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FACTORS AFFECTING BIOREMEDIATION OF PETROLEUM HYDROCARBONS BY BACTERIAL ISOLATES IN CONTAMINATED SOILS

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Abstract

This study was conducted to test the effect of different environmental conditions on the efficiency of the dominant bacterial isolation In contaminated soils on the consumption of crude oil By calculating the percentage of hydrocarbon consumption by bacteria as they were grown attemperatures of 20, 25, 30, 35, and various pH values of 4, 5, 6, 7, and 8.Two nitrogen sources were tested to find out the best nitrogen source for bacterial growth. Two phosphate sources were also tested to find out the best phosphate source for bacterial growth. The results revealed that a pH of 8 is optimal for these bacteria to consume hydrocarbons. For bacterial growth and biodegradation, the nitrogen supply $NaNO_3$, the phosphate sources K_2HPO_4 and KH_2PO_4, and the incubation temperature of 35°C are ideal.. Thus, we conclude from this study that environmental conditions are among the most important influences on the effectiveness of the biodegradation process and the removal of pollutants from soil contamination sites.

Keywords: oil-contaminated soil, biodegradation, bioremediation, petroleum hydrocarbons

1. Introduction

The development of technology in the twentieth century led to a sharp increase in oil consumption around the world. As oil is extracted in huge quantities from the ground, the extracted oil is transported by means of land transportation(Lai, 2001). It is transported freely or by long pipelines to different regions where it is exploited as a source of energy and raw material essential in various industries for many compounds and chemical products, and during the various stages of dealing with oil from extraction, transportation, and processing, errors may occur and lead to environmental pollution(Alsulami *et al.*,2014). The oil industry is behind many of the environmental problems that the soil is exposed to, resulting from spills and

contamination of soil surfaces with petroleum materials. Man's negligence and his pursuit of modern technology have disturbed the natural balance of the surrounding environment (Abdul-Ameer *et al.*, 2019). As a result, it contributed to the pollution of the water and air, as well as the degradation of agricultural soil. Because it has such negative effects on both people and the environment, soil pollution with petroleum is a significant issue that is currently receiving attention on a global scale. Numerous chemical and physical procedures have been employed to remove petroleum pollution from soil as there is an increasing interest in protecting ecosystems. However, these techniques are pricey(Farid *et al.*,2020).

This is what makes thinking about finding solutions to get rid of the seriousness of this pollution in a healthy way one of the most important things to do to ensure the health and safety of the environment. It was discovered that the best method for analyzing oil is to utilize bacteria that can utilise the carbon molecules in the oil as a source of energy. This way, oil pollution of the soil can be prevented, such as its use in marine oil slicks, and there are many types of bacteria that have the enzymatic ability to use petroleum hydrocarbons as nutrients, converting them into carbon dioxide and water, along with cellular materials such as proteins and nucleic acids, and thus the dissolution of crude oil hydrocarbons and their decomposition, which is a great benefit to transform them into other varieties that are less toxic and less dangerous (Couto *et al.*, 2010; Liu *et al.*, 2010).

2. Materials and methods

2.1. Sampling

I followed the method (Latha and Kalaivani, 2012). to collect samples of soil contaminated with crude oil surrounding the damaged conveyor pipe in the Al-Faw area, south of Basra Governorate, where the soil were taken 5-15 cm below the surface of the soil.

2.2. crude oil

The Basra crude oil utilized in the bio-fracturing research was acquired from the Basra Oil Company's PS1 station in the northern Rumaila site, and it was stored in sterile, dimly lit bottles in the refrigerator until laboratory tests were performed on it.

2.2. Isolation bacteria

1 g of soil was extracted, put in a 250 ml beaker with 100 ml of MSM media made in the lab that included 1% crude oil, and then cultured for 10 days in a shaker incubator at 150 rpm and 30 °C (Chaillan *et al.*, 2004). then then 0.1 ml of were withdrawn and

distributed on Petri dishes containing Nutrient Agar medium and incubated for 24 hours at 37 °C .

2.3. Identification of isolates

The isolated colony's shape and pigmentation, as well as its staining responses, were studied.

2.4. . Isolation of DNA

Use of a wizard genomic DNA extraction kit from Promega.

2.5. 16S_rRNA gene sequences

The isolated strains' taxonomic characteristics were determined by looking at the 16S rRNA gene. Using the CTAB technique, the bacterium's whole DNA was isolated (Winnepenninckx et al., 1993). The bacterial 16S rRNA loci were amplified using the domain-specific forward primer Bac27 F (5, -AGAGTTTGATCCTGGCTCAG-3,) and the all-purpose reverse primer Uni 1392R (5, - GGTTACCTTGTTACGACTT -3,). A total of 50 µl were used for the amplification reaction, of which 2 µl were used for forward and reverse transcription as well as 2 µl of DNA in accordance with the procedure. (Miyoshi *et al.* 2005)

2.6. Amplification of 16S_rRNA gene

Observe the PCR series program for bacteria. initial denaturation at 95 °C for five minutes and one cycle (denaturation at 95 °C for thirty seconds) 30 seconds of annealing at 55 C 30 seconds of extension at 72 C a 35 cycle Final prolongation 72 C 1 cycle takes 5 minutes. By electrophoresis of the PCR amplification products in 1% agarose gel generated with the previously prepared conditions, the amplified genes' PCR amplification products were confirmed. In the laboratories of the Korean Macrogen Company, purification and analysis of the DNA sequences of the 16S_rRNA ribosomal gene were done with the goal of diagnosing bacterial isolates for the current study.

2.7. Preparing of bacterial inoculum

Nutrient broth was prepared, inoculated with bacterial isolates, and incubated in the Sheker incubator for 24 hours at a temperature of 30 °C and a rotational speed of 150 revolutions per minute.

2.8. screening first for oil degradation

50 ml of sterile mineral salt media (Table 1) containing 1% v/v crude oil was added to each of the 250-ml conical flasks before being infected with bacteria. (Rahman *et al.*,2003). Uninoculated flasks were used as controls for the experiment, which was performed twice to account for abiotic losses. For 10 days, all flasks were incubated at 30 °C. Gas chromatography was used to determine residual crude oil concentrations(Röling WFM *et al*,.2004).

Table1. Mineral salt medium

The Substances	Quantity
NaCl	0.5
KH_2PO_4	1
$K_2HPO_4.3H_2O$	1
NH_4NO_3	1
$MgSO_4.7H_2O$	0.025
$(NH_4)_2SO_4$	0.2

2.9. Growth of isolated bacteria on crude oil

The technique was used (Patel *et al.*, 2012.) 100 mL of prepared MSM medium was introduced to 250 mL conical flasks, along with 1 mL of a bacteria culture, before the pH was adjusted and 1% of crude oil was added to determine how sensitive various bacterial species were to crude oil cracking. at 7, and then for 10 days at a rotating speed of 150 rpm and a temperature of 33. On the consumption of hydrocarbons, the impact of variations in pH, temperatures, and phosphate and nitrogen sources was investigated. The growth of the bacterial isolate under research was tested at various temperatures of 20, 25, 30, and 35 degrees Celsius. And examine the impact of various values of PH 4, 5, 6, 7, and 8. Each sample had its pH adjusted before being incubated at 30°C for 10 days. When these bacteria were cultured on crude oil, multiple sources of phosphate and nitrogen were utilized for the same incubation period to assess their capacity to grow and degrade at the start of the experiment(Al-Dossary *et al* .,2020).

2.10. Oil Degradation

A mineral salt medium was used, and 250-ml conical flasks containing 50 ml of medium were used to examine oil degradation. Media were inoculated with bacteria and shaken at 150 rpm in an incubator for 10 days at 30 °C. (Oudot, 1984).

2.11. Estimating the percentage of removal

Utilizing an equation, removal percentage was estimated:

$$\textbf{Degradation\%}=\frac{mg\ of\ crud\ oil\ control-mg\ of\ crud\ oil\ test}{mg\ of\ crud\ oil\ control}\times 100$$

2.12. Crude oil extraction from test flask

To extract petroleum hydrocarbons from the liquid media after the end of the incubation period, the method described by Chaillan *et al*., (2004) was followed with some modifications, as the MSM liquid growth medium was transferred to the separation funnel and chloroform was added at a ratio of 1:1, then the separation funnel was shaken well for more than Once the pressure of the gas generated inside the separation funnel was removed, the sample was left to settle and separate into two layers: the layer at the top contains water and the medium, and the layer at the bottom contains petroleum hydrocarbons. anhydrous sodium, which absorbs water and any remaining impurities from the sample and receives the liquid coming from the separation column in a glass beaker.

3. Results and Discussion

3.1. Identification of bacteria

Some of the DNA molecules that surfaced as a result of ultraviolet light were used to identify the bacterium that first appeared. The outcomes of bacterial isolate DNA amplification by polymerase chain reaction (PCR) were also provided. In comparison to the DNA ladder, the base size employed by the gene primers (F-27 and R-1392) was 1500 base pairs (bp) Fig (2). The 16S rRNA ribosomal gene's nitrogenous base sequences were to be processed by the Korean business. where the outcomes of bacterial isolation were displayed after being compared using the BLAST algorithm. the DNA nucleotide sequences of PCR-amplified genomic DNA and identify related species *Bacillus pumilus* accession No. OP703527. Fig (1).

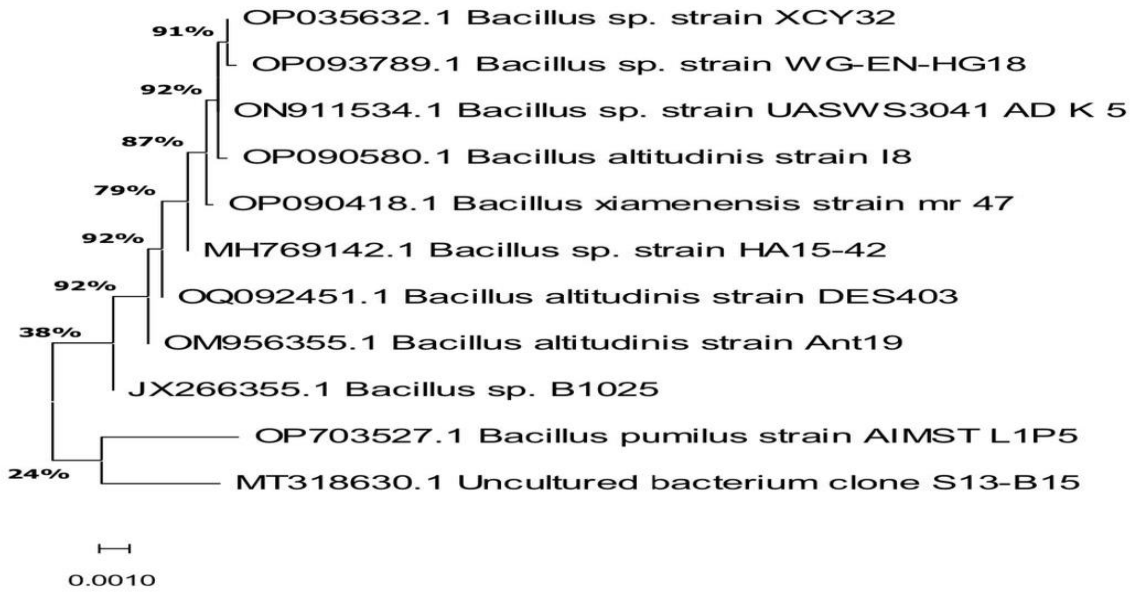

Fig . 1.phylogenetic tree of the bacteria strain

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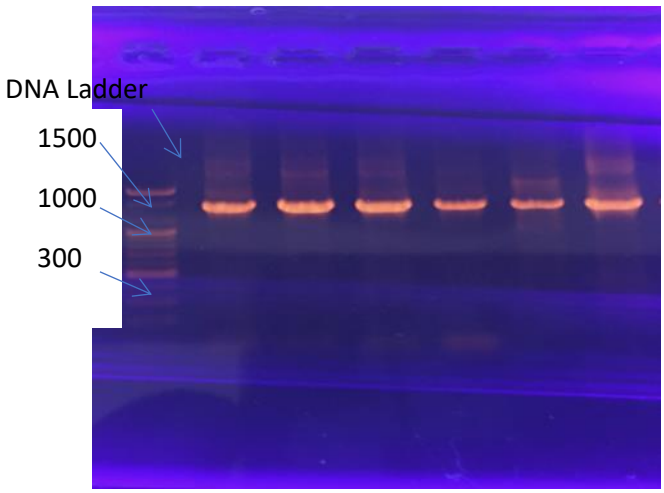

Fig. 2.DNA from FB1 strain pure culture was extracted, and the PCR reaction was analyzed using agarose gel electrophoresis.

3.2. Biostimulation of TPH degradation

The procedure of biostimulation includes modifying the environment to encourage the development and enzymatic activity of the preexisting microorganisms. The ideal circumstances, which are 35°C, pH 8, and nitrogen source$NaNO_3$ and phosphate source.

Bacillus pumilus had the highest rate of degradation (69%). This species' capacity to manufacture multiple types of degradative enzymes in large quantities may boost its capacity to break down TPH compounds. To investigate the impact of temperature, pH, nitrogen source, and phosphate source on the biodegradation process, the bacterium B. pumilus was used. total hydrocarbons throughout a 10-day period. One of the most crucial requirements The temperature that can impact how quickly bacteria multiply is (Delille, Coulon, & Pelletier, 2004). In this study, B. pumilus was grown of temperatures between 20 and 35 degrees Celsius, with the optimum temperature for growth at 35 degrees Celsius. And the degradation is at 20 °C, with the biodegradation of TPH at 15% for treatment. At 20 °C, the degradation increased slightly to 26%, while at 30 °C, the degradation increased significantly to 59%. to cure at 35 °C, which presents 62% degradation. This is consistent with the studies of Venosa & Zhu (2003) and Vinothini *et al.*, (2015), in addition to the fact that heat has a role in the physical and chemical change of the hydrocarbon compounds that make up crude oil, as well as the fact that in environments with a temperature ranging from medium to high, the degrading process is better. and faster than cold environments with low temperatures Fig (3).

The bacteria results showed that raising the pH of the mineral salt medium of *Bacillus pumilus* to 8 improved the growth of bacteria and the bio degradation process of crude oil, as the percentage of cracking increased to 62% Fig (4).

Also, the pH has a clear effect on the biodegradation. On the one hand, the pH affects the enzymatic system of the oil- degrading microorganisms directly. Each type of microorganism needs a pH that is suitable for the effective performance of its enzymatic activity and growth, in addition to its indirect effect on the nature of the pollutants and their chemical composition and its availability in the environment (Atlas, 1981).

The findings demonstrated that the addition of the inorganic nitrogen source, $NaNO_3$, to the mineral salt medium of *Bacillus pumilus* enhanced bacterial growth as opposed to the other nitrogen source, peptone, which had no such effect which had no such effect at 63%. Fig (5).

This is in line with research on the best sources of nitrogen, its impact on bacterial growth, and how that relates to the biodegradation process by Wu *et al.*, (2016) and Wang *et al.*, (2018).

The analysis revealed that after the 10-day incubation period, the two phosphate sources—NaH_2PO_4 and Na_2HPO_4—that were added to the mineral salts medium for bacteria did not significantly improve the bio-cracking of crude oil; instead, the cracking rates were 28% and 25%, respectively, compared to the phosphate source in the special mineral salts medium. The Bacillus sp. FB1 strain, with a crushing rate of 58%, had the highest rate Fig (6).

This is in line with studies on the utilization of various phosphate sources and their impact on bacterial growth and the biodegradation process conducted by (Enon *et al.*,2011 and Nkeng *et al.*,2012)

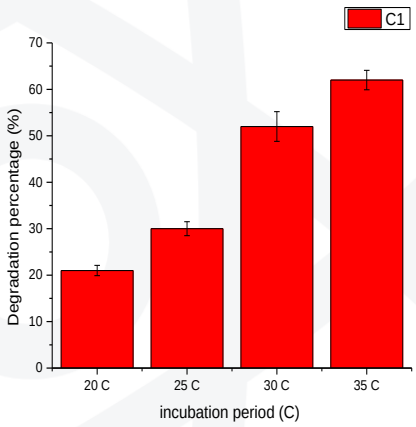

Fig (3) effect of temperature

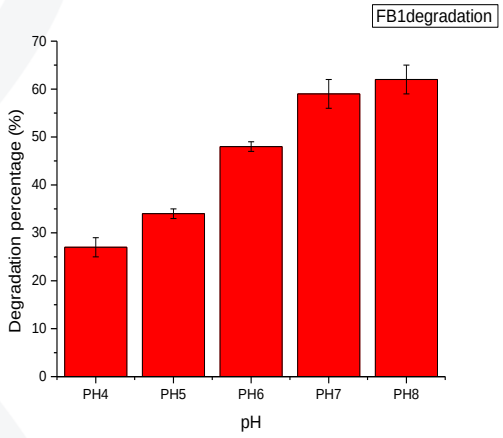

Fig (4) effect of the pH

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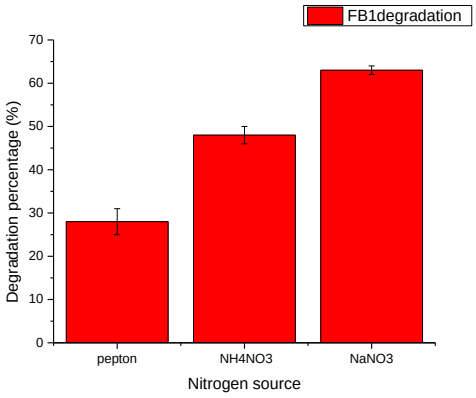


Fig (5) effect of nitrogen sources

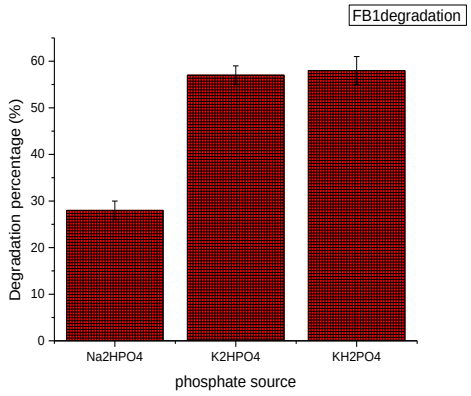
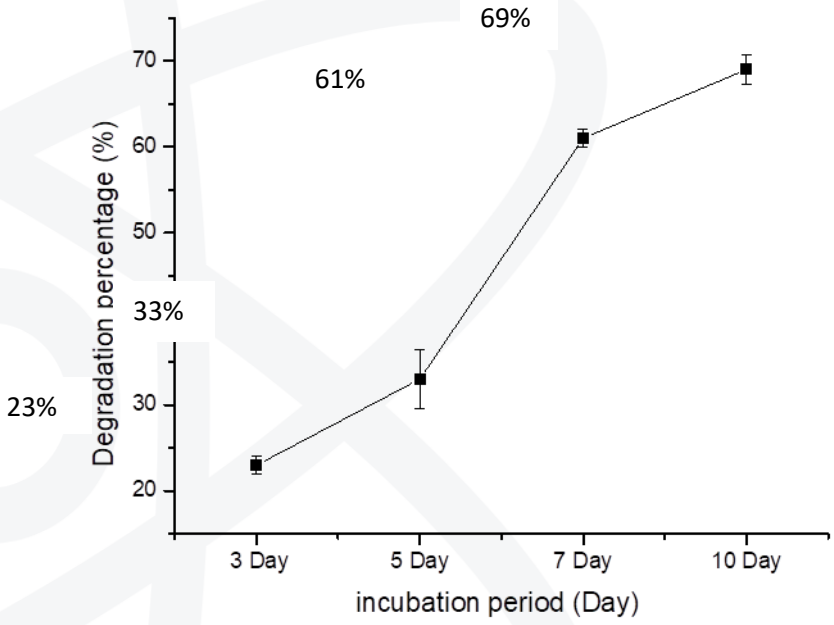


Fig (6) effect of phosphate sources

3.3. Optimization of TPH degradation using *B. pumilus*

The bio biodegrading rate was increased to 69% at the end of the 10-day incubation period when the best conditions, which had been tried and tested to improve the biodegrading process, were used. These circumstances included the phosphate supply KH2PO4 and K2HPO4, the nitrogen source NaNO3, the pH of 8, and an incubation temperature of 35 C.According to Tahseen *et al.* (2016), who demonstrated in their study that adding nutrients to oil-contaminated soil enhanced the growth of oil-breaking bacterial strains, increased their enzymatic activity, and gave the bacteria more effective performance in removing oil pollutants in a shorter amount of time, the study concurs with their findings Fg.7.



Fg.7. Biodegradation test in growth-friendly conditions

4. CONCLUSIONS

The bacteria B. pumilus showed strong degradation ability after 10 days of incubation and was able to process 63% of the total petroleum hydrocarbons. Biostimulation has a significant effect, as represented by the conditions of temperature, pH, nitrogen, and phosphate supply, which have an impact on the biodegradation process, as more than 69% of the total petroleum hydrocarbons decompose after 10 days of incubation. Our study indicates that B. pumilus may act as a biodegrading agent, and PH bioremediation can be used to clean up petroleum-contaminated soils.

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