International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.8, pp 245-249, 2015

Microbial Degradation of crude oil by *Bacillus* sp. - A Bioremedial approach

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Abstract: Water contamination due to oil leads the formation of hazardous environment for the aquatic living systems. The basic reason for this type of pollution is the immediate discharge of waste from household, improper treatment of waste water from industries etc. Among these, effluent from petroleum industries exhibited most deterious effect. Bioremediation is the simple and eco-friendly mechanism to solve this environmental issue. In this study, *Bacillus* sp. SM-5 collected from petroleum contaminated soil and used for degradation study. Maximum degradation (79 %) was found at 35 °C, pH at 6.5 with 1 % of crude oil after 30 d of incubation. But percentage of degradation was decreased with increasing oil concentration.
Keywords: Crude oil, *Bacillus* sp., oil degradation, temperature, pH.

Introduction

Crude oil contamination of water and soil poses a severe ecological and environmental threat. This problem is of great concern to the oil industry. Oil refineries generate huge volume of oily sludge and effluent during the refining of crude oil[1] . Extensive petroleum hydrocarbon exploration activities often result in the pollution of environment which will lead to disastrous consequences for the biotic and abiotic components of the ecosystem[2,3] . Improper disposal of this oil contaminated water causes serious hazard to the ecosystem. The traditional treatment of oily wastewater, such as containment and collection using floating booms, adsorption by natural or synthetic materials, etc., cannot degrade the crude oil thoroughly[4] . Thus biodegradation or bioremediation is the promising and effective alternative approach to environment cleanup. Bioremediation employs microorganisms capable of degrading toxic contaminants[5,6] . Augmenting the contaminated site with an appropriate inoculum of microorganisms is a promising technique to enhance the biodegradation of hydrocarbons[1] . Biodegradation by microorganisms is more favourable than chemical treatment for dealing with oil pollution since the microbes modify crude oils in beneficial ways and the end products are environmentally safe to all living things[7] .

Petroleum hydrocarbon can be degraded by various microorganisms such as bacteria, fungi and yeast[8] . Biodegradation has been considered as a natural process in the microbial world as carbon and energy source for their growth and takes a key role in the recycling of materials in the natural ecosystem[9] , hence the microbes will utilize this crude oil as organic carbon and energy sources. The enzymes produced by the microorganisms depolymerise these toxic polymers to non-toxic oligomers or monomers. The microbial utilization of hydrocarbon was highly dependent on the chemical nature of the components within the petroleum mixture and environmental determinants[10] . Furthermore, the application of bacterial isolates in degrading crude oil involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate[11] .The objective of this preliminary study was to determine the optimum conditions for the biodegradation of crude oil.

Experimental

Collection of sample

The crude oil contaminated soil sample was collected from oil refineries, Tamil Nadu, India, form 2-3 cm depth and kept in sterile container for further use.

Bacterial isolation and Screening

Isolation and screening of bacteria was done by enrichment culture techniques. One g of soil sample was mixed with 10 ml of saline water and kept for homogenization for 10 min. The soil suspension was serially diluted and plated on mineral salt medium (MSM) containing 1 % (v/v) of crude oil. The composition of mineral salt medium as follows (g/L): Urea 2.0; Na_2HPO_4 3.61; $(NH_4)_2SO_4$ 2.0; KH_2PO_4 1.75; $MgSO_4.7H_2O$ 0.20; $CaCl_2.2H_2O$ 0.05; $FeSO_4.7H_2O$ 0.01; $CuSO_4.5H_2O$ 0.005; H_3BO_3 0.01; $MnSO_4.5H_2O$ 0.01; $ZnSO_4.7H_2O$ 0.07; MoO_3 0.01; n-hexadecane 10, pH adjusted at 7. The MSM oil plates were incubated at 37 °C for 48 h. The maximum grown colonies were and pure culture was maintained at 4 °C.

Identification of bacterial isolates

The isolated bacteria were identified based on their shape, morphology and physiological characters. The physiological characters are evaluated by some biochemical test, such as, indole test, citrate utilization test, methyl red test, Voges-Proskaeur test. The results of these test was compared with Bergey's Manual of Systematic Bacteriology[12].

Biodegradation of oil

The experiment was carried out in 500 ml Erlenmeyer flasks filled with 250 ml of sterile mineral salt medium with 1 % crude oil. The bacteria were inoculated. The flasks were then incubated in a shaker at 150 rpm at 37 °C for 30 d. At every 5 d intervals, the total hydrocarbons in the treatments were determined spectrophotometrically[13] . Sample (5 ml) was mixed with equal volume of carbon tetra chloride to extract hydrocarbons from the sample. The extracted hydrocarbons were detected spectrophotometrically at 420 nm. A standard curve prepared using known concentrations of crude oil was used to estimate the amount of hydrocarbons in the sample. Degradation was estimated as the difference between the initial and final concentrations of total hydrocarbons.

Effect of temperature, pH and concentration of crude oil on biodegradation

The percentage of degradation was influenced by temperature. The rate of biodegradation of crude oil was estimated different temperature, ranges from 25 – 45 °C. The bacterial isolate was incubated at each specified temperature in MSM media with 1 % crude oil for 30 d. The effect of pH was evaluated by incubating the culture at optimum temperature with various pH (4-9) for 30 d. The pH of MSM media with crude oil (1 %) was maintained using 1N HCl and 1M NaOH. To maintain the pH, citrate – phosphate buffer (pH 4– 6), phosphate buffer (pH 7 and 8), and carbonate – bicarbonate buffer (pH 9) were used[14] . Every 5 d interval pH was checked. The optimum pH for maximum oil degradation was determined. Similarly the influence of concentration of crude oil was studied by varying the concentration of crude oil (1, 5, 10, 15 %) in MSM at optimum temperature and pH for same incubation period. All the experiments were done in triplicates.

Results and Discussion

It is particularly important to address oil polluted waters as soon as possible as the contamination can have the potential to damage fishery resources and affect the health of those animals and humans that consume contaminated fish[15]. Degradation of hydrocarbons by the microorganisms is more environmentally benign than the physical and chemical methods in term of its end products. Thus bioremediation is the better options for oil cleanup. In this study, bacteria was isolated from oil contaminated soil samples, identified and used for biodegradation studies. The efficacy of bioremediation was enhanced by optimizing the environmental factors.

Screening and identification of bacterial isolate

The potent crude oil degrading bacteria was screened by enrichment culture technique using mineral salt medium. The isolated pure culture was identified to belong to the genera *Bacillus* based on the morphological and physiological characters (Table 1). The degrading strain was gram-positive, motile bacteria. It shows positive test for oxidase, catalase, Voges- Proskauer and negative test for urease, indole. The identified isolate was used for crude oil degradation.

Table 1. Morphological, physiological and biochemical characteristics of the isolated strain SM-5.

Characters	Results
Morphology	Rod shaped, gram +ve
Motility	+ve
Catalase	+ve
Oxidase	+ve
Voges- Proskauer	+ve
Hydrolysis of starch	+ve
Hydrolysis of urea	-ve
Formation of Indole	-ve
Nitrate reduction	+ve

Biodegradtion of crude oil

Crude oil degradation by *Bacillus* sp. was carried out for 30 d of incubation. Studies on the effect of temperature on bioremediation are shown in Figure 1. Maximum crude oil degradation was found at temperature of 35 °C. But decrease or increase in temperature from the optimum value reduces the microbial degradation. This may be due to the inactivity of the microbial enzymes responsible for degradation. Similar result was observed for pH (Figure 2). pH 6.5 was found more favourable by *Bacillus* sp. for hydrocarbon degradation.

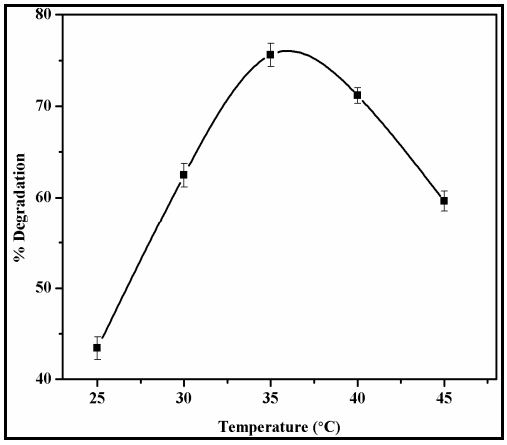

Figure 1. The effect of temperature on degradation of crude oil by *Bacillus* sp.

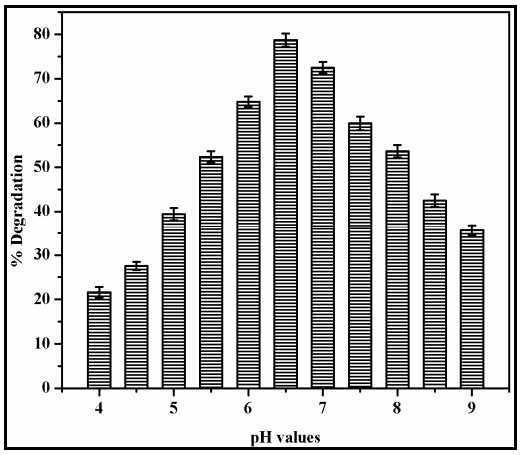

Figure 2. The effect of pH on degradation of crude oil by *Bacillus* sp.

The effect of crude oil concentration on bioremediation was evaluated and presented in Figure 3. Four different concentrations (1- 15 %) were tested. *Bacillus* sp. showed significant result at 1 % crude oil concentration. The degradation potential decreases at higher concentrations of crude oil.

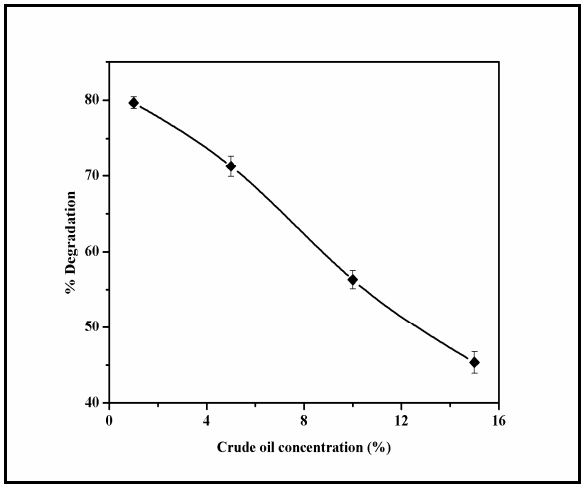

Figure 3. The effect of concentration of crude oil on degradation

Conclusion

Bioremediation is a simple and environment-safe approach to clean-up water contamination. The present study demonstrates that the oil contamination can be reduced by microbial degradation. Here *Bacillus* sp. was showed significant deterioration of crude oil at optimum temperature and pH. It suggests that the use of microorganisms under optimum conditions is a fast and effective method of biodegradation of crude oil.

References

1. Hadibarata T, Tachibana S. Microbial Degradation of Crude Oil by Fungi Pre-Grown on Wood Meal. Interdisciplinary Studies on Environmental Chemistry — Environmental Research in Asia, 2009, 317-322.
2. Ockoh AI. Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. Biotechnol Mol Biol Rev., 2006, 1(2); 38-50.
3. Mueller JG, Resnick Sm, Sheltton ME, Pritchard PH. Effect of inoculation on the biodegradation of wathered Prudhoe Bay crude oil. J Indst Microb., 1992, 10; 95-102.
4. Ollis D. Slick solutions for oil spills. Nature. 1992, 358; 453-454.
5. Bossert I, Bartha R. The fate of petroleum in soil ecosystem. In Petroleum Microbiology, ed. by Atlas, Macmillan, New York, U.S.A, 1984, 435-473.

6. Eriksson M, Dalhammar G, Borg-Karson A-K. Aerobic degradation of hydrocarbon mixture in natural contaminated potting soil in indegenous microorganisms at 20°C and 6°C. Appl Microbiol Biotechnol., 1995, 51; 532-535.
7. Hamzah A, Rabu A, Azmy RFHR, Yussoff NA. Isolation and Characterization of Bacteria Degrading Sumandak and South Angsi Oils, Sains Malays., 2010, 39(2); 161-168.
8. Chaillan F, Fleche AL, Bury E, Phantavong Y, Grimont P, Saliot A, Oudot J. 2004. Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. Res Microbiol., 2004, 155; 587-595.
9. Albertsson AC, Andersson SO, Karlsson S. The mechanism of biodegradation of polyethylene. Polym Degrade stab., 1987, 18; 73-87
10. Atlas RM, Bartha R. Stimulated biodegradation of oil slicks using Oleophilic fertilizers. Environ Sci Tech., 1973, 7; 538-541.
11. Vidali M. Bioremediation: An overview. Pure Appl Chem., 2001, 73; 1163-1172.
12. Sneath PHA, Mair NS, Sharpe ME, Holt JG. Bergey's Manual of Systematic Bacteriology, vol. 2. Williams and Wilkins, Baltimore, US, 1986, 965-1599.
13. Odu CT, Nwoboshi LC, Esuruoso OF. Environmental Study of the Nigerian Agip oil Company Operation Areas. in Proceedings of the International Seminar on the Petroleum Industry and the Nigerian Environment, Kaduna, Nigeria 1985, 274-283.
14. Colowick SP, Kaplan NO. Methods in Enzymology, Academic Press, New York 1995, 142-155.
15. Margaret MK, John ES. Assessing Exposure of Marine Biota and Habitats to Petroleum Compounds. Analytical Chemistry News & Features, 1998, 186-192.

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