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Investigation of Rhodococcus Equi Effects On Crude Oil from Biological Degradation Aspects by SARA, FT-IR and GC Technique

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Abstract

Today, many measures have been taken to eliminate oil pollution, including the use of natural and synthetic adsorbents, mechanical methods, catalytic failure, and so on. There is still a long way to go in order to find an affordable and cost effective way, and it requires a variety of researches. Basically, one of the relatively new approaches in various industries, especially in the oil industry, is to use environmentally friendly technologies. One of the most widely used methods is the utilization of bacteria, which is considered as a modern method. In this study, after identifying the Rhodococcus equi and preparing it from the microbial bank (PTTC), it was added to the crude oil and tested by SARA, FT-IR and GC's analysis. This study indicates that the Rhodococcus equi has the potential to use various hydrocarbon groups as source of energy in different fraction in crude oil. Accordingly, such a bacteria is suitable for bioremediation in oil polluted sites and oil refinery for removing hydrocarbon pollutants from the environment.

Keywords: Heavy Oil, Bioremediation, FT-IR, SARA, Rhodococcus equi

Introduction

The soil is in danger by the chemical and petrochemical industries, as well as the oil refinery industries[1]. The soil is particularly affected by oil spills and its morphological and chemical properties change, thus having a negative effect on the activity and function of living organisms and the environment[2]. Today, various methods are used, such as polymer, catalytic and burning methods to eliminate oil waste. However, there are environmental friendly methods, such as the microbial method, which are good substitute for these methods. One of these methods is a bioremediation. Bioremediation is a process in which contaminants are degraded in controlled conditions by micro Organisms. Also, their concentrations are lower than the amount of considered legally. In addition, microorganisms, plants and fungi also carry out bioremediation. Biodegradation is used to destroy chemicals in the soil, underground water, sewage, sludge, industrial wastes and gases. These days oil and oil products have grabbed attention[3]. The existence of biological species in areas that is full of oil pollution indicates a special ability in these microorganisms. Using biological methods is practically affordable and natural. Microorganisms are able to use petroleum as a source of carbon and energy for their growth; So, today, use of biological methods has surpassed other methods in many cases[4]. In this study, for the first time we use Rhodococcus equi to eliminate oil residue in environment. also, growth of bacteria in oil does not require medium, so it can be used for wide areas which are polluted by petroleum. The R. equi belongs to the Actinomyces of the Nocardia group. The R. equi is a species of aerobic, non-spore, Non-motile and gram-positive bacteria that is more phylogenic in nature than coriognobacterium and mycobacterium[5]. The R. equi species can be identified in different environment such as soil, rock, underground water, aqueducts, plants and animals[6]. Today, biotechnology has become an important major especially in terms of improving environmental conditions and industrial wastewater treatment. We have chosen R. equi species for their ability to deg-

rade chemical contamination. It is anticipated that a large number of potential antibiotic producers will be included[7]. Although Rhodococcus species are generally known as low pathogenicity, they cause disease in animals, plants, and humans too[8]. Infections caused by R. equi are generally caused by inhalation, direct inoculation and oral intake[5].

First, the R. equi is widely distributed in nature, and is found especially in soil and vegetarian feces. Second, one of the materials which this bacterium needs to grow is carbon that is present in crude oil. Finally, the possibility of this species to grow in soil is analyzed. Given that the oil is completely enclosed by soil, therefore carrying all organic and inorganic materials, it can be a good option for bacteria growth. Eventually, the three points mentioned above, presence in the soil, presence in animal wastes and its nutritional sources, led to the study of the effect of this bacterium on crude oil[9].

In this study, we used SARA, FT-IR and GC methods. Saturate, aromatic, resin and asphaltene (SARA) is an analysis method that separates crude oil components according to their polarizability and polarity. The saturate fraction consists of nonpolar hydrocarbon including straight-chain (n-Paraffins), branched, and cyclic saturated hydrocarbons. Aromatics, which contain one or more aromatic rings, are slightly more polarizable[10]. Resines and asphaltenes fractions, have polar substituents. The difference between resins and asphaltenes is that asphaltenes are more insolable than resin. [11].

Gas Chromatography, namely GC, is one of the methods that used to analyze volatile substances in the gas phase which results in separation of the entities as a cause of gettig partitioned between two different phases, a stationary phase and a mobile phase[12].

In this study, there are several important features, first to mention is that we do not use medium, so it is suitable for large scale. Second to mention is that R. equi effects resin and asphaltene fraction is same as saturate and aromatic fractions.

Materials & Methods

Many microorganism have the ability to utilize hydrocarbons as sole source of carbon as energy for metabolic activities[13]. In this way, we investigate R. equi on crude oil from the well No. 43 of Gachsaran oil field. At the beginning, we order the R. equi from microbial bank (PTCC), it was a white powder. We mixed it with serum physiology to create McFarland standards. In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions [14]. Then we transferred it to blood agar medium and put it in incubator for 24 hours in 30oC. After a day, colonies grew up and already for use. We got some colonies and mixed it with serum physiology again to create McFarland standards. Then we mixed 500 Lambda (λ) R. equi with 15 ml crude oil and mixed them for 20 minutes with shakers and put samples in incubator in 30oC for 10 days. During these days, each 24 hour we get the samples out of the incubator to check them and mixed them with shaker.

SARA test results after 10 days before and after the treatment regarding the bacterial effects on crude oil are given in Table 1.

Table 1: SARA fractionation of oils before and after treatment with bacteria

	Before Treatment	After Treatment	Remarks
Saturate (%)	58	45	-13
Aromatic (%)	17	33	+16
Resin (%)	5	8	+3
Asphalthene (%)	20	14	-6

Table 1 illustrates that the bacterium has affected the entire fraction. The saturation result, as well as the asphaltene fraction, are decreased by 13% and 6%, respectively. Also, the aromatic fraction and resin are increased by 16% and 3%, respectively.

Evidence shows that this bacterium used more straight-run alkane compounds as source of energy, because straight-run alkane bond is weak and it is easier for bacteria to use saturate fraction. Also, it has effects on the asphaltene section to release the heterocyclic carbon materials on the aromatic and resin section.

Gas Chromatography Interpretation

To evaluate the bacterium's function on the samples of crude oil, we use Gas Chromatography (GC) to evaluate saturation fraction before and after the treatment. Since crude oil is a good source of nutrition for bacteria, comparison of the chromatograms obtained from the sample (before and after the treatment) can provide proper information on the bacterial functions in the crude oil sample.

The spectrum obtained from the gas chromatography analysis is shown in (Fig. 1). According to the spectrum, the distribution of normal hydrocarbon starts from C12 to C33 in the spectrum field. The gas chromatography analyzed the saturate fraction after applying bacterial effects on the crude oil sample. The spectrum obtained from this test is shown in (Fig. 1).

Evidence suggests that the distribution of the standard alkane components is different from the sample, before the treatment. The reduction of the saturated hydrocarbon components concentration, especially alkanes from C14 to C21 in the field of the spectrum shows that the bacterial effects on the

saturated fraction are more than other fractions and that the bacteria use the saturate compound as the source for energy.

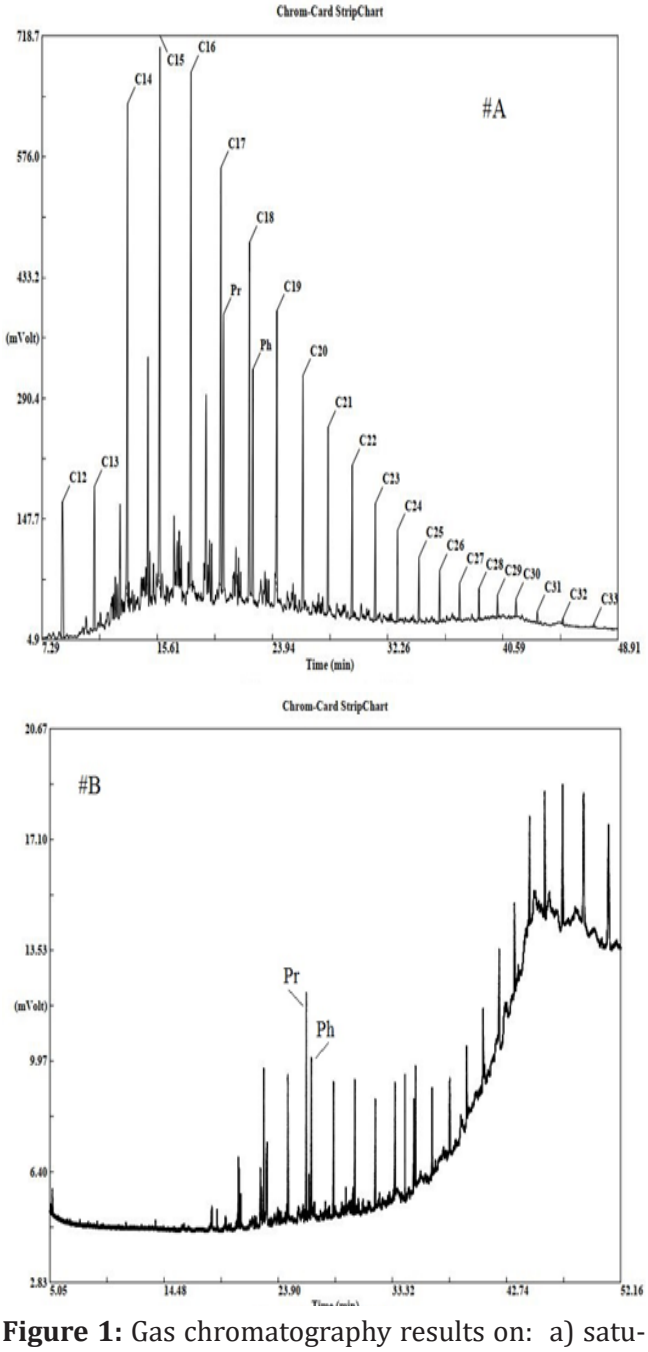

Figure 1: Gas chromatography results on: a) saturate fraction of Gachsaran oil before treatment with bacteria, b) after treatment with bacteria.

According to the Fig. 1, we can identify C12 to C33 from chart "a" which is without bacteria. However, chart "b" illustrates the sample of crude oil affected by bacteria that leads to producing C34 and C35 with low peaks, and eventually, the peaks are terminated with C35. The two graphs clearly show the elimination of light hydrocarbon structures especially peaks C14 to C20.

FT-IR Analysis

The FT-IR analysis shows which molecules with what structure is present in a sample [15]. In general two types of infrared spectrometers exist, one of them is dispersive and another one is the Fourier transform (FT) instruments that is used for Identification organic compounds in the range of 4000 cm-1 to 400 cm-1 [16]. Below are the FT-IR results of the samples before and after treatment (Fig. 2 to Fig. 4).

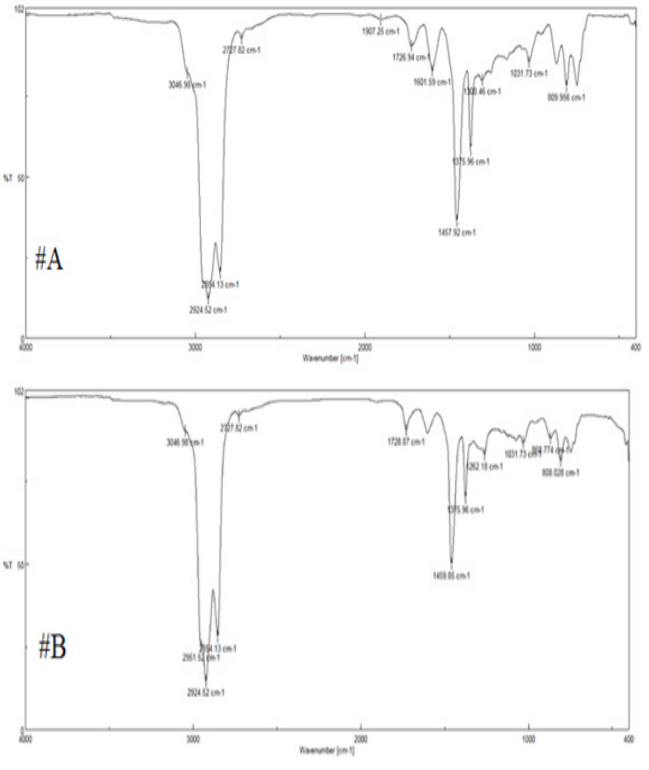

Figure 2: FT-IR result for aromatic fraction; A) aromatic fraction of the Gachsaran oilfield sample before treatment without bacteria, B) after treatment with bacteria.

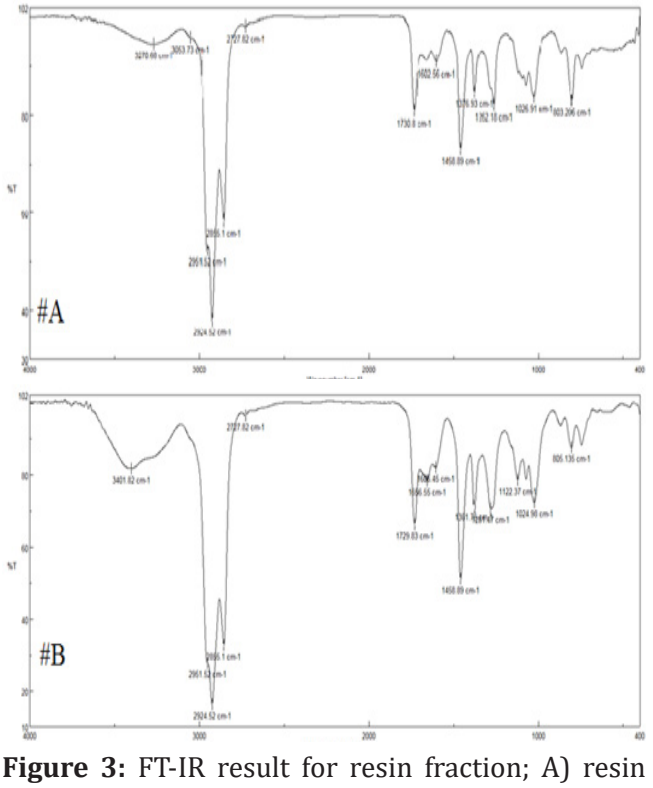

Figure 3: FT-IR result for resin fraction; A) resin fraction of the Gachsaran oilfield sample before trea-

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tment without bacteria, B) after treatment with bacteria.

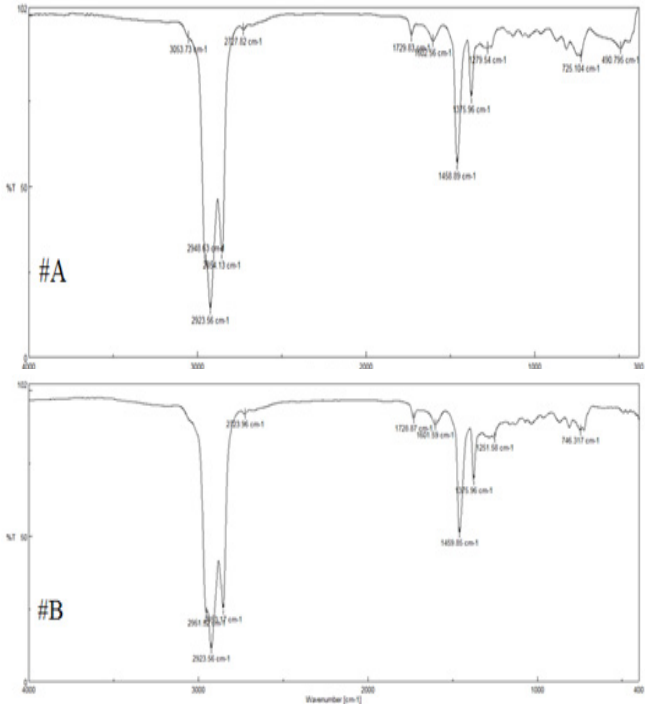

Figure 4: FT-IR asphaltene for aromatic fraction; A) asphaltene fraction of the Gachsaran oilfield sample before treatment without bacteria, B) after treatment with bacteria.

These figures illustrate that the aromatic fraction of the hydroxy- group and amine group with the small bound carbons are created and that the long chain groups such as OH and NH2 have increased gradually. Also, the pick number 1262 cm^(-1) after treatment shows that the oxygen – sulfur from sulfate oxide group are created. On the other hand, after treating the pick number 2951 cm^(-1), making hydrogen – carbon with strong structure from aldehyde hydrogen group with weak bounds was considered. These changes represent the alcohol functional oxidation to aldehyde (Fig. 2). The aromatic charts indicate that the carbonyl group are disappearing gradually, the picks from 1726 cm^(-1) ato 809 cm^(-1) confirms such evidence (Fig. 2). Biodegradation has occurred in the Resin fraction, then the smaller molecule with OH and NH functional groups are isolated from the asphaltene complex. Such changes have caused an increase in the concentration of alcohol, amine, and phenol (Fig. 3).

Eventually, the asphaltene charts primarily show that the hydrogen and nitrogen structures are disappeared, and that the peaks less than 1729 cm^(-1) have increased. These peaks include alcohol and aromatic compounds with hydrogen structure. Also, the tensile strength of the alcohol and phenol groups have increased; in addition, the smaller alkane chain compounds are isolated from the asphaltene fraction (CH and CH2), (Fig. 4).

Result and Discussion

This study indicates that R. equi have the ability to utilize hydrocarbons as sole source of carbon. Also, these results are similar to the results of Ekanem, J O and Ogunjobi (2017), which shows that the Pseudomonas sp., Bacillus sp and Nocardia sp are the best to be able to utilize the N-alkane and polyaromatic. With the FT-IR technique, it is proven that the bacteria in various oil fraction uses the structure of oxygen molecules, sulfur, nitrogen, and hydrocarbons with weak bonds.

The GC results (Fig. 1) shows that this bacteria have used all hydrocarbon in saturate fraction which in accordance with the study by Ekanem, J O and Ogunjobi, A A (2017), which has investigated the hydrocarbon degradation potential of 7 bacteria isolated from spent lubricating oil contaminated soil. This study shows that these bacteria have the ability to degrade saturate fractions after 21 days, especially the aliphatic (n-alkanes) and polymathic hydrocarbon fractions. The GC result of the mentioned study confirms our results too.

The aromatic fraction illustrates decreased aromatic compounds such as C=C, however, the O-H and C-H with weak bond have increased which shows that this bacteria separates/breaks/detaches this type of compound from the rezin and asphaltene fractions (Fig. 2).

Also, the Resin fraction shows the smaller molecule with OH and NH functional groups isolated from the asphaltene complex. Also, oxygen-sulfur from sulfate oxide group, carbonyl group and phenol groups increased too.

Finally, asphaltene results indicate that long chain groups such as OH and NH2 are deleted. However, the sulfate oxide group, carbonyl group and phenol groups have increased alike the Resin fraction.

Conclusion

To sum up, many microorganisms have the ability to utilize hydrocarbons as sole source of carbon as energy for metabolic activities and one of them is the R. equi. Evidences show that R. equi successfully affected the crude oil sample. Hence, it is suitable for bioremediation in oil polluted sites and oil refinery for removing hydrocarbon pollutants from the environment.

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REFERENCES

1. Bashkin V.N., Kalinina I.E., Galiulin R.V., Galiulina R.A. Diagnosis of hydrocarbon contamination of soil and its bioremediation by analyzing dehydrogenase activity. Protection of the environment in the oil and gas complex; 6: 4-6, 2009.
2. Pleshakova E.V. Ecological and functional aspects of microbial remediation of petropolluted soils. Abstract of the thesis of the Dr. of biological sciences. Saratov; 47 p, 2010.
3. Donaldson, E. C. (Ed.). Microbial enhancement of oil recovery-recent advances (Vol. 31). Elsevier, 1991.
4. Banat, I. M. Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: a review. Bioresource technology, 51(1), 1-12, 1995.
5. Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L. & Pfaller, M. A.Nocardia, Rhodococcus, Gordonia, Actinomadura, Streptomyces and other aerobic actinomycetes. Manual of clinic microbiology. Conville & Witebsky. 9th edn, pp. 515-542, 2007.
6. Gürtler, U., Fuchs, P., Stangelmayer, A., Bernhardt, G., Buschauer, A & Spruss, T. Construction and validation of a microprocessor controlled extracorporal circuit in rats for the optimization of isolated limb perfusion. Arch Pharm (Weinheim) 337, 672–681, 2004.
7. Teramoto, K., Kitagawa, W., Sato, H., Torimura, M., Tamura, T. & Tao, H. Phylogenetic analysis of Rhodococcus erythropolis based on the variation of ribosomal proteins as observed by matrix-assisted laser desorption ionization-mass spectrometry without using genome information. J Biosci Bioeng 108, 348–353, 2009.
8. Bell, K. S., Philp, J. C., Aw, D. W. & Christofi, N. The genus Rhodococcus. J Appl Microbiol 85, 195–210, 1998.
9. Stephen H. Gillespie and Peter M. Hawkey. Principles and Practice of Clinical Bacteriology, Second Edition. John Wiley & Sons, 2006.
10. Tianguang Fan, Jianxin, Jill S.Buckley. 2002. Evaluating Crude Oil by SARA Analysis. Society of Petroleum Engineers Journal; SPE 75228.
11. Fan T, Wang J, Jill S. 2002. "Evaluating Crude Oils by SARA Analysis", Journal of Society of Petroleum Engineers, Proceeding of SPE/DOE Improved Oil Recovery Symposium, 13–17.
12. Robert L. Grob, Eugene F. Barry. 2004. 'practice of gas chromatography', 4th ed. a John Wiley & sons, inc.
13. Ekanem, J O, Ogunjobi,A A. Hydrocarbon degardation potentials of bacteria isolated from spent lubricating oil contaminated soil. Journal Sciences and environmental management .Vol.21 (5) 973-979; 2017.
14. Cockerill, Franklin R.; et al. (2012). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—9th Edition. CLSI. p. 12. ISBN 1-56238-784-7, 2012.
15. Brian C. Smith. 2011. "fundamentals of fourier transform infrared spectroscopy", 2nd ed. CRC press Inc.
16. Pavia, D. L., Lampman, G. M., Kriz, G. S., and Vyvyan, J. A. 2008. Introduction to spectroscopy. Cengage Learning.f.

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