils is between 1000 and 30000 ppm. Diesel oil sulfur content can be around 5000 ppm even though in recent years it has been reduced to less than 15 ppm (Boniek et al., 2015; Del Olmo et al., 2005). According to the reports from United Nations Environmental Program (UNEP), countries such as Iran, Egypt, and Iraq are using diesel fuels with sulfur levels higher than 5000 ppm. In addition to low sulfur, mature crude oil is going to be exhausted in the reservoirs and refineries have to

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handle the highly immature, sulfur- and nitrogen-rich, heavy crude oils (containing high concentrations of sulfur, nitrogen, and several metals, particularly nickel and vanadium) soon (Jiang et al., 2014).

Further, SO_2 emission through fossil fuel combustion is a major source to generate acid rains and air pollution. Regulatory standards against the sulfur content of petroleum products such as gasoline and diesel oil are becoming stricter due to the need for environmental protection. The current desulfurization technology, hydrodesulfurization (HDS), is utilized with chemical catalysts under severe conditions (extremely high temperature and pressure) (Caro et al., 2007; Guerinik and Al-Mutawah, 2003; Li et al., 2005a). There are also many other options: selective adsorption, catalytic oxidative desulfurization and biodesulfurization (BDS), to desulfurize gasoline and/or crude oil, yet none has been scaled up for commercial use yet (Maass et al., 2015; Nuhu, 2013).

Up to 70% of the sulfur in fossil fuels is found as refractory polynuclear aromatic sulfur heterocyclics (dibenzothiophene (DBT) and substituted DBTs), which are particularly recalcitrant to HDS (Constanti et al., 1994); therefore, biocatalytic desulfurization (BDS) may offer an attractive alternative or may be used complementary to HDS due to its mild operating conditions, low costs, and greater reaction specificity afforded by the nature of biocatalysis (Breysse et al., 2003; Constanti et al., 1994; Li et al., 2005b). Two major pathways of DBT metabolism are Kodama and 4S pathways, even though various alternatives may be present and different groups of microorganisms use various enzymatic reactions to utilize DBT as carbon, sulfur, or carbon and sulfur sources (Konishi and Aruhashi, 2003; Mohebali et al., 2006; Papizadeh et al., 2010; 2011; Young et al., 2006).

Dibenzothiophene, a tricyclic organosulfur heterocycle compound, exists as a colorless solid powder when purified. Because of high density (1.252 g/cm^3) and water-insolubility, it precipitates in aquatic media. There are also other polycyclic aromatic hydrocarbons, like as Phenanthrene, Pyrene, Anthracene, and Benzothiophene, with the above-mentioned features. Water-insolubility and high density are two main characteristics of these hydrocarbons, reducing their bioavailability, making them recalcitrant to biological degradation. Hence, they may be biodegraded more easily when solved in organic solvents (Papizadeh et al., 2010; 2011). Also, the biodegradation and biodesulfurization of such hydrocarbons in aquatic media can be catalyzed by many microorganisms, unable to resist the toxic effects of gasoline and/or other fuels. Furthermore, the biodegradation and biodesulfurization rate of a given microbial strain is very different in mono-phase aquatic and oil-water biphasic systems, thus it is recommended to consider the reaction of a given biodegrading strain in model oils, gasoline, or other fuels.

A small number of bacterial strains, belonging to the genera of *Paenibacillus* (Konishi and Aruhashi, 2003), *Corynebacterium* (Luo et al., 2003), *Rhodococcus* (Castorena et al., 2002; De Carvalho et al., 2007; Del Olmo et al., 2005; Konishi et al., 2005), *Pseudomonas* (Alcon et al., 2005; Hou et al., 2005; Liu et al., 2015), *Gordonia* (formerly *Gordona*) (Jia et al., 2006; Kim et al., 2004; Maghsoudi et al., 2000), *Microbacterium* (Li et al., 2005a; Mezcua et al., 2007; Papizadeh et al., 2010), *Mycobacterium* (Li et al., 2005b; Li et al., 2007), and *Arthrobacter* (Seo et al., 2006), are known to be able to remove sulfur from DBT via a sulfur-specific pathway or the extended "4SM" pathway. Until now, most of the discovered DBT desulfurizing microorganisms have been gram-positive bacteria, though *Stenotrophomonas*

NISOC-04 (Papizadeh et al., 2011), *Pseudomonas delafieldii* R-8 (Luo et al., 2003), *Pseudomonas putida* (Alcon et al., 2005), *Pseudomonas stutzeri* (Hou et al., 2005), *Klebsiella* sp. *LSSE*-H2 (Li et al., 2008) and *Enterobacter* sp. (Liu et al., 2015) have been reported as gram negative strains, able to biodegrade C-S or C-C bonds of DBT (Jiang et al., 2014).

Enterobacter sp. strain NISOC-03 as a member of *Enterobacteriaceae* is resistant to the toxic effects of gasoline and its DBT utilization behavior is to some extent unique. This study has dealt with DBT metabolism of *Enterobacter* sp. strain NISOC-03; another gram negative DBT desulfurizing bacterium, discovering some critical features of the medium culture that influence the desulfurization rate.

MATERIALS AND METHODS

Culture medium, Enrichment, and DBT metabolism

Mineral Salt Medium (MSM) was prepared from 3.2 g KH_2PO_4, 5.1 g K_2HPO_4, 2 g NH_4Cl, 0.6 g $MgCl_2$, 0.001 g $FeCl_3$, and 0.001 g $CaCl_2$ dissolved in a litter of double distilled deionized water (DDW). Final pH=6.8 was adjusted by 20% NaOH titration (Papizadeh et al., 2010). Desirable carbon and sulfur sources were also added as is mentioned in the enrichment procedure.

Biphasic system (liquid paraffin-aquatic). 0.2 g of soil sample, collected from Ahvaz oil fields, was inoculated into 98 ml MSM in a 250 ml Erlenmeyer flask. Two g/L of sodium benzoate was added as carbon source. Furthemore, 2 ml of liquid paraffin, containing 100 mM of authentic DBT, was added to enrich the microorganisms able to uptake DBT from the liquid paraffin layer. Flasks were incubated at 30°C to encourage pellicle formation by the mostly aerobic microorganisms, capable of uptaking DBT from the hydrophobic phase. After 20 days of incubation, a piece of the pellicle, developed at the medium's interface (between liquid paraffin and water), was inoculated into fresh medium. After 2 weeks of incubation in the same conditions, a piece of the pellicle was removed, serially diluted, and plated on 1.5% agar-solidified MSM, containing 2 g/L benzoate and 0.5 mM $MgSO_4$ as the carbon and sulfur sources, respectively. Cultures were incubated at 30°C until development of representative single colony (14 days) (Papizadeh et al., 2010).

Bait enrichment. Glass slides, immersed in crude oil containing 100 mM authentic DBT were buried in soil samples to encourage colonization of resistant crude oil and DBT metabolizing microorganisms, being incubated at 30°C. After 10 days of incubation, the visible film that developed on the slides was inoculated into a 250 ml Erlenmeyer flask with 96 ml MSM containing 2 g/L of benzoate as carbon source. Also 2 ml of gasoline containing 100 mM authentic DBT was added to the medium and incubated in an orbital shaker (150 rpm, 30°C). After 10 days of incubation, 0.5 ml of the culture medium was inoculated into the same medium to be incubated at the same conditions. Eighty µl of the third transfer was streaked on solidified MSM containing 2 g/L of sodium benzoate and 0.5 mM $MgSO_4$ as carbon and sulfur sources, respectively. After 14 days of incubation at 30°C, the representative single colonies were obtained and purified (Papizadeh et al., 2011).

Enrichment in aquatic medium. An amount of 0.2 gram of the soil sample was added to 100 ml of MSM medium, enriched with 2 ml of filter sterilized 80 mM DBT stock in ethanol, and was incubated (for 150 rpm at 30°C). After 10 days of incubation, 50 µl of culture was subcultured into the same fresh medium, and after 2 subcultures 50 µl of the material was streaked on solidified MSM, containing 2 g/L of sodium benzoate and 0.5 mM $MgSO_4$ as carbon and sulfur sources, respectively. After 14 days of incubation at 30°C, the visible single colonies were purified (Liu et al., 2015).

Purified colonies were inoculated into separate flasks, containing 100 ml of MSM with 1 mM DBT or 2-HBP as the sole source of carbon to detect biodegradation (starting cell concentration of 12×10^4 cell/ml). DBT biodesulfurization ability of the strains was studied in the presence of glucose, glycerol, ethanol, and benzoate (final concentration of 3 g/L) as carbon sources and DBT as sulfur source in a simple aquatic medium. DBT was added from an acetone or ethanol solution. Also the ability of the strains to metabolize DBT was investigated in a liquid paraffin/water biphasic medium.

Analytical procedures

Most of the examinations and analytical assays were repeated three times (Papizadeh et al., 2010; 2011). In case of GC-SCD analysis, some samples were analyzed repeatedly to reach logical data.

Metabolite extraction. Method A: An amount of 0.5 ml of the culture medium was acidified to pH ≤ 2.0 by titration of 50% HCl. 0.5 ml ethyl acetate was added and the mixture was stirred for 5 min. Then, after a 5 min interval to separate the phases, ethyl acetate was removed. Extraction with the same method was repeated 3 times with all of the ethyl acetate aliquots centrifuged (5000 rpm, 10 min). Upper 2/3 volume of the ethyl acetate was removed and dried at 50°C. Finally, 0.5 ml of ethanol was added and analyzed. Method B**:** One ml of the culture medium was centrifuged (3000 rpm, 10 min) and supernatant was acidified to pH ≤ 2.0 by 50% HCL titration. The supernatant was extracted three times by ethyl acetate as mentioned above.

Gas chromatography. Gas Chromatography (GC) was set up and performed to detect DBT utilization. Ethyl acetate extracted samples, which were monitored to determine the concentration of DBT and its sulfurous metabolites, using a GC equipped with a sulfur chemiluminescence detector (SCD). One µl of the sample was injected into a column, with an interior diameter of 0.32 mm, height of 30 m, and 0.015 mm of film CP-SIL 5CB for sulfur. The column operated at 1.7 ml/ min volume velocity of helium carrier gas. The injector and detector temperatures were maintained at 275°C and 800°C respectively, while the column temperature remained at 200°C, according to different organic sulfur compounds until the appearance of the characteristic peak (Mezcua et al., 2007).

Identification

16S rDNA sequence-based Identification. 16S rDNA of the isolate was amplified in a PCR amplification, as described in previous works [9, 10]. Nearly complete fragment of 16S rDNA was amplified, using FD1 and RD1 primers and the product was sequenced with the same primers from both 5′ and 3′ ends by Genefanavaran Biotechnology Corporation. Using CLC main workbench and Geneious packages, the full 1500 bp 16S rDNA was assembled and studied in order to find its phylogenetic affiliation.

Phenotypic Identification. Identification of the isolate was performed based on phenotypic methods as follows: gram staining and reaction, oxidase and catalase reactions, urea hydrolysis, indole production, methyl red, Voges-Proskauer, oxidative-fermentative metabolism, motility, optimum pH, temperature and salinity for growth, nitrate reduction, citrate utilization, and hydrogen sulfide production in TSI medium.

RESULTS AND DISCUSSION

Culture Medium, Enrichment, and DBT Metabolism

It was shown that strain NISOC-03 did not use up DBT as carbon source in culture media, containing DBT and MgSO4 as carbon and sulfur sources, respectively. Also, it has been highlighted that the carbon source is the most limiting factor in most natural environment niches and the introduction of hydrocarbons to the environment encourages hydrocarbon-

degrading microorganisms to produce higher biomass via nutrients, available in the soil. Other microorganisms, which are probably unable to utilize hydrocarbons, may lose nutrients to produce biomass, even though these conditions occur just in the vicinity of the glass slides, used as baits. Adding the glass slides into the liquid medium introduced hydrocarbon-resistant/degrading microorganisms. What is more, unshaken incubation along with an organic layer, present on the aquatic phase encouraged the hydrocarbon-degrading microorganisms to develop as a pellicle at the interface of aquatic and hydrophobic phases (Papizadeh et al., 2010; 2011).

Bi-phasic enrichment culture by a hydrophobic carbon source and an aquatic phase, enriched by macro and micro nutrients, was shown to be suitable for selecting microorganisms, resistant to toxic hydrocarbons and solvents. Unshaken incubation and the organic layer present on the aquatic phase, can encourage the hydrocarbon-degrading microorganisms to develop as a pellicle at the interface of aquatic and hydrophobic phases (Li et al., 2008; *Papizadeh et al., 2010; Papizadeh and Roayaei Ardakani, 2010*).

By means of the traditional enrichment and extraction procedures described above, we obtained many bacterial and fungal colonies, almost unable to tolerate and metabolize DBT in pure cultures. Actually, the water insoluble Poly-Aromatic Hydrocarbons (PAHs), such as DBT, phenanthrene, anthracene, and pyrene have specific gravities higher than water and precipitate in aquatic solutions. These compounds can be biodegraded in simple enrichment cultures but considering their scarcity in aquatic solutions, microorganisms should either attach physically to these molecules or produce bio-molecules to solve trace amounts of them in the vicinity of the cell membrane. Obviously this kind of metabolism progresses pretty slowly (Ollivier and Magot, 2005). Nonetheless, a very low concentration of DBT and other PAHs may solve in aquatic solutions and can be utilized, though it cannot afford the growth demands. Besides, the toxicity of a given hydrocarbon is quite limited as long as it is not dissolved and it reduces the selectivity of the designed medium (Ollivier and Magot, 2005).

In the presence of DBT and glucose as sulfur and carbon sources, *Enterobacter* sp. strain NISOC-03 utilized 19.6% of DBT, producing an insignificant amount of 2-HBP as indicated by Gibb's assay (at the 240^{th} hour of incubation; Figs. 1 and 2). About 1 µM of 2-HBP was detected after 96h of incubation, which increased up to 4 µM as analyzed at the 240^{th} hour of the process. Gibb's assay showed that the produced 2-HBP was diluted after this period and 2-HBP started to disappear during the stationary phase of growth (t_{240h}-t_{312h}). In comparison, in the presence of benzoate and DBT as the carbon and sulfur sources, the rate of the DBT biodesulfurization was 3.26 times higher than that of glucose (Figs. 3 and 4). In the presence of benzoate, *Enterobacter* sp. strain NISOC-03 used up about 63.9% of DBT, producing 1 µM of 2-HBP after 12h, and along with DBT utilization and biomass production, accumulating 2-HBP itself (27 µM after 96h). Five µM of sulfate was detected only at this growth phase as barium chloride photometry showed. Such a DBT consumption pattern and 2-HBP production, witnessed during the log phase of growth was followed by an accelerated 2-HBP dilution to disappear into the medium during stationary phase of growth (t_{120h}-t_{168h}) (Fig. 3). Also, adding concentrations of carbon and sulfur sources by two and three folds had no impact on the 2-HBP dilution which occurred always during the stationary growth phase. Additionally, there were no significant changes from adding glycerol as the carbon source and the DBT metabolism was comparable to the results, achieved from glucose.

It was shown that adding benzoate (2 g/l) as carbon source as well as DBT (final concentration of 1mM) from an ethanol stock, in a way that the ethanol concentration was limited within 2-3% V/V, led to the highest utilization level of DBT. Moreover, replacing ethanol with acetone or diethyl ether, as the solvent for DBT, to be added to the aqueous phase decreased the rate of DBT utilization.

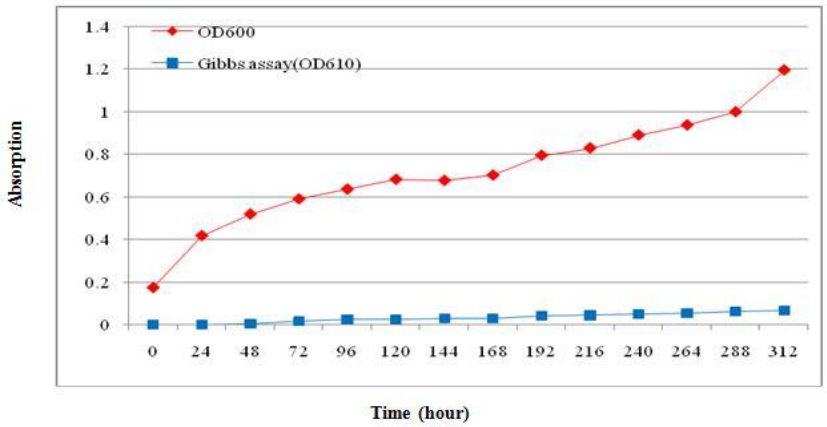

Fig. 1. DBT utilization and 2-HBP production by *Enterobacter* sp. strain NISOC-03 in the presence of Glucose and DBT (dissolved in Acetone) as carbon and sulfur sources

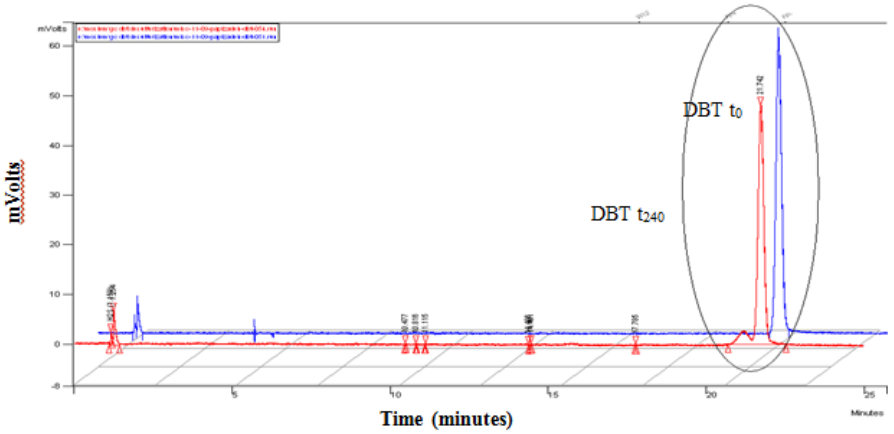

Fig. 2. Utilization of 19.6% of 0.8 mM DBT after 240h incubation by *Enterobacter* sp. strain NISOC-03 in the presence of glucose as the sole carbon source

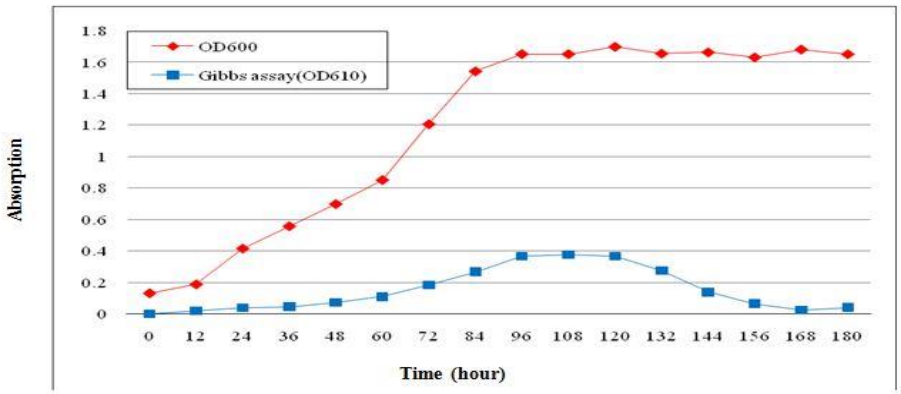

Fig. 3. DBT utilization and 2-HBP production by *Enterobacter* sp. strain NISOC-03 in the presence of benzoate and DBT (dissolved in Ethanol) as carbon and sulfur sources

UV spectrophotometry studies showed that the presence of DBT as the sole sulfur source affected absorption at wavelengths 285 nm and 323 nm without detecting any significant absorption change in the presence of DBT as the sole carbon or carbon and sulfur sources (Fig. 5). UV spectrophotometery showed that during stationary growth phase, absorption at wavelengths specific for phenyl phenol, declined. It was also concluded that the 2-HBP, produced during the exponential growth phase, was mineralized during the stationary phase. Furthermore, biomass measurement of MSM-sulfate, MSM-DBT, and sulfur-free MSM flasks, containing glucose as carbon source, showed that DBT affected biomass production effectively, and that such an influence was not detected in the presence of DBT as carbon or carbon and sulfur sources (Fig. 6). Additionally, it was shown that physico-chemical properties of the used solvent (ethanol or acetone), especially its boiling point value, can affect the DBT utilization, 2-HBP production, and growth pattern (Figs. 7 and 8).

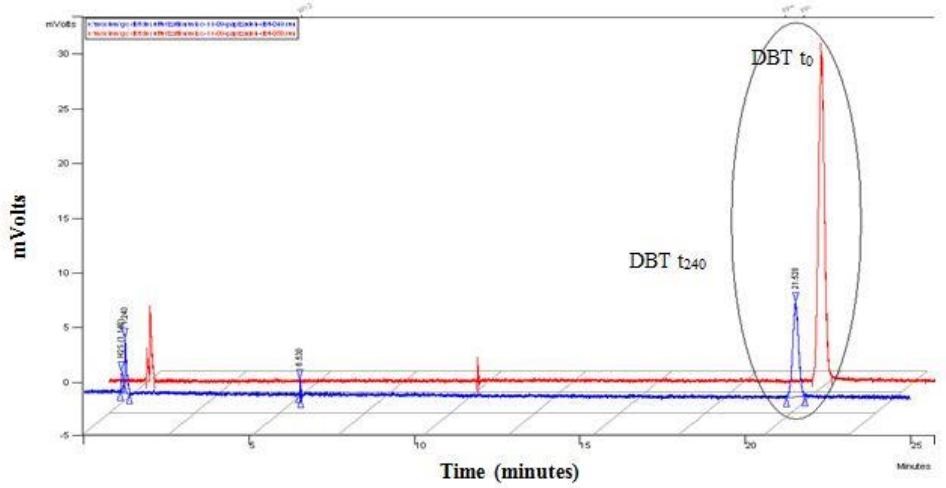

Fig. 4. Utilization of 64% of 0.8 mM DBT after 240 hours of incubation by *Enterobacter* sp. strain NISOC-03 in the presence of benzoate as the sole carbon source

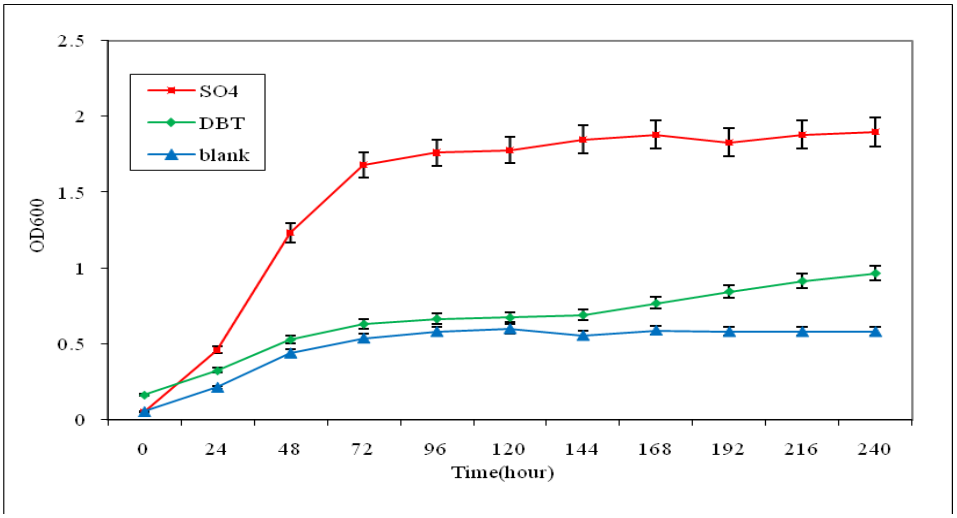

Fig. 5. Growth pattern of *Enterobacter* sp. strain NISOC-03 in the presence of sulfate, DBT, and in sulfur-free blank flask (glucose as carbon source)

Using 64% of 0.8 mM DBT as sulfur source must result in the production of 0.512 mM of 2-HBP, but by adopting optimized extraction techniques, only about 0.27 mM of 2-HBP was detected (at the end of log phase), and this concentration was diluted to disappear into the medium, during the stationary growth phase. By aging the culture and nutrient exhaustion of culture medium, apparently the strain started to utilize 2-HBP as carbon source. Another reason behind this might be higher water solubility of 2-HBP as well as its lower specific gravity. Such properties increase the vulnerability of 2-HBP to the biodegradation.

Considering the intracellular metabolism of DBT in the majority of reported strains, it is assumed that the presence of benzoate, and probably other phenol-like compounds, can increase the uptake and/or transformation rate of DBT via co-metabolism or a metabolic pathway, encouraged in the presence of phenol-like chemicals. Such acceleration of DBT biodesulfurization was detected even in the concomitant presence of ethanol or benzoate with glucose. Furthermore, ethanol and acetone are solvents that increase DBT solubility in aquatic medium, though in terms of their boiling point values, ethanol evaporates more slowly and is a more favorable solvent. Thus, ethanol and benzoate chemistry might cause the differences in biomass production, illustrated in Figures 1, 3, 7, and 8. In the presence of benzoate, DBT utilization was triggered after 24h of lag phase, followed by a 48h long log phase, during which an accelerated DBT utilization and 2-HBP production was detected. Most of DBT utilization and 2-HBP production were detected from the middle to the end of log phase (Figs. 1 and 3) (Kim et al., 2004).

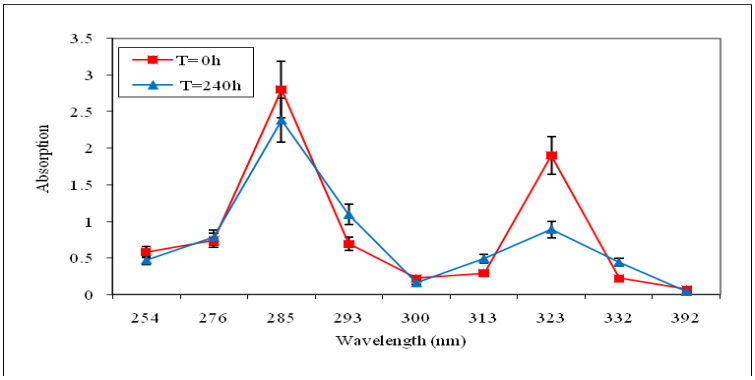

Fig. 6. Decline in absorption at 285 nm and 323 nm shows DBT utilization by *Enterobacter* sp. strain NISOC-03

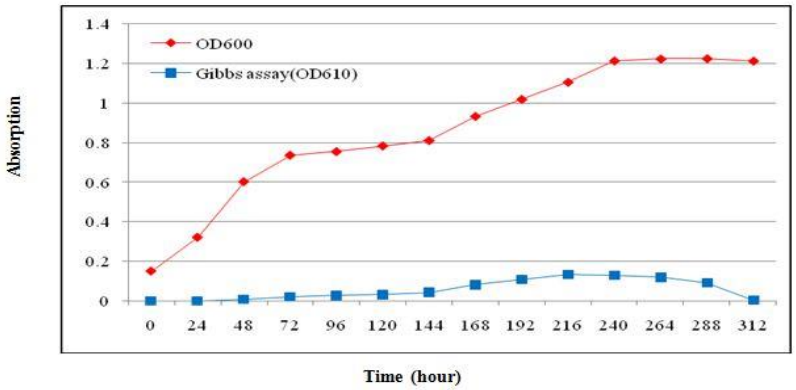

Fig. 7. DBT utilization and 2-HBP production by *Enterobacter* sp. strain NISOC-03 in the presence of benzoate and DBT (Dissolved in acetone) as carbon and sulfur sources

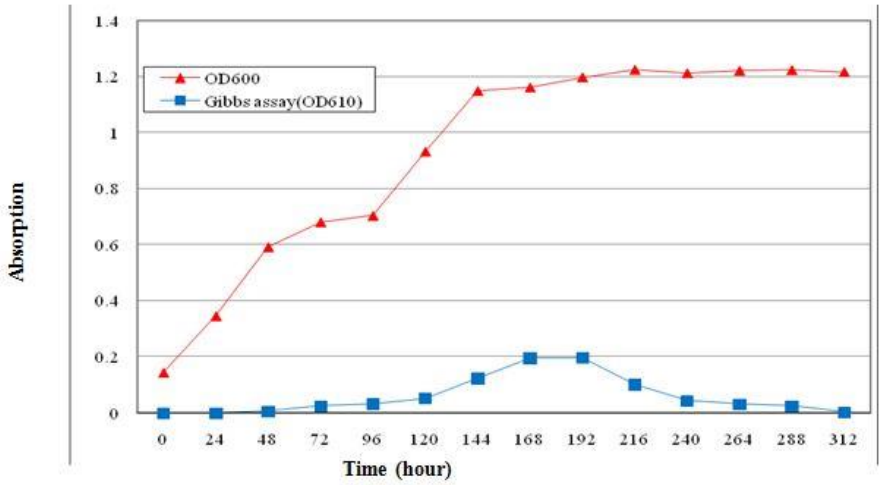

Fig. 8. DBT utilization and 2-HBP production by *Enterobacter* sp. strain NISOC-03 in the presence of Ethanol and DBT (Dissolved in Ethanol) as carbon and sulfur sources

Identification

As a result of aligning the 16S rDNA sequence with 187 sequences of *Enterobacteriaceae* family (obtained from Ribosomal Database Project (RDP) version 10) the isolate was identified as a member of the genus *Enterobacter*. Strain NISOC-03 is characterized as a motile, Citrate, Urease, Methyl red, and Voges-Proskauer positive facultative anaerobic gram negative nitrate reducing coccobacili. The 16S rDNA gene sequence of *Enterobacter* sp. strain NISOC-03 was deposited in Gene bank under the accession number: HQ419280.

CONCLUSION

The present study showed that there are various alternative pathways by which polyaromartic hydrocarbons, like DBT, can be used up by microorganisms. Such a diverse metabolic network, eukaryotic and prokaryotic units included, enables the soil microbiome to biodegrade and mineralize varied recalcitrant chemicals. Besides, it was demonstrated that the efficiency of a given strain to biodegrade a given compound could not be simply evaluated and the efficiency was highly dependent on the environmental parameters. Also, the chemistry of the culture medium was highly critical for the evaluation of biodegradation efficiency. Thus, many of the strains that had been assumed unable to metabolize a given compound, may be highly biodegrading in the desirable conditions and vice versa.

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REFERENCES

Alcon, A., Santos, V.E., Martin, A.B., Yustos, P. and Garcia-Ochoa, F. (2005). Biodesulfurisation of DBT with Pseudomonas putida CECT5279 by resting cells: Influence of cell growth time on reducing equivalent concentration and HpaC activity. Biochem. Eng. J., 26, 168–175.

Boniek, D., Figueiredo, D., dos Santos A.F.B. and de Resende Stoianoff M. (2015). Biodesulfurization: a mini review about the immediate search for the future technology. Clean Technol. Environ. Policy., 17, 29–37.

Breysse, M., Djega-Mariadassou, G., Pessayre, S., Geantet, C., Vrinat, M., Pérot, G. and Lemaire, M. (2003). Deep desulfurization: reactions, catalysts

and technological challenges. Catal. Today., 84, 129–138.

Caro, A., Boltes, K., Let´on, P. and Garc´ıa-Calvo, V. (2007). Dibenzothiophene biodesulfurization in resting cell conditions by aerobic bacteria. Biochem. Eng. J., 35, 191-197.

Castorena, G., Sua.rez, C., Valdez, I., Amador, G., Fernandez, L. and Le Borgne, S. (2002). Sulfur-selective desulfurization of dibenzothiophene and diesel oil by newly isolated Rhodococcus sp. strains. FEMS Microbiol. Lett., 215, 157-161.

Constanti, M., Giralt, J. and Bordons, A. (1994). Desulphurization of Dibenzothiophene by bacteria. World J. Microbiol. Biotechnol., 10, 510–516.

De Carvalho, C.C.C.R., Fatal, V., Alves, S.S. and Fonseca, M.M.R. (2007). Adaptation of Rhodococcus erythropolis cells to high concentrations of toluene. Appl. Microbiol. Biotechnol., 76, 1423–1430.

Del Olmo, C.H., Santos, V.E., Alcon, A. and Garcia-Ochoa, F. (2005). Production of a Rhodococcus erythropolis IGTS8 biocatalyst for DBT biodesulfurization: influence of operational conditions. Biochem. Eng. J., 22, 229–237.

Guerinik, K. and Al-Mutawah, Q. (2003). Isolation and characterization of oil-desulphurizing bacteria. World J. Microbiol. Biotechnol., 19, 941–945.

Hou, Y., Kong, Y., Yang, J., Zhang, J., Shi, D. and Xin, W. (2005). Biodesulfurization of dibenzothiophene by immobilized cells of Pseudomonas stutzeri UP-1. Fuel., 84, 1975–1979.

Jia, X., Wen, J., Sun, Z., Caiyin, Q. and Xie, S. (2006). Modeling of DBT biodegradation behaviors by resting cells of Gordonia sp. WQ-01 and its mutant in oil–water dispersions. Chem. Eng. Sci., 61, 1987 – 2000.

Jiang, X., Yang, S. and Li, W. (2014). Biodesulfurization of model compounds and de-asphalted bunker oil by mixed culture. Appl. Biochem. Biotechnol., 172, 62–72.

Kim, Y.J., Chang, J.H., Cho, K., Ryu, and Chang, H.W., Y. K. (2004). A Physiological Study on Growth and Dibenzothiophene (DBT) Desulfurization Characteristics of Gordonia sp. CYKS1. Korean J. Chem. Eng., 21, 436-441.

Konishi, J. and Aruhashi, M.K. (2003) 2-(20-Hydroxyphenyl) benzene sulfinate desulfinase from the thermophilic desulfurizing bacterium Paenibacillus sp. strain A11-2: purification and characterization. Appl. Microbiol. Biotechnol. 62, 356–361.

Konishi, M., Kishimoto, M., Omasa, T., Katakura, Y., Shioya, S. and Ohtake, H. (2005). Effect of sulfur sources on specific desulfurization activity of Rhodococcus erythropolis ka2-5-1 in exponential fed-batch culture. J Biosci Bioeng., 99, 259–263.

Li, W., Zhang, Y., Wang, M.D. and Shi, Y. (2005a). Biodesulfurization of dibenzothiophene and other organic sulfur compounds by a newly isolated Microbacterium strain ZD-M2. FEMS Microbiol. Lett., 247, 45–50.

Li, F., Xu, P., Feng, J., Meng, L., Zheng, Y., Luo, L. and Ma, C. (2005b). Microbial Desulfurization of Gasoline in a Mycobacterium goodii X7B Immobilized-Cell System. Appl. Environ. Microbiol., 71, 276–281.

Li, F., Zhang, Z., Feng, J., Cai, X. and Xu, P. (2007). Biodesulfurization of DBT in tetradecane and crude oil by a facultative thermophilic bacterium Mycobacterium goodii X7B. J. Biotechnol., 127, 222–228.

Li, Y.G., Li, W.L., Huang, J.X., Xiong, X.C., Gao, H.S., Xing, M. and Liu H.Z. (2008). Biodegradation of carbazole in oil/water biphasic system by a newly isolated bacterium Klebsiella sp. LSSE-H2. Biochem. Eng. J., 41, 166-170.

Liu, L., Guo, Z., Lu, J., and Xu, X. (2015). Kinetic model for microbial growth and desulphurisation with Enterobacter sp. Biotechnol lett., 37(2), 375-381.

Luo, F., Xing, J.M., Gou, Z.X., Li, S., Liu, H.Z. and Chen, J.Y. (2003). Desulfurization of dibenzothiophene by lyophilized cells of Pseudomonas delafieldii R-8 in the presence of dodecane. Biochem. Eng. J., 13, 1–6.

Maghsoudi, S., Kheirolomoom, A., Vossoughi, M., Tanaka, E. and Katoh, S. (2000). Selective desulfurization of dibenzothiophene by newly isolated Corynebacterium sp. strain P32C1. Biochem. Eng. J., 5, 11-16.

Maass, D., Todescato, D., Moritz, D.E., Oliveira, J.V., Oliveira, D., Ulson de Souza, A.A., et al. (2015). Desulfurization and denitrogenation of heavy gas oil by Rhodococcus erythropolis ATCC 4277. Bioprocess Biosyst. Eng., 38, 1447–1453.

Mezcua, M., Fern´andez-Alba, A.R., Rodr´ıguez, A., Boltes, K., Leton, P. and Garc´ıa-Calvo, E. (2007). Chromatographic methods applied in the monitoring of biodesulfurization processes. Talanta., 73, 103-114.

Mohebali, G., Ball, A.S., Rasekh, B. and Kaytash, A. (2006). Biodesulfurization potential of a newly isolated bacterium, Gordonia alkanivorans RIPI90A. Enzyme Microb. Technol., 40, 578-584.

Nuhu, A.A. (2013). Bio-catalytic desulfurization of fossil fuels: a mini review. Rev Environ Sci Bio-Technol., 12, 9–23.

Ollivier, B. and Magot, M. (2005). Petroleum microbiology. (USA: Blackwell).

Papizadeh, M. and Roayaei Ardakani, M. (2010). Bio filtration of volatile sulphurous hydrocarbon-polluted air by hydrocarbon degrading Pseudomonas NISOC-11, J Biotechnol., 150, 209-210.

Papizadeh, M., Roayaei Ardakani, M., Ebrahimipour, G. and Motamedi, H. (2010). Utilization of dibenzothiophene as sulfur source by Microbacterium sp. NISOC-06. World J. Microbiol. Biotechnol., 26, 1195-1200.

Papizadeh, M., Roayaei Ardakani, M., Motamedi, H., Rasouli, I. and Zarei, M. (2011). C-S targeted biodegradation of dibenzothiophene by Stenotrophomonas sp. NISOC-04. Appl. Biochem. Biotechnol., 165, 938-948.

Seo, J., Keum, Y., Cho, I.K., and Q. Li, X. (2006). Degradation of dibenzothiophene and carbazole by Arthrobacter sp. P1-1. Int. Biodeterior. Biodegrad., 58, 36-43.

Young, R.F., Cheng, S.M. and Fedorak, P.M. (2006). Aerobic Biodegradation of 2, 2_-Dithiodibenzoic Acid Produced from Dibenzothiophene Metabolites. Appl. Environ. Microbiol., 72, 491–496.