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Full Length Research Paper

Hydrocarbon-degrading bacteria isolation and surfactant influence on the growth of organisms: A case study in Ibadan, Nigeria

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Hydrocarbons are substantially insoluble in water, often remaining partitioned in the non-aqueous phase liquid (NAPL). However, there had been little or no attempts to advance the bioavailability of hydrocarbons through the use of surfactants. This study was conducted based on the need to isolate hydrocarbon degrading bacteria and to establish the effect of surfactants on the growth of organisms. Ten organisms were isolated and classified into five genera based on their physiological, morphological and biochemical characteristics. These genera include *Pseudomonas, Bacillus, Micrococcus*, *Flavobacterium* and *Corynebacterium*. In determining the effect of surfactant on isolated organisms, *Bacillus* strain and *Corynebacterium* strains were enhanced by palmitic acid. Detergent was found to have stimulatory effect on *Bacillus* and *Pseudomonas*. There is a significant difference between separate applications of palmitic acid and detergent on the samples with respect to the growth of *Micrococcus* sp. ($p < 0.01$). There was also a significant difference between the applications of detergent and control on the selected samples with respect to *Flavobacterium* sp. ($p < 0.001$). Surfactant which stimulated bacterial growth is highly recommended in bioremediation, although the use of improved strains may be preferable.

Key words: Hydrocarbons, surfactants, biodegradation, pollutants, microoganisms.

INTRODUCTION

Surfactant was coined by Antara products in 1950. They are blend of surface-active agents and are organic compounds that are amphiphilic. This implies that they have both hydrophobic groups (their "tail") and hydrophilic groups (their "heads"). Consequently, they can dissolve in both organic solvent and water. They are produced by microorganisms such as *Rhodococcus,* hence are called biosurfactants (Rosenberg and Ron, 1998; Mulligan, 2005).

Hydrocarbons are organic compounds, which have carbon and hydrogen as their constituent elements and mostly found in petroleum gas, coal, etc. They are released into the soil environment as industrial chemicals due to mechanical malfunction of equipment, human errors, ineffective incineration practices, leakage, improper disposal practices, corrosion, and accidental spillage (Morgan and Watkinson, 1989; Cerniglia, 1992). This often led to soil contamination, causing very serious damage to the environment. Heavily contaminated soil contains a different type of non-aqueous phase liquid (NAPL) which are seen as droplets on the surfaces of the soil (Chy, 2003).

Contaminated sites are often revitalized through biodegradation because of its economic viability and environmental sustainability. Biodegradation processes

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often occur as a result of the dissolution of the key contaminants in an aqueous solution (Wodzinski and Coyle, 1974; Grimberg and Aitken, 1995). However, the major shortcomings of the biodegradation processes are that hydrocarbons may not be accessible to the bacteria, may not be soluble in water, and could end up remaining partitioned in the non-aqueous phase liquid (NAPL). Based on these, it will therefore, be important to enhance the bioavailability of hydrocarbons through the utilization of surfactants. This has always been an area of interest to researchers and is becoming too worrisome to policy and decision-makers.

In recent years, several methods of biological remediation of oil-contaminated soil have become very significant due to their ecological safety. This has prompted researchers to develop intriguing methodologies which are aimed at solving the menace of biological degradation of hydrocarbons in the water and in the soil. One of the common methods is the bioaugumentation process. This is the introduction of specifically selected association of micro-organisms into the fluid for the purpose of degrading the various classes of the hydrocarbon pollutants. Another technique includes the activation of indigenous oil-oxidizing micro flora into the fluid to provide an optimal condition for its growth. This process is known as biostimulation. However, the most effective method is the biotechnology process which involves the combination of the bioaugumentation and biostimulation processes such as the case of incurporating biofertilizer containing microbial cultures and inorganic nutrients into a fluid. In order to augment the biovailability of hydrocarbon pollutants through microbial cells absorption, surfactants are primarily used to permit desorption and solubilization of the petroleum hydrocarbons (Mills et al., 1978; Deschenes et al., 1995; Volkering et al., 1995).

Biosurfactants produced by a number of microorganisms are becoming imperative for biotechnology-oriented products for industrial and medical purposes (Das, 2001; Mulligan, 2005). Both chemical and biological surfactants have the tendency to boost the pseudo-solubility of petroleum-oriented mechanisms in water (Chy, 2003; Pekdemir et al., 2005). In order to effectively reduce the interfacial tension of crude oil and water, surfactants are often applied (Al-Sabagh, 2000; Liu et al., 2004). In some cases, the microbial population could adopt the surfactants used for bioremediation and transform them to growth substances which could lead to an increase in the level of the biomass. This could eventually cause an increase in the effectiveness of the containment removal. The efficiency of substrate on contaminant biodegradation and its potency may vary due to the level and characteristics of the microbial species present in the oil (Aronstein and Alexander, 1992).

There are three degrees of hydrocarbon metabolism, which results in biochemical oxygen demand (BOD). In hydrocarbon polluted water, these are; oxidation, biodegradation and mineralization (Makkar and Cameotra, 1997; Cameotra and Makkar, 2004). In this study, we explored possibility of isolating hydrocarbon-degrading bacteria and determined the effect of surfactant on the growth of the organism on selected sites in an urban city in Nigeria, sub-Saharan Africa.

MATERIALS AND METHODS

An experimental set-up was used to investigate the various characteristics of the surfactants and the microorganisms. Due to the level of complexity in terms of number and accessibility of the target locations, refuse dump sites, automobile mechanic workshops and petrol stations were purposefully selected in Ibadan metropolis. At these locations, soil samples were collected and also collected from the rhizosphere of plants using sterile test tubes. Screening of hydrocarbon degrading bacteria, isolation of bacteria, characterization of hydrocarbon utilizers using Austine et al. (1977) method, Gram reaction test, motility test, catalase test, sugar fermentation test, triple sugar iron test, citrate agar test, nitrate reduction test, methyl red test and vogues-proskauer test were conducted in the laboratory using standard methods. Effects of surfactant on isolated organisms and determination of bacterial growth were also carried out. Equipment and apparatus used included: autoclave, beaker, bijou battles, conical flasks, conical wool, cover slip, hot air oven, incubator, measuring cylinder, microscope, petri dishes, pipettes, refrigerator, slides, test tubes, water bath, shaker, weighing balance and wire loops. The media and reagents used included: bromoscresol blue solution, MacConkey, methyl red solution, nutrient agar, peptone water and Gram stain.

RESULTS

Bacteria isolation

A total number of 10 bacterial organisms were isolated from the soil and based on the morphological biochemical and physiological characteristic properties, five different genera were chosen as representative organisms from the 10 isolated organisms. The morphological, physiological and biochemical properties of the isolates are presented in Table 1. Based on the characteristic features observed, the identified organisms were *Pseudomonas, Micrococcus, Bacillus, Flavobacterium* and *Corynebacterium*.

Effect of surfactant on isolated organism

Tables 2 to 6 show the effect of surfactant on isolated organisms as determined on the spectrophotometer. *Bacillus* and *Corynebactrium* strains were enhanced by palmitic acid. Detergent was found to have stimulatory effect on the *Bacillus* and *Pseudomonas.*

Table 7 shows the effect of palmitic acid and detergent on growth of microorganisms (*Bacillus* sp*., Corynebacterium* sp*., Micrococcus* sp*., Flavobacterium* sp.,

Table 1. Morphology and biochemical characteristics of the isolates.

Lab Ref number	Colony morphology nutrient agar	Deoxycholate agar	Microscopic identification	Sugar fermentation														Suspected organism
				Catalase	Mortility	Fructose	Galactose	Glucose	Lactose	Maltose	D-Mannitol	Sucrose methyl red test	Vogues proskauer test	Nitrate reduction test	Triple sugar Iron test	Citrate test		
Pdo2	Circular, creamy and entire		Gram positive rod, spores with chains	+	+	+	+	+	+	+	+	+	+	-	+	Acid H_2S gas	+	*Bacillus* sp*p*
RBdo4	Circular, puncti form creamy and entire		Gram positive rods in chains with spores	+	+	+	+	+	+/-	+/-	+	+	+	-	-	Acid	+	*Bacillus* sp*p*
Peo8	Circular, creamy and entire		Gram Positive rods in chains with spores	+	+	+	-	+	-	+	+	+	+	-	-	Acid alkaline	+	*Bacillus* sp*p*
Peo9	Rhizoid and circular colonies	Circular and puncti form colonies	Gram Positive short rods.	+	-	+	+	+	-	-	-	-	+	-	+	Acid alkaline gas	+	*Corynebacteriu m* spp
RAeo5	Translucent circular and entire		Gram negative short rods in cluster	+	-	+	+	+	+	+	+	+	-	+	-	Acid alkaline H_2S	+	*Flavobacterium* spp.
MWdo6	Blue-green Circular and flat colonies		Gram negative slightly curved rods	+	+	+	+	+	+	+/-	+	+	+	-	-	Acid alkaline H_2S and gas	-	*Pseudomaons* sp*p*
RBeo10	Irregular, green flat colonies		Gram negative slightly curved rods	+	+	+	+	+	+	+/-	+	+	+	-	-	Acid alkaline H_2S and gas	-	*Pseudomaons* spp
RAdo3	Circular, pigmented yellow colonies		Gram positive cocci in clusters	+	+	+	+	+	+	-	+	+	+	-	-	Acid and gas	-	*Micrococcus* spp
MWeol	Circular and punti form colonies		Gram positive cocci in clusters	+	+	+	+	+	+	+/-	+	+	+	-	+	Acid and alkaline	-	*Micrococcus* spp
RAeo7	Circular and punti form colonies	Puncti form and raised	Gram positive cocci in clusters	+	+	+	-	+	-	+	+	+	+	-	+	Acid and alkaline	-	*Micrococcus* spp

Lab Ref =Laboratory reference number; + = positive; - = negative; +/- weakly positive.

Table 2. Effect of palmitic acid and detergent on growth of *Bacillus* sp.

Concentration (mg/ml)	O.D. (660 nm)		
	Palmitic acid	Detergent	Control
0.05	0.410	0.536	0.354
0.10	0.265	0.073	0.354
0.20	0.396	1.251	0.354
0.50	0.376	0.144	0.354

O.D.= Optical density.

Table 3. Effect of palmitic acid and detergent on growth of *Corynebacterium* sp.

Concentration (mg/ml)	O.D. (660 nm)		
	Palmitic acid	Detergent	Control
0.05	0.741	0.372	0.451
0.10	0.475	0.166	0.451
0.20	1.206	1.194	0.451
0.50	0.851	0.318	0.451

O.D.= Optical density.

Table 4. Effect of palmitic acid and detergent on growth of *Micrococcus* sp.

Concentration (mg/ml)	O.D. (660 nm)		
	Palmitic acid	Detergent	Control
0.05	0.207	0.076	0.381
0.10	0.254	0.073	0.381
0.20	1.274	0.987	0.381
0.50	0.487	0.282	0.381

O.D.= Optical density.

Table 5. Effect of palmitic acid and detergent on growth of *Flavobacterium* sp.

Concentration (mg/ml)	O.D. (660 nm)		
	Palmitic acid	Detergent	Control
0.05	0.367	0.137	0.385
0.10	0.490	0.175	0.385
0.20	1.290	0.125	0.385
0.50	0.568	0.150	0.385

O.D.= Optical density.

Table 6. Effect of palmitic acid and detergent on growth of *Pseudomonas* sp.

Concentration (mg/ml)	O.D. (660 nm)		
	Palmitic acid	Detergent	Control
0.05	0.286	0.913	0.325
0.10	0510	0.093	0.325
0.20	0.315	0.139	0.325
0.50	0.256	0.151	0.325

O.D.= Optical density.

Table 7. Statistical tests of the effect of palmitic acid and detergent on growth of microorganisms (*Bacillus* sp., *Corynebacterium* sp*., Micrococcus* sp*., Flavobacterium* sp*., Pseudomonas* sp.).

Parameter	Paired difference					Test	d.f	Significant
	Mean	Standard deviation	Standard error	95% Confidence Interval				
				Lower	Upper			
***Bacillus* sp.**								
Palmitic acid versus detergent	-0.140	0.503	0.252	-0.940	0.662	-0.553	3	0.619
Palmitic acid vs control	0.008	0.066	0.033	-0.097	0.113	0.235	3	0.829
Detergent versus control	0.147	0.540	0.270	-0.712	1.006	0.545	3	0.624
***Corynebacterium* sp.**								
Palmitic acid versus detergent**	0.306	0.218	0.109	-0.040	0.652	2.811	3	0.067
Palmitic acid versus control**	0.367	0.301	0.152	-0.115	0.849	2.425	3	0.094
Detergent vs control	0.062	0.463	0.231	-0.675	0.798	0.266	3	0.808
***Micrococcus* sp.**								
Palmitic acid versus detergent*	0.201	0.066	0.033	0.097	0.305	6.176	3	0.009
Palmitic acid versus control	0.175	0.494	0.247	-0.612	0.961	0.706	3	0.531
Detergent vs control	-0.027	0.433	0.216	-0.715	0.662	-0.122	3	0.910
***Flavobacterium* sp.**								
Palmitic acid versus detergent**	0.532	0.429	0.214	-0.151	1.215	2.481	3	0.089
Palmitic acid versus control	0.294	0.416	0.208	-0.368	0.955	1.413	3	0.253
Detergent vs control*	-0.238	0.021	0.011	-0.272	-0.204	-22.243	3	0.000
***Pseudomonas* sp.**								
Palmitic acid versus detergent	0.018	0.450	0.225	-0.698	0.734	0.079	3	0.942
Palmitic acid versus control	0.017	0.115	0.057	-0.166	0.199	0.292	3	0.789
Detergent vs control	-0.001	0.393	0.197	-0.627	0.625	-0.005	3	0.996

*Significant at 5%; **significant at 10%.

Pseudomonas sp.). The data shows that there is a significant difference between separate applications of palmitic acid and detergent on the samples with respect to the growth of *Micrococcus* sp. ($p < 0.01$). Moreover, significant difference was also found between the applications of detergent and control on the selected samples with respect to *Flavobacterium* sp. ($p < 0.001$). However, the following pairs showed significant difference at 10%: palmitic acid versus detergent (*Corynebacterium*), palmitic acid versus control (*Corynebacterium*), palmitic acid versus detergent

(*Flavobacterium* sp.).

DISCUSSION

In the search for hydrocarbon-degrading bacteria, ten isolates were recovered which were both Gram positive and Gram negative bacterial. The characteristic features showed that they belong to the following genera; *Bacillus, Pseudomonas, Micrococcus, Flavobacterium and Corynebacterium* (Billingsley et al., 1999a and b). The recovery of these organisms from soil was evidenced in the work of Austine et al. (1977) which observed that hydrocarbon degrading bacteria have the ability of utilizing hydrocarbon as a sole of carbon source and can be isolated from aquatic and terrestrial environment, if the area is polluted with hydrocarbons.

Further results of this study showed that *Bacillus* sp. at concentration 0.1 mg/ml was inhibited by palmitic acid and growth was enhanced at concentration of 0.2 mg/ml. The addition of palmitic acid also enhanced the growth of the organism *Corynebacterium*. The presence of a surfactant had a stimulatory effect on the growth of *Pseudomonas* strain when a hydrocarbon was used as a carbon source. This is contrary to the work of Bilingsley et al. (1999a) which used Igepal co-630, in the biodegradation of 4 PCB congeners 2,4,2,4-chlorobipheny1 (VBP), and was found to inhibit the growth of the *Pseudomonas* LB-400. However, the isolation of *Flavobacterium, Bacillus, Micrococcus and Pseudomonas* is in line with the findings of Mills et al. (1978) where they reported that these organisms including *Achromobacter, Acinetobacter* and *Arthrobacter* are known for their capability to degrade hydrocarbon.

It was also observed that the surfactant, palmitic acid stimulated the growth of *Bacillus* and *Corynebacterium* species. The obtained detergent was inhibited by all the organisms apart from *Bacillus and Pseudomonas* at concentration of 0.05 mg/ml. This is in line with the research conducted by Almeida et al. (2004) which indicate that the use of surfactant in bioremediation provided stimulatory, inhibitory or neutral effect on the bacterial degradation of the oil component. This is dependent on the species of the microbial cell and the type of the surfactant involved. *Pseudomonas* species was found to degrade engine oil. This view is supported by the work of Tanner et al. (1991). They noted that *Pseudomonas* species can degrade heavy hydrocarbon and improve oil recovery of a contaminated area. Overall, the use of surfactant in remediation of contaminated sites is very important. Surfactant which stimulates bacterial growth is highly recommended in bioremediation, although the use of improved strains may be preferable.

There is a need to understand in details the general characteristics (genetics, physiology and biochemistry) of biosurfactants-producing strains. This should be in view of improving the process and the technology. For process optimization, detailed information on pollutants and optimum conditions for surfactants and degradation to occur must be provided. It is anticipated that in the future, additional work will be done to provide in depth understanding into the study of the solubility of the pollutants, and other associated factors affected by the biosurfactants. This may be required in order to expand beyond the current literature and provide for a wider application in the field.

REFERENCES

Almeida PF, Moeira RF, Almeida RCC, Guimaras AK, Carvalho AS (2004). Selection and improvement of Microorganisms to improve oil recovery, Engine Life Sci. 4:319-325.

Al-Sabagh AM (2000). Surface activity and thermodynamic properties of water soluble surface based on 1, 3-dicarboxymethoxybenzene used for enhanced oil recovery. Polym. Adv. Technol. 11:48-56.

Aronstein BN, Alexander M (1992). Surfactants at low concentration stimulate biodegradation of adsorbed hydrocarbon in sample of aquifer sands and soil slurries. Environ. Toxicol. Chem. 11:1227-1233.

Austine JC, Bouchez-Naitali M, Rakatozafy H (1977). Diversity of bacterial strain degrading Hydrocarbon in relation to the mode of substrate uptake, J. Appl. Microbiol. 86:421-428.

Billingsley KA, Backus SM, Ward OP (1999a). Effect of surfactant solubizaton on biodegradation of polychlorinated biphenyl cogeners by pseudomonas LB 400. J. Appl. Microbiol. Biotechnol. 52:255-260.

Billingsley KA, Backus SM, Ward OP (1999b). Remediation of PCB in soil by surfactant washing and biodegradation in the wash by Pseudomonas sp. LB 400. Biotechnol. Lett. 24:1827-1832.

Cameotra SS, Makkar RS (2004). Recent application of bisurfactants as biological and immunological molecules. Curr. Opin. Microbiol. 7:262-266.

Cerniglia CE (1992). Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3:351-368.

Chy W (2003). Remediation of contaminated soils by surfactant-aided soil washing; Practical period Hazard toxic Radioact. Waste Manage. 7:19-24.

Das M (2001). Characterization of de-emusification capabilities of a *Micrococcus* sp. Biore, Technol. 79:15-22.

Deschenes L, Lafrance P, Villeneure JP, Samson R (1995). The effect of an anionic surfactant on the mobilization and biodegradation of PAHs in a creosote-contaminated soil. J. Hydrol. Sci. 40:47-484.

Grimberg SJ, Aitken MD (1995). Biodegradation Kinetics of Phenanthrene solubilized in surfactant inicelles. In Hinchee RE, Brockman FJ and Vogel CM(ed); Microbial processes for bioremediation, Battelle Press, Columbus, Ohio, pp. 59-66.

Liu Q, Dong M, Zhoua W, Ayub M, Zhang YP, Huangs S (2004). Improved oil recovery by adsorption-desorption in chemical flooding. J. Pet. Sci. Engin. 43:75-86.

Makkar RS, Cameotra SS (1997). Utilization of molasses for biosurfactant production by two Bacilus strains at thermophilic condition. J. Am. Oil Chem. Soc. 74:889.

Mills AL, Breuel C, Colwel RR (1978). Enumeration of petroleum degrading marine and terrestrial microorganism by the most probable method. Can. J. Microbiol. 24:552-557.

Morgan P, Watkinson RJ (1989). Hydrocarbon Degradation in soils and methods for soil biotreatment. Crit. Rev. Biotechnol. 8:305-333.

Mulligan CN (2005). Environmental application of biosurfactants. Environ. Pollut. 133:193-198.

Pekdemir T, Copur M, Urum K (2005). Emulsification of Crude oil-water systems using biosurfactant. Process Saf. Environ. Protect. 83(B1):38-46.

Rosenberg E, Ron EZ (1998). Surface active polymers from the genus Acinetobacter. In: Kaplan DL. editor Biopolymers from Renewable Resources. Springer-Verlag. Berlin, pp. 281-291.

Tanner RS, Udegbunam EO, McInerney MJ, Knapp RM (1991). Microbially enhanced oil recovery from carbonate reservoir. J. Geomicrobiol. 9:169-195.

Volkering F, Van de Wiel R, Brueure AM, Van Andel JG, Rulkens EH (1995). Biodegradation of polycyclic aromatic hydrocarbons in the presence of nonionic surfactants. In: Hinchee RE, Brockman FJ, and Vogel IM (ed). Microbial processes for bioremediation. Battle press, Columbus, Ohio, pp. 145-151.

Wodzinski RS, Coyle JE (1974). Physical state of phenanthrene for utilization by bacteria. Appl. Microbiol. 27:1081-1084.