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Research Article

SURVEYING THE POTENTIAL BIODEGRADATION OF ASPHALTENE AND TARBALL IN KHARK OIL ZONE BY *BREVIBACTERIUM IODINUM* BACTERIUM

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ABSTRACT

There are broad areas of tide planes across the Persian Gulf covered with tarball. Sedimentation of tarball can be abundantly found in the tide Coast of Khark Island located in the north half of Persian Gulf waters due to several oil platforms as well as oil extraction and locating operations threatening the life of coastal residents, tourism and marine resources. One of the methods to remove these pollutants includes the use of biological procedures. The present study aims at finding a microorganism from the environments contaminated with tarball which is capable to decompose Tarball and its heavy constituent, asphaltene. This was conducted by *Brevibacterium iodinum* separated from Khark Coast which was separated and purified after sampling from 7 stations and was then identified through PCR and 16SrRNA sequence specification methods. All sampling and analyzing stages were based on MOOPAM Standard. The initial screening was conducted by measuring absorption through spectrophotometry (OD). The effect of pH factors (6,7,8), food ratio (1:1, 1:10, 10:1) on the performance of *Brevibacterium iodinum* microorganism was examined. Optimal conditions were examined and the results were evaluated by variance analysis and Danken test. Final measurement of biodegradation performance was conducted by SARA analysis GC/FID technique. According to the results, extensive distribution of n-Alcans (saturated materials) in the Tarball of Khark tide coasts, biodegradation of n-C10 to n-C14 and n-C24, reduction of n-C16, n-C17, and n-C18, aromatic materials and asphaltene were proved by *Brevibacterium iodinum* within a one-month time interval and in a food ratio of 10:1, 1:10 and pH: 6 and pH: 8.

Keywords: *Biodegradation, Tarball, Asphaltene, Persian Gulf, Khark, Saturated Materials, Aromatic Materials*

INTRODUCTION

Despite improving the quality of man's life, rapid advancement of science and technology in modern communities has been followed by consequences such as environmental contaminations. The contamination caused by oil materials is one of the most important factors threatening the environment. That part of the oil released to the sea that reaches the coast includes heavy hydrocarbons which are changed into tarball and position throughout the coast in different sizes. Tarball are spherical and quite dark. They become solid and sticky as the result of weathering. Their sizes range from a few millimeters to tens of centimeters and their densities include 1 to 100 per square meter (Chandru *et al.*, 2008). Asphaltene is the highest consequent of tarball. Color of the tar ball highly depends on the availability of asphaltenes. Asphaltenes are complex compositions with high molecular weights. In other words, they are the heaviest constituents of raw oil consisting of aromatic rings (polyaromatic) and benzene often known as NSO compositions because they consist of nitrogen (N), Sulfur (S) and Oxygene (O) atoms some of which substitute the carbon of aromatics ring. Moreover, complex organic compositions including nickel and vanadium are available in asphaltenes (Evdokimov, 2005).

Sedimentation of organic hydrocarbon components and especially asphaltene is one of the most important problems of oil upstream and downstream industries resulting in difficulties in oil formations of reservoir, operation equipments of production, oil transmission pipelines and storage. On the other hand, if tarball reach the coast, they are distributed by coastal waves. They penetrate into the underlying layers and can remain in the environment for months and years causing severe and chronic toxicity in living creatures followed by gradual death of creatures (Chandru *et al.,* 2008). For this reason, surveying the potential biodegradation of oil, asphaltene and tarball using native microorganisms is of high importance (Fiocco and Lewis, 1998).

Biodegradation is a natural process through which microorganisms change and analyze the pollutants into other compositions and change the quality of oil in deep reserves and resources. Biodegradation affects on the molecular composition and arrangement and physical properties of oil compositions (Liao *et al.,* 2009; Thomas and Lunel, 1993; Margesin, 2000).

The most important step during biodegradation process is to find the microorganisms which are capable to remove and decompose oil hydrocarbons on an enzyme basis (Prince and Clark, 1996) which are extended throughout different water and dry environments (Kasai *et al.,* 2002; Barathi and Defranca, 2001). Some groups are found among bacteria, fungi and ferments which are capable to decompose asphaltene and tarball in water and dry environments. In marine environments, bacteria are generally considered as the main elements analyzing hydrocarbons among microbial population of environment. Fungi allocate less percentage of the decomposing microfelor in the contaminated marine environments (Capelli *et al.,* 2001; Subama *et al.,* 2002). In 1985, Kvenvolden *et al.,* evaluated tarball and biological decomposition of this oil composition and reflected the reduction of their biodegradation against raw oil (Hegazi *et al.,* 2004). Zekin, Chaalal proved that bacteria use heavy hydrocarbon components as nutrition materials during biodegradation of asphaltene and provide different chemicals such as organic acids and regeneratives (Zekri *et al.,* 2003). In 1995, Wang reported that biodegradation of tarball from Baffin Island in Canada played an important role in the cleaning of that zone. As of 1990 up to date, extensive researches have been conducted in relation to the compositions constituting tarball and their biodegradation. Several factors affect biodegradation capability of oil compositions in marine environments among which pH, temperature and nutrition materials may be referred (Xu and Obbard, 2003). It is especially important to be aware of the factors affecting the level of biodegradation in order to specify a fundamental strategy to design systems for biocleaning and improvement of microbial population (Sharma and Pant, 2001). This research aims at examining the potential biodegradation of asphaltene and tarball and also examining the effect of some of the environmental factors on their biodegradation level in Khark Island, Persian Gulf, by the bacterial strain separated from this zone taking into consideration biosafety. Khark Island is located in the north half of Persian Gulf waters within 30 Miles (57 km) of northwest of Boushehr Port and between 50º and 17" to 50 º and 20" longitude and 29º and 12" to 29º and 17" latitude (Geographical Organization of Armed Forces; 2002). Considering the relatively high range of oil activity in Khark Island including excavation, extraction, drilling, storage and transportation, sedimentation of tar lumps can be abundantly found in the Island which is a regional pollutant as well.

MATERIALS AND METHODS

Sampling was conducted in August 2010 from the inter-tidal beaches of Khark Island. Based on the initial data and regional inspection around the island, 2 stations in which the accumulation of tarball was higher than other zones were selected for sampling from tarball, sedimentation and water and sampling was then conducted. The situation of stations has shown in Table1.

Table 1: Geographical Coordinates and Specifications of Sampled Stations

Station No.	Local Name of Zone	Coastal Specifications	Latitude	Longitude
1	Opposite Azarpad Station	Rocky and Stepwise	N14/8´ 13 29	E10/56´ 18 50
2	Discharge of the wastes of factories	Rocky and Sandy	N50/6´ 13 29	E56/4´ 17 51
3	Abandoned Jetty	Rocky	N40´ 12 29	E29/9´ 18 50
4	Sailors' Club	Sandy	N55/15´ 15 29	E11/2´ 19 50
5	Offshore Jetty	Rocky	N10´ 13 29	E20 50
6	Beach Club	Rocky and Sandy	N22´ 16 29	E49/5´ 17 50
7	Urban Zone Adjacent to Airport	Rocky	N´ 0/5´ 16 29	E1/3´ 17 50

Sterilized spatulas were used for sampling from tarball. Taken samples were placed inside pre-sterilized glass containers (Hegazi *et al.,* 2004). Water sampling was conducted by using 1.5-liter pre-sterilized glass containers. A sterilized shovel was used for sedimentation sampling (Chandru *et al.,* 2008). After sampling, containers of samples were placed inside boxes containing pieces of ice. After transferring to the laboratory until conducting the test, the samples were kept in 4º centigrade (MOOPAM, 1999).

Separation of Microorganisms

Sedimentation and water were added to Sea Water Medium Broth (S.W.M.B) in order to separate microorganisms from the samples of tarball (Standard method, 2005). Samples were placed in an Incubatory shaker with 160 rpm/m weathering and 30º of centigrade (Lia *et al.,* 2005). Purification of microorganisms was conducted by consecutive cultures.

Screening the Analysis of Asphaltene and Tarball

A fresh overnight culture was prepared from each organism. 10 of the cultured bacteria were used for initial screening of capability of microorganisms in degradation of tarball using sink embedding method. Sinks were embedded in each of the plates in which tundale asphaltene and tarball as well as asphaltene and tarball dissolved in toluene were added as witnesses. Then, formation and non-formation of prohibited zone was examined and microorganisms with the potential of degradation of asphaltene and tarball were bored. The microorganisms selected in the previous stage were placed in the vicinity of asphaltene and tarball in the fluid culture medium and their ODs were measured with three repetitions. Based on the comparison of OD amount, microorganism capability to degrade tarball was evaluated.

Optimizing Biodegradation of Asphaltene and Tarball

Two parameters, namely nutrient ratio and pH were evaluated for determination of optimal conditions.

Evaluation of Nutrient Ratio: In this stage, three ratios of nitrogen to phosphor (N:P) were examined. Each microorganism needs to nitrogen, phosphor and energy sources to grow. Like other bacteria, degrading bacteria need such a source as well. In this examination, the ratio of variables including 1:1, 1:10, and 10:1 were examined in three time intervals including 2 days, one week and one month. K2HPO4 was used as phosphor source (Haritash *et al.,* 2009).

Evaluation of pH Effect: Most of the environmental bacteria can grow in neutral limits pH. Alkaline or acidic pHs affect on the growth trend of the aforesaid bacteria. Culture mediums Nos. 6, 7, and 8 were prepared to determine optimal amount of pH and were evaluated within three time intervals, namely two days, one week and one month (Haritash *et al.,* 2009).

Optimization for each bacterial strain was conducted in the presence of asphaltene and tarball in all factors in the same conditions and specified times. During the test, different solvents of asphaltene and tarball were used which included Toluene, xylene, benzene, toluene, ethylbenzene, and xylene. Toluene was the best solvent according to the tests. At first, SWMB environment was prepared with different ratios of nutrient (N:P) and the aforesaid pHs. Then, microorganisms were inseminated into flask with the same concentrations to an amount of 1% according to Nim McFarland Opacity Test. At the end, certain concentrations of asphaltene and tarball were separately added to the flasks and the flasks were then placed on incubators equipped with shakers with a weathering severity of 160 rpm and in a temperature of 30ºC. The amounts of OD in the intervals of 48 hours, one week and one month were read with UV spectrophotometer in a wavelength of 600 nanometers. The results of performance of microorganisms in the degradation of asphaltene and tarball were prepared by SARA and GC/FID analyses (Evdokimov, 2004). Oil slices including saturated and aromatic materials, resin and asphaltene were prepared to examine the effect of microorganisms on tarball through SARA method. Considering the obtained results, the amount of hydrocarbons was measured in the witness and bacteria-treated samples to detect the changes during the test period (Evdokimov, 2004). At the same time, the samples were evaluated by GC/FID as well. Gas chromatography is used for separation, identification and measurement of organic compositions. The detectors used in GC are different. Use of flame ionization detector (GC/FID) is more common. This detector only responds to organic compositions except for carbonyl and carboxyl carbons. It functions up to 400ºC. It is very stable and has a high sensitivity. Moreover, hydrogen, nitrogen and helium gases are used as carrier gases (Chandru *et al.*, 2008). The sample in the one month interval was transferred to GC/FID machine and was compared to witness sample.

Identification of Microorganisms Degrading Asphaltene and Tarball

Preliminary identification was conducted by using initial tests such as gram staining and biochemical assays including oxidase, catalase, use of sugars, spore production, movement, and aerobic and anaerobic tests. Then, molecular identification was conducted by proliferating a part of 16SrDNA gene. PCR program included gene amplification with denaturation temperature of 94ºC for one minute, primer binding temperature of 55ºC for one minute, and proliferation temperature of 72ºC for one minute with 35 cycles. The product obtained from PCR was loaded in 1% agarose gel and pb 400 band of agarose gel was then sent for sequence determination. The obtained sequence hermology was examined with the sequence available in the gene bank using Blast software packages (Holt *et al.*, 1998).

RESULTS

Results of Separation and Purification of Microorganisms from the Samples of Tarball, Sedimentation and Seawater

After separation of seawater microorganisms, sedimentation and tarball of Khark Zone, Persian Gulf with 120 bacterial colonies were purified. Then, the separated strains were examined through microscopic and macroscopic studies. The colonial strains were 3-4 mm in diameters, convex, flat surfaces, complete edges, opaque or shiny, and with cream, white or orange colors. After gram staining, bar, basil, cocci, spherical, coco basil and bent shapes were observed in the microscopic examination of bacteria. Polymorphic condition of bacteria was quite obvious during purification stage and after that. Different gram-positive and gram-negative bacteria were observed among the separated bacteria. Of course, the number of gram-negative bacteria was higher than gram-positive.

Results of PCR Molecular Identification

Figure 1: Results of the Electrophorus Gel Image of the Separated Strain (PCR)

This sequence was identified based on NCBI International Blast Bank under tracking code No. ….. in relation to *Brevibacterium iodinum* species.

The Effect of pH and Nutrient (N:P) on Biodegradation Process of Asphaltene and Tarball

The results of the effect of pH and N:P ration on the growth of *B.iodinum* in the vicinity of asphaltene and tarball are shown in figures 2 and 3.

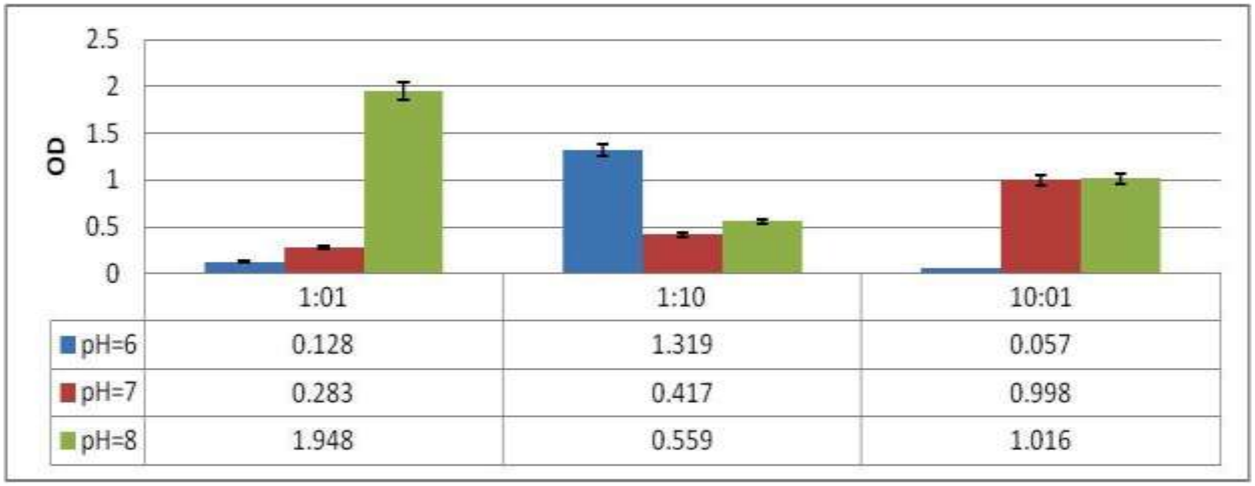

Figure 2 Examination of pH Effect in the Process of Asphaltene Biodegradation by *Brevibacterium iodinum* for one month

Considering figure 1, the best nutrition ratio for *B. iodinum* in the vicinity of asphaltene is 1:1, and the best pH is 8 within a one month time interval. Evaluation of statistical analysis of date obtained from measurement of OD amount, *Brevibacterium* in the vicinity of asphaltene, showed in 1:1 and 1:10 ratios had no significant difference ($p>0.05$), but no significant difference was found with 10:1 ratio. As for pH effect, it was found that there were significant differences in the amounts of OD of the bacterium in 6, 7, and 8 pHs.

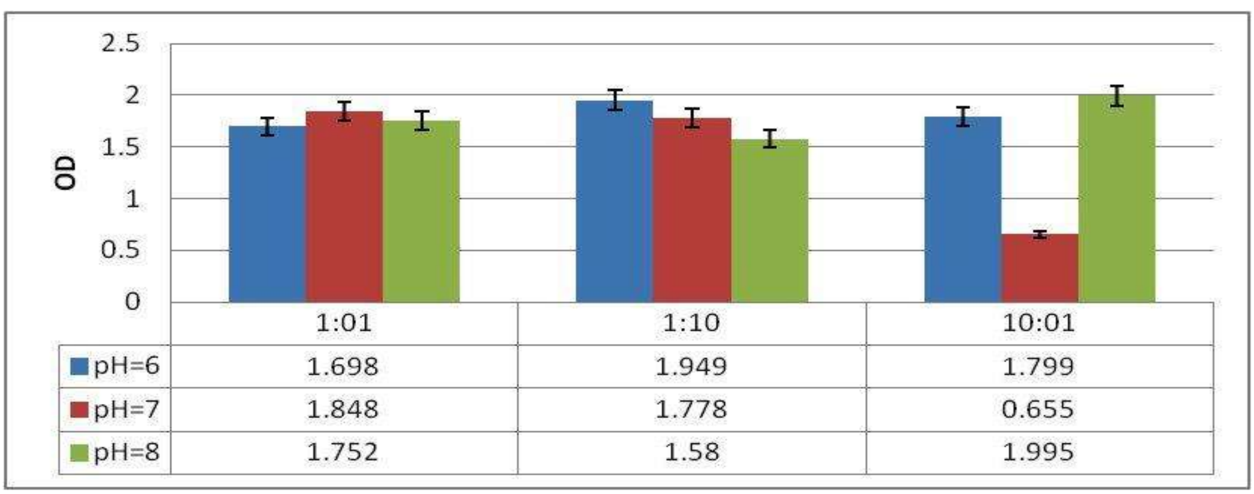

Figure 3: Examination of pH Effect in the Process of Tarball Biodegradation by *Brevibacterium iodinum* for one month

Considering figure 3, the best nutrient ratios for *B. iodinum* in the vicinity of tarball include 1:10 and 10:1 and the best pH: 8and6 within one month time interval. Considering statistical analysis of data by variance

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analysis, it was specified that the amounts of OD for *B. iodinum* in the vicinity of tarball in 10:1 and 1:10 nutrient ratios, no significant difference was found, but a significant difference was found with 1:1 ratio ($p>0.05$). As for evaluation of pH, the amounts of OD in pHs 6 and 8 had no significant difference, but the amount of OD in pH 7 was significantly different as compared to the two other pHs.

Results of SARA Analysis

In the initial analysis for the composition of tarball in different stations of Khark Island using SARA analysis it was found that saturation materials were 25.6 to 59%, aromatic materials were 17.5 to 28.5%, resin materials were 11 to 9.7%, asphaltene was 2.8 to 17.6% and insoluble materials of toluene were 0 to 28.3%. The results obtained from SARA analysis in the performance of *B. iodinum* in degradation of tar lumps are shown in figure 4.

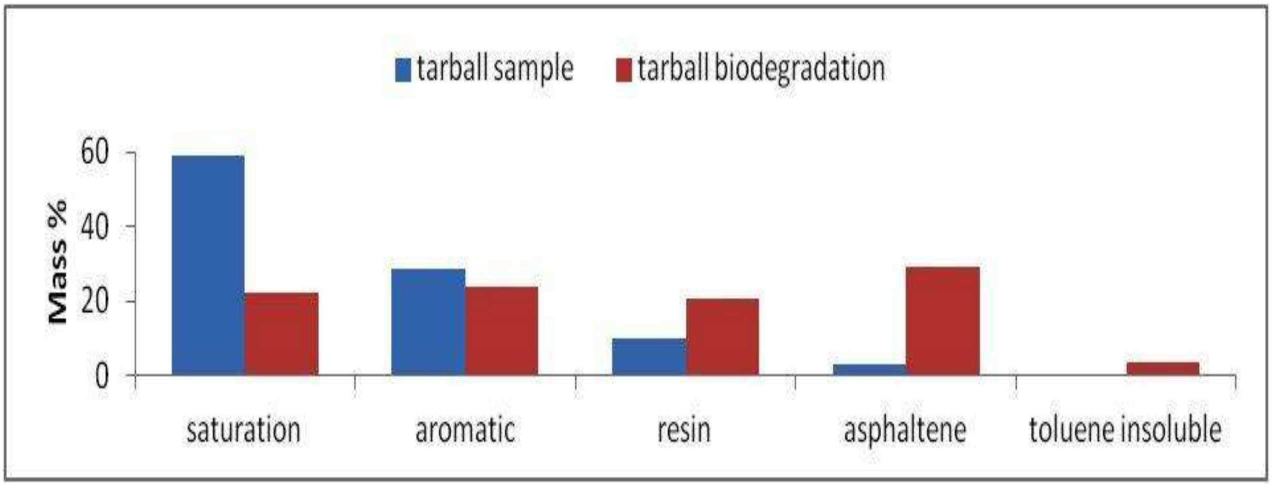

Figure 4: Comparison of compositions of tar lumps before and after biodegradation by Brevibacterium iodinum with optimal conditions (pH:6, N:P: 1:10)

Results of GC/FID Analysis

Considering figure 5 (a), (b) and (c) related to the GC/FID conducted on tarball sample under the influence of *B. iodinum* within a one month interval, n-alkanes C10, C11, C12 and C14 have been removed completely. As it is seen, a series of n-alkanes are produced and after passing a one month interval in the vicinity of bacterium, n-C24 is removed completely and the peaks related to n-C16, n-C17, and n-C18 are decreased. Comparison of the three figure indicates consumption of a major part of tar clump by *B. iodinum*.

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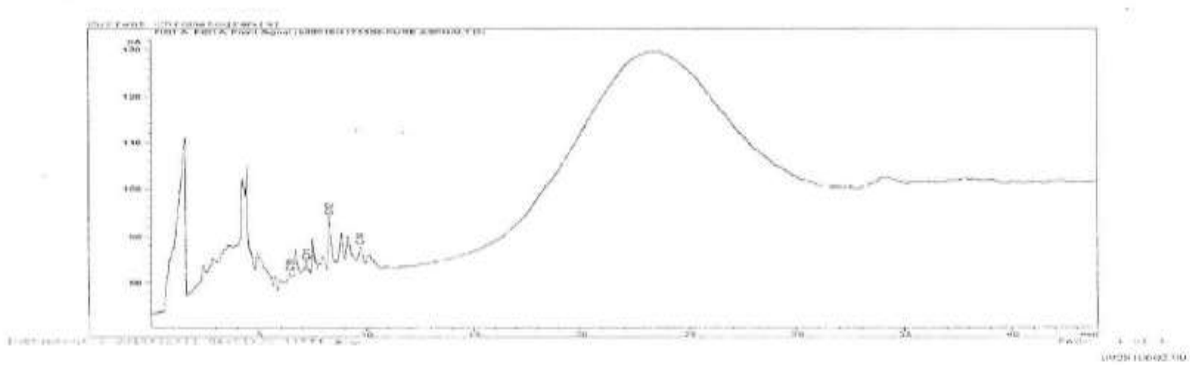

Figure 5(a): GC/FID Chromatogram of the Witness Tarball

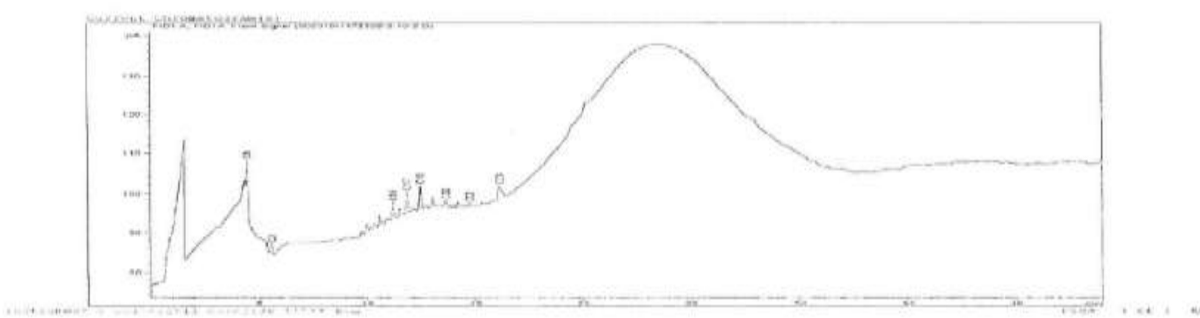

Figure 5(b): GC/FID chromatogram of the tarball treated by *Brevibacterium iodinum*

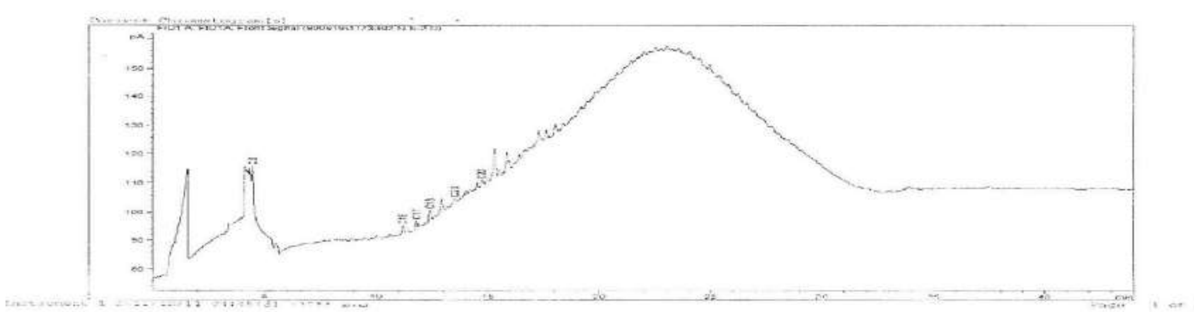

Figure 5(c): GC/FID chromatogram of the tarball treated by *Brevibacterium iodinum*

Figure (a) is related to separation of the peaks of tarball witness samples during different retention times. In diagram (a), C10, C11, C12, and C14 peaks are observed in time intervals 6, 7, 8, and 10, respectively. In diagram (b), tarball sample under the influence of *B.iodinum*, the peaks related to intervals 6, 7, 8, and 10 are removed. Moreover, n-alkanes of carbon, namely C16, C17, C18, C20, C22, and C24, were generated in time intervals 10, 11, 12, 13, 15, and 16, respectively. In diagram (c), tarball sample under the influence of *B.iodinum* is within a one month interval which was examined by SARA analysis and the results were examined according to both analyses. According to the diagram, n-alkane of carbon C24 is removed and the peaks for intervals 10, 11, and 12 (C16, C17, and C18) are decreased. On the whole, the results obtained from GC/FID and SARA analysis indicate the degradation of saturated materials or n-alkanes (saturated linear hydrocarbons) which included the highest percentage for compositions constituting tarball.

DISCUSSION

Comparison of the performance of different procedures for cleaning the environment from different pollutants and seeking for sustainable and cheap procedures resulted in the development of biocleaning technology. In fact, the antiquity of biocleaning utilization using microorganisms in order to clean and treat the pollutants in the environment goes back to 600 BC. Aquatic and terrestrial ecosystems have naturally emerged mircroorganisms which are capable to degrade oil hydrocarbons (Kasai *et al.,* 2002; Vasudevan and Barathi, 2002). In 2004, Hegazi *et al.,* evaluated tar clumps and biological degradation of this oil composition by separating microorganisms and reflected their biodegradation as compared to raw oil. In 2010, Essien *et al.,* and Robert *et al.,* successfully separated the bacteria degrading tar clips formed as the result of oil fall of Santa Barbara Channel in California. Moreover, extensive researches have been made by the scientists in relation to biodegradation of asphaltenes among which the research made by Lioa *et al.,* in 2009 may be referred (Hegazi, 2004; Essien *et al.,* 2010; Robert *et al.,* 2010). In this process, separation of the bacterium degrading asphaltene and tarball from a zone with a long record of contamination with oil and oil products was conducted. In the present study, *B.iodinum* was almost capable to consume a major part of asphaltene and tarball as its carbon source. In 1974, Atlas examined the use of bacteria in analyzing oil and its derivatives and introduced *B.iodinum* as the effective factor in analyzing heavy compositions of oil. After one month, the highest opacity (OD:600nm) for *B.iodinum* species was calculated at 2 in the environments of mineral basic salines along with asphaltene and tarball. In this study, increase of OD was considered as a criterion for the capability of the separated bacterium in asphaltene and tarball analysis (Talaie *et al.,* 2008; Ghazali, 2004). In 2010, Talaie *et al.,* considered changes and increase of OD as the omission percentage of oil compositioins by microorganisms. Environmental conditions for microorganisms should be optimized for a successful biocleaning. The selected range during optimization process was considered at pH:6,7,8. Considering the obtained results, *B.iodinum* successfully biodegraded tarball and asphaltene with pH:6 and pH: 8, respectively. But in acidic pHs, a higher amount of OD was found as compared to alkali conditions. pH reduction during degradation period indicates chemical changes on the oil compositions made by bacterial enzymes. Biodegradation of oil hydrocarbons by the bacterium results in production of bi-products and sometimes organic acids. These compositions reduce pH of the environment. pH reduction of environment confirms growth and activity capabilities of the bacterium. In the researches made by Bola (2006), it was specified that pH reduces during the period and this is the reason for release of compositions as the result of raw oil degradation (Bola, 2006). For this reason, in this test, such a bacterial species with less pH equal to 6, progressed the degradation to the next stages. It should be noted that pH of the culture medium is only considered as a criterion in providing optimal conditions for degradation of oil compositions and it cannot individually serve as a suitable index for investigating the capability of bacteria in analyzing oil compositions. Moreover, optimal pH for biodegradation is variable considering the types of bacterial and oil composition. As for the effect of nutrients on biodegraation process of asphaltene and tarball, it was found that increase of phosphate and nitrate will facilitate the degradation of asphaltene and tarball(Westlake *et al.,* 1978). examined oil degradation in the soils of Northwest Canada where nitrogen and phosphoric fertilizers had been added to them. In that study, the number of bacteria increased and a rapid degradation and reduction occurred in the amount of n-alkanes and isopropanoids. The study showed that nitrogen and phosphor could be used as nutrients to facilitate microbial destruction of oil hydrocarbons(Head *et al.,* 2003). Moreover stated that in most surface and subsurface environments, nitrogen and phosphor are sometimes the restricting nutrients. Further to this, nitrate reduction was considered as a restricting factor for the growth of bacteria on oil compositions(Oldenburg *et al.,* 2006). In other studies stated that nutrients controlled biodegradation rate of oil compositions. The results obtained from the present research shows the effectiveness of different conditions of nutrients ratios in bioanalysis of asphaltene and tarball. In this research, SARA analysis and GC/FID chromatography were conducted to examine the condition of tarball hydrocarbons in the culture medium. Considering the results of SARA analysis and GC/FID chromatography, the separated bacterium in this study was capable to degrade saturated and aromatic materials available in the tarball(Owens *et al.*, 2002). In similar studies used GC/FID chromatography technique to analyze tarball in the coasts of Pacific Ocean in North America and to separated saturated hydrocarbons, especially n-C8, n-C17, n-C18, n-C40(Hegazi *et al.*, 2004). Moreover used GC/FID chromatography technique to analyze the compositions of coastal tars contaminating the coast of Alexandria. The obtained results, similar to those of this research, indicate the extensive distribution of n-alkanes. Moreover, biodegradation results of the research conducted by Hegazi *et al.*, were similar to those of this research, in the manner that biodegradation reduced the ratio of n-alkanes n-C17 (Prystan) and n-C18 (Fytan). In the examination made to evaluate biodegradation and anthracene degradation, GC/FID chromatography technique was also used(Liao *et al.*, 2009). In a similar research examined this measurement method in the oil samples containing asphaltene in the coasts of Liahohe Basin and used the data obtained from GC to describe the procedure of change and biodegradation of asphaltene. They specified asphaltene with a series of n-alkanes from C7 to C30. They proposed that biodegradation could affect the composition and structure of asphaltene. According to the results, Evdokimov, 2005 used SARA analysis and stated that upon increase of asphaltene percentage during biodegradation, saturated and aromatic materials will reduce and resin volume will increase. Biodegradation products such as carboxylic acids, phenols and alcohols not only are mixed with oil, but also they can be linked to asphaltenes and form new asphaltene molecules. That is why total amount of asphaltene sometimes increases during biodegradation. Of course, it is likely in this situation that saturated hydrocarbon sections decrease upon increase of biodegradation level. This is in conformity with the omission of n-alkanes and enrichment in lighter alkanes. In this situation, n-alkanes are consumed first and fatty acids are produced. Again, the linking of carboxylic acid to resin components changes solubility and an asphaltene is formed. Fatty acids of long chain produced in the preliminary stages of biodegradation are first placed in asphaltenes and increase the ratio of long chain species and finally, as advances of biodegradation, they become shorter (Evdokimov, 2005).

REFERENCES

Atlas RM (1995). Petroleum biodegradation and oil spill bioremediation. *Marine Pollution Bulletin* **31** 178-182.

Barathi S and Vasudevan N (2001). Utilization of petroleum hydrocarbons by Pseudomonas fluorescens isolated from petroleum contaminated soil. *Environment International* **26** 413-416.

Bola O (2006). Hydrocarbon degrading potential of petroleum hydrocarbons by Pseudomonas fluorescens isolated from petroleum contamirated soil. *Environment International* **26** 413-416.

Capelli SM, Busalmen JP and Sanchez SR (2001). Hydrocarbon bioremediation of a mineral – base contaminated waste from crude oil extraction by Indiagenous bacteria. *International Biodeterioration and Biodegradation* **47** 233-238.

Chandru K, Zakaria MP, Anita S, Shahbazi A, Sakari M, Bahry PS and Mohamed CAR (2008). Characterization of alkanes, hopanes and polycyclic aromatic hydrocarbons (PAH) in tar-balls collected from the East coast of peninsular. *Malaysia Marine Pollution Bulletin* **56** 950-962.

Clark RC and Macleod WD (1977). Inputs,transport mechanism and observed concentrations of petroleum in marine environment. In: *Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms Nature and Fate of Petroleum* edited by Malins DC (Academic press, NY) **1**.

Essien JP and YHah A (2005). Growth profile and hydrocarbonoclastic potential of microorganisms isolated from tarballs in the Bight of Bonny, Nigeria. *World Journal of Microbiology and Biotechnology* **21**(6-7) 1311-1322.

Evdokimov IN (2005). Bifurcated *correlations of the properties of crud oils with their asphalten content*.

Fuel **84** 13-28.

Fiocco RJ and Lewis A (1998). Oil spilt dispersants. *Pure and Applied Chemistry* **61** 24-42.

Ghazali FM, Rahman RNZA, Salle AB and Basri M (2004). Biodegradation of hydrocarbons in soil by microbial consortium. *International Biodeterioration and Biodegradation* **54** 61-67.

Haritash AK and Kaushik CP (2009). Bidegradation aspects of polycyclic Aromatic Hydrocarbons (PAH), *Journal of Hazardous Materials* **169** 1-15.

Head IM, Jones DM and Larter SR (2003). Biological activity in the deep subsurface and the origin of heavy oil. *Nature* **426** 344-352.

Hegazi AH, Anderson JT, Abu Elgheit MA and EL-Gayar MSh (2004). Source diagnostic and weathering indicators of Tar-balls utilizing acyclic, polycyclic and s-heterocyclic components. *Chemosphere* **55** 1051-65.

Kasai Y, Kishira H, Sasaki T, Syutsubo K, Watanabe K and Harayama S (2002). Predominant growth of Alcanivorax strains in oil contaminated and nutrient supplemented sea water. *Environmental Microbiology* **4** 41-147.

Lia Q, Kang C and Zhang C (2005). Waste water produced from an oilfield and continuous treatment with and oil-degrading bacterium. *Process Biochemistry* **40** 873-77.

Liao Y, Geng A and Huang H (2009). The influence of biodegradation on resins and asphaltenes in the liaohe basin. *Organic Geochemistry* **40** 312-320.

Margesin R (2006). Potential of cold_ adapted microorganism for bioremediation of oil polluted Alpine soils. *International Biodeterioration and Biodegradation* **46** 3-10.

Prince RC and Clark JR (1994). Bioremediation of the Exon Valdez oil spill: monitoring safety and efficacy. In: *Hydrocarbon Remediation* edited by Hinchee RE, Alleman BC, Hoepple RE and Miller RN (Lewis publisher, Boca Raton, USA).

Robert J, Hostettler DF, Baker R, Lorenson DT and Dougherty J (2004). Geochemical characterization of tarballs on beaches along the California coast. *Organic Geochemistry* **35**(6) 725-746.

Talaie AR, Jafaarzadeh N and Talaie MR (2008). Optimization biodegradation of floating diesel fuel contaminated wastewater using the tagochi method. *Journal of Water andWastewater* **20**(3) 57-68.

Thomes R and Lunel T (1993). The Braer incident dispersion in action, in: proceedings of the sixteen Arctic Marine oil spill program technical seminal, Edmonton, Alberta, Canada.

Zekri AY and El-mehaideb R (2003). Steam / bacteria to treatment of asphaltene deposition in carbonate rocks. *Journal of Petroleum Science and Engineering* **37** 123-133.