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Using *Pseudomonas aeruginosa* to Treat Soil Polluted with Gasoline

Raad Abd Al-Hadi Neif*, Ithar Kamil Abbas Al-Mayaly

Department Of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Pseudomonas aeruginosa was isolated from three soils contaminated with gasoline which leak from electricity generators that used in different regions in Baghdad. The regions choices to collect the contaminated soils were Al-Dora, Al-Jadryia and Tunis quarter. The bacterial isolate is identified according to international biochemical methods and through the identification and selection the *P.aeruginosa* in order to use to degrade the gasoline also it was found that the optimum temperature and pH were 35°C and 9 to the *P.aeruginosa*, as well as using Fourier transmission infrared (FTIR) technique in order to test the ability of isolated bacteria to biodegrade the gasoline in order to use this bacteria in biodegradation for contaminated soils with gasoline. And results of using FTIR technique appended the degradation by *P.aeruginosa.* Results of (FTIR) technique referred to that the *P.aeruginosa* appeared an ability of degradation in the first day comparing with control , and some materials were appeared because of degradation such as Alcohol , where in third day the biodegradation by *P.aeruginosa* was very clear , also an increasing in peak area which refers to many compounds as sodium, Al-dehyd and alkanes . In fifth day, an increasing in biodegradation was observed in alkanes while in the eighth day - as in the tenth day -appeared an increasing in biodegradation.

Keywords: Biodegradation, *P. aeruginosa* , gasoline

استعمال بكتريا *Pseudomonas aeruginosa* لمعالجة التربة الملوثة بمادة الكازولين

رعد عبد الهادي نايف*، ايثار كامل عباس الميالي

قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

عزلت بكتريا *P.aeruginosa* من ثلاثة مواقع للترب الملوثة بالكازولين المستخدم في المولدات الكهربائية في مناطق مختلفة في بغداد و المناطق التي تم اختيارها لجمع الترب الملوثة (الدورة والجادرية وحي تونس) و العزلات البكتيرية شخصت وفق الطرائق البايوكيميائية المعتمدة عالميا و من خلال التشخيص تم اختيار عزلت الـ *P.aeruginosa* لكي تستخدم في تكسير الكازولين كذلك وجد ان درجة الحرارة المثلى و الرقم الهيدروجيني هي 9 و 35 °C بالاضافة الى استخدام تقنية طيف الاشعة تحت الحمراء (FTIR) لاختبار قدرة البكتريا المعزولة على تفكيك الكازولين لغرض اختبار قدرتها على تحليل الكازولين و استعمالها في المعالجة الحيوية للتربة الملوثة بالكازولين . واشارت نتائج تحاليل تقنية الـ (FTIR) بأن عزلة *P.aeruginosa* اظهرت قدرة للتكسير في اليوم الاول مقارنة مع Control وظهرت المواد نتيجة التفكك مثل الكحول و الفينولات و في اليوم الثالث كان التكسير الحيوي واضحا بواسطة *P.aeruginosa* و لوحظ زيادة في المناطق القمية و التي تشير الى مركبات عديدة مثل مركبات الصوديوم و الالديهايد و الالكانات كذلك في اليوم الخامس لوحظ زيادة في المعالجة الحيوية في الالكانات بينما في اليوم الثامن متفقاً مع اليوم العاشر حيث اظهرت زيادة في التكسير الحيوي .

*Email: raad.hadi22@yahoo.com

Introduction:

Gasoline is one of the pollutants which are harmful for ecosystems if it increased more than the acceptable level in soil [1]. In many contries gasoline has colloquial name derived from that of the chemical benzene-eig(German Benzen, Dutch benzene, Italian benzene, polish benzyra, Chilebencina, thaibayusin, Greek venzin. Romanian benzina, Swedish benzin and Arabic binzin).

Several methods can be employed to remove gasoline from soil vopour extraction pump and treat and dioremedation. There is no general rule to choose the best treatment for contaminated sites [2].

Each case must be analyzed individually evaluating its particularities physical treatments separate soil and contaminants without destroying or chemically modifying them but have many limitations such as high cost [3].

Biological processes on the other hand is a promising technology for clean-up these contaminants mainly due to its simplicity and cost-effectivenees as compared to the other alternatives [4]. Bioremediation can be considered as a technology to treat contaminated sites by the use of biological agents able to modify or decompose target pollutants, It uses the action and addition of indigenous microorganism. The maximum benefit of this processes is the mineralization of the pollutant leading ultimately to the formation of CO_2, H_2O and biomass [5].

Material and Methods:

Soils contaminated with gasoline:

Bacteria are isolated from polluted soils with gasoline obtained from three polluted soils with generators gasoline which were taken from Al-Dora, Al-Jadryia and Tunis quarter in Baghdad.

Isolation of strain from polluted soils:

Polluted soil (10 gm) was humidified with 25 ml of Nutrient agar medium. The bacteria was separated from the soil particles by gentle shaking of 1 g soil dry weight with 10 ml of sterile water for 30 minutes after sedimentation [6]. The supernatant suspension was used to prepare appropriate dilutions for $1\times10^{-4}$with sterile water aliquots of 0.2 ml were spread on the nutrient agar medium [7]. The plates were incubated at 25°C for 5 days, the bacteria was allowed to spread until purification [8]. The morphology of the bacterial strain was determined by Gram staining and they were then streaked in petri dishes. The isolated bacterial cultures were identified according to biochemical tests and API system [9].

The optimum conditions of cultivations:

In order to know the optimum conditions for the isolated bacteria, it was cultivated with different temperature which including in 30°C, 35°C, 40°C and 45°C and pH 5, 7 and 9 (Table 1, 2, 3, 4 ,5 and 6).

Casoline degradation in liquid culture:

In order 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 50ml 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 8 days [10] and the ability of the isolated bacteria to biodegrade gasoline was determinate by using flourier transmission infrared (FTIR).

Emulsification index test (24%)

In each flask, 100 ml of modified mineral salt medium containing 2000 mg /1 gasoline inoculated with overnight (18h) of *P. aeruginosa* (O.D=0.5) After 8 days of incubation at 30°C, 5ml of filtered medium was transferred to 10ml test and mixed by vortex at high speed for 2 min left to stand for 24 hours. Emulsification index given as percentage of height of emulsified layer (cm) divided by total height of the liquid column (cm) multiplying by 100 [11].

Measurement surface tension

Modified mineral salt medium 100 ml containing 2 ml gasoline (pH. 7.2) sterilized by autoclaving and inoculated with overnight (18h) bacterial culture after 8 days of incubation in a shaker incubation at 30°C and 150 rpm. The pellets were removed by centrifugation at 1000 for 10 minutes. Culture free cells were used for surface tension measurement using asigma 703 D DU-Nouy-Ring tension meter. All measurement were carried out at room temperature 27°C [12]

Results and Discussion:

Using the biochemical tests to identify of isolates and explained in Table-1. The most of isolates species in polluted soils that taken from Al-Dora, Al-Jadryia and Tunis quarters *Staphylococcus aures*, *E.coli* and selective *P.aeruginosa* in order to be used in gasoline degradation [13]. The tested soil samples were filled with gasoline fuel which used in generators of electricity so the saturation with gasoline may limit the kind of microbial content [14]. The difference in microbial population is a reflection of many factors such as nutrients and oxygen levels and minerals [15].

The factors such as temperature, pH and others are depended to know the optimum conditions of cultivation [16].The results indicated that the 35°C and pH 9 was the optimum conditions for *P. aeruginosa*, Table 2,3,4,5, and 6. FTIR spectra refer to the contribution of bacteria to biodegradation.
The gasoline used as control Figure-1 the control sample showed degradation bands places of H-Banded Al-cohol, phenol at 3500 cm^{-1} (3200-3600) and amide C=O stretching at 2000 cm^{-1} (16300-1680) of alkanes and degraded band of aromatic compounds. Figure-2 illustrates that the degraded bands in the range of wave number 3500 cm^{-1} (3355.41) which refer to the O-H stretching for oxime C=N and alkanes. While in three day increasing in the peaks area at region of 3000 cm^{-1}(2923.55) shows CH and OH and decrease in the range of wave number (1758, 1459 and 1376) which refer to C=O, NO_2 compounds and alkanes Figure-3. In fifth day observed more increasing in biodegradation refers to C-H also in eighth day Figures-4 and-5. In tenth day refers to more increasing in amine which refers to N-H with appeared other compounds as Aldehyde, C≡N, C≡O and bending O-H and this is an indicating to ability of the *P.aeruginosa* for the biodegradation of aromatic compound Figure-6.
Results of FTIR showed these changes of gasoline groups give a potential evidence that *P.aeruginosa* has good effect on biodegradation of gasoline [17].

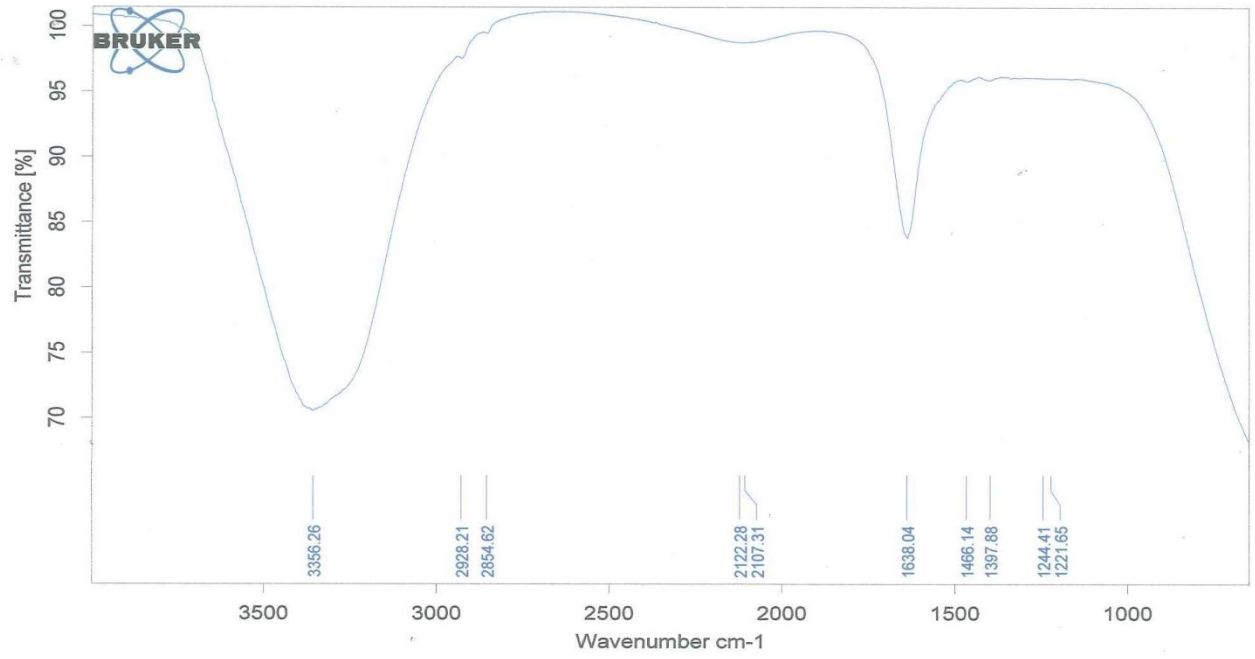

Figure 1- FTIR Spectra gasoline for Control

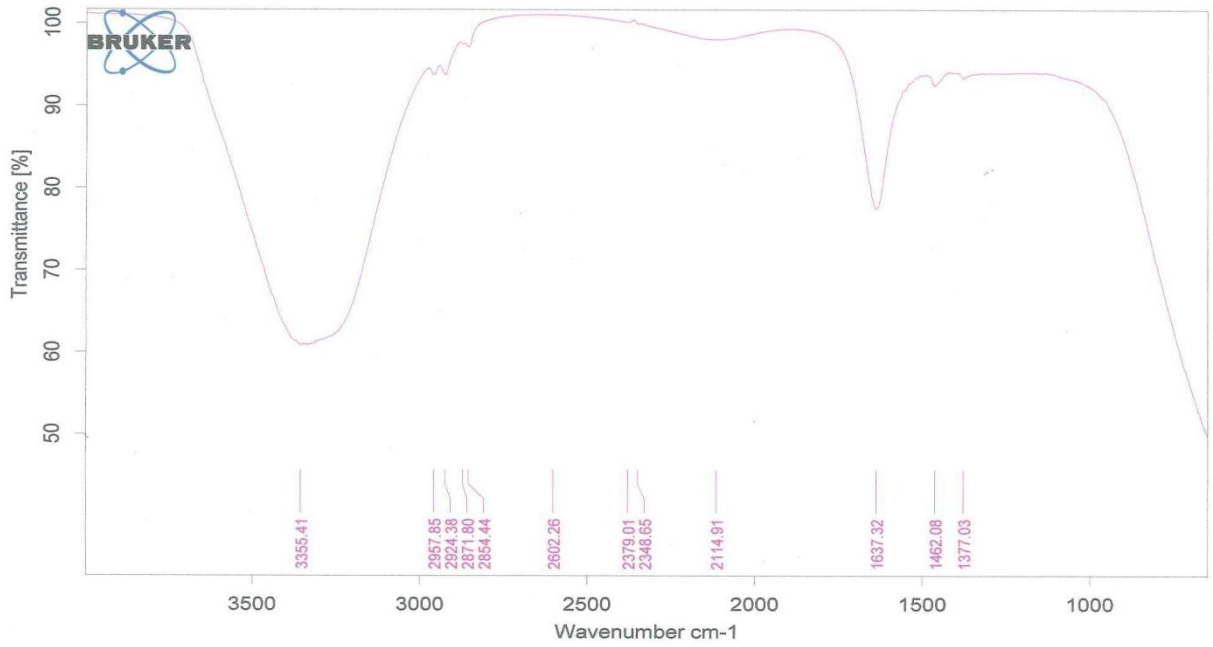

Figure 2- FTIR Spectra gasoline for after bio treatment for 1 day with *P.aeruginosa*

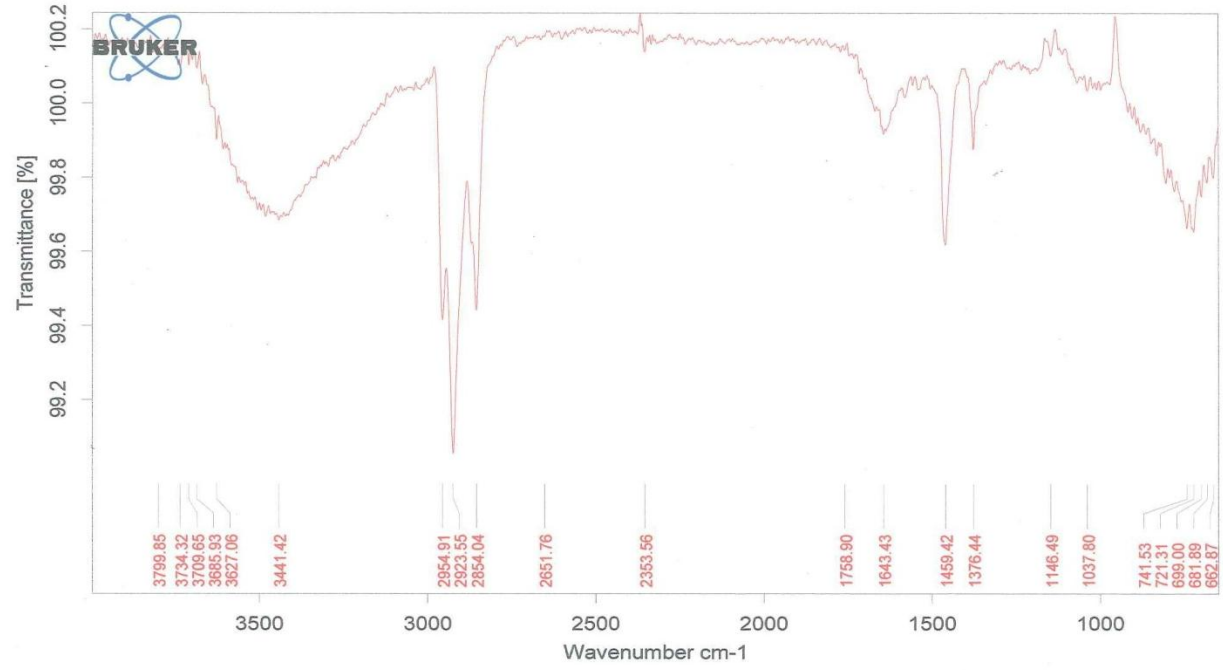

Figure 3- FTIR Spectra gasoline for after bio treatment for 3 days with *P.aeruginosa*

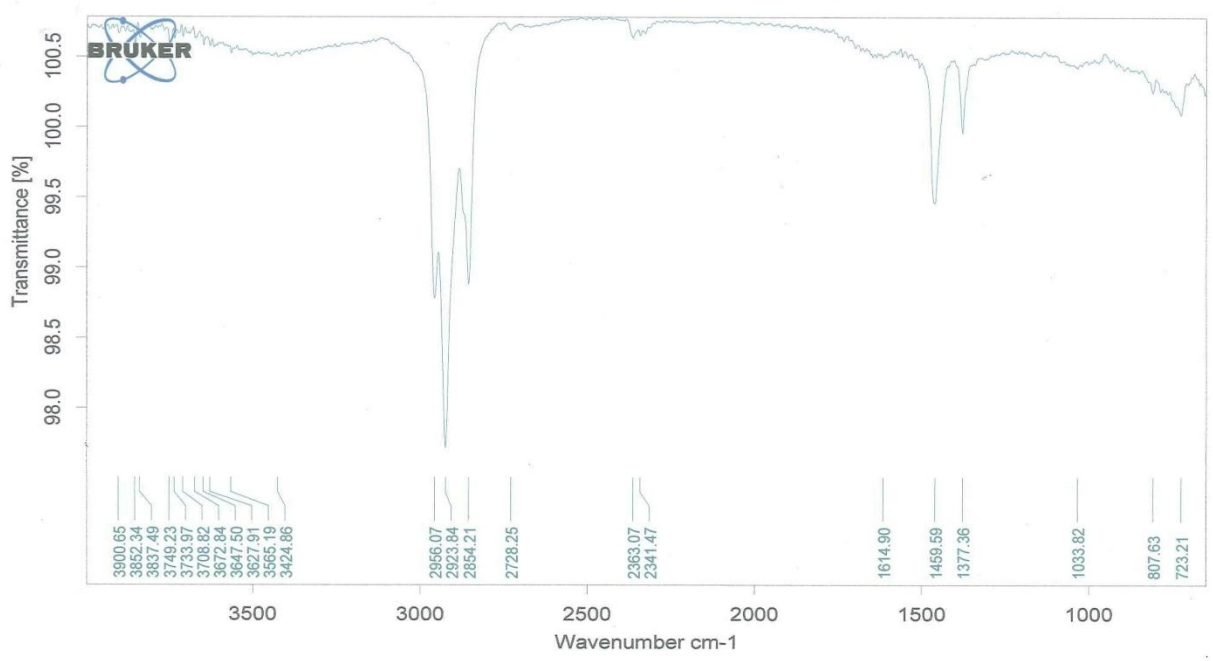

Figure 4- FTIR Spectra gasoline for after bio treatment for 5 days with *P.aeruginosa*

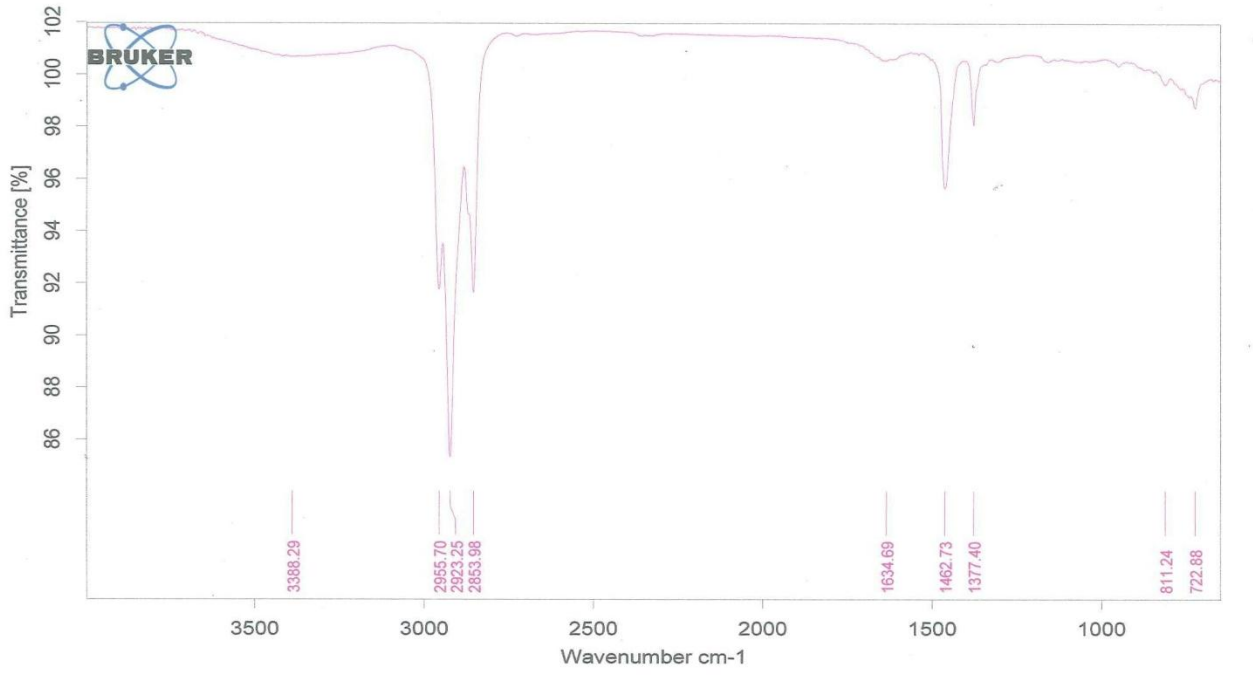

Figure 5- FTIR Spectra gasoline for after bio treatment for 8 days with *P.aeruginosa*

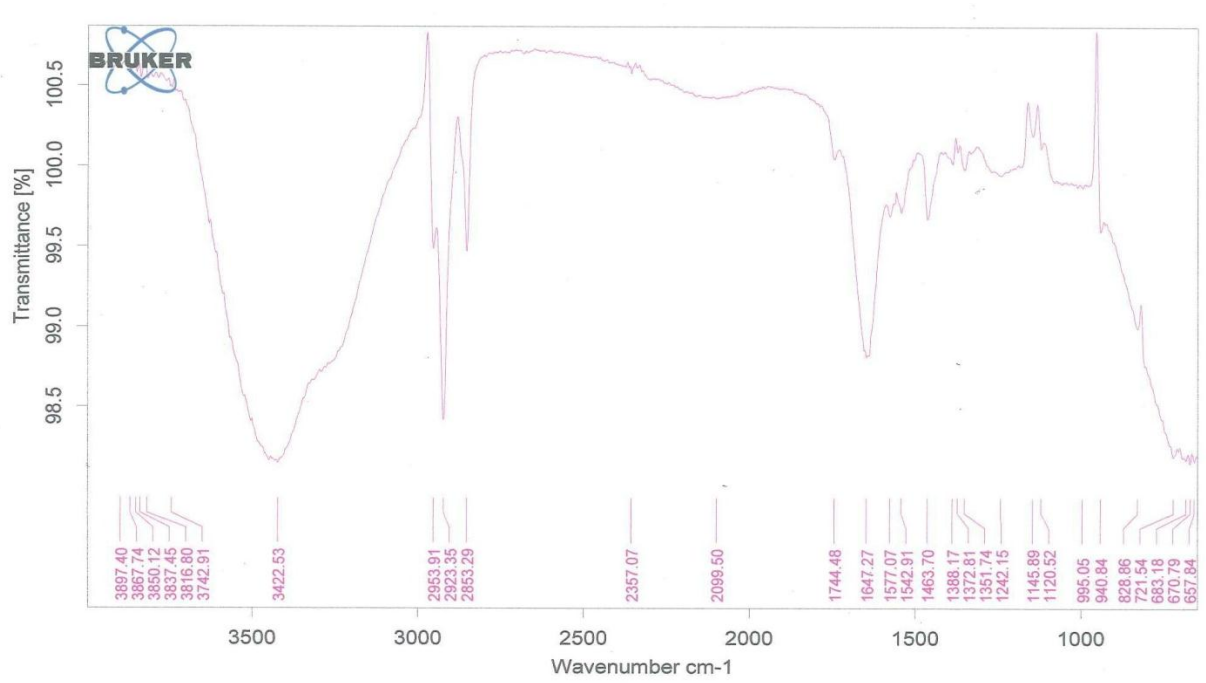

Figure 6- FTIR Spectra gasoline for after bio treatment for 10 days with *P.aeruginosa*

Emulsification activity

Emulsification was taken as an indicator for gasoline biodegradation capacity of the bacteria . The biosurfactants produced by the bacteria would emulsify the hydrophobic phase the results of emulsions activity test of *P.aeruginosa* 60% for gasoline emulsification . The bacteria have produced biosurfactant to increase the bioavailability of gasoline resulting in enhanced growth and biodegradation of Contaminants by gasoline – degraders [18] Figure-7. The result of E24 test for *P.aeruginosa* are closely to results of previous Study [19].

The results indicated that these bacteria showed emulsified producer biosurfactant production the high emulsification activity due to the production of extracellular water soluble biosurrfectent by the isolate during the incubation that is utilizing the crude oil as sole carbon source and energy [20].

Surface tension

The surface tension of culture free cells of *P.aeruginosa* was measured after 8 days of inenbation . The results showed that the biocmulsification produced by bacterial isolates lowesr the Surface tension of the Media to 32.26 MN/M compared to the control 44.6 MN/M. Similar result was found by [21].

Which their study was about biosurfactants production by *P.aeruginosa* FR using Palm Oil , they found this bacteria was able to reduce surface tension of the tested media Figure-8.

The biosurfactant which produced by the isolates caused a reduction in surface tension *P. aeruginosa* showed a good result of surface tension and that is agree with the result found biosurfact ant produced by *P. aeruginosa*. Critical Micelle concentration (CMC) very important in surface tension reduction because effect of the increase the surface tension as well as reduction was also effected by rotation speed whereas mentioned that rotation Velocity affected in surface tension reduction when they applied different rotation speed and the lowest surface tension measurement obtained was with 100-150 rpm [22].

These results were supported by the results that found greater bio surfactants production as the sole carbon source at 100 rpm, which in turn influenced the surface tension reduction [23].

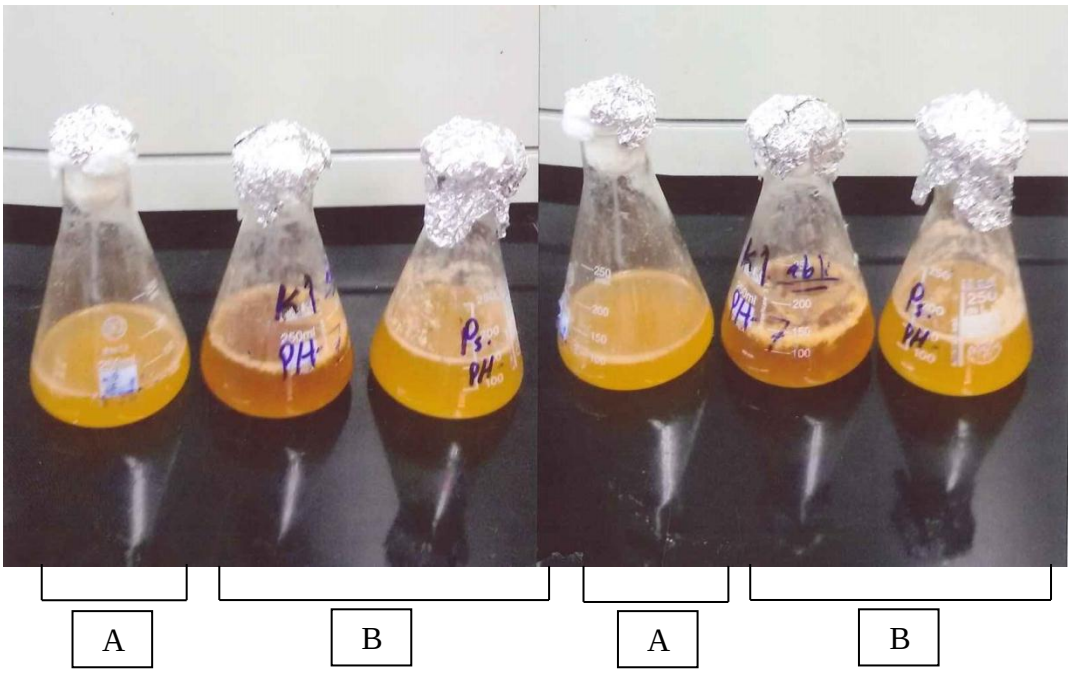

Figure 7- Bacteria growth after 8 days

A = Control

B = growth of bacteria (*P.seudomouas*). In pH 5 and 7.

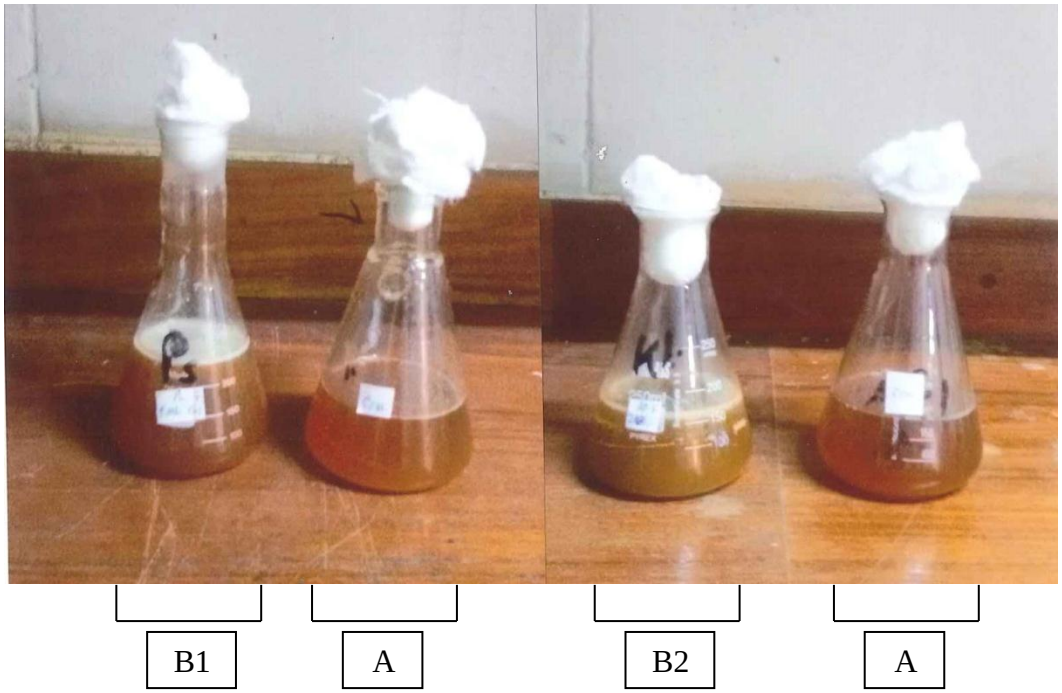

Figure 8- Bacteria growth after 30 days

A = Control , B = growth of bacteria after 7 days.

B1 = *P.aernginosa*

Table 1- Effect of pH (5 , 7 & 9) in Optical density rate (nm) for *P. aeurginosa* in 30 ^{0}C

pH	Days								LSD value
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	
5	0.051	0.156	0.056	0.147	0.702	0.554	0.385	0.565	0.226
7	0.732	1.121	1.235	1.051	1.279	1.193	0.733	0.162	0.382
9	0.965	0.963	0.878	0.830	0.652	0.415	0.743	0.814	0.294
LSD value	0.315	0.475	0.362	0.427	0.366	0.372	0.363	0.351	---

Table 2- Effect of pH (5 , 7 & 9) in Optical density rate (nm) for *P. aeurginosa* in 35 ^{0}C

pH	Days								LSD value
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	
5	0.090	0.904	1.158	1.077	1.406	1.285	1.480	1.505	0.519
7	1.871	1.648	2.049	1.776	1.305	1.458	1.556	1.606	0.362
9	0.328	0.238	0.323	0.313	0.318	0.303	0.083	0.219	0.175
LSD value	0.509	0.462	0.471	0.425	0.397	0.449	0.382	0.559	---

Table 3- Effect of pH (5 , 7 & 9) in Optical density rate (nm) for *P. aeurginosa* in 40 ^{0}C

pH	Days								LSD value
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	
5	0.254	0.096	0.211	0.152	0.076	0.100	0.143	0.121	0.107
7	0.067	0.141	0.072	0.044	0.024	0.037	0.053	0.077	0.046
9	0.428	0.741	0.494	0.667	0.756	0.648	0.441	0.568	0.215
LSD value	0.296	0.374	0.226	0.349	0.382	0.371	0.266	0.309	---

Table 4- Effect of pH (5 , 7 & 9) in Optical density rate (nm) for *P. aeurginosa* in 45 ^{0}C.

pH	Days								LSD value
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	
5	0.123	0.167	0.350	0.195	0.328	0.389	0.424	0.446	0.216
7	0.300	0.386	0.362	0.378	0.369	0.136	0.363	0.332	0.152
9	0.147	0.298	0.038	0.105	0.016	0.045	0.015	0.164	0.083
LSD value	0.133	0.162	0.208	0.142B	0.172	0.166	0.216	0.194	---

Table 5- Effect of Temp. (30,35,40 & 45) in Optical density rate (nm) for *P.aeurginosa* in pH =7

Temp. ^{0}C	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
30	0.732	1.121	1.235	1.051	1.279	1.193	0.733	0.162
35	1.871	1.648	2.049	1.776	1.305	1.458	1.556	1.606
40	0.067	0.141	0.072	0.044	0.024	0.037	0.053	0.077
45	0.300	0.386	0.362	0.378	0.369	0.136	0.363	0.332
LSD value	0.425	0.393	0.447	0.389	0.357	0.366	0.405	0.372

Table 6- Effect of Temp. (30,35,40 & 45) in Optical density rate (nm) for *P.aeurginosa* in pH =9.

Temp. ^{0}C	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
30	0.965	0.963	0.878	0.830	0.652	0.415	0.743	0.814
35	0.328	0.238	0.323	0.313	0.318	0.303	0.083	0.219
40	0.428	0.741	0.494	0.667	0.756	0.648	0.441	0.568
45	0.147	0.298	0.038	0.105	0.016	0.045	0.015	0.164
LSD value	0.267	0.356	0.275	0.261	0.246	0.274	0.214	0.268

References:

1. Akpor, O.B., Lgbinosa, O.E. and Lgbinosa, O.O. **2007**. Studies on the effect of petroleum hydrocarbon on the microbial and physic-chemicals characteristics of soil. *African journal of bio technology*. 6(16):1939-1943.
2. Alexander, M. *Biodegradation and Bioremediation*. **1994**. Academic Press. p: 302.
3. Bodour, A. A., Gerero – Barajas, C. and Maier, M. **2004**. Structure and characterization of flavor lipids , a novel class of Bio surfactants produced by flavor lipid sp. Strain MTNII, *App and Env. Microbial*, 10 (6), pp: 1114 – 1120.
4. Cameotra, S. S. and Makkar, R. S. **2010**. Bio surfactant – enhanced bioremediation of hydrophobic pollutants, *Pure Appi*. Chem., 82(1): 97 – 116, USA.
5. Cirelli, A.F., Ojeda, C., Castro, M.J.L. and salgot, M. **2008**. Surfactauts in sludge. Ameuded agriculture soils: A review. *Environ. Chem. Lett.*, 6:135-148.
6. Englert, C.J., Kenzie, E.J. and Dragun, J. **1993.** *Bioremediation of petroleum products in soil. In principles and practices* for petroleum contaminated soils. Ed .E.J. Calabreseund P.T. Kostechi. Lewis Publishers, Chelsea, MIPP. 112-115, 121.
7. Haris, J.O. **1962**. Microbiological studies of oil and gas pipelines. *Techndogy bulletin*. pp: 135.
8. Jacobucci, D.F.C., Vasconcelos, C.K., Matsunra, A.B., Falconi, F. A. and Durant, L.R. **2001.** Degradation of diesel oil by bio surfactant. Producing Bacterial strains contaminated soil sediment and water, *Environ. Chem.* 8(1), 31-34.
9. Kaczorek, E., Moszynska, S. and Olszanowski, A. **2011.** Modification of cell surface properties of *Psendomouas alcaligenes* S_{22} daring hydrocarbon biodegradation, *Biodegradation*, 22, pp: 359-366.
10. Long, G.M. **1993**. Clean up Hydrocarbon contamination effectively, *Chem. Eng. Prog*., 8:58-67.
11. Lopez, M.E., Rodrignez, M.T., Rios. E.L., Esparza, F.G., Gharez., G., Rodriguez, V. and Barrera. J.C. **2007**. Fungi and bacteria isolated from two highly polluted soils for hydrocarbon degradation. *Acta Ghim. Slor. (technical paper)*, 54:201-209.
12. Minai, T., Her Fatmanesh, D., Azari – Dehlkordi, A. and Minnoi, S. **2006.** Effect of salinity on biodegradation of aliphatic fractions of crude oil in soil – *Biological Sciences*. 9 (8):1531 – 1535.
13. Natioual Reaserarch council (NRC). **2003.** *Oil in the Sea III: Inputs, Fates, and Effects*. National Academies press, Washington, D.C.
14. Oliveira, A. J, Falconi, R., Saweki,K. and soza. **2006.** Bio surfactants production by *pseudomonas aeruginosa* FR using Palm oil, Appl. *Biochemistry and Biotechnology*, 131:727 – 737.
15. Pacwa – Plciniczak, M., Plaza, G. A., Seget , S. P. and Gameotra S. S. 2011. Environmental applications of bio surfactants: Recent Advances. *Int. J. Mol. Sci*., 12:633 – 654.
16. Plaza, G. A., Lukasik, K., Wypych, J., Nate cz- Jaweki, G., Berry, C. and Brigmon, R. L**. 2008.** Biodegradation of crude oil and distillation products by bio surfactant – producing bacteria. *Pol J Environ Stud*. 17 (1): 87 – 94.
17. Prince, R. C.**1996.** Petroleum spill bioremediation in marine environments. Critical reviews microbiology, 19:217 – 242.
18. Rezende, R. P., Maciel B. M., Dias, J. T. and Souza, F. O. **2012**. Microbial outlook for the bioremediation of crude oil contaminated environments, Introduction to Enhanced oil Recovery (EOR) processes and Bioremediation of oil contaminated sites, Dr. Laura Romero Zeron (Ed.), In Tech, pp: 245 – 260.
19. Schneider, D.R.**1990.** Bioremedation de reiduos organicos" Galgany go conference, Haztech caudq (Western).
20. Thenmozhi, R., Nagasathya, A. and Thajuddin, N. **2011.** Studies on biodegradation of used engine oil by consortium cultures. *Advances in environmental biology*. 5(6):1051-1057.
21. Tuleva, B.J., Inanov, G.R. and Chi istora, N.E. **2002.** Biosurfactant production by anew *Pseudomonas putida* strair , *Z Nzturforsch* 57 C. 356-360.
22. Van Hamme , j. D., Singh, A. and Ward , O. P. **2003** . Recent Advances in petroleum Microbiology . *Microbiol. Mol. Rev*. 67: 503 – 549.
23. Yasin, A. R. **2013.** Biodegradation of polluted water with crude oil discharged from South Baghdad power plant. M.Sc. Thesis College of Science for University of Baghdad, Baghdad, Iraq.