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Degradation of Polyethylene Using Bacteria from Waste Dump Sites in Obio/Akpor Local Government Area in Rivers State, Nigeria

Ezekwu Chizike [a*], Williams Janet Olufunmilayo [a] and C. J. Ugboma [a]

[a] Department of Microbiology, Faculty of Science, Rivers State University, P.M.B. 5080, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

All around the world today, different types of plastics are used for packaging materials, especially in form of low density polyethylene (LDPE). The aim of the study was to degrade polyethylene using bacteria from waste dump sites in Alakahia and Ogbogoro of Obio/ Apkor Local Government Area, Rivers State. Soil samples were randomly collected from Alakahia and Ogbogoro dump sites in Obio/Apkor Local Government Area, in Rivers state. Polyethylene degraders were determined using microscopy and weight loss methods. The total heterotrophic bacterial counts recorded were 3.03 x 10^6 CFU/g and 1.12 x 10^6 CFU/g for Ogbogoro and Alakahia samples. The total count of *Pseudomonas* recorded were 5.0 x 10^4 CFU/g and 4.9 x 10^4 CFU/g for Ogbogoro and Alakahia sample. There was no significant difference ($P \leq 0.05$) within the counts of the samples. The

**Corresponding author: Email: chizikeezekwu2018@yahoo.com;*

bacteria isolated from the soil collected from the dumpsites were *Bacillus* sp, *Klebsiella* sp, *Pseudomonas* sp, *Staphylococcus* sp, *Serratia* sp, *Escherichia coli*, *Shigella* sp, *Salmonella* sp. The identified bacteria were *Pseudomonas proteolytica* NR025588.1, *Pseudomonas aeruginosa* CP0296051 *and Bacillus circulans* CP026031.1. From the result of the degradative ability by the individual microorganism using weight loss measurement, *Pseudomonas aeruginosa* CP0296051 showed a greater percentage of degradation of polyethylene of 5% to 27.03% (after 150 days) followed by *Pseudomonas proteolytica* NR025588.1 which recorded percentage degradation of 6.0% to 23.28% (after 150 days) while *Bacillus circulans CP02603.1* recorded the least percentage of 5% to 19.9% (after 150 days). The result of degradative potential of the microbes in consortium set ups showed that the consortium of *Pseudomonas aeruginosa* CP0296051 and *Bacillus circulans* CP02603.1 (B8+B6) showed the highest degradation potential producing degradation percentage range of 8.7 to 46.80% after 150 days of the study and the least degradation potential of polyethylene was observed in *Pseudomonas aeruginosa* CP0296051 and *Pseudomonas proteolytica* NRO255SS.1 (B6+B3) with the percentage range of 5% to 27.03% after 150 days. From the study, *Pseudomonas* sp, *Bacillus* sp and their consortium showed higher potential to degrade low density polyethylene (LDPE) hence the prospect in remediation of polyethylene.

Keywords: Polyethylene; degradation; LDPE; Pseudomonas proteolytica.

1. INTRODUCTION

Polyethylene have been defined as the polymers in which when heating, becomes mobile and can be cast into moulds. They are said to be non-metallic compounds and the materials that are made from them can be pushed into any desired shape and size. They are classified into different categories such as LDPE (Low Density Polyethylene), LLDPE (Linear Low Density Polyethylene), and HDPE (High Density Polyethylene). Among these, LDPE plays a vital role and it is a thermoplastic made from the monomer ethylene. It was the first grade of polyethylene produced in 1933 by Imperial Chemical Industries (ICI) using a high pressure process via free radical polymerization. During the past three decades, polythene materials have been increasingly used in food, clothing, shelter, transportation, construction, medicals, and recreation industries. Polythene are advantageous as they are strong, light weighted and durable [1]. These plastics are characteristically inert and are resistant to microbial attack, leading to their accumulation in the environment. Recently, the biodegradation of plastic waste and the use of microorganisms to degrade the polymers have gained notable importance because of the inefficiency of the chemical and physical disposal methods used for these pollutants as well as the environmental problems they cause. Microorganisms play a significant role in the biological decomposition of materials (Shah et al*.,*2004).

Biodegradation has played a key role in reducing the molecular weight of the polymer by naturally occurring microbes like bacteria, fungi and actinomycetes isolated from different environments [2] (Sivan et al*.,* 2006). There have been a lot of literatures on degradation of many types of plastics, all of them are starch blended. There has not been much reports on microorganisms' ability to degrade pure form of polyethylene. On the other hand, bacteria and fungi have the capacity to degrade various types of plastic through the secretion of specific extracellular enzymes (i.e. oxygenases, lipases and esterases) [3].

Most of these activities (indiscriminate dumping of plastic waste) are done ignorantly, paucity of information, lack of awareness, understanding, and advocacy on waste management. Thus, it is important to investigate the capacity of bacteria from waste dump sites in Obio/ Akpor Local Government Area, Rivers State to degrade polyethylene, which is now a fast growing city in Nigeria.

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples

Soil samples were collected from surface soil within 0-15 cm depth into sterile black polyethylene bags using a hand auger from two different dumpsites. The dumpsites include the Ogbogoro dumpsite which is located in Ogbogoro town, and a dumpsite at Alakahia town both in Obio-Akpor Local Government Area in Rivers state. The coordinates of the dumpsites are 4.842524^{o}N, 6.942053^{o}E for Ogbogoro dumpsite, while that of Alakahia dumpsite is 4.886874^{o}N, 6.923765^{o}E.

2.2 Culture of the Samples

One gram (1g) of the soil sample was transferred into test tube containing 9ml sterile normal saline and 10-fold serial dilution was carried out to dilution 10^{-6}. Aliquot (0.1ml) of 10^{-2} was inoculated on pre-dried cetrimide agar plates and nutrient agar plates. Plates were inoculated in duplicates and were spread using sterile bent glass rod before they were incubated at 37℃ for 24-48 hours. After the incubation, plates were observed for growth and the colonies were counted for enumeration of bacterial population in the soil samples. Pure cultures of bacteria were obtained by aseptically streaking representative discrete colonies of different morphological characteristics which appeared on the cultured plates onto freshly prepared sterilized Nutrient agar plates and were later incubated at 37^{o}C for 24hours. The bacterial isolates were preserved at 4^{o}C in 10% glycerol in bijou bottles for later use (Cheesbrough 2006).

2.3 Identification of Bacterial Isolates

The bacterial isolates obtained from the samples were characterized and identified based on their cultural microscopic and biochemical characteristics and according to the schedule depicted by the Bergey's Manual of Determinative Bacteriology. The isolates were characterized genotypically by cloning and sequencing the 16S rRNA. The genomic DNA from a pure culture of each isolate was extracted and purified for PCR amplification of the 16S rRNA sequence using the Big Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa.

2.4 Screening of Polyethylene Degrading Microorganisms

Polyethylene beads were weighed and aseptically transferred to mineral salt medium and inoculated with each bacterial isolates from the waste dump sites to determine ability to degrade polyethylene beads using (weight loss method) as shown in Fig. 2. Control experiments were maintained with polyethylene bead in the microbe-free medium. Different flasks were kept in shakers incubator for 30 days. After the incubation, the polyethylene bead were collected, disinfected with ethanol, shade dry and then weighed for final weight. The percentage weight loss was determined by gravitational method [4].

2.5 Determination of Polyethylene Degradation using Weight loss Method

This was determined according to the method described by Amadi et al., [4] with slight modification. In this technique, equal gram (0.3g) of LDPE beads was first sterilized with 70% alcohol. To quantify the extent of LDPE degradation by the isolates, 100 µL saturated culture (1×10^{8} cells/mL) of the respective isolates were inoculated into 100 mL of sterile mineral salt medium supplemented with 0.3g (six beads) of sterile LDPE beads. In the control set, an equal weight of the LDPE beads was incubated for a similar length of time without the introduction of microbial isolate. All the growth media including control were incubated at 35°C for different length of time as per the requirement of the experiments (150 days). After the incubation, the LDPE beads were recovered, washed with sterile distilled water, and air-dried. Weight loss of the LDPE strips were measured accordingly using the formula:

$$\text{Weight Loss (\%)} = \frac{InitialWeight - FinalWeight}{Initial\ Weight} \times 100 \quad (1)\ [4]$$

Fig. 1. Polyethylene Beads

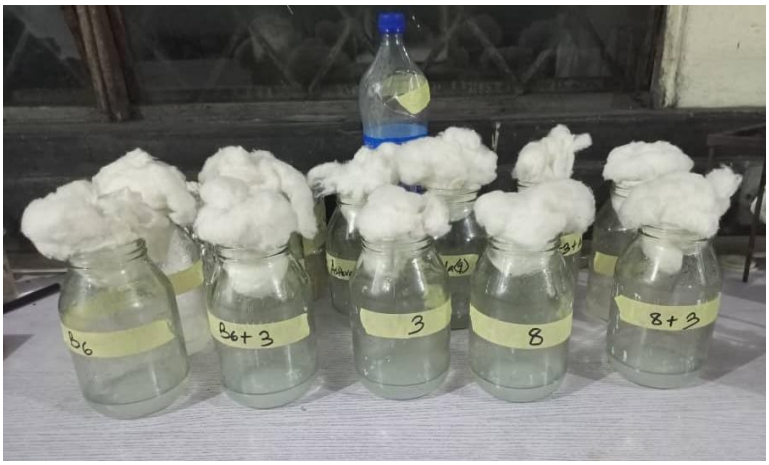

Fig. 2. Degradation setup

2.6 Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 26 was used to analyze the mean and standard deviations of the microbial counts and the % weight loss of the LDPEs. The percentage occurrence was also calculated. The data were summarized using descriptive statistics for tabulation. Analysis of Variance (ANOVA) was used to test for significant difference while Duncan was used to separate means. The significant level used was 95% confidence interval (*P≤0.05*) (Akintoku et al., 2017).

3. RESULTS AND DISCUSSION

Plastics (such as polythene and polypropylene) are not easily degraded. Some these plastics are disposed in waste dumps that remain dormant in their initial state. Biodegradable plastics have been seen as alternatives to non-biodegradable plastics because biodegradable plastics can easily be degraded by microbes. Microorganisms play an important role in the biological decomposition of materials, including synthetic polymers in the natural environment [5].

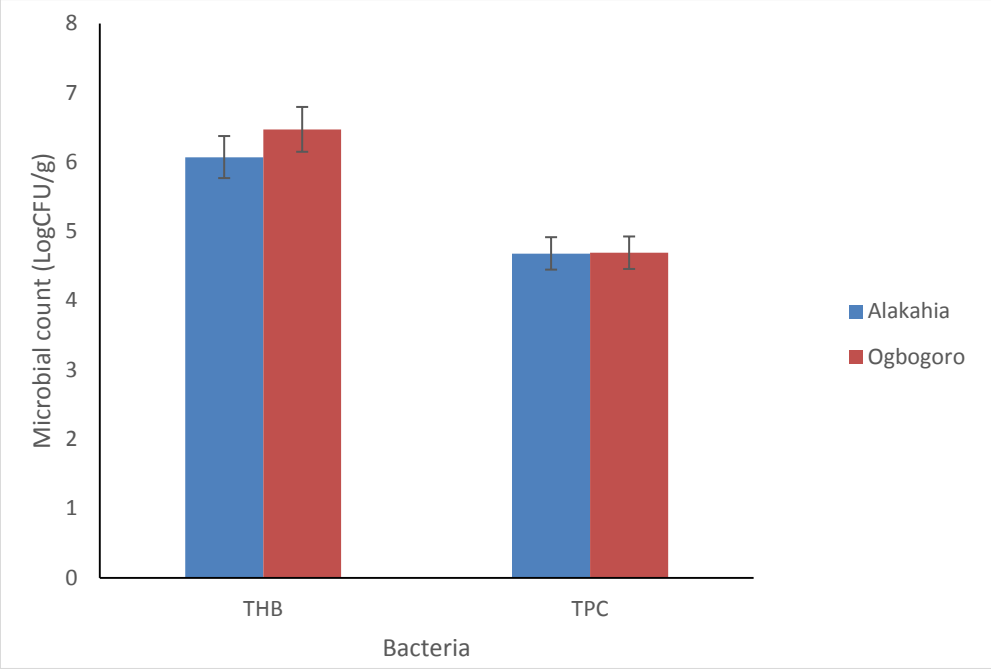

Fig. 3. Mean bacteria counts of dump sites (Log_{10}CFU/g)

The total Heterotrophic Bacterial (THB) Count of the soil samples collected from the two different locations were 1.2×10^6 CFU/g (*Alakahia dumpsite*) and 3.0×10^6CFU/g (*Ogbogoro dumpsite*). There was no significant difference ($P \leq 0.05$) for the Mean bacterial counts. The THB count recorded in this study is lower than that the total heterotrophic bacterial count recorded in the study of Williams and Hakam [6] which reported total heterotrophic count of 2.42×10^7 CFU/g to 2.9×10^8 CFU/g from some dumpsites in Rivers state. However, it is similar to the count recorded by the study of Oshoma et al. [7] in which a heterotrophic bacteria count was recorded as $4.1\pm0.3\times10^4$CFU/g to $1.02\times10^7\pm0.4$ CFU/g in a study of microbial analysis of top soil from municipal dumpsites in Benin City.

The total Count of *Pseudomonas* obtained from the soil sample collected from the two dump sites studied were recorded as 4.8×10^4 CFU/g (*Alakahia dumpsite*) and 5.0×10^4 CFU/g (*Ogbogoro dumpsite*) as shown in Fig. 3. There was no significant difference ($P \leq 0.05$) for the Mean *Pseudomonas* count in both dump sites. Several studies of microbial determination of dumpsites in Nigeria have reported *Pseudomonas* with higher frequency of occurrence (Oshoma et al*.,* 2015).

The bacteria isolated from the soil collected from the dumpsites were *Bacillus* sp, *Klebsiella* sp, *Pseudomonas* sp, *Staphylococcus* sp, *Serratia* sp, *Escherichia coli*, *Shigella* sp and *Salmonella* sp. These bacteria are similar to those reported by Williams and Hakam [6], Oshoma et al. (2015) in the microbial analysis of dumpsites. Bacteria reported in this study have been reported as potential pathogens and these microorganisms in the dumpsites can be attributed to the disposal of different waste including fecal waste in the environment [8]. These microorganisms use waste constituents as nutrients hence their growth [9]. These microbes also produce enzymes such as DNase, Hyluronidase, staphylokinase, staphylolysin, streptokinsase among others that help degrade waste materials at dump sites [6]. Exoenzymes from microorganisms first breakdown the complex polymers giving short chains or monomers that are small enough to permeate through the cell walls to be utilized as carbon and source of energy through a process known as polymerization [10,11].

Following the screening for the potential to degrade polyethene, *Pseudomonas proteolytica* NR025588.1, *Pseudomonas aeruginosa* CP0296051, *Bacillus circulans* CP026031.1 showed higher potential for polyethylene degradation by weight loss (as shown in Table 1) and they were used in further degradation study of low density polyethylene. *Pseudomonas* sp, *Bacillus* sp, *Aspergillus* sp, and *Rhodotorula* sp has been reported by Merina and Santosh [12] for their ability to undergo polymerization process of low density polyethylene. Studies have also

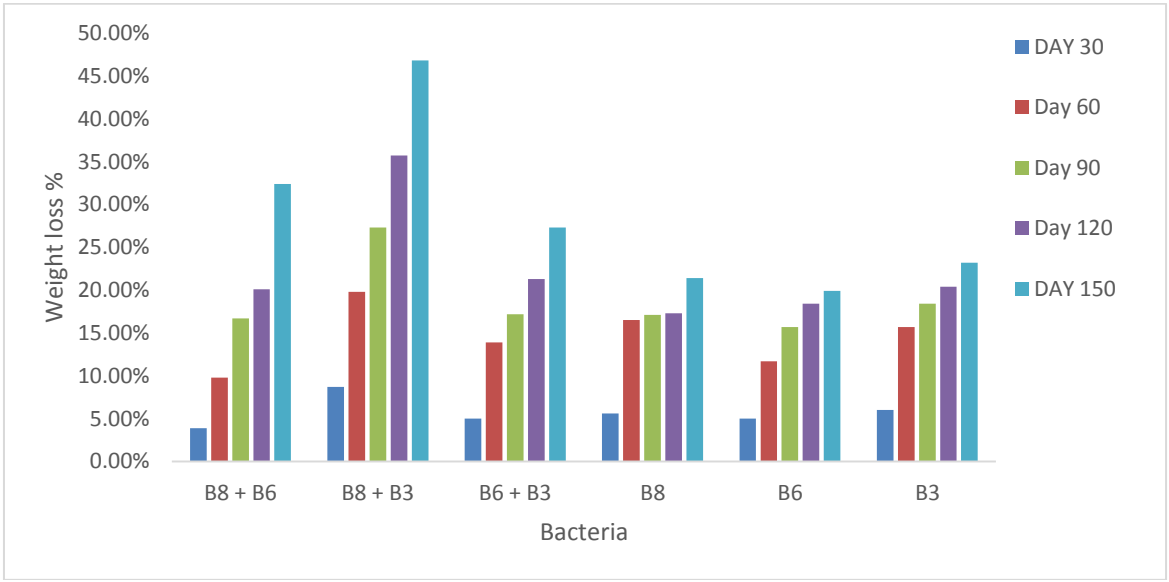

Fig. 4. Bacterial degradation of polyethylene bead

Key: B8 = *Bacillus circulans,* ***B3 =*** *Pseudomonas aeruginosa* ***B6 =*** *Pseudomonas proteolytica*

Table 1. Screening of bacterial Isolates for their capacity to degrade polyethylene bead using the weighing method

Description	Initial weight(g)	Final weight (g)	Weight loss	Weight loss (%)
Bacillus sp	0.3	0.2832	0.0168	5.6
Klebsiella sp	0.3	0.298	0.002	0.6
Pseudomonas sp	0.3	0.2851	0.0149	5.0
Staphylococcus sp	0.3	0.292	0.008	2.6
Serratia sp	0.3	0.298	0.002	0.6
Escherichia coli	0.3	0.293	0.007	2.3
Shigella sp	0.3	0.293	0.007	2.3
Salmonella sp	0.3	0.295	0.005	1.6
Pseudomonas sp	0.3	0.2822	0.0178	6.0

shown that *Pseudomonas aeruginosa* releases alkane hydroxylases, which showed 30% LDPE degradation. The depolymerases enzymes, which are extracellular help in cleaving the complex synthetic polymers into monomers and dimers hence, the microbes utilize these short-structured oligomers as source of carbon. *Bacillus* is also known to secrete enzymes that catalyzes the proteolysis of low-density polyethylene [13].

A simple and quick way to measure the biodegradation of polymers such as polyethylene is by determining the weight loss. Microorganisms that grow within the polymer lead to an increase in weight due to accumulation while the loss of polymer integrity leads to weight loss [14-16]. Degradation is initiated at the surface of the polymer hence; weight loss is proportional to the surface of the polymer (Dudu et al., 2015). From the result of the degradative ability by the individual microorganism (Fig. 4) using weight loss measurement, the bacteria *Pseudomonas aeruginosa* CP0296051 showed a greater percentage of degradation of polyethylene with percentage of 5% to 27.03% (from month1 to month 5) followed by *Pseudomonas proteolytica* NR025588.1which recorded percentage degradation of 6.0% to 23.28% (from month 1 to month 5) while the bacteria*, Bacillus circulans* CP02603.1 recorded the least percentage degradation of polyethylene of 5% to 19.9% (from month 1 to month 5). The higher potential of *pseudomonas* degrade polyethylene can be attributed to their ability to produce biosurfactant (Duddu et al., 2015) and their alkane hydroxylases enzyme production which helps to break the polymer to monomers used by the organism [13]. Biosurfactant is reported to be one of the mechanisms of degradation of low density polyethylene (Duddu et al., 2015).

The result of degradative potential of the microbes in consortium set ups showed that the consortium of the bacteria, *Pseudomonas aeruginosa* CP0296051 and *Bacillus circulans* CP02603.1 (B8+B6) showed the highest degradation potential producing degradation percentage range of 8.7 to 46.80% from month1 to month 5 of the study and the least degradation potential of polyethylene was observed in *Pseudomonas aeruginosa* CP0296051 and *Pseudomonas proteolytica* NRO255SS.1 (B6+B3) with the percentage range of 5% to 27.03% from month 1 to month 5. In comparison with the individual degradation potential, it can be deduced from this study that the consortium of microorganisms especially the consortium *Bacillus* and *Pseudomonas* result in higher degradation of polyethylene. According to the study of Robert et al*.* [17], the consortium of five strains containing *Pseudomonas* and *Bacillus* species grew synergistically in the presence of polyethylene terephthalate and the cleavage product bis (2-hydroxy ethyl) terephthalic acid (BHET) as sole source of carbon. This is also in line with the study of Ogunbayo et al*.,* [18] in which consortium of microorganisms was reported to have higher degradation efficacy. The higher degradation by consortium of these organisms can be attributed to the synergistic activity of these microorganisms in degradation of polyethylene. In the study of Meyer-ciruentes et al. [19], it was understood that the biodegradation of plastic or polyethylene is achieved synergistically by labor division among specialized microorganisms.

4. CONCLUSION

This study revealed that some bacterial isolates with the ability to degrade low density polyethylene can be isolated from dumpsites as shown in thus study. From the study,

Pseudomonas sp and *Bacillus* sp showed the potential to degrade low density polyethylene (LDPE). The study also showed a higher degradation potential in a consortium, *Bacillus* and *Pseudomonas* compared to others which can be related to their synergistic activities, hence their prospect in remediation of polyethylene.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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