Bioremediation On Oil Spills Using Bacterium (*Pseudomonas Putida*)

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Abstract- This research work is designed to evaluate the capacity of the bacterial species, Pseudomonas putida in bioremediating and biodegrading of hydrocarbons which are the major constituents of petroleum oil. The bacterial species was checked in the laboratory condition, in order to determine and test for its capabilities in bioremediation process in oil spills. The total counts and cfu of the bacterial species result were carried out after first and second weeks of inoculation, and was found to be significant. Solvent extraction method was primarily used to determine the percentage of oil. The Pseudomonas putida degraded 6.425% of the oil after two weeks. The results showed that Pseudomonas putida has the capabilities of degrading and bioremediating hydrocarbons contaminants from water bodies. Bioremediation is advantageous due to its time and cost saving than physical method, also unlike chemical method; no foreign or toxic chemicals are added to the site.

Indexed Terms- Oil Spills, Hydrocarbons, Bioremediation, Pseudomonas putida

I. INTRODUCTION

Global pollution is increasing, due to the variations in natural and anthropogenic activities leading to contamination of various terrestrial and aquatic ecosystems with heavy metals,inorganic and organic compounds. Controlled and uncontrolled discharge of solid and liquid wastes, use of agricultural fertilizers, herbicides, insecticides and sewage disposal, explosives and accidental or intentional spillages, are some of the main contributors of alarmingly increased content of various contaminants in the biosphere. Industries such as textiles, electroplating, tannaries and refinaries are recognised as a serious environmental threat all over the world (Tariq *et al.*, 2005; Safiyanu, *et al.*, 2015).

Accidental or intentional oil spills has a deep impact on the environmental pollution.Oil spills from oil tankers and from distant oil spills, have been recognized as a major environmental hazard. The spilled oil is believed to destroy the habitat of seabirds, marine mammals and fish.The thick and gummy crude oil discharges can cause immediate harm to fish and wildlife, degrade oceans and coastal habitats, and over time, even threaten human health (Agarwal *et al.*, 2002; Narayani, 2010; Safiyanu *et al.*, 2015).

Environmental contamination by crude oil is relatively common because of its widespread use and its associated disposal operations and accidental spills. The term petroleum is referred to an extremely complex mixture of a wide variety of low and high molecular weight hydrocarbons. This complex mixture contains saturated alkanes, branched alkanes, alkenes, napthenes (homo-cyclic and hetero-cyclic), aromatics (including aromatics containing hetero atoms like sulphur, oxygen, nitrogen, and other heavy metal complexes), naptheno-aromatics, large aromatic molecules like resins, asphaltenes, and hydrocarbon containing different functional groups like carboxylic acids, ethers, etc. Crude oil also contains heavy metals and much of the heavy metal content of crude oil is associated with pyrrolic structures known as porphyries (EPA, 2006; Singh, 2007; Nilanjana and

Preethy, 2011; Shukla and Cameotra, 2015; Safiyanu *et al.,* 2015).

There are mechanical, chemical, and biological methods for clean-up oil spills. Mechanical methods include booms, skimmers, and truck vacuums. Chemical methods include dispersants, surface washing agents, and surface collecting agents. Biological methods (Bioremediation) are the use of microbiological cultures, enzyme additives, and nutrient additives to increase the rate of biodegradation of the contaminants (EPA, 2006; Nilanjana and Preethy, 2011; Safiyanu *et al.,* 2015).
Hydrocarbons in the environment are primarily biodegraded by bacteria and fungi. The reported efficiency of biodegradation ranged from 6% to 82% for soil fungi, 0.13% to 50% for soil bacteria and 0.003% to 100% for marine bacteria. Many scientists reported that mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons (EPA, 2006; Nilanjana and Preethy, 2011; Safiyanu *et al.,* 2015).

Bioremediation can be used by bacterial species, plant species (a process called *phytoremediation)* and fungal species (a process called *mycoremediation*) (Saptakee, 2011; Sarang *et al.,* 2013; Safiyanu *et al.,* 2015).

Bioremediation using bacterial species can include using *Pseudomonas species* which are potent bacteria that are capable of degrading hydrocarbons from petrol and diesel, thereby reducing the impact of oil spills. *Pseudomonas alcaligenes* is capable of breaking down polycyclic aromatic hydrocarbons while *Pseudomonas mendocina* and *Pseudomonas putida* can remove toluene. *Pseudomonas veronii* can degrade large number of aromatic organic compounds. These oil-based compounds are eaten up by the bacteria as they utilize them as substrates for carrying out metabolism. These microorganisms occur in abundance in water bodies and soil and are effective in cleansing oil spills. With an increase in density of these microorganisms, the process of bioremediation is also accentuated. Other Bacteria that help in bioremediation are *Achromobacter, Flavobacterium, Acinetobacter, etc* (EPA, 2006; Sarang 2013; Olanipekun, 2015; Safiyanu *et al.,* 2015).

Pseudomonas putida has the ability to degrade and remove toluene and other monocyclic aromatic hydrocarbons, e.g., benzene, toluene and xylene. The bacterial degradation of aromatic hydrocarbons, normally involves the formation of a diol followed by cleavage of aromatic ring and formation of diacid such as *cis-cis* muconic acid (Sarang *et al.,* 2013; Olanipekun *et al.,* 2015). The aim of the research is to evaluate the capacity of the bacterial species (*Pseudomonas putida*) in bioremediating and biodegrading hydrocarbons which are the major constituents of petroleum oil.

II. MATERIALS AND METHODS

- Culture and Engine Oil Collection: A pure culture of the *Pseudomonas putida* was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Sector-39A, Chandigarh-160036, India, While the Engine Oil was obtained from Tugal pur Market, Greater Noida, Knowledge park III, UP, India.

- Screening Test

After revival of the culture, by subculturing, plating and incubation.The incubations was done at appropriate temperature and under condition recommended by MTCC for the culture. Growth of the bacterial species (*Pseudomonas putida*) was visible withing 3 days, in a petridishes. After that, a Broth medium and Bushnell haas medium (medium salt media) was prepared, and then screening tests were carried out. The screen test was done as described by (Al-nasrawi, 2012). After the screened tests of the microbes, experimental analysis was carried out.

- Experimental Analysis

Two kinds of experiment were set up; Experimental set up (A) and Control set up (C).
Experimental set up was used by bacterial inoculum (*Pseudomonas putida*).
1ml of the species was taking and inoculated into 100ml of Bushnell haas medium with 10ml of engine oil into a 250ml conical flask and was incubated in room temperature (27^0C) at 160rpm for 2weeks in BOD incubator.

At 1st and 2nd Week; Viable count of the microbe, determination of degradation level of hydrocarbons by solvent extraction method and weighed by weighing balance of the engine oil and other physico-chemical parameters like temperature and PH were determined.

- Control set up: In this case, no inoculation of the microbe, only the 100ml of Bushnell haas medium with 10ml of engine oil into a 250ml conical flask were incubated in room temperature at 160rpm for 2weeks. Also, the determination of physico-chemical parameters were determine as in experimental set up, but no positive result were found, due to absent of inoculations of the microbial culture.

The degradations capabilities of the bacterial species was checked after 1st and 2nd weeks respectively for degradation efficiencies of the organism, by applying serial dilutions plate counts method and solvent extraction method., this is accordance with (Jayashree *et al;* 2012).

- Determination of Microbial Viable Counts (1st and 2nd weeks)

A serial dilutions plate counts method was carried out, as in accordance with, (Amund *et al.*, 1994; Umanu *et al.*, 2013).

- Solvent Extraction Method

This was done as described by (Jayashree *et al.*, 2012) and the rate of oil degradation was expressed in grams as well as in percentage.

Percentage of residual oil and oil degradation is calculated by the below formula:

$$\% \text{ of residual oil} = \frac{\text{Weight of the remaining oil sample}}{\text{Weight of the control oil sample}} \times 100$$

$$\% \text{ of oil degraded} = \frac{\text{Weight of the degraded oil sample}}{\text{Weight of the control oil sample}} \times 100$$

- Determination of PH

The PH of the incubation broths (Bushnell haas media) were determined by PH meter.

- Determination of Temperature

The incubation of the broths (Bushnell haas media) were incubated in B.O.D INCUBATOR at room temperature (27^0C).

RESULTS AND DISCUSSION

The results of the data analyzed were presented in the tables.

Table 1: Viable counts at 1st week

Dilution factor	*Pseudomonas putida*
10^{-1}	200
10^{-2}	184
10^{-3}	163

Table 2: Viable counts at 2nd week

Dilution factor	*Pseudomonas putida*
10^{-1}	254
10^{-2}	201
10^{-3}	187

Table 3: Bacterial species counts

Organisms	1st week	2nd week
Pseudomonas putida	$6.1133x10^4$cfu/ml	$6.988x10^4$cfu/ml

Table 4: PH of the Broth medium inoculated with the microbe

Organisms	PH
Pseudomonas putida (bacteria)	8

Table 5: Mass (Weight) of the samples (engine oils)

Sample (engine oil) Used	Weight in Gram (g)
Control	8.00
Bacteria (*Pseudomonas putida)*	7.486

Table 6: Percentage of oils degraded

Organisms	Percentages
Pseudomonas putida	6.425

Table 1 and 2; showed the total bacterial counts and cfu result were after first and second weeks of inoculation.
Table 3; showed the bacterial species in first and second week.
Table 4; showed the optimum PH for the growth of the microbe in the broth medium.
Table 5; showed the mass (weight) of the control and test samples.
Table 6; showed the percentage of oil degraded by the bacterial species.

From the above tables, Bacterial species (*Pseudomonas putida*) was checked in the laboratory condition, in order to determine and test for its capabilities in bioremediation process in oil spills. The total bacterial counts and cfu result was carried out after first and second weeks of inoculation, and was found to be significant. Solvent extraction method was primarily used to determine the percentage of oil degraded. *Pseudomonas putida* degraded 6.425% after two weeks, this shows *Pseudomonas putida* was found to have the capability of degrading hydrocarbons, which is in accordance with (Atlas, 1992; Amund and Nwokoye, 1993; Jones *et al.,* 1970; Adebusoye *et al.*2007; Lal and Khanna 1996; Daugulis and McCracken, 2003). This research is also in accordance with (Sarang *et al.,* 2013; Olanipekun *et al.,* 2015) studies; *Pseudomonas putida* has the ability to degrade and remove toluene and other monocyclic aromatic hydrocarbons, e.g. benzene, toluene and xylene. The bacterial degradation of aromatic hydrocarbons, normally involves the formation of a diol followed by cleavage of aromatic ring and formation of diacid such as *cis-cis* muconic acid (Sarang *et al.,* 2013; Olanipekun *et al.,* 2015).

CONCLUSION

Microorganisms, most especially bacteria and fungi have the capacity of bioremediating and biodegrading of hydrocarbons which are the major constituents of petroleum oil and other xenobiotics, e.g., heavy metals, which even at low concentrations, can be toxic to humans and other forms of life. There are three methods to clean up oil spills; physical, chemical and biological (bioremediation), but bioremediation is advantageous due to its time and cost saving than physical method, also unlike chemical method, no foreign or toxic chemicals are added to the site.

REFERENCES

[1] Adebusoye, S.A., Ilori, M.O., Amund, O.O. Teniola, O.D. and Olatope, S.O. (2007). Microbial degradation of petroleum hydrocarbons in a polluted tropical stream, *World Journal of Microbiology and Biotechnology*, Volume 23(8):1149–1159.

[2] Agarwal, S. K. (2002). *Environmental biotechnology,* department of botany, government college, Kota, india. pp:267-289.

[3] Al-Nasrawi, H. (2012). Biodegradation of Crude Oil by Fungi Isolated from Gulf of Mexico.*J Bioremed Biodegrad* Volume 3(143). Doi:10.4172/2155-6199.1000147. Florida State University,USA.

[4] Amund, O.O. and Nwokoye, N. (1993). Hydrocarbon potentials of yeast isolates from a polluted Lagoon. *Journal of Scientific Research and Development*. Volume 1:65–68.

[5] Atlas, R.M. (1992). *Encyclopedia of Microbiology.* Baltimore, Md, USA: Academic Press. Petroleum microbiology. pp:363-369.

[6] Daugulis, A.J. and McCracken, C.M. (2003). Microbial degradation of high and low molecular weight polyaromatic hydrocarbons in a two-phase partitioning bioreactor by two strains of *Sphingomonas* sp. *Biotechnology Letters*. 25(17):1441–1444.

[7] EPA. *International journal of environmental research and public healt*h (2006). ISSN 1660-4601 int. *J. Environ. Res. Public health***,** *6*, 1393-1417. *www.mdpi.com/journal/ijerphsidual* phytotoxicity.

[8] Jayashree, R., Evany, N., Rajesh, P.P. and Krishnaraju, M. (2012). Biodegradation capability of bacterial species isolated from oil contaminated soil. *J. Acad. Indus. Res.* **Vol. 1(3)** August 140 ©Youth Education and Research Trust (YERT) ISSN: 2278-5213.

[9] Jones J., Knight, M. and Byron, J.A. (1970). Effect of gross population by kerosene hydrocarbons on the micro flora of a moorland soil. *Nature*. 227: p. 1166.

[10] Lal, B. and Khanna, S. (1996). Degradation of crude oil by *Acinetobacter calcoaceticus* and *Alcaligenes odorants*. *Journal of Applied Bacteriology*. 81(4):355–362.

[11] Narayani, K. (2010): *journal on impacts of oil spills.www.buzzle.com/articles.*

[12] Olanipekun, O.O., Ogunbayo, A.O., Nwachukwu, S.C.U. and Bello, R.A. (2015): Comparative Study of Microbial Activities and Biodegradation-Abilities of Undefined Consortium in Some Hydrocarbon Contaminated Sites in the Niger Delta, Nigeria. *Journal of Environmental Protection*, 6, 138-145.

[13] Safiyanu I, Abdulwahid Isah A, Abubakar US and Rita Singh M. (2015). Review on Comparative Study on Bioremediation for Oil Spills Using Microbes. *Research Journal of Pharmaceutical, Biological and Chemical Sciences (RJPBCS) 6 (6)* PP. 783-790.

[14] Safiyanu, I., Sani, I. and Rita, S. M. (2015). Review on Bioremediation of Oil Spills Using Microbial Approach. Volume **3,** ISSN: 2347-6532. *International Journal of Engineering & Scientific Research http://www.ijmra.us.* Indexed & listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A.

[15] Saptakee, S. (2011): *journal on bioremediation for oil spills.www.buzzle.com/articles.*

[16] Shukla, A. and Cameotra, S, S. (2015*): Hydrocarbon pollution*: effects on living organisms, remediation of contaminated environments, and effects of heavy metals co-contamination on bioremediation. Institute of microbial technology, Chandigarh, India. *J Pet Environ Biotechnology, 6:217*

[17] Tariq, S. R., Shah, M. H., Shaheen, N., Khalique, A., Manzoor, S. and Jaffar, M. (2005): Multivariate analysis of selected metals in tannery effluents and related soil. *Journal of hazardous materials*, 122, 17-22.

[18] Umanu, G., Akpe, A.R. and Omoikhudu, A.R. (2013). Oil degradation assessment of bacteria isolated from used motor oil contaminated soils in ota, Nigeria. *I.J.A.B.R,* VOL. 3(4) pp: 506-513 ISSN 2250 – 3579