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Compare the effect of *Rhodococcus erythropolis* and *Rhodococcus equi* on crude oil by FT-IR technique

Abstract

The anthropogenic activities of the fossil fuel industry are a key contributor to environmental pollution, producing more than one billion tons of waste sludge annually. This sludge is a complex water-oil emulsion containing toxic levels of polycyclic aromatic hydrocarbons and heavy metals that causes severe damage to the ecosystem and public health. Bioremediation exploits the catabolic machinery of microbes to convert hydrocarbons into non-hazardous forms.

In this study *Rhodococcus erythropolis* and *Rhodococcus equi* were prepared from Persian Type Culture Collection (PTCC), they were added to crude oil, and then the samples were put for 10 days in an incubator. After a specific time, the effect of bacteria on crude oil was investigated by Fourier-transform infrared spectroscopy (FT-IR). FT-IR results show that compounds in polar fractions increased while non-polar fractions decreased. This study shows both bacteria have a suitable effect to break organic matter.

Keywords: bioremediation, FT-IR, crude oil, *R. erythropolis sp, R. equi sp*

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Arash Rasti,[1] Mohammad Ali Riahi[2]
[1]Department of Petroleum, Science and Research Branch, Islamic Azad University, Iran
[2]University of Tehran, Institute of Geophysics, Iran

Correspondence: Mohammad Ali Riahi, University of Tehran, Institute of Geophysics, Tehran, Iran, Email mariah@ut.ac.ir

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Introduction

World oil demand was seen rising by 5.5 million b/d in 2021 and by 3.3 million b/d in 2022, the IEA said, surpassing its pre-pandemic levels by 200,000 b/d to 99.7 million b/d. During the fourth quarter of 2021, the IEA said global demand "defied expectations" rising by 1.1 million b/d to 99 million b/d, an upward revision of 345,000 b/d compared to its previous report. During the fourth quarter of 2021, the IEA said global demand "defied expectations" rising by 1.1 million b/d to 99 million b/d, an upward revision of 345,000 b/d compared to its previous report.[1] Makes the processing of petroleum, which is a complex mixture of organic liquids called crude oil and natural gas, and the generation of petroleum waste important issues. Industrial petroleum waste treatment is an important study area in environmental engineering. For instance, oil spills have the potential to cause significant impacts on the environment, natural resources, and livelihoods. Hence the reason oil spill prevention should be practiced as a way of life. Unfortunately, mishaps do occur, and the effects, whether short-term or long term depends to a significant degree on the success and efficiency of the response, and how it is mitigated. Nonetheless, the success of a response more often may not only be adjudged by the responsible party or the responders but by the public and, to a certain extent, the media.[2] The wastes from Petroleum industries and refineries mainly contain oil, organic matter, and other compounds.[3] The treatment of this waste can be carried out by physical, chemical, and biological treatment processes. Treatment of petroleum wastes has two stages, firstly, the pre-treatment stage reduces grease, oil, and suspended materials. Secondly, an advanced treatment stage is to degrade and decrease the pollutants to acceptable discharge values.[4] Among the various known methods to deal with petroleum waste, biodegradation technology is considered the most accepted and eco-friendly method. The bioremediation process proceeds due to the action of potential microorganisms without utilizing any kind of heat. Based on the utilization of oxygen in the biodegradation of the toxic compounds (organic in nature), two types of biodegradation processes are recognized, viz., aerobic biodegradation (proceeds only in the presence of oxygen) and anaerobic biodegradation (proceeds only in the absence of oxygen).[5]

Rhodococcus

Members of the genus *Rhodococcus* have metabolic versatility and unique adaptation capacities to fluctuating environmental conditions, enabling the colonization of a wide variety of environments; they also play an important role in nutrient cycling and have potential applications in bioremediation, biotransformations, and biocatalysis. *Rhodococcus* spp. are mainly distributed in soil, water, and marine sediments, although some of them are also pathogens for humans, animals, and plants. Some *Rhodococcus* spp. are also pathogens for plants (*R. fascians*), animals, and humans (*R. equi*). *Rhodococcus* genus is featured by a broad metabolic versatility and environmental persistence supporting its clinical, industrial, and environmental significance. In particular, *Rhodococcus* strains have peculiar degradative capacities towards a variety of organic compounds, including toxic and recalcitrant molecules like chlorinated hydrocarbons.[13]

Rhodococcus erythropolis

R. erythropolis is a genus of aerobic, non-spore, non-motile, and gram-positive bacteria that is more phylogenetically similar to Corynebacterium and Mycobacterium.[6] *R. erythropolis* sp. has been identified from soils, groundwater, sea, plants, and animal waste.[7] Although *R. erythropolis* sp. is generally recognized as a low pathogenic disease, also causes disease in animals especially goats and horses.[8] *R. erythropolis* are generally caused by inhalation, direct inoculation, and *oral intake*. *R. erythropolis* found in the environment and it is especially found in the soil and herbivores' feces.[9]

Rhodococcus equi

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 corynebacterium and mycobacterium.[6] The *R. equi* species can be identified in different environments such as soil, rock, underground water, aqueducts, plants, and animals.[7]

FT-IR analysis

The FT-IR analysis shows which molecules with what structure are present in a sample.[10] In general, two types of infrared spectrometers exist, one of them is dispersive and another one is the Fourier transform (FT) instruments that are used for the identification of organic compounds in the range of 4000 cm-1 to 400 cm-1.[11]

Material and methods

Sample collection

A crude oil sample was collected from well No. 43 of the Gachsaran oil field. Crude oil was before treatment analyzed by SARA and FT-IR technique to compare with the treatment sample after 10 days. Moreover, *R. erythropolis* and *R. equi* were prepared from the Persian Type Culture Collection (PTCC).

Laboratory study

Bacteria were mixed with serum physiology to create McFarland standards. In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions.[12] then they were transferred to the blood agar medium separately and put into the incubator for 24 hours at 30°C. After a day, colonies grew up and they were all ready for use. We got some colonies and mixed them with serum physiology again to create McFarland standards.

The 500 Lambda (λ) *R. equi* was mixed with 15 ml crude oil and mixed for 20 minutes with shakers and put samples into an incubator at 30°C for 10 days. During these days, every 24 hours we get the samples out of the incubator to check them and mix them with a shaker. A similar process was performed for *R. erythropolis sp.*

Column chromatography

Column chromatography was used to determine the mass percentage of saturated, aromatic, resin, and asphaltene components during two steps before and after treatment. In this experiment, silica gel and alumina were used as fixed phases, and hexane, toluene, chloroform, and ethyl acetate as mobile phases.

Column chromatography results for *R. erythropolis sp.*

The SARA test shows that *R. erythropolis sp.* has affected non-polar fractions and the amount of asphaltene and resin fractions decreased by 2 and 3 percent relatively, while the amount of aromatic increased by almost 13 percent. It is notable that the saturation fraction is reduced by more than 8 percent Table 1.

Table 1 The results of the SARA test before and after treatment for *R. erythropolis sp*

Fractions	Before treatment	After treatment	Rate of change
Saturate %	58	50	-8
Aromatic %	17	30	+13
Resin %	5	2	-3
Asphaltene %	20	18	-2

Column chromatography results for *R. equi sp.*

According to Table 2, *R. equi sp.* affects crude oil in a similar way. The SARA test illustrates that the amount of asphaltene and saturate fractions decreased by about 6 and 13 percent while aromatic and resin fractions increased by more than 16 and 3 percent Table 2.

Table 2 The results of the SARA test before and after treatment for *R. Equi sp*

Fractions	Before treatment	After treatment	Rate of change
Saturate %	58	45	-13
Aromatic %	17	33	+16
Resin %	5	8	+3
Asphaltene %	20	14	-6

FT-IR analysis

R. erythropolis sp. results

Results of the FT-IR experiment for aromatic, resin, and asphaltene, before and after treatment for *R. erythropolis sp.* presented in Figure 1-3, respectively.

R. equi sp. results

At the bottom, there are FT-IR results of samples before and after treatment for *R. equi sp.* from Fig. 4-6.

Discussion

R. erythropolis sp. FT-IR interpretation

In aromatic fractions (Figure 1), the aldehyde, isocyanide, and isocyanide groups are eliminated and sulfur, Nitrogen, and Carbon are released like gas NH_3, CO_2, and SH_2 are produced (1907^{cm-1}). Likewise, carboxylic acids are removed (1308^{cm-1})._Reduction of 1308^{cm-1} and 1031^{cm-1} shows structures like C-O and C-N from aliphatic compound decreased, while various structures like aromatic esters were made (1257^{cm-1}).

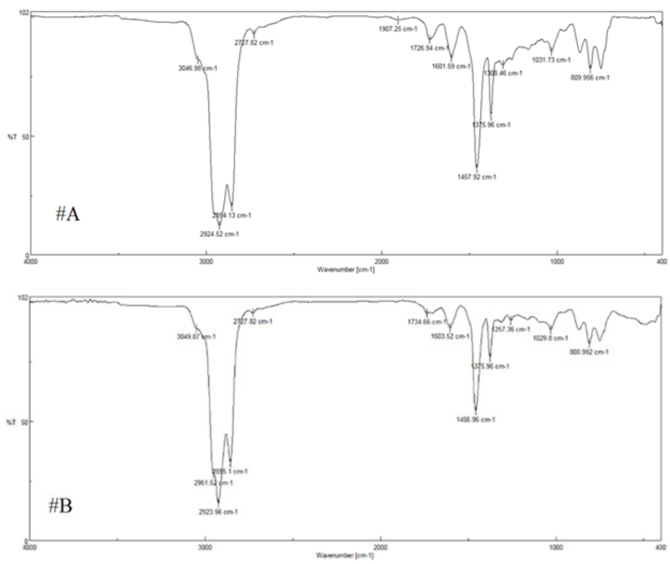

Figure 1 FT-IR results for aromatic fractions, before (A) and after treatment (B).

According to the evidence, Amine groups slowly decreased (1024^{cm-1} and 1254^{cm-1}). Table 1 shows that the aromatic fraction 13 percent increased and it's due to breaking and separating aromatic rings from resin and asphaltene fractions.

In resin fractions (Figure 2), the amount of O-H increased while hydrocarbon with a small chain (C-H) with peak number 3053^{cm-1} was removed. Also, Peak number 1118^{-1} also appeared, which shows sulfoxide compounds were made.

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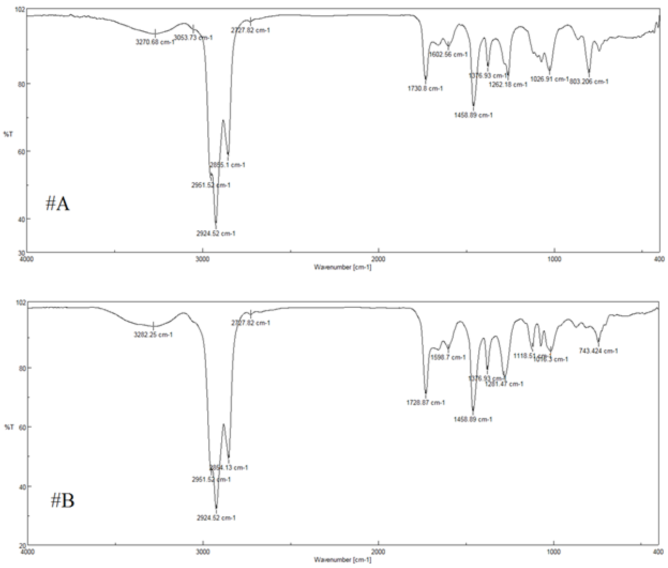

Figure 2 The FT-IR spectrum for resin fractions, before (A) and after treatment (B).

According to the above definition, the reason for increasing O-H structures is breaking the hydroxyl group from resin fraction; and the reason for the decline in the C-H structures is due to biological destruction. In other words, *R. erythropolis* sp. could use hydrocarbon with a small chain, hydroxyl group, and carbonyl group as a power source.[14]

In asphaltene fractions (Figure 3), a small chain of hydrocarbon (C–H) with small bounds is increased (3053^{cm-1}). Likewise, a bunch of groups of asphaltene fractions like amides, aromatics, phenol, ketone, and alcohol compounds are increased, except sulfoxide compounds which decreased (1118^{cm-1}).

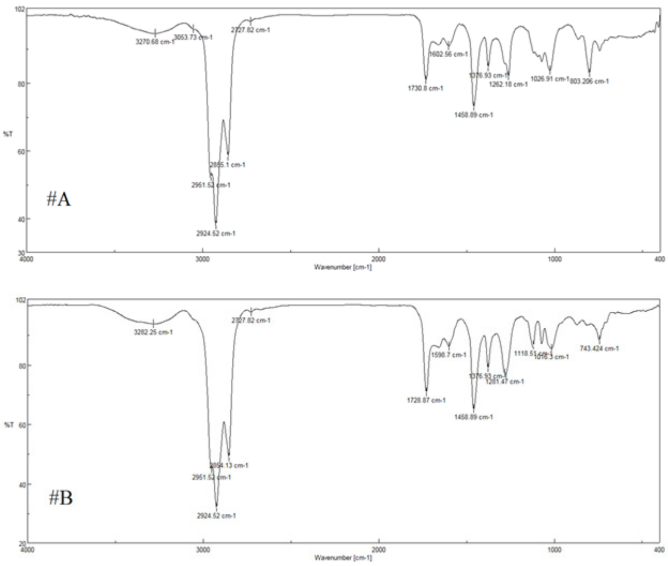

Figure 3 The FT-IR spectrum for asphaltene fractions, before (A) and after treatment (B).

R. equi FT-IR interpretation

In the aromatic fraction (Fig. 4), the number of alkanes is decreased and the aldehyde, isocyanide, and isocyanide groups are eliminated and sulfur, Nitrogen, and Carbon are released like gas NH_3, CO_2, and SH_2 are produced (1907^{cm-1}). Moreover, the number of aromatic rings, alcohol, and sulfoxide compounds decreased. It is notable that the amount of phenol compounds is increased slowly. These changes represent the alcohol's functional oxidation to aldehyde.

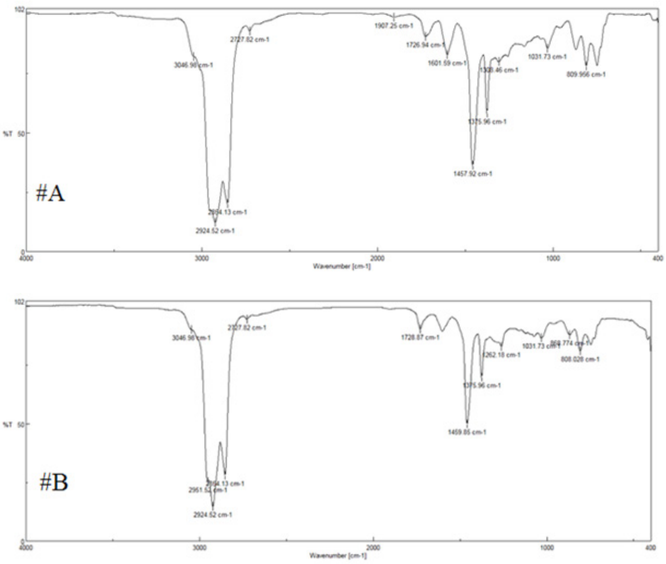

Figure 4 FT-IR results for aromatic fraction;A) aromatic fraction of Gachsaran oil before treatment without bacteria, B) after treatment with bacteria.

Biodegradation occurred in the resin fraction (Figure 5), then the smaller molecule with OH and NH functional groups was isolated from the asphaltene complex. The achievement of these changes is to increase the concentration of alcohol, amine, and phenol (Figure 3).

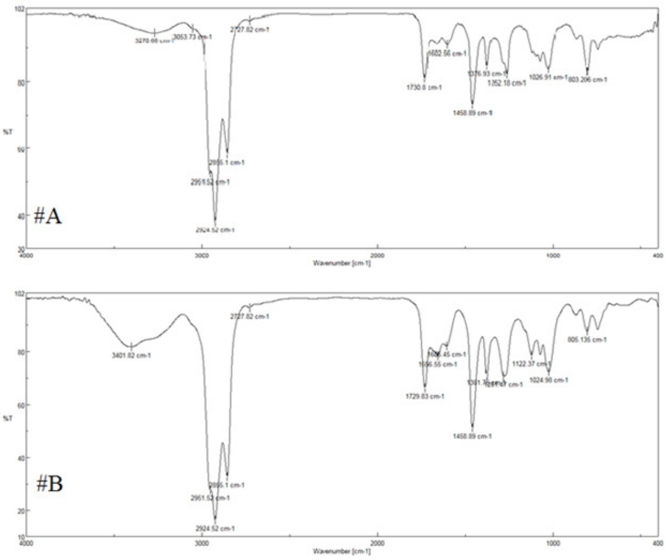

Figure 5 FT-IR results for resin fraction; A) resin fraction of Gachsaran oil before treatment without bacteria, B) after treatment with bacteria.

In asphaltene fractions (Figure 6), however a small chain of hydrocarbon (C–H) with small bounds and C-C structures connected to asphaltene fraction has broken, but_peak numbers 2727^{cm-1} and 2854^{cm-1} which are related to hydrocarbon with a small chain, are stable before and after treatment and it shows bacteria has less effect on these compounds.

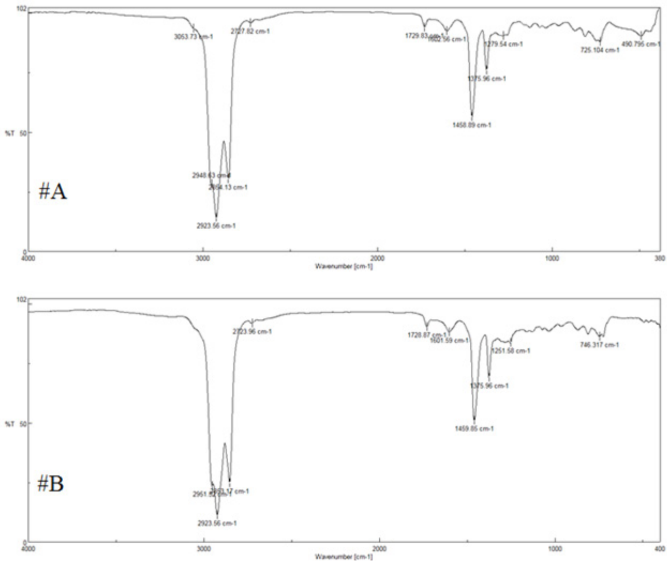

Figure 6 FT-IR asphaltene for aromatic fraction; A) asphaltene fraction of Gachsaran oil before treatment without bacteria, B) after treatment with bacteria.

Conclusion

Based on the chromatographic column results, it can be observed that both bacteria exhibit similar patterns of weight changes. The analysis of column chromatography demonstrated a significant impact of both *R. erythropolis sp*. and *R. equi sp*. on the saturate fraction, which served as a potent energy source. Conversely, the breakdown of resin and asphaltene compounds led to an increased percentage of the aromatic fraction for both bacterial species. This indicates their capability to efficiently degrade heavy hydrocarbon compounds and generate lighter hydrocarbons for energy production. Overall, both bacteria display commendable proficiency in the degradation and decomposition of petroleum products. However, *R. erythropolis* exhibits a superior capacity for breaking down asphalt structures.

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Conflicts of interest

The authors declare there are no conflicts of interest.

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