**Isolation of plastic degrading microorganisms from Clifford University Dumpsites**

 **ABSTRACT**

The accumulation of plastic waste on the environment poses a significant threat to ecosystem and human health, necessitating effective mitigation strategies. This study explores the potential of microorganism in bioremediating plastic pollution. Soil samples were collected from Clifford University dumpsites, and microorganisms were isolated using serial dilution techniques. The ability of these microorganisms to degrade plastic was assessed using polyethylene as substrates. Characterization of the isolated microorganism through morphological and biochemical analysis revealed *Streptococcus* sp., *Pseudomonas* sp., *Bacillus* sp., *Proteus* sp*.*, *Staphylococcus aureus* and *Micrococcus* sp. with the capacity to degrade plastics. Further degradation study of the Poly ethylene by determination of Weight Loss revealed *Micrococcus* sp. *Bacillus* sp. and *Streptococcus* *sp*. with higher potential for plastic degradation and *Micrococcus* sp. with highest significant potential of 3.8% weight loss after 28 days of incubation. This research contributes to the development of sustainable solutions for plastic waste management by highlighting the potential of indigenous microorganism in bioremediation.

**Keywords: Plastics, Degradation, Bioremediation, Polyethylene**

**1. INTRODUCTION**

Environmental pollution is an important issue that needs utmost attention. Human activities have greatly polluted the environment and have created more harm to it than in past centuries. The natural balance of the planet earth has been disrupted by excessive cutting down of trees, burning of fossil fuels and littering of waste products such as plastics to mention but a few. Some parts of the environment are gradually becoming uninhabitable by man due to plastic accumulation resulting mainly from human activities. Its effect on the water, air, soil, plants and animals is threatening (Rana & Rana, 2020).

Plastic wastes cause death of marine lives by entanglement and ingestion, food web is disrupted by broken plastics and its pellets, its litter destroys the primary habitat of new emerging life forms (Singh & Gupta, 2014; Rana & Rana 2020) and it do not break down easily and therefore remain in the environment for a very long time. Their resistance to degradation poses significant environmental challenges with millions of tons of plastic waste accumulating on the environment-the land and water bodies. Traditional methods of plastic waste disposal, such as land filling, incineration, and recycling, have limitations and adverse environmental effects. Consequently, there is a growing interest in bioremediation, specifically the use of microorganisms to degrade plastics in a sustainable manner (Dela Tore *et al*., 2018; Azizi *et al.,*2024). Microbial degradation of plastics involves several steps, typically starting with the colonization of the plastic surface by microorganisms, followed by the enzymatic breakdown of the polymer into smaller molecules, and finally the mineralization of these molecules into carbon dioxide, water, and biomass (Urbanek *et al*., 2020). Dumpsite present a complex matrix of organic and inorganic materials, creating unique ecological niches where microbial communities can evolve. The constant influx of plastic waste provides selective pressure for the emergence of microorganisms capable of utilizing plastics as a carbon and energy source (Rillig, 2012).

Microorganisms isolated from dumpsites are particularly valuable due to their natural adaptation to plastic-rich environments. These organisms have likely evolved efficient enzymatic pathways to degrade plastics, offering potential for high degradation rates and robustness under variable environmental conditions. The isolation of plastics degrading microorganisms from dumpsites holds promise for addressing the global plastic waste crisis. By leveraging the natural capabilities of these microorganisms, it is possible to develop sustainable and effective strategies for plastic waste management, reducing the environmental impact of plastics and contributing to a circular economy.

This study therefore aims to isolate plastic degrading bacteria from soils taken from various dumpsites in Clifford University, Owerrinta, Ihie Campus, Abia State, Nigeria.

**2. MATERIALS AND METHODS**

**2.1 Collection of Samples**

 Soil samples were collected from three dumpsites in Clifford University which includes; female hostel dump site, Cafeteria dump site and Creche dump site with a sterile spatula and sterile sample bottles. The samples were transported immediately to Microbiology laboratory.

**2.2 Isolation of Microorganism**

Tenfold serial dilutions were conducted separately on each of the soil samples for the isolation of microorganisms. One millimeter (1 ml) from the stock test tube was collected and transferred into the second test tube and this process was repeated up to 10‾6 respectively for the three soil samples. Using Pour Plates method, 0.1ml of the diluted samples (10−3, 10−4, and 10−5) in duplicates, were transferred to different sterile petri dishes and then mixed with a Nutrient Agar which was prepared according to the manufacturer’s instruction. This mixture was carefully and gently rocked to ensure that the samples were distributed evenly within the medium. The petri dishes were incubated in an incubator at 37⁰C for 24hours. This process was performed separately for each of the soil samples used for this experiment.

**2.3 Identification of bacterial isolates**

This was done by carrying out colonial morphology (which includes shape of colony, elevation of colony, edge of colony and pigmentation). Representative colonies after being differentiated on the basis of morphology and color were then sub-cultured on a Nutrient Agar to obtain pure cultures by repeated streaking and then on slant for future studies. Colonies were counted from the different mixed culture plates before subculturing of the representative colonies. The purified cultures were identified using microscopic observation of their Gram staining activities and also by using other biochemical method. The biochemical tests carried out include Sugar fermentation tests, Catalase test, Coagulase, Indole, Citrate and Oxidase.

**2.4 Screening of Polyethylene (PE) degrading strains**

The liquid polyethylene medium (LPEM) in which PE was the sole carbon source containing 0.7g 0f KH2PO4, 0.7g of K2HPO4, 0.7g of MgSO4\_7H20, 1.0g of NH4NO3, 0.005g of NaCl, 0.002g of FeSO4.7H20, 0.002g of ZnSO4.7H2O, and 0.001g of MnSO4.H2O (per 1000ml) which were weighed using a standard Mettler analytical balance as suggested by SAI (1996) was prepared and a carbon free source agar solid medium, Agar polyethylene medium (APEM) was also prepared by adding 15g agar to 1000ml of liquid basal medium and then the pH was adjusted to about 7.0. All media were autoclaved at 1210C for 20 minutes. The homogenate was inoculated into the LPEM (containing 1% PE) and cultured at 260C using a thermostat incubator with shaker (ZHP – 100 model) which was set at (220r/min) the set up was allowed to stay for 21 days (3 weeks) at room temperature. The colonies grown on the plate and liquid medium were considered as primary screening colonies for PE- degrading bacteria.

**3.6. Further Degradation study of PE Degrading Strains by determination of Weight Loss**

 The Liquid basal medium (LPEM) in the various conical flasks, were sterilized and the primary screening organisms were introduced aseptically from the existing culture plate into the conical flasks according to the labelling by using a sterile wire loop to collect a colony of each organism into their respective conical flasks. Immediately after this, weighed PE plastic pellets were then introduced into all the LPEM conical flask before been placed inside an incubator at 370C. At different time interval, the conical flasks were all brought out from the incubator and with a clean clinical glove, the pellets were retrieved, dried using a thermostat oven at very low temperature and then reweighed to notice if there has been any lose in pellets weight. The same was done for the control which contained no PE degrading strains. The weight loss percentage of the plastic sample was calculated using the formula Usha *et al*., (2011):

% Decrease of plastic weight= R1 – R2/R1 ×100

Where:

 R1 = initial weight of plastic pellet

R2 = final weight of plastic pellet

**3. RESULTS**

After 24hours culture of the various samples obtained from different dumpsites at Clifford University and then sub culturing of the bacteria isolates, the following bacteria isolates with unique characteristics were isolated from the dumpsites, *Streptococcus* sp *Pseudomonas* sp*,* *Bacillus* sp,  *Proteus* sp*.* *Staphylococcus aureus* and *Micrococcus* sp as shown in Table 1 and 2. These bacteria isolates were identified based on their macroscopic (cultural) and microscopic characteristics.

*Micrococcus* sp*.* shows the highest degrading power of PE as compare to other isolates in this study as revealed in Table 3 and 4.

Isolates like *Pseudomonas sp., Proteus sp., Bacillus sp*3*., Bacillus sp*2*., Staphylococcus aureus* and *Micrococcus sp*. had abundant growth (+++) on solid media while some like *Streptococcus sp*1. and *Bacillus sp*1*.* showed a moderate growth (++) on the medium and *Streptococcus sp*2 showed a scanty growth (+) on the solid medium.

**Table 1. Isolate cultural characteristics and growth pattern on solid media**

|  |  |  |  |
| --- | --- | --- | --- |
| Location | Isolate code | Cultural characteristics | Growth pattern |
| Cafeteria | CW1CW3 | White, circular and flatWhite, filamentous &dry | ++++++ |  |
| Crèche  | CRY1CRM1CRM2CRBR1 | Yellow, risen, smooth & spherical Milky, circular and flatMilky and punctiformBrown, flat & circular | +++++++++ |  |
| Female hostel | HWIHW2HBR1 | White and filamentousWhite and smoothBrown and punctiform | ++++++++ |  |

Key: Abundant growth (+++), moderate growth (++), scanty growth (+), and no growth (-)

**Table2. Biochemical tests and gram staining results of bacterial isolates from the dumpsites in Clifford University**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Isolate code | Gram stain | Shape  | Cat | Oxi | Coa | Ind | Cit | H2S | *Gas*  | Possible organisms |
| HBRI | G+ | Cocci | - | - | - | - | + | - | - | Streptococcus sp |
| CWI | G+ | Cocci | + | - | - | - | - | - | - | *Micrococcus sp* |
| CW3 | G+ | Cocci  | + | + | + | - | - | - | - | *Staphylococcus aureus* |
| HWI | G+ | Rod | - | - | - | - | + | - | + | *Bacillus sp* |
| HW2 | G+ | Rod | + | - | - | - | + | - | - | *Proteus* sp |
| CRY1 | G+ | Cocci  | - | - | + | - | - | - | - | *Streptococcus* sp |
| CRM1 | G- | Rod  | + | + | - | - | + | - | - | *Pseudomonas* sp |
| CRM2 | G+ | Rod | + | - | - | - | - | - | - | *Bacillus* sp |
| CRBR | G+ | Rod | + | - | + | - | + | - | - | *Bacillus* sp |

Key: positive (+), negative (-), gram positive (G+), gram negative (G-), catalase (cat), oxidase (oxi), coagulase (coa), Indole (ind), citrate (cit) and hydrogen sulphide (H2S).

**Table 3: Plastic pellets weight loss after 14days**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S/N | ISOLATES | INITIAL WEIGHT(g**)** | FINAL WEIGHT(g**)** | WEIGHT LOSS (%) |
| 1. | *Pseudomonas* sp. | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps35.jpg | 0.151 | 0.658 |
| 2. | *Bacillus* sp.1 | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps38.jpg | 0.156 | 0.637 |
| 3. | *Streptococcus* sp1 | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps41.jpg | 0.169 | 1.744 |
| 4. | *Streptococcus* sp2 | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps44.jpg | 0.160 | 0.000 |
| 5. | *Proteus* sp | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps47.jpg | 0.164 | 0.000 |
| 6. | *Bacillus* sp3 | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps50.jpg | 0.156 | 1.887 |
| 7. | *Staphylococcus aureus* | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps53.jpg | 0.156 | 0.000 |
| 8 | *Bacillus* sp2 | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps56.jpg | 0.157 | 0.633 |
| 9. | *Micrococcus* sp | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps59.jpg | 0.152 | 2.564 |
| 10. | Control | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps62.jpg | 0.152 | 0.000 |

Table 4: ANOVA TABLE after 14 days

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SOV | df | SS | MS | Fcal | Fcrit(α, 0.05) |
| Total | 29 | 10.174 | - | - |  |
| Treatment | 9 | 7.097 | 0.786 | - |  |
| Error | 20 | 3.077 | 0.154 | 5.104 | 2.393 |

LSD = 0.67

**Table 5.: Plastic pellets weight after 28days**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S/N | ISOLATES | INITIAL WEIGHT(g**)** | FINAL WEIGHT(g**)** | WEIGHT LOSS (%) |
| 1. | *Pseudomonas* sp. | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps35.jpg | 0.150 | 0.136 |
| 2. | *Bacillus* sp.1 | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps38.jpg | 0.156 | 0.637 |
| 3. | *Streptococcus* sp1 | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps41.jpg | 0.168 | 2.326 |
| 4. | *Streptococcus* sp2 | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps44.jpg | 0.159 | 0.625 |
| 5. | *Proteus* sp | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps47.jpg | 0.163 | 0.625 |
| 6. | *Bacillus* sp3 | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps50.jpg | 0.154 | 3.145 |
| 7. | *Staphylococcus aureus* | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps53.jpg | 0.155 | 0.641 |
| 8 | *Bacillus* sp2 | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps56.jpg | 0.155 | 1.899 |
| 9. | *Micrococcus* sp | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps59.jpg | 0.150 | 3.846 |
| 10. | Control | C:\Users\HP\AppData\Local\Temp\ksohtml13640\wps62.jpg | 0.152 | 0.000 |

Table 6 : ANOVA TABLE after 28 days

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SOV | df | SS | MS | Fcal | Fcrit(α, 0.05) |
| Total | 29 | 33.286 | - | - |  |
| Treatment | 9 | 23.176 | 2.575 | - |  |
| Error | 20 | 10.108 | 0.505 | 5.099 | 2.393 |

LSD = 1.213

**4. DISCUSSION AND CONCLUSION**

Microbial community found in dumping sites characterized by substantial plastic contamination, exhibits the potential to break down both natural and synthetic substances while utilizing them as a carbon source (Maclean *et al*., 2021). In this study, *Streptococcus* sp1, *Micrococcus* sp, *Staphylococcus aureus*, *Bacillus* sp1, *Bacillus* sp2, *Bacillus* sp3, *Pseudomonas* sp, *Proteus* sp and *Streptococcus* sp2 were isolated from the Clifford University dump sites in Isiala-Ngwa North L.G.A of Abia State. This is in agreement with other reported work. Akerele *et al*. (2022), reported isolating similar organisms from different dump sites in Lagos. They reported *Staphylococcus aureus*, *Stretococcus sp*, *Bacillus sp* and *Escherichia coli* as being able to degrade a higher percentage of plastic material. *Pseudomonas* and *Bacillus* genera have been reported to possess the ability to degrade polyethylene and polypropylene (Amobonye *et al.*, 2021; Giocomucci *et al*., 2019). However, the isolates reported by Nademo et al., 2023 seems different from the organisms as presented in this study. Nademo *et al*., 2023 in their report isolated two different species of *Methylobacterium*, which includes *Methylobacterium radiotolerans* and *Methylobacterium fujisawaense* and also *Lysinibacillus fusiformis*. The reason for the variation in isolates may be attributed to distance and different environmental factors.

In the PE biodegradation studies, the weight loss method was used to determine microbial consumption of polymers (Das and Kumar 2015; Jamil *et al*., 2017). In this study, the percentage of PE weight loss was calculated, and the highest value after 14 days of incubation was recorded by *Micrococcus* sp followed by *Bacillus* sp and *Streptococcus sp* as shown in Table 3 above. After 28 days of incubation, the highest value was still recorded by *Micrococcus sp* followed by *Bacillus sp* and then *Streptococus sp* (Table 4). The result of this work shows that *Micrococcus sp, Bacillus sp and Streptococcus sp* possess a better potential to degrade plastics especially PE with *Micrococcus* showing a higher degrading potential than others. Akerele et al. (2022) has also reported *Micrococcus sp* showing the highest degrading ability than other microorganisms he isolated. Javid *et al*. (2024) identified seven bacteria isolates efficient in plastic degradation which included *E. coli, Corynebacterium* sp, *Micrococcus* sp, *Azotobacter* sp, *Pseudomonas* sp, *Staphylococcus* sp and *Bacillus* sp. *Micrococcus* sp demonstrated notable reduction in white plastic after 28 days of incubation. Kalia and Dhanya (2022) reported *Bacillus fusiformis* with the potential to degrade LDPE after 30 days of incubation which is in agreement with this study that shows *Baccillus* sp ability to cause a reduction of 1.9% after 28 days of incubation.

Therefore, the microorganisms, *Micrococcus* *sp, Bacillus sp, Streptococcus* s*p* could be enhanced and used for remediation of different environments polluted by plastics materials.

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