

Degradation of Crude Oil by Microbial Populations of Lagos Lagoon Water Microcosms

Olumide Omotosho

Biological Institute, National Research Tomsk State University

Lenina Ave 36, Tomsk, Russia, 634050

Olumide.Omotosho@stud.tsu.ru

INTRODUCTION & AIM

The contamination of aquatic environments by petroleum hydrocarbons is a significant environmental concern driven by its increasing demand globally. Nigeria faces severe pollution issues due to petroleum-related activities, necessitating remediation efforts. Bioremediation, the use of microorganisms to degrade pollutants, offers a promising solution for the remediation of oil-polluted environments.

This study focuses on the isolation and characterization of hydrocarbon-degrading microorganisms from Lagos Lagoon and evaluates their potential for bioremediation.

RESULTS & DISCUSSION

Table 1: Typical physiological and biochemical characteristics of the bacterial isolates

| Characteristics | THUB-1 | THUB-2 | THUB-3 | THB-1 | THB-2 | THB-3 | THB-4 | THB-5 |
|-----------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Colony form | Circular |
| Pigmentation | Creamy | Creamy | Yellow | Yellow | Creamy | Creamy | Creamy | Purple |
| Optical properties | Opaque | Opaque | Translucent | Opaque | Opaque | Translucent | Translucent | Opaque |
| Colony size | Punctiform | Moderate | Small | Small | Moderate | Moderate | Punctiform | Small |
| Cell shape | Bacilliform |
| Motility | + | + | + | + | + | + | + | + |
| Gram stain | - | - | - | - | - | - | - | - |
| Endospore staining | - | - | - | - | - | - | - | - |
| AcidFast staining | - | - | - | - | - | - | - | - |
| Hydrolysis of starch | + | - | - | - | - | - | - | - |
| Oxidase test | + | + | + | + | + | + | + | + |
| Citrate test | + | + | + | + | + | + | + | + |
| O/F test | + | + | + | + | ND | + | + | + |
| Indole test | - | + | + | + | + | + | + | - |
| Catalase test | + | + | + | + | + | + | + | + |
| H ₂ S production | + | - | - | - | - | - | - | - |
| Glucose | A | - | - | + | + | - | - | - |
| | G | + | - | + | + | - | - | - |
| Fructose | A | + | - | + | + | + | + | + |
| | G | + | - | + | + | + | + | + |
| Galactose | A | + | + | + | - | + | + | + |
| | G | + | + | + | - | + | + | + |
| Maltose | A | + | - | - | - | - | - | + |
| | G | + | + | + | + | + | + | + |
| Lactose | A | + | - | - | - | - | - | - |
| | G | + | - | - | - | - | - | - |

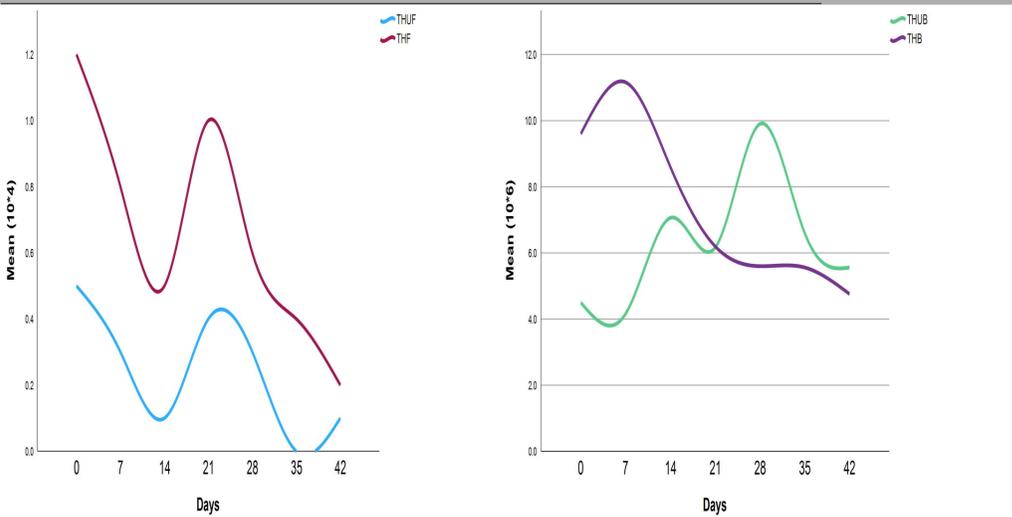


Figure 1: Growth profile of the total heterotrophic fungi on potato dextrose media and hydrocarbon utilising fungal isolates in carbon free mineral (CFMM) supplemented with Escravos light crude oil (1% V/V) as sole carbon and energy source over a 42-day period.

Figure 2: Growth profile of the total heterotrophic bacteria on nutrient media and hydrocarbon utilising bacterial isolates in carbon free mineral (CFMM) supplemented with Escravos light crude oil (1% V/V) as sole carbon and energy source over a 42-day period.

| Isolate | Doubling time | Specific growth Rate (day ⁻¹) | Degradation rate % | Degradation Rate (%/day) | Half life t _{1/2} (d) |
|---------|---------------|---|--------------------|--------------------------|--------------------------------|
| THUB | 0.702 | 0.987 | 93.853 | 2.235 | 10.433 |

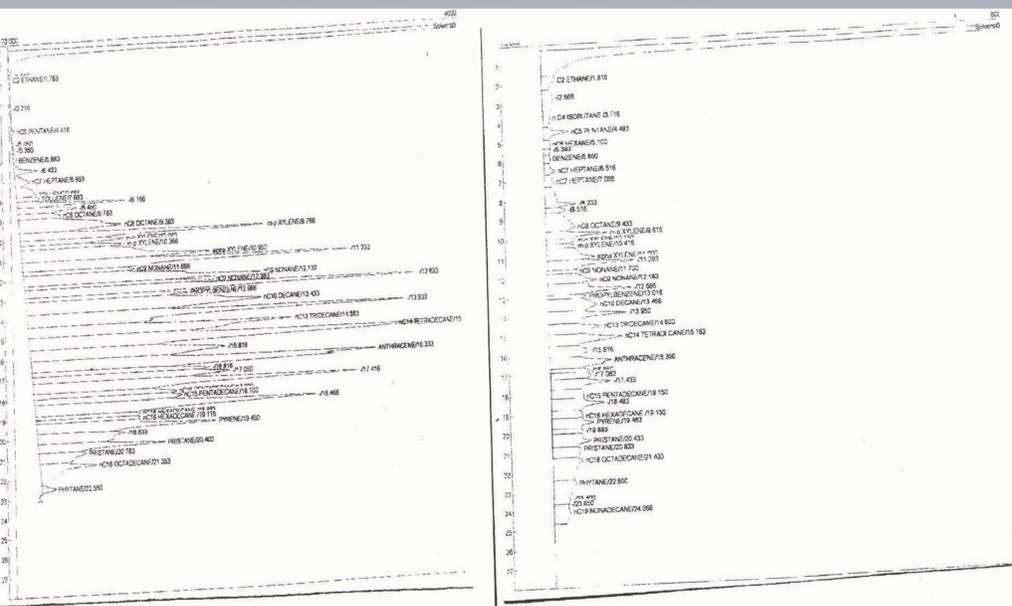


Figure 3: Chromatographic fingerprints of n-hexane extract of lagoon water microcosm crude oil (Escravos light) from Day 0 (left) and Day 42 (right).

METHOD

Sample, Media, and Culture:

- Water samples were collected from Lagos Lagoon at Mile 2, Lagos (6.3979° N, 3.4006° E).
- Samples were taken from three different points, mixed, and transported to the laboratory at 4 °C.
- Escravos blend crude oil was used in the study.

Physicochemical Analysis:

- The pH and temperature were measured using an Adwa pH meter and a mercury bulb thermometer, respectively.
- Other parameters like total organic carbon, total hydrocarbon content, nitrate, phosphate, and sulphate were determined using standard procedures.

Microcosm Experiment:

- 21 flasks containing 50 mL water samples were incubated with 1 mL sterile crude oil for 42 days at 28 ± 2 °C.
- Sub-samples were taken periodically on days 0, 7, 14, 21, 28, 35 and 42 to determine microbial counts and degradation rates.

Microbial Enumeration:

- Microbial populations were determined using the standard plate count method. Bacteria and fungi were enumerated on nutrient agar and potato dextrose agar, respectively. Hydrocarbon-utilizing bacteria were grown on mineral salt medium.

Isolation and Characterization:

- Microbial isolates were identified based on colony morphology and biochemical tests. Various biochemical assays, including Gram staining, endospore staining, catalase test, oxidase test, and carbohydrate utilization tests, were performed.

Gas Chromatography Analysis:

- Residual crude oil was analysed using a gas chromatograph equipped with a flame ionization detector (GC-FID). The degradation rate constant and half-life were calculated based on the residual oil data.

| Hydrocarbon Fractions | Retention Time | | Peak Area | | Residual HC % after 42 days |
|--------------------------------|----------------|--------|------------|-----------|-----------------------------|
| | Day 0 | Day 42 | Day 0 | Day 42 | |
| Solvent | 0.350 | | 66048.1245 | | |
| n C ₂ -Ethane | 1.783 | | 165.1750 | 698.4785 | 422.87 |
| n C ₄ -Isobutane | | 3.716 | | 368.3480 | |
| n C ₅ -Pentane | 4.416 | 4.483 | 454.7055 | 755.7730 | 166.21 |
| n C ₆ -Hexane | | 5.100 | | 295.9940 | |
| Benzene | 5.883 | 5.850 | 546.1380 | 274.7760 | 50.31 |
| n C ₇ -Heptane | 6.983 | 6.516 | 2712.3720 | 440.7210 | 16.248 |
| n C ₇ -Heptane | | 7.066 | | 470.6380 | 17.351 |
| Toluene | 7.666 | | 4403.6760 | | |
| Toluene | 7.883 | | 2580.8640 | | |
| n C ₈ -Octane | 8.783 | | 5483.9540 | | 39.546 |
| n C ₉ -Nonane | 9.383 | 9.433 | 26472.8400 | 2168.7340 | 8.192 |
| m-p- Xylene | 9.766 | 9.816 | 26434.2710 | 1464.4600 | 5.540 |
| m-p- Xylene | 10.083 | 10.150 | 12399.1850 | 673.2315 | 5.429 |
| m-p- Xylene | 10.366 | 10.416 | 10022.4635 | 577.0980 | 5.758 |
| Alpha - Xylene | 10.950 | 11.000 | 41888.5010 | 2220.9590 | 5.302 |
| n C ₉ -Nonane | 11.666 | 11.700 | 11264.1000 | 591.2900 | 5.249 |
| n C ₉ -Nonane | 12.133 | 12.183 | 46545.1790 | 2001.4295 | 4.299 |
| n C ₉ -Nonane | 12.383 | | 16543.3470 | | |
| Propylbenzene | 12.966 | 13.016 | 16858.7245 | 736.4340 | 4.368 |
| n C ₁₀ -Decane | 13.433 | 13.466 | 64158.7110 | 2774.6935 | 4.324 |
| n C ₁₁ -Tridecane | 14.583 | 14.600 | 55155.9055 | 2301.3700 | 4.172 |
| n C ₁₄ -Tetradecane | 15.166 | 15.183 | 76652.3750 | 2873.3645 | 3.748 |
| Anthracene | 16.333 | 16.350 | 70269.8240 | 2652.4195 | 3.774 |
| n C ₁₅ -Pentadecane | 17.900 | | 17351.5355 | | 8.684 |
| n C ₁₅ -Pentadecane | 18.100 | 18.150 | 19786.4720 | 1506.8270 | 7.615 |
| n C ₁₆ -Hexadecane | 18.883 | | 11545.6335 | | 10.185 |
| n C ₁₆ -Hexadecane | 19.116 | 19.150 | 8964.2610 | 1175.9480 | 13.118 |
| Pyrene | 19.450 | 19.483 | 32380.7390 | 1812.7730 | 5.598 |
| Pristane | 20.400 | 20.433 | 18612.4670 | 2077.7090 | 11.162 |
| Pristane | 20.783 | 20.833 | 10656.3380 | 1545.3750 | 14.501 |
| n C ₁₈ -Octadecane | 21.383 | 21.433 | 12463.4045 | 2763.9970 | 22.176 |
| Phytane | 22.550 | 22.600 | 1633.2290 | 1790.3665 | 109.621 |
| n C ₁₉ -Nonadecane | | 24.066 | | 1372.0810 | |

Table 2: Percentage degradation of hydrocarbon fractions at inoculation in 2 lagoon water microcosm at day 0 and day 42.

CONCLUSION/ FUTURE WORK

The study demonstrated the significant potential of the isolated microbial strains in degrading petroleum hydrocarbons. The mixed microbial cultures exhibited synergistic interactions, enhancing the overall degradation process. These findings highlight the potential application of these microorganisms in bioremediation strategies for oil-polluted environments. Future research will focus on optimizing environmental conditions to maximize the efficacy of these microbial consortia in bioremediation.

REFERENCES

Hassanshahian, M., & Cappello, S. (2013). Crude oil biodegradation in the marine environments. In *InTech eBooks*. <https://doi.org/10.5772/55554>

Al-Dhabaan, F. A. (2019). Morphological, biochemical and molecular identification of petroleum hydrocarbons biodegradation bacteria isolated from oil polluted soil in Dhahran, Saud Arabia. *Saudi Journal of Biological Sciences*, 26(6), 1247-1252. <https://doi.org/10.1016/j.sjbs.2018.05.029>