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Microbial degradation of waste oil sludge found near the automobile service station

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ABSTRACT

Biodegradation is a process in which microorganisms are committed to transform toxic chemicals into less toxic or harmless. Three bacterial strains, *Escherichia coli (E. coli), Micrococcus sp.,* and *Clostridium sp.* were isolated from contaminated soil found near the automobile service station and tested for their ability to degrade the waste oil sludge found near automobile service station. The results collectively indicating that these bacterial strains have considerable potential for bioremediation of waste oil sludge. The present study also demonstrates that, among the three organisms, *E. coli* showed the best oil degrader, when compared to other two bacterial species.

Key words: Oil pollution, Sludge, Biodegradation, Bacterial strains.

INTRODUCTION

Oil pollution is a serious environmental problem throughout the world. This pollution may be caused due to various activities in oil exploration that include geophysical explorations, drilling of wells, pressure control and management of oil and natural gas gushing from the well, transportation and refining of crude oil etc. Crude oil is a homogenous but complex mixture of hundreds of different hydrocarbons, which widely vary in their characteristics. It is reported that certain microorganisms are capable to degrade these hydrocarbons in various forms (Klug and Markovetz, 1971). These microorganisms are widely distributed in nature where such compounds are present (Atlas 1981; Deka *et al.,* 1993). Environmental degradation may cause due to sudden increase of hydro carbonaceous compounds into the natural ecosystem as a result of seepage or leakage. In order to develope a strategy for microbial degradation of hydrocarbons, it is necessary to isolate specific microbes and to test the efficiency of the isolates in degradation of hydro carbonaceous compounds likely to present in oil contaminated site before application to the field (Janiyani *et al.,* 1993).

Soil is accidentally contaminated by petroleum fuel spills are classified as hazardous (Bartha and Bossert, 1984). When the amounts of contaminated soil are large, the currently accepted disposal methods of incineration or burial in secure chemical land fuels can become prohibitively expensive. Land treatment disposal of oil refinery sludge's has been practiced for decades with generally good results (Bartha, 1986). A number of other treatment methods are also available to reduce such pollutants. Among them, the bioremediation is the most attractive, simple, environmentally acceptable and economically feasible treatment method (Black *et al.,* 1994).

Bioremediation has been defined as a biological response to environmental abuse (Hamer, 1993). It is concerned with biological restoration of historically contaminated sites and with the clean up of areas contaminated either accidentally or incidentally (Hamer, 1993; Baker, 1994). In the present investigation an attempt has been made to isolate and identify the microbial population of waste oil sludge found near the automobile service station and also to study the effect of selected microbes in the biodegradation of waste oil sludge.

MATERIAL AND METHODS

Sample collection

Oil contaminated soil sample was collected from the waste disposal site of an automobile service station, at Thanjavur. To eliminate the water content and to facilitate handling, the sludge was air-dried. The air-dried sludge was stored at 5º C in a sterilized and sealed glass container. Collected soil samples were spread out thinly on a pof stout paper and subjected to oil dry in shade free from dust. During drying, the lumps of soil samples were broken and screened through 2 mm serve plant root, pebbles and other unwanted materials were discarded. The samples were kept in clean polythene bags for analysis.

Isolation and identification bacterial strains

Sterile specific medium was prepared and sterilized at 121^0 C for 20 minutes at 15 lbs. To this flask 2% v crude oil and 1 gm of soil sample was inoculated separately in 250 ml conical flask. Then the flasks were placed in shaker incubator at 30^0 c to 32^0 c for a period of seven days. 1 ml of broth from each conical flask of the above were taken and repeated as above. From each of second batch flask 1ml of broth was transferred into test tubes containing 9ml sterile water and shake vigorously to mix uniformly. Serial dilution was made up to 10^4, 10^5 and 10^6 dilutions from each of respective tubes.

Nutrient agar was prepared and sterilized at 121ºC for 20 minutes at 15 lbs. Then the plating was done in sterile Petri dishes 0.1 ml of sample was spreaded, on nutrient agar media from respective dilution as above. The plates were incubated for 7 days at 30º to 2º C. Colonies developed on plate were isolated and inoculated into the plates containing nutrient agar and used as pure culture for study.

The colony morphology was studied based on the colour, shape, size and margin of the colonies. Motility test were also performed. Gram's staining, Indole test, Methyl Red Test, Voges Proskauer test, Citrate Utilization test, Catalase test, Oxidase test, Urease test and Triple Sugar iron test were performed according to the standard methods (Mac Faddin, 1980).

Biodegradation

Mineral Nutrient Medium was prepared and sterilized at 121ºC for 20 minutes at 15 lbs and then poured into the three conical flasks.

Pure culture inoculation

The sterilized media was kept for cooling and then one loop full of isolated bacterial species from the plate of pure culture was inoculated into 3 conical flasks. One set of conical flask was kept without inoculation of bacterial species (i.e. Control). The flasks were kept in shaker incubator at 30°C to 32°C.

Determination of broth and crude oil

Solvent extraction method was used for analyzing the total oil and broth contents. Residual crude oil after degradation was extracted in petroleum ether. (Boiling point 60°C) up to 28 days for a period of intervals of 7 days. After extraction petroleum ether was evaporated at 60° C and then OD was taken for both the control and the sample.

Determination of Biomass

Treated sample from each conical flask was taken in a test tube then the samples were filtered by using Whatmann No: 1 filter paper. Then, the settled biomass was taken on a preweighed Whatmann No: 1 filter paper and placed in an hot air over at 50º C for 15 minutes and weighed by an electronic balance. The dry biomass weight was calculated from the initial and final weight of the filter papers.

RESULTS AND DISCUSSION

Oil is the source of energy for human and a significant environmental pollutant. In addition to accidental contamination of ecosystems by oil spills,

vast amounts of oily sludge generated in refineries from accumulated oily waste materials in the bottom of storage tanks and water–oil separation systems pose great challenges because of the expense of disposal (Ferrari *et al.,* 1996; Vasudevan and Rajaram, 2001).

Isolation and Identification of Bacterial species from oil sludge

Three different types of bacterial cultures *(Escherichia coli (E. coli), Micrococcus sp., Clostridium sp.)* were isolated and purified in nutrient agar plates for identification **Table (1)**.

Physical Analysis

This was studied by observing the colony morphology of the bacterium. Here three colonies were pale yellow, creamy white and rhizoid white colonies grown over the nutrient agar medium. Hanging drop method was carried out motility test. Here the three cultures are non-motile.

Biochemical Analysis

Three bacterial species had taken up the primary stain and appeared in red colour. So the three bacterial species were identified as negative type of bacteria. After the addition of Kova's reagent at the top there was no ring formation in all cultures and it indicates negative results. After addition of methyl red indicator to the cultures, presence of red colour in S1 and S2 showed positive results and the culture remained yellow colour in S3 this showed as negative results. After the addition of Barritts reagent I, Barritts reagent II the preference of pink colour change occurred in all cultures. So it

Table 1: Identified Bacterial Species

S. No.	Samples	Organisms
1.	S1	*Micrococcus sp.*
2.	S2	*E. coli*
3.	S3	*Clostridium sp.*

Table 2: Results of the biochemical Analysis

S. No.	Name of the Biochemical Test	Result S1	S2	S3
1.	Motility	Non motile	Non motile	Non motile
2.	Gram Staining	-ve cocci	-ve rod	-ve rod
3.	Indole Test	-ve	-ve	-ve
4.	Methyl Red Test	+ve	+ve	-ve
5.	Voges Proskauer Test	-ve	-ve	-ve
6.	Citrate Utilization Test	+ve	-ve	+ve
7.	Catalase Test	+ve	+ve	+ve
8.	Oxidase Test	-ve	-ve	-ve
9.	Urease Test	+ve	+ve	+ve
10.	Triple Sugar Iron Test	K/A	K/A	K/A

Note: S1 - *Micrococcus sp.* S2 - *E. coli* S3 - *Clostridium sp.*

Table 3: Degradation of hydrocarbon from refinery sludge by the test bacterial species at different time interval over control (in Mineral Media with 2gm sludge) units alues in gram

Name of the Organisms	Control	Days of inoculation 7th	14th	21st	28th
Micrococcus sp.	0.37	0.41	0.59	0.057	0.055
E. coli	0.37	0.80	0.041	0.044	0.064
Clostridium sp.	0.37	0.64	0.062	0.093	0.090

indicates negative results. The inoculated citrate agar slants showed that there was a presence of royal blue colour in S1 and S3 and it indicates the positive results. Addition of 3% H_2O_2 to the culture, showed the presence of bubble formation occurred in all the cultures and it indicates as positive results. After the addition of 2.3 drops of cultures the purple colour was not appeared in all the cultures on the oxidase disc. So it indicates as negative results. The culture inoculated urease agar slants showed that the presence of pink colour in S1, S2, S3 and it indicates the positive results. After incubation period, the alkaline slant and acid butt was produced in all the three cultures and is indicated positive results (Table 2).

Leahy and Colwell (1990) have reported biodegradation of petroleum oil by *Achromobacter, Arthrobacter Acinetobacter, Alcaligenes, Bacillus, Flavobacterium, Nocardia, Pseudomonas* and *Rhodococcus.* Okerentugba and Ezeronye (2003) reported the bacterial isolates (*Bacilus sp. Micrococcus sp. and Rhizopus sp.*) from two rivers and refinery effluent to degrade two Nigerian crude oils.

Table 4: Biomass

Name of the Organisms	Control	Days of inoculation			
		7th	14th	21st	28th
Micrococcus sp.	1.028	0.55	10.64	0.17	1.041
E. coli	1.028	0.42	10.33	0.54	1.033
Clostridium sp.	1.028	0.47	10.77	0.07	1.035

Note:
Mineral Nutrient Medium + 2% sludge + *Micrococcus* sp.
Mineral Nutrient Medium + 2% sludge + *E. coli*
Mineral Nutrient Medium + 2% sludge + *Clostridium* sp.

Bio degrader

The result of degradation of hydrocarbon was presented in Table 3 and 4. From these results degradation percentage was calculated. The degradation of hydrocarbon was determined by talking Biomass and OD values of each broth cultures supplemented with oil refinery sludge. The bioremediation is a treatment process that use naturally occurring microorganism to breakdown or degrade hazardous substances into less toxic or non-toxic substances certain microorganisms can digest organic substance such as fuels or solvents that are hazardous to humans. Using microbial inoculants is a common practice, which enhances the rate of biodegradation (Eriksson *et al*., 1995; Lal and Khanna, 1996). Microbes breakdown the organic contaminants into harmless products – mainly carbon dioxide and water.

It was revealed that the degradation of hydrocarbons was increased gradually with increasing the incubation time of the bacteria. Degradation was found rapid after 7 days in all the bacterial species and found maximum at 28 days of incubation. However, after 21 days incubation the rate of degradation was not rapid. It may be rapid up to a certain time period. At four weeks of incubation period the *E. coli* showed the lowest values, which was considered to be the most efficient hydrocarbon degrader among the three bacterial species. The weight of biomass also exhibited the increased level in *E. coli,* which confirmed the best degrader. To identify the degraded compounds of hydrocarbon further investigation is necessary. Moreover, bacterial species can be used to recovery of hydrocarbons from tank bottom sludge of refinery for which field trial is also necessary.

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