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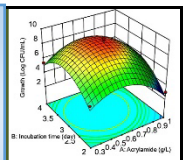

Biodegradation of Used Engine Oil by *Pseudomonas* sp. Isolated from an Automobile Workshop's Soil

Abubakar Muhammad[1] and Faggo Abdullahi Adamu[1]*

[1]Department of Microbiology, Faculty of Science, Bauchi State University Gadau, P.M.B 065, Bauchi, Nigeria.

*Corresponding author:
Faggo Abdullahi Adamu
Department of Microbiology,
Faculty of Science,
Bauchi State University Gadau,
P.M.B 065, Bauchi,
Nigeria.
Email: abdullahiadamufaggo@basug.edu.ng

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ABSTRACT

Environmental pollution caused by used engine oil has been on the increase as a result of accidental or deliberate discharge of the oil. Used engine oil is relatively recalcitrant to biodegradation due to the high concentrations of metals from the wear and tear of engine parts. Several physical and chemical methods were employed for the remediation of used engine oil-contaminated soil, but bioremediation remains the most economical especially in remediating polluted soils. Therefore, this work aimed to isolate and screen efficient used engine oil-degrading bacteria from serval automobile workshops located at Gadau town, Itas Gadau, Bauchi state, Nigeria. Soil samples were collected from three different locations and were used to isolate, and screen used engine oil-degrading bacteria. The best degrader was *Pseudomonas* sp. with 82% degradation of 1% (v/v) of used engine oil after 5 days of aerobic incubation. The result further indicated that degradation occurs best at 1% (v/v) and no degradation was recorded at 3% (v/v) of used engine oil. *Pseudomonas* sp. can be employed in the field remediation of used engine oil-contaminated soil.

INTRODUCTION

Automobile workshops generate millions of gallons of used engine oil containing toxic compounds which are released untreated into the environment [1]. The release of such toxic substances into the environment has been identified as a major threat to the drinking water and soil ecosystem [2]. In modern days, the use of engine oil among vehicle and machine users is continuously increasing [1]. Moreover, there are three possible sources of used engine oil pollution in the environment: mechanical operations, and deliberate and accidental spillages [3]. These three sources have remained the main sources of concern and threat to environmental safety.

In addition, engine oil contains both aliphatic and aromatic hydrocarbons which may change into highly toxic compounds when used recent studies have shown that fresh engine oil improved earthworm growth, indicating its less toxic nature while the used engine oil was found to be lethal [4–6]. This is a link to the presence of heavy metals and polycyclic aromatic hydrocarbons (PAHs) by-products [3]. PAHs have been known to be carcinogenic, mutagenic and teratogenic, with adverse effects on both nervous and immune systems [7].

Consequently, the indiscriminate disposal of used engine oil not only contaminates the environment but also introduces highly recalcitrant as well as toxic PAHs compounds that may cause serious health implications to both humans and animals [3,7]. The recalcitrant nature of PAHs is due to their hydrophobic, hydrophilic and less soluble nature which might affect the complete removal of used engine oil from the environment [8]. Thus, effective remediation methods need to be investigated. There are a number of physicochemical techniques for the remediation of engine oil and other petroleum products such as diesel and kerosine, conversely, all methods employed have been identified with certain advantages and disadvantages [2,9]. Biodegradation is considered one of the best alternatives for the reclamation of engine oil-contaminated soil.

Biodegradation, as a form of bioremediation, is cost-effective, efficient, safe and eco-friendly [10]. It employs different organisms such as fungi, algae and bacteria, or their products (enzymes and biomolecules) to degrade petroleum products. Among the various agent used, bacteria are the most widely reported [2,11–13]. Bacteria have been reported by many researchers as being superior to other organisms in biodegradation [5]. For example. bacteria such as *Pantoea*

wallisii [1] *Ochrobactrum pseudintermedium* [14], *Streptomyces ginkgonis* , *Pseudomonas* sp. [15] *Ruegeria* sp., *Pseudoalteromonas* sp., *Acinetobacter* sp. and *Exiguobacterium* sp. [5], *Bacillus licheniformis* [16] were successfully used for the degradation of used engine oil.

Although several researchers have reported the success of used engine oil degradation by different bacteria both singly and consortium in Nigeria and other parts of the world, this work is the first of its kind to test check the presence of used engine oil degraders in northern Bauchi state, Katagum zone, Bauchi state, Nigeria. Therefore, this work aimed to isolate used engine oil degraders in an automobile workshop in Itas Gadau local government, Bauchi State, Nigeria.

MATERIALS AND METHODS

Isolation and Identification

Soil samples were collected from three different automobile workshops within the town, Itas Gadau Local Government of Bauchi state, Nigeria. Bacterial isolates were obtained by serially diluting the samples following standard protocol [17]. Briefly, 1 g of the soil sample from each soil was serially diluted and plated onto nutrient agar plates which were followed by incubation at 37 ^{o}C for 24 hrs. The colonies formed were repeatedly subcultured until pure isolates were obtained. The isolates were identified using colonial morphology, Gram reaction, and conventional biochemical tests [18].

Screening of used engine oil-degrading bacteria

The isolated bacteria were screened for their potential to utilize used engine oil using 250 ml Erlenmeyer flasks each containing 150 ml nutrient broth supplemented with 1% used engine oil (v/v). The inoculated flasks were incubated on the orbital shaker (170 rpm) at room temperature for 4 days. Cell growth was measured as optical density at 600 nm (OD_{600}) and was used as a parameter for screening the used engine oil degrading potential of the isolates using a spectrophotometer [19].

Effect of used engine oil concentration on biodegradation

Nutrient broth supplemented with 1%, 2% and 3% used engine oil was inoculated by the best isolates to monitor the effect of used engine oil concentrations. Both the inoculated flasks and the control were shaken (120 rpm) and incubated at room temperature. The effect of used engine oil concentration was investigated by collecting and analyzing residual used engine oil samples at regular 24-hour intervals for five days [19].

RESULTS AND DISCUSSION

The results revealed that, out of the 5 isolates, *Pseudomonas* sp., *Bacillus* sp. and *Corynebacterium* sp. were the best degraders with *Pseudomonas* sp. outperforming the other isolates and was chosen for further studies. The results revealed that *Pseudomonas* sp. was able to degrade 60-82% of the used engine oil within 5 days of incubation (**Fig. 1**). The superiority of *Pseudomonas* species over *Bacillus* sp. in hydrocarbon degradation has been well established by many researchers [20–22]. For example, [13] isolated and screened 5 bacterial isolates for their ability to utilize spent engine oil.

The authors found that *Pseudomonas putrefacience* CR33 (68%) had the best degradation potential compared with other isolates, while *Bacillus coagulans* CR31 (45%) had the least potential. [15] screened 19 isolates for their potential to utilize 0.5% $_2$T spent engine oil and discovered that *Pseudomonas* sp. GD18 was the best candidate based on its increasing optical density (OD_{600}) in M9 broth. Nevertheless, *Bacillus* sp. has been used with great success in the biodegradation of used engine oil. [16] employed marine biosurfactant-producing bacterium, *Bacillus licheniformis* LRK1 for the degradation of 1% used engine oil by inoculating the mineral salt medium with 2% of the isolates at 35 °C for 21 days. It was observed that the isolate degraded 24.23% of the oil after 21 days of incubation. Similarly, *Bacillus* sp. such as MOSUL1-4 was found to be very effective in used engine oil degradation [23]. Recent reports have indicated that used engine oil degradation can be improved by using biosurfactant and bioemulsfying bacteria such as *Ocrobactrum pseudintermedium* strain C1[14]. Thus, employing different nitrogen sources and optimizing operational parameters were reported to improve used engine oil degradation [24].

The quest for more efficient isolates for bioremediating the used engine oil-contaminated soil has continued to receive much attention. Several researchers have isolated *Pseudomonas* sp. [3,12] *Bacillus* sp. [25] *Citrobacter freundii* and *Ochrobactrum anthropic* [26] with great success for the degradation of used engine oil. *Pseudomonas* and bacillus species not only efficient engine oil degraders, but also have been used to degrade crude oil and various petroleum fractions such as diesel [21,22,27]. [28] screened 36 isolates for their ability to degrade used engine oil and concluded that only four isolates: *Pseudomonas azotoforman, Bacillus velezensis, Pseudomonas brenneri* and *Bacillus flexus*, have better potential. Similarly, [29] tested the capacity of 5 isolates including *Pseudomonas* sp., *Bacillus* sp., *Corynebacterium* sp., *Aeromonas* sp. and *Acetobacter* sp. for used engine oil degradation.

Incubation time has been one of the most important factors affecting biodegradation. Bacterial consortium such as *Pseudomonas* sp., *Mycobacterium* sp. and *Arthrobacter* sp. was used for the treatment of used engine oil. It was found that the consortium was able to degrade 66% of the aliphatic and 47% aromatic fractions of the used engine oil within 60 days of incubation [30]. However, [31] shown that a bacterial consortium (*Pseudomonas aeruginosa, Acinetobacterium calcoaceticum* and *Flavobacterium sp.*) was able to degrade as high as 90% of the used oil within 28 days of incubation, thus, reducing the degradation time. Several researchers have focused on not only isolating efficiently used engine oil degraders but also how to reduce degradation time. [12] demonstrated that *Pseudomonas aeruginosa* was able to completely degrade spent engine oil within 12 days of incubation, indicating the influence of time on the degradation. Similarly [32] reported that 65mg/L of the used engine was degraded by *Methylobacterium mesophilicum* within 12 days of incubation. In addition, [3] found that extending the incubation time to 21 days led to a 91% increase in the degradation of used engine oil by *Pseudomonas* sp. Therefore, optimization of incubation time, nitrogenous sources, as well as operational parameters, will improve degradation efficiency.

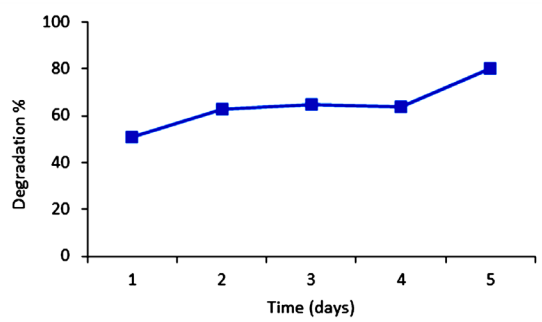

Fig. 1. *Pseudomonas* sp. degradation of used engine oil (1% (v/v) over time.

In order to test the impact of used engine oil concentration on degradation, 1%, 2% and 3% were supplemented to the growth media. The results revealed that the culture that was amended with the 1% used engine oil had the best degradation percentage followed by 3% and the least was recorded in 2% used engine oil concentration (**Fig. 2**). Hydrocarbon concentration has been reported to influence degradation efficiency [21,22]. [26] investigated the effect of used engine concentration on degradation by a single culture of *Ochrobactrum anthropic* HM-1 and mixed culture (*Ochrobactrum anthropic* HM-1 and *Citrobacter freundii* HM-2). The finding confirmed that the best degradation percentage (72%) was achieved at 1% (v/v) concentration.

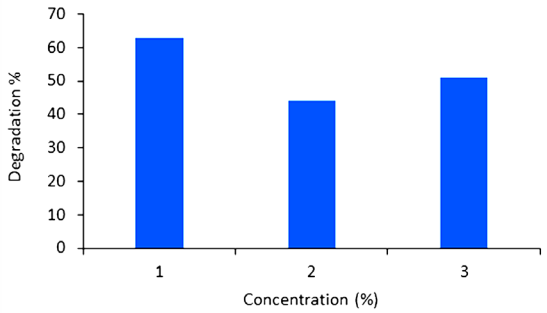

Fig. 2. Effect of used engine oil concentration on degradation by *Pseudomonas* sp.

In addition, [22] tested the efficiency of *Pseudomonas putida* on 1%, 2% and 3% of diesel. They found that *P. putida* grows best at 1% diesel concentration perhaps due to its low toxicity. In a different study,[33] investigated the efficiency of *Pseudomonas* and *Rhodococcus* to degrade various concentrations (0.5%, 1% and 1.5%) of used engine oil. The authors found that all the isolates degraded about 80% of each concentration. [28] screened *Pseudomonas azotoforman, Bacillus velezensis, Pseudomonas brenneri* and *Bacillus flexus*, for their ability to degrade used engine oil on nutrient agar and MSA agar containing 0.2-5% crude oil concentrations. They discovered that out of 42 engine oil components amended in the degradation medium, 26 were completely degraded by the isolates using GC-MS technique.

Various bacterial groups including *Bacillus megaterium, Enterobacter aerogenes and Bacillus cereus Raoultella sp.* [34] were employed for the degradation of used engine oil. *Streptomyces ginkgonis* KM-1–2 was used for the degradation of used engine oil using the gravimetric method. The isolate was degraded by 76% and used engine oil over 28 days of incubation. [23]. The extent of degradation is determined by hydrocarbon concentration and usually, microbes degrade at lower concentrations rapidly while the higher concentration was reported to be lethal for many soil microorganisms [35]. Therefore, the higher degradation observed in 1% used engine oil concentration in this study might be attributed to its low toxicity.

CONCLUSION

The quest for efficiently used engine oil-degrading bacteria has been on the increase. Therefore, in this study out of the 5 isolates tested, *Pseudomonas* sp., *Bacillus* sp. and *Corynebacterium* sp. were the best degraders with *Pseudomonas* sp outperforming the other isolates and was chosen for further studies. The results revealed that *Pseudomonas* sp. was able to degrade 82% of the used engine oil within 5 days of incubation. A further study shows that out of three different concentrations of used engine oil (1%, 2% and 3%), the bacterium performed best at 1% concentration. In this study, the incubation time, used engine oil concentrations as well as bacterial type were found to influence the degradation efficiency. The current study includes further optimization of the bacterium and studying the effect of heavy metals on the degradation of used engine oil by this bacterium.

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