

BIODEGRADATION EFFICIENCY OF *BACILLUS sp* ISOLATED FROM OIL CONTAMINATED SOIL IN LIBYA

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Abstract: Thirteen bacterial isolates were isolated from oil contaminated soil samples of oil field (59 Jallo) in Libya four of them showed increased growth turbidity (O.D 600nm). From the above isolated *Bacillus sp* was taken for the present study. Biodegradation activity was tested by liquid and solid CFMM enriched by petroleum (1%,2%) and benzene (1%, 2%). The growth potential of isolated *Bacillus sp* on petroleum and benzene was observed which was depended on time and concentration of carbone source. Biodegradation activity was determined also by liquid culture characterization.
Key word: oil biodegradation ; oil contaminated soil; bacteria isolation; benzene; bioremediation

1. INTRODUCTION

Due to widespread use of petroleum products, the number of petroleum contaminated sites has abounded. Natural attenuation, which relies on in situ biodegradation of pollutants, has received a large amount of attention, especially for petroleum contamination. (Mashreghi and marialigeti, 2005, Okoh.A.I.,2006., Obire,O. and I.v.Okudu 1997 Abed, R.*et al* 2006) Biodegradation is a biologically catalyzed reduction process of complex chemicals. This process, performed by a variety of bacteria, fungi and yeasts, transforms potentially toxic compounds into non-toxic compounds to obtain energy and nutrients (Alexander,1994; Scragg,2001)

Oil spills have become a global problem particularly in industrialized and developing countries, apparently inevitable spillages, which occur during routine operations of crude oil production, refining, distribution and as consequence of acute accidents, have generated continuous research interest in this field. (Mittal and Singh, 2009) It is well known that microbial degradation of spilled hydrocarbons is a major technique in the natural decontamination process therefore, various bacteria degrading hydrocarbons have been isolated. (Gomare and Lahane, 2011) Most of the microorganisms in the environment are alkane-degraders (Roubal and atlas, 1978; Foght *et al.*, 1990), Isolates that can degrade both aliphatic and aromatic hydrocarbons that are highly desirable; especially since crude oil comprises mostly alkanes (Atlas, 1981) monocyclic hydrocarbons (Scragg, 2001), aromatic hydrocarbons (Foch and Westlake, 1987), resins and asphaltene (Leahy and Colwell, 1990). The aim of this study was to explore the biodegradation efficiency of isolated bacteria (*Bacillus sp*) from Libyan oil contaminated soil sample.

2. MATERIAL AND METHOD

2.1 Collect the samples

Thirteen Soil samples were collected from several locations at 59 Jalu oil field in Libya, samples collected at a depth within 5cm from the surface of the soil. The soil samples were collected in pre-sterilized sample bottles, then carefully transferred to the laboratory and stored at 4°C aseptically before processing.

2.2. Isolation and identification of Bacteria

Isolation was performed by soil dilution, 1 g from soil was suspended separately in 10 ml of distilled water, and a serial dilution-agar plating technique on CFMM agar with 1% benzene as carbon source. The plates were incubated at 37°C for 24hrs. For purification of the isolates, selected colonies of bacteria were transferred from mixed culture plates onto respective CFMM agar plates containing crude oil and incubated at 37°C for 24hrs, before stored at 4°C for examination.

Carbon Free Minimum Medium (CFMM) used in this study composed of (g / L): NH₄NO₃ 3.0, CaCl₂.7H₂O 0.005, KH₂PO₄ 0.8, Na₂HPO₄ 2.2, MgSO₄.7H₂O 0.01. (Ebrahimi *et al.*, 2012).

The organisms were further identified using the Phoenix. The isolates were characterized based on cultural characteristics, cell morphology and biochemical characteristics.

2.3. Biodegradation test

Utilization of hydrocarbon sources were detected in CFMM broth supplemented with (1%,2%) benzene, (1%,2%) petroleum .In broth assay , the inculation was performed in 250ml flasks ,flask containing 100ml CFMM without carbon source as (-) control , and another flasks containing 100ml CFMM with carbone source ,benzene for one set and petroleum for another set. Bacterial inoculums was added to each flasks and inoculated .

Bacterial growth activity considered an indicator for utilization and bioremediation of benzene and petroleum .The microbial count was monitored by the change of turbidity of culture by spectrophotometer (OD 600nm).

3.RESULTS

Table 1. Characterization of isolated bacteria

Isolated	Colony color	Colony size	Colony form
<i>Bacillus pumilus</i>	Beige	Small	irregular
<i>Staphylococcus vitulins</i>	Yellow	Medium	Circular
<i>Paenibacillus alvei</i>	Orang	Small	Circular
<i>Bacillus circulans</i>	Orang	Small	Circular

Table 2. Biochemical tests

Test	result
Gram stain	+
Oxidase test	+
Catalase test	+
Indole ring	+
Motility test	+

Positive= (+) Negative = (-)

Table 3. Antibiotics sensitivity

Antibiotic	Inhibition zone diameter
Vancomycin	3.4 cm
Tetracyclin	1.4 cm
Amoxicilli	1.3 cm
Chloramphene	1 cm
Penicillin G	-
Ampicillin	-
Clindamycin	-

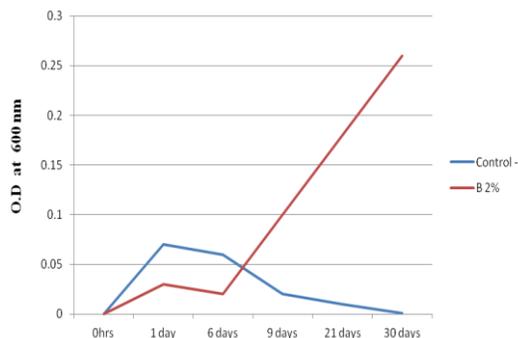
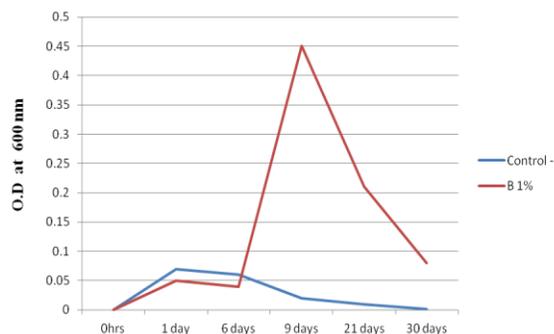


Figure 1. The growth curve (O.D values) of *Bacillus pumilus* in Benzene and control (-) at different incubation time

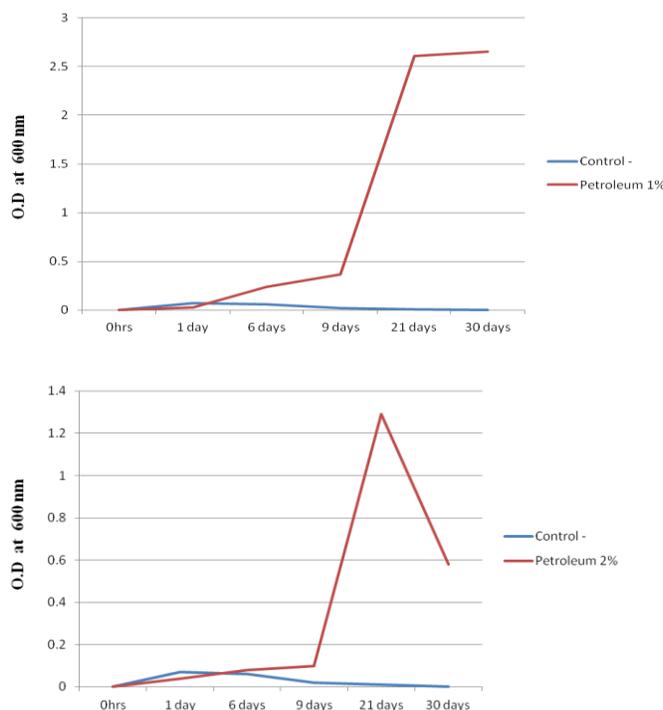


Figure 2. The growth curve (O.D values) of *Bacillus pumilus* in Petroleum and control (-) at different incubation time

4. DISCUSSION

Survival of microorganisms in petroleum hydrocarbons medium after their inoculation is a key deciding factor in the rate of biodegradation of hydrocarbon either in soil or in liquid phase (Ramos et al., 1991; Burger, 1993; Aouad and Abbouni, 2012; Kishore and Ashis, 2006). Since, all the bacteria in the present study were isolated from a petroleum contaminated soil and water sample, they survived and adapted the oil contaminated soil/ liquid environment very easily as also reported by other authors (Rahman et al., 2003; Sugiura et al., 1997). This was evident from result increase in the population of *Bacillus* as compared to control.

In the flasks with *Bacillus pumilus* increase absorbance was related with the microbial growth as well as with the increase of water solubility of petroleum hydrocarbons as a result of transformation of petroleum due to the bacterial degrading activity. (Ilyina et al., 2003)

Decreasing of bacterial population in some cases may be explained by composition of hydrocarbons and its concentration which at initial step bacteria

faced with a shock and stress and gradually adaptation mechanism in bacteria and induction of some mechanism which help to survive and growth in new condition (Abd-Elsalam et al., 2009) cause an increasing trend in bacterial number. Reversely in some condition such as high concentration of hydrocarbons and its toxicity furthermore limitation of oxygen and nutrition cause to inhibit biodegradation (Leahy and Colwell, 1990). Increasing time of incubation usually cause to increase of bacterial growth (Abd-Elsalam et al., 2009; Shafiee et al., 2006; Joeng-Dong et al., 2005) while in other experiments at the end of incubation period were reduced the number of microorganisms (Mehrasbi et al., 2003) (Ebrahimi et al., 2012).

Biodegradation proceeds most quickly when the oil is emulsified into small droplets (Munn, 2004) (Aouad and Abbouni, 2012).

The presence of oil-degrading organisms in the polluted soil and water is clear indication that the indigenous microbes were carrying out their metabolic activity. The activities of these microorganisms could be responsible for the bioremediation of the environment.

5. CONCLUSION

In present study the utilization or degradation potential of benzene and petroleum by isolated bacteria from petroleum pollution sites was performed in soil and liquid assay. The isolated bacterial strains were identified as: *Bacillus pumilus*, *Staphylococcus vitulins*, *Bacillus circulans* and *Paneobacillus alvei*, from the four isolated bacteria *Bacillus pumilus* was screened for their growth capacity in media contained petroleum or benzene as sole carbon source using spectrophotometer CE 7500 at O.D 600nm, the results show that *Bacillus pumilus* is are capable to grow in CFMM that were contained with benzene or petroleum as sole carbon source which was evidence of hydrocarbons biodegradation. The activity of these bacteria could be responsible for the bioremediation of the polluted environment with oil spills

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