THOMAS ADEWUMI UNIVERSITY, OKO, KWARA STATE
Science | Technology | Medicine

**Thomas Adewumi University Journal of Innovation, Science and Technology (TAU-JIST)**

***ISSN: 3043-503X***

*RESEARCH ARTICLE*

# DEGRADATION OF CRUDE OIL (ESCRAVOS LIGHT) BY BACTERIA ISOLATED FROM MILE TWO LAGOON LAGOS, NIGERIA

*Olayinka Odunola Usman & Oluwafemi Sunday Obayori*

***Department of Microbiology, Faculty of Life Science, Ahmadu Bello University, Zaria***

***Department of Microbiology, Faculty of Science, Lagos State University, Ojo***

***Corresponding Author E-mail: olayinka.usman@yahoo.com***



**ARTICLE DETAILS**

***Article History:***
Received 02 July 2024
Accepted 05 October 2024
Available online 10 December 2024

**ABSTRACT**

*Four objectives were formulated and, four research questions were answered in the study. The population was forty-Crude oil is a major pollutant of many ecosystem including aquatic system such as rivers and lagoons. The use of these systems as waterways and discharge from runoff and indiscriminate disposal make such water veritable receptors of hydrocarbon pollutants. Clean up of polluted environments requires adequate information about the properties of the site as well as the biodegradation capacity of the microbial community. This research study examined the effectiveness of pseudomonas aeruginosa isolated from polluted water sample collected from a lagoon situated at Mile two, Lagos State Nigeria to ascertain the effective degradation of crude oil (escravos light). The physicochemical parameters of the polluted water sample such as organic matter content colour, odour was analysed. The biochemical characteristics of the pseudomonas aeruginosa was identified using Analytical profile index Microbact 12E. Further test was carried out showing pseudomonas aeruginosa was positive for endospore staining which was located at its terminal. There was no biosurfactant production and beta haemolysis. The degradation rate was evaluated by residual oil hydrocarbon capacity using a spectrophotometer (Beckman Du700), total viable count in CFU/g and the pH which decreases significantly within 16 days from 2.800 to 1.193,3×10⁸ to 2×10⁵ and converted to percentage respectively.*

**KEYWORDS**

*Crude oil, Pseudomonas Aeruginosa, Degradation, Lagoon, Nigeria*

## 1 INTRODUCTION

Crude oil is composed of thousands of components that can be categorised into saturated hydrocarbons, aromatic hydrocarbons, resins, and asphaltenes. These products are the major sources of energy for industry and daily life. It is also a major source of raw materials for many chemical products, such as plastics, paints, and cosmetics. The oil spill is heavily concentrated around offshore production sites, major shipping routes, and refineries and frequently exceeds the self-purification capacity of receiving waters. Which makes oil floating in water technically difficult to contain and collect. Oil spills pollute groundwater and surface water, which can be destructive to various forms of marine life. Microorganisms such as bacteria are very effective in the biodegradation of crude oil. They are equipped with metabolic machinery to use petroleum products as a carbon and energy source. Carbon dioxide and water are released as byproducts.

**Quick Response Code** | **Access this article online**

**Website:** https://journals.tau.edu.ng/index.php/tau-jist

**DOI:** https://doi.org/10.5281/zenodo.15003281

Cite the Article: *Olayinka Odunola Usman & Oluwafemi Sunday Obayori. (2024). Degradation Of Crude Oil (Escravos Light) By Bacteria Isolated From Mile Two Lagoon Lagos, Nigeria*

They utilise oxygen as the primary electron acceptor. The degradation proceeds more rapidly, and it is considered effective.

Bacteria and other microorganisms are highly efficient in the biodegradation of crude oil. They have metabolic machinery that allows them to consume petroleum products as a source of energy and carbon. As byproducts, carbon dioxide and water are emitted. Their principal electron acceptor is oxygen. The degradation is thought to be effective and is happening more quickly. Numerous variables might cause microbes to co-metabolise or use contaminants as a substrate; consequently, it is useful to identify key elements for successful pollution cleanups by comprehending the catabolic pathways, mechanisms, and relevant enzymes. The biodegradation of crude oil is influenced by a number of environmental conditions, including salinity, temperature, pH, nutrients, oxygen availability, pressure, and light. Hydrocarbon-degrading bacteria must be present in sufficient numbers is a prerequisite for an effective bioremediation. Hence this study seeks to assess the biodegradation potential of *pseudomonas aeruginosa* on crude oil.

## 2 MATERIAL AND METHODS

### 2.1 Sample Collection

Thirty water samples were taken in Lagos State, Nigeria, from the Mile Two Lagoon. Using sterile, labelled sampling bottles, water samples were randomly taken from three separate sampling locations. Samples taken from the three sampling stations were blended into a composite sample in a 3L Erlenmeyer flask, covered with a new polythene bag, and promptly transferred to the microbiology laboratory, Lagos State University Ojo, for further investigation. The physico-chemical properties of the water sample, such as pH, organic matter content, biological oxygen demand, chemical oxygen demand, total suspended solids, colour, odour, available phosphorus, and potassium, were determined using the standard methods (Tijjani et al., 2014; Olsen et al., 2006).

### 2.2 Microbiological analysis of the water sample

The total heterotrophic bacteria counts were enumerated by serially diluting the water sample from the appropriately labeled Erlenmeyer in physiological saline at dilution $10^{-2}$ -$10^{-9}$ and 0.1 mL of aliquots of each dilution was spread into nutrient agar plates in triplicates. The plates was incubated at room temperature, (27 ± 2°C) for 5 days.

Similarly, the population of hydrocarbon utilizing bacteria was estimated on mineral salts medium formulated by Habe *et al.,* 2009. The medium contained (in g/L) $K_2HPO4$, 0.38 g; $KH2PO_4$, 0.6 g; $NH_4Cl$ 1 g, $FeCl_3$, 0.05 g, $MgSO_4$ .$7H_2O$, 0.20 g and Agar 15g. The pH of the medium was adjusted to 7.2 for bacterial estimation. The mineral salts medium was fortified with nystatin (50µg/mL) to suppress fungi growth and sterile crude oil served as the sole carbon and energy. Plates were counted after incubation at room temperature for 7 days.

### 2.3 Isolation and screening of Pseudomonas aeruginosa

Five milliitre of water sample was added to 45ml of mineral salts medium broth and the suspension was incubated with a rotary shaker at 150rmp at room temperature 27ºC until there was turbidity in 7 days. *Pseudomonas aeruginosa* was isolated by plating out dilutions on Luria bertani agar. The colonies appear was further sub cultured by plating out on crude oil mineral salts medium agar. Morphological and biochemical characterization of *Pseudomonas aeruginosa* was further analysed and stored in nutrient broth: glycerol in ratio of 1:1.

### 2.4 Biodegradation studies

Standardization of inoculum

Resuscitation of the *pseudomonas aeruginosa stored* in nutrient broth and glycerol was harvested and cultured in Luria Bertani agar. The colonies was suspended in 4ml sterile normal saline and the turbidity was adjusted to match 0.5 MacFarland turbidity standard which was equivalent to $1.5\times10^8$ bacterial density (Atta *et al.,* 2009).

Biodegradation studies

An aliquot of one milliliter (1ml) of the inoculant was added to fourty nine milliliter of Mineral Salts Medium. The experiment set up was incubated at an ambient temperature 27 ±2°C on an orbital shaker at 150 revolution per minute for 16 days. Flasks with mineral salts medium and crudeoil was inoculated with heat killed cells as control. At interval of 4 days, the mineral salts medium containing crudeoil as a sole source of carbon was dissolved with tuolene and extracted to assess the residual crudeoil by taking the absorbance at 600nm with spectrophotometer and comparing it with the control setup. Hydrocarbon utilizing bacterial counts was enumerated in which 1ml of the suspension was dissolved into 9ml of sterile normal saline and serial dilution was done up to $10^{10}$. An aliquot of 0.1ml was taken at appropriate dilutions of ($10^{-6}$, $10^{-7}$, $10^{-8}$) and spread using a sterile bent glass rod aseptically into Luria Bertani agar and incubated at 27ºC for 24 hours and changes in pH of the culture was evaluated (Atta *et al.,* 2009).

## 3. RESULTS

### 3.1. Physicochemical Analysis Of The Mile 2 Lagoon Water Sample

The table below shows the physicochemical analysis of the mile 2 lagoon water sample.

**Table 1. Physicochemical analysis of Mile 2 lagoon water sample.**

| S/N | Parameters | Polluted water sample |
|---|---|---|
| 1 | pH | 6.9 |
| 2 | Odour | Foul |
| 3 | Colour | Pink |
| 4 | Available phosphorous | 1.223 |
| 5 | Potassium | 2.321 |
| 6 | Biological oxygen demand | 15.0 |
| 7 | Chemical oxygen demand | 13.2 |
| 8 | Total suspended solids | 165 |
| 9 | Total nitrogen | 16.0 |

**Table 2: Microbiological analysis of the mile 2 lagoon water sample.**

| Microbiological parameters | Amount (×10⁶cfu/g) |
|---|---|
| THB | 2.50±0.222 |
| THUB | 4.10±0.149 |

Keys: THB- total heterotrophic bacteria, THUB-total hydrocarbon utilizing bacteria

**Table 3: Morphological and Biochemical characterization of *Pseudomonas aeruginosa***

| Parameters | Results |
|---|---|
| Margin | Entire |
| Elevation | Raised |
| Appearance and colour | Dull and cream |
| Shape | Circular |
| Size | Moderate |
| Optical density | Opaque |
| Cellular morphology | Cocci |
| Gram staining | - |
| Catalase | + |
| Motility | + |
| Oxidase | - |
| Spore staining | + |
| Manitol | + |
| Lactose | + |
| Fructose | - |
| Galatose | - |
| Maltose | + |
| Nacl tolerance control | + |
| Beta heamolysis | Beta |
| Emulsification I ndex | 87% |
| Analytical profile index | 98% *Pseudomonas aeruginosa* |

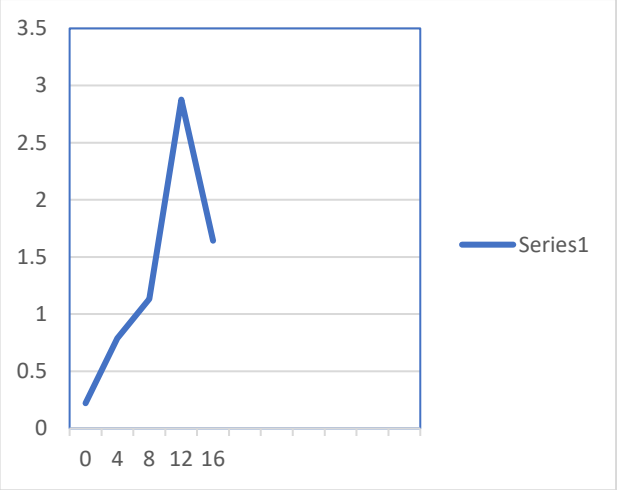


**Figure 1:** Percentage reduction of crude oil in mineral salts medium (broth) inoculated with *Pseudomonas aeruginosa* at room temperature at 27ºC.

## DISCUSSION

In a contaminated environment, the primary mechanism for the biodegradation of hydrocarbons is bacterial activity or bacterial consortium activity. Individual bacteria can usually break down a small variety of hydrocarbons. Crude oil is the only source of carbon and energy that bacteria can metabolise and thrive on. One of the most adaptable groups of organisms engaged in the hydrocarbon breakdown process is Pseudomonas aeruginosa. Pseudomonas aeruginosa was shown in this research investigation to be able to break down crude oil (escravos light) that was isolated from Mile Two Lagoon in Lagos State, Nigeria. During the degradation studies, there was a positive correlation between an increase in cell biomass and an increase in oil degradation. The study's contaminated water sample's physiochemistry revealed low levels of nutrients like phosphorus, potassium, and nitrogen.

Total hydrocarbon degrading and heterotrophic bacteria in polluted water sample was $2.50\times10^6$ cfu/g and $4.10\times10^6$ cfu/g respectively (Table 2).

Colonial morphology of *Pseudomonas aeruginosa* showed margin, elevation, appearance, shape and size. It was entire for margin and the appearance was dull. The biochemical characterization showed it was positive for spore and motile. It is very difficult to understand the degradation mechanism especially for complex hydrocarbons however some researchers have revealed the metabolic pathway for the degradation of some hydrocarbons bacteria population found to degrade crude oil is not minimal and it has been reported that bacteria has a little effect in the degradation of hydrocarbon.

*Pseudomonas aeruginosa* grew well on crude oil and effective degradation took place with stationary phase attained within 12 days and complete disappearance of crude oil on the basic of total viable count pH and residual oil concentration with the aid of spectrophotometer and optical density was measured at 87%, this is similar to the work of Adelowo (2004) that reported 73% degradation of crude oil within 16 days.

The degradation of crude oil in this study showed a decrease in pH from 5.3 to 3.2, this indicates that crude oil degradation is accompanied with lowering of pH, this may be connected with the fact that degradation of hydrocarbon compounds usually leads to the production of organic acids which invariably leads to lowering of pH, however the slight decrease in pH may be accounted for that the medium is buffered.

The spectrophotometer analysis of the residual hydrocarbon concentration showed crude oil was degraded by *Pseudomonas aeruginosa* in 16 days. The reduction in peak between days 0 to 16 reduced drastically as degradation increases with rapid growth shows that the organism metabolized crudeoil effectively.

## CONCLUSION

*Pseudomonas aeruginosa* isolated from mile 2 polluted lagoon could metabolize crude oil as a sole source of carbon and energy with constant growth and turbidity. The biodegradation of petroleum and other mixed hydrocarbons in the environment is a complex process whose quantitative and qualitative aspects depends on the rich nature and diversity of the microbial community and amount of hydrocarbon present, the ambient and seasonal environmental conditions.

## REFERENCES

Adebusoye, S.A., Ilori, M.O., Amund, O.O. Teniola, O.D and Olatope, S.O., (2008).Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. World journal of Microbiology and Biotechnology. **23 (8):** 1149-1159.

Adelowo, B., and Igbinosa, E (2004) Studies on the effect of petroleum hydrocarbon on the microbial and physic-chemical characteristics of soil. African journal of Biotechnology. **6(16) :**1939-1943.

Atta, H.I, (2009) Studies on petroleum hydrocarbon degradation by bacteria in soil. Master thesis Department of Microbiology, Ahmadu Bello University, Zaria.

Bundy J., Paton G.I. and Campbell C.D., (2004): combined microbial community level and single species bioreactor responses to monitor recovery of oil polluted soil. *Journal of Soil Biology and Biochemistry* **36:**1149-1159.

Habe, H., Ashikawa Y., Saiki Y., Yoshida T.,Nojiri H. and Omori, T (2009).*Sphingomonas* sp strain carrying carbozole Dioxygenase gene degrades chlorinated dibenzo-p-dioxins in soil. *Journal of European Microbiology*, **211:**43-49.

Ilori, M.O., Amobi C.J and Odoche, A.C (2010) factors affecting biosurfactant production by oil degrading *Aeromonas* sp isolated from a tropical environment. *Chemosphere* **61:**985-992.

Iwabuchi, N, Sunairi M., Urai, M, Itoh ,C., and Anzai Y(2002) extracellular polysaccharides of *Rhodococcus* stimulate the degradation of crudeoil by indigenous marine bacteria. *Journal of applied Environmental Microbiology*. **68:**2337-2343.

Nweke, C.O, Okpokwasili, G.C., and Abel H.K, (2008): effects of bioremediation treatments on the bacterial population of soil at different depth, Nigerian journal of Microbiology **18:**362-372.

Obayori, O.S, Ilori, M.O, Adebusoye S.A, Oyetibo G.O and Amund O.O (2008): pyrene degradation potentials of pseudomonas species isolated from polluted tropical soils World journal of microbial biotechnology **24:**2639-2646.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (2006). Estimation of available phosphorous and Ph in contaminated soils by xtraction with sodium bicarbonate and electrode. USDA circular.**939:**1-19.

Okoh, A.L, Okoro, M.N, and Obi, G.H, (2006): Biodegradation of bonny light oil in soil microcosm by some bacterial strain isolated from crude oil flow station. *Journal of African Biotechnology* **2:**104109.

Said, B.O., Goni, M.S and Duran R, (2008): Characterization of aerobic polycyclic aromatic hydrocarbon degrading bacteria from Bizerte lagoon sediments in Tunisia. *Journal of Applied Microbiology* **10:**988-998.

Tijjani, M.B. (2014). Impact of Tannery Effluents on the Quality of Environmental Samples and bioremediation potentials of Microbial Isolates. **234:**34-56