academicJournals

Vol. 13(11), pp. 1243-1249, 12 March, 2014
DOI: 10.5897/AJB2013.13151
ISSN 1684-5315 ©2014 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Improving oil biodegradability of aliphatic crude oil fraction by bacteria from oil polluted water

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Received 13, August 2013; Accepted 26 February, 2014

Water samples were collected from three oil polluted stations, two replicates for each station, from southern region of Shatt Al-Arab estuary, and southern of Basrah city during the period from September to October 2011. The mineral salts medium was used to isolating oil biodegrading bacteria. Four bacterial species were identified according to their morphological and biochemical profiles as: *Aeromonas hydrophila*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. The percentage of biodegrading ability of *B. subtilis* and the mixture of these bacteria to n-alkanes and isoprenoids (pristine, phytane) were measured and compared with control. Crude oil is used as a sole source of energy and the incubation period was 24 days, the hydrocarbons loss are detected each 6 day interval using capillary gas chromatography. Bacterial species were exposed to biological mutation by using Maillard reactions to improve the n-alkanes and isoprenoids (pristine, phytane) biodegradability. For this, a mixture of glucose-lysine in a concentration of 4 M was used to mutate *B. subtilis* and *A. hydrophila* while for *P. aeruginosa* and *P. fluorescens* a mixture of glucose-arginine in a concentration of 9 M. Biodegradability percentage was increased for *B. subtilis* from 60.6 to 92.5% and ranged from 37 to 72.3% for the other species. Also the bacterial mixture biodegradability for oil increased from 78 to 87.5%.

Key words: Oil biodegradation, *Aeromonas hydrophila*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, n-alkanes, Maillard reactions.

INTRODUCTION

The hydrocarbons and petroleum constitute one of the main environmental pollutants. The petroleum contains thousands of individual hydrocarbons and related compounds. Their main components are saturated (n- and branched-chain alkanes and cycloparaffins rings), aromatic and polynuclear compounds (PAHs) and resins and asphaltenes (Rosa et al., 2006).

Bioremediation of the environment polluted by crude oil relies on the fact that indigenous microbial population can biodegrade most of the hydrocarbons present in oils, mineralizing them into carbon dioxide and water (Fritsche and Hofrichter, 2008).

It is uncommon to find organisms that could effectively degrade both aliphatic and aromatics possibly due to differences in metabolic routes and pathways for the degradation of the two classes of hydrocarbons (Fritsche and Hofrichter, 2008). However, some reports have suggested the possibility of bacterial species with propensities fordegradation of both aliphatic and aromatic hydrocarbons simultaneously (Salam et al., 2011).

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Figure 1. The three oil polluted locations (Sampling stations 1, 2 and 3) from southern part of Shatt Al-Arab estuary, southern of Iraq.

Applications of genetically engineered microorganisms (GEMs) in bioremediation have received a great deal of attention to improve the degradation of hazardous wastes under laboratory conditions. The genetically engineered bacteria showed higher degrading capacity. However, ecological and environmental concerns and regulatory constraints are major obstacles for testing GEMs in the field. These problems must be solved before GEM can provide an effective clean-up process at lower cost (Das and Chandran, 2011). The heat-induced reaction of amino groups of amino acids, peptides, and proteins with carbonyl groups of reducing sugars such as glucose, results in the concurrent formation of so-called Maillard reaction products (MRPs) (Friedman and Mottram, 2005).

The aim of this study at first step was to isolate local bacterium (bacteria) which has the ability to degrade crude oil (n- alkanes) individually or in mixed culture. The second step is improving their abilities for degradability after mutating them by using of Maillard reaction products as mutagens.

The exposure of organisms to ultraviolet light or treated with nitrous acid has been employed with relative successes. Such mutants, under optimal growth conditions, could possess enhanced petroleum degradation potentials than their parents (Idise et al., 2010).

MATERIALS AND METHODS

Water sampling

Water samples were collected from three oil polluted locations from southern part of Shatt Al-Arab estuary, southern of Iraq (Figure.1).

Water samples were collected in sterile 500 ml glass bottles. The samples were placed on ice until returned to the laboratory.

Isolation and identification of bacterial species

1 ml of each sample was cultured in a conical flask containing 100 ml mineral salt medium (MSM), the composition of the medium was: 0.3 g KCl, 1.0 g K_2HPO_4, 0.5 g KH_2PO_4, 0.5 g $MnSO_4.7H_2O$, 0.2 g $CaCl_2.2H_2O$, 30 g NaCl, and 0.01 g $FeSO_4.7H_2O$ (Fujisawa and Murakami,1980) with 0.1 ml crude oil (Provided from Al-Shua'aba Refinery) and 1000 ml H_2O. Decimal dilution of 7-21 days grown culture was cultivated at 30ºC for 24 h. Pseudomonas agar base (Himedia) was used to isolate *Pseudomonas* species, Ampicillin Dextrin agar (Himedia) was used to isolate *A. hydrophila* and Luria-Bertani agar (Himedia) was used to isolate *B. subtilis*.

Presumptive colonies were subcultured on nutrient agar and were subjected to Gram stain, oxidase, catalase, nitrate reduction, motility, gelatin liquefaction, endospore, indole, methyl red , VogesProskauer, citrate utilization, growth at 4ºC, growth at 41ºC, fluorescens and H_2S tests.

Degradability study of crude oil

1 ml of *B. subtilis* broth culture as well as the bacterial mixture was incubated separately in 250 ml Erlenmeyer flasks containing 50 ml of MSM at 20 FID C for 24 days with shaking at 120 rpm using cooling incubator shaker (GermanySartorius Stidem-Certomat). All the experiments were carried out in four duplicates, each flask was taken out from the incubator in 6 days intervals for estimation of residual crude oil.

Extraction of residual crude oil

Residual crude oil was extracted by liquid-liquid extraction as described by Adebusoye et al. (2007). After removing the aqueous phase with separating funnel, the residual oil was dried in the oven at 40ºC to remove CCl_4.

The aliphatic fraction was separated by using separation column (25 cm length, 3 cm diameter) containing 8 g silica gel over a little amount of wool cotton (Farid, 2006). The residual oil dissolved with 25 ml of n-hexane and poured in the separation column and drawn off the aliphatic fraction in 50 ml beaker. Control flasks were also extracted similarly, n-alkanes hydrocarbons were estimated by FID gas chromatography (Agilent Chem Station).

Maillard reaction products (MRPs)

Four mixtures of amino acid and reduced monosaccharide were prepared: (glucose+lysine; glucose+arginine; fructose+lysine and fructose+arginine) in different concentrations from 0.1 to 10 M (Kitts et al., 1993). Equals volumes of amino acids and sugars were heated (after adjusting pH to 9) by autoclaving at 121°C for 1 h, the final brown products used to induce bacterial mutations.

Mutagenesis of isolates using MRPs

Mutant was initiated by using the method reported by Defontaine et al. (1999), pure isolates of each bacterial type was cultivated on Brain Heart agar by spreading method. Then 1 cm diameter well was made in the center of each culture medium filled with 0.1 ml of MRPs then incubated at 35ºC for 24 h, the colonies appear in the inhibition zone around the wells considered as mutants. Degradation study was repeated again by using mutant strains as described previously to distinguish the changes in the biodegradability after and before mutation.

RESULTS

The effective mixture of MRPs (glucose+Lysine) is at the concentration 4 M which lead to induced a random mutation in *B. subtilis* and *A. hydrophila* while the effective mixture to *P. aeruginosa* and *P. fluorescens* was (glucose-arginine) at the concentration 9 M. The concentrations of n-alkanes (C9-C28) with isoprenoid-spristine and phytane were calculated by comparing with standards solutions. Figure 2 shows gas chromatography results of n-alkanes of control sample. Biodegradation of n-alkanes by bacterial mixture at zero day, before and after mutation was shown in the results of gas chromatography (Figure 3). The biodegradation percentage was improved from 78 to 87.5 % (Table 1).

Figure 4 Shows the gas chromatography results of n-alkanes of crude oil incubated with *B. subtilis* at zero day, before and after mutation.The biodegradation percentage was improved from 60 to 92.5% (Table1). Gas chromatography results of n-alkanes of crude oil for days 6 and 18 were not reported.

DISCUSSION

Crude oil biodegrading microorganisms which are able to utilize crude oil as a sole source of energy distribute widely in different environments; air, water and soil (Magot, 2005). In the present study, four bacterial species have been identified according to morphological and biochemical profile in accordance with Holt et al. (1994) and De Vos et al. (2009). They were capable of oil degradation as a source of carbon that matches with many studies (Hamzah et al., 2010; Uğur et al., 2012; Malik and Ahmed, 2012). The results show that the bacteria degrading in the beginning, the lower and higher hydrocarbon chains while the middle chains were degraded later and these results are in accordance with Bello (2007).

Mineral salts medium has been used in many studies (Farid, 2006; Del'Arco et al., 1999; Salam et al., 2011) in spite of simple variations in its composition and concentrations, such as the addition of nutrients and biostimulators for enzymes which are involved in the biodegradation mechanisms.

Adding crude oil as a sole source of carbon is not sufficient because, microorganisms need another nutrients so that nitrogen and phosphorus can been added in high concentrations to the medium, but sulfur, iron, magnesium and sodium are added in low concentrations, Atlas(1984). Oil layer phenotypic changes in the test flasks were due to the growth of the studied isolates and this confirms the ability of bacteria to utilize crude oil and transform it to small droplets. This is evidence that

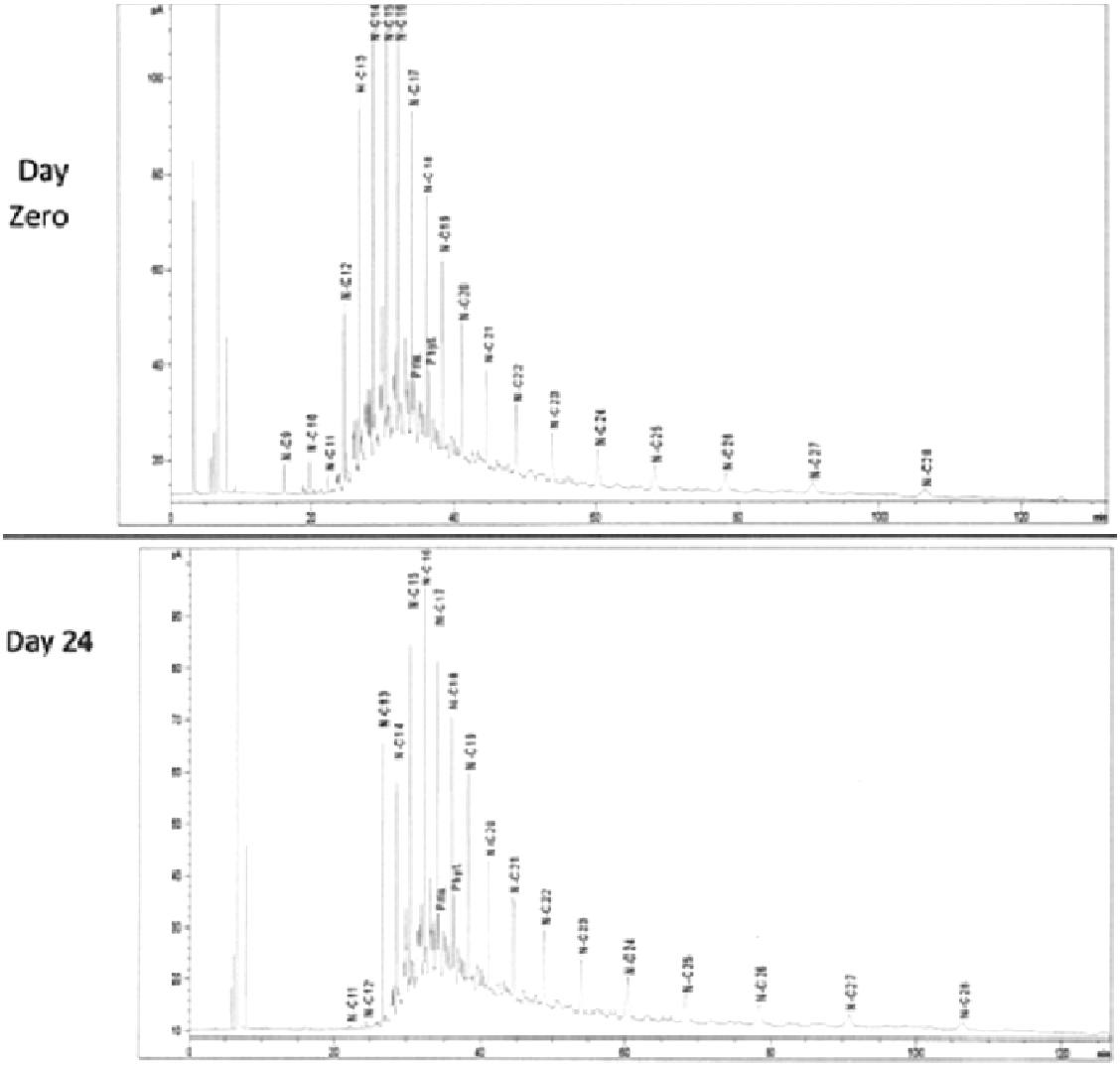

Figure 2. Capillary gas chromatography results of n-alkanes of control flasks at day zero and day 24.

the isolates emulsified the crude oil due to bioemulsifiers which are produced by oil degrading bacteria in the test flasks and no changes in the control flasks that match with Naser (2000).

The results of gas chromatography showed that the n-alkanes with short chains (C9-C12) are most degradable in early stages of incubation while longer chains (C14-C26) were degraded in the later stages. Malik and Ahmed (2012) suggested that the compounds with low molecular weights (C8-C12) dis-appear even in control flasks as a result of evaporation.

Heated food systems contain hundreds of chemical compounds, some being mutagenic, for example: reductones, dicarbonyles, pyrozines and furan (Powrie et al., 1986). The heat-induced reaction of amino groups of amino acids, peptides, and proteins with carbonyl groups of reducing sugars such as glucose results in the concurrent formation of so-called Maillard browning products or melanoidins (Friedman and Mottram, 2005). In this study, Maillard reaction products were used as mutagenic agents for the bacterial types to improve the ability of this bacterium to utilize aliphatic fraction of crude oil.

As a results of the great diversity of these products , each one affects in a different way on DNA, for example, phenols remove purines especially guanine so that site will be empty and there are four chances to link opposite nitrogen base of DNA during multiplication that leads to a change in nitrogen base sequence.Change in the genetic code resulting in a change in the nature of the formed enzymes (Al-Dalali,1994).

Mutation improved biodegradabilityof *B. subtilis* about 32% that leads to raised bacterial mixture biodegradability 9.5% (Table1) with statistically significant differences. These positive changes in bacterial ability may be induced due to formation of new enzymes involved in this process or increment in the enzymes activity. Testing this mutant strain in the field may show good possibility for using it in bioremediation later on.

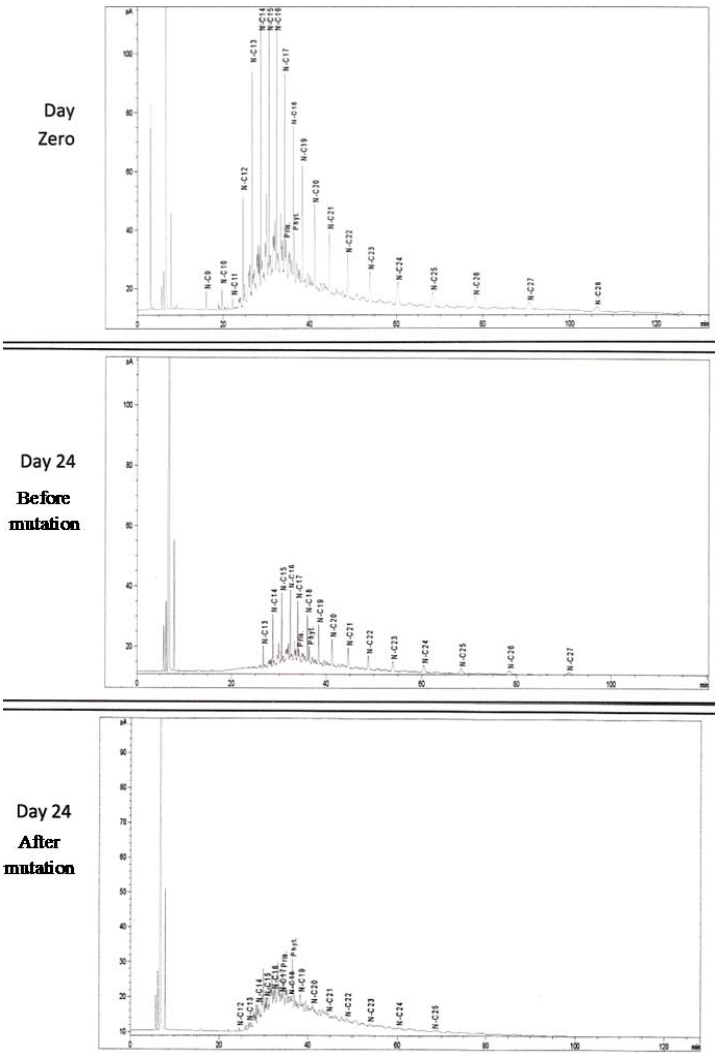

Figure 3. Capillary gas chromatography results of n-alkanes of bacterial mixture at day zero, day 24 before mutation and day 24 after mutation.

Table 1. Biodegradation percentage (BD %) of Bacillus subtilis and mixture before and after mutation

Day	Before mutation (BD%)	After mutation (BD%)
Bacterial mixture		
Zero	0	0
day 6	48.81	14.77
day 12	65.59	24.49
day 18	71.64	84.17
day 24	78	87.5
Bacillus subtilis		
Zero	0	0
day 6	16.76	46.6
day 12	20.9	63.58
day 18	49.64	72.13
day 24	60.6	92.5

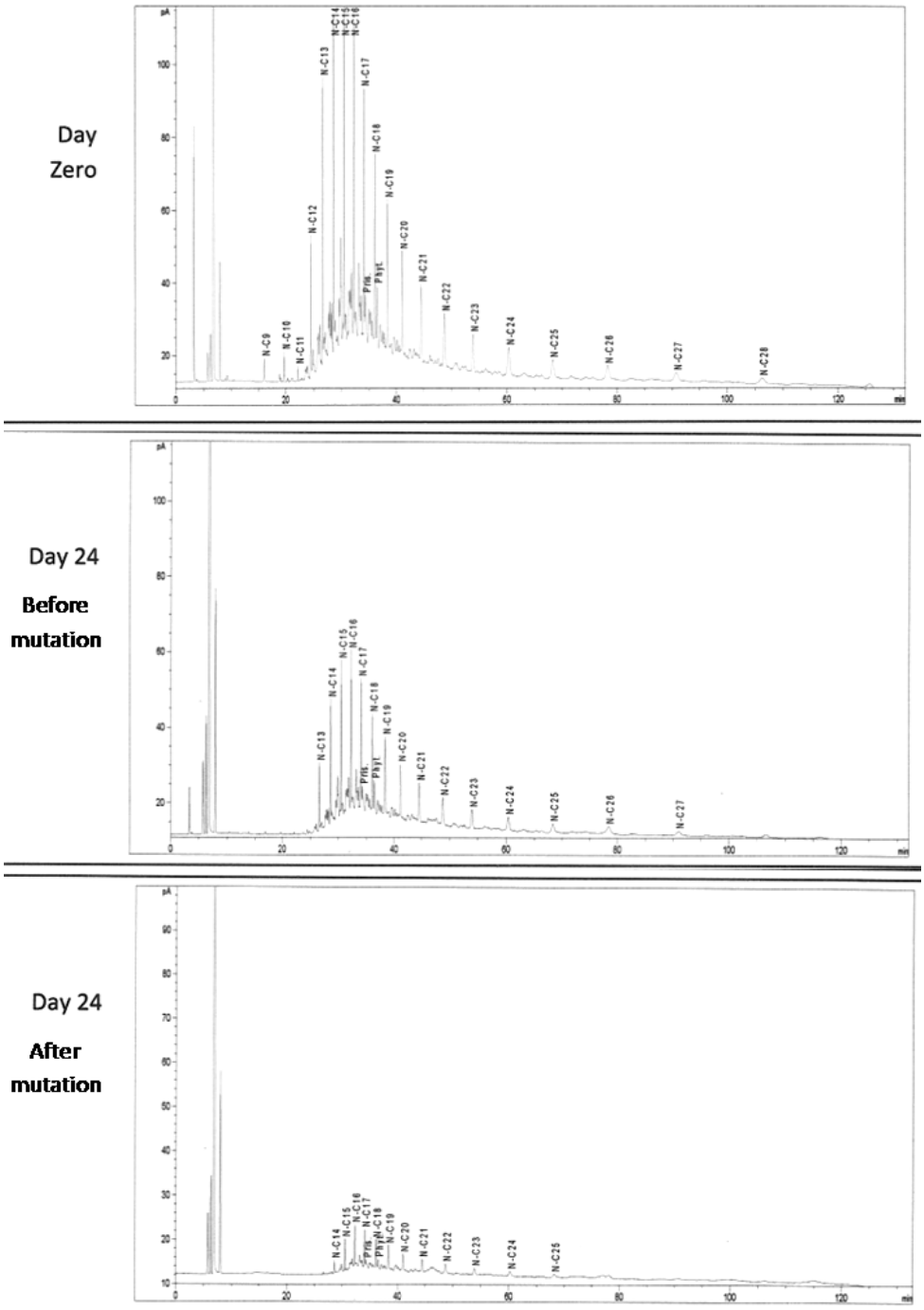

Figure 4. Capillary gas chromatography results of n-alkanes of Bacillus subtilis at day zero, day 24 before and after mutation.

REFERENCES

Adebusoye SA, Ilori MO, Amund OO, Teniola OD, Olatope SO (2007). Microbial degradation of petroleum hydrocarbons in a polluted tropical stream.World J. Microbial. Biotechnol. 23:1149-1159.

Al-Dalali BK (1994). Fundamentals of biochemistry,Book home for printing and publishing -Ministry of Higher Education and Scientific Research ,University of Mosul(Arabic).

Atlas RM (1984). The fate of Petroleum in Marine Ecosystem. Petrol Microbiol. N.Y 272.

4-Bello YM (2007). Biodegradation of Lagoma crude oil using pig dung. Afr J of Biotechnol. 6: 2821-2825.

Das N, Chandran P (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnology Research International. 2011(ID 941810).

Defontaine A, Bouchara JP, Declerk P, Planchenault C, Chabasse D, Hallet JN (1999). *In-vitro* resistance to azoles associated with mitochondria1 DNA deficiency in *Candida glabrata* .J. Med. Microbiol.

48:663-670.
Del'Arco JP, de Franca FP (1999). Biodegradation of crude oil in sandy sediment. Int. Biodeter . Biodeg. 44:87-92 .
De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB (2009). Bergey's Manual of Systematic Bacteriology, 2nd Edition, Volume three, The Firmicutes.
Farid WAA (2006). Effect of plant extracts on the microbial degradation of petroleum. PhD. Thesis Bioldept colleducation Basra Uni Iraq.
Friedman MA, Mottram DS (2005). Biological effects of millard browning products that may affect acrylamide safety in food. Chemistry Safety ofAcrylamide in Food 561:135-156.
Fritsche W, Hofrichter M (2008). Investigation of the bioremediation potential of aerobic zymogenous microorganisms in soil for crude oil biodegradation. Biotechnology 11(2):144.
Fujisawa H, Murakami M (1980). Method for screening hydrocarbon-oxidizing bacteria in the sea.JShimonosekiuviv Fish. 28(101-108).
Hamzah A, Rabu A, Azmy RF, Yussoff NA (2010). Isolation and Characterization of Bacteria Degrading Sumandak and South Angsi Oils. SainsMalaysian. 39(2):161-168.
Holt JG, Krieg NR, Sneath PAH, Stanly JT, Williams ST (1994).Bergey's Manual of Determinative Bacteriology. 9th ed. Williams and Wilkins. Baltimor. USA.
Idise OE, Ameh JB, Yakubu SE, Okuofu CA (2010). Modification of *Bacillus cereus* and *Pseudomonas aeruginosa* isolated from a petroleum refining effluent for increased petroleum product degradation. Afr. J. Biotechnol. 9 (22):3303-3307.
Kitts DD, Wu HC, Stich HF, Powrie WD (1993). Effect of glucose -lysine Miallard products on bacterial and mammalian cell mutagenesis. J. Agr. Food Chem. 41:(2353-2358).
Malik ZA, Ahmed S (2012). Degradation of petroleum hydrocarbons by oil field isolated bacterial consortium. Afr. J. Biotechnol. 11(3):650-658.
Naser RB (2000). Bacteriological and Genetic study of hydrocarbons utilizing *Pseudomonas* spp. MSc. Sci. College, Baghdad Uni. Iraq (Arabic)
Magot M (2005). Indigenous microbial communities in oil fields. In: Ollivier B, Magot M, eds., editors. Petroleum microbiology. ASM, Washington, DC. pp. 21-34.
Powrie WD, Wu CH, Molund VP (1986). Browning reaction systems as sources of mutagens and antimutagens. Environ Health Perspect. 67:47-54.
Rosa M, Silva P, Rodríguez AÁ, de Oca1José MGM, Moreno DC (2006). Biodegradation of crude oil by *Pseudomonas aeruginosa* at 18 strain. Tecnologia Química. 26(1):70-77.
Salam LB, Obayori OS, Akashoro OS (2011).Biodegradation of Bonny Light Crude Oil by Bacteria Isolated from Contaminated Soil. Int. J. Agr. Biol. 13(2):245-250.
Uğur A, Ceylan Ö Aslım B (2012). Characterization of *Pseudomonas* spp. from Seawater of the Southwest Coast of Turkey. J. Biol. Environ. Sci. 6(16):15-23.