Determination the Efficiency of Biological Treatment of Oil Pollutant by Gas Chromatography Technique

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Abstract

Petroleum hydrocarbon especially in the form of crude oil has been a veritable source of economic growth to society from the point of view of its energy and industrial importance. For these reasons, the petroleum oil can cause environmental pollution during various stages of production, transportation and refining as well as spilling accident. Petroleum hydrocarbons pollution, ranging from soil, ground water to marine environmental, become an inevitable problem in the modern life. There are many various petroleum hydrocarbons clean-up technologies such as biological method, this method is efficient and economical compare with chemical and physical methods. The purpose of this study was to explore and study the efficiency of the fungi *Aspergillus Niger* which isolated from soil contaminated with crude oil in biodegradation of crude petroleum oil and hsing Gas chromatography technique to determine efficient of biological treatment. The results of this study show that the efficiency of fungi *Aspergillus Niger* to degrade petroleum oil hydrocarbon was 89% after 30 days of bioremediation treatment.

Key word: Biodegradation, Biological Treatment and Chromatography Technique

تحديد كفاءة المعالجة البيولوجية للملوثات النفطية باستخدام تقنية كروموتوغرافيا الغاز

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الخلاصة

يعد وجود البترول مبدأ أساسيا من مبادئ النمو الاقتصادي ومصدرا مهما من مصادر الطاقة للعديد من الصناعات مما جعل منه واحدا من أهم الملوثات البيئية خلال عمليات الإنتاج والنقل والتكرير فضلا عن حوادث التسرب النفطي وشملت تأثيراته التربة والمياه والنظام البيئي مما دفع بالمهتمين بسلامة البيئة إلى استخدام العديد من التقنيات لإزالته، منها التقنيات البيولوجية التي تتميز بالكفاءة والفعالية وكونها طريقة اقتصادية بالمقارنة مع الطرق الكيميائية والفيزيائية. يهدف البحث إلى عزل فطر *Aspergillus niger* من التربة العراقية الملوثة بالنفط الخام واختبار كفاءته في إزالة النفط الخام فضلا عن استخدام تقنيات التحليل الكروموتوغرافي في تحديد كفاءة المعالجة البيولوجية. وبينت نتائج الدراسة كفاءة فطر *Aspergillus niger* في تفكيك الملوثات الهيدروكاربونية النفطية وبنسبة بلغت 89% بعد مرور 30 يوما على المعالجة البيولوجية.

الكلمات المفتاحية: التحلل البيولوجي، المعالجة البيولوجية و تقنية الكرموتوغرافي

Introduction

The increasing contamination of the environment by dangerous, toxic substances is a worldwide problem. Nowadays, routine and accidental spillages of petroleum derivative compounds are contaminating the air, soil, rivers, seas, and underground water(Vieira *et al*., 2009). Petroleum hydrocarbons are one of the most frequently encountered pollutants in the environments habitats due to the increased usage of petroleum as the principle source of energy (Yu-Ying, 2011). Petroleum hydrocarbon especially in the form of crude petroleum-oil has been a veritable source of economic growth to society from the point of view of its energy and industrial importance.(Anthony and Okoh, 2006). There are many various petroleum hydrocarbons clean-up technologies. These can be categorized in three general schemes: chemical, physical and biological (Kermanshahi *et al.*, 2005). Bioremediation is believed to be one of the main processes used in the cleaning-up of contaminated soil and groundwater. This method is efficient and economical (Obire and Nwaubeta, 2001). The uptake of hydrocarbon by microorganisms is possible in three different mechanisms: hydrocarbon dissolved in the aqueous phase, direct cell contact and the uptake of hydrocarbon drops, and the uptake of pseudosolubilized hydrocarbon droplets by using biosurfactant (Bouchez *et al.* 1995; Kim *et al.*, 2002). It is known that the main microorganisms consuming petroleum hydrocarbons are bacteria and fungi as a method of biodegradation. Biodegradation using fungi have drawn little attention in the past two decades since most of the biodegradation researches focused mainly on the use of bacteria.

Fungi produce enzyme that breakdown and degrade a wide range of recalcitrant pollutants such as polyaromatic hydrocarbons, chlorophenols, and pesticides (Bumpus *et al.*, 1985) .In addition, fungi have advantages over bacteria such as fungal hyphae that can penetrate contaminated soil to reach the PAHs that have spread beyond the top layer of the soil (April *et al.*, 2000). Besides, the fungi are capable to grow under environmental conditions of stress, for example: environment with low pH values or poor in nutrients and with low water activity. (Yateem *et al.*, 1998). Assessing the petroleum damage to environment and natural resources caused by accidental release of crude oil requires the design of appropriate and reliable chemical analytical methods for oil samples collected in the study area. The analytical data and results will provide essential information to document oil exposure pathways, to determine extent and degree of oiling ,to evaluate the long term impact of spilled oil ,to estimate recoverability of the injured resources, and to suggest effective clean-up strategies. Analytical methods and techniques for oil analysis have made major advances in recent years and the development continues .

Gas chromatography (GC), mass spectrometry (MS) , ultraviolet(UV), infrared spectroscopy(IR),fluorescence spectroscopy, supercritical fluid chromatography (SFC), and hyphenated techniques such as GC-MS, GC-FTIR , SFC-GC are used as techniques to evaluate oil analysis. Usually the biological method have been used such as turbidity, biomass and spectrophotometric to determine the efficiency the biological activity of removing petroleum pollutant which considered as not accurate in analysis.

The purpose of this work is to explore and study the efficiency of fungi in biodegradation of crude petroleum-oil by using the gas chromatography to determine type and amount of the hydrocarbon compounds which are presented in crude petroleum oil.

Material and Methods

Chemicals

Hexane, Chloroform, n-Pentane (all >99% purity) and Methylene Chloride (98% purity) were purchased from Fluka (Steinheim, Germany) and Methanol (99% purity) (analy gainland chemical company), Silica Gel (35-70 mesh ASTM) Merck. Crude petroleum oil was obtain from south of Iraq which had chemical compositionas showed in the Table (1).

Table (1) Various Fractions of Crude Petroleum –Oil

Total Petroleum Hydrocarbons (TPH)			
Insoluble %	**Soluble %**		
Asphaltene	**Alkane**	**Aromatic**	**NSO**
12.2	58	17	12.8

Culture medium of Microorganism

The culture medium used throughout these studies consisted of:(1 g/L) Anhydrous Potassium Hydrogen Orthophosphate (K_2HPO_4); (1 g/L) Anhydrous Potassium Dihydrogen Orthophosphate (KH_2PO_4) ; (1g/L) Anhydrous Sodium Hydrogen Orthophosphate (Na_2HPO_4); (1g/L) Ammonium Nitrate (NH_4NO_3); (0.02g/L) Calcium Chloride 2-hydrate ($CaCl_2 . 2H_2O$); (0.2g/L) Magnesium Sulfate ($MgSO_4. 7H_2O$); (0.05 g/L) of Iron Chloride ($FeCl_3$); (0.1%)Tween 80.

Experimental Procedure Biodegradation method

The fungi *Aspergillus niger* which was used in the experiment was isolated from soil contaminated with crude petroleum-oil by ollowing the reference (Griffin, 1972).

Replicates samples (n=3) were grown in 250ml Erlenmeyer flasks containing 100ml of culture medium. Then 1 gm of crude petroleum–oil was added to reach a final concentration of 1 %(w/v). Three agar plugs ($1cm^2$) of the 24hr pure culture of *Aspergillus niger* were inoculated into the culture medium and then incubated at 37ºC for 10, 20 and 30 days . Control' tubes were prepared without the microorganism.

Chemical Analysis

Crude petroleum-oil was extracted from the mixture of *Aspergillus Niger* and culture medium by using sequentially extraction. In this extraction, 50ml of hexane followed 50ml of methylene chloride then 50ml of methanol: chloroform (1:1) were used, sequentially.

All of the three extracts were pooled and dried at room temperature by evaporation of the solvents under a gentle nitrogen stream in a fume hood. The dried-extract was dissolved in n-pentane (10ml) in order to separate it into soluble and insoluble (asphaltene) fractions. After that, the soluble fraction was evaporated in order to calculate the weight of residual (aliphatic, aromatic, and NOS compounds). In order to purify the residual, it was redissolve in n-pentane (10ml) and loaded on the top of a silica gel column (35-70 mesh) (2cm × 22cm). After 5-10 mints a calculated volume of n-pentane (12ml) was added to receive the purified-residual. In order to use a calculated concentration, the purify residual was dried at room temperature and weighted. Then it was dissolved in appropriate solvent and injected into (GC) apparatus. The same procedure was repeated with the control tubes.

Chromatography(GC)

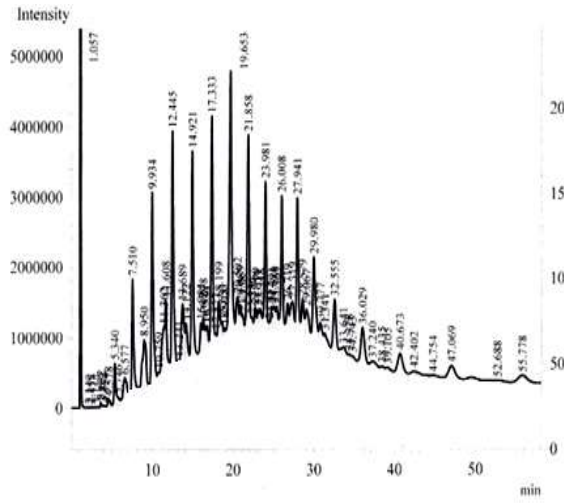
The fraction was analyzed by Gas Chromatography (GC) shimadzu, using a FID detector. The column was packed SE 30 (L3m, ID 2mm).The column temperature was 80-250 ºC for 20min rate 5cc/min. The injector temperature was 260ºC and detector temperature 270ºC.

Results and Discussion

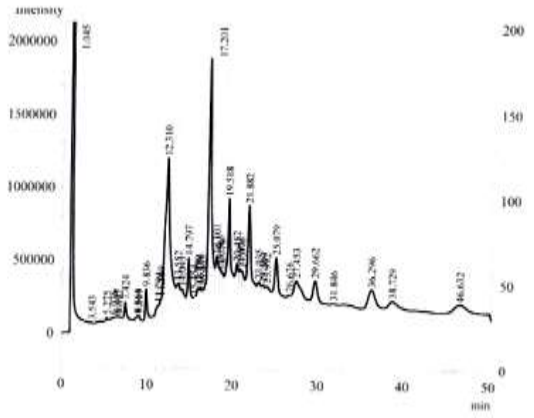
In this work, gas chromatography was used to evaluate the purified residue. Figure(1) shows the chromatograms of gas chromatography analysis of crude oil control(A), and purified residue after 10,20 and 30 days of biodegradation (B, C, and D).

In the period between 10-30 days, the biodegradation causes a dispersion of the oil, resulting a changes\

\ in the original feature of the crude petroleum-oil. It has been found that, a reduction of some hydrocarbon compounds and disappearance of others were occurred with increasing the biodegradation time.



(A)



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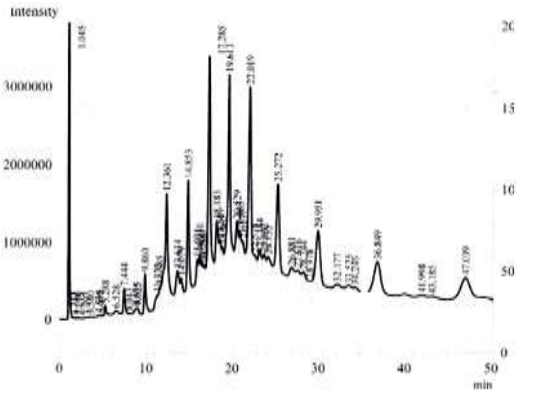

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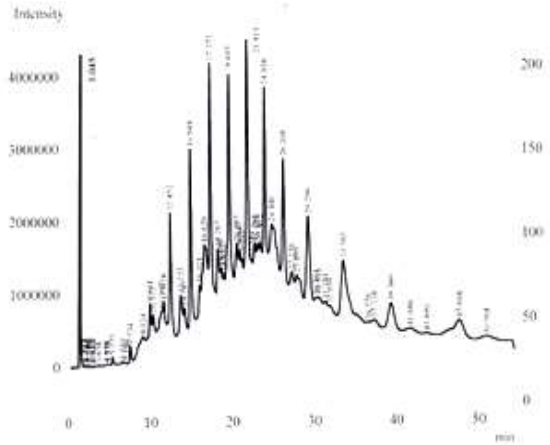


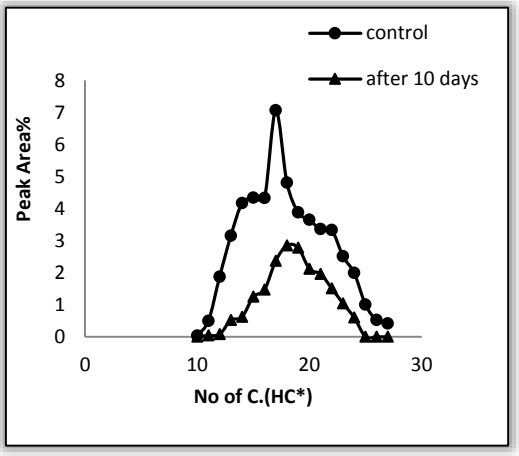
(D)

Figure(1) Gas Chromatographic Analysis of Crude Oil after Clean up. (A)Control and (B),(C),(D) after 10,20 and 30 Days Biodegradation Respectively

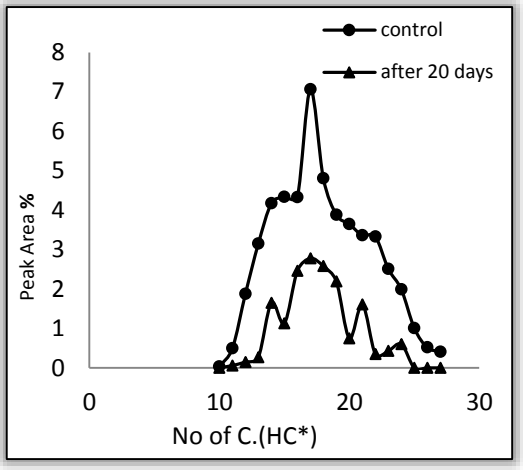
Related to the phenomena of disappearance of some hydrocarbons, it might be related that microorganisms can produce biosurfactants through biodegradation process. Microorganisms can survive and able to utilize the contaminant itself for growth on hydrocarbons that were insoluble in water. This surfactants enhance organic removal by raising the solubility of the hydrocarbons thereby making more of them available for degradation and facilitating transport of the hydrocarbons across the cell membrane of the

microorganisms (Khan**,** *et al.*2006). While the reduction of other hydrocarbons might be related to the petroleum oil as a complex mixture of aliphatic fraction, consisting of straight chain, branched chain , poly cyclic aromatic hydrocarbons, NSO and asphaltene fraction (Jain, *et al.* 2005), therefore a single microbial has only limited capacity to degrade all the fractions of hydrocarbons present, and the complex mixture of petroleum hydrocarbons might be influence each other's biodegradation the effects may go in negative as well as positive direction (Wackent. 1996).

The degree of oil degradation was calculated in the total petroleum hydrocarbon degradation, by comparing the total area of the chromatograms containing *Aspergillus niger* with those of the controls (Xie *et al*., 1999). Figure (1) shows the big changes in the crude composition. As expected, the biodegradation was occurred significantly. It was well documented that *Aspergillus niger* is able to degrade and resolved component of crude oil.



(A)



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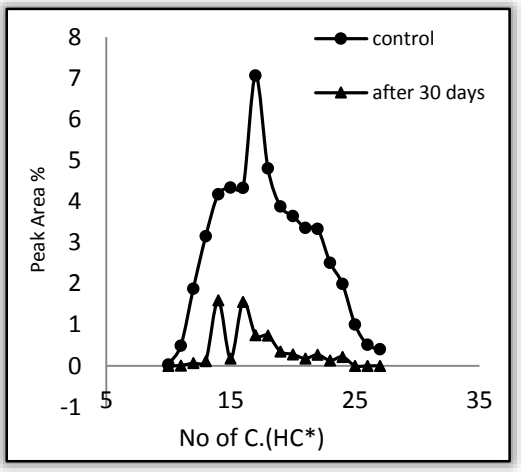


(C)

Figure(2) Comparison of Percentage of Peak Area between (HC*) and the Control (A, B, and C after 10,20 and 30 Days Biodegradation, Respectively)

Here in, HC* refers to n-paraffinic compounds (n-C10-C27), and HC** refers to isoparaffinic cycle, and aromatic compounds. The percentage of peak area was calculated by dividing the area of each individual peak on the total peak area of HC* or HC** in order to evaluate the biodegradation degree of *Aspergullus niger.*

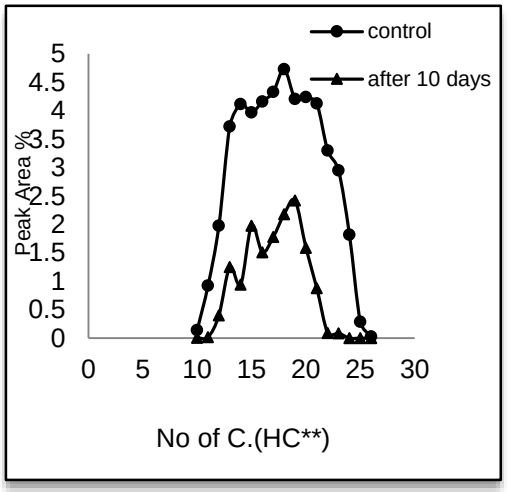
Figure(2) shows the relation between the percentage of peak area for normal paraffinic compounds(HC*) and the number of carbon atoms for both control and purified residue after 10, 20 and 30 days of biodegradation.

After 30 day, it is so clear that all the fractions (HC*) were completely utilized by Aspergillus *niger* except C14, C16, C17 and C18 which were suffered from drastic reduction.

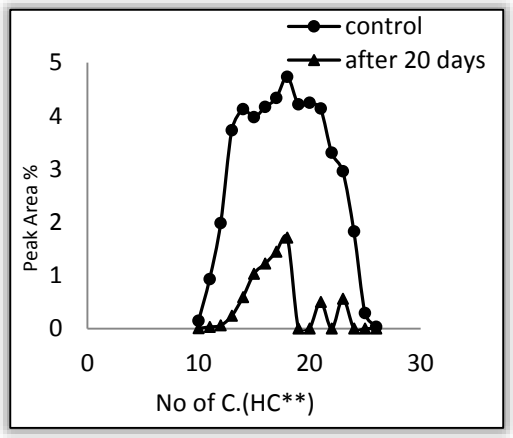
Figure (3) shows the relation between the percentage of peak area (C_{10}-C_{11} and C_{11}-C_{12}….etc)((HC**) and the control after 10,20 and 30 days of biodegradation.

Comparison of the percentage of the peak area between (HC**) and the control after 30 days of biodegradation indicated that, all fractions of isoparaffinic and aromatic compounds are removal.

Figure (2) as compared with figure (3) indicated that, the degradation isoparaffinic and aromatic compounds are more than normal paraffinic.



(A)



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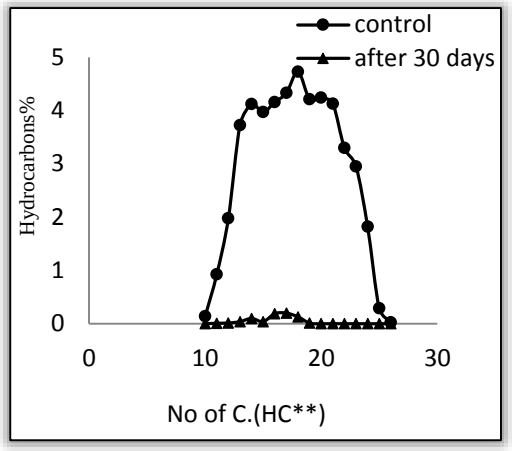

(C)

Figure(3) Comparison of percentage of peak Area Between (HC**) and the Control (A, B, and C after 10,20 and 30 Days Biodegradation, Respectively)

Biodegradation using fungi have draw little attention in the past two decades since most of the biodegradation researchers focused mainly on the use of bacteria. Some reports indicate that the isolation of a pseudomonas strains degrade both aliphatic and aromatic fractions more than 90% after 21 days for two pseudomonas isolates (mixed culture) (Salam, *et al*., 2011), while crude oil degradation rates of only 60 to 66% were reported by Adebusoye *et al*., (2007) for pure culture strains isolated from polluted tropical streams after 20 days of incubation. In the present study, The results reported more than 89% degradation of crude oil by *Aspergillus niger* which were isolated from contaminated soil after 30 days of incubation.

The data presented in this paper was summarized in Table (2) which showed that the total biodegradation percentage of crude petroleum-oil after 10,20 and 30 days of biodegradation were 29,53 and 89%. after 10,20 and 30 days using the fallouing equation.

$$\textbf{Biodeg.}\,\% = \frac{\text{crude oil control} - \text{crude oil degradeed}}{\text{crude oil control}} \times 100$$

Table(2)Biodegradation Percentage of Crude Petrpleum Oil with Different Period Time

Period of Treatment	10days	20days	30days
Percentage of Biodegradation	29	53	89

Conclusions

Here in, fungi *Aspergillus niger* was isolated from the petroleum-contaminated soil in order to use this fungi to degrade the crude petroleum oil. It has been found that the efficiency of *Aspergillus niger* is time- dependent. The degradation rate increases with period of degradation to reach an efficiency of 89% after 30 days. The potential of GC to detect the oil fractions was used to determine the degradation efficiency of *Aspergillus niger*. At the same time, it has been noticed that isoparaffinic fractions and compounds(HC**) was reduced more than n-paraffinic compounds and fraction (HC*). Comparing with other works, our study calculates and compares both of (HC*) and (HC**) compounds and fractions while other works calculated for the crude petroleum –oil as one fraction. We believe that this work can add an advance step to the petroleum industry.

Recommendations

Using of some techniques which are more efficient such as GC-mass and HPLC in order to determine quantity and quality of lower concentrations for all oil's fractions, besides using other type of fungus or mixing more than one fungus for more degradation to oil's fractions.

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