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Effect of Physical Parameters on Biodegrading Activities of Some Bacteria Isolated From Spent Motor Oil Contaminated Soil at Umuahia, Abia State

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Abstract

Indiscriminate discharge of spent motor oil (SMO) in the environment has become major environmental contaminants as they contain polycyclic aromatic hydrocarbon (PAHs) which are extremely toxic, carcinogenic and mutagenic to human, animals, and plants. Consequently, the need to expand economical and practical remediation technologies for spent engine oil contaminated sites is evident. The study assessed the effect of temperature and pH on biodegrading activities of bacteria isolated from the study area was assessed. Soil sample were collected from the study area from a depths 0 cm-20 cm using a hand held auger instrument to isolate hydrocarbon utilizing bacteria using serial dilution method. The influence of temperature (20°C, 30°C, 40°C, 50°C) and pH (5.5, 6.5, 7.5 and, 8.5) on degradation of spent engine oil by the bacterial isolates was studied. Each strain was incubated in mineral salts media supplemented with 2% spent engine oil. The bacterial culture was inoculated in conical flaks containing 100 mL of broth oil mineral salts media. Control conical flasks containing the same amount of MSM and spent engine oil but without bacterial culture were prepared. All flasks were incubated for 28 days. N-hexane was used to extract the residue oil. The optical density was read at 600 nm wavelength with UV-Visible spectrophotometer. The pH was adjusted at 5.5, 6.5, 7.5 and 8.5 using 1 N HCl/1 N NaOH. The result of the study reveal that fifteen bacteria genera were isolated and identified by biochemical technique from the study area and the effect of temperature and pH on biodegradation of spent engine oil revealed that the bacteria isolates gave the best degradation at 30°C-40°C and at a pH range of 6.5-7.5.

Keywords: Hydrocarbons; Spent motor oil; Spectrophotometer; Optical density; Auto mechanic workshop

Introduction

The Nigeria economy is mainly dependent on revenue from petroleum and petro-chemicals. Spent lubricating oil is one of the petro-chemicals reported to be a major and most common soil contaminant in Nigeria, obtained after servicing and subsequent draining of used oil from automobiles engines, it contains potentially toxic polycyclic aromatic hydrocarbons and heavy metals [1]. The existence of various kinds of auto-mobiles and machines has brought about an expansion in the utilization of motor oil. Release of utilized motor oils contaminates our natural habitat with hydrocarbon. Hydrocarbon pollution of air, soil and freshwater by polycyclic aromatic hydrocarbons (PHAs) draws in open consideration in light of the fact that numerous PAHs are harmful, mutagenic, and cancer-causing [2].

Spent motor oil can result to extraordinary harm to sensitive environs and soil microorganisms. Significant volumes of soil have been debased by utilized oil in numerous nations of the world, particularly industrialized countries. In Nigeria, contamination of the surface and underground water by oil and solid wastes is far reaching, in this way rendering water inadmissible for man's utilization.

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present. Many microorganisms have the ability to utilize hydrocarbons as sole sources of carbon as energy for metabolic activities and these microorganisms are widely distributed in nature. Microbial utilization of hydrocarbons depends on the chemical nature of the compounds within the petroleum mixture and on environmental determinants [3]. The fate of petroleum hydrocarbons in the environment is largely determined by abiotic factors which influence biodegradation of the oil. Factors which influence rates of microbial growth and enzymatic activities affect the rates of petroleum hydrocarbon biodegradation. In one environment petroleum hydrocarbons can persist indefinitely, whereas under another set of conditions the same hydrocarbons can be completely biodegraded within a relatively few hours or days [4].

Temperature affects petroleum biodegradation by influencing the physical nature and chemical composition of the oil, rate of hydrocarbon metabolism by microorganisms, and composition of the microbial community [4,5].Extremes in pH, as can be observed in some environments, would therefore be expected to have a negative influence on the ability of microbial populations to degrade hydrocarbons [4].

Since biodegradation of hydrocarbon is a natural process limited by several physical factors [6] our aim is to isolate bacteria present in the spent engine oil contaminated soil in the study area and determined the optimum temperature and pH that enhance the biodegrading activities of the isolates.

Materials and Methods

Study area and sample collection

The soil samples were collected from Ohiya mechanic Village one of the largest functioning mechanic villages in Umuahia, Abia State (Latitude: 5°26' and 5°35'N, Longitude: 7°03' and 7°05'E). The soil sample was collected using auger-boring instrument from a depth of 0 cm-20 cm into a polythene bag con-

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tainer which was then transported to the laboratory for microbial analysis.

Isolation, characterization and identification of isolates

10 fold serial dilutions were used to isolate hydrocarbon utilizing bacteria. The Bacterial isolates were characterized based on colonial and cell morphology, growth on differential/selective media and biochemical tests which include Gram's reaction, indole tests, methyl red, Voges-Proskauer, citrate utilization, Urea test, utilization of different types of sugars, oxidase and catalase tests. Pure cultures of bacterial isolates were identified on the basis of their colonial morphology, cellular morphology and biochemical characteristics according to the taxonomic scheme of Bergey's Manual of Determinative Bacteriology, as reported by [7].

Determination of the effect of temperature on biodegradation of spent motor oil

The influence of temperature (20°C, 30°C, 40°C, 50°C) on degradation of spent engine oil by bacterial isolates was studied. Each strain was incubated in mineral salts media supplemented with 5% spent motor oil. The bacterial culture was inoculated in conical flaks containing 100 mL of broth oil mineral salts media. Control conical flasks containing the same amount of MSM and spent engine oil but without bacterial culture were prepared. All flasks were incubated for 14 days. N-hexane was used to extract the residue oil. The optical density was read at 620 nm wavelength with UV-Visible spectrophotometer [8].

Determination of the effect of pH on biodegradation of spent motor oil

The effect of hydrogen ion concentration (pH) on growth and degradation of 5% spent motor oil was studied. Mineral salts medium with spent motor oil was prepared adjusting the pH at 5.5, 6.5, 7.5 and 8.5 using 1 N HCl/1 N NaOH. Each strain was incubated in mineral salts media supplemented with 5% spent motor oil. 2% (v/v) of the bacterial culture was inoculated in two conical flaks containing 100 mL of broth oil mineral salts media at different pH (5.5, 6.5, 7.5 and,8.5) set in triplicate. Control conical flasks containing the same amount of MSM and crude oil but without bacterial culture were prepared. All flasks were incubated at 37°C in an orbital shaker at a speed of 200 rpm for 14 days. The residual crude oil was extracted with 50 ml n-hexane. The optical density was read at 620 nm wavelength with UV-Visible spectrophotometer.

Result and Discussion

Isolation, characterization and identification of isolates

Results from the biochemical characteristics of the isolates shows that a total of fifteen isolates were obtained belonging to five genera (Table 1). Four isolates were identified as Bacillus species, two were *Acinetobacter* species, three were *Pseudomonas* species and three were *Citrobacter* species while three were Micrococcus species

This is in agreement with the findings of that isolated *Bacillus spp, Pseudomonas spp* and *Micrococcus spp* from oil contaminated sites of mechanical workshops in Lagos state Nigeria and the result of that isolated *Acinetobacter* species from oil contaminated sites of mechanical workshops in Sokoto metropolis, Nigeria [9,10].

s/n	Bacteria isolate	Slant	Butt	Glucose	Lactose	Gas	H2S	Indole	Motility	Oxidase	Citrate	Urease	Catalase	VP	Methyl red	Organism identified
1	A2	B	A	_	_	_	_	_	+	_	_	_	+	_	+	*Bacillus spp*
2	B1	B	B	_	_	_	_	_	+	+	+	_	+	_	+	*Pseudomonas spp*
3	B2	B	A	+	_	_	_	_	_	_	+	_	+	_	_	*Acinetobacter spp*
4	A1	B	A	_	_		+	_	_	+	+	+	+	_	+	*Micrococcus spp*
5	D1	B	A	+	_		_	+	+	+	+	+	+	_	+	*Citrobacter spp*
6	B1	B	B	_	_	_	_	_	+	+	+	_	+	_	+	*Pseudomonas spp*
7	E1	B	A	+	_		_	+	+	+	+	+	+	_	+	*Citrobacter spp*
8	A3	B	A	_	_	_	_	_	+	_	_	_	+	_	+	*Bacillus spp*
9	C2	B	A	_	_		+	_	_	+	+	+	+	_	+	*Micrococcus spp*
10	B3	B	A	+	_	_	_	_	_	_	+	_	+	_	_	*Acinetobacter spp*
11	E2	B	A	+	_		_	+	+	+	+	+	+	_	+	*Citrobacter spp*
12	D3	B	A	_	_		+	_	_	+	+	+	+	_	+	*Micrococcus spp*
13	C1	B	B	_	_	_	_	_	+	+	+	_	+	_	+	*Pseudomonas spp*
14	C3	B	A	_	_	_	_	_	+	_	_	_	+	_	+	*Bacillus spp*
15	D2	B	A	_	_	_	_	_	+	_	_	_	+	_	+	*Bacillus spp*
Indication of symbols: += Positive result & -= Negative result																

Table 1: Biochemical tests and identification of hydrocarbon degrading bacteria.

Effect of temperature on biodegrading activities of the bacteria isolate

Ecological factors have been stated to effect the degradation of contaminants by microorganisms [11].Temperature influences hydrocarbon degradation by affecting the physical and chemical structure of the oil, rate of uptake of hydrocarbons by microorganisms and composition of the microbial community [5]. (Figure 2) [12] Reported that at low temperatures, the viscosity of the oil is increased, alkanes volatilization reduced, and also decreased in water solubility, thus delaying and decreasing the commencement of biodegradation. Hydrocarbon degrading bacteria grow optimally in a range of temperature ranging from 27°C to 37°C. Growth decreases intensely at higher temperature. In this study, the degrading activities of the isolated bacteria as shown in Figure 1, *Pseudomonas* alongside *Acinetobacter*, *Monococcus* and *Citrobacter* showed high growth at 30°C, While Bacillus showed maximum growth at 40°C whereas The bacteria isolates shows less growth at low temperature 20°C as well as high temperature 50°C [13]. Reported that high temperatures increase the rates of hydrocarbon uptake to a maximum in the range of 30 to 40°C. Above the temperature of 40°C, degradation of hydrocarbons decreased, which may be attributed to membrane toxicity of hydrocarbons. Extreme Temperature can render microorganisms inactive. The optimum temperature for effective hydrocarbon degradation is normally within the range of 20°C-40°C. Higher temperatures cause proteins denaturation and alter the organism's membrane permeability [13,14].

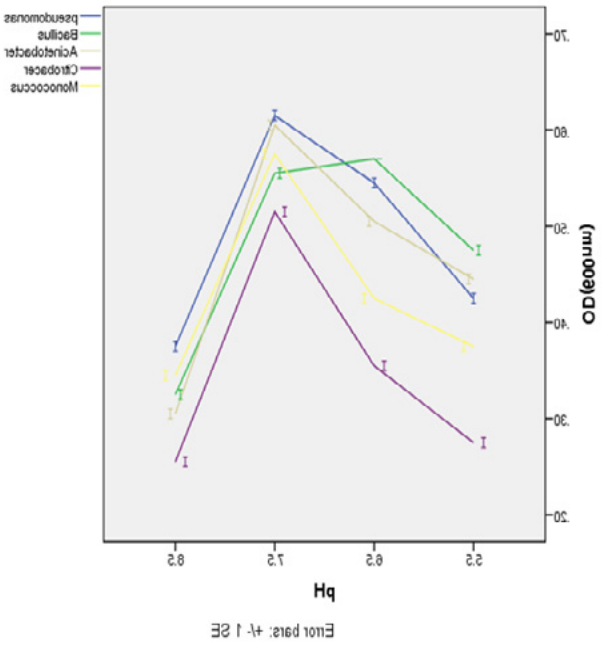

Figure 2: Effect of pH on Biodegrading activities of Isolated Bacteria Strain.

The result is in agreement with [8] who noted the maximum degradation was at 35°C compared with 15°C and 45°C. Similar data were obtained by [15] who recorded optimal biodegradation at 35°C for individual bacteria and mixed degrading bacterial consortium. Earlier reported that further increases in temperature showed a drop in oil degrading potential of the isolates. This may probably resulted from a gradual inactivation of the enzymes responsible for the degradation of the pollutants.

Effect of pH on biodegrading activities of isolated bacteria strain

A pH near to neutrality usually favours organisms for hydrocarbon degradation. pH affects many bacterial enzymes, used for functions such as membrane synthesis leading to many problems such as membrane structure and inability to degrade [4,15].

In this study, the optimal pH that supported growth of bacteria ranged from 6.5 to 7.5. *Pseudomonas*, Bacillus, *Acinetobacter*, *Monococcus* and *Citrobacter* showed highest growth at pH 7.5 (Figure 2). All the bacteria isolate shows very low growth at low pH (5.5) and at high pH (8.5). Several researchers like [16] who noted that most hydrocarbon degraders grow optimally in soils of neutral pH [15]. Recorded the optimal biodegradation at pH 7 for individual and mixed degrader bacterial consortium. Reported pH 7 as the optimal range for hydrocarbon degradation, while [8] noted the maximum degradation at 7.5 among 6.5, 7.5, 8.5 and 9.5 except *Flavobacterium* which shows maximum degradation is at 8.5. Similarly, [17] observed an optimal pH of 7.8, in the range 5.0 to 7.8, for the mineralization of oily sludge in soil [18]. Reported that extremes in pH have an adverse influence on the hydrocarbons degradation ability of microbial populations. Findings indicate that alkaline pH besides nutrient and low water content of some of the soils, are likely to limit oil biodegradation rates [19].

Conclusion

The study concluded that oil degrading bacteria can be isolated from soil directly contaminated with engine oil or similar pollutants. The soils within the premises of automobile workshops are good sources of hydrocarbonclastic bacteria notably; *bacillus spp, Pseudomonas spp, Acinetobacter spp, Monococcus spp* and *Citrobacter spp.* Physical parameters such as temperature and pH to a large extent have effect on biodegrading activities of microbes. The study shows that high rates of degradation were observed at an optimum temperature (30°C-40°C) and pH around neutrality (6.5-7.5).

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