Pak. J. Biotechnol. Vol 15 (4) 991-1001 (2018)
www.pjbt.org

ISSN Print: 1812-1837
ISSN Online: 2312-7791

EFFICIENCY OF SOME BACTERIAL CONSORTIA IN THE BIODEGRADATION OF PETROLEUM SOIL-CRUDE OIL

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Article received 15.10.2018, Revised 16.12.2018, Accepted 21.12.2018

ABSTRACT

Two soil textures, clay soil (CS) and sandy loam soil (SLS) were used among a simple microcosm system, and artificially spiked with 2% crude oil. The bioremediation process was continued for 60 days at 30°C for soil treated with 6 active bacterial hydrocarbon degraders. Results of the microcosm experiment showed that the total bacterial counts were higher in SLS than the CS for all treatments at different times (20, 40 and 60 days). The mixed bacterial strains (*Bacillus licheniformis* RdI17, *Pseudomonas nitroreducens* RdI14, *Bacillus subtilis* ssp. *subtilis* GH5, *Sphingobacterium thalpophilum* QBII6, *Pseudomonas nitroreducens* RdI14 and *Enterobacter cloacae* subsp. *dissolvens*) in consortium T5 was more efficient in biodegradation during the time course of experiment than other consortiaT3 and T4. Regarding the three consortia, T3, T4 and T5 results showed that the highest soil respiratory rate was 14 days post incubation during microcosm experiment and being 41.86 and 39.44 mg CO_2/100 g.dw.soil for T3, 46.70 and 41.86 mg CO_2/100 g.dw.soil for T4, and 49.12 and 46.70 mg CO_2/100 g.dw.soil for T5 in the case of SLS and CS, respectively. Overall the GC analysis showed that the highest biodegradation of crude oil in microcosm experiment was achieved with 98.891, 79.102 and 88.724% in sandy loam soil and 93.289, 77.112 and 67.921% in CS due to bacterial consortia application.

Keywords: Biodegradation, Bacterial consortia, Petroleum, Microcosm, GC analysis.

INTRODUCTION

It is known that petroleum is complex in structure, consisting of hydrocarbons and some organic compounds, which also include some organometallic metals (Hamme *et al.* 2003, Mahalingam and Sampath 2014). It has been proven that petroleum and its products are of great importance to modern societies, which consume large quantities of it each year (IOGP 2014). As the necessities increase in our lives, the rate of hydrocarbon pollution increases on a daily basis, making environmental pollution resulting from these compounds important to consider how to contentiously search for its reduction (Ward *et al.* 2009, Azubuike *et al.* 2016). This makes these compounds continuously enter the environmental as natural products or as pollutants by anthropogenic activities from the oil and petroleum industries (Joy *et al.*, 2017). Pathak *et al.* (2017) reported that approximately 70 million tons of aromatic hydrocarbons includeing benzene, toluene, ethylbenzene and xylene were produced. These aromatic hydrocarbons were considered as major pollution problem in terrestrial and ecosystems. Some investigators (Mondri and Lin 2007, Wu *et al.*, 2008, Lloyed and Cackette 2011) showed that plants, animals, human and microorganisms are suffering from the toxicity of petroleum oil or its products when they released to the environment. Plants are susceptible to oil exposure due to phytotoxic nature of hydrocarbons, and immobilization of nutrients in the soil. Inherent mutagenic properties of some hydrocarbons and their low degradation rates require special attention to remediate these pollutants (Udo and Fayemi 1995, Head *et al.*, 2006, Haghollahi *et al.*, 2016).

Environmentally degradation of the crude oil or its products is possible through several techniques, *i.e.*, chemical (Chu and Kwan, 2003), physical (Costes and Druelle 1997, Frick *et al.*, 1999), and biological (Hamme *et al.*, 2003). Soil remediation includes mechanical, burying, evaporation, dispersion and washing (Dindar *et al.*, 2013, Jahangeer and Kumar 2013, Sawadogo *et al.* 2014), but these technologies were found to be expensive, incomplete decomposition of contaminates, difficulty in handling, high energy input and production of toxic byproducts (El-Naas *et al.* 2014, Sawadogo *et al.* 2014, Azubuike *et al.* 2016). Das and Chandron (2011) showed that biodegradation of pollutants by microbes has received significant interest as mankind attempts to reduce contamination and construct a pollution free environment.

Microorganisms were able to grow on hydrocarbon, and bacteria remain qualitatively and quantitatively as biological agents for petroleum bioremediation (El-Naas *et al.*, 2014), but crude oil contains various compounds; a single strain might not be able to degrade all components of the crude oil. Generally, the bacterial consortia showed better results due to their synergetic effects (Boopathy 2000, Zhang *et al.*, 2010). Therefore, the present study aimed to investigate the efficiency of some bacterial consortia in the bioremediation of

petroleum contaminants in two soil types commonly applied in Egypt.

MATERIALS AND METHODS

Soil samples: Two soil textures, clay soil (CS) and sandy loam soil (SLS), were collected from private farms in Sharkia Governorate, Egypt. Soils were not previously contaminated with any petroleum hydrocarbon compounds and characterized as healthy soils. The samples were artificially contaminated with Arabian light crude oil, at 2% in all experiments, obtained from Petroleum Company, Egypt for 60 days at 30ºC

Soil analyses: Physiochemical analyses of soils used *in situ* bioremediation experiments were determined according to the methods of Page *et al.*, (1982) and Embrapa (1997).

Bacterial strains: Six effective bacterial hydrocarbon degraders isolated from petro-contaminated sites in Egypt, characterized, identified and evaluated by Fahmy (2017) were used. These bacterial strains are *Bacillus licheniformis* RdI17, *Pseudomonas nitroreducens* RdI14, *Bacillus subtilis* subsp. *subtilis* GH5, *Bacillus atrophaeus* GH6, *Sphingobacterium thalpophilum* QBII 6, and *Enterobacter cloacae* subsp. *dissolvens*. *In vitro* efficiency of the tested bacteria in bioremediation of crude oil was conducted in two experiments.

Preparation of the consortia: A chosen of 6 active bacterial hydrocarbon degraders were grown on tripticase soy broth (Biolife, Italy) for 24 h at 30˚C. These bacteria were used for inocula preparation individually or in consortia after adjusting each bacterial inoculum with sterile deionized water to give a bacterial cell count of 1.0×10^5 cfu/g soil. The inoculation volume was 40 mL/kg soil for each.

Seven treatments were designed:

T1 Soil + *Bacillus licheniformis* RdI17

T2 Soil + *Pseudomonas nitroreducens* RdI14.

T3 Soil + three mixed bacterial strains: *Bacillus licheniformis* RdI17, *Bacillus subtilis* subsp. *subtilis* GH5 and *Bacillus atrophaeus* GH6.

T4 Soil + three bacterial strains: *Sphingobacterium thalpophilum* QBII6, *Pseudomonas nitroreducens* RdI14 and *Enterobacter cloacae* subsp. *dissolvens*.

T5 Soil + mixture of the six mentioned bacterial strains.

T6 Soil without any bacterial inoculum and contained NPK and Tween 80 and used as a positive control.

T7 Soil without any bacterial inoculum or nutrients as a negative control.

Each 1.0 kg soil was artificially spiked by adding 20 mL of sterilized crude oil. The crude oil was mixed into the soil plus non-ionic Tween 80 surfactant at the rate of 0.2%. The nitrogen and phosphorus correction were performed using $(NH_4)_2SO_4$ (6080 mg/kg of soil dw) and KH_2PO_4 (973 mg/kg of soil dw) solutions, respectively. Thus, the nutrients ratio (C:N:P) was adjusted to became 100:15:1 as used by Mariano *et al.* (2007).

Soil microcosms: In all treatments, the water content was adjusted to 55% of the field-holding capacity, and the moisture content lies within the interval recommended by Dibble and Bartha (1979). This technique was conducted in the first experiment using specific plastic jars. For each soil, 21 jars covered with lids (5 cm diameter openings) were used as microcosms, each containing one kg sieved soil. The seven soil treatments, which carried out in three replicates, were incubated at 30ºC for 60 days.

Determination of total counts of heterotrophic bacteria: Total heterotrophic bacteria were counted using the pour plate technique as recommended by Wollum II (1982) using (Tripticase soy agar, Udeani *et al.*, 2009). Representative soil samples were periodically collected at 20, 40 and 60 days. The numbers of microorganisms were calculated per one gram dry weight of soil (g.dw.soil).

Gas chromatograph analysis: Ten grams of each of contaminated soil at zero time and the end of each experiment were subjected to Soxhlet extraction for 8 h in duplicate using n-hexane. The extracted hydrocarbon was dehydrated over a Na_2SO_4 column and concentrated to 1.0 mL by rotary evaporation. The total hydrocarbon quantification was calculated by difference in weight after GC analysis based on the methods of Fredericks and Brooks (1956) and DasAshis and Mukherjee (2007).

Determination of soil respiration: This respirometery experiment was performed as a complement experiment; second one, using the same condition used above. The CO_2 analyses were used to measure the total amount of hydrocarbons mineralized during biodegradation of crude oil to CO_2 (as a final product) according to the methods of Isermeyer (1952) and Mariano *et al*. (2007) with few modifications: A specific laboratory unit consists of one liter wide mouth glass jar, Pyrex, with a special lid, which was provided with an inner rubber ring and two clips from outside to insure airtight sealing was used. In all treatments, the water content of 100 g of each soil was adjusted to 55% of the field-holding capacity. This

experiment was conducted for 60 days at 30°C for each tested soil using biometer flasks. The inoculation concentration from each microorganism was 4.0 mL/100g soil. The produced CO_2 was trapped in 100 mL KOH and measured by titrating the residual KOH with a standard solution of HCl (0.1 N). This determination was periodically conducted after periods of 3.0, 7.0, 14, 28, and 60 days.

Statistical analyses: It worth to mention that each treatment was carried out in three replicates and the results were statistically analyzed by CoStat version 6.311 Copyright(c) 1998-2005 CoHort Software,http: //www.cohort.com.

RESULTS AND DISCUSSION

Physiochemical analyses of soil textures: Data in Table (1) show that the main physiochemical characteristics of the soil texture analysis proved the presence of two soil types CS and SLS. The studied soils have EC less than 4 dSm-1and pH less than 8.5, therefore it considers normal soil and free from salinity and sodicity problems. Cations found, listed in the order of decreasing proportion, were $Na^+ > Ca^{2+} > Mg^{2+} > K^+$.

On the other hand, chloride was the major anion. Anions found, listed in the order of decreasing proportion were $SO_4^{2-} > Cl^- > HCO_3^-$. Also, they have low content in organic matter (13.20 9.50 g kg^{-1} for CS and SLS texture soil, respectively). Regarding to available nutrients, studied soil contain 70.60, 19.4 and 189.0 mg kg^{-1} for N, P and K, respectively for CS texture soil and 44.5, 17.3 and 87.5 mg kg^{-1} for N, P and K, respectively, for SLS texture soil.

Regarding to soil types, it is necessarily, initially, to mention that soil texture has a major effect on the physical and chemical characteristics of soils and affects soil behavior especially retention capacity for moisture and nutrients (Kogbara *et al.*, 2015), since they also mentioned that there is little information on biodegradation of crude oil in different soil texture.

Bacterial total count (BTC) of tested soil: Data in Table 2 show the changes of BTC in the tested soil treated with crude oil for determination of bacterial abilities in the biodegradation. In control treatment (T7), the BTCs were 0.16×10^8 and 0.13×10^8 cfu/g.dw.soil in the case of SLS and CS, respectively, in the start of the experiment.

Table 1: Physiochemical analyses of soils used *in-situ* bioremediation experiments.

Characters	Values of physical analysis	
Clay %	38.70	11.80
Silt %	15.70	10.20
Sand %	45.60	78.00
Textural class	Clay	Sandy loam
$CaCO_3$(g kg^{-1})	00.93	11.50
Values of chemical analysis		
pH (1:2.5)	07.83	07.79
EC dSm^{-1}	01.97	00.53
Na^+	11.20	00.59
K^+	00.70	00.25
Ca^{2+}	04.90	01.91
Mg^{2+}	02.20	02.41
CO_3^{2-}	Nil	Nil
HCO^{3-}	01.40	02.20
Cl^-	06.80	00.55
SO_4^{2-}	11.80	02.30
Organic matter (g kg^{-1})	13.20	09.50
Available nutrients (mg kg^{-1} soil)		
N	70.60	44.50
P	19.40	17.30
K	189.0	87.50

Results also showed that the BTC was higher in all samples of SLS than that found in the case of CS. This might be due to the soil texture which has a major effect on the physical and chemical characteristics of soils, also the effects of soil behavior especially retention capacity for moisture and nutrients (Kogbara *et al.*, 2015). Furthermore, Abdel-Moghny *et al*., (2012) reported that the efficiency of remediation depends on the soil type and it was much higher for the sand than the CS.

By time, the BTCs were continuously increased in both soils compared to the experiment start up. This was due to the growth patterns of the natural microbiota in the tested soils contaminated with 2.0% crude oil, since they needed long time for adaptation and utilizes the contaminated compounds as substrates. The BTCs were increased when the nutrient agents and Tween 80 were added to the two tested soils compared with the control treatment (T6) as they increased from 1.5 to 2.0 folds at all sampling times as compared to T7. Results of increasing in the BTCs and consequently the biodegradation abilities of the native microbiota owing to nutrient additives were in agreement with that reported by Koshlaf and Ball (2017). The nutrient additives were proved to be very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus and in some cases iron (Das and Chandran, 2011).

Table 2: Total bacterial counts during the biodegradation of crude oil (2%) as affected by soil types and inoculation treatments.

Treatments		(10^8 × cfu/g.dw.soil)							
		0d		20d		40d		60d	
		SLS	CS	SLS	CS	SLS	CS	SLS	CS
T1	Axenic culture of long rod	0.180	0.140	1.200	0.700	1.300	0.900	2.010	1.600
T2	Axenic culture of short rod	0.170	0.130	1.300	0.330	1.600	0.980	2.020	1.800
T3	Consortium No.3 (3 strains)	0.220	0.160	1.800	0.880	6.100	4.400	5.800	3.800
T4	Consortium No.4 (3 strains)	0.180	0.140	1.700	1.100	6.900	3.400	4.440	2.400
T5	Consortium No.5 (6 strains)	0.230	0.180	4.700	2.070	8.100	7.440	6.500	4.700
T6	Nutrient agents addition	0.170	0.130	0.410	0.290	1.160	0.910	1.800	1.480
T7	without any addition	0.160	0.130	0.210	0.140	0.660	0.500	0.980	0.970
LSD 0.05		0.003	0.011	0.013	0.009	0.003	0.009	0.008	0.011

SLS: Sandy loam soil. CS: Clay soil. d: Days post treatment.

Regarding to the individual inoculation as previously shown in Table (2) using axenic cultures, namely: *Bacillus licheniformus* RdI17 (T1) and *Pseudomonas nitroreducens* RdI14 (T2), there were a considerable differences in the heterotrophic BTCs in the two soil types compared to control treatments (T6 and T7), and the greatest bacterial population were found at 60 days being 2.01-2.02×10^8 and 1.60-1.80×10^8 cfu/g in the case of SLS, and CS, respectively.

In treatments T3, T4 and T5, where three types of bacterial strains of bacilli, three bacterial short rods and the 6 mixed strains, respectively, were bio-augmented to the tested soil types in microcosm experiment, clearly revealed that the BTCs continuously increased from the start of incubation up to 40 days in three treatments T3, T4 and T5 being 6.10, 6.90 and 8.10×10^8 cfu/g in the case of SLS, respectively, and 4.40, 3.40 and 7.44×10^8 cfu/g in the case of CS, respectively. BTC was decreased during days between 40 to 60 days in the three treatments. It was noted that BTC was significantly increased when the soils seeded with consortium T5 followed by consortia T4 and T3 compared with the other treatments T1, T2, T6 and T7.

Therefore, the experimental results showed that the mixed bacterial strains (6 different microbes) in consortium T5 were more efficient than other consortia, T3 and T4, which resulted in enhancing the BTC and consequently extent the rate of crude oil biodegradation for both tested soils (Chaineau *et al.,* 2005, Ijah *et al*., 2008, Jasmine and Mukherji 2014). These results in harmony with that of Chaineau *et al.,* (2005) who reported that with the metabolic capacity a single strain of bacteria is not able to degrade all the components found within crude oil. Al-Wasify and Hamed (2014) supported these results as they found that in nature biodegradation of crude oil typically involves consortia of microbes. Furthermore, since crude oil contains various compounds, a single strain might not be able to degrade all components of the crude oil. In general, the combined bacterial consortium showed better results due to their synergetic effects (Boopathy 2000).

The highest BTCs were recorded after 40 days from incubation for SLS, being 6.10, 6.90 and 8.10×10^8 cfu/g for consortia T3, T4 and T5, whereas, it was 4.40, 3.40 and 7.44×10^8 cfu/g for CS and consortia, respectively. In this respect, it is well known that treating soil with high clay content is a great challenge since that kind of particle has a deleterious effect on mass transfer, blocking air and water from passing, thus affecting the aerobic microbial process. However, the organic pollutants attach to the soil matrix, reducing their bioavailability to microorganisms (Cunningham and Philp 2000). Young and Crawford (2004) reported that fine textured soil tend to harbor larger microbial population than coarser soil, as organic matter and nutrients can attach to the significantly higher surface area associated with clay and silt particles. Recently, Haghollahi *et al*., (2016) stated that the presence of sand in the soil is advantageous in bioremediation and the low bioremediation in clay could be due to inefficient oxygen transfer in the soil. Fine-grained clay with high surface area formed a sticky texture in the presence of water, blocking efficient oxygen transfer through the soil. Sandy soils on the other hand, were more porous than clays. Higher porosity allows better oxygen transfer in the soil, which is essential to biodegradation of hydrocarbon. Larger pores provide also enough space for microbial growth.

Determination of soil respiration: Monitoring of CO_2 produced per soils mass used in this experiment during 60 days-assays as affected by soil type and inoculation with individual and consortia are shown in Table 3. In control treatments, (T7 and T6) the production of CO_2 gas at the start of the experiment, after 3 days of incubation, were 1.93 and 6.37 mg CO_2 /100 g.dw.soil and 1.13 and 4.76 mg CO_2 /100 g.dw.soil, in case of SLS and CS, respectively. Progressive increases in the amounts of CO_2 evolved from both soil types were thereafter observed up to 28 days of incubation

being 12.82 and 15.24 mg CO_2 /100 g.dw.soil in case of SLS and 10.81 and 13.23 mg CO_2 /100 g. dw.soil in case of CS, respectively. On 28 days of incubation, CO_2 production decreased up to the end of the experiment. This trend of CO_2 production, in the two soils, was quite nature, since it is well known, that the CO_2 production is proportional to the percentage of substrate biodegraded and mineralization studies involving quantifying CO_2 evolution can provide excellent information on the biodegradability potential of crude oil (Baptista *et al.,* 2005, Mariano *et al.,* 2007, Dutta and Singh 2017).

Experimental results also revealed that there was a great-differences between microbial respiration evolved from T7 and T6 as indicated by CO_2 production. The calculated average daily rate of CO_2 production was higher in T6 than in T7 being 0.94 and 0.84 mg CO_2/day and 0.67 and 0.61 in T7 in the case of SLS and CS, respectively. In contrast, T7 showed a small response in both of soil types, probably due to the ability of the indigenous microbiotes present and slow transformation to assailable forms and its immediate incurporation of bacterial biomass, and this could be supported by results of (Sabate *et al.,* 2004). Meanwhile, the addition of nutrients was found to be necessary to enhance the biodegradation oil pollutants (Choi *et al.,* 2002, and Jahangeer and Kumar 2013).

As for axenic bacterial cultures, the respiretory activity of the tested two strains in T1 and T2 treatments was found higher in T2 in which *Ps. nitroreducens* RdI14 was applied in microcosm system than that recorded in the case of T1, where *B. licheniformus* RdI17 was inoculated in microcosm Jars. The calculated average daily rates of CO_2 production in the two tested treatments (T1 and T2) were 1.56 and 1.46 mg CO_2/100 g.dw.soil and 1.68 and 1.61 mg CO_2/100 g.dw.soil in the case of the SLS and CS, respectively.

Table 3: Carbon dioxide evaluation (mg CO_2/g.dw.soil) during the biodegradation of crude oil (2%) as affected by soil type and inoculation treatments.

Treatments	Days post treatment (d)										Average daily rate (mg CO_2/day)	
	3 d		7 d		14 d		28 d		60 d			
	SLS	CS	SLS	CS	SLS	CS	SLS	CS	SLS	CS	SLS	CS
T1	13.63e	10.40e	22.50e	20.08e	24.92e	22.50e	20.08e	21.62e	12.82e	13.52e	1.560e	1.460e
T2	14.03d	12.82d	22.91d	22.10d	27.34d	24.52d	22.50d	22.91d	14.20d	14.28d	1.680d	1.610d
T3	27.34b	17.66b	39.44c	37.02c	41.86c	39.44c	24.92c	27.34c	15.24c	16.45c	2.480c	2.290c
T4	25.73c	13.83c	44.28b	40.65b	46.70b	41.86b	29.76b	30.17b	15.35b	17.66b	2.690b	2.400b
T5	29.76a	21.86a	47.27a	44.28a	49.12a	46.70a	32.33a	32.18a	17.34a	19.12a	2.920a	2.730a
T6	06.37f	04.76f	10.40f	09.19f	12.42f	10.40f	15.24f	13.23f	12.82f	12.31f	0.940f	0.840f
T7	01.93g	01.13g	05.16g	05.36g	10.16g	08.39g	12.82g	10.81g	10.98g	10.40g	0.670g	0.610g
LSD 0.05	0.0101	0.0085	0.0101	0.0093	0.0101	0.0093	0.0101	0.0101	0.0101	0.0101	0.0101	0.0101

SLS: Sandy loam soil. CS: Clay soil.

In bacterial consortia studies, where three different association strains of mixing were used to study their microbial respiration, due to the biodegradability efficiency of crude oil by the applied cultures. The tested three consortia showed the highest respiratory rates after 14 days of incubition being 41.86 and 39.44 mg CO_2/100 g.dw.soil from T3, 46.70 and 41.86 mg CO_2 /100 g.dw.soil from T4 and 49.12 and 46.70 mg CO_2/100 g. dw. soil from T5 in the case of SLS and CS, respectively. This result was in agreement with that reported by Dutta and Singh (2017) who reported that there was a progressive increase in the amount of CO_2 produced for the first 12 days, after which CO_2 production decreased and their results showed that 136.36% CO_2 released after 12days incubation. Also, the calculated average daily rates of CO_2 evaluation in T3, T4 and T5 were 2.48 and 2.29 mg CO_2/day, 2.69 and 2.40 mg CO_2/day, 2.92 and 2.73 mg CO_2/day in the case of SLS and Cs, respectively. This means that T5 was the best treatment in biodegradation of crude oil owing to the present of 6 active bacterial strains recorded as hydrocarbon degraders. These results were similar to that found by Ghazali *et al.* (2004), Sathishkumar *et al.* (2008), Zhang *et al*. (2010), Dutta and Singh (2017). In the same trend the advantage of employing mixed cultures as opposed to pure cultures in bioremediation have also been demonstrated, and it could be attributed to the effects of synergistic interactions among members of the association. It is possible that one species removes the toxic metabolites (that otherwise may hinder microbial activities) of the species preceding it. It is also possible that second species are able to degrade compounds that the first are able to degrade only partially (Alexander 1999 and Ghazali *et al.,* 2004).

Again, consortium T5, a bacterial formulation consisting 6 hydrocarbon bacterial degraders as mentioned before in the previously experiments, could be effectively degraders crude oil in soil bioremediation processes under field conditions. However, the types of soil, hydrocarbon mixtures, and hydrocarbon degraders may determine the rate and extent of crude oil bioremediation. As for

the effect of soil type in bioremediation proc-ess, it is interesting to mention that this effect is among the less investigated factors affecting bio-remediation (Kogbara *et al.,* 2015 and Haghollahi *et al.,* 2016), since they stated that fine grained soils like clay have low permeability and retired oxygen and nutrients transport in the soil.

Also, it is clear to notice that the soil respiration (SR) values (CO_2 evolved from each soil due to the treatments used) were higher in SLS than in CS at all sampling times as well as the calculated average daily rate of CO_2 production, as an expected results. In this trend, generally, the values of the major soil properties in the tested soil were widely different. Hence, it could be assumed that soil type would be large assailable for observed differences. Also, this difference may be attributed to the interaction between metabolic activity of the indigenous microorganisms and bio-augmented consortia due to the treatments or mixed with the crude oil presented and its composition taking into account the physical and chemical properties of each soil during the time course of this experiment. Also, the CO_2 emissions were in the highest values after 14 days of incubation in most treatments (T1, T2, T3, T4 and T5), in which the active hydrocarbon degraders were bio-augmented individually (T1 and T2) and in mixed association (T3, T4 and T5). This means that the present microbiota utilized rapidly from petroleum hydrocarbon fractions, especially lighter ones, then decreased substantially with the time elapsing to 60 days of incubation, where the residues fractions become more difficult to degrade on account of the increase in the heavier fractions.

The obtained results as regards to the effect of soil type on bioremediation process used in this experiment were in line with those reported by Kogbara *et al.,* (2015) who reported that, bacterial numbers declined significantly in the fine soil textures after petroleum contamination but were either unaffected or increased significantly in the coarser soil textures, and hydrocarbon biodegradetion efficiency increased with silt content among soil grouping such as fine and coarse soils but not necessarily with increasing silt content of soil. Also, Haghollahi *et al.,* (2016) stated that the efficiency of bioremediation was affected by the soil type (p value <0.05). The removal percentage was the highest (70%) for the sandy soil with the initial TPH content of 69.62 g/kg, and the lowest for the CS (23.5%) with the initial TPH content of 69.70 g/kg. In the same connection, Abdel-Moghny *et al.,* (2012) found that the efficiency of remediation depended on the type of the soil and it was much higher for the sand than for the clay. This difference can be explained by much looser structure of the soil particles in the sandy soil and much higher stickiness and plasticity of clay. Overall, from this study it could be inferred that consortium T5, in which 6 active hydrocarbon degraders could be used as a model consortium to use for bio-augmentation and cleanup process of petro-contaminated soils.

Gas chromatograph analysis: The chromategraphic measurements in this study, based on peak areas of GC profiles, of crude oil HC as affected by soil type and inoculation with individual and/or mixed bacterial consortia *in-situ* bioremediation experiment are given in Table 4 and illustrated in Figs. 1, 2 and 3. The chromatograms of the control and the treatments (the partially degraded crude oil by the tested seven treatments) are present below. In control treatments, T6 and T7, negligible amounts of crude oil HC were lost after 60 days from incubation being 28.038% and 34.500 % in the case of SLS and 0.559% and 22.498% in the case of CS, respectively. On the other hand, the percent (%) of residual crude oil HC in the soil were relatively high being 71.961% and 65.499% in the case of SLS, and 99.440% and 77.501% in the case of CS, respectively. These differences may be explained on the base of the variations in the relative capabilities of the indigenous microorganisms, present in the tested soils, to biodegrade the used crude oil as shown in T7, and due to the addition of nutrients as given in T6.

As for axenic bacterial cultures, as presented in treatments T1, and T2, it is clear from the results that the tested hydrocarbon bacterial degraders, *B. licheniformis* RdI17 and *Ps. nitroreducens* RdI14 attack the crude oil components efficiently and caused moderate degradation of crude oil components. The values of this degradation were found to degrade ca. 58.665% and 65.019% in the case of SLS and 54.681% and 63.261% in the case of CS, respectively. The moderate degradation ratios of the crude oil recorded in this study by the individual strains were in agreement with those reported by many authors. Subathra *et al.,* (2013) mentioned that 3 efficient crude oil bacterial isolates of *B. substilis*11, *Ps. Aeruginosa*15 and *Ps. putida*18 were selected out 113 bacterial isolates, and the quantitative analysis of biodegradation rate of crude oil 1.0 % was 55% and it was recorded by *Ps. aeruginosa* 15 isolate. In the same trend, Yang *et al.,* (2014) found that two hydrocarbon degrading bacteria, which were identified as *Pseudomonas* spp. (Ptr3 and Ptr20), showed 80 and 69% degradation ratios aromatic hydrocarbons, respectively. Recently, El-Hanafy *et al.,* (2016) stated that 23 crude oil degrading

bacteria were isolated and only four isolates were selected and identified and they reported that two strains namely S-5 (*Pseudomonas* sp., 95%) and b-4 (*Nitrotireductor* sp., 70%) were the most effective ones in degrading crude oil, and by using a spectrophotometer and gas chromatography mass spectrometry, degradation more than 90% of the crude oil after two week of cultivation in Buschnell-Haas medium.

Table 4: Biodegradation efficiency of crude oil using individual and/or mixed bacterial consortia during the incubation periods at 30˚C for 60 days, as determined by GC analysis.

	Treatments	% of residual crude oil		% of degraded crude oil	
		CS	SLS	CS	SLS
T1	Axenic culture of long rod	41.334	45.318	58.665	54.681
T2	Axenic culture of short rod	34.980	36.738	65.019	63.261
T3	Consortium No.3 (3 strains)	11.275	32.078	88.724	67.921
T4	Consortium No.4 (3 strains)	20.887	22.897	79.102	77.112
T5	Consortium No.5 (6 strains)	01.108	06.710	98.891	93.289
T6	Nutrient agents addition	65.499	77.501	34.500	22.498
T7	without any addition	71.961	99.440	28.038	00.559

SLS: Sandy loam soil. CS: Clay soil.

Regarding co-inoculation as shown in treatments T3, T4 and T5, where three different consortia were used to determine their biodegradation abilities of crude oil. Data showed that when T3, T4 and T5 were used, approximately 88.724, 79.102 and 98.891% hydrocarbons depletion were recorded in the case of SLS, and 67.921, 77.112 and 93.289% were obtained in the case of CS. This means that consortium T5 caused an extensive biodegradation of crude oil owing to the presence of 6 active hydrocarbon degraders in its formulation. The mixed bacterial consortium as shown in T5 achieved a maximum crude oil biodegradation. These results were in agreement with those reported by Ghazali *et al.*, (2004) and Sathishkumar *et al.*, (2008). Also, Zhang *et al.*, (2010) reported that about 87.5% TPHs of crude oil were degraded by consortium of seven strains.

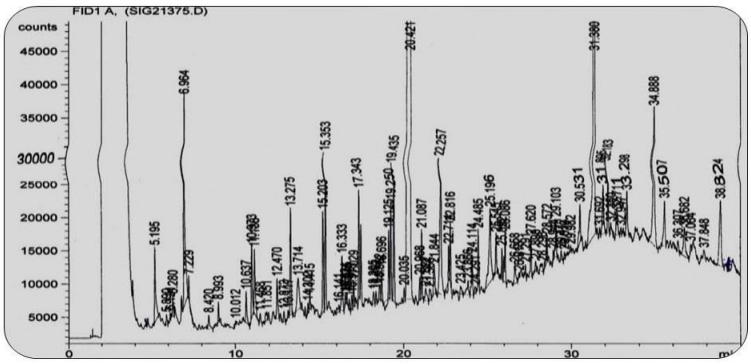


Figure 1: GC chromatograms standard hydrocarbon crude oil.

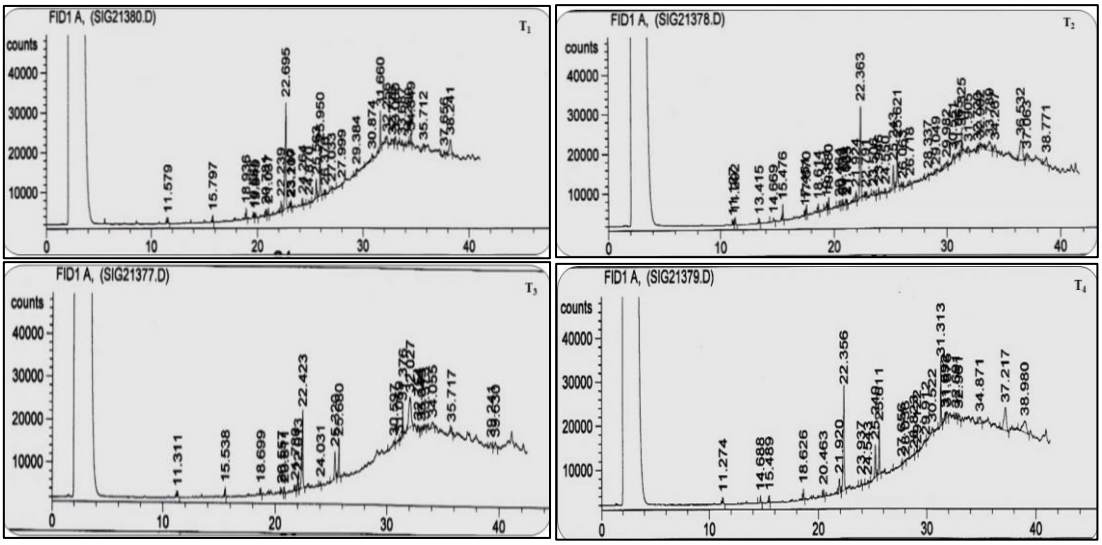


Figure 2: GC **chromatograms of biodegraded crude oil in CS of four treatments (T1 – T4).**

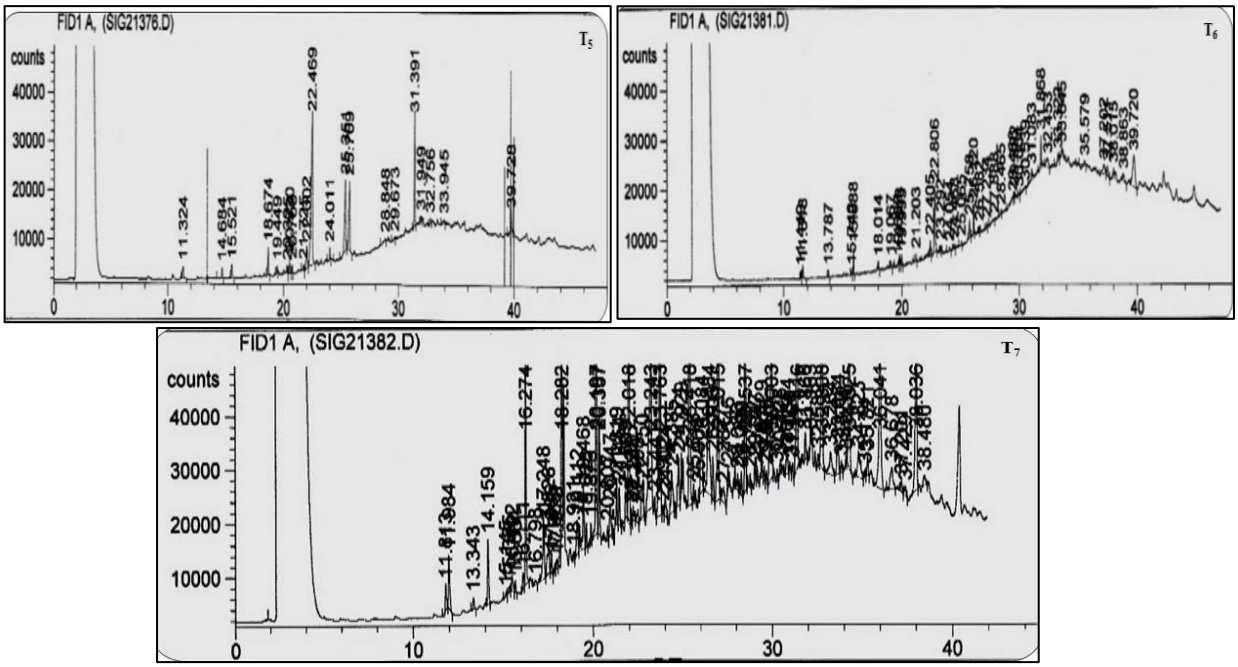


Figure 3: GC chromatograms of biodegraded crude oil in CS of three treatments (T5- T7).

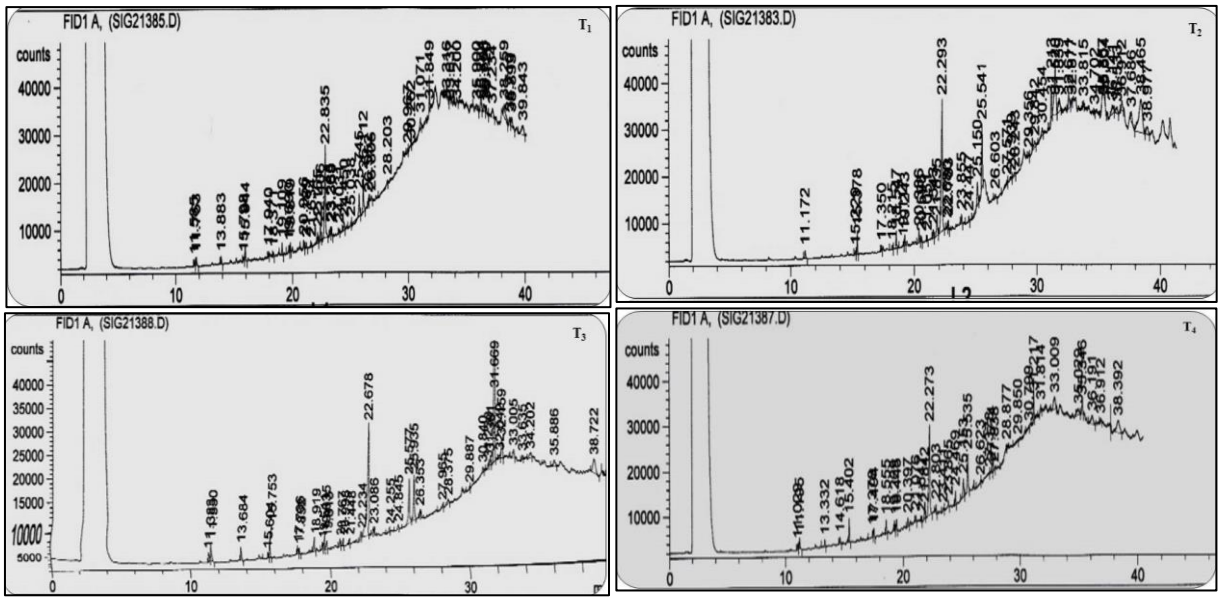
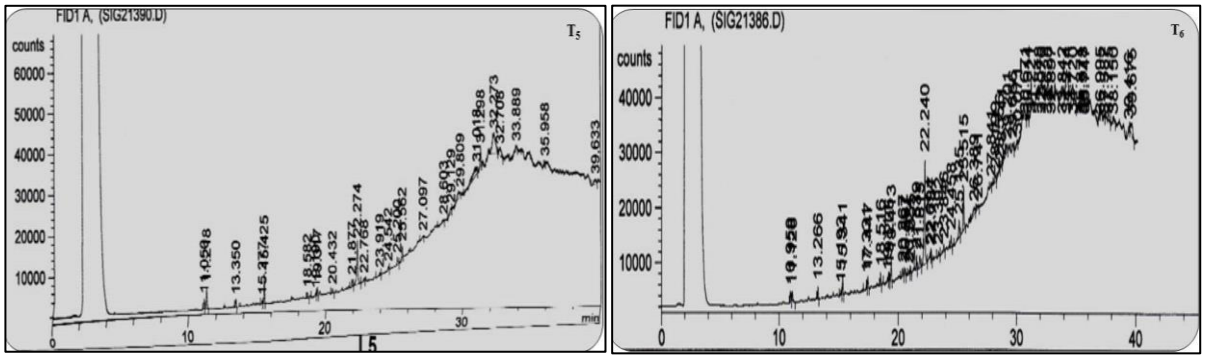


Figure 4: GC chromatograms of biodegraded crude oil in SLS among four treatments (T1 – T4).



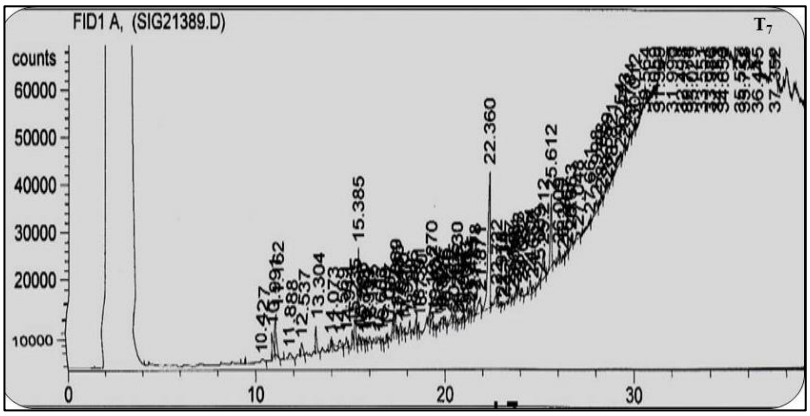


Figure 5: GC chromatograms of biodegraded crude oil in SLS among three treatments (T5– T7).

Concerning soil types used in microcosm experiment, it is clear from the results that the fine textured soil with clay content had lower biodegradation ratios of crude oil in all tested treatments than that found in the course textured soil with sand content. This is because that the percentage of crude oil bioremediation in SLS was higher than that recorded in CS used, by all treatments employed. Similar result was obtained by Kogbara *et al.,* (2015) who reported that after 6 weeks, the hydrocarbon losses ranged from 42-99% during the study petroleum degradation in different textural classes, and SLS had the highest, while the CS had the least THC reduction. Also, Baptista *et al.,*(2005) monitored biodegradation of the crude oil in the contaminated CS, and they found that the best removals of organic matter (50%), oil and grease (37%) and total petroleum hydrocarbon (45%) were obtained in the bioreactors in which the highest CO_2 production was achieved. Degradation was calculated on the basis of the reduction in peak area in GC chromategrams as compared to its corresponding control.

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