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Biodegradation of kerosene in soil by a mixed bacterial culture under different nutrient conditions

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Abstract

The present investigation dealt with the biodegradation of kerosene in soil using a mixed bacterial culture under two different treatment setups, treatment 1 (T1) having low nutrient concentration as compared to treatment 2 (T2). Each treatment contained 4% (w/w) kerosene in soil as a sole carbon source. After 6 weeks of incubation, T1 and T2 exhibited 27±3% and 65±7% kerosene degradation, respectively. The highest bacterial growth was observed in T2 with a significant reduction in nutrient content of soil over 2–3 weeks of incubation. Overall, 46±12% and 54±24% of nitrogen, 36±3% and 43±3% of phosphorus and 24±2% and 35±2% of potassium content of the soil were depleted under T1 and T2, respectively. The present work has defined nutrient requirements for kerosene oil degradation and has opened avenues for its remediation from contaminated soil.

Keywords: Kerosene; Biodegradation; Bacteria; Soil; Nutrients

1. Introduction

The use of large quantities of petroleum products has resulted in a high level of environmental pollution. Oil spills caused by blowouts, leakage from tanks and dumping of waste petroleum products, for example, lead to an elevated loading of petroleum hydrocarbons in soil, which results in significant decline in the quality of soil and makes it unfit for use. Crude oil is an extremely complex mixture of aliphatic and aromatic hydrocarbons, including volatile components of gasoline, petrol, kerosene, lubricant oil and solid asphaltene residues. The kerosene fraction poses the greatest pollution problem (Solano-Serena et al., 2000).

To control the environmental risks caused by petroleum products, various new regulations have been introduced and, at the same time, research focusing on the bioremediation of contaminated soils has been boosted. The dominant mechanism that breaks down these petroleum products is biodegradation, which is carried out by natural microbial populations (Kokub et al., 1990; Margesin and Schinner, 2001a). Recently, this biotechnique has been employed successfully for the clean up of sand contaminated with crude oil (Khalid et al., 2005). For this purpose, some investigators have suggested the addition of specific hydrocarbon degraders to enhance the rate of biodegradation process (Horowitz and Atlas, 1980).

Crude oil, as well as other commercial hydrocarbons, could be considered extensively biodegradable in soils. Differences in the extent of hydrocarbon degradation have been reported in the literature (Morgan and Watkinson, 1989; Leahy and Colwell, 1990; Bossert and Compeau, 1995; Huesmann, 1997), depending on soil and hydrocarbon source type, concentration of total hydrocarbons and oxygen and nutrient availability.

Generally, hydrocarbons in kerosene are not inhibitory to microbial activity (Song et al., 1990; Aelion and Bradley, 1991) and biodegrade significantly under aerobic conditions, provided that sufficient amounts of essential nutrients are present (Raza et al., 2006, 2007a).

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Considerable information on the microbial degradation of defined sole hydrocarbons is available in the literature, but less is known on the biodegradability of some petroleum commercial products (Sugiura et al., 1997) such as kerosene (Wongsa et al., 2004).

Keeping this problem in view, an effort has been made to clean up soil contaminated with kerosene by using a mixed bacterial culture. The purpose of the present study was to investigate and enhance the rate of biodegradation of kerosene in soil by nutrient addition.

2. Materials and methods

2.1. Kerosene and chemicals

Kerosene purchased from a local petroleum products market was used as carbon source, in mineral salt medium (MSM). All the chemicals used were of analytical grade and used as such without further refinement.

2.2. Amendment of soil

Soil collected from three different agricultural fields was thoroughly mixed in equal proportions to form a composite soil. This soil was amended with sand (20%, w/w), obtained from the river Chenab, Pakistan. The soil/sand mixture was sieved to remove stones and gravel and sterilized at 121 °C for 15 min. This soil was termed "amended soil". Physico-chemical and microbial analyses, moisture content, nitrogen, phosphorus and potassium (NPK) and viable bacterial count were carried out. The analyses showed that the amended soil did not fulfill the nutrient (NPK) requirements for an efficient biodegradation process (Table 1). Therefore, salts of these elements were added in the form of MSM to provide the proper nutrients for the biodegradation process.

2.3. Mixed bacterial culture preparation

Previously isolated, characterized, oil-degrading bacterial strains of *Pseudomonas putida* 27, *P. putida* 6-B and *P. putida* 300-H (Tahseen, 2002), *Pseudomonas aeruginosa* EBN-8 mutant (Iqbal et al., 1995), *P. putida* 300-B mutant and *P. putida* 33 (Raza et al., 2007a) were subcultured on separate nutrient agar plates and incubated at 37 °C for 24 h. Single colonies of these microorganisms were transferred separately into Erlenmeyer flasks (250 ml) containing 100 ml of the nutrient broth medium (0.8%) and incubated for 24 h at 37 °C on an orbital shaker (Ogawa, Seiki, OSI-503 L, Japan) at 100 rpm. Aliquots (50 ml) of each culture medium were centrifuged (Beckman, J2-HS, USA) at 9820*g* for 15 min. The cell pellets were washed and resuspended in autoclaved normal saline (0.89% NaCl in distilled water) and the optical density (OD) at 660 nm (Spectro UV–vis RS Spectrophotometer, USA) was adjusted to 0.7 with normal saline. Five milliliters of each cell suspension was mixed in a sterilized screw-capped test tube. The resultant cell suspension was the mixed bacterial culture.

Table 1
Physico-chemical and microbial characteristics of the amended and experimental soils

Parameters	Units	Amended soil	Experimental soil	
			Treatment 1	Treatment 2
Nitrogen	mg kg^{-1}	637±45	742±50	1687±95
Phosphorus	mg kg^{-1}	21±1	142±10	1238±75
Potassium	mg kg^{-1}	81±3	281±30	2067±95
Bacterial viable cells	CFU g^{-1} soil	<10	3.8×10^8	3.8×10^8
Kerosene	%, w/w	Nil	4±0.2	4±0.3
Moisture	%, w/w	5±0.2	30±2	30±2
Sand	%, w/w	20±1	20±1	20±2

Each value is a mean of three replicates and ± indicates standard deviation among them.

2.4. Enrichment of mixed bacterial culture

The mixed bacterial culture (1 ml) was transferred to MSM containing kerosene (2% w/v) in a 250 ml Erlenmeyer flask and incubated on an orbital shaker (100 rpm) at 37 °C. After 48 h, growth was observed and the culture (1 ml) was shifted to fresh MSM containing 3% (w/v) kerosene. In the same way, the culture was transferred to serially increasing concentrations of kerosene up to 5% (w/v). The mixed bacterial composition was determined at the end of each enrichment; it contained all the component strains in the final enrichment (5% kerosene, w/v). Cells were recovered by centrifugation (9820*g* for 15 min) and resuspended in normal saline to set an OD of 0.7 at 660 nm. This inoculum was used at 4% (v/w) to study biodegradation of kerosene in soil.

2.5. Experimental design to study the effect of nutrients on kerosene biodegradation

Eighteen 250 ml Erlenmeyer flasks were prepared for each treatment (treatment 1 (T1) and treatment 2 (T2)) and control (C1 and C2). In each flask, 96 g of amended soil (sterilized soil and sand mixture as mentioned in Section 2.2) was contaminated and mixed well with 4 g of autoclaved kerosene (4% w/w). Flasks under T1 had C:N, C:P and C:K ratios of 45:1, 238:1 and 120:1, and flasks under T2 had the same nutrients but their ratios were 20:1, 27:1 and 16:1, respectively. Autoclaved modified MSM (Bushnell and Hass, 1941) was used to study the effect of nutrients on kerosene biodegradation; this contained (g l^{-1}): KH_2PO_4, 1.0; K_2HPO_4, 1.0; NH_4NO_3, 1.0; $MgSO_4$, 0.2 and $CaCl_2$, 0.02. This MSM composition was 1×. MSM with 10 times the salts concentration of 1× was termed 10×. Thirty milliliters of 1× and 10× MSM were added to flasks under T1 and T2, respectively; thus, T2 contained 10 times the nutrient (NPK) concentration. Flasks were inoculated with mixed bacterial culture (4 ml) in both treatments, closed with cotton wool plugs and incubated in the dark at 37 °C for 6 weeks. There were also parallel sets of flasks of C1 and C2 having the same composition of nutrients and incubating conditions, but without inoculum. The soil in these flasks was the experimental soil and its physico-chemical and microbial characteristics are shown in Table 1. The bacterial population (~3.8×10^8 CFU g^{-1} of soil), kerosene and water contents were the same in T1 and T2. There were six sets, each containing three flasks, for each treatment (T1 and T2) and control (C1 and C2). The water content of soil (in flasks) was adjusted with sterile distilled water to the maximum water-holding capacity (30–50%). In order to avoid anaerobic conditions, contents of the flasks were mixed thoroughly every second day. The experiments were performed for 6 weeks and samples (one set of three flasks of each treatment and control) were taken on a weekly basis and analyzed for different physico-chemical and microbial parameters.

2.6. Total bacterial count determination

Viable bacterial population in soil was determined by the plate count method (Cappuccino and Sherman, 1996) as follows: 1 g of each soil sample was shaken for 15 min in autoclaved normal saline. After settling for 30 min, appropriate dilutions up to 10^{-8} of the suspension were made in autoclaved normal saline, and 100 µl evenly distributed over the surface of nutrient agar plates, incubated at 37 °C overnight and colonies counted.