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Isolation of Dibenzothiophene Utilizing Bacteria and Determining the Optimal Conditions for Utilizing by P13 Isolate

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ABSTRACT

The aim of this study was obtaining efficient bacteria capable of utilizing Dibenzothiophene that found in petroleum and its derivatives. For this purpose oil contaminated soil samples with a history of oil pollution were collected from 40 different sites in Iraq. Sixty three bacterial isolates were obtained and these isolates capable to utilize dibenzothiophene (DBT). Results showed that most of these isolates capable to grow on DBT. The growth density of bacterial isolates in presence of glycerol (C-source) was more than growth density without presence of glycerol. Twelve efficient isolates (M3, M7, M16, M20, P4, P13, P14, S21-1, S22, S26, S34 and S37, however, P13 isolate was the best one), (cleaved both C-C and C-S bonds of DBT) were candidate for identification. These 12 isolates were identified as: *Staphylococcus* spp. [6 isolates, (including P13 isolate)], three of them were *Staphylococcus aureus*. *Micrococcus* spp. (2 isolates) and one isolate for each genus of *Neisseria* sp., *Pseudomonas* sp., *Corynebacterium* sp. and *Bacillus* sp. Optimum conditions for utilization of DBT by P13 isolate were investigated. It was found that these conditions are growing this bacterium in basal salt medium (pH 8) containing 0.6 mM DBT and incubated with shaking (150 rpm) at 35°C for 3 days. The GC/MS analysis for the P13 isolate showed that this isolate utilize the DBT as sulfur and carbon source.

Key words: Petroleum, organosulfur compounds, dibenzothiophene, utilization

INTRODUCTION

Bioremediation is defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition i.e., yeast, fungi or bacteria to clean up contaminated soil and water (Strong and Burgess, 2008) are an attractive process due to its cost effectiveness and the benefit of pollutant mineralization to CO_2 and H_2O (Da Cunha, 1996). It also provides highly efficient and environmentally safe cleanup tools (Margesin, 2000). Crude oil continues to be used as the principal source of energy and play an important role in the global environmental pollutant consideration. On the other hand, oil will remain as a major source of energy in the next several decades, because reliable alternative energy consumption has not yet been substituted (Trindade *et al*., 2005; Al-Saleh and Obuekwe, 2005). At specific sites where the contaminants are petroleum products, the spectrum of necessary professional expertise is greatly expanded. However, four important aspects are necessary in bioremediation studies and these include microbial composition, contaminant type, geology of polluted site and chemical conditions at the contaminated site (Aichberger *et al*., 2005). Among the major hydrocarbon products, benzene is of major concern as it is stable, water miscible, highly mobile, poisonous and cancer-causing aromatic compound. Successful degradation of benzene by microorganisms in an aerobic environment has been reported, however, under anaerobic conditions its rate of biodegradation is observed to be very slow and poor (Singh *et al*., 2009; Vogt *et al*., 2011). Liu *et al.* (2010) reported that the poor quality of crude oil currently can obviously result in the high sulfur contents of oil products, which can lead to corrosion, catalyst poisoning, environmental pollution and other negative consequences. All fossil fuels contain variety of organic and inorganic sulfur compound. Sulfur usually accounts for around 0.03-7.89% (w/w) of crude oil but depending on the sulfur content of any given crude oil supply (Mohebali *et al*., 2007). The common bacterial genera exploited for benzene bioremediation are *Pseudomonas*, *Bacillus* (Mukherjee and Bordoloi, 2012), *Acinetobacter* (Kim and Jeon, 2009), Gammaproteobacteria (Sei and Fathepure, 2009) and *Marinobacter* (Berlendis *et al*., 2010). The other bacterial species identified for diesel biodegradation were *Pseudomonas aeruginosa* (Mariano *et al*., 2010) and *Staphylococcus aureus* (Shukor *et al*., 2009). The aim of the current study was to obtained a potential biocatalytic (bacterial isolates) for bioremediation of dibenzothiophene (DBT).

MATERIALS AND METHODS

Isolation of bacteria: The microbial selection procedures were performed in Basal Salt Media (BSM) constituted of minerals (K_2HPO_4 (4 g), Na_2HPO_4 (4 g), NH_4Cl (2 g), $MgCl_2{\bullet}6H_2O$ (0.2 g), $CaCl_2{\bullet}2H_2O$ (0.001 g) and $FeCl_3{\bullet}6H_2O$ (0.001 g) per liter of distilled water pH 7.2) and DBT as a sole source of sulfur. One hundred milliliter of BSM were dispensed in the 250 mL Erlenmeyer flasks supplemented with 0.1 mM of DBT and Glycerol (10 mM) was used as the carbon source, autoclaved at 121°C for 15 min. The 1% (w/v) of soil samples were added to the flasks and incubated at 30°C with shaking (150 rpm) for 4 days. Samples of 0.1 mL of appropriate dilutions was spread onto plates of LB agar plates, incubated at 30°C for 24 h. A single colony was picked with a sterile loop to prepare a pure subculture in a fresh LB agar plates by streaking. The purity of the isolated colonies was checked by microscopic examination. Pure isolates were growing in BSM medium containing DBT and glycerol to ensure their ability to utilize DBT.

Testing bacterial utilization of DBT either as the sole S-source or as the S and C-source: The BSM medium supplemented with (10 mM) glycerol as the carbon source and 1 mM of DBT as the only source of sulfur (medium A), that sterilized by autoclaving. The same medium was prepared but without glycerol (medium B) to test the ability of the isolates to utilizing the DBT as sole source of carbon and sulfur. One hundred milliliter of medium A or B were dispensed in conical flasks (250 mL) and inoculated with bacterial isolates. The inoculated flasks were incubated at 37°C under shaking (150 rpm) for 4 days. Bacterial growth was determined by measuring the optical density at 580 nm.

Selection the efficient isolates that capable of utilizing DBT compound: The efficient bacterial isolates capable of utilizing DBT as sulfur and carbon source were chosen depending on their growth (isolates that gave high OD_{580}) in medium (B) at 37°C for 4 days.

Identification of bacterial isolates: The selected isolates were subjected to the morphological, cultural and biochemical tests for their identification. The morphological characterization involved culturing the isolates on LB (Luria-Bertani) agar plates for studying appearance of the colonies. Following that, smears from the colonies were gram stained to study the isolate cells shapes and gram reaction. Biochemical characterization of the isolates was based on the results of Catalase, Oxidase, Indole, Methyl red, Voges-Proskauer, Citrate utilization, Triple Sugar Iron (TSI) and Motility tests. These tests were performed according to the Bergey's Manual of Determinative Bacteriology (13).

VITEK2 system for identification the efficient isolate: It is consists of 64 biochemical tests and 20 antibiotic tests. The VITEK2 system was used in this study to identify of P13 isolate.

Determination of optimal conditions for utilizing of DBT by P13 isolates: Optimization experiments were carried out through dispensed of 100 mL of BSM in 250 mL Erlenmeyer flasks, inoculated with bacterial isolates. The flasks were incubated in shaker incubator (150 rpm). After incubation period, bacterial growth density was determined for each sample by measuring the OD_{580}.

Effect of incubation time: In order to determine the optimum incubation time, the Basel Salts Medium (BSM) (pH 7.2) supplemented with 0.1 mM DBT was inoculated and incubated at 37°C for different times (24, 48, 72, 96 and 168 h).

Effect of pH: The BSM supplemented with 0.1 mM DBT was prepared at different pH values (6.5, 7, 7.5, 8, 8.5, 9 and 9.5) to determine the optimum pH. Then cultures were incubated at 37°C for 3 days.

Effect of temperature: The BSM (pH 8) supplemented with 0.1 mM DBT was inoculated and incubated at different temperatures (25, 30, 35, 40, 45 and 50°C) for 3 days. Optimum temperature was subsequently employed.

Growth at different DBT concentration: Dibenzothiophene (DBT) was added to BSM (pH 8) at different concentrations (0.025, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mM), then inoculated and incubated at 35°C for 3 days.

Bioremediation of diesel by resting bacterial cells: The P13 isolate was grown in 500 mL of BSM containing 0.6 mM DBT (as sole S-source) at 35°C. The culture with OD_{580} of 1.602 were centrifuged (10,000 rpm, 4°C for 15 min) to harvest the cell pellets, which were washed twice with BSM and resuspended in 500 mL DBT-BSM and incubated at 35°C for 48 h.

The resulting cell pellets were washed once and then suspended in 50 mL of BSM lacking DBT to prepare bacterial cell suspension. The cell suspension of each culture was dispensed in two conical flasks (50/250 mL) and 25 mL of diesel (diesel fraction of 4650 ppm total sulfur) was added to each flask.

This flask in addition to non inoculated flask, served as control for biotic losses were incubated under shaking (150 rpm) at 35°C for 7 days. After incubation the cultures were centurifuged to separate bacterial cell, DBT and diesel layer were extracted from cell free supernatant. One milliliter of culture supernatant, from cultures growing with diesel was extracted with 3 mL of ethylacetate, this solvent was evaporated by lyophilizer and the residue was dissolved in 100 μL ethanol. The total amount of sulfur contained in the diesel layer and the selective detection of the sulfur species were traced by GC/MS analysis.

The identity and quantity of end products of extracted samples were determined by using Gas Chromatography Mass-Spectrometer (GC/MS). The GC/MS analysis was conducted with capillary ultra 2 columns (25 mm length, 0.32 mm inner diameter, 0.52 µm film thickness and 3.2 cm flow). Helium gas (high purity) was used as a carrier gas. The temperature program for the analysis of 2-hydroxybiphenyl was 75°C (1 min isothermal), 75-200°C (20°C/min), 200-280°C (15°C/min) and 280°C (6.42 min isothermal).

RESULTS

Isolation of bacterial isolates: A total of 40 soil samples were collected from different places in Iraq with a history of oil or its derivatives pollution. The samples showed clear good growth after 4 days of incubation in BSM constituted of minerals and 1 mM of DBT as sole source of sulfur with glycerol (10 mM) was used as the carbon source, inoculated with 1% (w/v) of soil samples at 30°C. After that, these cultures were plated on LB to get on pure isolates. Sixty three bacterial isolates were obtained and these isolates tested for their capability to utilize DBT by culturing them on two types of media (A and B). The growth patterns and utilization of DBT with and without glycerol (carbon source) in BSM were tested at 37°C incubation temperature. The obtained results (Table 1) showed that most bacterial isolates capable to grow on DBT in presence or without presence of glycerol (carbon source).

Table 1: Growth density of bacterial isolates in basel salt medium (pH 7) supplemented with 0.1 mM of DBT along 4 days of incubation with shaking (150 rpm) at 37°C

Bacterial growth (OD_{580} nm)					
Isolate No.	Medium A	Medium B	Isolates No.	Medium A	Medium B
M1	1.124	0.702	P9b	1.100	0.629
M2	0.971	0.731	P11	1.130	0.545
M3	1.170	1.140	P12	0.424	0.382
M4	1.136	0.802	P13	1.468	1.253
M5	1.044	0.734	P14	0.850	0.820
M6	1.478	0.683	P15	1.224	0.810
M7	2.005	1.150	P16	1.324	0.806
M8	1.986	0.722	P17	1.030	0.790
M9	1.050	0.812	P18	1.402	0.642
M10	1.990	0.756	P19	1.214	0.680
M2b	1.002	0.803	P20	0.771	0.525
M9b	1.858	0.730	S21-1	1.790	1.200
M11	0.739	0.487	S21-2	0.939	0.625
M12	0.617	0.537	S22	1.244	1.054
M14	1.186	0.562	S23	0.118	0.054
M15	0.839	0.372	S24	0.754	0.361
M16	1.192	1.198	S25	0.785	0.663
M17	1.404	0.803	S26	0.895	0.878
M18	0.904	0.303	S27	0.607	0.056
M19	0.955	0.292	S28	0.697	0.066
M20	1.090	0.982	S29	1.240	0.420
P1	0.586	0.487	S30	0.517	0.450
P2	0.763	0.131	S31	0.336	0.231
P3	0.994	0.158	S32	0.927	0.481
P4	1.080	0.964	S33	0.586	0.217
P5	0.828	0.498	S34	0.983	0.833
P6	0.584	0.213	S35	0.670	0.401
P7	0.711	0.207	S36	1.272	0.580
P8	0.812	0.728	S37	1.302	0.840
P9	0.860	0.527	S39	1.394	0.055
P10	0.581	0.543	S40	0.645	0.198
P2b	0.520	0.390			

Results (Table 1) showed also that four isolate (S23, S27, S28 and S39) were unable to grow on DBT only (medium B). This means that these isolates need a simple C-source (e.g., glycerol) to support their growth. The growth of bacterial isolates in medium (B), which not contained C-source, might be attributed to ability of these isolates to utilize DBT as C and S-source or utilize ethanol as C-source, because DBT in this medium was dissolved in ethanol.

Testing bacterial utilization of DBT either as the sole S-source or as the S and C-source: Gibb's reagent (2,6-dichloro-p-benzoguinone-4-chloroimine), which detects the formation of corresponding phenolic end-products resulting after desulfurization of DBT was used to screen this activity in our DBT utilizing bacterial isolates. All the 63 isolates were subjected to Gibb's assay after 4 days of incubation. The results showed that three isolates have the ability to desulfurizing DBT and converted it to 2-HBP or other phenolic end products and gave blue color in the presence of Gibb's reagent.

Selection the efficient isolates that capable of utilizing DBT compound: Twelve efficient isolates (cleaved of both C-C and C-S bonds of DBT) were candidate for subsequent study. The twelve isolates (M3, M7, M16, M20, P4, P13, P14, S21-1, S22, S26, S34 and S37, however, P13 isolate was the best one) showed respectable growth density in medium (B) (high OD_{580}) compared with other isolates throughout the incubation period (Table 1).

Identification of bacterial isolates: Twelve isolates were selected for identification according to their ability to utilize DBT, as S-source only or S and C-source. These isolates were identified depending on morphological, cultural and biochemical characteristics. Results indicated in Table 2 show that these isolates were characterized as *Staphylococcus* spp. (6 isolates, including P13 isolate), three of them were *Staphylococcus aureus*. *Micrococcus* spp. (2 isolates) and one isolate for each genus of *Neisseria* sp., *Corynebacterium* sp., *Pseudomonas* sp. and *Bacillus* sp. The morphological, cultural and biochemical characteristics for these isolates (Table 2) were as prescribed by Bergey and Holt (2000) and in agreement with Holt *et al.* (1994).

Table 2: Morphological, physiological and biochemical characteristics of Bacterial isolates

	Isolates			
Characters	M3	M7	M16	M20
Colony color	Cream to yellow	Cream	Cream to yellow	Yellow
Cell shape	Cocci	Cocci	Cocci	Cocci
Gram stain	+	-	+	+
Catalase	+	+	+	+
Oxidase	N	+	+	N
Motility	-	-	+	-
Urease	W	+	-	W
Gelatinase	-	-	-	-
Nitrate reduction	+	-	-	+
Citrate utilization	-	+	-	-
MR	+	-	+	W
VP	-	+	-	-
Indole	-	-	-	-
Mannitol	+	-	-	+
Bile salt	N	N	N	N
King A	N	N	N	N
King B	N	N	N	N
Growth on Cetrimide	N	N	N	N

Table 2: Continue

	H_2S	-	-	-	-
	CO_2	-	+	-	-
	Slant	Acid	Acid	Alkaline	Acid
TSI	Butt	Acid	Acid	Acid	Acid
		Isolates			
Characters		P13	P14	S22	S21-1
Colony color		Cream	Cream	Cream to yellow	Yellow
Cell shape		Cocci	Cocci	Cocci	Rod
Gram stain		+	+	+	-
Catalase		+	+	+	+
Oxidase		N	N	N	+
Motility		-	-	-	+
Urease		W	+	-	-
Gelatinase		N	N	-	-
Nitrate reduction		-	-	-	+
Citrate utilization		+	+	-	+
MR		-	W	+	-
VP		W	+	+	W
Indole		-	-	-	-
Mannitol		+	+	-	-
Bile salt		+	N	N	N
King A		N	N	N	+
King B		N	N	N	+
Growth on cetrimide		N	N	N	+
	H_2S	-	-	-	-
	CO_2	-	+	-	-
	Slant	Acid	Acid	Alkaline	Acid
TSI	Butt	Acid	Acid	Acid	Acid
		Isolates			
Characters		S34	S37	P4	S26
Colony color		White	Yellow	Cream	White
Cell shape		Spore bearing bacilli	Cocci	Cocci	Rod
Gram stain		+	+	+	+
Catalase		+	+	+	+
Oxidase		+	N	N	N
Motility		-	-	-	-
Urease		-	W	-	N
Gelatinase		+	-	N	-
Nitrate reduction		+	+	-	+
Citrate utilization		+	-	+	+
MR		-	W	-	-
VP		+	W	-	+
Indole		-	-	-	-
Mannitol		-	+	-	+
Bile salt		N	N	N	N
King A		N	N	N	N
King B		N	N	N	N
Growth on cetrimide		N	N	N	N
	H_2S	-	-	-	-
	CO_2	-	-	-	-
	Slant	Alkaline	Acid	Alkaline	Acid
TSI	Butt	Alkaline	Acid	Alkaline	Acid

+: Positive result, -: Negative result, N: Not tested, W: Weak test, TSI: Triple sugar iron, MR: Methyl red, VP: Vogas-Proskauer *Staphylococcus* spp.: P4, P13, P14, *Staphylococcus aureus*: M3, M20, M37, *Neisseria* sp.: M7, *Bacillus* sp.: S34, *Pseudomonas* sp.: S21-1, *Corynebacterium* sp.: S26, *Micrococcus* spp.: M16, S22

VITEK2 system for identification P13 isolate: The *Staphylococcus* sp. (P13) was unidentified by this system. Although, the isolate was pure and the test was repeated several time in two different centers used VITEK 2 system. To shed more light on the identification of P13 isolate, it is recommended to subjecte this isolate to molecular identification.

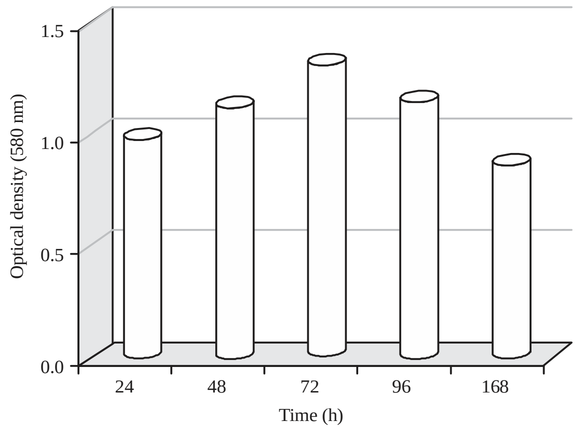

Fig. 1: Effect of incubation time on P13 isolate grown in basel salt medium (pH 7.2) in shaker incubator (150 rpm, 37°C)

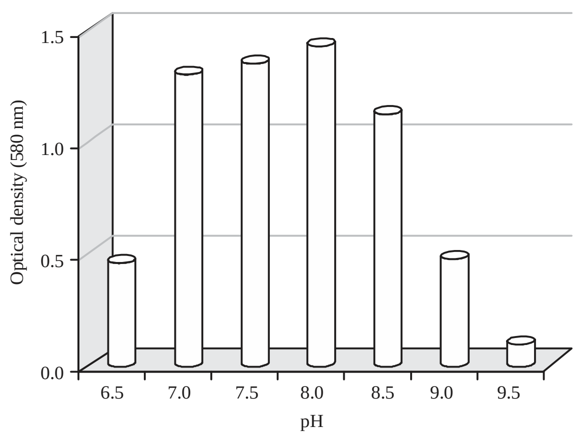

Fig. 2: Effect of pH on DBT utilization by P13 isolate grown in basel salt medium containing 0.1 mM of DBT in shaker incubator (150 rpm, 37°C) for 3 days

Determination of optimal conditions for utilizing of DBT by P13 isolate

Effect of incubation time: The P13 isolate was grown and incubated at different duration time (24, 48, 72, 96 and 168 h) to determine the optimum time for bacteria growth. The obtained results (Fig. 1) show that the highest optical density of bacterial growth was after 72 h. Three days of incubation in BSM supplemented with 0.1 Mm of DBT at shaking (150 rpm) at 37°C and the bacterial growth was decreased gradually after this time.

Effect of pH: Basel Salt Medium (BSM) supplemented with 0.1 mM DBT was prepared at different pH values (6.5, 7, 7.5, 8, 8.5, 9 and 9.5) in an attempt to determine the optimum pH required for growth of P13 isolate. Results presented in Fig. 2 elucidate that an optimum growth was occurred at pH 8, the optical density for bacterial growth was reached 1.442. While bacterial growth was decreased at other pH values compared with growth at pH 7.5 and 8. This mean that both bacteria preferred slightly alkaline media.

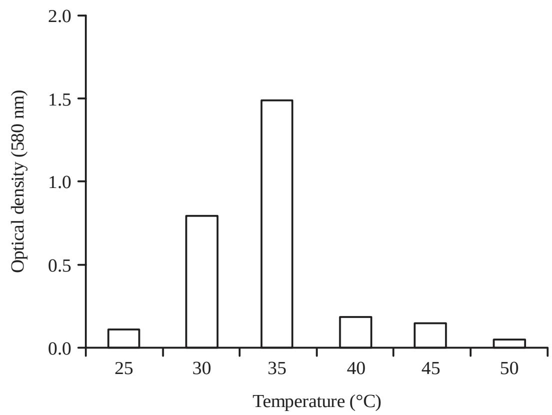

Fig. 3: Effect of temperature on DBT utilization by P13 isolate grown in basel salt medium (pH 8) containing 0.1 mM of DBT in shaker incubator (150 rpm) for 3 days

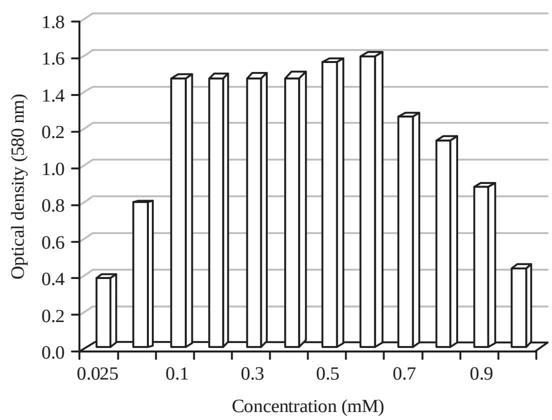

Fig. 4: Effect DBT concentration on P13 isolate at 35°C for 3 days

Effect of temperature: The P13 isolate was grown at different temperatures (25, 30, 35, 40, 45 and 50°C). Results shown in Fig. 3 declared that the optical density (OD_{580}) of bacterial growth at 35°C was 1.485. This bacteria exhibited highest cell densities only around 35°C and decreased above and below this temperature. Therefore, this temperature was suggested as the optimum temperature for bacterial growth.

Growth at different DBT concentrations: Different concentrations (between 0.025-1 mM) of DBT were used as S-source to grow P13 isolate in order to determine the optimum concentration. Results in Fig. 4 indicated that the optimum concentration for bacteria growth was 0.6 mM, the Optical Density (OD_{580}) of bacterial growth, at this concentration was 1.615 for P13 isolate. Figure 4 showed also that gradual increasing of DBT concentration accompanied with increasing of bacterial growth, until reached to its optimum at a concentration of 0.6 mM, while DBT concentrations higher than 0.6 mM caused gradual decrease of bacterial growth.

Detection and verifying the chemical nature of biodesulfurizing end products by GC/MS analysis: The GC/MS analysis for the extract of diesel (control) and sample of culture supernatant

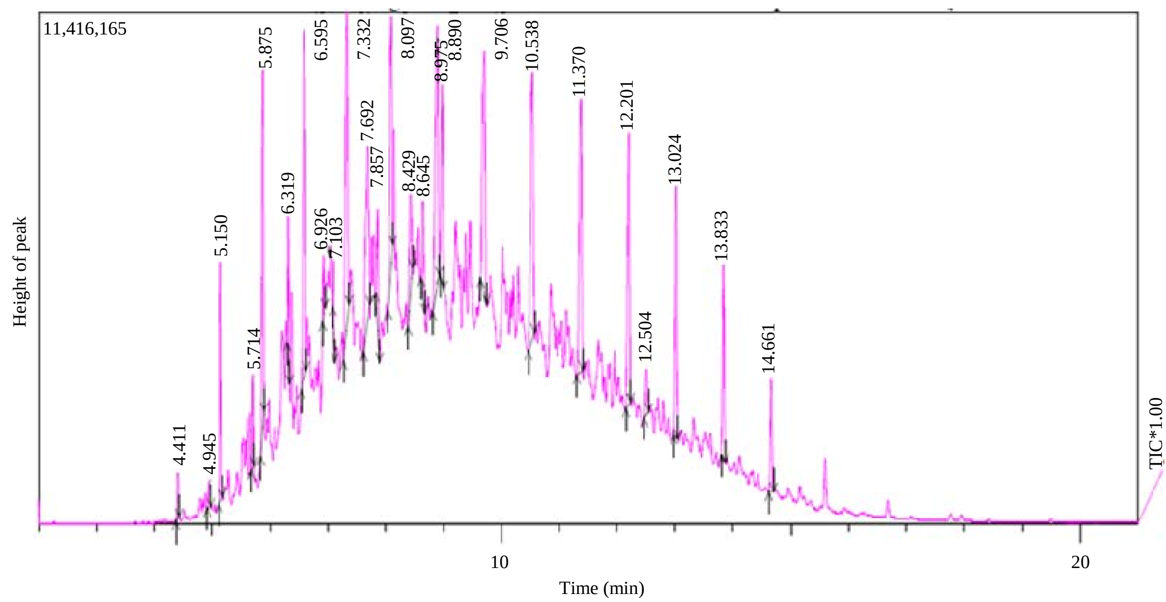

Fig. 5: GC/MS chromatograms of P13 isolate diesel-BSM supernatant extracted culture. That gives 25 peaks

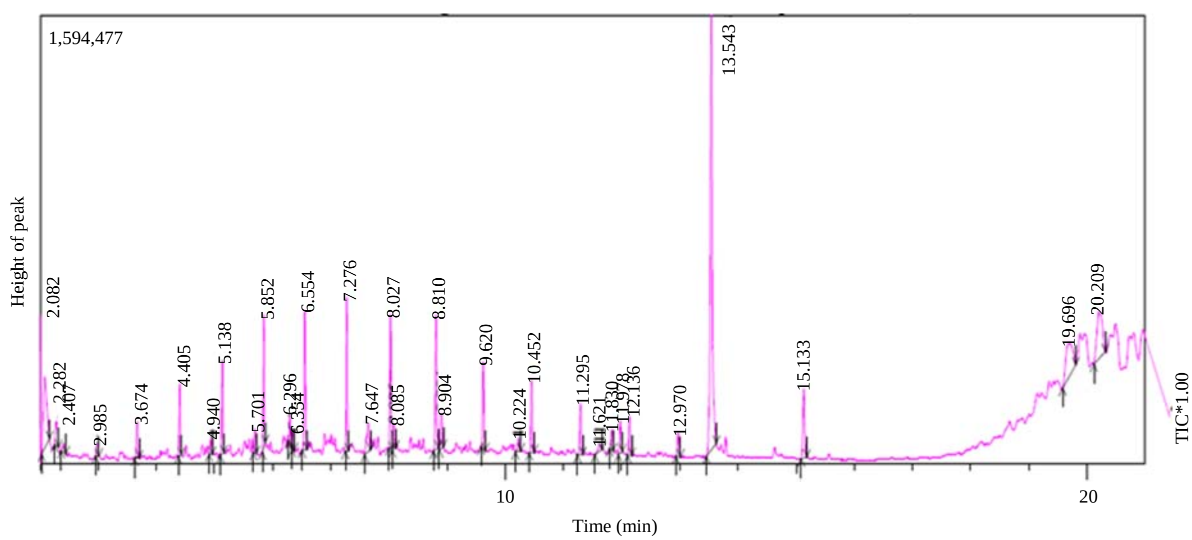

Fig. 6: GC/MS chromatogram of diesel-BSM (control) extract. That gives 32 peaks

extracted after growth of P13 isolate (Fig. 5), which gave 25 peaks. Analysis of diesel (control) gave 32 peaks (Fig. 6), no one of these peaks contain a DBT but instead contain other sulfur containing compounds like Thiazole, 2, 5-diethyl-4-methyl, 3-(Benzoylthio)-2-methylpropanoic acid and 2-Isopropyl-4, 5-dimethylthiazole, at a retention time 8.025, 8.083 and 9.617, respectively (Fig. 7). All these compounds that have sulfur atom were degraded by P13 isolate to give less complex compounds in the form of carbon chain like n-Eicosane (appeared at the retention time 8.642) which is result from diesel degradation by P13 isolate (Fig. 8).

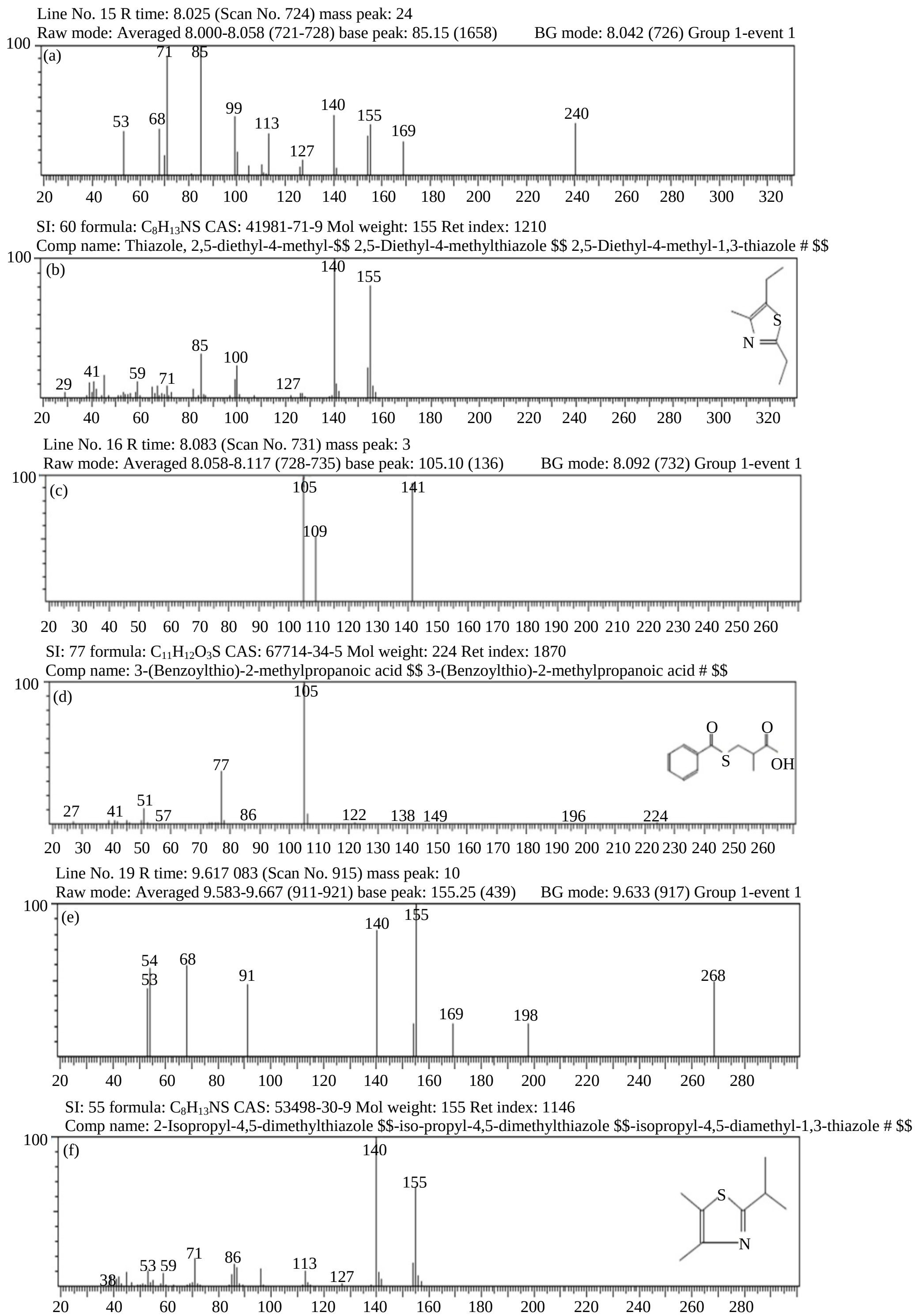

Fig. 7(a-f): GC/MS analysis showing the mass spectrum of Thiazole,2,5-diethyl-4-methyl (molecular mass, 155) with retention time 8.025, the mass spectrum of 3-(Benzoylthio)-2-methylpropanoic acid (molecular mass, 224) with retention time 8.083 and the mass spectrum of 2-Isopropyl-4,5-dimethylthiazole (molecular mass, 155) with retention time 9.617

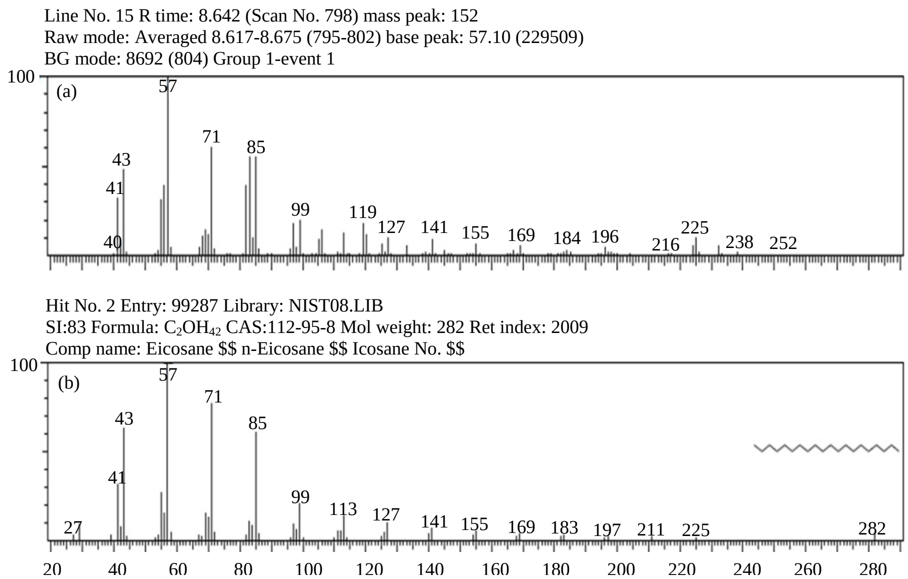

Fig. 8(a-b): GC/MS analysis showing the mass spectrum of n-Eicosane (molecular mass, 282) with retention time 8.642

DISCUSSION

In the present study, bacteria harboring the capability of utilizing petroleum from nature was isolated, soil samples with a history of oil pollution were chosen because most of the indigenous isolates living in these soils have acquired adaptable mechanisms. Sixty three bacterial isolates were obtained and the growth density of these bacterial isolates in presence of glycerol (in medium A) was more than growth density without presence of glycerol (in medium B). This may be attributed to the fact that glycerol is a simple source of carbon helps the bacteria to grow faster and stronger than in its absence (Kenny *et al*., 2012). From these 63 bacterial isolate 57 bacteria isolate was able to utilize DBT (three isolate was desulfurize DBT and four was unable to utilize it without carbone source) and the inability of these isolates for desulfurizing DBT might be due to; firstly, a bacteria attacked the aromatic skeleton of the compound and utilizing carbon and sulfur in DBT, so no 2-HBP will formed, it would be concluded that almost all bacterial isolates growing on DBT attacked this compound via cleavage of both C-C and C-S bonds in a manner similar to degradation by the common Kodama pathway (Gupta *et al.,* 2005; Mohebali and Ball, 2008). Secondly, the isolates (that gave negative Gibb's results) may have other specific enzymes that attack the 2-HBP and degraded it to less complex compounds and utilizing 2-HBP as carbon source (Al-Sayegh, 2005) and that what we need. This diversity in utilization might be attributed to physiological and genetic properties of these isolates. However, it is difficult to predict which molecular change can be expected by a specific microbe, since each group of microorganisms, even various strains of one genus can alter a selected molecule differently (Zipper *et al*., 1998). Many studies showed that, the maximum hydrocarbon degrading potential followed by *Staphylococcus* spp. (Borah and Yadav, 2012). Degradation activity of the hydrocarbon from environmental samples revealed that *Staphylococcus aureus* was the potent degraders of hydrocarbons (petrol and diesel). The ability of these isolates (*Staphylococcus* spp.) to degrade hydrocarbons was clear evidence that their genome harbors the relevant degrading gene (Shukor *et al*., 2009; Jyothi *et al*., 2012). Also, *Micrococcus* showed potential degrading of petroleum hydrocarbon (PHC), benzene and diesel when isolated from oil-contaminated soil (Prakash *et al*., 2014). The usage of petroleum hydrocarbon products increases soil contamination with diesel and engine oil is becoming one of the major environmental problems. Bioremediation provides an effective and efficient strategy to speed up the clean up processes. For this purpose, isolate some of the indigenous hydrocarbon degrading microorganisms i.e., *Staphylococcus* spp. and *Corynebacterium* sp. was important step. (Borah, 2011). It was reported that *Neisseria elongate* showed potential degradation of crude oil by more than 50%. Maximum growth of the isolates in the mineral salt medium (pH 7) occurs at 37°C and shaking (130 rpm) (Mukred *et al*., 2008).

CONCLUSION

Soil contaminated with hydrocarbons and oil derivatives were rich with dibenzothiophene (DBT) utilizing bacteria. Twelve of the efficient DBT utilizing isolates were belonged to *Staphylococcus aureus* (3 isolates), *Staphylococcus* spp. (3 isolates), *Micrococcus* spp. (2 isolates) and one isolate for each of *Neisseria* sp., *Pseudomonas* sp., *Corynebacterium* sp. and *Bacillus* sp. The most efficient one was unidentified (P13 isolate). The optimum conditions to utilize DBT by P13 isolate are growing this bacterium in Basel salt medium (pH 8) containing 0.6 mM DBT and incubated with shaking (150 rpm) at 35°C for 3 days.

The P13 isolate have a good ability to utilize the DBT as sulfur and carbon source. Also, showed a very good ability to consume sulfur from diesel-BSM.

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