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The Ability Of *Klebsiella Pneumonia* And *Klebsiella Oxytoca* To Degrade Oil Waste

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Abstract *. Crude oil contamination is one of the major environmental problems, it generated processing water pollution by hydrocarbon. Microorganisms have been used to remove or reduce the effects of environmental pollutants as bioremediation agents and the fact it is environmentally friendly. This study isolated two Klebsiella strains, Klebsiella pneumonia and Klebsiella oxytoca from wastewater of the North refineries company in Baiji according to the morphological and biochemical characteristics.This work aimed to evaluated the capacity of two Klebsiella strains to degrade the petroleum hydrocarbons efficacy, which excreted from wastewater of the North refineries company. The results showed that the Klebsiella oxytoca exhibited a higher hydrocarbon degradation capacity of 67.75% compared to Klebsiella pneumonia, which recorded 45.81%. While mixed culture (Klebsiella oxytoca and Klebsiella pneumonia) was recorded a biodegradation rate of 63.35%. The results showed that efficacy of Klebsiella oxytoca as biodegradation for oil wastewater was 30% higher than other strain.*

1. INTRODUCTION

Crude oil pollution is a rising environmental concern on a global scale (Singh *et al.*, 2020). Hydrocarbon pollution is a significant environmental issue resulting from several human activities associated with the petrochemical industry (Almansoory et al., 2019). Petroleum wastes as well as crude oil are discharged toward the environment due to activities such as extraction, processing, transportation, storage, accidents, illegal dumping of crude oil wastes, and leaks from deteriorated tanks and pipelines. The principal source of water and soil pollution is the discharge of hydrocarbon pollutants into the environment, whether it occurs accidently or when untreated before being dispensed. (Adebusoye *et al*., 2007).

Bioremediation techniques must be developed to degrade hydrocarbons and used to reduce the harmful impacts that petroleum hydrocarbons produce (Singh and Chandra, 2014). The technology of bioremediation is more effective, economical, labor-efficient, reliable, eco-friendly and adaptable. This technology, which works with live things like plants and microbes, is depended on harmful or degradation of toxic organic compounds into harmless compounds (Varjani, 2017; Alfaify *et al*., 2020)

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There are two basic methods used in bioremediation schemes: bio-stimulation which involves supplemental nutrients or bio-surfactants and bio-augmentation by adding microbes (Mrozik and Piotrowska-Seget , 2010). It is well known that micro-organisms possessing enzymatic capabilities use petroleum and petroleum hydrocarbons serve as energy and carbon sources (Thapa *et al.*, 2012). Several bacterial species break down hydrocarbons in solution by emulsifying them. They do this by creating biosurfactants, which are active surface agents that help the cells stick to the substrate(Hassanshahiant *et al.*, 2012). Bhattacharya *et al.* ,2003 reported on the catabolic ability of *Klebsiella* strains are gram-negative bacteria to break down hydrocarbons, especially polyaromatic hydrocarbon (PAH). The purpose of this work is to isolate bacterial *Klebsiella* strains that can be used in bioremediation of hydrocarbon contaminated water, and describe the ability of two *Klebsiella* strains and efficiently to degrade crude oil and its related products under laboratory conditions. There may be potential uses for this research in the remediation of hydrocarbon contamination.

2. METHODOLOGY

2.1. **Sample collection**

Samples of industrial wastewater polluted with oil waste were collected from various places in the water flow from which oil waste is released to the North Refineries Company in Al-Baiji, transported to the laboratory in sterilized glass bottles and stored at 4 C˚, until they were used to isolate the bacteria.

2.2. Isolation and identification of bacteria

Bacteria were isolated from oil wastewater by using dilution method, which was prepared from 1 ml of the wastewater sample in a test tube with 9 ml of normal saline solution to obtain the required concentration, then it was taken from a dilution of 103 and spread on nutrient agar and incubate at 37 C˚ for an hour, according to (Olukunle ,2013). Bacteria were identified according to the morphological characteristics, the shape and cell size, and their Gram stain interaction test when compared to Bergey´s manual (Murray *et al.*, 2003), and using biochemical tests (Catalase, oxidase enzyme production, acylation of gelatin, H_2S gas production, starch decomposition, nitrate consumption, motility test, indole test, hemolysis, ability to ferment sugars). Two species of bacteria belonging to the *Klebsiella* genus were identified and the isolated were preserved on nutrient broth at refrigerator temperature.

2.3 Measuring of quantitative loss% of hydrocarbon compounds

The measure of oil waste decomposition rate by using the gravimetric method to calculate the percentage of hydrocarbon consumption by calculating the Residue amount of hydrocarbon

compounds by measuring the difference between the amount of oil waste weight added to the culture medium used before and after the bacterial growth used in the study. The percentage rate of decomposition by Separately and synergistically was calculated for the bacterial isolates, and statistical analyses were performed for these results.

The infrared spectra of bacterial samples analyzed belonging to the *Klebsiella* genus in this study were measured by Shimadzu FT-IR 8400 spectrophotometer ranged 400-4000cm-1 using KBr carried out at Chemistry department / College of Science/Tikrit University.

3. RESULTS AND DISCUSSION

Two *Klebsiella* species were used to analyze petroleum residues in the waste water, they were distinguished from each other using the tests outlined in Table 1. The findings indicated the presence of Klebsiella species. The isolates exhibited characteristics of Gram-negative, facultative anaerobic, non-motile short rods, non-sporing. Biochemical tests revealed that the isolates were catalase positive, oxidase negative, methyl red test negative, Vogus-Proskauer and glucose test positive, and Urease test positive. Additionally, the isolates displayed diverse reactions to other biochemical tests. The two medically significant species, Klebsiella pneumoniae and Klebsiella oxytoca, can be distinguished based on their ability to synthesize indole from tryptophan. K. oxytoca is classified as indole positive. (Maslow *et al.*, 2003).

Table 1. *Biochemical tests of the Klebsiella species at the present study.*

Bacteria isolation	**Biochemical tests**											
	Gram stain	Catalase	Oxidase	H2S	Gelatin	Nitrate	Urease	Methyl red	Indole test	Motion test	Glucose	Lactose test
Klebsiella pneumoniae	-	+	-	-	-	+	+	-	-	-	+	+
Klebsiella oxytoca	-	+	-	+	+	+	+	-	+	-	+	+

The removal of hydrocarbons through microbial degradation has been regarded as an efficient and eco-friendly method, a various microorganisms isolated from contaminated locations can degrade hydrocarbons under aerobic and facultative anaerobic conditions (Kumar & Ghosh, 2022). The *Klebsiella* bacteria used were studied for their ability to consume

hydrocarbon content. It was found that the *Klebsiella oxytoca* exhibited a higher hydrocarbon degradation capacity compared to *Klebsiella pneumonia* and a mixed culture (*Klebsiella oxytoca+ Klebsiella pneumonia)*, which recorded a lower degradation rate. The rate ability of the *Klebsiella oxytoca* to the hydrocarbon degradation is 67.75% whereas *Klebsiella pneumonia* is 45.81%, while the mixed culture of bacteria was recorded of 63.35% for the hydrocarbon degradation (Table 2).

Table 2. Hydrocarbon degradation percentage in waste water using Klebsiella.

Bacteria isolates	Wt. of untreated Crude oil (cm^3)	Remaining wt. of un-degradation (cm^3)	Wt. of degradation petroleum waste (cm^3)	Degradation rate %
Klebsiella pneumonia	0.8447	0.4577	0.3870	45.81
Klebsiella oxytoca	0.8447	0.2724	0.5723	67.75
Klebsiella pneumonia + Klebsiella oxytoca	0.8447	0.5352	0.6335	63.35

Chamkha et al. (2011) observed that Klebsiella oxytoca has the ability to break down a diverse spectrum of aliphatic hydrocarbons, specifically those with carbon chain lengths ranging from C13 to C30. In their study, Mohamed et al. (2012) demonstrated that K. oxycota, obtained from crude petroleum oil samples, exhibited the ability to metabolize four distinct polycyclic aromatic hydrocarbons (PAHs): benzene , phenanthrene, pyrene, and fluoranthene. The infrared spectroscopy technique (IR) was used to determine the various bonds in the compounds. It was found that the chemical bonds, including stretching vibrations of C-H and C-C bonds with less frequency appearance, fall within the range of 4000-1300 cm^{-1}. The second effect is bending vibrations, which involve changes in the positions of bonds without their disappearance. All the oil residue samples gave similar absorption spectra, indicating that the groups and bonds in the compounds are similar.

Several studies have confirmed the use of this technique in studying crude oil degradation (Al-Asadi, 2000; Al-Jubouri, 2005). Additionally, (Al-Obaidi, 2003) pointed out changes in the positions of atoms and bonds, indicating the role of bacterial isolates in the degradation of aromatic compounds. Multiple peaks were observed for aromatic hydrocarbons and phenols in crude oil treated with *Klebsiella pneumonia* and *Klebsiella oxytoca*, as shown in Figure1,

compared to Figure (2,3) which represent the degradation of oil residue samples by the bacterial species *Klebsiella pneumonia* and *Klebsiella oxytoca*, and synergistically as mixed culture using this technique.The results show a significant change in the absorption positions of the bonds, but differences in the intensity of the peaks in the overall shape of the spectra were observed. The two spectra mentioned above were sufficient to demonstrate that there were differences in the spectra for degradation using this biological method.

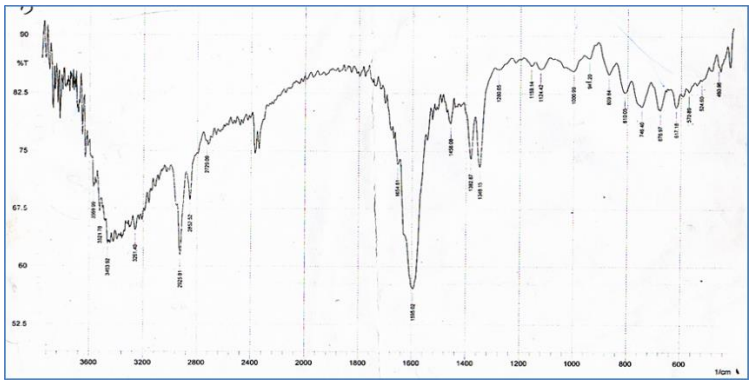

Figure 1. IR spectrum of the oil residue sample before the treatment by bacterial isolates.

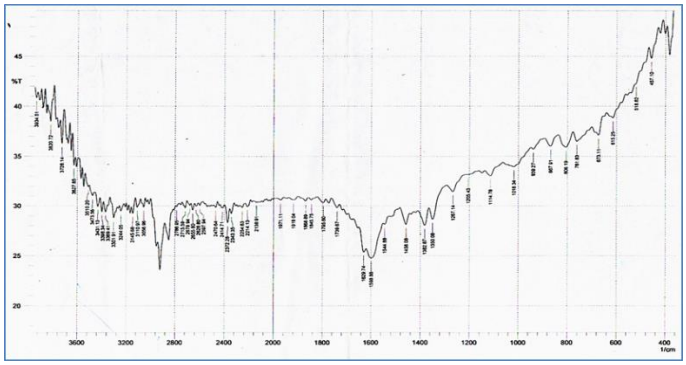

Figure 2. IR spectrum of the oil residue sample after treatment by *Klebsiella oxytoca*.

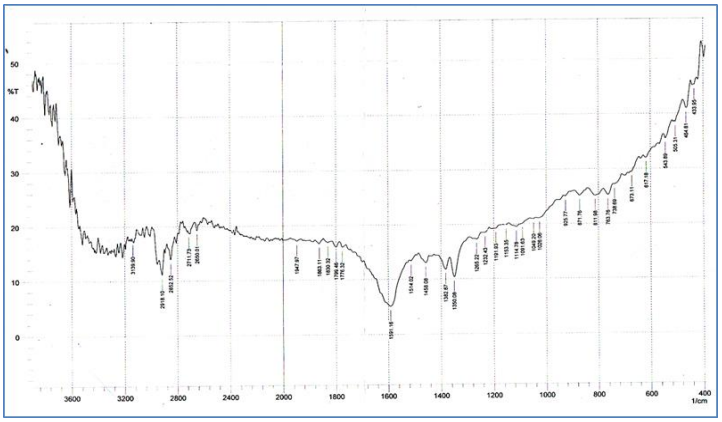

Figure 3. IR spectrum of the oil residue sample after treatment by *Klebsiella pneumonia*.

4. CONCLUSIONS

The degradation efficacy of *Klebsiella oxytoca* for oil wastewater like petroleum hydrocarbons was a 30% greater than the *Klebsiella pneumonia*. While a degradation rate of 63.35% was recorded for the hydrocarbon content degradation in the mixed culture of bacteria. This result showed that these *Klebsiella* strains would need to break down petroleum hydrocarbon contaminants as the eco-friendly technology clean-up (bioremediation). So, strains producing bio-surfactants may play an important role in petroleum hydrocarbon biodegradation, it enhancing biodegradation process.

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