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Bioremediation of diesel contaminated soil through microbial flora

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Abstract

Microbial degradation of spilled oil is one of the major routes of the natural removal of contaminants from the environment. Biodegradation gradually destroy oil spills by the help of microorganisms. In the present work the indigenous microorganisms from the oil contaminated area were isolated. Contaminant compounds transformed by these isolates through reactions that take place as a part of their metabolic process were studied. The result of the present study showed the bioremediation of hydrocarbon contaminated soils, which exploits the ability of micro organisms to degrade and/or detoxify organic contaminations.

Keywords:.*Bioremediation, oil spills, microorganism, degradation, diesel, contaminants.*

Introduction

The quality of life on earth is linked inextricably to the overall quality of the environment (Vidali, 2001). Petroleum products are used as fuels, solvents and feedstock in the textile, pharmaceutical and plastic industries; Petroleum is a complex mixture of hydrocarbons and other organic compounds, including some organometallo-constituents. Petroleum constituents represent: saturates, aromatics, resins and asphaltenes (Harayama,*et al*. 2004). Petroleum derived diesel is composed of about 75% saturated hydrocarbons (primarily paraffins including n, iso and cycloparaffins and 25% aromatic hydrocarbon (including naphthalenes and alkylbenzenes). The average chemical formula for common diesel is $C_{12}H_{33,}$ ranging from approx $C_{10}H_{20}$ to $C_{15}H_{28}$ (Riser-Roberts, 1992). Petroleum continues to be used as the principle source of energy; however, despite its important usage, petroleum hydrocarbon also poses as a globally environmental pollutant (Plohl*et al*., 2002). Oil spills especially in soil contamination have prompted research on cost-effective, environmentally benign clean upstrategies (Margesin and Schinner, 2001). Microbial degradation of spilled oil is one of the major routes of the natural removal of contaminants from the environment (Prince, 1993). Microbial degradation of petroleum is influenced by a number of factors, including seasons, history of previous exposure of the given environment to oil, temperature, sediment type and medium used for the isolation of organisms (Calomiris*et al*., 1976; Colwell and Walker, 1977). Microbial degradation appears to be the most environmental friendly method of removal of oil pollutant since other methods such as surfactant, washing and incineration lead to introduction of more toxic compounds to the environment (Oboh*et al*., 2006). This study was aimed at assessing the hydrocarbon utilizing bacterial species in an effort to develop active microbial species with characteristics that could be of relevance in bioremediation of petroleum contaminated oil spills.

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Material and Methods

Sampling

Survey of city was done and heavily contaminated diesel site was selected. Freshly collected soil samples were collected from four different locations from different depths, with the help of sterilized instrument. The soil samples were brought to laboratory under ice cold conditions.

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Soil microbial counts (APHA, 1985)

Heterotrophic plate count (HPC) was done by standard pour plate dilution agar technique using R_2A medium.

Identification of bacterial isolates (Mac Faddin, 1980)

The bacterial isolates were identified on the basis of morphological, cultural and biochemical characteristic with the help of the Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984) and Probabilistic Identification of Bacteria (PIB) computer kit (Bryant, 2003).

Screening of diesel degrading bacterial isolates microtiter plate count method (Medhi and Giti, 2008; Bento *et al*., 2005)

Microtiter plate assay was used to screen out diesel degrading bacterial isolated and analyzed with the help of ELISA reader (Thermoelectron corporation multiskan Ex.) at 450 nm using a UV spectrophotometer.

Assessment of potential of screened bacterial species (Whyte *et al*., 1998)

To determine the range of alkanes utilized by bacterial isolates, the growth of the organisms on different concentration of diesel fuel, containing 1-8% diesel oil were observed spectrophotometrically at 660 nm using a UV spectrophotometer (Systronics, model no. 118).

Results and Discussion

The morphological and biochemical characterization of the bacterial isolates obtained from different sites revealed the following genera: *Micrococcus varians, M. agilis, M. mucilaginosis, Staphylococcus saprophyticus, S. epidermidis, Celibiosococcus* spp., *Streptococcus anguis*. All the isolates were non-motile, cocci shaped and gram positive. Most were Vogesprouskauer positive, urease and citrate utilizers.Out of 25 isolates, 5 isolates with highest Optical Density value were screened out. These isolates were *Stapylococcusxylosus* (BGCC#753), *Micrococcus agilis* (BGCC#764), *Micrococcus varians* (BGCC#766), *Staphylococcus saprophyticus*-3 (BGCC#768) and *Celibiosococcus*spp. (BGCC#775).Diesel hydrocarbon utilization potential of five bacterial isolates was checked at different concentrations (1 to 10%) of diesel hydrocarbon in the medium (Fig. 1). *Staphylococcus xylosus* (BGCC#753) showed maximum growth on 1% diesel oil while *Micrococcus agilis*(BGCC#764) and *Micrococcus varians* (BGCC#766) on 8% diesel oil. *Staphylococcus saprophyticus* (BGCC#768) and *Celibisococcus* spp. (BGCC#775) did not exhibit proper growth at 5%, 8% and 10% but showed maximum growth at 1% of diesel oil.The results showed that *Micrococcus agilis* (BGCC#764) could utilized a higher percentage of diesel oil while high percentage decreased the growth of all the other isolate. The pH change in the culture growing medium was also studied for each isolates. The results showed pH decrease from 7.2 to 6.29 (Fig. 2).

Fig: 1 Change in total viable count during the growth in Bushnell Hass media containing different concentration of dieseloil

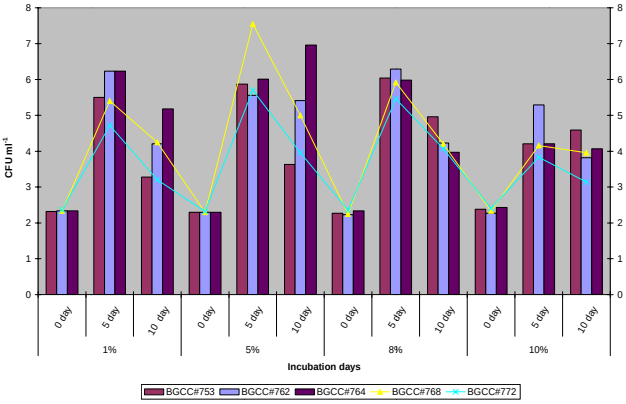
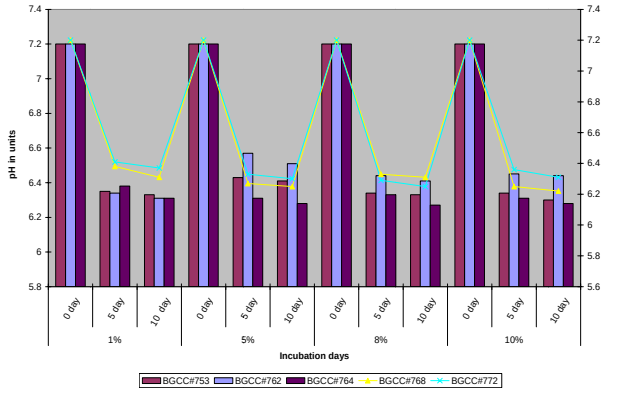


Fig: 2 pH change during the growth of diesel utilizing bacterial isolates grown in Bushnell Hass medium containing different concentration of diesel oil



Since all the bacteria in the present study was isolated from a petroleum contaminated oil sample, some of them survived and adapted the oil-contaminated environment very easily as also reported by other authors (Rahman *et al*., 2003; Das and Mukherjii, 2007).Twenty five bacterial isolates were obtained from diesel contaminated soil samples. The predominant flora was composed of *Micrococcus* spp., *Staphylococcus* spp., and *Celibiosococcus* spp. Bacteria belonging to these genera have been described as petroleum degraders or even as hydrocarbon degraders by (Marin *et al*., 1996).Soil bacterial diversity, as estimated by phylotype richness and diversity of all the soil variables examined, soil pH was, by far the best predictor of both soil bacterial diversity and richness. The lowest level of richness was observed in acidic soil.Microtiter plates have been already extensively used in applied research. Microbial diversity offers an immense field of environment friendly options for mineralization of contaminants or their transformation into less harmful non-hazardous compounds.

Conclusion

The overall results show that the bioremediation of diesel is an important process which improves the soil fertility by minimizing the toxic effect of it. The organisms evaluated in this work showed *Micrococcus* sp. were able to utilize hydrocarbons as the sole carbon source. Based on the results obtained from the laboratory study, biodegradation could be considered as a key component in the clean-up strategy developed in the future for the treatment of oil-sludge contamination. Some of the pure culture were versatile and persisted throughout the utilization process. Further studies are necessary on more interesting bacterial species and strains. However, it is advantageous and profitable to use native microorganism cultured from areas with historical contamination for degradation of hydrocarbons. This approach is likely to reduce or eliminate the initial lag phase, which can be long and optimize overall process time.

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