ISSN: 0067-2904
GIF: 0.851

Biodegradation of Aromatic and Alkanes Compounds in Contamination Soils with Gasoline by *Klebsiella pneumoniae*

Raad Abd Al-Hadi Neif*, Ithar Kamil Abbas Al-Mayaly
College of Science, University of Baghdad, Baghdad, Iraq.

Abstract

This study investigated the potential of bacterial culture in bioremediation of gasoline pollutant soils. *Klebsiella pneumonia*e has shown a tremendous ability in bioremediation of gasoline. *K.pneumoniae* was isolated from three electrical generator pollutant soils with gasoline in different regions from Baghdad (Abu-Graib, Al-Khadra quarter and Al-Seleikh region. Bacteria was isolated and identified according to biochemical tests, with optimum temperature at 35°C and pH=5.

FTIR spectrum was tested the ability of the *K.pneumoniae* to biodegrade the gasoline according to the peak areas, which appeared and referred to degrade amino compounds at wave number 3000 cm^{-1} (2955.23, 2923.47) which refer to the C-H with amines compounds and decreasing at wave number 1500cm^{-1}1458.77 and 1377.26 also appeared more degraded at regions of 3000 cm-1 (2955.31, 2922.97 and 2853.79) which represent alkanes C-H and amine groups.

Keywords: Biodegradation, *K .pneumoniae*.

التحلل الحيوي للمركبات الأروماتية والألكانات في التربة الملوثة بالكازولين بوساطة بكتريا *Klebsiella pneumoniae*

رعد عبد الهادي نايف*، إيثار كامل عباس الميالي
كلية العلوم، جامعة بغداد، بغداد، العراق.

الخلاصة

تمت دراسة قدرة المزارع البكتيرية في المعالجة الحيوية للتربة الملوثة بمركبات الكازولين وقد اظهرت العزلة *K.pneumoniae* قدرة عالية في المعالجة الحيوية . عزلت *K.pneumoniae* من ثلاث ترب ملوثة بالكازولين بالقرب من المولدات الكهربائية ومن مختلف المناطق في بغداد (ابو غريب، حي الخضراء، الصليخ). شخصت العزلة البكتيرية وفقاً للاختبارات البايوكيميائية. وقد وجد ان الحرارة المثلى والرقم الهيدروجيني للبكتيريا المعزولة 35°C و الـ pH=5. تقنية طيف الاشعة تحت الحمراء (FTIR) اختبرت قدرة *K.pneumoniae* على تكسير الكازولين وفقاً لمناطق القمة التي أظهرت وأشارت إلى تكسير الكازولين عند الموجة 3000 cm−1والتي تشير إلى C – H ونقصان عند الموجة 1500 cm−1 في حين ازداد التكسير الحيوي عند الموجة 3000 cm−1 والتي تمثل الالكانات C – H ومجاميع الامينات.

*Email: raad.hadi22@yahoo.com

Introduction

The gasoline is a liquid petroleum in its unrefined state contains a lot of hydrocarbons , wide scale production transport , and disposal of petroleum globally has made it a major contaminant in both prevalence and quantity in the environment .

Bioremediation is being used or proposed as a treatment option at many aromatic compounds contaminated sites [1, 2]. The effectiveness of bioremediation is often a function of the microbial population and how it can be enriched and maintained in an environment. Microorganisms with ability to degrade gasoline are ubiquitously distributed in soil environments [3].

Contaminated land sites are health hazards for human being and thus are unsuitable for housing or agriculture. The down ward migration of pollutants from the soil into the ground water causing a problem in developing countries where ground water was used for drinking without any prior treatment [4]. Microbial degradation appears to be the most environmentally friendly method for removing of gasoline pollutant since other methods such as surfactant washing and incineration lead to liberated more toxic compounds to the environment gasoline degrading microorganisms are widely distributed in soil environments [5].

Materials and Methods

- **Collection of pollutant soils**

  Native gasoline bacteria were isolated from pollutant soils with gasoline obtained from three polluted soils which were taken from (Abo-Geab, Al-Khadra quarter and Al-Seleikh) in Baghdad.
- **Isolation and identification of isolates :**

  10 gm of pollutant soil was humidified with 250 ml of mineral medium (MMSM). isolates were separated from the soil particles by shaking 1gm dry weight with 10ml of sterile water for 30 minutes then prepare dilutions from 1×10^{-1} to $1\times10^{-5}$with sterile water. Aliquots of 0.2 ml were spreaded , nutrient agar medium and incubated at 25 for 7 days [6].

  The morphology of the bacterial strains was determined by streaking pure colony on nutrient agar plate to identified according to growth of bacterial isolates and identify by biochemical tests and API system [7].
- **The determination of optimum conditions :**

  Experments were done determination the optimum conditions for the best temperature degree (30 , 35 , 40 and 45° C and pH (5,7 and 9) to degraded gasoline .
- **Mesurment gasoline degradation :**

  Gasoline was collected and preparing for biodegradation by *k.pneumoniae* after treatment 1gm of pollutant soil with (1 – 3) (acetone – hexane) and evaporation the solvent at 25 ° C , then added bacterial suspension (1ml) sterile media (with gasoline extraction) , and incubated at (35° C) for 8 days , with 150 rpm [8].

  The efficiency of the isolated to biodegrade gasoline was determinate by using (Fluori Transmission Infrared) spectram technique [9].

Results and Discussion

Results of study refers to the biochemical tests which explained the most predominant bacteria in polluted soils that taken from Abo-Grab, Al-Seleik and Al-Khadra quarter was *K.pneumoniae* [10].

(1) Lactose fermentation test

MacConkey agar plates were inoculated with bacteria and incubated at 37°C for 24 hour's. gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. The appearance of red or pink colonies is the indication of a positive result. [11].

(2) Oxidase test

One full loop of the bacterial growth was transported by sterile wooden stick to a filter paper saturated with oxidase reagent. Appearance of blue color of bacterial growth after 10-60 seconds considered as a positive result [12].

(3) Cetrimide agar

This medium was used as selective media for isolation of *P.aeruginosa* sp. Plates were streaking by bacterial isolates inoculated at 37°C for 24 hours [13]

(4) Catalase test

One full loop of bacterial colonies was transported to the microscope slide 1-3 drops of 3% hydrogen peroxide were added directly to the colonies. A positive reaction is indicated by bubbling production [14].

(5) Nitrate reduction test

Nitrate broth tubes inoculated by bacteria were incubated at 37°C for 24 hours, few drops of salfinal amide reagent and N(1-naphthyl)- ethylene diaminedihyrochloride were added to each tube. Appearance of pink- red color is appositive result. No color change, indicates as negative [15].

(6) Indole test

Tryptone broth tubes inoculated with by bacteria and incubated at 37°C for 24 hours. The addition of the appropriate kovac's reagent produced a cherry-red ring floating above the culture medium if positive test the negative is indicated by the lack of color change in the top of the tube after the addition of kovac's reagent [16].

(7) Methyl red.

Vogesproskaner reaction sterile tubes containing MR-vpmedium were inoculated with 0.1ml of 18 h. culture growth and incubated at 28°C for 48h.

(8) Motility test

Semisolid medium was incubated by stabbing and incubated at 37°C for 24 hours: spreading turbidity from and stab-line or turbidity throughout the medium was considered as positive result.

(9) Urease test

The surface of urea agar slant was streaked with a loop ful of a pure culture bacteria and incubated at 37°C for 24 hr. production of intense pink-red color on the slant which may penetrate into the butt was considered a positive.

Data showed that the optimum temperature and pH for growing and degrading gasoline by *K.pneumoniae* were 35℃ and pH 5 table-1, -2, -3 and -4 by measuring oD at (540) .

The results of (FTIR) explain the effect of *K.pneumoniae* to degrade the structures of gasoline as following:

Figure-1 appeared a significant increasing of the bands which observation in the range of wave number 3000 cm^{-1} (29, 55, 23, 2923.47) refers to amino C-H compounds and stretching with decreasing at ware number 1500 cm^{-1} 1458.77 and 1377.26 . Also more degraded at region of 3000 cm^{-1}(2955.31, 2922.97 and 2853.79) which represent alkanes C-H with amine groups and decreasing in region 1462.06 and 1377.24 and more decreasing in region 722.86 which refers to the benzene ring Figure-2 .

Figure-3 appear degraded C-H and H-Bonded alcohol phenol and decreasing in region (1377.32, 1542.40 and 1635.44). Which refers to C=N aromatic C-C and bending O-H as well as benzene ring also.

Figure-4 refers to more increasing in amine which refers to N-H with other compounds as aldehyde C≡N, C≡O and bending O-H and this is an indicating to ability of *K. pneumonia* for biodegradation of aromatic compounds in gasoline [17].

The biodegradation potential of *K.pneumoniae* was detected in liquid medium containing 1% gasoline for 7 to 14 days of incubation compared with control, IR analysis of gasoline's structures was done [18].

The obtained results showed that the isolate has the ability to modify the compounds of gasoline a degrade decreasing in the area of peak on 7 day to 14 days compared with control. Figure -4 and -5.

Results of the study agree with Tuleva [19] which refers to *Pseudomonas putida* isolate to degraded in the region of 3000 cm^{-1} after 14 days of incubation at 30°C in liquid medium. While the results disagree with [20] using mixed of isolate in degrade the alphatic group at 2930 cm^{-1+} after 7 days at 32°C. as well as used FTIR analysis to determine the biodegradation rate of short and long alkanes by single culture of bacteria and the results indicated that 97% of substrate was degraded by mixed culture while only 22% by signal culture when inoculation in liquid culture after 20 days at 30°C .

Table 1-Effect of pH (5 , 7 & 9) in optical density rate at 540 (nm) for *K. pneumoniae* in 30 ^{o}C

pH	Days								LSD value
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	
5	0.308	0.240	0.178	0.142	0.073	0.184	0.2104	0.258	0.155
7	0.854	0.103	0.633	0.506	0.799	0.598	0.573	0.493	0.362
9	0.384	0.715	0.699	0.774	0.394	0.413	0.645	0.574	0.216
LSD value	0.209	0.215	0.348	0.274	0.355	0.282	0.238	0.219	---

Table 2-Effect of pH (5 , 7 & 9) in optical density rate at 540 (nm) for *K. pneumoniae* in 35 ^{o}C

pH	Days								LSD value
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	
5	0.756	1.648	2.049	1.776	1.305	1.458	1.556	1.606	0.418
7	0.144	0.137	0.173	0.224	0.200	0.220	0.189	0.180	0.155NS
9	0.146	0.097	0.030	0.189	0.175	0.115	0.200	0.214	0.113
LSD value	0.268	0.416	0.385	0.466	0.372	0.357	0.361	0.477	---

Table 3-Effect of pH (5 , 7 & 9) in Optical density rate at 540 (nm) for *K. pneumoniae* in 40 ^{o}C

pH	Days								LSD value
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	
5	0.102	0.153	0.107	0.098	0.065	0.0417	0.072	0.119	0.088NS
7	0.051	0.216	0.062	0.116	0.057	0.071	0.041	0.091	0.062
9	0.253	0.752	0.624	0.631	0.708	0.521	0.632	0.741	0.255
LSD value	0.144	0.289	0.258	0.352	0.262	0.215	0.288	0.246	---

Table 4-Effect of pH (5 , 7 & 9) in optical density rate at 540 (nm) for *K. pneumoniae* in 45 ^{o}C

pH	Days								LSD value
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	
5	0.132	0.475	0.196	0.203	0.267	0.211	0.211	0.234	0.207
7	0.116	0.429	0.057	0.038	0.011	0.192	0.041	0.153	0.255
9	0.493	0.035	0.681	0.541	0.291	0.261	0.317	0.233	0.304
LSD value	0.236	0.272	0.293	0.264	0.138	0.165 NS	0.172	0.167 NS	---

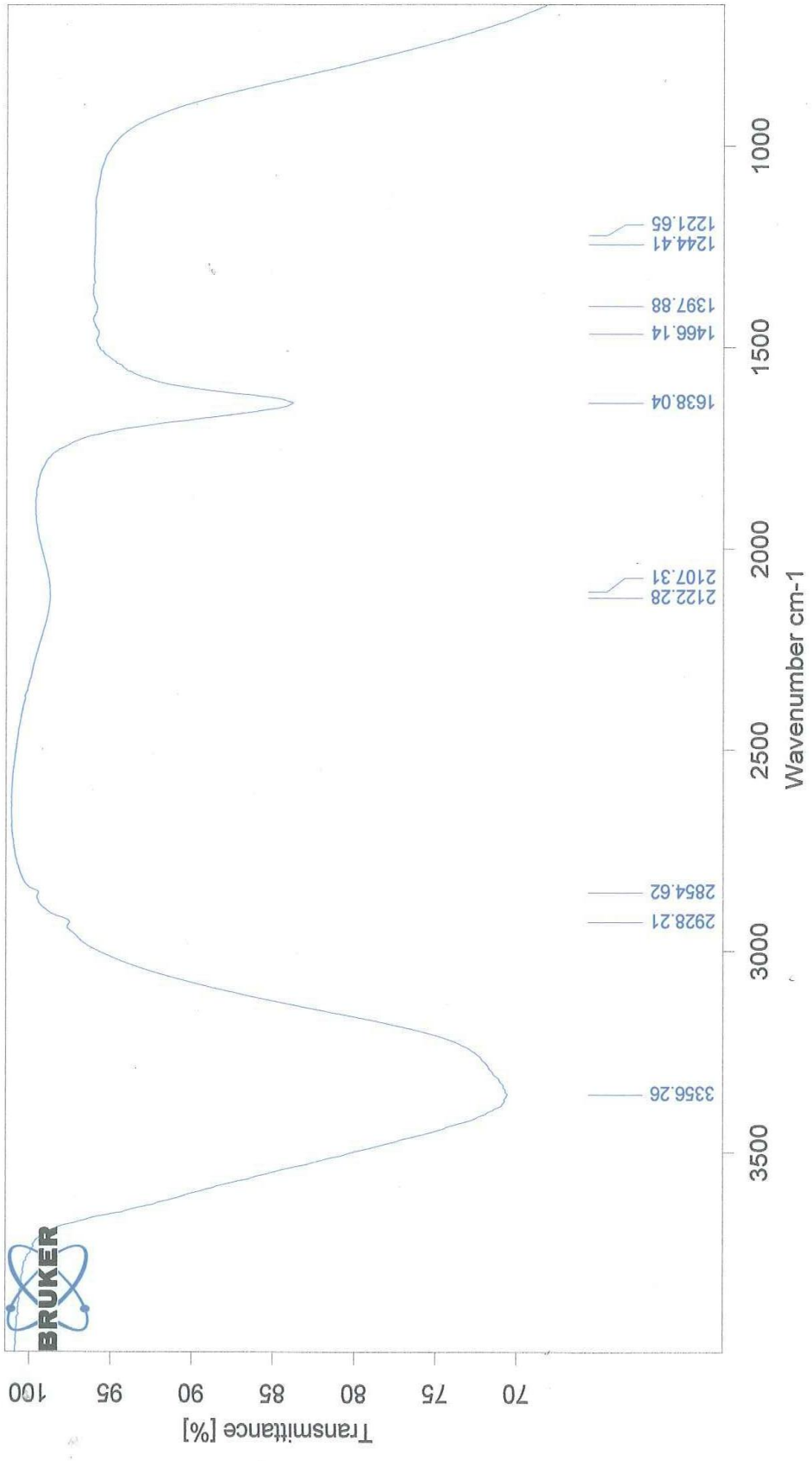

Figure C-FTIR Spectra gasoline for Control

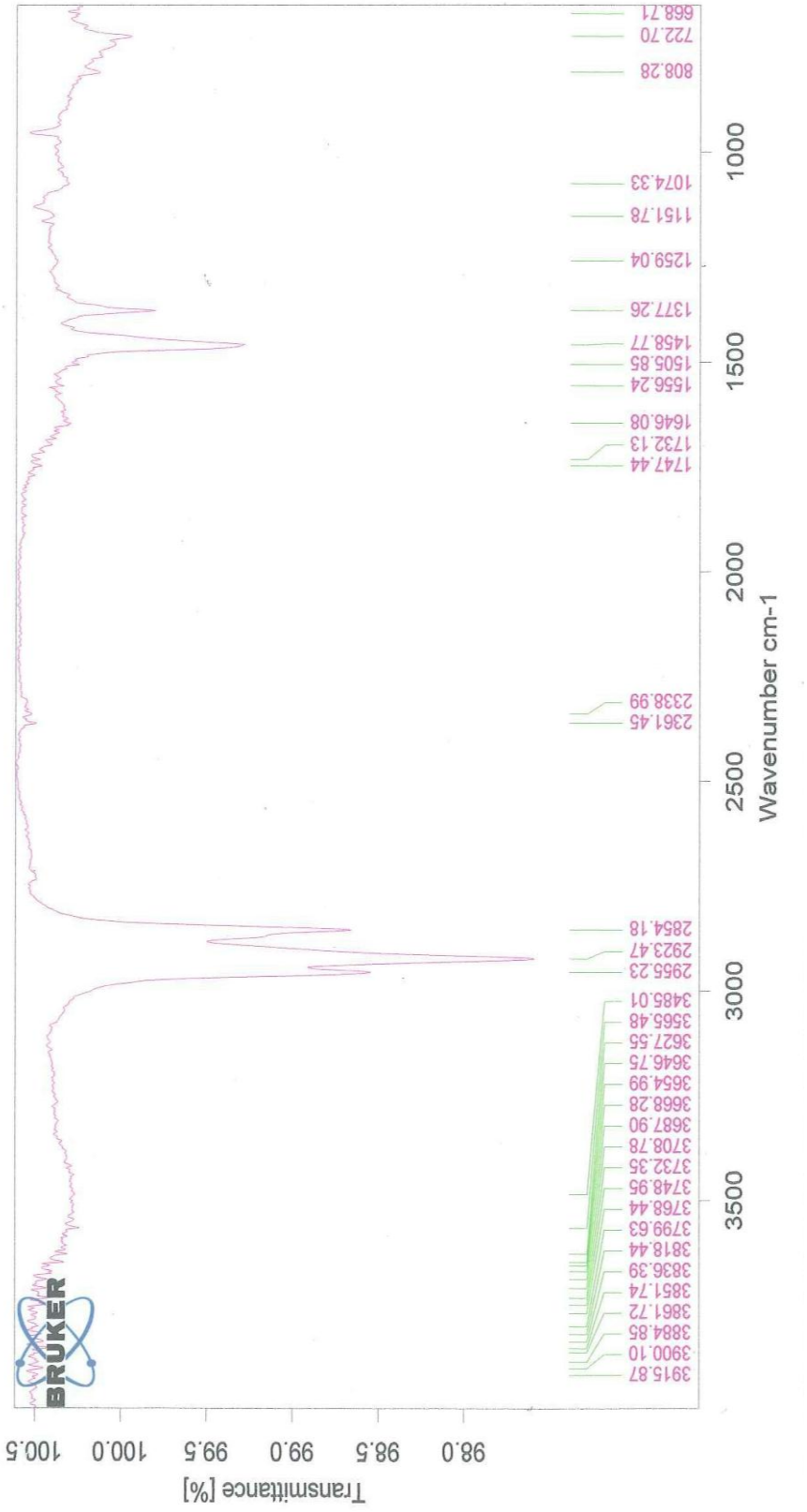

Figure 1- FTIR Spectra gasoline after bio treatment for 1 day with *K.pneumoniae*

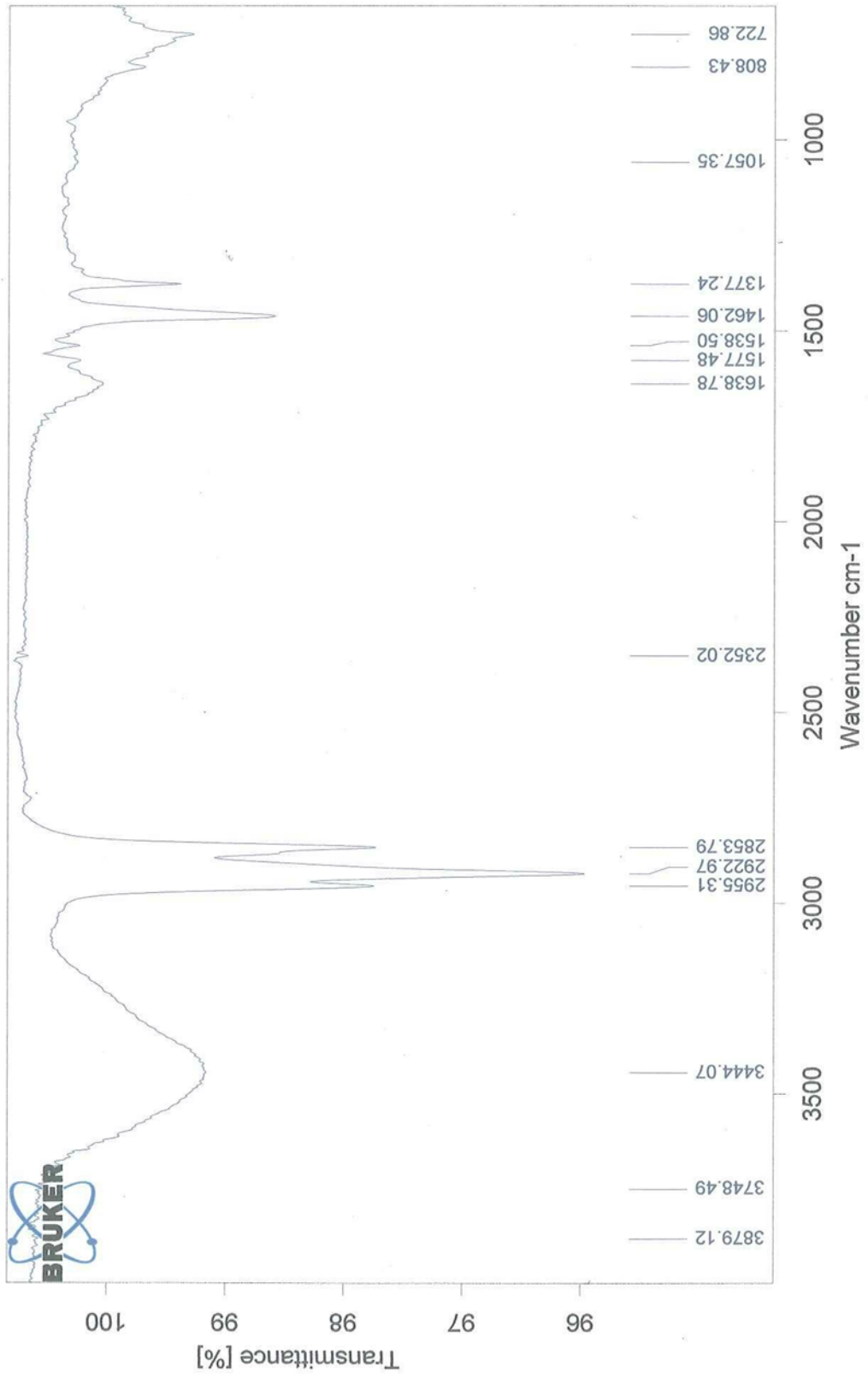

Figure 2 - FTIR Spectra gasoline after bio treatment for 3 days with *K.pneumoniae*

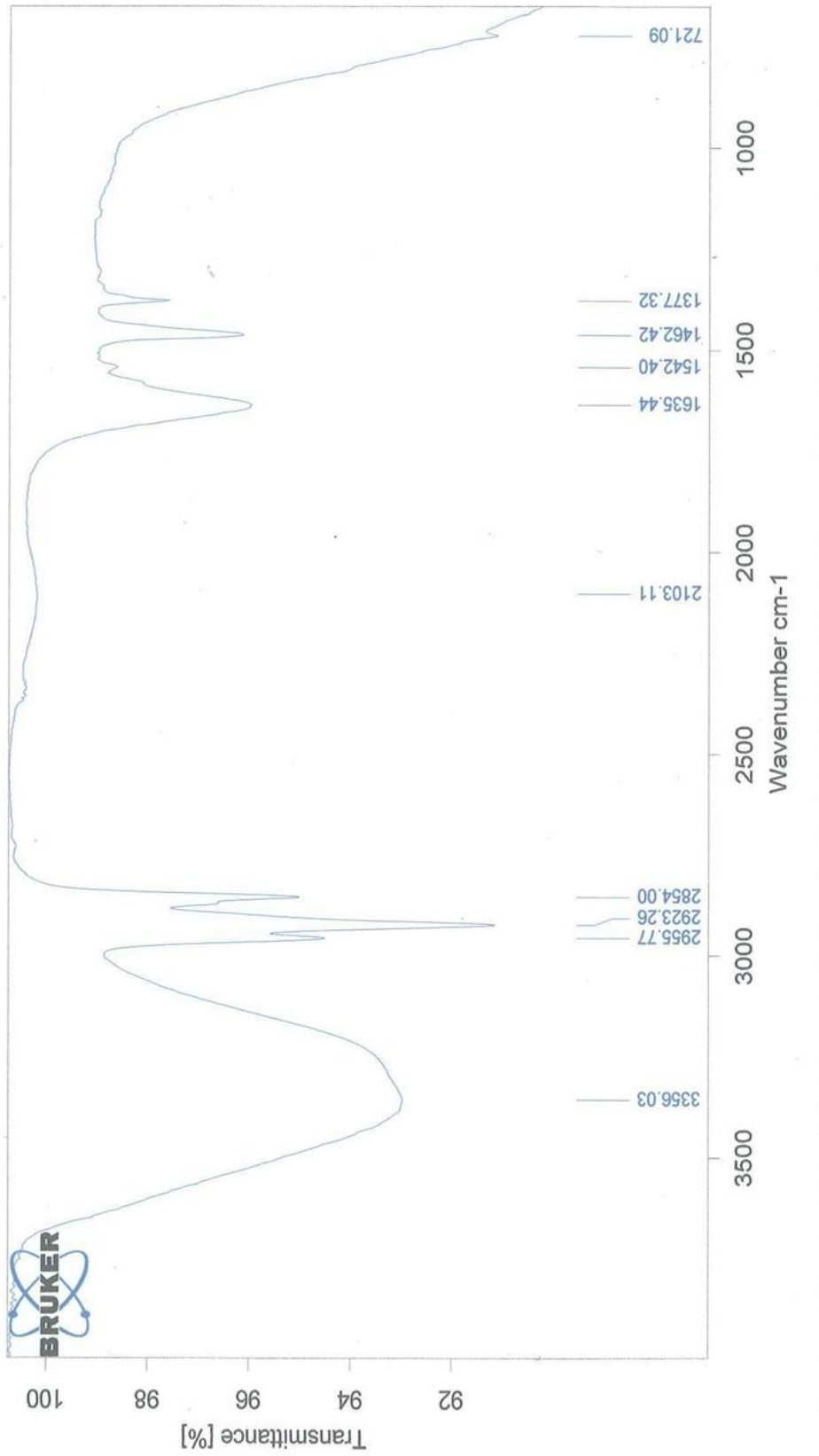

Figure 3- FTIR Spectra gasoline after bio treatment for 5 days with *K.pneumoniae*

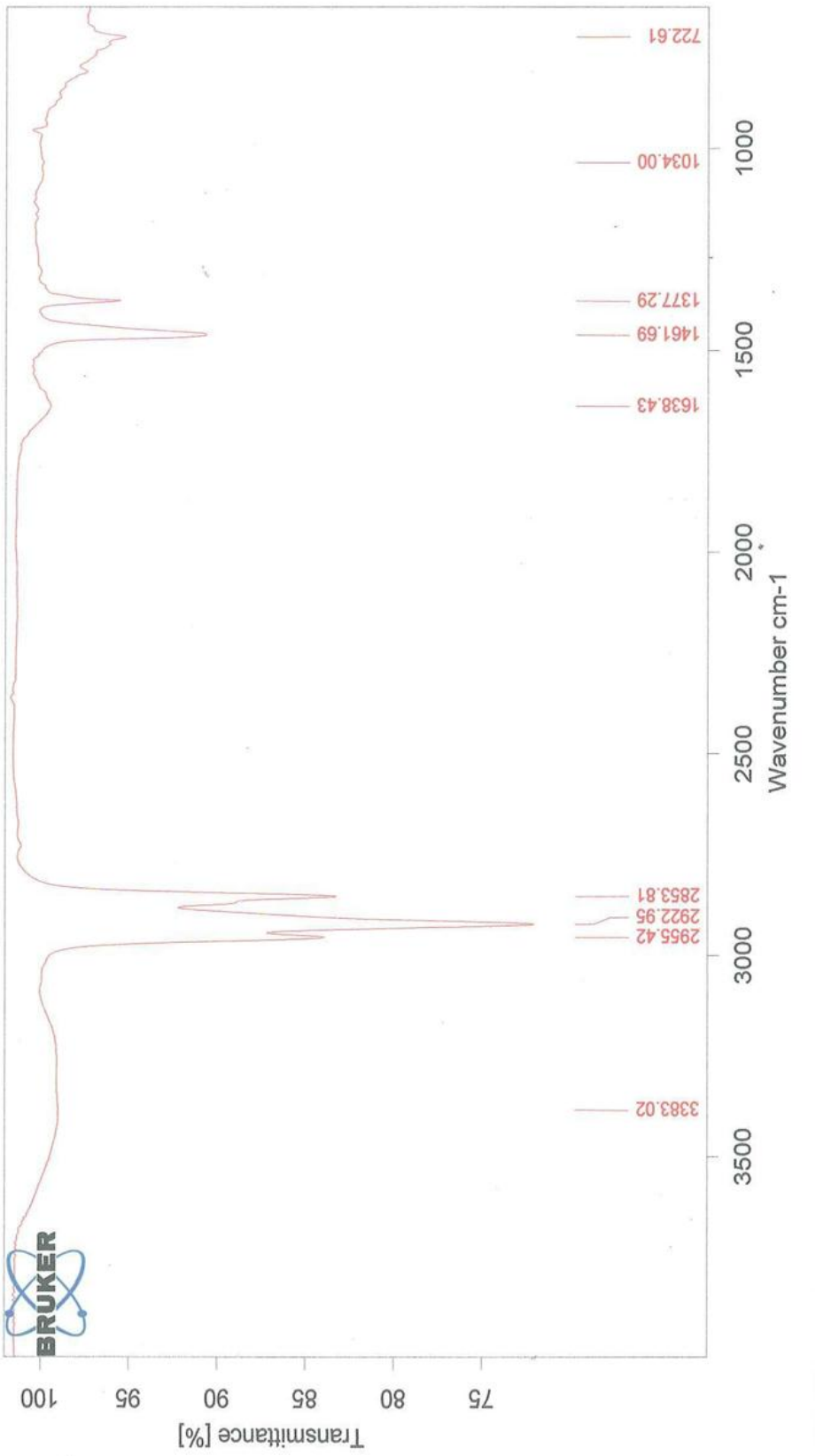

Figure 4 - FTIR Spectra gasoline after bio treatment for 8 days with *K.pneumoniae*

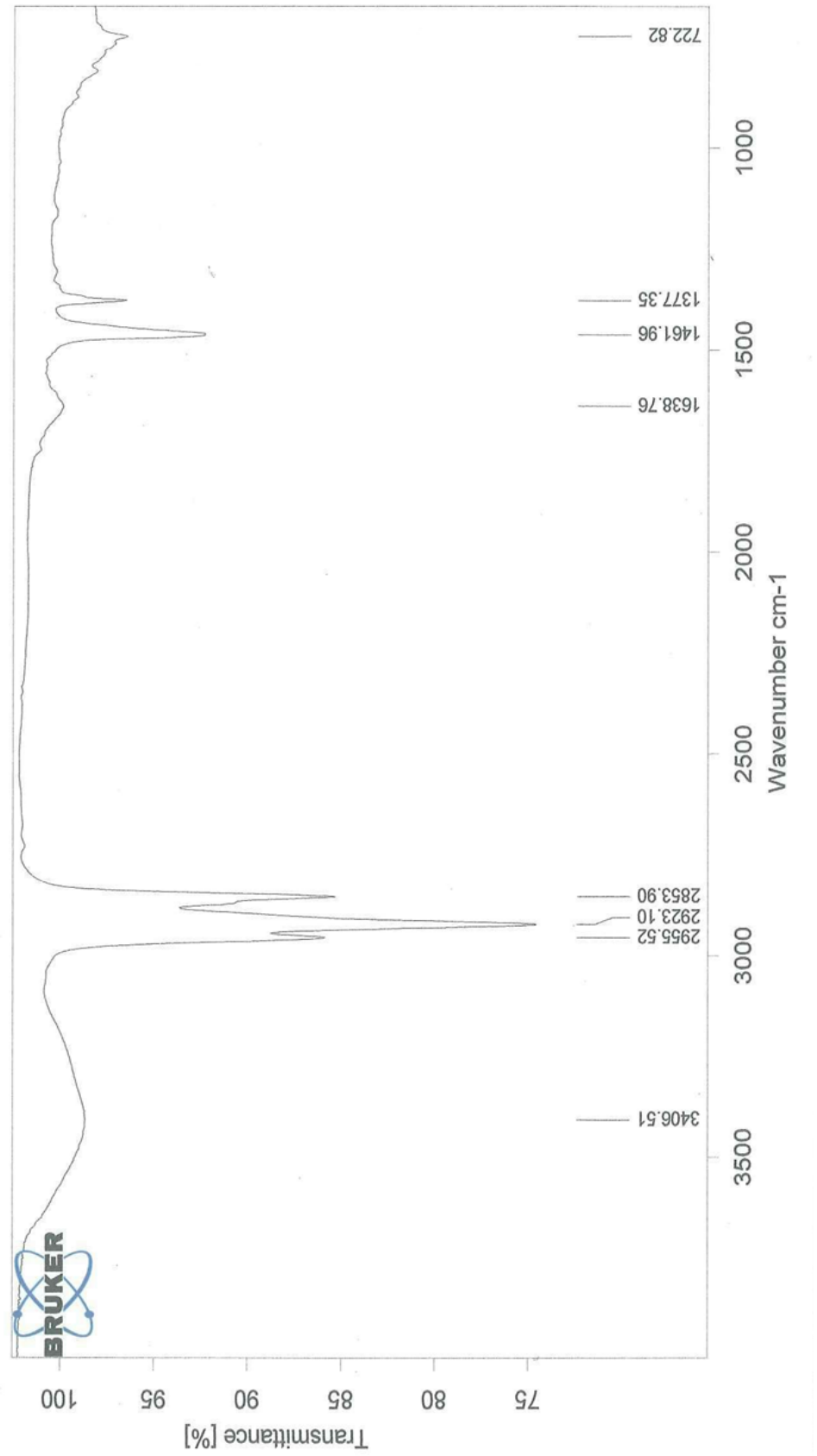

Figure 5- FTIR Spectra gasoline after bio treatment for 10 days with *K.pneumoniae*

References

1. Atlas, R. M. and Bartha, R. **1973**. Abundance, distribution and oil degradation potential of Microorganism in Raritan Bay. *Environ. Pollut*. 4: 291 – 300.
2. Brown, L. M. **2010**. Community dynamics and phylogentics of bacteria fouling Jet A and JP – 8 aviation Fuel. *Int. Biodeterior. Biodeg.* 64, pp: 253-261.
3. Van Hamme, J. D., Singh , A. and Ward , O. P. **2003** . Recent advances in petroleum microbiology . *Microbiol. Mol. Biol.* Rev. 67, pp: 503 – 549.
4. Harley, J.P. **2005**. *Laboratory Exercises imicrobiology* . Sixth Edition , Mc Grow Hill, New York, USA.
5. Adenipekun, C. O. and Fasidi, I. O. **2005**. Bioremediation of Oil – Polluted soil by Lentinus subnudus , a Nigerian White – rot fungus . *Afr. J. Biotechnol*., 4(8) : 796 – 798.
6. Johnson, A.R., Wick, L.Y. and Harms, H. **2005**, Principles of microbial PAH-degradation is soil. Environ. Poll 133:71-84.
7. Johnson, M.T. **2007**, Identification of the Enterobacteriaceae. Indiana University. India.
8. U. S. Environmental Protection Agency (EPA). **1989**. *Bioremediation of Hazardous waste sites work shop*. Washington, DC: CERI-89-11.
9. Vidali, M. **2001**. Bioremediation An over view , *Pure Appl. Chem*., 73(7): PP 1163 – 1172.
10. Hazen, T. C., Dubinsky, E. A., Desantis, T.Z. and Andersen , G. L. **2010**. Deeposea oil plume Enriches Indigenous oil – Degrading Bacteria. *Science express.*, 330(6001) : 204 – 208. USA.
11. Tortora, G. J. , Funk, B. Rand case , C. L. **1995** . Microbiology . An Introduction , fifth Edition . The Benjamin/Cumming publishing , Co., Inc., Red Wood city , CA, pp: 70 – 90.
12. Atlas, R. M., Parks, L. C. and Brown , A. E.**1995**. *laboratory Manual of Experimental Microbiology* , Mosby. USA.
13. Stolp, H. and Gadkari, D.**1981**. Nonpathogenic members of the genus pseudomonas . In the Prokaryotes . 1, pp: 719 – 74I. Edited by Starr, M. P., Stolp, H., Truper H. G., Balows, A. and Sclegel, H. G. Berlin: Springer.
14. Isenberg , H. D. **2004** . *Clinical Microbiology procedures handbook*. 2nd edition, Sections: 7.1 , 7.2, 7.3 , 7.4, 7.6 ASM press, Washington , DC.
15. Macfaddin, J. F. **2000**. *Biochemical test for identification of medical bacteria , 3rd ed. Lippincott* Williams & Wilkins, Philadelphia , USA.
16. 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.
17. Tuleva, B.J., Inanov, G.R. and Chi istora, N.E. **2002.** Biosurfactant production by a new *Pseudomonas putida* strair Z Nzturforsch 57 C. 356-360.
18. Wang, Q., Zhang, S., Li, Y. and Klassen, W. **2011**. potential Approaches to Improving Biodegradation of Hydrocarbons for Bioremediation of crude oil pollution. *J. Envir. Protect.*, 2 , 47 – 55.
19. Plaza, G. A., Lukasik, K., Wypych, J., Nate C.Z.- 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.
20. Lehmann, V. **1998**. Bioremediation: Asolution for polluted soils in the southl. *J. Biotechnology and Development Monitor*, .34 , pp 12 – 17 .