

Biodegradation of hydrocarbons from crude oil by *pseudomonas putida*

[Business](#), [Industries](#)



Biodegradation of Hydrocarbons from Crude Oil by Pseudomonas putida A
Project done under the guidance of Dr. K. Bharathi Department of

Biotechnology. Submitted to the faculty Of Department of Biotechnology

National Institute Of Technology, Warangal (A. P) Submitted By Febin P.

Nalpady, Anzal Rahman, Shruti Sharma, Sindhuja Nandiraju, Giraboina

Kranthi Kumar NATIONAL INSTITUTE OF TECHNOLOGY WARANGAL (A. P)

(DEEMED UNIVERSITY) 2010-2011 DEPARTMENT OF BIOTECHNOLOGY

NATIONAL INSTITUTE OF TECHNOLOGY, WARANGAL (A. P) CERTIFICATE This

is to certify that the project entitled "....." carried out by,

bearing roll no., final year B. Tech, Biotechnology, during academic year

2010-2011, is a bonafide work submitted to the National Institute of

Technology, Warangal in partial fulfillment of the requirements for the

requirements for the award of the Degree of Bachelor of Technology. Guide :

Dr. K Bharathi Dept. of Biotechnology NIT Warangal ACKNOWLEDGEMENT

With great pleasure and deep sense of gratitude, we take this opportunity to

express our sense of indebtedness to Dr. K Bharathi, our project guide for

accepting us under her good self to carry out this project work, and providing

us his invaluable guidance and constant encouragement at each and every

step throughout the progress of this project. To be sincere it was an

inextinguishable treasure of pleasure for us to work under her excellent

guidance. I would also like to thank the faculty of our department, Dr. P

Sreenivasa Rao, Mr. Onkara Perumal and Mr. K. Narasimhulu.

They were a pillar of strength for us and encouraged us to do our best. Name

Roll no Table of Contents 1. Abstract 2. Introduction 2. 1 Bioremediation 2. 2

<https://assignbuster.com/biodegradation-of-hydrocarbons-from-crude-oil-by-pseudomonas-putida/>

The conventional techniques of remediation

2. 3 Advantages of Bioremediation
2. 4 Microbes that are useful for bioremediation

3. Review of literature

3. 1 Microbial degradation
3. 2 Biodegradation of petroleum hydrocarbons
3. 3 Factors affecting Degradation
3. 4 Mechanism of Petroleum Hydrocarbon Degradation

4. Brief outline of the project work

5. Materials and Methods

5. 1 Preparation Of Nutrient Broth
5. 2 Preparation of Nutrient Agar Slants from the Bacterial Strain
5. Preparation of SubCultures of Bacterial Strain
5. 4 Centrifugation of Crude Oil
5. 5 Subculturing Of Petri Plates with oil
5. 6 Biodegradation Studies
5. 7 Gravimetric Analysis

6. Results

6. 1 Growth Analysis of Pseudomonas Putida
6. 2 Gravimetric Analysis

7. Discussion

8. References

1. ABSTRACT Oil spills have become a serious problem with the ever-increasing resource exploitation, transportation, storage, and accidental leakage of oil. Several techniques, including physical, chemical, and biological methods, are used to recover spilled oil from the environment.

Bioremediation is a promising option for remediation since it is effective and economic in removing oil with less undue environmental damages. However, it is a relatively slow process and the degree of success depends on a number of factors. These factors include the existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population and the environment factors are type of soil, temperature, pH, the presence of oxygen and nutrients. This project aims to study the degradation extent of the pseudomonas putida on oil.

The microbial strain used is procured from NCL pune. 2. Introduction In quantitative terms, crude oil is one of the most important organic pollutants in marine environment and it has been estimated that worldwide somewhere between 1. 7- 8. 8? 10⁶ tons of petroleum hydrocarbons impact marine waters and estuaries annually. Reports have been appearing since last three decades on the biodegradability of crude oil by bacteria which can use hydrocarbons as source of carbon and energy. A way to mitigate the effects of oil spills is bioremediation. 2. 1 Bioremediation

It is a process by which chemical substances are degraded by bacteria and other microorganisms. The use of these microorganisms has been successfully applied for the treatment of waste and wastewater in controlled systems. Several research studies have recently been performed to investigate the use of bioremediation for oil-spill cleanup in seawater, freshwater and terrestrial areas. The technique has been found to have a potential for broad applications in terrestrial and freshwater environments for treating soils and sediments contaminated with oil and other substances, as well as for coastal environments impacted by oil spills.

Water is a more sensitive medium than soil and requires different remediation techniques. Spills to surface water are easier to clean up than spills to groundwater, for obvious reasons. It is not only much harder to see the extent of the contamination, but also to remove the source of the contamination as, for example, a leaking underground storage tank. 2. 2. The conventional techniques of remediation . The conventional techniques

used for remediation have been to dig up contaminated soil and remove it to a landfill, or to cap and contain the contaminated areas of a site.

The methods have some drawbacks. The first method simply moves the contamination elsewhere and may create significant risks in the excavation, handling, and transport of hazardous material. Additionally, it is very difficult and increasingly expensive to find new landfill sites for the final disposal of the material. A better approach than these traditional methods is to completely destroy the pollutants if possible, or at least to transform them to innocuous substances.

Some technologies that have been used are high-temperature incineration and various types of chemical decomposition (e. g. , base-catalyzed dechlorination, UV oxidation). They can be very effective at reducing levels of a range of contaminants, but have several drawbacks, principally their technological complexity, the cost for small-scale application, and the lack of public acceptance, especially for incineration that may increase the exposure to contaminants for both the workers at the site and nearby residents. . 3

Advantages of Bioremediation Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques and can often be carried out on site. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the timescales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate.

Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result. Because bioremediation seems to be a good alternative to conventional clean-up technologies research in this field, especially in the United States, rapidly increasing. Bioremediation has been used at a number of sites worldwide, including Europe, with varying degrees of success.

Techniques are improving as greater knowledge and experience are gained, and there is no doubt that bioremediation has great potential for dealing with certain types of site contamination. Unfortunately, the principles, techniques, advantages, and disadvantages of bioremediation are not widely known or understood, especially among those who will have to deal directly with bioremediation proposals, such as site owners and regulators. 2. 4

Microbes that are useful for bioremediation The biodegradation of petroleum in the marine environment is carried out largely by diverse bacterial populations, including various *Pseudomonas* species.

The hydrocarbon-biodegrading populations are widely distributed in the world's oceans; surveys of marine bacteria indicate that hydrocarbon-degrading microorganisms are ubiquitously distributed in the marine environment. Generally, in pristine environments, the hydrocarbon-degrading bacteria comprise < 1% of the total bacterial population. These bacteria presumably utilize hydrocarbons that are naturally produced by plants, algae, and other living organisms. They also utilize other substrates,

such as carbohydrates and proteins. When an environment is contaminated with petroleum, the proportion of hydrocarbon-degrading microorganisms increases rapidly. In particular, in marine environments contaminated with hydrocarbons, there is an increase in the proportion of bacterial populations with plasmids containing genes for hydrocarbon utilization. The proportion of hydrocarbon-degrading bacterial populations in hydrocarbon-contaminated marine environments often exceed 10% of the total bacterial population.

Review of Literature 3. 1 Biodegradation of petroleum hydrocarbons

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present.

Petroleum hydrocarbons can be divided into four classes: the saturates, the aromatics, the asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and the resins (pyridines, quinolines, carbazoles, sulfoxides, and amides) [R. R. Colwell, J. D. Walker, and J. J. Cooney, " Ecological aspects of microbial degradation of petroleum in the marine environment,]. Different factors influencing hydrocarbon degradation have been reported by Cooney et al.

One of the important factors that limit biodegradation of oil pollutants in the environment is their limited availability to microorganisms. Petroleum hydrocarbon compounds bind to soil components, and they are difficult to be removed or degraded [S. Barathi and N. Vasudevan], " Utilization of petroleum hydrocarbons by *Pseudomonas fluorescens* isolated from a petroleum-contaminated soil]. Hydrocarbons differ in their susceptibility to microbial attack. The susceptibility of hydrocarbons to microbial degradation

can be generally ranked as follows: linear alkanes > branched alkanes > small aromatics > cyclic alkanes [J. J.

Perry, " Microbial metabolism of cyclic alkanes," in Petroleum Microbiology].

Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be degraded at all. 3. 2 Microbial degradation

Microbial degradation is the major and ultimate natural mechanism by which one can cleanup the petroleum hydrocarbon pollutants from the

environment [1-3] The recognition of biodegraded petroleum-derived

aromatic hydrocarbons in marine sediments was reported by [Jones et al].

They studied the extensive biodegradation o alkyl aromatics in marine

sediments which occurred prior to detectable biodegradation of n-alkane

pro? e of the crude oil and the microorganisms, namely, Arthrobacter,

Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas, and

Rhodococcus were found to be involved for alkylaromatic degradation.

Microbial degradation of petroleum hydrocarbons in a polluted tropical

stream in Lagos, Nigeria was reported by Adebusoye et al. Nine bacterial

strains, namely, Pseudomonas ? uorescens, P. aeruginosa, Bacillus subtilis,

Bacillus sp. , Alcaligenes sp. , Acinetobacter lwo? , Flavobacteriumsp. ,

Micrococcus roseus, and Corynebacterium sp. were isolated from the

polluted stream which could degrade crude oil.

Hydrocarbons in the environment are biodegraded primarily by bacteria,

yeast, and fungi. The reported e? ciency of biodegradation ranged from 6%

to 82% for soil fungi, 0. 13% to 50% for soil bacteria, and 0. 003% to 100%

[6] for marine bacteria. Many scientists reported that mixed populations with

overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil in soil, fresh water, and marine environments [8]. Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment [7].

Several bacteria are even known to feed exclusively on hydrocarbons [9]. Floodgate [36] listed 25 genera of hydrocarbon degrading bacteria and 25 genera of hydrocarbon degrading fungi which were isolated from marine environment. A similar compilation by Bartha and Bossert [6] included 22 genera of bacteria and 31 genera of fungi. In earlier days, the extent to which bacteria, yeast, and filamentous fungi participate in the biodegradation of petroleum hydrocarbons was the subject of limited study, but appeared to be a function of the ecosystem and local environmental conditions [7].

Crude petroleum oil from petroleum contaminated soil from North East India was reported by Das and Mukherjee . *Acinetobacter* sp. Was found to be capable of utilizing n-alkanes of chain length C₁₀-C₄₀ as a sole source of carbon [6]. Bacterial genera, namely, *Gordonia*, *Brevibacterium*, *Aeromicrobium*, *Dietzia*, *Burkholderia*, and *Mycobacterium* isolated from petroleum contaminated soil proved to be the potential organisms for hydrocarbon degradation [9]. The degradation of poly- aromatic hydrocarbons by *Sphingomonas* was reported by Daugulis and McCracken .

Fungal genera, namely, *Amorphoteca*, *Neosartorya*, *Talaromyces*, and *Graphium* and yeast genera, namely, *Candida*, *Yarrowia*, and *Pichia* were isolated from petroleum contaminated soil and proved to be the potential organisms for hydrocarbon degradation [Singh et al.] also reported a group of terrestrial fungi, namely, *Aspergillus*, *Cephalosporium*, and *Pencillium* which were also found to be the potential degrader of crude oil hydrocarbons. The yeast species, namely, *Candida lipolytica*, *Rhodotorula mucilaginosa*, *Geotrichum* sp, and *Trichosporon mucoides* isolated from contaminated water were noted to degrade petroleum compounds [5].

Though algae and protozoa are the important members of the microbial community in both aquatic and terrestrial ecosystems, reports are scanty regarding their involvement in hydrocarbon biodegradation. [Walker et al.] isolated an alga, *Prototheca zop?* which was capable of utilizing crudeoil and a mixed hydrocarbon substrate and exhibited extensive degradation of n-alkanes and isoalkanes as well a aromatic hydrocarbons. Cerniglia et al. observed tha nine cyanobacteria, ? ve green algae, one red alga, one brown alga, and two diatoms could oxidize naphthalene.

Protozoa by contrast, had not been shown to utilize hydrocarbons. 3. 3

Factors affecting Degradation A number of limiting factors have been recognized to affect the biodegradation of petroleum hydrocarbons, many of which have been discussed by Brusseau. The composition and inherent biodegradability of the petroleum hydrocarbon pollutant is the ?rst and foremost important consideration when the suitability of a remediation approach is to be assessed. Among physical factors, temperature plays an

important role in biodegradation of hydrocarbons by directly affecting the chemistry of the pollutants as well as affecting the physiology and diversity of the microbial flora. Atlas [4] found that at low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons were reduced, delaying the onset of biodegradation.

Temperature also affects the solubility of hydrocarbons [8]. Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperature. shows that highest degradation rates that generally occur in the range 30–40°C in soil environments, 20–30°C

in some freshwater environments and 15–20°C in marine environments. Venosa and Zhu [11] reported that ambient temperature of the environment affected both the properties of spilled oil and the activity of the microorganisms. Significant biodegradation of hydrocarbons have been reported in psychrophilic environments in temperate regions. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus, and in some cases iron [8]. Some of these nutrients could become limiting factor thus affecting the biodegradation processes.

Atlas [11] reported that when a major oil spill occurred in marine and freshwater environments, the supply of carbon was significantly increased and the availability of nitrogen and phosphorus generally became the limiting factor for oil degradation. In marine environments, it was found to be more pronounced due to low levels of nitrogen and phosphorous in seawater

[10]. Freshwater wetlands are typically considered to be nutrient deficient due to heavy demands of nutrients by the plants. Therefore, additions of nutrients were necessary to enhance the biodegradation of oil pollutant.

On the other hand, excessive nutrient concentrations can also inhibit the biodegradation activity [11]. Several authors have reported the negative effects of high NPK levels on the biodegradation of hydrocarbons especially on aromatics [10]. The effectiveness of fertilizers for the crude oil bioremediation in subarctic intertidal sediments was studied by Pelletier et al. . Use of poultry manure as organic fertilizer in contaminated soil was also reported , and biodegradation was found to be enhanced in the presence of poultry manure alone. Maki et al. reported that photo-oxidation increased the biodegradability of petroleum hydrocarbon by increasing its bioavailability and thus enhancing microbial activities.

3. 4 Mechanism of Petroleum Hydrocarbon Degradation

The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. Figure 2 shows the main principle of aerobic degradation of hydrocarbons [11]. The initial intracellular attack of organic pollutants is an oxidative process and the activation as well as incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidases.

Peripheral degradation pathways convert organic pollutants step by step into intermediates of the central intermediary metabolism, for example, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, acetyl-CoA, succinate, pyruvate. Sugars required for various biosyntheses and growth are synthesized by

gluconeogenesis. The degradation of petroleum hydrocarbons can be mediated by specific enzyme system. Figure 3 shows the initial attack on xenobiotics by oxygenases.

Other mechanisms involved are (1) attachment of microbial cells to the substrates and (2) production of biosurfactants [12]. The uptake mechanism linked to the attachment of cell to oil droplet is still unknown but production of biosurfactants has been well studied. 4. Brief outline of the project work:

1. Procurement of oil Samples.
2. Procurement of *Pseudomonas putida* strain.
3. Sub-culturing the microbe in nutrient rich media for checking viability..
4. Culturing microbes on a mineral salt media containing only crude oil as a carbon source.
5. Biodegradation studies.
6. Gravimetric analysis

Materials and Methods Soil Samples - Samples(500g) contaminated with oil used for hydrocarbons utilizing microorganisms, were collected from Nhava Sheva port in Mumbai(where a recent oil spill has took place). Crude Oil - Crude Oil is procured from an Oil production site of ONGC. Bacterial Strain - *Pseudomonas Putida* PS-I strain procured from NCL Pune. 5. 1 Preparation Of Nutrient Broth For preparation of nutrient agar, malt extract, yeast extract, Potassium dihydrogen phosphate and dextrose is required. Malt extract and yeast extract is generally used as a nutritious agent. Potassium dihydrogen phosphate i. . KH_2PO_4 is used as a buffering agent to maintain the pH. Dextrose is generally used as a carbon source because dextrose inhibits the growth of other micro-organisms. AUTOCLAVE is a device to sterilize equipment and supplies by subjecting them to high pressure steam at 121°

C or more. Machines in this category largely operate by utilizing pressurized steam and superheated water. To sterilize culture media, rubber material, gowns, dressing, gloves etc. are used. It is particularly useful for materials which cannot withstand the higher temperature of hot air oven. CHEMICALS REQUIRED:- For 1000ml,

Malt extract -- 10 gm Beef Extract -- 4 gm K_2HPO_4 -- 1 gm Magnesium sulphate -- 1 gm Sodium Chloride -- 0.5 gm pH -- 7.0 Agar -- 15%

PROCEDURE:- For preparation of 100ml of nutrient broth, around 100ml of double distilled water was taken in a conical flask. Malt extract, yeast extract, KH_2PO_4 and dextrose was weighed as per the composition mentioned above and added to the conical flask.

The conical flasks are to be shaken so well so that all the chemicals should dissolve. pH was checked using pH meter and adjusted to 7.0 using NaOH and HCl. The volume was made to 100ml by adding double distilled water. The above solution i. e. nutrient agar along with the Petri-plates was autoclaved at 15 psi and 15 minutes. Now the solution was allowed to cool down to ready to pour condition. PRECAUTIONS:- The autoclave should be done at 15 psi and 15 min. The pH should be maintained at 7.0.

Preparation of Nutrient Agar Slants from the Bacterial Strain

For the preparation of Slants, Flame the inoculating loop to redness by holding it pointed down into the flame, starting near the handle and then moving the loop into the flame. This technique sterilizes the loop and, if wet with a culture, heats up the loop without spattering bacteria into the air and

onto the surrounding area. Let the loop cool a minute. A hot loop will damage the bacteria cells. Using the fingers of the "loop hand" remove the cap from the stock culture tube and flame the tube mouth. Do not set the tube top down on the table.

Insert the cooled sterilized loop into the culture tube being careful to not touch the sides of the tube. Touch the loop to the culture. You need not scrape a visible amount from the culture. Hold the tube as horizontal as possible to preclude particles from the air settling into the tube. But do watch out for any condensate in the bottom of slant cultures. Don't let this fluid wash across the face of the culture. Remove the loop being careful again to not touch the tube sides. Flame the tube mouth and replace the cap.

Remove the cap of the broth tube. Flame the top.

Remember to hold the top in your fingers. Insert the loop into the Slant tube filled with agar and shake to remove the bacteria. Withdraw the loop, flame the tube mouth and replace the cap. Resterilize the inoculating loop and place it on the table. Never place a contaminated loop on the table. If there is any liquid in the bottom of the slant tube avoid sticking the loop into this condensate.

5. 3 Preparation of SubCultures of Bacterial Strain

The Nutrient Broth Cultures are inoculated with the bacterial strain from the nutrient agar slant as detailed below. PROCEDURE Light your Bunsen burner.

In one hand hold both the Nutrient Broth culture to be inoculated and the nutrient slant agar. Loosen the tube caps. In your other hand hold the inoculating loop. Flame the inoculating loop to redness by holding it pointed

down into the flame, starting near the handle and then moving the loop into the flame. This technique sterilizes the loop and, if wet with a culture, heats up the loop without spattering bacteria into the air and onto the surrounding area. Let the loop cool a minute. A hot loop will damage the bacteria cells. Using the fingers of the "loop hand" remove the cap from the stock culture tube and flame the tube mouth.

Do not set the tube top down on the table. Insert the cooled sterilized loop into the slant tube being careful to not touch the sides of the tube. Touch the loop to the culture. You need not scrape a visible amount from the culture. Hold the tube as horizontal as possible to preclude particles from the air settling into the tube. But do watch out for any condensate in the bottom of slant cultures. Don't let this fluid wash across the face of the culture. Remove the loop being careful again to not touch the tube sides. Flame the tube mouth and replace the cap. Remove the cap of the broth tube. Flame the top.

Remember to hold the top in your fingers. Insert the loop into the broth and shake to remove the bacteria. Gently shake the broth culture. This inoculated broth culture is incubated at room temperature for 72 hours and the bacteria is allowed to grow in the broth medium.

5. 4 Centrifugation of Crude Oil

Centrifugation is a process that involves the use of the centrifugal force for the separation of mixtures with a centrifuge, used in industry and in laboratory settings. More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis.

The precipitate (pellet) gathers on the bottom of the tube. The remaining solution is properly called the "supernate" or "supernatant liquid". The Crude Oil is Centrifuged at a speed of 5000 rpm for a period of ten minutes. The Contaminants in the oil are collected at the bottom of the tube in the form of pellets. These pellets can be removed by filtration using a filter paper. Now the concentrates oil which is free from impurities is collected in a flask and gently shaken. Spectrophotometric Analysis Optical density, measured in a spectrophotometer, can be used as a measure of the concentration of bacteria in a suspension.

As visible light passes through a cell suspension the light is scattered. Greater scatter indicates that more bacteria or other material is present. The amount of light scatter can be measured in a spectrophotometer. Typically, when working with a particular type of cell, you would determine the optical density at a particular wavelength that correlates with the different phases of bacterial growth. Generally we will want to use cells that are in their mid-log phase of growth. Typically the OD600 is measured.

5. 5 Subculturing Of Petri Plates with oil % of crude oil is mixed with 100 ml of Nutrient broth medium. The 1. 5g of agar is added to the medium and Nutrient Agar(with 1% crude oil) is prepared. Now take 6 Petri dishes. Open one of the dishes. Take the nutrient agar to be added and Swab the agar, barely pressing, side to side on the entire surface. The dish is closed immediately after swabbing to prevent contamination. The dish is sealed with tape around the edges to prevent contamination. Repeat the same procedure for the other dishes. Put

the dishes in an incubator for 4 days to allow some growth. 5. 6

Biodegradation Studies

Laboratory Biodegradation studies were carried out under optimized conditions for assessing the biodegradation potential of the *Pseudomonas putida* PS-I Strain. After the desired interval of time, the petriplates were taken out and the bacterial activities were stopped by adding 1% N HCl. For the extraction of crude oil from these plates, 50ml of culture broth was mixed with 50 ml of acetone : petroleum ether (1: 1) in a single separating funnel and shaken vigorously to get a single emulsified layer and acetone was added then to it and shaken gently to break the emulsification which resulted in three layers.

Top layer was a mixture of Petroleum ether crude oil and acetone. Clumping cells were formed in the middle layer and the bottom layer contains acetone, water and biosurfactant in soluble form. The lower two layers were separated out while the top layer containing petroleum ether mixed with crude oil and acetone is taken out in a fresh beaker. The extracted oil is passed through anhydrous sodium sulphate in order to remove the moisture. The petroleum ether and acetone were evaporated on a water bath leaving us with the dry oil clump. 5. 7 Gravimetric Analysis

Gravimetric analysis describes a set of methods in analytical chemistry for the quantitative determination of an analyte based on the mass of a solid. the analyte must first be converted to a solid by precipitation with an appropriate reagent. The precipitate can then be collected by filtration,

washed, dried to remove traces of moisture from the solution, and weighed. The amount of analyte in the original sample can then be calculated from the mass of the precipitate and its chemical composition. Gravimetric analysis is performed on the dry oil clump collected after the water bath.

It is done by weighing the quantity of residual oil left after biodegradation in a tared vial. The mass of this crucible is subtracted from the initial mass of the 1% of oil that is added in the petridishes giving the amount of oil that is degraded due to the biological activity of the *Pseudomonas putida* strain.

6. 1 Growth Analysis of *Pseudomonas Putida*: The culture which was obtained in test tube slants was further sub cultured in conical flasks in a LB medium and the growth analysis was done to check the viability of the culture obtained. The growth kinetics plot was obtained by measuring the O.D. using a visible spectrophotometer and recording the reading at regular intervals. The Graph was then plotted.

6. 2 Gravimetric Analysis: Biodegradation studies were conducted for 15 days and gravimetric analysis was done after every five days. The biodegradation effect was seen from the 5th day onwards. Laboratory biodegradation studies on crude oil by

<i>Pseudomonas putida</i> No. Of Days	Initial Concn	Final Concn	Difference	Degradation (%)
5 days	1. 431 ± . 57	1. 325 ± . 46	0. 106 ± . 11	7. 4
10 days	1. 453 ± . 71	1. 198 ± . 38	0. 255 ± . 34	17. 54
15 days	1. 398 ± . 68	0. 936 ± . 31	0. 62 ± . 28	33. 04

7. Discussion It can be seen that the degradation percentage of oil has increased from mere 7. 41 in the first 5 days to a good 33. 04 percentage towards the 15th day, from this it is clearly understood that *Pseudomonas putida* is an ideal organism for bioremediation

programmes. Moreover this rate of degradation has been obtained under normal conditions without any aid from surfactants or fertilizers. Hence there is scope for achieving much greater rates by using the above mentioned methods of fertilizing or adding surfactants. 8. BIBLIOGRAPHY (1). U. S. Environmental Protection Agency (1990).

Interim Report, Oil Spill Bioremediation Project. U. S. Environmental Protection Agency, Office of Research and Development, Washington (2). T. Cairney. Contaminated Land, p. 4, Blackie, London (1993). (3). R. B. King, G. M. Long, J. K. Sheldon. Practical Environmental Bioremediation: The Field Guide, 2nd ed. , Lewis, Boca Raton, FL (1997). (4). Atlas, Ronald M. (1995). Petroleum Biodegradation and Oil Spill Bioremediation.

Marine Pollution Bulletin 31, 178-182 (5) Hoff, Rebecca Z. (1993).

Bioremediation: an overview of its development and use for oil spill cleanup. Marine Pollution Bulletin 29, 476-481. 6). Irwin, Patricia (1996). To clean up environmental spill, know your medium. Electrical World 37-40. (7).

Swannell, Richard P. J. ; Lee, Kenneth; McDonagh, Madeleine (1996). Field Evaluations of Marine Oil Spill Bioremediation. Microbiological Reviews 60, 342-365 (8). Radwan, S. S. ; Sorkhoh, N. A. ; El-Nemr, I. M. ; El-Desouky, A. F.

(1997). A feasibility study on seeding as a bioremediation practice for the oily Kuwaiti desert. Journal of Applied Microbiology 83, 353-358. (9). P. E.

Flathman, D. Jerger, J. E. Exner. Bioremediation: Field Experience, Lewis, Boca Raton, FL (1993). 10). J. G. Mueller, C. E. Cerniglia, P. H. Pritchard.

Bioremediation of Environments Contaminated by Polycyclic Aromatic Hydrocarbons. In Bioremediation: Principles and Applications, pp. 125-194,

Cambridge University Press, Cambridge (1996). (11). P. J. S. Colberg and L. Y. Young. Anaerobic Degradation of Nonhalogenated Homocyclic Aromatic Compounds Coupled with Nitrate, Iron, or Sulfate Reduction. In *Microbial Transformation and Degradation of Toxic Organic Chemicals*, pp. 307–330, Wiley-Liss, New York (1995). (12). A. S. Allard and A. H. Neilson. Oil Eating Microbes 39, 253–285 (1997).