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Ex-Situ Bioremediation of Diesel Polluted Wastewater in Tropical Hot Climate

O.A. Olu-Arotiowa, M.O. Aremu and A.O. Alade
Department of Chemical Engineering, Ladoke Akintola University of Technology,
Ogbomoso, Nigeria

Abstract: Ex-situ Bioremediation of diesel-polluted wastewater had been investigated in this study. Three microbes namely *Baccillus subtilis, Pseudomonia aerigunosa,* both bacteria and *Penicillilum funiculosum*, a fungus, isolated from the wastewater collected from a refinery sewage tank were used. The cultured micro-organisms were used to degrade 1.5 mL of diesel oil in 7 mL minimal salt medium for 20 days and samples were taken and analysed every 5 days. Bioremediation was achieved by all the organisms but at different rates. The results showed that out of the three organisms tested, the fungus *Penicilium funiculosum* has the best degrading ability with 0.2 and 0.46 mL residual oil at 15 days and 20 days, respectively, while *Bacillus subtilis* and *Pseudomonas aeruginosa* (both bacteria) has the same degrading ability of 0.7 mL residual oil after 20 days.

Key words: Bioremediation, wastewater, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Penicilium funiculosum*

INTRODUCTION

The idea of bioremediation of oil spills using aerobic bacteria has been experimented with since 1940's (Bragg and Roger, 1994). Bioremediation involves the manipulation of an organism's biochemical pathways for the molecular degradation of oil polluting a medium (Amadi *et al*., 1993). These microbes convert the hydrocarbon into carbondioxide and water with a release of energy and cell mass which are essential to the microbial growth, development and activities (Atlas, 1995). The environment in which the contaminants exist influences the type of organism that can be used (Borja *et al*., 1995). In cold climate, (0-15°C), Psychrophylic organism would be effective and conversely in hot climate (>25°C) thermophylic organism would be effective. Also pH and heavy metals concentration are other factors that can be controlled through bio-stimulation to optimize bioremediation (Nwauchukwu *et al*., 1998).

This research work was carried out in Ogbomoso, South West Nigeria, which is in a tropical forest region with a temperature range of 25- 30°C. Wami and Ogoni (1997) made use of *Pseudomonas* species as a biodegrader in the kinetic study of microbial activities of crude oil polluted soil, while Sanni and Ajisebutu (2003) worked on oil-polluted soil. This study however, is focused on bioremediation of a diesel polluted water surface.

MATERIALS AND METHODS

Collection of wastewater sample: Wastewater sample used for this study was collected from Kaduna refinery sewage tank, in Kaduna State of Nigeria.

Cultivation of microorganism colonies: The wastewater sample was serially diluted and plated out using pour-plate technique. The serial dilution involves putting 1 mL of water sample in 9 mL of distilled water in a test-tube. The tube was shaken vigorously to allow for thorough mixing. One mililitre of this mixture was further diluted with 9 mL of distilled water in another test-tube and this process continued to reduce the microbial load. The diluted water sample was used to inoculate a mixture of the minimal salt medium and a little amount of diesel inside sterilized petri-dishes which were then incubated at 28°C for 7 days. After 7 days of incubation, mixed colonies of various microorganisms were evident on the petri-dishes. These colonies were subjected to sub-culturing and characterization to get distinct colonies of each microorganisms present.

Characterization and identifications of organism: The organisms stored in the broth were duplicated and a part was sent to KVL/RMR industrial consultants for characterization and identification of the organisms. The bacteria were characterized based on Bergey's manual of bacteriology. Table 1 shows how the bacteria were identified.

Corresponding Author: O.A. Olu-Arotiowa, Department of Chemical Engineering, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Table 1: Biochemical characterization of isolates

Probable identified organism	Grain strain	Cell morphology	Catalase	Oxidase	Casein hydrolysis	Gelatin hydrolysis	Methyl red	Nitrate red
B.s	+	R	+	+	+	+	+	+
P.a	-	R	+	+	-	+	+	+
Hydrogen sulphide production	15°C	45°C	60°C	3°C	Coagulase	Urease	p.H. 3.9	p.H. 9.2
+	+	-	-	-	+	+	-	+
-	-	+	-	-	-	-	+	+
Probable identified organism	Arginase	SH	Growth 40% NaCl	Citrate	Motility	Indole	Glucose	Fructose	Maltose
B.s	+	-	+	+	+	-	+	-	+
P.a	+	+	+	+	+	-	+	-	-
Dul		Mant			Inositol				V/P
+		+			-				-
+		-			-				-

Key: R =Rod, + = Positive, - = Negative, d = doubtful, *B.s = Bacillus subtilis, P.a = Pseudomonas aerigunosa V/P= Voges Prokaver*

Table 2: Preparation of minimal salt ager

Chemical	Concentration (g L^{-1} H_2O)
KH_2P0_4	4.7
K_2HP0_4	0.50
$MgS0_4$	0.50
$CaCl_2$	0.10
$FeS0_4$	0.10
$NaHN0_3$ (Urea)	0.50

(sterilised in autoclave at 121°C for 15 min, pH 7.0)

Table 3: Micro organisms isolated from refinery water and their biodegradabilities

	Residual oil (mL)				
Organism	Day 0	Day 5	Day 10	Day 15	Day 20
*Bacillus subtilis**	1.5	0.9	0.85	0.85	0.7
*Pseudomonas aerigunosa**	1.5	0.9	1.1	0.85	0.7
Penicillium funiculosum#	1.5	0.85	0.75	0.76	0.46

*Bacteria, #Fungus

Sub culturing of isolates: Seven gram of Nutrient agar powder was added to 250 mL of distilled water in a flask. The flask with its content was sterilized inside the autoclave at 121°C for 15 min after which the medium was allowed to cool and dispensed into petri-dishes previously sterilized. Distinct colonies that grew on the Minimal Salt Medium (MSM) were then streaked into the surface of the Nutrient agar plates for bacteria and on Potato Dextrose broth for fungi. The plates were then incubated at 30°C for 24 h. Pure colonies from the Nutrient agar and Potato Dextrose broth plates were then stored on Nutrient agar slant and Potato Dextrose agar slant. Three different organisms, namely *Bacillus subtilis*, *Pseudomonas aerigunosa*, both bacteria and *Penicilium funiculosum*, a fungus, were isolated and used for the degradation experiment.

Preparation of minimal salt agar: Minimal Salt Medium (MSM) was prepared by weighing the salts shown in Table 2 into 1500 mL of distilled water.

Preparation of inoculum: Thirteen gram of nutrient broth was dissolved in 1000 mL of water in a conical flask and sterilized in the autoclave at 121°C for 15 min.After cooling to room temperature, 80 mL of the nutrient broth media and 20 mL of minimal salt agar were measured into a 250 mL conical flask. This was then inoculated with pure culture which has been incubated for 24 h at 37°C (a day old culture). The inoculum was then put inside the incubator shaker set at 30°C for 48 h for proper growth of the organism. 1.0 mL of the suspension of the organism was used for biodegradation.

Biodegradation of diesel oil: Seven mililiter of MSM was dispensed into 15 bottles and 1.5 mL of diesel oil was added as the sole carbon source. The medium was sterilized inside the autoclave at 121°C for 15 min and then inoculated with 1.0 mL of the suspension of each of the three isolates. Four bottles were inoculated with each organism. One each was kept to serve as control for each organism. All the bottles were then incubated at 28°C for 20 days.

Sampling for each residual oil was carried out at 5, 10, 15 and 20days. This was done by selecting one bottle for each isolate on the sampling date.

Analysis of residual oil: The oil-water mixture and measured amount of N-Hexane were mixed in a separating funnel and the oil-hexane phase was separated and allowed to stand for some minutes for the hexane to vaporize. The residual oil was determined by measuring cylinder.

RESULTS AND DISCUSSION

Results showed that while *Bacillus subtilis* shows a declining sequence (Table 3) with a minimum of 0.7 mL of diesel undegraded at day 20, *Pseudomonas aerigunosa* shows an oscillating sequence of 1.1 mL at day 10 and minimum of 0.7 mL in day 20. However, the fungus, *Penicillum funiculosum,* shows a declining sequence in the quantity of diesel from days 5 (0.85 mL) to day 15 (0.26 mL). The amount of residual diesel rises again after day 15 to give 0.46 mL in day 20. From this, it could be deduced that *Penicillium funiculosum* has

the best degrading ability and must be used for 15days after which new fungi must be introduced. The oscillating sequence exhibited by *Pseudomonas aerigounosa* may be due to some biochemical activities in the cell of the organism.

CONCLUSION

Three microorganisms, *Bacillus subtilis, Pseudomonas aerigonisa,* both bacteria and *Penicillium funiculosum,* a fungus, were isolated and found capable of degrading diesel oil-polluted water at different rates. From an experimental amount of 1.5 mL initial diesel oil in day 1, *Penicillum funiculosum* was left with 0.26 mL of residual oil at a peak period of 15 days while *Bacillus subtilis* and *Pseudomonas aerigunosa* were left with 0.7 mL of the residual oil after 20 days.

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