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RESEARCH ARTICLE

ISOLATION, IDENTIFICATION, DEGRADATION POTENTIAL OF KEROSENE AND CRUDE OIL DEGRADING MICROBES

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ABSTRACT

Hydrocarbon pollutants in contaminated soils can potentially be degraded by microbial activity. The potentiality of microbes as agents of degradation of several compounds thus indicates biological treatment as the major promising alternative to attenuate environmental impact caused by pollutants. The present study was designed to identify microorganism present in the agricultural contaminated kerosene and crude oil treated soil sample and study their degradation. Serial dilution of 1gm of treated soil samples showed distinct colonies at 10^{-4}, 10^{-5} and 10^{-6} dilution and found to identified 5colonies. Colonies subjected to biochemical test revealed that they were *Acinetobacter baumamii, Azotobacter tropicalis, Bacillus subtilis, Corynebacterium variabilis, Flavobacterium lutescens.* The pH of the kerosene and crude oil also increased as the degradation percentage increased. The isolated organism were analysed for hydrocarbon degradation with 4ml kerosene and 4ml of crude oil in minimal salt medium. The degrada tion of the kerosene and crude oil was increased from 0th day of 0.78% to 20.22% in 16th day and for crude oil degradation increased from 0th day of 0.43% to 13.48% in 16th day.

INTRODUCTION

Hydrocarbon pollutants in contaminated soils can potentially be degraded by microbial activity. The potentiality of microbes as agents of degradation of several compounds thus indicates biological treatment as the major promising alternative to attenuate environmental impact caused by pollutants (Nwaeke and Okpokwasili, 2003). Microbial breakdown of hydrocarbon pollutants is generally a very slow process, but it could be optimized to enable the rate of microbial transformation proceed more rapidly. Optimum biodegradation can only occur if the right environmental conditions such as pH, temperature, nutrients and relevant microbial consortia are present. Conditions such as temperature and microbial composition cannot be influenced in real practical bioremediation situations except on ex-situ bioremediation programs. However, conditions such as pH and nutrient availability can be optimized to enhance speedy indigenous microbial breakdown of contaminants if these conditions are predetermined. Conditions suitable for effective biodegradation processes vary from place to place because soils vary in their physical, chemical and biological composition and properties. Knowledge of such conditions that could be influenced will enhance the ordinarily slow natural attenuation process towards speedy bio-remediation.

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Crude oil is a complex mixture of hydrocarbons, composed of aliphatic, aromatic and asphaltene fractions along with nitrogen, sulfur and oxygen-containing compounds. The constituents of these hydrocarbon compounds are present in varied proportions resulting in high variability in crude oil from different sources. Contamination of soils and aquifers by oil spills is a persistent and widespread pollution problem ravaging almost all compartments of the environment and imposing serious health implication and ecological disturbances. This problem has become compounded in recent years by increasing sabotage and vandalization of pipelines by restive oils communities particularly in the Niger Delta area of the country. If exposure is prolonged and the concentration of the oil is high, liver or kidney disease may develop, bone marrow damage is possible and the risk of cancer is increased (Baars, 2002). A lot of cosmetics, paints, inks, drugs, fertilizers and plastics as well as myriad of other items contain petroleum products. A gram of soil may contain up to a billion bacteria, a million algae, 100 thousand protozoa and 10km of fungi hyphae. Therefore the present study aims to isolate the kerosene and oil degrading bacteria from the agricultural soil.

MATERIALS AND METHODS

Agricultural soil samples were collected from in around Namakkal district. Soil samples were contaminated with kerosene and petrol by 4% v/w in 100 gm soil samples and

maintained for 16 days. Soil samples were collected after 1st, 6th, 11th and 16th days for enumeration of bacterial population.

Enumeration of bacterial population

Nutrient agar (NA) medium was prepared according to manufacturer's instruction, sterilized and poured into Petri dishes. One gram of soil sample was diluted serially until fifth dilutions and 0.1ml aliquot from the fifth dilution was inoculated on the freshly prepared media, incubated at 370C for 24 hours and observed for growth. Colonies were counted and recorded as colony forming units per gram of soil (cfu/g). Isolates were subcultured repeatedly to obtain pure isolates and characterized according to the methods described by Holt *et al.* (1994).

Bacterial characterization

The bacterial species that were isolated from the soil samples were characterized further using morphological, physiological and biochemical properties that included Gram reaction, shape, indole production test, Methyl red test, Voges Prosakaeur test, Citrate test, Nitrate reduction, Catalase test, Oxidase test, Glucose utilization test, Triple sugar ion test and urease test were determined according to standard methods of Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994)

Determination of rates of utilization of refined petroleum products by 0.1 ml soil bacteria

Minimal salts medium (MSM) of Zajic and Supplisson (1972) containing; 0.27g K2HPO4, 0.6g NH4Cl, 0.03g MgSO4.7H2O, 0.015g NaCl, 0.0015g NaSO4.7H2O and 150ml distilled water with 1% refined petroleum product (petrol, diesel, kerosene) as the only source of carbon. The MSM was inoculated with 0.1ml of five time's serially diluted agricultural soil samples. The setup was incubated at 30^0C for six (16) days. Turbidity produced as a result of microbial growth was monitored visually at 1st, 6th, 11th and 16th days incubation period and their absorbance reading at 540nm on spectrophotometer were determined.

RESULTS

Biochemical test

Four isolates (P1, P5) showed negative gram staining and five isolates showed positive (P2, P3, P4) in which two cocci (P1 was cocci and others are rod shaped bacteria (P2, P3, P4, P5) were observed. Indole test showed positive cherry red colour in the isolates P2, other organisms are negative. Methyl red test showed a positive result a red or pink in colour in the isolates P2, and P3, negative in P1 and P4. Voges-proskauer test showed a positive result brown or red colour in the isolates, negative in P1, P3, P4, and positive in P2. Citrate utilization test showed a positive result deep Prussian blue in the isolates positive in P1, P2, and negative in P3 and P4 (Table.1). Urease test showed a positive result pink colour in the isolates P8, P6 and P2 negative in P1, P3 and P4. Triple sugar iron agar test showed a positive result red colour in the isolates P4 and, negative in P1 and P3. Nitrate reduction test showed a positive result red colour in the isolates P2, P4 and negative in P1, P3, P5. Catalase test showed a positive result in air bubbles to adding of hydrogen per oxide in the isolates P1to P6. Oxidase test showed positive result blue colour in the isolates P2, P3 and negative in P1, and P5

ISOLATION OF MICROORGANISMS

Kerosene

The result of the bacterial count show that kerosene treated soil had the highest count of *Acinetobacter baumamii* was increased in 1st day and decreased in 16th day they are 20.76% and 2.63%. *Azotobacter tropicalis* was increased in 1st day and decreased in 16th day they are 14.28% and 3.72%. *Bacillus substilis* was increased in 16th day and decreased in 1st day they are 15.48% and 4.27%. *Corynebacterium variabilis* was increased in 6th day and decreased in 11th day they are 5.33%

Table 1. Biochemical analysis of isolated strains

S.No.	Bacterial species	Gram stain	Shape	Indole	Methyl red	VP	Citrare	Catalase	Oxidase	Nitrate	Glucose	Tsi	Urease
P1	*Acinetobacter baumannii*	-	Cocci	-	-	-	+	+	-	-	+	-	-
P2	*Azotobacter tropicalis*	+	Rod	+	+	+	+	+	+	+	+	+	+
P3	*Bacillus subtilis*	+	Rod	-	+	-	-	+	+	-	-	-	-
P4	*Corynebacterium variabilis*	+	Rod	-	-	-	-	+	-	+	-	+	_
P5	*Flavobacterium lutescens*	-	Rod	-				+		-	-		

and2.58%. *Flavobacterium lutescens* was increased in 16th day and decreased in 1st day they are 18.38% and 6.83%.

Table 2. Bacterial counts in soils treated with 4ml kerosene for 16 days (%)

Bacterial species	Days			
	1	6	11	16
Acinetobacter baumannii	20.76	11.38	7.37	2.63
Azotobacter tropicalis	14.28	8.45	5.93	3.72
Bacillus subtilis	4.27	8.37	14.47	15.48
Corynebacterium variabilis	2.78	5.33	2.58	2.71
Flavobacterium lutescens	6.83	15.28	17.26	18.38

Crude oil

The result of the bacterial count show that crude oil treated soil had the highest count of *Acinetobacter baumamii* was increased in 1st day and decreased in 16th day they are 18.37% and 4.31%. *Azotobacter tropicalis* was increased in 1st day and decreased in 16th day they are 12.38% and 2.56%. *Bacillus substilis* was increased in 16th day and decreased in 1st day they are 15.41% and 4.58%. *Corynebacterium variabilis* was increased in 11th day and decreased in 1st day they are 9.37% and3.27%. *Flavobacterium lutescens* was increased in 16th day and decreased in 1st day they are14.29% and 6.38%.

Table 3. Bacterial count of soils treated with 4ml crude oil (%)

Crude oil(4ml)	Days			
	1	6	11	16
Acinetobacter baumannii	18.37	12.16	8.76	4.31
Azotobacter tropicalis	12.38	9.25	6.78	2.56
Bacillus subtilis	4.58	8.34	12.78	15.41
Corynebacterium variabilis	3.29	4.83	9.37	6.36
Flavobacterium lutescens	6.38	10.17	13.36	14.29

pH of the kerosene and crude oil contaminated soil

The pH of the kerosene contaminated soil ranges from 4.47 to 6.22. The pH was acidic i.e., 4.47 in the 1st day and the pH was 6.22 in the 16th day. The pH of the crude oil contaminated soil ranges from 4.42 to 7.45. The pH was 4.42 in the 1st day and the pH was 7.45 in the 16th day. The pH was tremendously decreased during the 1st day and increased higher in the 16th day in kerosene and crude oil.

Degradation of kerosene and Crude oil in Agricultural soil

The kerosene contaminated soil were analysed for the degradation activity. The degradation percentage was low in the 1st day with 1.78% and the percentage of degradation was higher in the after 16 days with 22.20%. The crude oil contaminated soil were analysed for the degradation activity. The degradation percentage was low in the 1st day with 1.43% and the percentage of degradation was higher in the after 16th days with 15.47%

DISSCUSSION

From our study we found 9 different organisms identified using biochemical characterization *Acinetobacter baumamii, Azotobacter tropicalis, Bacillus subtilis, Corynebacterium variabilis, Flavobacterium lutescens, Micrococcus luteus, Plesiomonas shigelloides, Pseudomonas fluorescens*and *Xanthomonas oryzae.* In the findings of (Apkoveta *et al*., 2011). Microbiological analysis gave a total of twelve heterotrophic bacteria of which eight are hydrocarbon utilizers. The heterotrophic bacteria are *Alcaligenspp, Bacillus spp, Micrococcus spp, Chromobacteriumspp, Corynebacterium spp, Serratia spp., Pseudomonas spp, Cellulomonasspp, Proteus spp, Flavobacteriumspp, Norcardiaspp, and Alcaligen spp*. The eight hydrocarbon degrading bacteria are *Alcaligen spp, Bacillus spp, Chromobacterium spp, Corynbacteriumspp, Pseusomonasspp, Aeromonasspp, Serratiaspp and Flavobacterium spp*. Five hydrocarbon degrading fungi were also isolated and identified, they are: *tricodemaspp, penicillinium spp, Rhizopus spp, fusarium spp and Aspergillus.* The heterotrophic microorganism of the crude oil polluted soil was decreased in number in comparing to the unpolluted soil. In our study the pH of the crude oil contaminated soil was low in 1st day and there was increase in pH after 16th day due to degradation of the crude oil. In a study by (Akvopetva *et al*., 2011) the crude oil contaminated soil has reduced pH in comparing to the normal soil. This finding was similar to (Osuji and Nwoye 2007). The reduction in pH was due to the increased in acidity which makes a problem in agricultural soils because of metal cations are more soluble and available in the soil solution (McBride 1994).

Conclusion

The present study was designed to identify microorganism present in the agricultural contaminated kerosene and crude oil treated soil sample and study their degradation. Serial dilution of 1gm of treated soil samples showed distinct colonies at 10^{-4}, 10^{-5} and 10^{-6} dilution and found to identified 9 colonies. Colonies subjected to biochemical test revealed that they were *Acinetobacter baumamii, Azotobacter tropicalis, Bacillus subtilis, Corynebacterium variabilis, Flavobacterium lutescens,*. The pH of the kerosene and crude oil also increased as the degradation percentage increased. The isolated organism were analysed for hydrocarbon degradation with 4ml kerosene and 4ml of crude oil in minimal salt medium. The degradation of the kerosene and crude oil was increased from 0th day to16th day.

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