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Biodegradation of used engine oil using Actinobateria

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Abstract:-- **The objective of this work is to study the biodegradation of used lubrication oil by actinobacteria. The culture was collected from the soil samples taken from Muthupettai, a marshy land. Various strains of bacteria were extracted and introduced into the sample (used engine oil). The samples were given continuous movement and constant temperature in shaking incubator for a month. Result was observed by FTIR Method twice, at an interval of 15 days once. There was a prominent degradation of oil at the of 30 days and the superficial bacterial strain also identified.**

Keywords:-- **Used engine oil, Actinobateria, Banshell Hans meium, FTIR , Degradation of bonds.**

I. INTRODUCTION

Petroleum like fuels primarily consists of a complex mixture of molecules called hydrocarbons. In large concentrations, the hydrocarbon molecules that make up crude oil and petroleum products are highly toxic to many organisms including humans (Alexander 1994).

Used motor oil contains metals and heavy polycyclic aromatic hydrocarbons that could contribute to chronic hazards including mutagenicity and carcinogenicity (Boonchan et al.,2000, Hagwell et al.,1992, Keith et al., 1979).

The most rational way of decontamination of the environment loaded with petroleum derivatives is an application of methods based on mainly metabolic activity of micro organisms (Leahy et al.,1990)

Single cultures of bacteria have been found to be better than mixed cultures.(Okerentugba et al.,2003) and more recently, fungi have been found to be better degraders of petroleum than traditional bioremediation techniques including bacteria (Batelle 2000).

The application of bioremediation capabilities of indigenous organism to clean up pollutants is viable and has economic values (Bijofp 2003).

In the process of fulfilling the energy requirement for today's population, various natural resources have been exploited. But the principal source of energy continues to be petroleum hydrocarbon and hence a global pollutant. During accidental spills, action will be taken to remove or remediate the contaminant immediately, whereas in the gasoline and diesel stations the spills due to leakage may be small but continuos and prolonged. Because of its persistence, the chance for groundwater contamination is high.

Toxicity of crude oil includes liver necrosis, congestion of the liver, fat degeneration and dissociation of hepatocytes .Birds and animals in oil contaminated area are found to have black emulsion in the digestive tract with a petroleum odour. This leads to decrease in the absorption of nutrients and finally leads to death of these birds and animals due to rupture of capillaries and haemorrhage, heptacellular dissociation hemosiderosis, renal tubular necrosis and anemia.

The aromatics in these oils also have numerous adverse effects on the environment particularly to the local microbial flora.It was shown that alpha pinanene, limonene, camphene and isobornyl acetate were inhibitory to the micro organisms.

The phenolic and quininic naphthalene derivatives inhibited the growth of cells.Calder and Lader demonstrated that increasing amounts of naphthalene, 2-methyl naphthalene, pyrene and others resulted in an increased lag phase and lowered the growth rates of two bacteria growing on these compounds.

Contamination of soil by used lubricating oil is rapidly increasing due to global increase in the usage of petroleum products.Environmental pollution with petroleum and petro chemical products has attracted much attention in recent decades.

The presence of different types of automobiles and machinery has resulted in an increase in the use of lubricating oil spillage of used motor oils such as diesel or jet fuel

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contaminates our natural environment with hydrocarbon. Lack of essential nutrients such as nitrogen and phosphorus is one of the major factors affecting biodegradation of hydrocarbon by microorganisms in soil and water environment. Therefore the addition of inorganic or organic nitrogen rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process.

Concentration of petroleum hydrocarbon determines to a environment. High concentration of hydrocarbon can be inhibitory to micro organism and concentration at which inhibition occurs varied with the compound. High concentration can be associated with heavy undispersed oil slicks in water causing inhibition of oil bioremediation due to oxygen limitation or through toxic effects exerted by volatile hydrocarbons on micro organisms.

The drained old engine oil seems thick and black, it is because oil like fine red wine, ages and oxidises. The process of oxidation is naturally occurring in the oil, but the rate at which this happens depends on the following factors viz.,temperature, moisture, oxygen catalytic reactions conditions of the engine, quality of the base stock oil.

The illegal dumping of used engine oil is an environmental hazard with global ramification. Most mechanical methods to reduce hydrocarbon pollution is expensive, time consuming and depends mainly on excavating of these soil, treating in separate area or better treatment facilities. These treatments include incineration and burial in secure landfills.

Petroleum hydrocarbon, metabolizing microorganisms are widely distributed in nature. Petroleum hydrocarbons can be degraded by microorganisms such as bacteria, fungi, cyanobacteria and micro algae. However, bacteria play the important role in bioremediation.

Over the last two decades the microbiological investigation of total petroleum impacted environments have revealed the presence of a physiologically diverse microbial flora. Due to the wide variety of complex individual substrates constituting total petroleum hydrocarbons and different metabolic interactions, there are certain inherent difficulties in characterizing microbial communities impacted by total petroleum hydrocarbons.

The objective of this study is to determine the effects of oil contamination on biodegradation of used engine oil by actinobacteria.

MATERIALS AND METHODS

Collection of soil sample

Soil sample were collected from different habitats of Muthuppettai Marshyland. Each collection was made from 10-15 depth of the soil (saadoun,Gharaibeh, 2003).They were air dried for one week (Williams et al.,1972), crushed and sieved. The sieved soil were then used for actinobacteria isolation.

Preparation of soil suspension

Soil suspensions were prepared by the following methods:

Serial dilution of soil sample:

1 g soil sample from each different collection area, was vigorously shaking in 10 ml of sterile distilled water for 30 min on a shaker. Serial 1 in 10 dilutions were then made down to 10-6.

Centrifugation of soil sample

1 g of soil sample was mixed with 10 ml of sterile distilled water and centrifuge at 16oo rpm for 20 min (Rehacek, 1959).

Isolation of Actinobateria

Banshell Hans medium was prepared and sterilized a 1210C temperature, 15-psi pressure for 15 min in autoclave. Medium was poured on sterile petridishes once it reaches the tolerable temperature(450 C) and allowed to solidify. Spread plate technique was followed to isolate the actinobacteria. Each plate was received 0.2ml of 10-4,10-6or10-6 dilutions of the inoculums. The plates were incubated at room temperature and examined the plates weekly for three weeks.

Maintenane of Suspeted Actinobaterial isolates

Suspected actinobacterial isolates were maintained in Banshell Hans medium contained the following (g/l):tryptone,5.0;yeast extract,3.0;agar, 16.0 and pH was adjusted at 7.3.Inoculation of suspected actinobacterial isolates was done on solid medium surface and incubated the plates at room temperature for 7-10 days.The used engine oil samples were collected from various automobile Workshops in pudukkottai, Tamilnadu, India.

Broth was prepared by added 50 ml of distilled water to 0.16 g of Banshell Hans medium. The solution was sterilized by autoclaving.2 ml of sterilized used engine oil was measured and added to the broth in all the flasks. Actinobacterial culture was inoculated in all the flasks with the exception of the last flask which served as control.

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Esach of the flask was plugged with sterile cotton wool so as to ensure maximum aeration and prevent cross contamination. The flasks were then incubated at room temperature for 30 days.

The flasks were shaken constantly throughout the duration of the experiment to facilitate oil cell phase contract. The ability to degrade the petroleum products was measured everyday by visual method for the formation of pellets.

The flasks were agitated at 100 rpm room temperature. The used motor oil experiment was carried for a period of 30 day and the content of the each test including the control were harvested and subjected to hydrocarbon analysis by FTIR analysis.

FTIR analysis was done twice at an interval of 15 days.

Background to IR Analysis

The Electromagnetic Spectrum

Electromagnetic waves are waves that have both an electric and magnetic component. Well known examples of electromagnetic waves include Xrays, visible light, microwaves and even radio waves.

These waves all travel at the same velocity but differ in the wavelength and frequency bands used to describe them.Infra red radiation is part of the make up of the electromagnetic spectrum and covers electromagnetic waves wavelength between 0.00008 and 0.04.

Chemists have adopted a more convenient method of describing IR radiation in that it is described in terms of the number of waves that occur per cm. This number is called the WAVENUMBER and is actually a measure of wave frequency. Wave numbers are calculated by dividing 1 by the wavelength expressed in centimeters.

Infrared analysis only uses a portion of the IR spectrum known as “mid range infrared”.It is defined as infrared waves having wave numbers between 4000 and 400 cm-1 .

Molecular Vibrations and Infrared Absorption

The chemical bonds within organic molecules are in a state of continual vibration with bond stretching and contracting as well as bending relative to one another. When an infrared beam falls on a molecule, waves of specific frequencies are absorbed from the beam by the molecule. The actual frequencies of the waves absorbed depends on the types of bonds present in the molecule’s structure. Different types of bonds within the same molecule would absorb different frequency bands while several identical bonds would all absorb the same frequency bands and give rise to stronger absorption.

A Record of the frequencies at which infrared absorption takes place for an organic compound is a highly characteristic property of the compound and is called its INFRARED SPECTRUM. An infrared spectrum of a compound will reveal information about molecular structure as the existence of specific groups of atoms may be confirmed from the presence of their characteristic absorptions.

Modern FTIR spectrometers scan frequencies in an infrared beam and measure the radiant powers of frequencies after the beam has passed through and interacted with a sample in a sample cell.These values are compared against stored values of radiant powers obtained with an empty sample cell and absorbance values calculated for output in an infrared spectrum.

REVIEW OF LITERATURE

The used engine oil contaminated soil samples were collected from various automobile workshops in pudukkottai,Tamilnadu,South India. From the collected samples indigenous actinobacteria were isolated using Banshell Hans medium. Totally six isolates were isolated among which two were found predominant. The morphological and microscopical studies were done. Morphological studies of isolated actinobacteria showed different growth pattern on different media. Prelimnary hydrocarbon degradation analyses were done to evaluate the growth diameter of actinobacteria colonies on minimal media supplement with used engine oil. Turbidity assay using minimal medium was done to confirm the hydrocarbon degradation . The isolates which showed better results were seleted for furthert analysis. The gravimetric analysis revealed that the both isolates degraded used engine to great extent at 30 days. The FTIR spectrum of control and treated samples with actinobacterial isolates revealed differences in formation indicating microbial oxidation of hydrocarbons.When conditions such as pH requirement and nutrient availability are taken into consideration during a bioremediation project, the rate of microbial degradation could be conveniently achieved within a much a shorter time than even what was obtained in this study.The rigours of bioaugmenting contaminated soils for the purpose of bioremediation can be avoided and effective remediation still achieved if suitable conditions that will enhance indigenous microbial activities for optium degradation are satisfied.

Actinobacterial culture were isolated from soil sample collected from Muthuppettai Marshy land.

The isolates are Streptomyces hiveoruber, Lechevalieria aerocolonies, Streptomyces heliomycini, Dactylosporangium vinaceum, Lechevalieria flava Streptomyces flavomacrosporus. The isolates were grown in Banshell

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Hans medium.The cultures were maintained by subculturing the isolates and kept in incubator at 270C. The experiment was carried out in seven conical flasks. Broth was prepared using BH medium. The medium and used engine oil sample was sterilized by autoclaving. Cultures were inoculated in conical flasks with the exception of last, which serves as control. The set up was kept at rotary shaker at 100 rpm for 30 days.

The results were observed through FTIR gravimetric analysis.

In order to counter acid build-up from combustion by-products and oxidation, some of the additive package is over-based in order to neutralise these harmful acids occurring in the oil. One of the primary sources of acid in engine oil is usually the result of fuel dilution. Fuel also contains Sulphur impurities and this can react with water from combustion and condensation to form Sulphuric Acid. However, the level of Base Reserve in the oil depletes with time as it neutralises harmful acids and hence this is another reason to change the oil to ensure there is always sufficient Base Reserve to counter acid contamination.

When draining an engine oil, there is as much as 20% oil remaining residual in the block and cooler, and just 10% residual of severely degraded old oil will damage the new oil's additive package within hours. So if missed a service, have the oil changed, then run the engine for some time (50 miles or so) and then change thse oil again.

Older engines running on carburettors and points-based distributors or injector pumps generally have less efficient combustion than their modern counterparts. The engine goes off tune after a period of running until its next service with new points and plugs. Therefore the contamination from combustion on the oil is proportionately higher than on modern engine oils. Allied to better quality, cleaner fuels and base stocks and adpacs, the OCIs have increased significantly to as much as 30k miles and 24mths on some cars, albeit with a need for very expensive synthetic oil.

In recent years, various technologies have emerged in order to manage oil residues and effluents contaminated with hydrocarbons. Bioremediation is one of the most extensively used because of its low cost and high efficiency (Alxander, 1999; Van Hamme et al., 2003). Biodegradation of hydrocarbons by natural populations of microorganisms is the main process acting in the depurination of hydrocarbon-polluted environments. The mechanism has been extensively studied and reviewed (Atlas, 1984; Leahy and Colwell, 1990; Korda et al., 1997; Kanaly and Harayama, 2000; Vam Hamme et al., 2003; Pinzon-Martinez et al., 2010).

The utilization of n-alkanes by actinobacterial as a sole carbon and energy source has been reviewed by Obuekwe et al. (2005) and Ashraf and Ali, (2006). In many reports, bacteria have been identified as more efficient crude oil degraders than yeast.

On the contrary, there are scanty information that yeasts is better crude oil degraders than bacteria (Walker et al., 1978). Additionally, a consortium of symbiotic bacteria or supporting materials can be used to enhance the biodegradation process as described by Tejo-Hernandez et al. (2007); Nievas et al. (2008); Hii et al. (2009); Wang et al. (2010) and Zhang et al. (2010). Some reviews focused on the examination of factors, are including nutrients, physical state of the oil, oxygen, temperature, salinity and pressure influencing petroleum biodegradation rates, with a view to developing environmental applications (Atlas, 1981; Jonathan et al., 2003).

Factorial design techniques present a more balanced alternative to the one-factor-at-a-time approach to fermentation improvement (Haaland, 1989; Myers and Montgomery, 1995). Plackett-Burman design comprises one type of two level screening designs and can be constructed on the bases of factorial replication of a full factorial design (Plackett and Burman, 1946). This design allows reliable short listing of small number of ingredients for further optimization and allows to obtain unbiased estimates of linear effects of all the factors with maximum accuracy being the same for all the effects (Akhnazarova and Kafarov, 1982).

The ability of the bacteria isolate to biodegrade some aromatic derivatives hydrocarbons (naphthylamine, phenol, naphthalene ethyldiamine, phenanthrene, naphthalene 2-sulfonate and naphthalene) and aliphatic (n-hexane, n-heptane and n-pentadecane) was taken as an indication for the biodegradation potential of this isolate. Each hydrocarbon or derivative was tested at a concentration of 500 mg/l in NSW medium. Two percents of mother culture of the bacteria isolate pre-grown in GPY medium for O.N was used to inoculate different sets of NSW supplemented with different aromatic hydrocarbons separately. All bacterial cultures were grown at 30ºC for three days with agitation at 200 rpm. Bacterial growth was monitored spectrophotometrically as mentioned above. However, the residual non-degraded hydrocarbons were estimated by GC and HPLC. The degradation efficiency of the isolate was studied using different concentrations (500 to 3000 mg/l) for naphthalene and phenol under the same cultural conditions.

One major obstacle that has slowed the implementation of microbial enhanced oil recovery has been the difficulty in isolating and/or engineering microorganisms that can survive the harsh environment of the oil reservoir. A Natural isolate of petroleum oil contaminated area, motivated to investigate the potentiality of this isolate to be used for petroleum biodegradation in sea water.

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The ability of crude oil degradation by the investigated strain was monitored in different media with respect to cellular growth, where M5 containing glucose and yeast extract supported the growth, and consequently maximum oil consumption (7.7%). On the other hand, M3 which contained glucose and petroleum oil showed lower levels of oil consumption (6 %). However, the addition of yeast extract to the medium may enhance the biodegradation capacity. This could be due to the fact that yeast extract was enriched with amino acids and vitamins which acted as co-factors for enzyme productions (El-Helow et al., 2000; Pedersen and Nielsen, 2000; Konsoula et al., 2004).

Crude petroleum oil and hydrocarbon degradation has been analyzed for several fungal and bacterial species, showing variable potentialities (Ijah, 1998; Chaillan et al., 2004; Elshafie et al., 2007; Nievas et al., 2007). However, only few works refered to the isolation and characterization of yeast strains able to utilize hydrocarbons (Ijah, 1998). Therefore, this study focused initially on measuring the ability of the yeast strain A to degrade different aliphatic and aromatic hydrocarbons and some derivatives as sole carbon source in natural sea water medium.

Among tested compounds the investigated isolates showed a great ability to degrade most of them but, with distinctive preference for naphthalene and phenol.

Regarding the aliphatic hydrocarbons, the strain showed adequate potential of degradation which decreased with increasing the chain length.

The tested yeast could not metabolize or degrade naphthylamine and naphthalene 2- sulphonate, due to their lethal effect as reported by Nynes et al. (1968). The ability of Streptomyes hiveoruber and Dactylosporangium vinaceum to degrade long and short chain hydrocarbons could be attributed to having a very efficient degradative enzyme system. This was in agreement with the data reported by Ijah (1998). The utilization abilities of fungi to different hydrocarbons showed significant differences (Elshafie et al., 2007). Prince (1993) reported that microorganisms show a distinct preference for some hydrocarbons over other.

To evaluate the potency and tolerance of the selected strain to degrade different naphthalene and phenol concentrations, the natural sea water was supplemented with naphthalene and phenol at different concentrations (500-3000 mg/l). Strain A showed good potency in degradation and tolerance of naphthalene over phenol. At a concentration of 1500 and 1000 mg/ml of naphthalene and phenol the utilization reached 78 and 49.9%, respectively.

This work might eventually help in bioremediation studies to clean up marine oil spills in an affected environment by the use of marine yeast strains with desirable degradative activities. Bioremediation in expected to play an important role as environmentally safe and cost-effective response to marine oil spill.

The use of infrared spectroscopy for routine monitoring of oil lubricated components, breakdown products and contaminants has not been widely used in the past, although infrared studies of lubrication oils themselves have been performed for a number of years. The reason for this is that older dispersive infrared spectrometers would take several minutes to generate a spectrum of the oil and then considerable additional time would be needed to reduce and interpret spectral data.

Fourier Transform Infrared (FTIR) Spectrometer and the microcomputer is the combination in modern equipment which produces infrared spectra in seconds and sub one minute data reductions. Infrared analysis has become a powerful, practical analytical tool for used oil analysis.

To extract information from IR spectra of used oils, a basic knowledge of the process involved in oil degradation is required.

The lubricant in a combustion engine is operating in a very hostile environment, temperatures are high, and the lubricant is dispersed over a large surface area where it is exposed to chemically reactive process. In addition, oil is exposed to sources of internal and external contamination.

At elevated temperatures, oil exposed to oxygen from the air will oxidize to form a variety of compounds. The majority of these are carbonyl containing compounds such as ketones and carboxylic acids. Some of these compounds are dissolved by the oil, or remain suspended owing to dispersive additives in the oil ,carboxylic acids contribute to the acidity of the oil and deplete its basic reserves as neutralization takes place.

The progress of neutralization is obtained by monitoring the build up of atmospheric nitrogen and metallic sulfates in the oil respectively.

Infrared analysis on used engine oils can provide a great deal of information about what happens to an engine in service within a relatively short period of time. In a number of areas, infrared information is favoured over conventional oil analyses as it is regarded as having a great diagnostic value ,however, exercise caution when interpreting data as engine design and operating conditions play an important role. A series of results from consecutive samples in which trends are evident has far greater diagnostic value than results from a lone sample.

RESULTS AND DISUSSION

In this study the actinobacterial isolates with different morphological characteristics has been successfully isolated from different types of soil sample from marshy land of Muthuppettai, had increased growth rates in the media

containing petroleum products compared to only when minimal salt broth was used. This might be due to the fact that the actinobacterial isolates were able to use the hydrocarbons molecule by dismantling the long chains of hydrocarbon and carbon therby, converting petroleum into simpler forms or products that can be absorbed for the growth and nutrition of the bacteria.

After being completely broken down, the reaction releases carbon IV oxide, water and energy used to create cellular biomass.

The isolates Streptomyces hiveoruber and Dactylosporangium vinaceum showed promising result in preliminary gravimetric analysis of hydrocarbon degradation and they were selected for further FTIR analysis. The analysis at 30 days indicated that almost all the isolates have degraded the oil effectively. The FTIR analysis of untreated used engine oil revealed bands isolated actinobacteria .

S.hiveoruber showed strong bands at 3433 cm-1 indicating the presence of a peptide component resulting from the N-H stretching mode and at 2078 cm-1 resulting from the stretching mode of C=C Bond.The bonds at 1500-1600 shows the stretching of C=C and 690-720 C-H of aromatic rings.1123 cm-1 showed C-N stretching mode.

In D.vinaceum 2085.76 is stretched and it shows the presence of C=C alkyne.1401.76 the C=C alkene group is stretched at 1550 cm-1.

After 30 days the high degradation of hydrocarbon compounds by the microbial incubation, maximum bands observed after treatment with actinobacterial strains. Similar studies by Mohammad yunus et al, 2011 strongly supports the present work. Moreover FTIR spectroscopy can be a very useful tool in performing preliminary tests in order to predict remediation performance so as to select an appropriate approach for cleanup technologies.

Microbial degradation of oil has been shown to occur by attack on the aliphatic or light aromatic fractions of the oil. Although some studies have reported their removal at high rates under optimal conditions high molecular weight aromatics, resins and asphaltenes are generally considered to be recalcitrant or exhibit only low rates of biodegradation. Ijah and Antai reported high degradation of hydrocarbons in soil contaminated with 10% and 20% crude oil compared to those contaminated 30 and 40% crude oil which experienced partial degradation o hydrocarbons within a period of 12 months.

Rahan et al., reported that percentage of degradation by mixed bacterial consortium decreased from 78%to52% as the concentraton of oil increased from 1 to 10%.

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