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Crude Oil Degradation by Microorganisms in Soil Composts

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ABSTRACT

The hydrocarbon utilizing capabilities of *Micrococcus varians, Bacillus badius, Corynebacterium ulcerans* and *Corynebacterium amycolatum* isolated from soil composts were analysed. In the biodegradation experiment, utilization of the substrate by bacterial isolates resulted in increased population densities with simultaneous increase in emulsification index (EI_{24}) value from 30.0 to 48.84 and a decrease in residual crude oil concentration. The growth rate constants (μ) and mean generation (h) times of *Micrococcus varians, Bacillus badius, Corynebacterium ulcerans* and *Corynebacterium amycolatum* were 0.027 and 25.5, 0.025 and 27.5, 0.019 and 36.2, 0.023 and 30.0, respectively. The gas chromatographic analysis of the residual hydrocarbons in the liquid medium at the end of 30 days incubation showed that *Micrococcus varians* had the highest degradation rate of 93.10%, whilst *Bacillus badius, Corynebacterium ulcerans* and *Corynebacterium amycolatum* had biodegradation rates of 89.22, 88.22 and 90.82%, respectively. The residual hydrocarbon in soil microcosm after 30 days of biodegradation was 73.75, 75.81 and 76.94% for *Micrococcus varians, Corynebacterium amycolatum* and a consortium of the two isolates, respectively. A substrate specificity test of the isolates on different hydrocarbons showed that the isolates had good growth on anthracene and engine oil but poor growth on pyrene, toluene, naphthalene, dodecane and xylene.

Key words: Crude oil, biodegradation, compost, hydrocarbon, pollution, bacterial isolates

INTRODUCTION

Petroleum is a major source of energy globally. Wide scale production, transport, use and disposal of petroleum globally have made it a lead contaminant in both prevalence and quantity in the environment (Rahman *et al*., 2002). The release of these compounds poses a threat to water and soil ecosystems. Consequently, many techniques are being developed to clean up petroleum polluted environment. Biodegradation of hydrocarbon-contaminated soils which exploits the ability of microorganisms to degrade and/or detoxify organic contamination, has been established as an efficient, economic, versatile and environmentally sound treatment (Mehrasbi *et al*., 2003). The biological treatments are more efficient and cheaper than chemical and physical ones. In relation to biological treatment, the bioremediation technology is being employed for the degradation of crude oil in soil matrix through microorganisms able to transform petroleum hydrocarbons in less toxic compounds. However, the low solubility and adsorption are two major properties of high molecular weight hydrocarbons that limit their availability to microorganisms (Millioli *et al*., 2009). Compost contributes organic matter to the soil that may serve as a source of nutrient among other functions for the various microbes that inhabit the soil. Compost is a very rich source of nitrogen that maintains and enhances the fertility and productivity of agricultural soils. Studies carried out

both in microcosms and field experiments showed that organic amendments not only act by improving soil structure and serving as a source of nutrients, they can also strongly enhance the activities of microflora (Crecchio *et al.*, 2001). In this study, microorganisms with the capacity to utilize crude oil as carbon and energy source were isolated from soil composts. The biodegradation potential of the compost-inhabiting microorganisms was also estimated in order to assess the applicability of composts in bioremediation of hydrocarbon-polluted sites.

MATERIALS AND METHODS

Sample collection: Soil samples were collected randomly and homogenized from five different spots at Igando dumpsite (31N 0527762 and UTM 0726400; 31N 0527835 and UTM 0726326; 31N 0527778 and UTM 0726326; 31N 0527723 and UTM 0726340; 31N 0527894 and UTM 0726394) and Olusosun dumpsite at Ojota (31N 054566 and UTM 0728806; 31N 0541634 and UTM 0728963; 31N 0541562 and UTM 0728963; 31N 0541758 and UTM 0728893; 31N 0541732 and UTM 0728700), Lagos, Nigeria, respectively. The soil samples were collected at a depth of 0-10 cm below the soil surface into sterile McCartney bottles and transported to the laboratory. Physicochemical and microbiological analysis commenced immediately upon arrival in the laboratory.

Source of crude oil: The Escravos crude oil (dark brown in colour) used in this study was obtained from Chemistry Department, Faculty of Science, University of Lagos.

Physicochemical analysis of soil samples: The pH of the soil samples was determined with a pH meter (Jenway 3051) in 1:1 soil solution in distilled water. The moisture level, organic content, total nitrogen content, potassium content and available phosphorous were determined at the Department of Chemistry, University of Lagos, Nigeria as described previously (Obayori *et al.*, 2008; Onifade *et al.*, 2007; Jones *et al.*, 1983).

Microbiological analysis: The total heterotrophic bacterial and fungal counts were enumerated by plating aliquots (0.1 mL) of appropriate diluted soil samples on nutrient agar and potato dextrose agar containing streptomycin (1 mg 100^{-1} mL), respectively. Starch casein agar was employed in determining the population density of actinomycetes according to the method of Kuster and Williams (1964). The nitrogen fixing bacterial counts were estimated using the Ashby's mannitol agar. All inoculated plates were incubated aerobically at room temperature (30°C) counted after 48, 96, 120 and 168 h for bacteria, fungi, actinomycetes and nitrogen fixers, respectively. Similarly, the population of hydrocarbon-utilizers was estimated on Mineral Salts (MS) medium formulated by Kastner *et al.* (1995). The medium contained (in g L^{-1}) Na_2HPO_4: 2.13 g, KH_2PO_4: 1.30 g, NH_4Cl: 0.50 g and $MgSO_4.7H_2O$: 0.20 g. Sterile trace elements solution (1.0 mL L^{-1}) of Bauchop and Elsden (1960) was aseptically added to the medium after sterilization. The pH of the medium was adjusted to 7.2 and 5.6 for bacterial and fungal estimations,, respectively. The MS medium was also fortified with 1 mg 100 mL^{-1} of streptomycin for fungi. Sterile crude petroleum served as the sole carbon and energy source and made available to the cultures through vapour-phase transfer (Amund *et al.*, 1987). Plates were counted after incubation at room temperature for 5-7 days.

Crude oil utilization by the isolates: The ability of the isolates to utilize crude oil was confirmed by inoculating each isolate in separate cotton plugged 250 mL Erlenmeyer flasks containing sterile

Liquid Minimal Salts (MS) medium. The liquid MS medium and crude oil were autoclaved separately at 121°C for 15 min. Sterile crude oil which served as source of carbon and energy was added at 1% (v/v) to make up a final volume of 100 mL sterile liquid MS medium. Each isolate was subsequently inoculated in separate flask of the medium. Control flask containing the liquid MS medium and 1% (w/v) of crude oil but without organism was also prepared. The flasks were monitored and agitated daily for a period of 14 days. Isolates with the highest turbidity were selected for further study. An aliquot of each isolate was transferred to fresh medium containing 1% (w/v) of crude oil to re-confirm their ability to utilize crude oil.

Characterization and identification of the isolates: The isolates were identified on the basis of their cultural, cellular morphology and biochemical characteristics using API kit.

Biodegradation study: The biodegradation study was performed in liquid MS medium as described by Omotayo *et al.* (2011). The growth medium used was prepared as described above. The isolates were inoculated into each flask and incubated in the dark at about 30°C with constant shaking for 30 days. Total Viable Counts (TVC) and the emulsification index were monitored at 6 days intervals. The residual hydrocarbon content before and after biodegradation was analyzed by gas chromatography.

Emulsification index: In determining the emulsification index, 2 mL of the medium was centrifuged at 3000 rpm for 15 min to separate the cells. The supernatant was collected in a test tube while the cells were discarded. The emulsification stability of the isolates was determined by adding 2 mL of crude oil to the test tube containing the spent media. The tubes were properly vortexed at high speed for 2 min and allowed to stand for 24 h. The emulsification index was calculated as the height of the emulsion divided by the total height of supernatant with added oil multiplied by 100 (Abbasi and Amiri, 2008):

$$\text{Emulsificationindex}\,(EI_{24}) = \frac{\text{Height of emulsion}}{\text{Height of supernatant}} \times 100$$

Soil microcosm study: Biodegradation of the crude oil polluted soil sample by axenic and mixed cultures of the isolates was assessed. Two hundred grams of the soil was placed in 4 aluminum trays (10 cm diameter and height) and sterilized by autoclaving at 121°C for 1 h. The sterile soil samples were artificially contaminated with 10 mL crude oil to simulate 5% (v/w) crude oil pollution (Vidali, 2001). Two of the trays were each inoculated with 5 mL suspension of the pure isolates containing 10^9 CFU mL^{-1} of the isolates. A third tray was inoculated with bacterial consortium containing 2.5 mL of each inoculum preparation. A fourth tray was taken as control to determine natural attenuation; it had no inoculum. Sterile distilled water was added to the soil to achieve a moisture content of 30% of the water holding capacity of the soils (Vidali, 2001). The microcosms were kept in the glass-house to minimize loss of moisture via evaporation and monitored for 30 days. Residual hydrocarbon content of the soil microcosms were determined by gas chromatography at the end of the biodegradation experiment.

Gas chromatographic (GC) analysis: Gas chromatography was used to determine the residual hydrocarbon present in the media after 30 days of incubation. A standard profile was first obtained

by injecting 1 mL of the hydrocarbon standard into the GC and a chromatogram was generated to serve as a calibration window with which the test sample was analyzed. After generating the standard profile, 10 mL of the sample was extracted with 10 mL Hexane and was concentrated to 1 mL (test sample) from which 1 μL was injected into the GC and an equivalent chromatogram was generated. The peak areas of the standard and the test sample chromatogram were compared with respect to the concentration of standard of the sample (Odebunmi *et al*., 2002). This is given by:

$$\text{Concentration of hydrocarbon} = \frac{\text{Total peak of sample} \times \text{Concentration of standard}}{\text{Peak area of standard}}$$

Substrate specificity test of the isolates: The ability of the isolates to utilize pure hydrocarbon substrates was tested by adding 1% v/v of liquid and 1% w/v of solid hydrocarbons to minimal salts medium to which the isolates had been inoculated in Erlenmeyer flasks. The flasks were incubated with constant shaking at 30°C for 14 days. A control flask was set up for each hydrocarbon. Degradation was monitored by measuring increase in optical density at a wavelength (λ) 520 nm.

RESULTS

Physicochemical parameters of soil samples: The soil from Ojota (pH 6.16) was found to be more acidic compared to the soil from Igando dumpsite (pH 7.72) (Table 1), while the moisture content of the soil from Igando (12.8%) was higher when compared to Ojota soil sample (7.63%). The Total Organic Carbon (TOC), percentage nitrogen and phosphate content and salinity of Igando sample (10.20, 0.22, 0.11 mg kg^{-1} and 0.11%, respectively) were higher than those of Ojota sample (1.17, 0.06, 0.10 mg kg^{-1} and 0.06%, respectively). However, the nitrate content of Ojota (0.31) was slightly higher than Igando (0.22).

Enumeration of microbial populations: The microbial populations of Igando soil sample were higher compared to Ojota soil sample. The population per gram of hydrocarbon utilizers in Igando sample were also observed to be higher compared to that of Ojota sample. Likewise, the proportion of hydrocarbon utilizers in the Igando soil sample (0.37%) was found to be higher when compared to Ojota sample (0.34%). The population of nitrogen fixers and actinomycetes were also shown to be higher for Igando soil sample than Ojota soil sample (Table 2).

Table 1: Physicochemical parameters of the soil samples

	Sample source	
Parameters	Ojota	Igando
pH	6.16	7.72
Salinity (‰)	0.06	0.11
PO_4^{3-} (mg kg^{-1})	0.10	0.11
NO_3 (mg kg^{-1})	0.31	0.22
K (mg kg^{-1})	0.90	0.87
TOC (%)	1.23	3.99
Nitrogen (%)	0.06	0.51
Moisture (%)	7.63	12.8
Water holding capacity (%)	41	33

PO_4^{3-}: Phosphate content, NO_3: Nitrate content, TOC: Total organic carbon, K: Potassium content

Table 2: Microbial population of soil samples

	Sample source	
Microbial population	Ojota	Igando
Total heterotrophic bacteria	2.67×10^{8}	2.70×10^{8}
Total heterotrophic fungi	6.67×10^{5}	1.89×10^{6}
Total nitrogen fixers	3.53×10^{5}	1.17×10^{6}
Actinomycetes	8.70×10^{5}	1.63×10^{6}
Hydrocarbon utilizing bacteria	8.25×10^{6}	9.0×10^{6}
Hydrocarbon utilizing fungi	2.0×10^{5}	1.0×10^{6}
Percentage of bacterial hydrocarbon utilizers (%)	0.34	0.37

Values are in (CFU g^{-1})

Characterization and identification of crude oil-utilizing isolates: The isolates were identified using colonial morphology, biochemical tests and the use of API test kit. Tentative identification shows the isolates to be *Micrococcus* sp., *Corynebacterium* sp., *Bacillus* sp., *Enterobacter* sp., *Pseudomonas* sp., *Alcaligenes* sp., *Flavobacterium* sp., *Moraxella* sp., *Aeromonas* sp., *Acinetobacter* sp., *Aspergillus* sp. and *Penicillium* sp. However, *Corynebacterium ulcerans, Bacillus badius, Corynebacterium amycolatum* and *Micrococcus varians* were selected for further studies based on their capability to grow well in the crude oil used as carbon and energy source.

Biodegradation study: During the time-course analysis, the Total Viable Counts and Emulsification Index (EI_{24}) were monitored. The results are shown in Fig. 1. The population density of *Micrococcus varians* shows that there was a considerable period of lag phase of about 15 days before the isolate entered the exponential phase of growth. The population density of *Micrococcus varians* increased steadily from 7.00×10^{6} CFU to 7.40×10^{12} CFU mL^{-1} of medium in 21 days before reaching the stationary phase. The emulsification index rose steadily from 30 to 48.84. *Micrococcus varians* had a growth rate constant (μ) of 0.027 and grew with a mean generation time (g) of 25.5 h (Table 3).

The population density of *Bacillus badius* increased steadily in 6 orders of magnitude from 1.99×10^{5} CFU to 4.10×10^{11} CFU mL^{-1} of the medium in 24 days before reaching the stationary phase. The emulsification index rose steadily from 30 to 48.23. The growth rate constant (μ) of *Bacillus badius* in the liquid medium was 0.025 and it grew with a mean generation time (g) of 27.5 h (Table 3).

Corynebacterium ulcerans had a brief lag phase of about 3 days before entering the exponential phase of growth. The population density of the isolate increased from 1.96×10^{7} CFU to 1.20×10^{12} CFU mL^{-1} of the medium in 24 days before reaching the stationary phase of growth. The emulsification index rose from 30 to 48.80. *Corynebacterium ulcerans* had a growth rate constant (μ) of 0.019 and grew with a mean generation time (g) of 36.2 h (Table 3).

The population density of *Corynebacterium amycolatum* increased from 6.00×10^{5} CFU to 3.60×10^{11} CFU mL^{-1} of the medium before reaching the stationary phase. The emulsification index rose from 30 to 48.84. *Corynebacterium amycolatum* had a growth rate constant (μ) of 0.023 and grew with a mean generation time (g) of 30.0 h (Table 3).

The growth kinetics of the isolates is presented in Table 3. *Micrococcus varians* had the lowest doubling time (25.5 h) in the liquid medium followed by *Bacillus badius*,

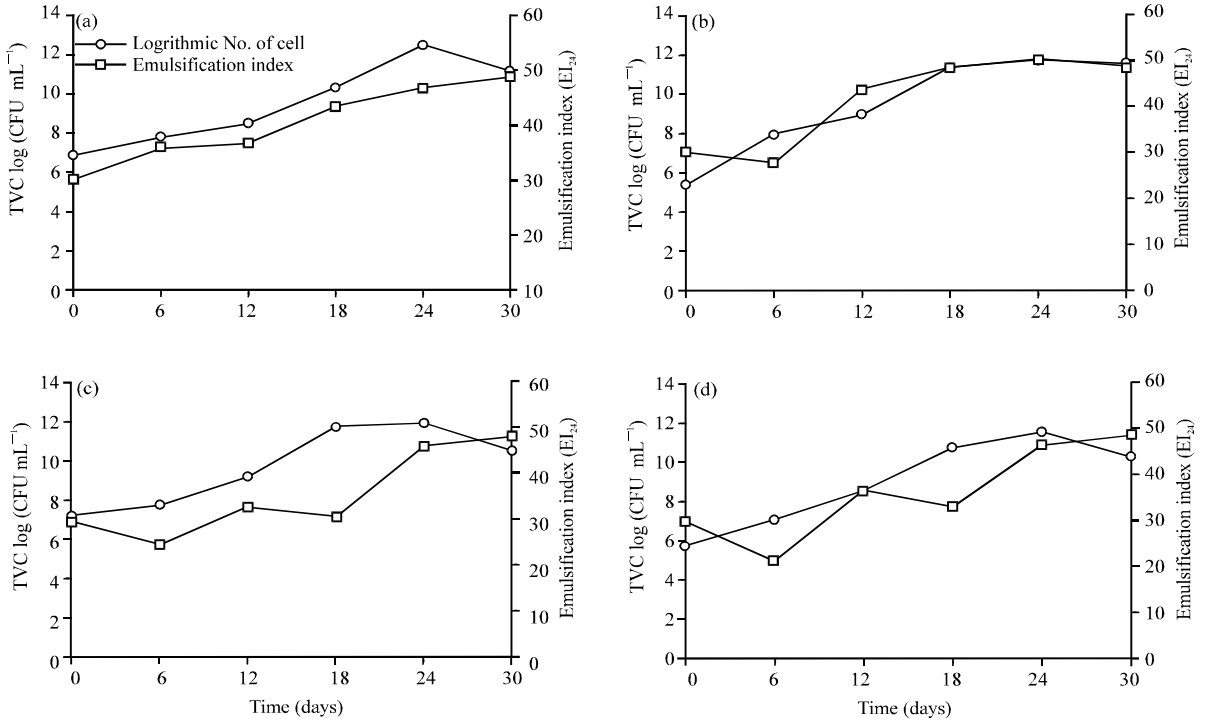

Fig. 1(a-d): Degradation of crude oil by (a) *Micrococcus varians* (b) *Bacillus badius* (c) *Corynebacterium ulcerans* and (d) *Corynebacterium amycolatum*

Table 3: Growth kinetics data of isolates in crude oil liquid culture medium

	Isolates			
Parameters	*Micrococcus varians*	*Bacillus badius*	*Corynebacterium ulcerans*	*Corynebacterium amycolatum*
Mean generation time (g) (h)	25.5	27.5	36.2	30.0
Growth rate constant (μ)	0.027	0.025	0.019	0.023

Corynebacterium amycolatum and *Corynebacterium ulcerans* with mean generation times of 27.5, 30.0 and 36.2 h, respectively. The highest number of generations was given by *Micrococcus varians* with 16.94 generations followed closely by *Bacillus badius* with 15.71 generations. *Corynebacterium ulcerans* and *Corynebacterium amycolatum* gave 9.95 and 9.60 generations, respectively.

Gas chromatographic profiles: The gas chromatograms of the biodegradation experiment after 30 days showed reduction in the concentration of residual hydrocarbon present in the media. The concentration of hydrocarbon present at the start of experiment was 3395.82 mg L^{-1}, the value of residual hydrocarbon in liquid cultures of *Corynebacterium ulcerans*, *Bacillus badius*, *Corynebacterium amycolatum* and *Micrococcus varians* were 400.15, 366.14, 311.59 and 234.24 mg L^{-1}, respectively at day 30 of the experiment. *Micrococcus varians* gave the highest degradation value of 93.10% followed closely by *Corynebacterium amycolatum* with a rate of 90.82%. *Bacillus badius* and *Corynebacterium ulcerans* gave degradation rates of 89.22 and 88.22%, respectively (Table 4).

Table 4: Residual hydrocarbon content in crude oil liquid culture medium at the end of 30 day incubation period

Sample	Residual crude oil (mg L^{-1})	Biodegradation (%)
Control	3395.82	0.00
Corynebacterium ulcerans	400.15	88.22
Bacillus badius	366.14	89.22
Corynebacterium amycolatum	311.59	90.82
Micrococcus varians	234.24	93.10

Table 5: Residual crude oil in Ojota soil microcosm at the end of 30 day biodegradation

Sample	Residual crude oil (mg kg^{-1})	Biodegradation (%)
Control, day 0 (uninoculated)	3629.78	0.00
Control, day 30 (uninoculated)	3167.36	12.69
Compost+*Micrococcus varians*	490.39	73.78
Compost+*Corynebacterium amycolatum*	415.82	75.85
Compost+*Micrococcus varians*+*Corynebacterium amycolatum*	374.69	76.98

Table 6: Substrate specificity of the isolates

	Growth of isolates			
Substrate	*Micrococcus varians*	*Bacillus badius*	*Corynebacterium ulcerans*	*Corynebacterium amycolatum*
Pyrene	+	+	+	+
Anthracene	+++	++	+++	+
Toluene	+	+	+	+
Naphthalene	+	+	+	+
Dodecane	+	+	+	+
Xylene	+	+	+	+
Engine oil	+++	+++	++	+

+: Poor growth (<0.2 μm), ++: Moderate growth (0.2-0.3 μm), +++: Excellent growth (>0.3 μm)

Gas chromatographic analysis was carried out on the microcosm to determine the amount of residual hydrocarbon in the soil after 30 days of degradation. The amount of hydrocarbon present in the control soil at day 0 was 3627.75 mg kg^{-1}, the residual hydrocarbon at day 30 in the control soil was 3167.36 mg kg^{-1} thus, 12.69% of the total hydrocarbon in the trays was lost due to natural degradation. The residual hydrocarbons in trays of *Micrococcus varians*, *Corynebacterium amycolatum* and consortium (*Micrococcus varians* and *Corynebacterium amycolatum*) were 490.39, 415.82 and 374.69 mg kg^{-1}, respectively. The consortium had the highest effective biodegradation rate (76.98%) followed by *Corynebacterium amycolatum* (75.85%) and *Micrococcus varians* (73.78%), respectively (Table 5).

Substrate specificity tests: The ability of the isolates to degrade different hydrocarbon substrates was observed for a period of 14 days at room temperature. The optical density of the liquid culture medium of the isolates was measured to determine the growth of the isolates in the broth medium. *Micrococcus varians*, *Bacillus badius* and *Corynebacterium ulcerans* had excellent growth on anthracene and engine oil but poor growth on pyrene, toluene, naphthalene, dodecane and xylene. *Corynebacterium amycolatum* however, had poor growth on all the hydrocarbons tested (Table 6).

DISCUSSION

In this study, microorganisms capable of degrading crude oil were isolated from active composted soil samples. The bacterial, actinomycetes and fungal populations obtained were higher in Igando sample compared to Ojota sample. The microbial population density of Igando soil sample was higher than that of Ojota probably because the soil from Igando contained more nutrients compared to the sample from Ojota. As shown in Table 1 the nitrogen content, phosphate and total organic carbon of Igando sample were relatively higher than that of Ojota study site. Anthropogenic impacts, such as changes in nutrient composition, have the potential to directly or indirectly affect the bacterial and fungal composition of the soil (Rousk *et al*., 2009). Likewise, Igando sample had more moisture which favours microbial growth than Ojota sample.

The proportion of hydrocarbon utilizers in the Ojota and Igando samples were 0.34 and 0.37%, respectively. It has been reported that population levels of hydrocarbon utilisers and their population within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons (Rahman *et al*., 2002). In unpolluted ecosystem, hydrocarbon utilizers generally constitute about 0.1% of the microbial community and in oil polluted ecosystems they can constitute up to 100% of the viable microorganisms (Rahman *et al*., 2002). The microbial populations quantitatively reflect the degree or extent of exposure of that ecosystem to hydrocarbon contamination (Atlas, 1981). The low proportion of hydrocarbon utilizers compared to the total heterotrophic population indicates that the soil ecosystem from which the samples were obtained probably had not been exposed to heavy and consistent crude oil pollution. The compost soil samples used in this study harboured hydrocarbon utilizers which include *Micrococcus* sp., *Corynebacterium* sp., *Bacillus* sp., *Enterobacter* sp., *Pseudomonas* sp., *Alcaligenes* sp., *Flavobacterium* sp., *Moraxella* sp., *Aeromonas* sp., *Acinetobacter* sp., *Aspergillus* sp., *Penicillium* sp. and other species. The flora reflects the diverse heterotrophic bacteria present in compost soil and the diversity could be as a result of the varied sources of the refuse, dumped at the sites. The genera *Micrococcus, Bacillus, Streptomyces, Actinomyces, Azotobacter, Aspergillus, Penicillium* and *Trichoderma* was mentioned by Ryckeboer *et al*. (2003) in their study as part of the microbial flora of compost. Several hydrocarbon degrading organisms have been isolated from diverse environments; soil and aquatic sources which are the two major environments affected by hydrocarbon pollution (Mittal and Singh, 2009) and their isolation is not restricted to hydrocarbon-bearing environments. The hydrocarbon utilizing isolates obtained in this study all had varied degree of degradation however, *Micrococcus varians, Bacillus badius, Corynebacterium ulcerans* and *Corynebacterium amycolatum* appeared to be the fastest growing species in crude oil. The study of Barathi and Vasudevan (2001) identified the above genera among hydrocarbon degrading microorganisms. Several other workers also reported on the above genera as hydrocarbon degrading microorganisms (Atlas, 1981; Leahy and Colwell, 1990; Banat *et al*., 2000).

The isolates showed different rates of growth in hydrocarbon liquid media. The population density of the isolates started from a range of between 1.99×10^{5} and 1.96×10^{7} CFU mL^{-1} to between 3.60×10^{11} and 7.4×10^{12} CFU mL^{-1}. *Micrococcus varians* had the highest growth rate constant (0.025) with a mean generation time of 25.5 h compared to the other isolates. This result agrees with that of Rahman *et al*. (2002) in which *Micrococcus* sp. had a very good growth on crude oil. This was followed closely by *Bacillus badius* with a mean generation time of 27.5 h.

The Gas Chromatographic (GC) analysis of the residual crude oil showed that *Micrococcus varians* demonstrated the highest degree of crude oil degradation (93.10%) by having the lowest residual crude oil (234.24 from 3395.82 mg L^{-1}) at the end of the 30 day incubation

period. *Bacillus badius, Corynebacterium ulcerans* and *Corynebacterium amycolatum* exhibited biodegradative capabilities of 89.22, 88.22 and 90.82%, respectively. The high rate of hydrocarbon degradation by *Micrococcus varians* could emanate from the massive growth and enzyme production responses during the growth of the isolate. This is supported by the reports of Bogan and Lamar (1996) which showed that extracellular enzymes of organisms are produced in response to their growth phases.

From the GC analysis of residual hydrocarbon present in the microcosms, the biodegradation rates of the *Micrococcus varians* (73.78%) and *Corynebacterium amycolatum* (75.85%) were less compared to the biodegradation rates in the liquid media (93.10 and 90.82%, respectively). This might be due to the bioavailability of the crude oil to the organisms. The bioavailability of hydrocarbons has been shown to greatly affect the extent of biodegradation of the hydrocarbons due to sorption onto soil particles (Leahy and Colwell, 1990). The bacterial consortium showed a degradation percentage (76.98%) of degradation of crude oil after 30 days of incubation.

The isolates had good growth on anthracene and engine oil as hydrocarbon substrates while they exhibited poor growth on the other hydrocarbons tested. The engine oil is a mixture of alkanes and it contains heavy chains (C_{18}-C_{40}). The alkanes are the most abundant compounds and are simpler to oxidize. Aliphatic hydrocarbons are degraded with greater speed but the key step involves oxidation of the molecule to increase their solubility (Silva *et al*., 2006). Biodegradation rates have been shown to be highest for the saturates, followed by the light aromatics, with high-molecular-weight aromatics and polar compounds exhibiting extremely low rates of degradation (Leahy and Colwell, 1990).

CONCLUSION

An effective degradation of crude oil would require simultaneous action of several metabolically versatile microorganisms with favourable environmental conditions such as pH, temperature and availability of nutrients. The high nitrogen content is a major factor that contributes to the degradation of hydrocarbon in composts. The organisms isolated in the course of this study showed appreciable degree of degradation of the crude oil used. The natural microbial community of the compost soil includes a variety of microorganisms that can degrade, alone or together, most crude oil components. Thus, bioremediation of oil polluted fields could be achieved using indigenous hydrocarbon utilizers of the compost soil and the process could be enhanced by supplementing the polluted environment with compost.

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