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ORIGINAL RESEARCH

# Biodegradation of Polyethylene Terephthalate (Pet Bottle) by Bacteria Isolated from Elepe Dumpsite at Ikorodu, Lagos, Nigeria

**Isiaka Adio Hassan**

*Department of Biological Science (Environmental biology Unit), Lagos State University of Science and Technology, Ikorodu, Lagos State, Nigeria.*

***Correspondence***
*Department of Biological Science (Environmental biology Unit), Lagos State University of Science and Technology, Ikorodu, Lagos State, Nigeria. PMB, 21606*

*Email: hassan.ia@lasustech.edu.ng or hisiaka2017@gmail.com*

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**Abstract**:
**Introduction**: The ever-increasing usage of plastic [Poly Ethylene Terephthalate (PET bottle)] in our day-to-day life is inevitable; hence need to find remedy to inundated waste generated from its usage.
**Aims**: The aim of this study is to biodegrade the pol-yethylene terephthalate (PET bottle) by bacteria iso-lated from contaminated soil with PET bottles.
**Materials and Methods**: Soil sample contaminated with the PET bottle was collected from Elepe dumpsite, Ikorodu, Lagos State. Five different bacte-ria (Pseudomonas alcaligenes, Pseudomonas putida, Pseudomonas fluorescence, Pseudomonas cepacia and Bacillus firmus were isolated from the soil using a spread plate method and were identified using colonial and cellular morphology, and biochemical characteri-sation. However the isolated and identified organisms were inoculated into the new sterile PET bottle in nu-trient broth and incubated for 10, 20, 30 days.
**Results**: The result of the degradation of the PET bottle in 10, 20, and 30 days by pseudomonas alcali-genes (11.43, 17.14 and 27.14 %); Pseudomonas putida (8.57, 14.28, and 22.86 %); Pseudomonas flu-orescence (9, 10.5, and 12.8 %); Pseudomonas ce-pacia (0, 0 and 0 %) and Bacillus firmus (0, 4.5, and 10.50 %) respectively. The Pseudomonas alcaligenes was found to be more effective than the rest four de-grading bacteria.
**Conclusion**: It can be concluded that Pseudomonas alcaligenes, Pseudomonas putida, Pseudomonas fluo-rescence, and Bacillus firmus can degrade PET bottle within 30 days, with the exception of Pseudomonas cepacia

**Keywords**: biodegradation, polyethylene tereph-thalate, bacteria, Elepe - dumpsite

All co-authors agreed to have their names listed as authors.

## 1. INTRODUCTION

The plastic production was started in the year 1907. The first synthetic plastic produced then was Bakelite [1]. Hence, started the beginning of the plastic industry and from then till now these industries are waxing stronger trying to meet the demand of the teeming population of the world. Now they are becoming a threat to our existence. According to Ritchie and Roser [1] they reported that the production of plastic rose to 2 million tonnes annually in 1950 and however, as at 2015, the production had increased to 381 million tonnes annually.

Plastic is a synthetic polymer. It consists of carbon, hydrogen, silicon, oxygen, chloride and nitrogen. It is derived from different sources of petrochemicals such as oil and natural gas. Plastics are extensively used because of their stability and durability. They are good sources of packaging material nowadays. They are very light in weight, durable, corrosion resistant and have high thermal and electrical insulation properties. There are different types of plastic, namely polyethylene (PE), Poly Ethylene Terephthalate (PET), nylons, Poly-Propylene (PP), Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyurethane (PUR) [2]. However there is no efficient method for the plastic disposal; hence, they often end up accumulating in the environment like water bodies and land, thereby posing an ecological threat to flora and fauna especially in water bodies [3].

Plastic can be degraded by a variety of mechanisms such as chemical, thermal, photo-oxidation and biodegradation, all of which take an extremely long time depending on the molecular weight of the polymer. It could take up to 1000 years for some types of plastic to degrade [4]. However, there is a need to develop methods of degrading these plastics in a very short time possible so as to clean the environment from their mess. Recycling of plastics is not always economically possible, so it becomes necessary to study the various methods of biodegradation of plastics. Plastic waste might eventually end up in composts along with other biodegradable waste. Studying the biodegradation of plastic in mature composts will help in understanding the eventual fate of such plastic waste [5]. Microorganisms can also play a vital role in this process, as over 90 genera of bacteria, fungi and actinomycetes have the ability to degrade plastic [6]. Generally, the biodegradation of plastic by microorganisms is a very slow process, and some microorganisms can't degrade certain plastics [7].

Biodegradation has been proven to be eco-friendly because of its non-toxic end products like $CO_2$, $H_2O$ and $CH_4$ [2]. In the previous studies an attempt had been made to use mixed microbial cultures or specific microorganisms (e.g. bacteria and fungi isolated from the natural environment) to degrade synthetic plastics into low molecular weight organics and subsequently mineralize into $CO_2$ [8; 9]. Many experiments proved that Pseudomonas putida has the ability to biodegrade many plastic materials such as; plastic bags, polythene bags, plastic cups, and milk covers; with milk cover being the most degradable substance [10]. Evidence also showed that P. putida cells of strain IRN22 were capable of fragmenting and biodegrading LDPE (powdered low-density polyethylene plastic) into polyhydroxyalkanoate polymers [11]. Although many studies have proven the effect of P. putida in degrading polyethylene plastic, but none has shown that it possesses PET degrading characteristics.

[12] found four isolates that degrade polyethylene film. Among them Streptomyces species degraded the highest amount of polythene films. [13] reported that the plastic materials in mangrove soil are rich in total heterotrophic bacterial counts (79.67 x 104) and the plastic materials have been colonized commonly by five species of bacteria (Pseudomonas species, Staphylococcus species, Moraxella species, Micrococcus species and Streptococcus species).

Furthermore, PET bottle hydrolyzing enzymes (PET hydrolases) have relatively low turnover rates and appear to be limited to a few bacterial phyla. Out of which most members belong to Gram-positive phylum Actinobacteria [14] and genera *Thermobifida* or *Thermomonospora* [15;16). Hence, there is a need to search for alternative bacteria that have high turnover rate of the PET hydrolase to degrade the pet bottle within a few days with higher percentage; so as to clear the large backlog of PET bottle waste in our society. Thus, a search for the bacteria capable of degrading the PET bottle can be the beginning of finding a solution to the problem of PET bottle accumulation in the environment. This study was undertaken at the Ewu-Elepe dumpsite to evaluate biodegradation of polyethylene terephthalate (PET bottle) by bacteria isolated from the dump site soil.

## 2. MATERIAL AND METHODS

### Study Location

Lagos state is operating four recognised (legalised) dumpsites namely, Olusosun at Ojota, Ewu - Elepe at Ginti Elepe, Epe at Epe and Igando at Alimosho. Ewu - Elepe dumpsite is situated at Ewu Elepe area in Ginti Estate, Ewu-Elepe, off Ijede road, Ikorodu Local Government in Lagos State, Nigeria. This dumpsite is where all forms of waste; be it medical or general waste are dumped. The site is surrounded by human settlement; though at onset it was a thick bush but as the time goes on; human settlement expands to meet the dumpsite. It lies between Latitudes 06° 35′ and 06° 36′ N and Longitude 003° 34′ and 003° 35′ E. It is almost eight hectares of land and has been in operation for almost fourteen years. The dumpsite is a swampy valley and refuse were dumped daily into the furrow of the valley, which has been filled to the brim of the valley. An average of 42, 326.41 M3 of wastes per month are dumped on this site [17].

### Sample Collection

Two samples of polyethylene terephthalate (PET bottle) were used for this experiment; one from the polyethylene terephthalate (PET bottle) contaminated soils

from the Elepe dumpsite. The other polyethylene terephthalate (PET bottle) was bought from a shop in Ikorodu as control. Thereafter, both samples were taken to the laboratory in Nigeria Institute of Medical Research (NIMR), Yaba, Lagos State.

## Isolation of Soil Bacteria

A 1g of pet bottle from the dumpsite was soaked in 100 mL of Phosphate-Buffered Saline (PBS) in a conical flask and shook vigorously so as to detach already clinched bacteria on the pet bottle. A 1 ml of inoculum from 100 mL pet bottle soaked in PBS was introduced with syringe into the 5 test tubes already contained 9 mL (PBS) each, by serial dilution and from the last test tube of 5, 1 mL of inoculum was taken and introduced into already prepared nutrient agar and spread by using a hockey stick, and incubated at 37℃ for 24 hours.

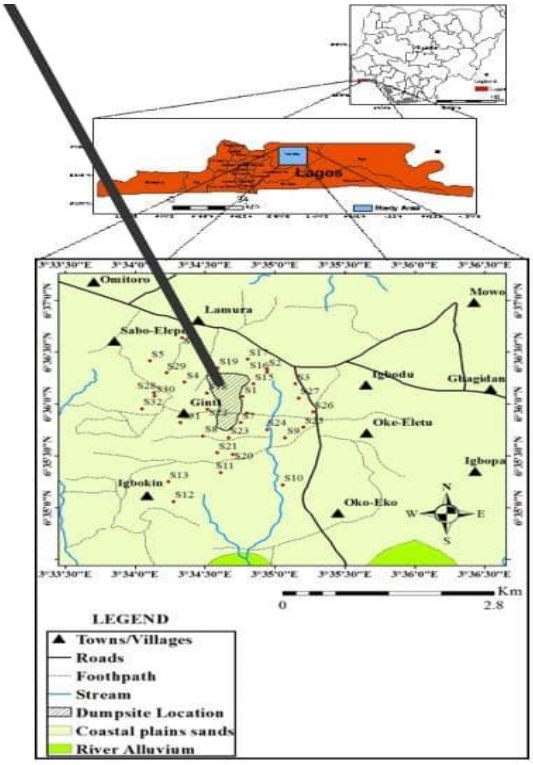


Figure1 shows the map of location of the Ewu – Elepe dumpsite
**Source**: [18]

## Identification of Soil Bacteria

The isolated bacteria were identified using colonial morphology (texture, appearance, elevation and colors); cellular morphology (shape, size and structure) were studied through microscope; and biochemical characterization (gram reaction, motility, catalase, oxidase, citrate, methyl red, Voges Proskauer, nitrate, indole, urease, glucose, xylose and lactose) were carried out using standard protocols.

## Biochemical Test

**Gram staining:** A clean grease free slide was used and a smear of the isolated bacterial culture was made on it with a sterile loop. The smear was air-dried and then heat fixed. Then it was subjected to the following staining reagents: The smeared glass slide was flooded with crystal violet for 1 minute, followed by washing with running distilled water. Again, flooded with Gram's Iodine for 1 minute, followed by washing with running distilled water. Then the slide was flooded with Gram's decolourizer for 30 seconds. After that the slide was counter stained with Safranin for 30 seconds, followed by washing with running distilled water. The slide was air dried and cell morphology was observed under oil immersion microscope. Purple colour indicates positive while the red colour indicated negative.

## Catalase test

A small quantity of 24 hours old culture was transferred into a drop of 3 % hydrogen peroxide solution on a clean slide with the aid of sterile inoculating loop. Gas seen as white froth indicates the presence of catalyst enzymes [19].

## Oxidase test

A piece of filter paper was soaked with drops of oxidase reagent. Sterile inoculating loop was used to pick a colony of the test organism and smeared on the filter paper. If the organism is an oxidizer; the phenylenediamine in the reagent will be oxidized to a deep purple colour [19].

## Urease test

A small quantity of 24 hours old culture was transferred and inoculated into a urease agar and incubated at 30℃ for 48 hours, the development of a red-pink colour indicates a positive result. [20].
Glucose test
This was carried out as described by [21], in order to test the ability of micro-organism to metabolize a large variety of sugar as carbon source. The medium used contains peptone 1.0 % fermentable sugar, 1.0 mL of phenol red indicator was added and 9 mL of the preparation was dispensed into a different test tube carefully, avoiding air bubbles. The tubes were sterilized at 121 o C for 15 minutes, and allowed to cool. A loopful of the each test organism was inoculated and incubated at 37 o C for 24 hours. The tubes were examined daily for colour change and the Durham tubes were examined for display of gas. Yellow colour (acid formation) indicates (positive) result. Red colour indicates negative result. The gas produced was accumulated in the inverted Durham tube, which easily ignites a glowing splinter.

### Indole test

The indole production was carried out as follows; One gram of peptone stuck sample was weighed and dissolved into 200 mL of distilled and sterilized water. It was shared into 10 test tubes and a loopful of organism was inoculated into each test tube and these tubes were incubated at 35℃ for 24 hours. The tubes were re-

moved from the incubator and 10 drops of Kovacs reagent were added to each tube and shaken gently. A deep red colour indicates indole positivity. A negative reaction remains colourless or light yellow.

### Citrate test

Agar plate (TSBA, Himedia) surface with bacteria culture incubated at 37 o C up to 48 hours. Changing the media colour from green to bright blue is a positive reaction.

### Motility test

A sterile needle was used to pick a loop of 24 hours old culture and was stabbed into nutrient agar in a glass vial. The vial was incubated at 37 ˚C for 24 - 48 hours. Non-motile bacterial growth confined to the stab line with definite margins without spreading to the surrounding area, while motile bacteria had diffused growth extending from the surface [22]. Positive motility test was indicated by a red turbid area, extending from the line of inoculation. A negative test was indicated by red growth along the inoculation line [20].

### Methyl red (MR)

An inoculum of the test organism was inoculated into methyl red broth and then incubated at 37°C for 48 hours after which a few drops of methyl red were added to the incubated medium. A red coloration indicates a positive test [23].

#### Voges Proskauer (VP)

The VP test was carried out using part of the medium for MR test, after which three drops of 6 % alpha napthol was added and followed by 0.5 mL of 40 % KOH. It was then agitated and allowed to stand for 30 minutes. A red colour indicates positive reaction [23].

#### Lactose test

An inoculum from a pure culture was transferred aseptically to a sterile tube of phenol red lactose broth. The inoculated tube is incubated at 35 – 37℃ for 34 hours and the results are determined. A positive test consists of a colour change from red to yellow indicating a pH change to acidic.

#### Mannitol

An inoculum from a pure culture was transferred aseptically to a sterile tube of phenol red of Mannitol broth. The inoculated tube was incubated at 35 - 37 o C for 34 hours and the results were determined; a positive test consists of a colour change from red to yellow; indicates a pH change to acidic

#### Bacterial Identification

Identification of bacteria isolates were done after colonial, cellular morphology and biochemical characterisations by checking the Bergey's Manual of Systematic Bacteriology for nomenclature.

### Surface Sterilization of Polyethylene Terephthalate (Pet Bottle)

The collected pet bottle from dumpsite were cut into small pieces and washed in the tap water. Thereafter, were sterilized with ethanol (70 %) and washed with distilled water, 0.1 % mercuric chloride and again washed with distilled water.

### Degradation of Polyethylene Terephthalate (Pet Bottle)

Nutrient broth was prepared following manufacturer's prescription and autoclaved at 121 ˚C for 15 minutes. A 200 mL of cooled nutrient broth was poured into sixteen different 250 mL sterile conical flasks each. The sterile pre-weighed pet bottle pieces (1 g) were aseptically transferred into sixteen different nutrient broths each. A loopful of each of five isolates *(Pseudomonas alcaligenes, Pseudomonas putida, Pseudomonas fluorescence, Pseudomonas cepacia and Bacillus firmus)* identified from dumpsite soil (pet bottle) was inoculated into each set of three 250 mL flask containing pet bottle pieces (1 g) and nutrient broth. One 250 mL flask containing pet bottle piece (1 g) and nutrient broth without bacterial isolates from dumpsite soil was maintained as control. These flasks were incubated at 37 ˚C for 10, 20 and 30 days. The pet bottle pieces were carefully removed from the culture by using forceps after different days of incubation. The collected pet bottle pieces were washed thoroughly in tap water, 70 % ethanol and then distilled water. The pieces were shade dried in the laboratory and later weighed for final weight. The data were recorded.

#### Determination of Degradation of Pet Bottle

The percentage of degradation of pet bottle pieces by different bacteria isolates were determined by calculating the percentage of weight loss of plastics. The percentage of weight loss was calculated by the following formula:

Percentage of weight loss =

$$\frac{initial\ weight\ -\ final\ weight}{initial\ weight} \times 100$$

## 3. RESULTS AND DISCUSSION

**Table 1. Colonial and Cellular Morphological Characterization**

| **Bacterial isolate** | **Shape** | **Size** (mm) | **Structure** | **Texture** | **Appearance** | **Elevation** |
|---|---|---|---|---|---|---|
| First (1) | Rod | 2.6 | Convex | Moist | light yellow | Straight |
| Second (2) | Rod | 4 | Convex | Moist | Brown | Flat |
| Third (3) | Rod | 2 | Circular | Moist | White | Flat |
| Fourth (4) | Rod | 3 | Convex | Moist | White | Flat |
| Fifth (5) | Rod | 2.5 | Circular | Moist | White | Flat |

**Table 2: Biochemical Characterisation**

| Bacterial isolate | Gram staining | Oxidase | Catalase | Citrate | Motility | Indole | Urease | Methyl Red | Voges Proskauer | Glucose | Mannitol | Lactose | Bacteria identified |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| First | -ve Rod | +ve | +ve | +ve | -ve | -ve | +ve | -ve | -ve | -ve | +ve | -ve | *Pseudomonas alcaligenes* |
| Second | -ve Rod | +ve | +ve | +ve | +ve | -ve | -ve | -ve | -ve | +ve | -ve | -ve | *Pseudomonas putida* |
| Third | +ve Rod | +ve | +ve | +ve | +ve | -ve | +ve | -ve | -ve | +ve | +ve | -ve | *Pseudomonas fluorescence* |
| Fourth | -ve Rod | +ve | +ve | +ve | +ve | -ve | +ve | -ve | -ve | +ve | -ve | -ve | *Pseudomonas cepacia* |
| Fifth | +ve Rod | -ve | +ve | -ve | +ve | -ve | -ve | -ve | -ve | +ve | -ve | +ve | *Bacillus firmus* |

**Table 3: Biodegradation of Polyethylene Terephthalate (PET BOTTLE)**

| Bacteria Identified | Days of treatment | Initial weight of polyethylene terephthalate (g) | Final weight of polyethylene terephthalate (g) | % of weight loss |
|---|---|---|---|---|
| | 10 | 1 | 0.89 | 11.43 |
| *Pseudomonas alcaligenes* | 20 | 1 | 0.83 | 17.14 |
| | 30 | 1 | 0.73 | 27.14 |
| | 10 | 1 | 0.91 | 8.57 |
| *Pseudomonas putida* | 20 | 1 | 0.86 | 14.28 |
| | 30 | 1 | 0.77 | 22.86 |
| | 10 | 1 | 0.91 | 9.00 |
| *Pseudomonas fluorescence* | 20 | 1 | 0.9 | 10.50 |
| | 30 | 1 | 0.87 | 12.80 |
| | 10 | 1 | 1 | 0 |
| *Pseudomonas cepacia* | 20 | 1 | 1 | 0 |
| | 30 | 1 | 1 | 0 |
| | 10 | 1 | 1 | 0 |
| *Bacillus firmus* | 20 | 1 | 0.96 | 4.50 |
| | 30 | 1 | 0.9 | 10.50 |

## DISCUSSION

In this study, five bacteria were isolated from plastic (PET bottle) containing soil and identified by their morphological and biochemical characterizations. Table 1 illustrates the colonial and cellular morphological characterization of the degrading bacteria 1, 2, 3, 4 and 5. The degrading bacteria culture showed variation in their morphological characterisations. Table 2 shows biochemical characterization of bacteria isolates with *Pseudomonas alcaligenes, Pseudomonas putida, Pseudomonas fluorescence, Pseudomonas cepacia and Bacillus firmus* identified as bacteria 1, 2, 3, 4 and 5 respectively. The results were comparable to the earlier finding of Kathiresan (2003), who reported that the polythene associated soils were rich in bacteria species.

Table 3 shows that after each 10 days, the organisms were growing and the colour of the broth culture got turbid. *Pseudomonas alcaligenes* at day 10 of treatment had an initial weight of 1.00 g and a final weight of 0.8950 g with 11.43 % of weight loss. At day 20 of treatment, with an initial weight of 1.00 g and a final weight of 0.83 g with 17.14 % of weight loss. At day 30 of treatment, with an initial weight of 1.00 g and a final weight of 0.73 g with 27.14 % of weight loss. It signifies that with an increase in incubation period (30 days) there is a dramatic increase in weight loss of Polyethylene terephthalate (Montazer et al., 2019).

At day 10 of treatment, *Pseudomonas putida* with an initial weight of 1.00 g has a final weight of 0.914 g with 8.57 % of weight loss. At day 20 of treatment, the initial weight of 1.00 g depreciated to a final weight of 0.857 g with 14.28 % of weight loss. At day 30 of treatment, the pet bottle of an initial weight of 1.00 g has a final weight of 0.77 g with 22.86 % weight loss (Saminathan et al., 2014).

*Pseudomonas flourescence* at day 10 of treatment depreciates the PET bottle (1g) from an initial weight to the final weight of 0.91g (9.00 % of weight loss). At day 20 of treatment, the pet bottle of an initial weight of 1.00 g reduced to a final weight of 0.900 g with 10.50 % of weight loss. However, at day 30 of treatment, it has an initial weight of 1.00 g and a final weight of 0.87 g and has 12.8 % weight loss. These results were compared to the result of Deepika and Madhuri (2015) during the study of biodegradation of polythene bags using bacteria isolated from soil.

Furthermore, in Table 3, polyethylene terephthalate was not degraded by *Pseudomonas cepacia*, which means it cannot break down the polymer chain of PET bottles after 30 days (Kyaw et al; 2012). Probably, if the

incubation period is increased to 60 days, *Pseudomonas cepacia* could degrade the pet bottle.
*Bacillus Firmus* at day 10 of treatment with a pet bottle had an initial weight of 1.00 g and a final weight of 1.00 g with 0 % weight loss. At day 20 of treatment, the pet bottle had an initial weight of 1.00 g and a final weight of 0.96 g, with 4.5 % weight loss. At day 30 of treatment, the pet bottle has an initial weight of 1.00 g and a final weight of 0.90 g with 10.5 % of weight loss. These results were compared to the findings of Deepika and Madhuri (2015) during the study of biodegradation of polythene bags using bacteria isolated from soil.

## 4. CONCLUSION

This study investigated the biodegradation of pet bottles by bacteria isolated from Elepe dumpsite, Ikorodu, Lagos State. Biodegradation of plastic waste using bacteria is a valuable plastic waste treatment that should be implemented to maintain the environmental problem caused by plastic waste. The overall investigation can be concluded that *Pseudomonas alcaligenes* exhibited significant polyethylene terephthalate (PET) degradation ability and it is promising in degrading the pet bottle in the nearer future than any bacteria used in this study. However, this study revealed that Pseudomonas alcaligenes were found to be more efficient than Pseudomonas putida for bioremediation of plastic material. In addition, *Bacillus firmus* did not show any depreciation on pet bottle after 10 days but at the end of the 30 days appreciable degradation was observed. The result of the study on the Pseudomonas cepacia revealed that there was no weight loss after 30days of incubation on pet bottle.

## COMPETING INTERESTS

No competing interest with any organization or anybody.

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