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Studies on the Biodegradation of Hydrocarbons Using *Pseudomonas Aeruginosa* and *Aspergillus Niger*

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Abstract

The biodegradable activities of Aspergillus niger (fungi) and Pseudomonas aeruginosa (bacteria) on two crudes were investigated. Petroleum oil sludge containing the microbes and the control were monitored at the end of the bioremediation period, the results obtained and compared. Among the test organisms Aspergillus niger showed more biomass production than Pseudomonas aeruginosa. There was rapid growth rate exhibited by fungi during 15–30 days.

Keywords: *Biosurfactant, hydrocarbon, automobiles, oil refineries*

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INTRODUCTION

Oil sludge is mainly generated from oil refineries, automobile industries and waste oil processors. Oil sludge contains several toxic hydrocarbon constituents, making the sites contaminated by it a major environmental concern because many of the constituents of oily sludge are carcinogenic and potent immunotoxicants[1].

Microbial remediation of a hydrocarbon-contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in the soil[2]. These microorganisms can degrade a wide range of target constituents present in oily sludge.

Bio degradation or biotic degradation or biotic decomposition is the chemical dissolution of material by bacteria or other biological means[3]. The term is often used in relation to ecology, waste management, biomedicine and the natural environment (bioremediation) and is now commonly associated with environmentally friendly products that are capable of decomposing back into natural elements. Organic material can be degraded aerobically with oxygen, or an aerobically, without oxygen[4]. A term related to biodegradation is biomineralisation, in which organic matter is converted into minerals. Bio-surfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process.

The purpose of this study is to explore and compare the efficiency and accuracy of degrading the hydrocarbon waste by the bacterial strain *Pseudomonas* and fungal strain *Aspergillu*s isolated from the soil samples collected from an Automobile industry (Tanjore) and from Hero Honda service centre in Trivandrum (Kerala).

MATERIALS AND METHODS

Sample Collection

Petroleum oil sludge samples were collected from the waste disposal sites of an automobile service station in Vallam (Tanjore), and from Hero Honda service centre in Trivandrum (Kerala). In order to eliminate the water content and facilitate easy handling, the sludge was air-dried.

Isolation of Microorganisms
One gram of the sludge was vortexed with 10 ml of distilled water and the suspension was allowed to settle down. The clear supernatant was used for further dilution.

Isolation of Bacteria
Two different oil sludge samples were serially diluted for plating by serial dilute spread plate technique.

Identification of Bacteria
Cell dimensions and morphology were determined on living cells by light microscope. Gram's staining technique was determined was carried out for differentiation. The hanging drop technique was followed to observe the motility of bacteria with which they were classified as motile and non-motile organism.

Identification and Isolation of Fungi
The fungi were identified by lactophenol cotton blue method. Place one loop full of fungal culture from place on to a clean dry slide and place one drop of lactophenol cotton blue onto it and mix well. Place a clean cover slip onto it without air bubble and observed under microscope.

Biodegradation Assay
One predominantly growing population of bacteria *Pseudomonas aeruginosa* and the fungi *Aspergillus niger* were selected for screening their role in biodegradation[5]. Mineral Salt Medium [MSM] which includes Na_2HPO_4 [2.2g], K_2HPO_4 [2.0g], NH_4NO_3 [0.1g], $MGSO_4$[0.02g], $CaCl_2$ [0.02g], few drops of 10% $FeCl_3$ and distilled water 1OOO ml, with p_H 6.0 was used for this study. Potassium phosphate was sterilized separately and added to the medium after cooling in order to avoid the formation of precipitate. The screened bacterial and fungal species were subjected to two different kinds of treatments.
The treated samples were analyzed for biomass, COD and oil and grease content in terms of biodegradation at different periods of incubation 5th and 10th day for bacteria, 15th and 30th day for fungi.

RESULTS AND DISCUSSION
From the soil sample 1 collected from an Automobile industry (Thanjur), and the soil sample 2 collected from Hero Honda service centre in Trivandrum [Kerala], 15 bacterial colonies were observed after 24 hours of incubation. Among the 15 colonies obtained dominant colonies were isolated and identified. *Pseudomonas aeruginosa* was isolated from soil sample 1 (Table 1).

Fungal colonies were observed after 2–3 days of incubation. Among the 7 colonies dominant colonies were isolated and identified. *Aspergillus niger* was isolated from soil sample 2. Between two different treatments, the medium containing sludge and yeast extract favored high yield of biomass, both in the case of bacteria and fungi. *Pseudomonas auruginosa* and *Aspergillus niger* were potential organisms that could be effectively degrade hydrocarbon contents of the soil. Biomass of the individual fungal and bacterial species are different growth stages was estimated the relation to the hydrocarbon treatments.

Among the test organisms *Aspergillus niger* showed more biomass production than *Pseudomonas aeruginosa*. There was rapid growth rate exhibited by fungi during 15–30 days (Table 1). Bacteria showed more growth at 5–10 days when compared to the first five days. Many microorganisms have the ability to utilize hydrocarbons as sole source of energy. The hydrocarbon utilizing microbes including fungi and bacteria degrade very high concentrations of oil that pollutes the soil environment. Diverse groups of microbial populations were frequently isolated from the soil polluted with hydrocarbons and the involvement of such organisms in the degradation has been well established[6].

Table 1: *Comparison of Biodegradation of Hydrocarbons Using Pseudomonas aeruginosa and Aspergillus niger.*

Biomass [mg/l]											
Pseudomonas aeruginosa						***Aspergillus niger***					
5^{th} day			10^{th} day			15^{th} day			30^{th} day		
C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2
298	270	265	310	345	332	287	260	254	302	296	280

The ability of the microorganisms to utilize hydrocarbons as sole source of energy and their ubiquitous nature of distribution have often been reported already[7]. There are more than 100 species belonging to 3O genera of bacteria are capable of degrading petroleum oil in different environments[8,9] listed 22 bacterial genera, 14 genera of fungi an algal genus having of the capacity to utilize petroleum hydrocarbons.

Zobell (1996)[10] compared the abilities of bacteria and fungi in the degradation of hydrocarbons. They studied *candid*a *sp.*, *Hensunula sp*., *Rhodotorula sp*. and found large numbers of pseudomonas sp are capable of utilizing petroleum hydrocarbons. Biomass of the *Pseudomonas aeruginosa* and *Aspergillus niger* in MSM.

CONCLUSION

The study concluded that microbial degradation can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation.

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