Evaluation of the effect of Physicochemical Parameters on the Biodegradation of spent engine oil using selected Oleophilic Bacteria

Olabisi Omolola Yusuf, Nkem Torimiro, Sunday A. Afolalu*, Abiodun A. Abioye, Moses E. Emetere, Samson O. Alayande

1Department of Microbiology, Obafemi Awolowo University. Ile-Ife, Nigeria
2Department of Mechanical Engineering, Covenant University, Ota, Nigeria.
3Department of Physics, Covenant University, Ota, Nigeria.

*Corresponding Author: sunday.afolalu@covenantuniversity.edu.ng

Abstract: Pollution emanating from the disposal of the auto-mechanic's workshop is of significant challenge globally. This study presents an evaluation of physicochemical parameters on Biodegradation of spent engine oil using selected Oleophilic bacteria. The effect of nitrogen sources (Casein, Urea, Ammonium Sulphate and Potassium Nitrate), pH (4-10) and temperature (25°C to 45°C) were varying on the Biodegradation of spent engine oil using isolated hydrocarbon degraders; Klebsiella sp., Acinetobacter sp., Pseudomonas sp., and Bacillus sp obtained from auto-mechanic's workshops. The assessment of the growth of these hydrocarbon degraders was assessed in Minimal Salt medium (MSM) supplemented with 1% Spent engine oil for seven days. The turbidity of the inoculated MSM was assayed using U.V. Spectrophotometer. The study revealed that the most preferred Nitrogen sources that supported the optimum growth of these hydrocarbon degraders were Casein, while the best pH was 8 and 9. The temperature that supported the optimal growth of the isolates varied with each strain which ranges between 35°C and 40°C. The growth of these bacterial isolates revealed that Biodegradation of the used engine oil is proceeding.

Keywords: Physicochemical; Biodegradation; Engine oil; Oleophilic; Bacteria

1. Introduction

Spent engine oil is described as lubricating oil used in internal combustion engines (ICE) and ejected after sometimes from the engine during the servicing of vehicles [1]. The chemical composition of lubricating oil becomes altered during its use in (ICE) as a result of the disintegration of additives, contaminants from products of combustion, contaminants from metal addition (wear and tear of engines) and by-products of combustion [2]. Hence, spent engine oil is a mixture of chlorinated biphenyls, chlorodibenzofurans, additives, heavy metals, petroleum hydrocarbon, especially Polyaromatic hydrocarbon (PAH) such as Benz (a)anthracene, benzo (a)pyrene, fluoranthene [3]. Obinna et al. [4] in his study analyzed the concentration of PAH in soil samples collected from selected Auto mechanic workshops.
reported six PAH components, which could be categorized as high health-risk compounds upon prolonged exposure [5]. Also, four out of the PAH compounds; benzo(a)anthracene, benzo(b)fluoranthene, chrysene, and benzo(k)fluoranthene were implicated as carcinogens [6]. The disposal of this spent engine oil is on the high side, and it is of significant concern globally. Reports have shown that approximately 20 million gallons of spent-engine fuel (SEO) are generated annually and disposed into the environment. This occurs in developing countries, most notably Nigeria [7, 8]. The implication of this continuous disposal of spent engine oil on the situation could be a future threat to the biotic and abiotic components of the environment. Disposed spent engine oil on the soil could indirectly disrupt plant-water-air relationship [9], affect the fertility of the land hence limiting plant[10, 11], cause considerable damage to soil microorganisms [12] and cause health defects especially among the artisans that come in contact with [4, 13]. In recent years, bioremediation has been the most adopted method that offers the possibility of disintegrating toxic complex compounds into harmless simpler compounds by exploring natural biological activity. This method overrides other methods due to its cost-effectiveness and sustainability. An aspect of bioremediation utilizes microorganisms by a natural process to transform environmental contaminants into harmless end products. Hence, this natural process is time-consuming [14].

However, in overcoming this limitation, some conditions were put in place to catalyze Biodegradation, which can be generally categorized into two; Bio-stimulation and bioaugmentation. Bio-augmentation is by adding indigenous/ engineered microorganisms to the hydrocarbon-impacted ecosystem to supplement the existing microbial population. At the same time, biostimulation involves the stimulation of natural activities by environmental modifications such as fertilizer addition, optimizing the temperature, pH, oxygen to increase rates of Biodegradation [15, 16]. Therefore, the addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is a practical approach to enhance the bioremediation process. Studies have reported various nutrient sources such as inorganic fertilizer, Urea, sawdust, compost manure, and biosolids [17, 18]. Ogunjobi and Ekanem [19] reported the highest Biodegradation of Nitrogen Phosphorus and Potassium (NPK) amended soil spiked with spent engine oil. Therefore, this study aims to compare the rate of Biodegradation of spent engine oil using selected bacteria by varying nitrogen sources, pH, and temperature in the Bushnell Hass medium.

2. Materials

Laboratory equipment and instruments Microfield portable autoclave (Heaton Composite Systems, Seattle, Washington), UV-VIS spectrophotometer (Model 721D), centrifuge (Model 800D), Colorimeter, closed incubator shaker, Mettler Toledo Weighing balance.

2.1 Chemical Composition of Minimal Salt Medium (MSM(g/L)): NaNO3 - 7 , KH2PO4 - 0.5, K2HPO4 - 0.5, KCl - 0.5, MgSO4·7H2O - 0.5, yeast extract – 0.1g glucose-10g. Trace elements (g/L) - MnSO4 - 0.5, FeSO4 - 0.01, CaCl2- 0.01, CuSO4 - 0.01, ZnSO4- 0.07. 5 mL of the trace element solution was added into the MSM.

3. Methodology

3.1 Preparation: The MSM was prepared following the method of Nwinyi et al. 2014 with modifications.
3.2 Isolation, Screening, and Characterization of the Bacterial Isolates

The four bacteria isolate used isolated from the Auto mechanic workshop in Ile-Ife, Nigeria, and was screened for hydrocarbon biodegradation potential in Bushnell Hass broth supplemented with 2% Spent engine oil of seven (7) samples for 20 days (Figure1). These bacteria were characterized based on traditional methods as *Klebsiella sp*, *Actinomycetes sp.*, *Pseudomonas sp.*, and *Bacillus sp*.

![Figure 1.0](image_url)

**Figure 1.0:** The flasks labeled C, D, E and F are the hydrocarbon degraders used in this study; *Klebsiella sp*, *Actinomycetes sp*, *Pseudomonas sp*, and *Bacillus sp* which degraded spent engine oil in Minimal Salt Medium after 20 days.

3.3 Standardisation of the Bacterial isolates

Nutrient broth containing 24 hours old culture of each of the bacterial isolates were spun in a centrifuge at 1000rpm for 15 minutes. The supernatant was decanted, and the residual cell pellets were washed in sterile normal saline. This was repeated thrice. The cell pellets of each of the bacterial isolates were standardized to 0.5 Mcfarland Standard at 590nm, and the inocula were used for degradation studies.

3.4 Optimization of Physico-chemical Parameters of Potential Hydrocarbon Degraders

The effects of various physical parameters such as pH, temperature, nitrogen sources on used engine oil degradation were conducted. The settings were standardized using one factor at a time and keeping the others constant.

3.5 Determination of optimal incubation temperature, pH, and Nitrogen sources

The optimal incubation temperature for maximum culture growth of each of the four test bacterial isolates was determined by incubating at various temperatures 25°C, 35°C, 40°C, 45°C for seven days keeping other conditions at their predetermined level. The negative control flask was also subjected to the same requirements for comparative study. Also, this was applied for pH ranging between 4 and 10, and Nitrogen sources; Organic nitrogen-Casein and Urea, Inorganic Nitrogen: Ammonium sulphate and Potassium Nitrate. Optical
density was measured at 24-hour intervals to determine the optimum temperature for growth [21, 22].

3.6 Statistical Analysis

The statistical analysis was done using the SAS software (version 9.0). Categorical variables were compared using the Chi-square exact test. P values less than or equal to 0.05 were considered insignificant.

4. Results and Discussion

4.1 Effect of Nitrogen Sources, pH and temperature on the Growth Curve of the Bacterial Isolates

The optimum pH, temperature, and preferential nitrogen sources for each of the hydrocarbon degraders were assessed in this study, as shown in the Table and Figures below.

4.2 Best Nitrogen Source for Optimal Growth of Hydrocarbon Degraders

Casein proved to be the most preferred Nitrogen source for the four hydrocarbon degraders; *Klebsiella* sp., *Acinetobacter* sp., *Pseudomonas* sp., and *Bacillus* sp with Optical Densities (O.D): 0.72, 0.88, 0.88, 0.82 respectively against the Control O.D: 0.48±0.1 as shown in Table 1.0

4.3 Best pH for Optimal Growth of Hydrocarbon Degraders

The most suitable pH which gave optimal growth for *Klebsiella* sp. was pH 8 (Figure 2A). The lowest increase was at pH 4 to 6. The optimal growth for *Acinetobacter* sp. was at pH 9, it showed tolerance for growth at pH 8 and 10, while the least growth was at pH 4 to 6 (Figure 2B). pH 8 showed the best performance for the growth of *Pseudomonas* sp., and the least increase was observed at pH 4 to 6, as shown in Figure 2C. Also, the pH which gave optimal growth for *Bacillus* sp. was nine while the least increase was observed at pH ten, as shown in Figure 2D.

4.4 Best Temperature for Optimal Growth of Hydrocarbon Degraders

The optimal growth temperature for *Klebsiella* sp. was 35°C, as shown in Figure 3A. The temperature for the optimal growth of *Acinetobacter* sp. was observed to be 40 °C (Figure 3B), the preferred temperature for the optimal growth of *Pseudomonas* sp. was observed at 40 °C. In comparison, the least increase was observed at 25 °C (Figure 3C). Moreover, the temperature for the optimal growth of *Bacillus* sp. was found to be 35 °C and 45 °C. (Figure 3D).
Table 1: Comparative analysis of potential hydrocarbon-degrading bacteria under different Nitrogen Source

| Nitrogen Source | Klebsiella sp.  | Acinetobacter sp. | Pseudomonas sp. | Bacillus sp. | Control |
|-----------------|-----------------|-------------------|-----------------|--------------|---------|
| Ammonium Sulphate | 0.51±0.15       | 0.74±0.29         | 0.57±0.15       | 0.73±0.23    | 0.47±0.12 |
| Casein          | 0.72±0.23       | 0.88±0.36         | 0.88±0.29       | 0.82±0.23    | 0.49±0.13 |
| Potassium Nitrate | 0.52±0.16      | 0.54±0.18         | 0.52±0.14       | 0.63±0.19    | 0.48±0.12 |
| Urea            | 0.64±0.20       | 0.59±0.14         | 0.51±0.13       | 0.67±0.18    | 0.49±0.11 |

Fig 2A: Comparative Growth Profile of Klebsiella sp. at varying pH

Fig 2B: Comparative Growth Profile of Acinetobacter sp. at varying pH
**Fig 2C:** Comparative Growth Profile of *Pseudomonas* 1sp. at varying pH

**Fig 2D:** Comparative Growth Profile of *Bacillus* sp. at changing pH

**Fig 3A:** Comparative Growth Profile of *Klebsiella* sp at varying temperature

**Fig 3B:** Comparative Growth Profile of *Acinetobacter* sp. at varying temperature
4.5 Discussion

Findings revealed that microorganisms have limited tolerance to some environmental conditions, which could also affect their optimal performance. These conditions include physical factors such as nitrogen, pH, and temperature, which play a significant role in controlling microbial growth and activity during hydrocarbon biodegradation. The influence of pH on soil degradation is essential, as most microbial species survive only within a specific pH range. It has been documented that the field for the Biodegradation of petroleum hydrocarbon is between pH 6 and 8 [23]. In this study, the best pH for growth for the hydrocarbon degraders used in this study pH of 8 and 9. This contradicts the study by Mahmood et al., [24], Dipti et al. [25] who obtained an optimum of pH 7 for the growth of *Pseudomonas* sp. and *Bacillus* sp. on spent engine oil.

Temperature plays an essential role in the Biodegradation of hydrocarbon and also controls the rate of enzymatic reactions. At low temperature, the viscosity of oil increases which result in low volatility of low molecular weight hydrocarbon and consequently delay the onset of Biodegradation [26]. In this study, it was observed that *Pseudomonas* (1) and *Bacillus* showed optimum growth rate at temperature 40°C and 45°C, respectively. This is in contrast with the study by Dipti et al. [25] and Borah and Yadah [27] who obtained optimum growth rate for *Pseudomonas* sp and *Bacillus* sp at 37°C and 35°C. Moreover, in this study, all four bacterial isolates showed low growth rate at 25°C. Ogbonna [28], in his research, reported that most bacteria found in soil, including many bacteria that degrade petroleum hydrocarbon, are mesophiles that have an optimum temperature ranging from 25°C to 45°C.

Nitrogen supplements act as a macronutrient for the synthesis of amino acids and nucleic acids, which enhance the growth of the bacterial isolates in the medium. It has been reported that ammonium and phosphate ions contributed positively to the production of oil-degrading enzyme "lipase" obtained from *Pseudomonas aeruginosa* SL-72 [29]. The addition of nutrients (Nitrogen) might be useful in increasing the Biodegradation of organic compounds because these amendments effectively stimulate bacterial growth [30, 31, 32]. In this study, it was observed that Casein was the most preferred Organic nitrogen that gave optimal growth rate for all the hydrocarbon degraders followed by ammonium sulfate. Moreover, Urea gave the lowest growth rate for all the bacteria isolates in this study. This supports the research by...
Mahmood et al. [24], who reported Urea as an unsuitable nitrogen source for the Biodegradation of spent engine oil. This subsequently implies that the growth rate of the bacterial isolates increased the rate of degradation. However, it could be acknowledged that the preference for nitrogen sources, pH, and temperature are relative concerning each of the microorganisms and the physicochemical parameters of the environment (terrestrial and aquatic) where they were isolated.

5. Conclusion

This study revealed that Casein, an organic nitrogen source, the enhanced growth rate of the hydrocarbon degraders than Urea, Ammonium sulfate, and Potassium nitrate, which has been broadly reported. This implies that it can accelerate the Biodegradation of spent engine oil. Therefore, it is expedient to explore products, especially biowaste/agro-waste, that has a high content of Casein for better results. Also, field study could be experimented using Casein as an Organic nitrogen source. Furthermore, the best pH and temperature could be adapted better to enhance bioremediation procedures, especially for ex-situ bioremediation in bioreactors.

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