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HYDROCARBON DEGRADING POTENTIALS OF INDIGENOUS BACTERIA ISOLATED FROM AUTO-MECHANIC WORKSHOPS AT MGBUKA-NKPOR, NIGERIA

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Abstract

Five bacterial isolates capable of utilizing used engine oil were isolated from auto-mechanic workshops at Mgbuka-Nkpor, Anambra State, Nigeria, using enrichment culture. The isolates were identified as *Pseudomonas* sp., *Acinetobacter* sp., *Corynebacterium* sp., *Bacillus* sp., and *Flavobacterium* sp., based on morphological and biochemical characteristics. The gravimetric analysis revealed that *Bacillus* sp., *Acinetobacter* sp., and *Pseudomonas* sp., were capable of utilizing 66.67%, 65.47%, and 58.33% of used engine oil respectively, under laboratory conditions at 30^{o}C and 120 rpm with modified mineral salt medium in a 14 day period. *Corynebacterium* sp. and *Flavobacterium* sp. showed a lower degradation potential of 53.57% under the same laboratory conditions and time, with modified mineral salt medium. Observations of turbidity showed that *Bacillus* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Corynebacterium* sp., and *Flavobacterium* sp. exhibited the highest optical density (O.D) of 1.204, 1.102, 0.901, 0.795 and 0.701, respectively, at 420nm over a 14 day period. An increase in oil degradation was correlated to an increase in cell number indicating that the bacterial isolates were responsible for the oil degradation. The results obtained demonstrate the oil biodegradation potentials of these isolates.

Key words: Biodegradation, hydrocarbon, used engine oil, Mgbuka-Nkpor, bioremediation.

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INTRODUCTION

Engine oil is a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents [1]. It is used to lubricate the parts of an automobile engine, in order to keep everything running smoothly [2]. The most important characteristic of the lubricating oil for automotive use is its viscosity. New motor oil contains a high percentage of fresh and lighter hydrocarbons that would be more of a concern for acute toxicity to organisms. Used motor oil contains more metals and heavy polycyclic aromatic hydrocarbons (PAHs) that would contribute to chronic hazards including mutagenicity and carcinogenicity [3]. Prolonged exposure and high oil concentration may cause the development of liver or kidney disease, possible damage to bone marrow and an increased risk of cancer [4-6]. In addition, PAHs have a widespread occurrence in various ecosystems that contribute to the persistence of these compounds in the environment [7]. The illegal dumping of used motor oil is an environmental hazard with global ramifications [8]. The release of oil into the environment causes environmental concern and attracts public attention [9].

The ability of microbes to degrade organic contaminants into harmless constituents has been explored as a means to biologically treat contaminated environments. It is the subject of many research investigations and real-world applications and is the basis for the emergent field of bioremediation [10]. The present study was therefore undertaking with a view to isolating and characterizing oil-degrading bacteria from used engine oil polluted soil and to assess the used engine oil degrading potentials of the isolates.

MATERIAL AND METHODS

Collection of soil samples

Soil samples were randomly collected using a precleaned hand scoop at a depth of 2-3 cm from auto-mechanic workshops that had a heavy spillage of used engine oil at Mgbuka-Nkpor, Anambra State, (6^o 9^1N 6^o 50^1E) Nigeria. They were homogenously mixed, placed into sterile bottle and transported immediately in cold storage container to the laboratory. The hydrocarbon (spent lubricating oil) used in this work was collected direct from the engine of 911 lorry (at Mgbuka-Nkpor) in a sterile container and transported to the laboratory. It was stored in the dark at 25^0C throughout the study to prevent algal growth.

Isolation and identification of engine oil degraders

Engine oil degrading bacteria were isolated from soil samples by enrichment culture on mineral salt agar [11]. The isolates were identified as described by Holt *et al*. [12].

Characterization of the degradation potential of isolates

The isolates were tested for engine oil utilization capabilities in mineral salt broth medium [13].

Determination of used engine oil biodegradation

The level of used engine oil degradation was determined using the gravimetric analysis [14, 15]. The percentage of engine oil remaining was calculated and compared to the control.

RESULTS AND DISCUSSION

Table 1: Variations in pH, optical density (OD) and total viable count values with time during the degradation of used engine oil with *Pseudomonas* sp.

Period of incubation (days)	pH	O.D (420nm)	Total bacterial count ($x\ 10^6$) cfu/ml
0	6.50	0.005	4.8
2	6.55	0.075	6.8
4	6.59	0.284	7.0
6	6.86	0.315	7.5
8	6.95	0.472	8.0
10	7.12	0.530	8.5
12	7.16	0.736	8.5
14	7.18	0.901	8.5

Table 2: Variations in pH, optical density (O.D) and total viable count values with time during the degradation of used engine oil with *Acinetobacter* sp.

Period of incubation (days)	pH	O.D (420nm)	Total bacterial count ($x10^6$) cfu/ml
0	6.50	0.012	6.0
2	6.61	0.065	9.5
4	6.68	0.251	10.2
6	6.72	0.440	10.5
8	7.07	0.583	10.9
10	7.32	0.797	11.0
12	7.32	0.908	11.0
14	7.38	1.102	11.0

Table 3: Variations in pH, optical density (O.D) and total viable count values with time during the degradation of used engine oil with *Corynebacterium* sp.

Period of incubation (days)	pH	O.D (420nm)	Total bacterial count ($x10^6$) cfu/ml
0	6.45	0.005	4.0
2	6.50	0.237	6.0
4	6.61	0.198	6.8
6	6.68	0.310	7.0
8	6.88	0.307	7.8
10	7.25	0.438	8.0
12	7.25	0.687	8.0
14	7.32	0.795	8.0

Table 4: Variations in pH, optical density (O.D) and total viable count values with time during the degradation of used engine oil with *Bacillus* sp.

Period of incubation (days)	pH	O.D (420nm)	Total bacterial count ($x10^6$) cfu/ml
0	6.48	0.015	5.6
2	6.51	0.252	7.0
4	6.62	0.316	9.0
6	6.68	0.460	9.8
8	6.84	0.660	10.2
10	7.05	0.898	10.8
12	7.15	1.054	11.0
14	7.18	1.204	11.0

Table 5: Variation in pH, optical density (O.D) and total viable count values with time during the degradation of used engine oil with *Flavobacterium* sp.

Period of incubation (days)	pH	O.D (420nm)	Total bacterial count ($x10^6$) cfu/ml
0	6.50	0.006	4.2
2	6.56	0.168	6.2
4	6.57	0.219	6.8
6	6.67	0.278	7.0
8	6.96	0.328	7.4
10	7.21	0.354	7.6
12	7.21	0.536	7.8
14	7.23	0.701	7.8

The results signified that the isolates: *Pseudomonas* sp., *Acinetobacter* sp., *Bacillus* sp., *Corynebacterium* sp. and *Flavobacterium* sp. increased the pH of the medium from acidic to slightly alkaline and could best degrade the hydrocarbon in engine oil at slightly alkaline pH (Tables 1-5). The O.D and viable count also showed that the isolates grew well in the growth media, demonstrating their potential for oil bioremediation.

The gravimetric analysis for the estimation of used engine oil degradation was shown (Table 6). Highest engine oil biodegradation was observed with *Bacillus* sp. (66.67%) and *Acinetobacter* sp. (65.47%). *Pseudomonas* sp. exhibited 58.33% degradation, while both C*orynebacterium* sp. and *Flavobacterium* sp. exhibited lower degradation of 53.57%.

Table 6: Estimation of used engine oil biodegradation

Weight of residual hydrocarbon (g)	Amount of hydrocarbon degraded (g)	Percentage (%) degradation	Bacterial isolates
0.35	0.49	58.33	*Pseudomonas* sp.
0.29	0.55	65.47	*Acinetobacter* sp.
0.39	0.45	53.57	*Corynebacterium* sp.
0.28	0.56	66.67	*Bacillus* sp.
0.39	0.45	53.57	*Flavobacteruim* sp.

Each value is the mean of 3 replicates

The result for the estimation of used engine oil biodegradation showed the differences in the ability of the isolates to degrade the used engine oil. The result also signified that *Bacillus* sp. could be best exploited for bioremediation of oil contaminated soil since it had the highest degradation potential among the isolates.

Some of these isolates have been reported as hydrocarbon degraders [16]. Mandri and Lin [17] reported the isolation of *Flavobacterium* sp., *Acinetobacter* sp. and *Pseudomonas aeruginosa* from engine oil contaminated soil. Ijah and Antai [18] reported *Bacillus* sp. as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples. It was postulated that *Bacillus* sp. are more tolerant to high levels of hydrocarbons in soil due to their resistant endospores [19].

Acinetobacter sp. is widespread in nature and can degrade a wide range of organic and inorganic compounds [20-23]. *Acinetobacter* sp. was among the best hydrocarbon degrading bacteria reported in this work, degrading up to 65.47% of the used engine oil, over a 14 day period (Table 6).

The pH values showed increase in pH from 6.50 to 7.18 for *Pseudomonas* sp., 6.50 to 7.38 for *Acinetobacter* sp., 6.45 to 7.32 for *Corynebacterium* sp., 6.48 to 7.18 for *Bacillus* sp. and 6.50 to 7.23 for *Flavobacterium* sp., over a 14 day period. This pH values fell within the range of the optimum value for oil degradation [24]. This signified that the isolated bacteria increased the pH of the medium from acidic to slightly alkaline and that bioremediation of oil polluted soil may be best achieved at slightly alkaline pH. This result was not in agreement with the work that observed a decrease in pH from 6.80 to 6.62 over a 10 day period with *Bacillus* sp. during the degradation of crude oil [25]. Adenipekun [26] observed a pH range of 6.65 to 7.15 during the degradation of oil contaminated soils with *P. tuber-regium*. Verstrate *et al.* [27] found optimal activity for microbial degradation at a pH 4.5 and 8.5.

Total plate count of the isolates showed increased growth in cell numbers from $4.8 \times 10^6 - 8.5 \times 10^6$ cfu/ml, $6.0 \times 10^6 - 11.0 \times 10^6$ cfu/ml, and $4.0 \times 10^6 - 8.0 \times 10^6$ cfu/ml for *Pseudomonas* sp., *Acinetobacter* sp. and *Corynebacterium* sp. respectively over a 14 day period of incubation (Tables 1-3). Counts of *Bacillus* sp. and *Flavobacterium* sp. increased from $5.6 \times 10^6 - 11.0 \times 10^6$ cfu/ml and $4.2 \times 10^6 - 7.8 \times 10^6$ cfu/ml respectively over a 14 day period of incubation (Tables 4,5). Mukred *et al.* [28] observed increased growth in the cell numbers from $243 \times 10^2 - 178 \times 10^3$ cfu/ml within 5 and 10 days and at the end of the 15 days decreased to 105×10^2 cfu/ml, during the degradation of crude oil.

In this study, *Bacillus* sp. had the highest degradation potential of 66.67% and highest optical density (O.D) of 1.204. *Acinetobacter* sp. had the degradation potential of 65.47% (O.D; 1.102), followed by *Pseudomonas* sp. with degradation potential of 58.33% (O.D; 0.901). *Corynebacterium* sp. and *Flavobacterirum* sp. achieved equal degradation potential of 53.57% and optical density of 0.795 and 0.701 respectively at 420nm (Tables 1-6). These findings indicate that an increase in oil degradation

was corresponding to an increase in cell number during the degradation process demonstrating the ability of utilizing engine oil as carbon substrate. This observation was not in agreement with the work which reported that the ability of microorganism to produce turbidity in culture media does not necessarily indicate efficient hydrocarbon – degrading potential [29].

Moreover, among the bacterial isolates, *Bacillus* sp. exhibited the highest degradation potential. This may be due to their resistant endospores. This shows that *Bacillus* sp. are actively involved in the natural degradation of oil- polluted environment. This was not in agreement with the report that spore-forming bacteria in general have negligible role in oil biodegradation [30]. The result was however supported by the works that species of *Bacillus* showed promise as good bioremediating microorganism [31,32].

CONCLUSIONS AND ACKNOWLEDGEMENT

The result obtained revealed that *Bacillus* sp demonstrated the highest degradation potential and therefore could be exploited for bioremediation of used engine oil polluted environment.

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