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Isolation of Microorganism from Natural Source for Bioremediation of Oil-spill

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ABSTRACT

Bioremediation is a process by which chemical substances are degraded by bacteria and other microorganisms. Several research studies have recently been performed to investigate the use of bioremediation for oil-spill cleanup in seawater, freshwater and terrestrial areas. In-situ bioremediation involves the treating the contaminated materials at the site by Biostimulation and Bioaugmentation. Biostimulation involves stimulation of indigenous microbial population by adding nutrients so that they become metabolically active and degrade the organic pollutants.Bioaugmentation involves Seeding with pollutant degrading bacteria which increase the rate of extent pollutant degradation. Pure cultures of microorganism were obtained by enrichment technique from the effluent-polluted soil collected from different location which had been contaminated over a period of time. After the Isolation of pure culture different Biochemical characterizations were analyzed further confirmation of the Microorganism. The oil contaminated water collected from Visakhapatnam port trust. Four Experiments were designed for treatment like oil, oil plus fertilizer, oil plus pure culture of bacteria and fertilizer, oil plus isolated bacteria and fertilizer were formed. Water samples were collected periodically and analyzed for bacterial counts. There was an increase in microbial count and rate of degradation of poly aromatic hydrocarbons in oil plus bacteria and fertilizer plot. The presence of hydrocarbons was detected by gas chromatography. Therefore Bioremediation by applying nutrients along with the microbes considered as an effective `tool' for combating oil spills.

Key words: *Biostimulation, Bioaugmentation, Polyaromatic hydrocarbon*

INTRODUCTION

Biodegradation of accidentally spilled crude oil in aquatic and terrestrial environments has been the subject of recent reviews [1, 2, 3, 4]. Crude oil accounts for majority of oil spills which gives a serious damage to marine life. Biological treatment of oil contaminated waste water is efficiently used nowadays [5]. Microorganisms are considered to be the only biological source for degradation of hydrocarbons [6]. Bacterial species have been shown to degrade hydrocarbon. The degradation was reported to be stimulated with fertilizer in laboratory [7, 8]. As compared to Genetic engineering microorganism (GEM) seeding of naturally adapted microbes is considered as an important approach in bioremediation [9].Thus, bioremediation is normally achieved by stimulating the indigenous microbiota (naturally occurring microorganisms).Stimulation is achieved by the addition of growth substrates, and nutrients resulting in an increase in contaminant biodegradation and biotransformation. This notion was confirmed in the large scale operation for bioremediation after the oil spill from the Exxon Valdez in Alaska, with the addition of nitrogen and phosphorus fertilizers [10].

Crude oil causes imbalances in the C-N ratio at the spilled site. For the efficient growth of bacteria, the C-N ratio should be around 60-100:1 [11, 12]. If it is slower or greater, the growth of bacteria will be retarded. Urea is used as a nitrogen source at the spilled site which stimulates the growth the microorganism and simultaneously accelerates the disappearance of hydrocarbons [13].

Microorganism was isolated from the contaminated sites by enrichment technique as well as the pure culture of *Pseudomonas putida* (MTCC-2445) obtained from IMTECH, Chandigarh was seeded with fertilizer for treatment of oil contaminated water. The physicochemical parameters of oil contaminated water selected for treatment were also characterized.

MATERIAL AND METHODS

Pure cultures of microorganism were obtained by enrichment technique from the effluent-polluted soil collected from different location which had been contaminated over a period of time. The cells were grown on a rotary shaker in 500 ml flasks each containing 250 ml of basal salts medium to which 0.5 gram of soil plus 0.5 ml of crude oil were added. The medium containing soil and crude oil was incubated at 30 ± 2 ºC under shaking (200 rpm).After 48 to 72 hr of growth samples from the flasks were streaked on plate which contains Basal salt media that had been solidified with 2% agar. The Plates were incubated at 30 ºC temperature. The colonies were grown on the plates were picked and streaked on new basal agar medium plate for isolation of pure culture of microorganism. At another 500 ml flask 0.5 gram of soil were added into of basal salt medium without crude oil and the medium was incubated at 30 ± 2 ºC under shaking (200 rpm) for 48 h.The crude oil were added at an initial concentration of 100 µl and slowly increased up to 1ml over a period of 30 days. After one weeks of enrichment, sub-samples from the flasks were streaked on plates containing basal agar medium and isolation of pure culture had been done by the same method which was already described. Further biochemical characterization was analyzed for identification of bacteria. Cells were recovered by centrifugation and re-suspended in distilled water. The oil contaminated water collected from Visakhapatnam port trust. The physicochemical parameters of the oil contaminated water were also characterized before treatment as shown in Table 1. Four Experiments were designed for treatment like oil, oil plus fertilizer, oil plus pure culture strain of bacteria and fertilizer, oil plus isolated bacteria and fertilizer were formed. Fertilizer Urea was added simultaneously as a Nitrogen source for the growth of bacteria at a rate of 1g/l.Water samples were collected periodically and analyzed for bacterial counts. Increase in the bacterial count observed from 0 to 15 days of treatment by sampling and serial dilution technique using agar spread plate method as shown in Table 2.

Approximately 5 ml of sample was extracted with 5 ml of CCl_4 and the extracts were evaporated to dryness. The final residue was re-dissolved in 5 ml of CCl4 and analyzed for GC profiles. The presence of hydrocarbons was detected by gas chromatography. The Schimadzu gas chromatograph was equipped with a flame ionization detector and a 10 ft stainless steel column containing chromosorb P pre-coated with 3% OV-1. The operation conditions were as follows: Oven initial temperature (50^0C); Oven final temperature (270^0C); Injection port temperature (90^0C); N_2 carrier gas flow rate (30 ml/min); H_2 gas pressure (1.5 kg/cm^2); Air pressure (2.0 kg/cm^2); Chart speed (1 cm/min); and Volume of sample injected (0.5 ml).

RESULTS AND DISCUSSION

The GC profiles of sample of four different experiments before bioremediation are represented in Fig. 1.The initial concentration of poly aromatic hydrocarbon (PAH) in four different experiment were 6.13, 6.12, 5.26, 5.02 respectively. Concentrations of Sample were obtained from the standard plot. Comparisons of the GC profiles 30 days after bioremediation are represented in Fig. 2. The final concentrations of PAH in four different experiments were

Table 1: Physicochemical parameter of water

Sl no	Parameter	oil spill
1.	pH	8.3
2.	EC (mS/cm)	2.80
3.	Nitrogen (mg/l)	1.24
4.	Phosphorus (mg/l)	3.9

The data are mean values of three different Experiments (Mean ± SE).

Table 2: Fertilizer effect on bacterial population at different experiment

Sl no	Treatment	0th Day	15th Day
1.	Control plus oil	211×10^4	215×10^4
2.	Oil plus fertilizer	208×10^4	220×10^4
3.	Oil plus pure culture of bacteria and fertilizer	180×10^4	204×10^4
4.	Oil plus isolated bacteria and fertilizer	176×10^4	202×10^4

The data are mean values of triplicate plate count.

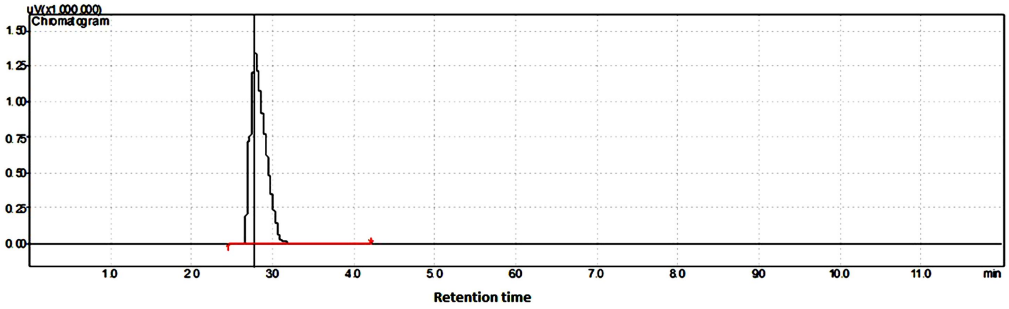

Fig 1.1 Oil (initial)

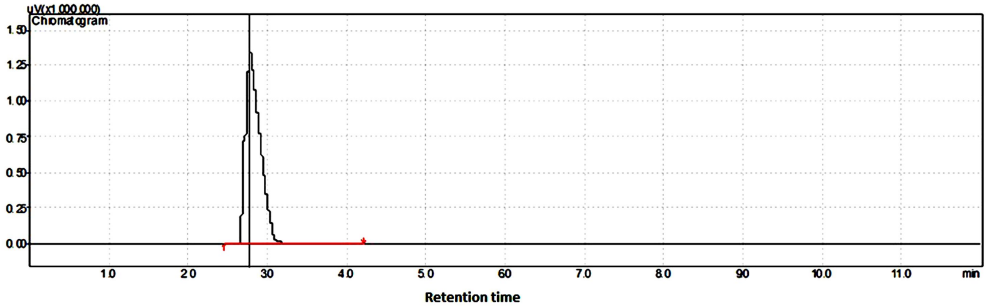

Fig 1.2 Oil plus fertilizer (initial)

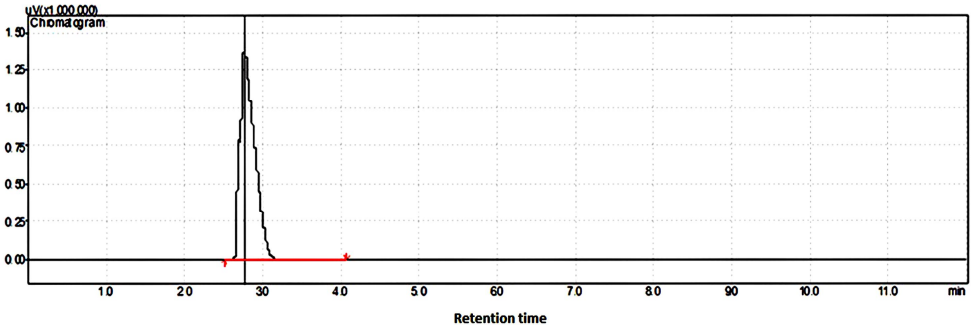

Fig 1.3 Oil plus pure culture strain of bacteria and fertilizer (initial)

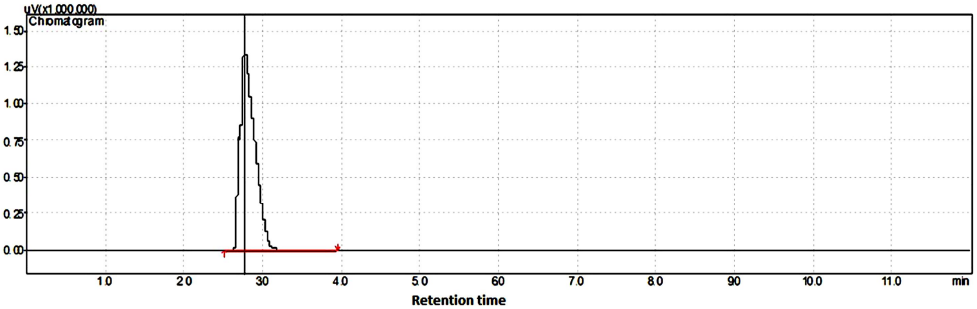

Fig 1.4 Oil plus isolated bacteria and fertilizer (initial)

Fig 1. Initial Gas chromatographic profiles of the crude oil for various experiments.

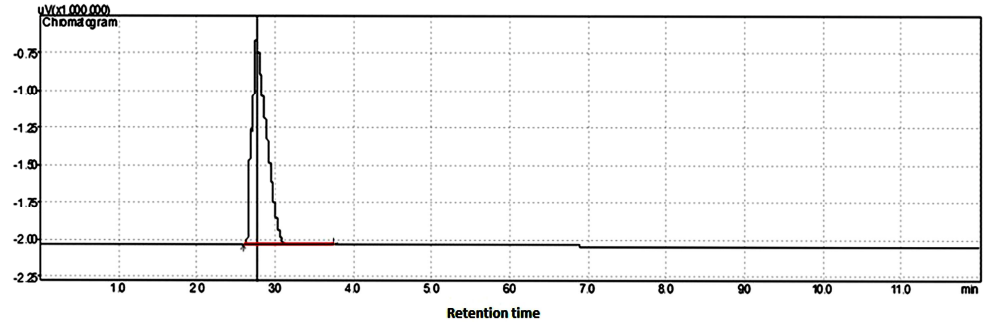

Fig 2.1 Oil (Final)

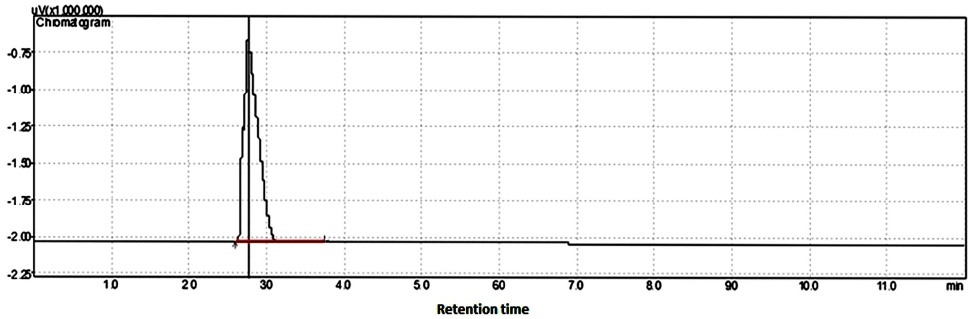

Fig 2.2 Oil plus fertilizer (Final)

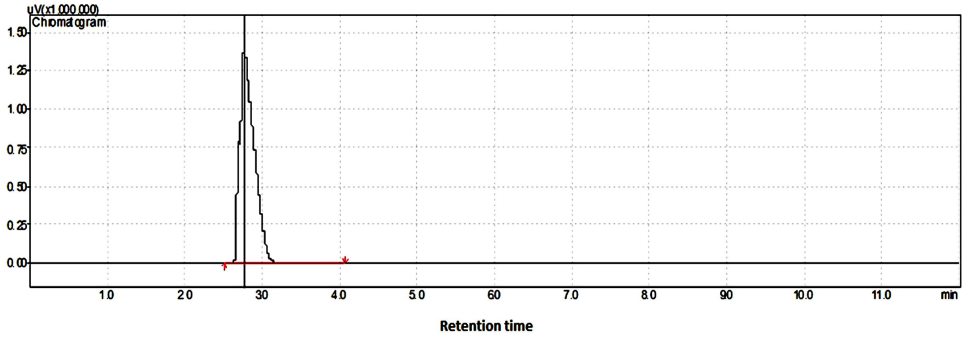

Fig 2.3 Oil plus pure culture strain of bacteria and fertilizer (Final)

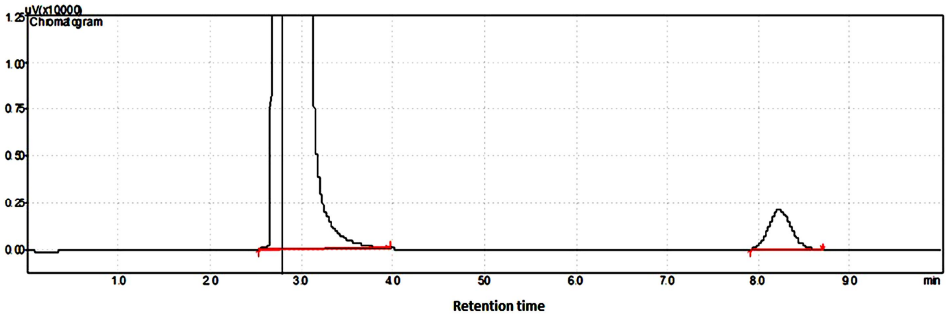

Fig 2.4 Oil plus isolated bacteria and fertilizer (Final)

Fig 2. Gas chromatographic profiles of the crude oil after 15 days for various experiments.

5.94, 5.92, 2.17 and 2.61 respectively. Application of fertilizer plus microbial inoculums resulted in a marginally higher rate of substrate utilization so the concentration of PAH gradually decreased after successful bioremediation. The utilization of crude oil is evident by the increase in the number of viable cells as well as in the rate of degradation at 15 days in oil plus bacteria and fertilizer plot. The degradation efficiency of PAH in oil plus pure culture strain of bacteria and fertilizer plot was 58.74% and in oil plus isolated bacteria and fertilizer plot was 48.00%. Therefore the microorganism isolated from natural source by enrichment technique utilize the PAH as efficient as the pure culture

strain of bacteria i.e. *Pseudomonas putida* (MTCC-2445). Therefore Bioremediation by applying nutrients along with the microbes considered as an effective `tool' for degrading oil spills.

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