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BIODEGRADATION OF DIESEL SPILL SOIL FROM CHENNAI HARBOUR

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Abstract

In the present study, soil sample was collected from diesel spill soil at Chennai harbour. Bacterial colony isolated from the soil was screened for their ability to grow in the medium containing diesel. The selected bacterial isolated was subjected to taxonomic identification and tentatively as *Bacillus* spp. *Bacillus* spp tested for their efficiency to degrade diesel with four different concentrations. The degradation of diesel was recorded by the measurement of carbon-di-oxide and biomass. Degradation was measured in 42 hours for 16 days. Degradation was maximum in 10%.

Keywords: *Biodegradation, toxicity, cirrhinus mrigala, dimethoate, diesel spill, contaminated soil.*

Introduction

Biodegradation is the partial or complete conversion of the compound of interest to its elements. The role of organisms, both micro- and macro-organisms, in biodegradation is complex. Also mean a potential risk to human health, as many of them carcinogens. Their persistence within the ecosystems is due to their low solubility and high sorption to soil, two features that limit their availability for the degrading microorganisms (Toledo *et al*., 2005). Microorganisms are actively involved in the degradation of naturally occurring and toxic substances such as petroleum hydrocarbons, pesticides etc. Aerobic biodegradation is the breakdown of organic contaminants by microorganisms when oxygen is present.

Aerobic bacteria use oxygen as an electron acceptor, and break down organic chemicals into smaller or organic compounds, often producing carbon dioxide and water as the final product. Petroleum hydrocarbon continues to be used as the principle source of energy and hence an important global environmental pollutant. Apart from accidental contamination of ecosystem, the vast amounts of oil sludge generated in refineries from water oil separation system and accumulation of waste oily materials in crude oil storage tank bottoms pose great problems because of the expensive disposal methods. (Ferrari *et al*., 1996; Vasudevan and Rajaram, 2001). Despite decades of research, successful bioremediation of petroleum hydrocarbons contaminated soil remains challenge.

Materials and Methods

Soil sample was collected from diesel spill area at Chennai harbor. The collected soil sample was sprinkled on Bushnell-Hass mineral salt medium containing 5% of diesel. Gram staining and biochemical tests like Catalase, Oxidase, Methyl Red, Vogus Proskauer, Indole production, Citrate utilization tests were carried out for strain identification. The isolate and reference strains were inoculated separately on Bushnell-Hass mineral broth containing different concentrations of diesel like 2.5%, 5.0%, 7.5%, and 10%. They were incubated at room temperature for a period of 16 days and the degradation was confirmed by analyzing the biomass, Carbon dioxide production and degradation products. The above parameters were measured at every 48 hours for 16 days. 1ml of the sample was taken and titrated against 0.1ml NaOH solution. Phenolphthalein was used as an indicator and appearance of pink colour is the end point. Turbidimetric method was done for estimating the biomass by measuring the turbidity at 600nm.

Results and Discussion

In the present investigation, the soil samples were collected from the diesel contaminated sites, because the capability of native bacterial population mineralize diesel hydrocarbons in diesel contaminated sites was confirmed by Ojo (2006), Okoh (2003), Emtiazi and Shakarami (2004). The bacterial colony was obtained at sprinkled plate on Bushnell-hass agar medium after 16 days of incubation at 37^0C. The present investigation was carried out to explicate the effects of crude oil concentration on biodegradation of crude oil by *Bacillus* spp. Bacillus spp is a Gram positive and rod shaped. It can easily be isolated soil environments because it is able to colonize multiple environmental niches by using natural compounds as energy source.

B. subtilis virulence genes allow the organism to proliferate in response to the given environmental demands. In addition, some *Bacillus* spp strains produce a yellow pigment, which is a virulence factor in this organism.

In the measurement of biomass, the isolated and reference strain showed increasing turbidity when measured in a colorimeter. It shows the increase in biomass during the treatment period by representing OD values. Changes in the turbidity of the medium during long treatment with various concentration of diesel by isolate and reference strain *Bacillus* spp are observed. In 2.5%, 5%, 7.5% concentration of diesel, growth is relatively low when compared to 10% concentration of diesel. The growth of isolated and reference strain *Bacillus* spp in various concentrations at different period, measured at 600nm for 16^{th} days (Figures 1 and 2). Therefore *Bacillus* spp strains are effective isolates in biodegradation of hazardous contaminates in the environment, so they can be used for biodegradation of contaminated soils.

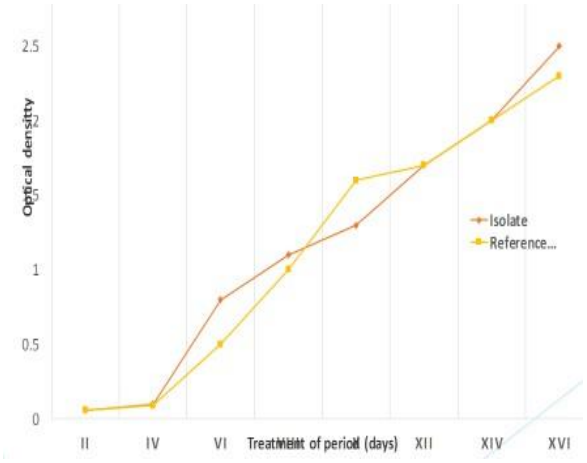

Figure 1. Growth of bacillus spp in 10% of die

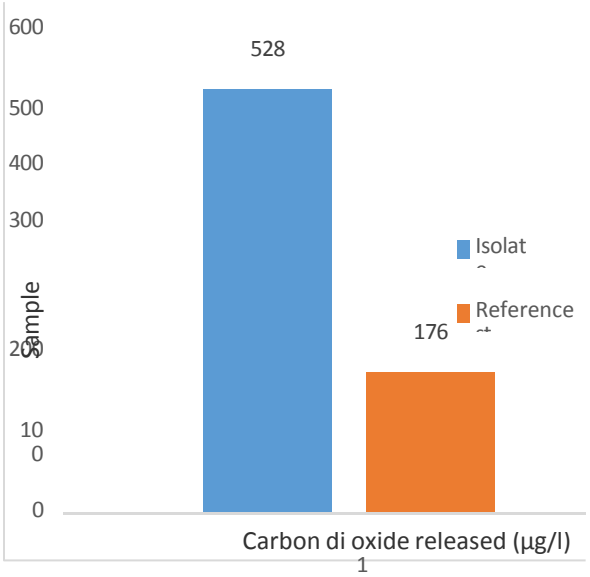

Figure 2. Quantity of carbon-di-oxide on 16th day of incubation

An increase degradation is correlated to an increase in cell number indicating the bacterial isolates were responsible for oil degradation (Mandri and Lin 2007). Therefore, diesel degradation was confirmed in the present work. The higher rate of growth observed at 10% concentration of diesel suggests the extent of dependency of *Bacillus* spp on diesel for its hydrocarbon requirement.

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