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Using of Some Bacterial Species to Treat Polluted Soils with Hydrocarbons.

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Abstract

Three bacterial species were isolated from three polluted soils with gasoline which leaks from electricity generators that used in different regions in Baghdad; the regions choices to collect the polluted soils were (Al-Shaab, Al- Jadryia and Al-Saydiya).

The bacterial species were identified according to international biochemical methods. It was found that these species were *Escherichia coli*, *Enterobacter aerogenes* and *serratia marcesens.*

It was found that the optimum temperature and pH were 37C°and 9 these were to cultivate *E.coli* and *S.marcesens*, while for *E.aerogenes* were 25 C° and 9.

FTIR (Fluori Transmission Infra Red) spectrum technique was depended to test the ability of isolated bacteria to biodegrade the gasoline in order to use these bacteria in bioremediation for polluted soils with hydrocarbons.

Introduction

Hydrocarbons are one of the pollutants which are harmful for ecosystems if it increased more than the acceptable level in water, soil, as well as air [1].

Biological cleaning procedures (bioremediation) depend on the fact that most organic chemicals are subject to enzymatic attack of living microorganisms. These activities are summarized under the term *biodegradation.* However, the end products of these enzymatic processes might differ drastically. For instance, an organic substance might be mineralized (i.e. transformed to carbon dioxide and water). It might also be converted to a product that binds to natural materials in the soil, or to a toxic substance[1] .

Essentially, there are three major categories of sites with polluted soils. (a) Sites that have been polluted by either spillage or leakage during production, handling, or use of industrial material Also the activities to gain raw materials, such as mining and oil drilling; (b) locations that have been used as disposal sites for diverse waste; (c) farmlands that have been excessively exposed to pesticides.(d) in Iraq (study region) from generators which used to generate electricity.
Contaminated land sites are health hazards for human beings and thus are unsuitable for housing or agriculture. The downward migration of pollutants from the soil into the groundwater is especially problematic in developing countries where groundwater is often directly used for drinking without any prior treatment [2].

Leticia (2006) discuses the potential of some actinomyces to degrade polycyclic aromatic hydrocarbons (PAH) and the effect of co-substrates, plants and other additives on their degradation and bioavailability [3].

Microbial degradation appears to be the most environmentally friendly method for removing of oil pollutant since other methods such as surfactant washing and incineration lead to the introduction of more toxic compounds to the environment. Hydrocarbon-degrading microorganisms are widely distributed in marine, freshwater, and soil ecosystems [4].The

ability to isolate high numbers of certain oil-degrading microorganisms from an environment is commonly taken as an evidence that those organisms are the active degraders of that environment [5]. Although, hydrocarbon degraders may be expected to be readily isolated from a petroleum-polluted environment, the same degree of expectation may be anticipated for microorganisms isolated from a total unrelated environment. There is an extensive body of knowledge on mineralization or degradation of hydrocarbons by microorganisms [6&7]

Materials and Methods

- Soils polluted with hydrocarbons:

Native hydrocarbonoclast bacteria were isolated from polluted soils with gasoline obtained from three polluted soils with generators gasoline, which were taken from (Al-Shaab; Al-Jadriyia and Al- Saydiyia) regions in Baghdad.

- Isolation of strains from polluted soils:

10 gm of polluted soil was humidified with 250 ml of mineral medium (modified mineral salts medium –MMSM-) which composed of: 4 gm NH_4NO_3; 4 gm KH_2PO_4; 0.2 gm $MgSO_4.7H_2O$ and 0.01 gm $CaCl.2H_2O$ per litre [8] .

The bacteria was separated from the soil particles by gentle shaking of 1 gm soil dry weight with 10 ml of sterile water for 30 minutes. After sedimentation, the supernatant suspension was used to prepare appropriate dilutions (from $1x10^{-1}$ to $1x10^{-5}$) with sterile water. Aliquots of 0.2 ml were spread –plated on the nutrient agar medium. The plates were incubated at 25 C° for 5 days. The bacteria were allowed to spread until purification and were then conserved in a refrigerator ready for use in the production of inocula for characterization and the hydrocarbon degradation experiments [9].

The morphology of the bacterial strains was determined by gram staining, and they were then streaked in Petri dishes. The isolated bacterial cultures were identified according to [10&11]

- The Optimum Conditions of Cultivation:

In order to know the optimum conditions for the three isolated bacteria; they were cultivated with different degrees of temperature which include :in 25 C° ; 37 C° and 40 C° and pH 5,7 and 9.

- Hydrocarbons Degradation in Liquid Culture:

To test the ability of isolated bacteria to biodegrade the gasoline, about 1 gm sample of polluted soil was taken and dissolved in (1 acetone: 3 hexane) and added to a flask of 250 ml, after the evaporation of solvents at 25° C, about 50 ml of sterile medium and 1 ml of the isolated bacteria was inoculated into each flask and then incubated at 35 C° and 150 rpm for 7 days [9]

The ability of the isolated bacteria to biodegrade gasoline was determinate by using FTIR (Fluori Transmission Infra Red) spectrum technique.

Results and Discussion:

According to [10&11] and the biochemical tests which done and explained in table (1) , the most predominant bacteria species in polluted soils that taken from Al-shaab, Al-jadriyia and Al-saydiya were *E.coli*; *Enterobacter aerognosa* and *Serratia marcesens*, respectively.

Usually, the tested soil samples were filled with gasoline fuel which used in generators of electricity So the saturated with gasoline may have effect to limit the kind of microbial content [12] .The difference in microbial population is a reflection of many factors such as nutrients and oxygen levels, temperature and availability of minerals [13].

Usually, the temperature, pH and other factors like available energy source, moisture availability and the appropriate depend to know the optimum conditions of cultivation [14], So, it was found that the optimum conditions to cultivate *E.coli* and *Serratia marcesense* were 37C° and pH9 but 25C°and pH9 were optimum conditions to cultivate *Enterobacter aerogenes* (Tables 2, 3& 4).

The results of Flouri Transmission Infra- red (FTIR) spectra explain the effect of bacteria to biodegrade the gasoline, as following:

Figure (1-A) represents the spectrum of control sample , the important bands absorbency of infra-red in 2931.80 ; 2897.08 ; 2877.79 and 2850.79 cm^{-1} , these wave numbers represents a strong and wide absorbency for blended frequency for NH_3^+ group in primary amino acids.

The frequency in the wave number 1720.50 cm^{-1}related to amine salts.also1458.18 cm^{-1} represents as strong absorbency for the symmetrical blended frequency of the amine salts. The nitroparfins appear two bands in 1377.17and 1357.89 cm^{-1} which related to asymmetrical and symmetrical frequency for NO_2.

There is a weak absorbency for the aliphatic amines in 1215.15; 1064.71 cm^{-1} . Three peaks of blended frequency in 887.26; 794.67 and 725.23 $cm^{-1}$are for C-H group. Also, there is absorbency for disulphides in 528.50 and 455.20 cm^{-1} .

Figures (1, B-E) explain that the symmetrical and a symmetrical frequency of 2974.23 and 2931.80 cm^{-1} related to alkanes group [15] while 2357.01 cm^{-1} represents the identical absorbency of stretch frequencies for OH and NH_3^+ groups.

The wave number 1361.74 cm^{-1} represents the blended frequency was related to carboxyl group while 1134.14 cm^{-1} was related to asymmetrical blended frequency for C-O group.

The disappear of 2357.01; 1064.71; 794.67 cm^{-1} is the main notice in the fifth day of the experiment. Also the appearance of other wave numbers in 1292.31; 1033.85; 786.96 cm^{-1} is very clear.

Usually these changes of hydrocarbon groups give good evidence that *E.coli* has good effect on biodegrade the gasoline [16] where in the seventh day 786.96 and 759.95 cm^{-1} were disappeared completely , also a clear appearance for 2966.52 ; 2977.94 and 2862.36 cm^{-1} can be recognized .

In the tenth day of the experiment (last day), many changes in absorbency of the hydrocarbon groups would occur like appearance of peak in 1716.65 cm^{-1} as a blended

frequency of N-H group, and there is a strong absorbency in 1219.01 cm^{-1} as result of the blended frequency for carboxyl group.

The symmetrical blended frequency for the aromatic ethers group is in 1056.99 cm^{-1} while the number wave 891.11 cm^{-1} represents the frequency for C-C group in alkaline.

The peak appears in 725.23 cm^{-1} is related to C-N group absorbency for isobutyramide.also, There is a bromine compounds frequency in 528.50 cm^{-1} and frequency of disulphides in 447.49 cm^{-1} .

Figures (2, A-D) represent the treat of *Enterobacter aerogenosa* with gasoline during ten days. It was found there are weak absorbency bands of C≡C, C ≡N and S-H groups in 2357.01 cm^{-1} and a blended frequency of C =O group of aliphatic Easters in 1716.56 cm^{-1} , also there is absorbency for C-H group in 786.96 and 763.81 cm^{-1} .

Simple change would occur in the fifth day of the experiment , some absorption would appear other disappears but all of them in the same region of the absorbency would appear in previous days; but in the seventh day, more than one peak of new absorbency regions would appear, especially in 3421.72 and 3309.85 cm^{-1} which represent acute strong absorbency of O-H group in phenols compounds.

Many changes in absorbency peaks would be clear in tenth of the experiment, these are usually related to the effect of bacteria activity with biodegradation process which has sure effect in initial new absorbency peaks [15&16].

Figures (3, A-D) represent the treat of *Serratia merscens* with 1m /50 ml of gasoline. It can by found that the absorbency peak in 794.67 cm^{-1} is sharper in first day comparative with control sample. This wave number represents a blended absorbency of C-H group for benzene compounds. The long chain of these compounds which led to prevent or reduce the bacterial enzyme activity [17] but its sharp shape is be more reduced in the fifth day and may disappear till the end of the experiment as a result of bacteria effect.

Many absorbency peaks would disappear in the fifth day which give a good sight to the change that occur in the chemical compound of gasoline. Also peaks disappear and other appears in the seventh day.

In the tenth day, there is absorbency frequency of C=O group of aliphatic Easter appear in 17166.66 cm^{-1} , a frequency of C-C group in 1465.90 cm^{-1} , other frequency of C-H group in 786.96 and 759.95 cm^{-1} [17].

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Table (1) Biochemical tests to identification the isolated bacteria

Test	Strain 1	Strain 2	Strain 3
Oxidase	–	–	–
Growth on Mac Conky agar	Pink colony	Pink colony	White colony
Indol reaction	+	–	*
Urease	*	–	
H2S	*	*	–
Citrate	*	*	+
Gram strain	–	–	–
Morphology	Bacilli-cocci	Short bacilli	bacilli

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Table (2): Bacterial density in 25C° and pH 5, 7and 9. (cellx10^5).

Bacteria	Time (days)					pH
	0	3	5	7	10	
E.coli	0.005	0.015	0.025	1	5	5
	0.005	0.015	1.5	5.5	7.5	7
	0.005	0.007	0.055	0. 175	25	9
E.aerogenes	0.005	0.006	1.5	15	20	5
	0.005	0.015	16	18	20	7
	0.005	0.2	0.5	15	50	9
S.marcesens	0.005	1	1.2	4	5	5
	0.001	2.4	5	7.5	25	7
	0.005	0.7	5	5.5	0.195	9

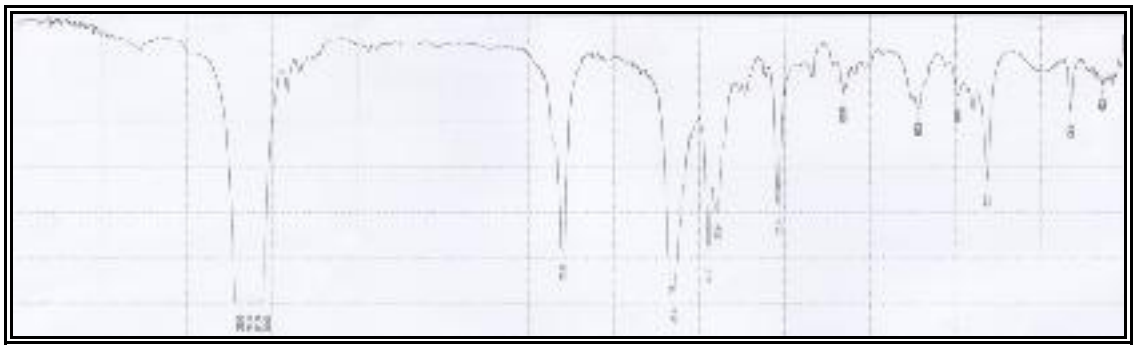
Table (3): Bacterial density in 37C° and pH 5 7and 9. (cellx10^5).

Bacteria	Time (days)					pH
	0	3	5	7	10	
E.coli	0.005	100	15	15	12	5
	0.005	200	350	150	50	7
	0.005	140	300	350	400	9
E.aerogenes	0.005	20	30	35	12	5
	0.005	20	10	3	3	7
	0.005	100	100	30	20	9
S.marcesens	0.005	3	1	zero	zero	5
	0.005	250	300	500	500	7
	0.005	125	150	400	500	9

Table (4): Bacterial density in 40C° and pH 5 7and 9. (cellx10^5).

Bacteria	Time (days)					pH
	0	3	5	7	10	
E.coli	0.005	0.03	6.5	10	6.6	5
	0.005	0.02	55	30	15	7
	0.005	0.12	45	33	20	9
E.aerogenes	0.005	0.007	2	2.5	3.5	5
	0.005	0.03	25	8	4.6	7
	0.005	100	100	30	25	9
S.marcesens	0.005	3.5	4.1	1.6	0.3	5
	0.005	0.5	2	5	5.5	7
	0.005	5.5	20	50	55	9

A



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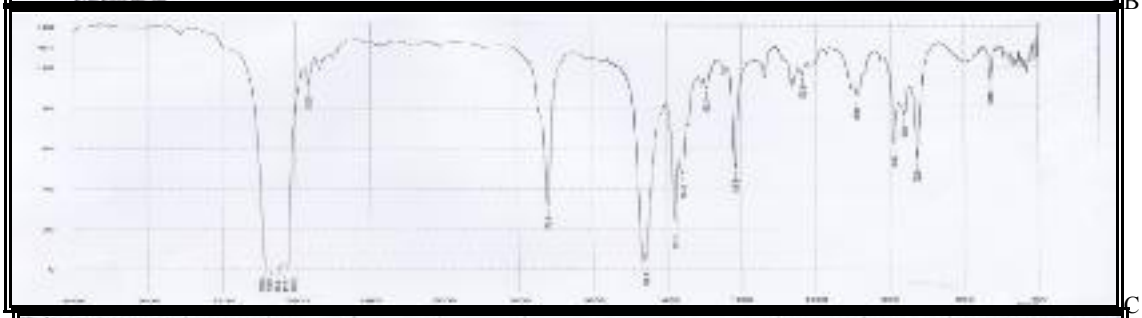

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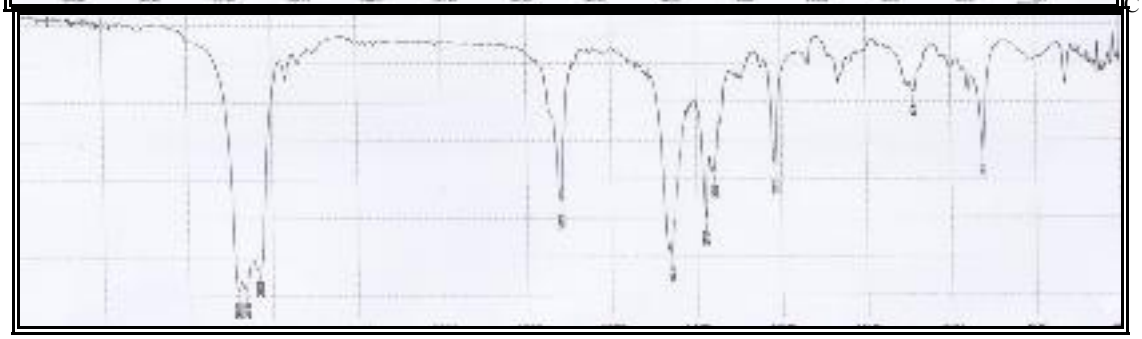

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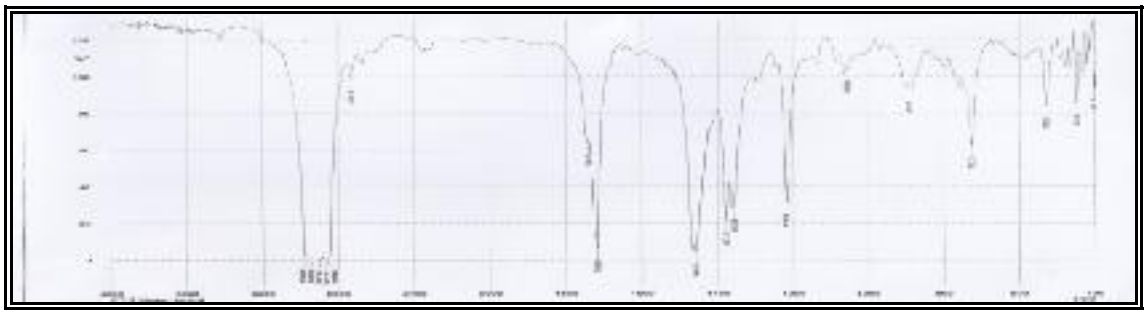
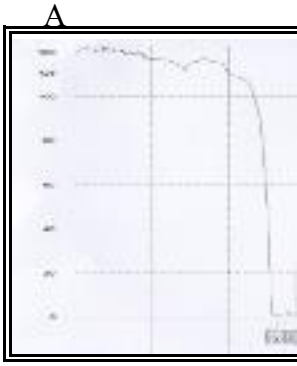


Fig. (1, A-E): FTIR Spectrum for gasoline with *E.coli*.where:
A= FTIR spectrum of gasoline without treat with *E.coli.* (Control sample)
B= FTIR spectrum of gasoline after 3 days of the treat with *E.coli.*
C= FTIR spectrum of gasoline after 5 days of the treat with *E.coli*
D= FTIR spectrum of gasoline after 7 days of the treat with *E.coli.*

A



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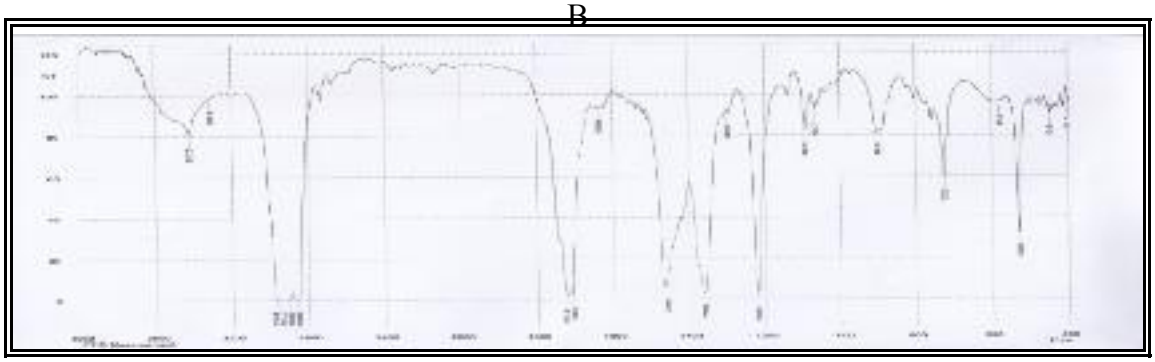

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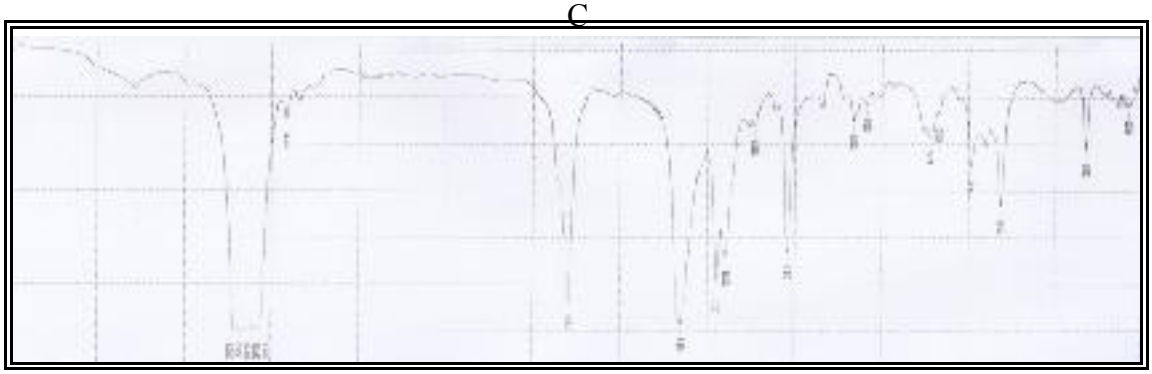
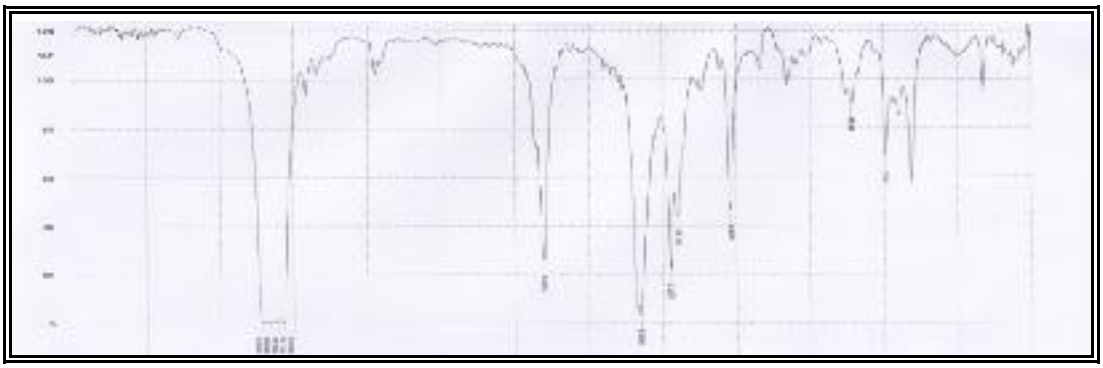


D

Fig. (2, A-D): FTIR Spectrum for gasoline with *Enterobacter aerogenes* where:
A= FTIR spectrum of gasoline after 3 days of the treat with *E. aerogenes*
B= FTIR spectrum of gasoline after 5 days of the treat with *E. aerogenes*
C= FTIR spectrum of gasoline after 7 days of the treat with *E aerogenes*
D= FTIR spectrum of gasoline after 10 days of the treat with *E aerogenes*.



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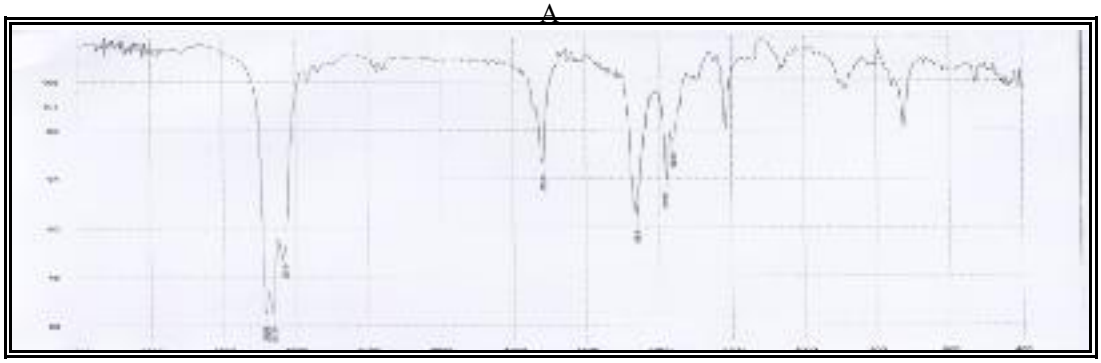

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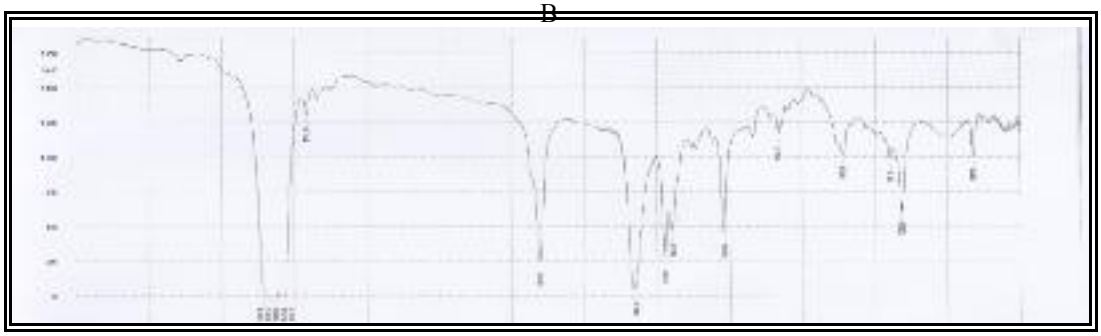

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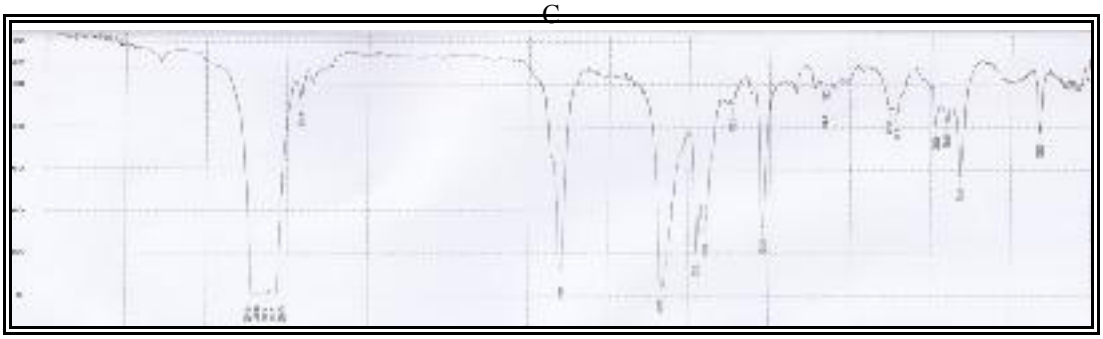

D

Fig. (3, A-D): FTIR Spectrum for gasoline with *S.marcesens* where:
A= FTIR spectrum of gasoline after 3 days of the treat with *S.marcesens*
B= FTIR spectrum of gasoline after 5 days of the treat with *S.marcesens*
C= FTIR spectrum of gasoline after 7 days of the treat with *S.marcesens*
D= FTIR spectrum of gasoline after 10 days of the treat with *S.marcesens*.

مجلة ابن الهيثم للعلوم الصرفة والتطبيقية المجلد 23 (3) 2010

استخدام بعض الأنواع البكتيرية لمعالجة الترب الملوثة بالهيدروكاربونات

إيـــثـــار كـــامـــل الـــمـيـــالـــــي
قسم علوم الحياة ، كلية العلوم ،جامعة بغداد

الخلاصة

عزلت ثلاثة أنواع بكتيرية من ثلاث ترب ملوثة بالكازولين المتسرب من المولدات المستخدمة عادة لتوليد الطاقة الكهربائية في مناطق مختلفة من بغداد(الشعب، والجادرية ،والسيدية) ، شخصت الأنواع البكتيرية المعزولة باعتماد الطرائق البايوكيميائية المعتمدة عالميا" ، وقد وجد إن هذه الأنواع كانت :
بكتريا *E.coli* و*E.aerogenes* ,و*S.marcesens* . ووجد أن درجة الحرارة المثلى و الرقم الهيدروجيني الأمثل لتنمية *E. coli*، و *S.marcesens* هما 37م°، و 9 بينما لبكتريا *E.aerogenes* كانت 25 م° و 9 .
تم اعتماد تقنية طيف الأشعة تحت الحمراء (FTIR) لاختبار قدرة الأنواع البكتيرية المعزولة على تفكيك الكازولين ، لغرض استعمال هذه البكتريا في المعالجة الحيوية للترب الملوثة بالهيدروكاربونات.