Biodegradation of Crude Oil Using Three Types of Bacteria Pseudomonas Fluoroscens, Bacillus Licheniformis and Sphingomonas Paucimobilis.

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Abstract

The biodegradation process is one of the best ways to remove organic pollutants with different organic concentrations that have a very dangerous impact on the ecosystem. Soil samples contaminated with crude oil were collected from eight oil producing sites of Basrah Governorate. In the present study three types of bacterial species were isolated from the soil contaminated with crude oil in the producing fileds. The bacterial species were diagnosis of Vitek device as *Pseudomonas fluoroscens*, *Bacillus licheniformis* and *Sphingomonas paucimobilis*, their ability to biodegrade crude oil was tested of the mineral salts medium. As single isolated for 10,20 and 30 days incubation. results showed that the *P.fluoroscens* the best with biodegradation ability at different incubation periods .

Keywords: Biodegradation, Bacteria, concentrations crude oil.

INTRODUCTION

The ecosystem is subject to imbalance due to many accidental leaks and spills of crude oil frequently during exploration, production, restorage of petroleum products and other industrial activities. Also, the release of petroleum hydrocarbons into the environment, whether by chance or due to human activities, is a major cause of annual water and soil pollution, which causes severe damage in many environments, including the environment of plants, animals and humans, and because of the toxicity of oil and the susceptibility of some of its components to solubility such as benzene, toluene, xylene and naphthalene. Exposure to high concentrations of petroleum hydrocarbon pollutants may lead to many dangerous diseases and carcinogens (Panda *et al*.,2013;Xenia and Refugio,2016).

The negative impact of oil pollution on the ecosystem leads to the exploration of several strategies to return sites contaminated with petroleum hydrocarbons to sites less harmful or to what they were . As the physical and chemical techniques currently used to treat oil spills are considered expensive and not environmentally friendly. Recently, a lot of efforts have been made to treat oil pollution by using natural processes that include biological and phytoremediation (Jahangeer and Kumar,2013) .

Bioremediation is a natural process in which microorganisms convert environmental pollutants into harmless end products to obtain carbon and energy sources, being a simple, easy to maintain, applicable to large areas and cost effective as it leads to the complete removal of petroleum hydrocarbon pollutants. Where this technology uses the metabolic ability of microorganisms such as bacteria, fungi and a few primary organisms to decompose

the crude oil spilled in the land and marine environments and turn it into harmless compounds. Physical and chemical such as aeration, pH, water holding capacity and ion exchange capacity (Varjani and Upasani,2012;;Abbasian *et al*.,2015;Nwogu *et al*.,2015).

Microorganisms have different mechanisms for adapting to different hydrocarbon compounds through the metabolism processes that follow them, as microorganisms have different mechanisms for decomposing hydrocarbons than Enzyme-catalyzed decomposition of organic and inorganic pollutants Whereas other types of microorganisms assist in this process, through their symbiotic relationships that lead to the release of glucose to aid the proliferation of degradation species of hydrocarbons or the secretion of surfactants to make the crude oil more soluble (Tang *et al*.,2012;Bak *et al*.,2015).

Bacteria are one of the most dynamic factors in the decomposition of petroleum hydrocarbons, as they act as major decomposers of polluted environments. Among the most efficient bacteria in bioremediation are *Pseudomonas , Bacillus , Alcaligenes , Mycobacterium Rhodococcus , Sphingomonas These genera hydrolyze aliphatic and aromatic hydrocarbons and use them as the sole source of carbon and energy. Fungi are also good decomposers of hydrocarbon compounds due to the nature of the extracellular enzymes that produce them. These complex enzymes degrade a wide range of pollutants, including petroleum pollutants.* (Nilanjam and preethy,2011).

MATERIALS AND METHODS

Sample collection

Samples soil collection contaminated with petroleum hydrocarbons, the surface layer of the soil was removed to a depth of (5-15 cm) were collected from eight different locations of oil fields contaminated soil was taken and placed in sterilized polyethylene bags for each site, then the soil samples were transferred to the laboratory and kept in the refrigerator at a temperature of 4 ° C until use (Latha and Kalaivani,2012) .

Isolation and Identification of Bacteria

Bacteria were isolated from soil contaminated with crude oil according to the method (Prathyusha *et al*.2016) Where 2.5 g of soil was weighed and added to 25 ml of Luria Bertani Broth medium and the suspension was using an electric shaker device (shaker) for 10 minutes and then incubated in the incubator at a temperature of 22 ° C for (24-48) hour Then take 1 ml of suspension and add to 10 ml of sterile distilled water and shake well. Then a series of dilutions was made from 10^{-1} to 10^{-6}, then 1 ml of each dilution was withdrawn and transferred to a sterile Petri dish, then Oil agar medium was added to it before solidified, and the dish was moved with a vertical movement for the purpose of homogeneity. Then the dishes were left to solidify and then incubated at 25°C for 24 hours, three replicates were used for each samples After that, the bacteria were diagnosed by the Vitek device .

The ability of Bacteria species to biodegradation oil

Used mineral salt medium (MSM) Which contains the following ingredients of NH_4NO_3 , K_2HPO_4 , KH_2PO_4 , $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, $FeSO_4.7H_2O$ By dissolving chemicals in a liter of distilled water, and then the pH was adjusted to 6.5, which was used to test the laboratory diagnosed bacteria on the biodegradation of crude oil. add 100 ml of MSM medium 250 ml Erlenmeyer flask, after the pH was adjusted to 6.5, and the flasks were sterilized in an autoclave. 1 ml of crude oil was added to the flasks used in the laboratory after sterilization,

as the flasks were inoculated with bacterial cultures with two replicates for each isolate and for each incubation period. The flask was incubated in the vibrating incubator at a temperature of 25 ℃ at a speed of 120 rpm for three periods of 10, 20 and 30 days with a control flask for the medium used to ensure that no contamination occurred.

Crude oil extraction

After the end of the incubation period, the activity of bacteria was stopped by adding 1 ml of HCl of 1N to extract petroleum hydrocarbons from the liquid media, as the method described before was followed Mittal & Singh,(2009), Where the liquid growth medium was transferred to the separating funnel and 80 ml of Ether Petroleum and Aceton mixture was added to it in a ratio of 1:1 to the separating funnel with shaking well for several times and the sample was left to settle to separate into two layers. The upper layer contains petroleum hydrocarbons and the lower layer (water + acetone) neglected the lower layer and passed the upper layer over the separation column that contains at the bottom of the glass wool and then a layer of anhydrous sodium sulfate, where it works to withdraw the remaining water and impurities from the sample and receive the descending solution from Separator column in glass flask . The solvent was vaporized the crude oil was then calculated gravimetrically according to Oudot (1984) .

statistical analysis

The study data was analyzed using a Complete Randomized Design (CDR). On the basis of a factorial experiment with three factors and their interactions using the readymade statistical program Genstat to test the significance of differences between the studied averages, the least significant difference (LSD) test was used at the level of significance ($p<0.05$).

RESULTS

Identification of Bacterial species

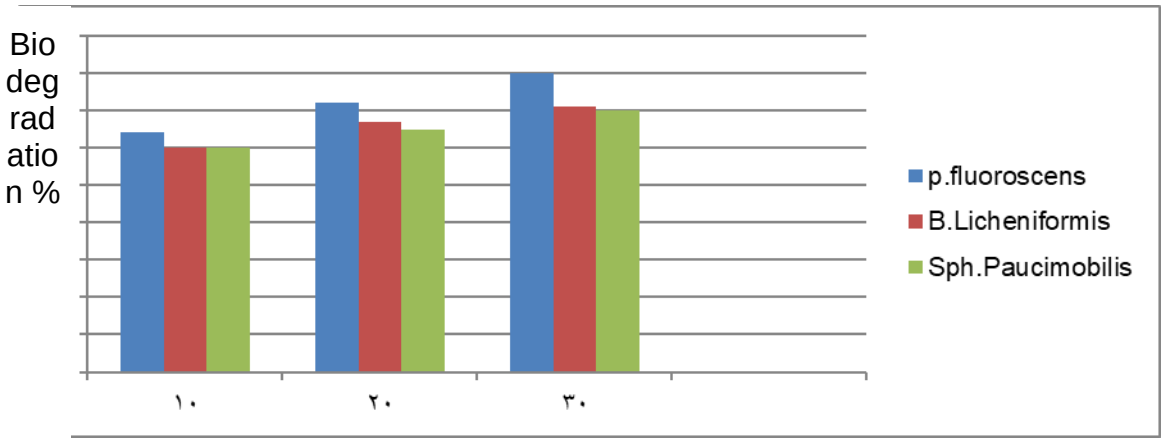
Most of the bacteria isolated from soils contaminated with petroleum hydrocarbons have varying ability to biodegradation petroleum hydrocarbons**.** Where bacterial isolates, when tested in a culture medium to which crude oil was added, proved the ability of these isolates to biodegrade petroleum hydrocarbons as *Pseudomonas fluorescens , Bacillus licheniformis , Sphingomonas paucimobilis*. Its ability to biodegrade crude oil in laboratory liquid media. During incubation periods of 10, 20 and 30 days and at a temperature of 30 ℃.

Determination of the ability of bacteria to Biodegradation crude oil in liquid media

The results of the statistical analysis and the table (1) showed There were significant differences at the level ($P < 0.05$) between the bacterial species that were biodegradation on petroleum hydrocarbons in liquid culture media and at incubation periods of 10, 20 and 30 days. The biomass of the bacterial species increased over the time incubation period, the more biodegradable the petroleum hydrocarbons .It was noted that the isolate *P.fluorecens* outperformed other species in terms of removal rates in *B. licheniformis* and *Sphingomonas paucimobilis*, they were less effective in terms of biodegradation. This confirms that the effect of the bacterial species and the different incubation periods had a distinct role in the biodegradation process of petroleum hydrocarbon compounds.

Table (1) Average total concentrations of residual petroleum hydrocarbons in media inoculated with bacterial species at different incubation times µg/ L

LSD for total overlap = 0.2804, LSD for time = 0.1402, LSD for coefficient = 0.1669



Shape (1) Shows the percentages of biodegradation of bacterial species in vitro, incubation time (days)

DISCUSSION

Microorganisms such as bacteria can be used in the bioremediation process to biodegradation certain compounds that contained in petroleum. Some types of bacteria that have these abilities were Pseudomonas , Sphingomonas , Bacillus (Haritash and Kaushik, 2009; Kafilzadeh *et al*., 2011; Nandiyanto *et al*., 2016) . Bacteria has an enzymatic capacity to associate with hydrocarbon, making it effective to use in the degradation process of hydrocarbon complexes in oil-contaminated environments .

Time effect rate	*Sph.paucimabilis*	*B.licheniformis*	*P.fluoresence*	control	Treatment / Time
36.282	26.774^{c}	26.455^{c}	24.265^{c}	67.626	10
28.485	20.211^{b}	18.923^{b}	16.033^{b}	58.774	20
18.764	12.525^{a}	11.884^{a}	8.383^{a}	42.265	30
0.2804	19.337	19.091	16.227	56.222	Bacterial effect rate

It is noted from figure one that the ability of bacteria *P. fluoroscens* to degradation petroleum hydrocarbons at different incubation times, was the highest in the biodegradation process of crude oil over the rest of the two isolates *B.lichenoiformis* , *Sph. Paucimobilis* . Bacteria *P. fluoroscens* outperformed in terms of total concentration of TPH at a significant level, the highest percentage was 8.383mg/L at 30 days of incubation period. Whereas, bacteria *B.lichenoiformis* came next in terms of total concentration, as they recorded 11.884 mg/L at 30 days of incubation period, while bacteria *Sph. Paucimobilis* were the least in the removal process by 12,355mg/L and at the same incubation period. Where the longer the incubation period, the better the process of biodegradation of petroleum compounds, and these results are consistent with (Al-Dossary *et al*., 2019).

Figure (1) also shows the superiority of isolation *P. fluoroscens* over the rest of the isolates in terms of bio-fracture, as it recorded 80% at 30 days of incubation period, while isolation

B.lichenoiformis came after it, which recorded 72%, while isolation *Sph. Paucimobilis* recorded 70% for the same incubation period, and this confirms the longer the incubation period, the longer it was. This study is applicable to (Al-Hawash *et al.,*2018) when using two isolates of fungi to biodegradation the alphatic compounds of crude oil. The biodegradation process is better in terms of removal possessed by other microorganisms are their ability to express hydroxylase enzymes, namely hydrocarbon oxidizing enzymes, such as alcohol dehydrogenase and alkane hydroxylase which play a role in the biodegradation of hydrocarbon (Parthipan *et al*., 2017).

CONCLUSION

This study demonstrated the ability of laboratory-selected bacteria isolates to successfully remove petroleum hydrocarbons, due to their specialized enzymes in the breakdown of petroleum hydrocarbons, which demonstrated the ability of these isolates to successfully biodegrade and rid soil contaminated with these toxic compounds.

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This technology confirms that biological treatment is the best in terms of biodegradation, less cost and less damage to the environment by aiding microorganisms in all forms of pollution that occur .

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