Advances in Environmental Technology 1 (2022) 59-71

Iranian Reasearch Organization for Science and Technology (IROST)

Advances in Environmental Technology

Journal home page: https://aet.irost.ir/

Isolation and identification of plastic degrading bacteria from dumpsites Lagos

Hilda. A. Emmanuel-Akerele*[1], Priscilla O. Akinyemi[1], Oritsematosan E. Igbogbo-Ekpunobi[2]

[1]*Department of Biological Sciences, Anchor University Lagos, Nigeria.*

[2]*Department of Microbiology, University of Benin, Nigeria.*

ARTICLE INFO

Document Type:
Research Paper

Article history:
Received 30 November 2021
Received in revised form
2 March 2022
Accepted 7 March 2022

Keywords:
Biodegradation
Microorganisms
Pollution
Polyethylene
Polystyrene

ABSTRACT

Plastic pollution is a threat to the environment because of its slow degradation rate and high usage. The continuous accumulation of these synthetic plastic wastes poses an ever-increasing threat to animals, humans, and the environment. The use of microorganisms to effectively degrade plastic waste can provide a solution to this problem. This study aims to isolate plastic degrading microorganisms from soils taken from the Alimosho local government area of Lagos State, Nigeria. The soil samples were collected from dumpsites filled with plastic and plastic materials. The effectiveness of the degradation of plastic materials was studied over six (6) weeks in broth and agar culture under laboratory conditions by the weight determination method. Physicochemical and microbiological analysis was carried out on the various soil samples using standard protocols. The biodegradation of polyethylene and polystyrene was done in-vitro using the microorganisms isolated from the soil. The following microorganisms were able to degrade a higher percentage of the plastic materials; *Staphylococcus aureus, Streptococcus sp, Bacillus sp,* and *Escherichia coli*. The total viable count for bacteria was within the range of 11.8×10^5 to 2.0×10^{10}CFU/g. *Staphylococcus aureus, Streptococcus sp, Bacillus sp,* and *Micrococcus sp* degraded plastic up to 25%, 31.2%, 25%, and 31.2%, respectively. These isolates may be used to actively degrade plastics, thereby reducing the rate of plastic pollution in our ecosystem.

1. Introduction

Plastics are non-biodegradable, strong, durable, moisture-resistant, lightweight polymers of carbon along with hydrogen, nitrogen, sulfur, and other organic and inorganic elements and are manufactured from fossil fuel, which is a non-renewable source [1]. They are a polymeric material that is synthetic or semi-synthetic and can be molded into any shape. Due to their versatility, durability, and lightweight, they are used in the packaging and production of different materials needed to make life easier. They have become substitutes for glass, metal, ivory, horn, silk, cotton, and natural rubber [2]. Basic materials used to produce plastics are derived from coal,

*Corresponding author: +2347031612082
E-mail: akatahhilda@gmail.com
DOI: 10.22104/AET.2022.5268.1428

natural gas, and oil [1]. They can be called "ubiquitous" because they can be found almost everywhere (construction companies, industries, corporate offices, our homes, schools, market, etc.). Due to the daily production and use of plastics, the disposal of used plastics is not done efficiently, thereby polluting the environment. Therefore, to prevent polythene accumulation, an adequate disposal system should be adopted [3]. Nonetheless, most synthetic plastics such as polyethylene, polypropylene, polystyrene, polyvinyl chloride, and polyethylene terephthalate are non-biodegradable, and their expanding collection in the climate poses a danger to the planet [2]. A few stages have been embraced to ameliorate each of these issues. The principal procedure includes the production of plastics with a high level of degradability. Plastic pollution occurs as a result of the wide variety of plastic products produced and used by consumers. Plastic pollution is the build-up or accumulation of plastic and plastic products in our environment, which is detrimental to the ecosystem [4]. Tons of plastics are being discarded into our environment by natural events and human actions, harming the health and survival of a population [5]. Plastic accumulation can cause harm to the land, streams, and seas. It is assessed that 1.1 to 8.8 million tons of plastic waste enter the sea from waterfront networks (coastal bodies and oceans) each year [2]. It is estimated that as of 2013, 86 million tons of plastic marine garbage has accumulated in the sea, with a presumption that 1.4% of worldwide plastics delivered from 1950 to 2013 has entered the sea and has amassed there [5]. Biodegradation is a process whereby microorganisms break down organic substances into smaller compounds through metabolic or enzymatic processes [2]. Organic substances that can be biodegraded include polystyrene, polyethylene, polyvinylchloride, and other plastic products. Plastic biodegradation is an environmentally friendly method to degrade polyethylene as it does not generate any harmful by-products [6]. Through the process of degradation, hazardous toxic materials are made less toxic or non-toxic [5]. Microorganisms break down these materials into a smaller form for them to feed on. This serves as nutrients, carbon, and an energy source. When microorganisms degrade plastics through enzymatic actions, they cause cleavage of the polymer chains into monomers and oligomers, which are now further absorbed by the microbial cells to be metabolized [5]. The problem of plastic degradation has made plastic a major focus in solid waste management. The accumulation of these plastics in the environment is causing great damage to our ecosystem. Aquatic organisms are being suffocated due to excess plastic in the water bodies. Low-density polyethylene (LDPE) is one of the major sources of environmental pollution. Polyethylene is a polymer made of long-chain monomers of ethylene. The worldwide utility of polyethylene is expanding at a rate of 12% per annum, and approximately 140 million tonnes of synthetic polymers are produced worldwide each year [7]. With such a huge accumulation of polyethylene in the environment, their disposal evokes a big ecological issue [5]. There is an urgent need to find a solution to this problem. This study aims to isolate plastic degrading microorganisms from soils collected from several dumpsites and landfills in the Alimosho local government area of Lagos State.

2. Materials and methods

2.1. Study area

Alimosho is a local government area in the Ikeja Division, Lagos State, Nigeria. It is the largest local government in Lagos, with 1,288,714 inhabitants. It is home to a good number of the working population in Lagos who live on the mainland and work on the Island. Soil samples were collected from different dumpsites and landfills in Igando. The Soluos community is situated in the Ikotun/Igando local council development area of the Alimosho local government in Lagos State, Nigeria. Three dumpsites are located in the Soluos community known as Soluos 1 (Closed), Soluos 2, and Soluos 3 (open), as shown in Figure 1. Soluos 1 covers about 7.8 hectares of land and is located on (N06° 34. 307′, E003° 15. 211′), Soluos 2 covers approximately 3.2 hectares and is located on (N06° 34. 286′, E003° 15. 146) of land, and Soluos 3 covers about 5 hectares and is located on (N06° 33. 897′, E003° 15. 082′) of land [8]. The Soluos landfills are located at the extreme east-west area of metropolitan Lagos in Alimosho local government. They are among the five active landfills currently

operated by Lagos Waste Management Authority (LAWMA) [8]. It is reported that Soluos 2 and 3 landfills received 81,388 metric tonnes of MSW out of a total of 239,282 metric tonnes landfilled in December 2011 [8]. They receive waste from the entire Lagos metropolis; the wastes are of different types, including domestic, commercial, and industrial sources.

Fig.1. A map of the landfill. Source: [8]

2.2. Sample collection

A total of 16 samples were collected between June and August 2021 from three different dumpsites located at Igando, Alimosho LGA. The soil samples were specifically collected from areas populated with plastic bottles and nylon. The samples were collected using a soil auger at a depth of 10cm, transferred into a well-labelled sample collection tube, and transported to Anchor University's microbiology laboratory for analysis.

2.3. Preparation of media

All the media (nutrient agar (HiMedia, India) and mineral salt medium (HiMedia, India) were prepared according to the manufacturer's instructions. Serial dilution was carried out on the soil samples using 1g of each soil, and the bacteria isolation technique was done using the pour plate method [9].

2.4. Physico-chemical Analysis

The physico-chemical analyses were carried out on the soil samples to determine the level of acidity/ alkalinity of the soil, organic matter and moisture content, total dissolved solids, and conductivity.

2.4.1. pH

To check the pH, the electrode (BANTE-510, China) of the pH meter was calibrated with standard buffer solutions with known pH values. 20g of the soil sample was diluted into 100ml of distilled water and stirred for 10 min. Then, the pH value was recorded [10].

2.4.2. Organic matter

The process of checking the organic matter was done according to [11]. Sixteen crucibles were dried in the oven at 105 °C for 24 h and cooled in the desiccators. After cooling, the weights of the crucible were taken separately, and 1g of each soil sample was added to the different crucibles. The samples were heated on a furnace for 30 min at 115

°C with occasional stirring. They were transferred into desiccators to cool down, and the weight was recorded.

2.4.3. Moisture content

Soil moisture content is the available water present in the soil that is necessary for plant growth and the organism's survival. Sixteen crucibles were dried in the oven at 105°C for 24 h and heated until the constant weight was reached, after which their weights were taken separately. 1g of each soil sample was weighed into each crucible. The samples were dried in an oven at 105°C for 24 h. The crucibles were made to cool down in the desiccators, and the weight of each crucible was recorded [11].

2.4.4. Conductivity and total dissolved solids (TDS)

100g of the soil sample was diluted into 200ml of distilled water, stirred for 5 min, and allowed to sit for 30 min. The solution was filtered into another clean beaker and measured using the conductivity meter (BANTE-510, China). The conductivity and TDS values were recorded [10].

2.5. Microbiological analysis

The spread plate method was used to culture the organisms aseptically. Nutrient agar was used to culture the bacteria, while mineral salt medium was used to screen the isolated organism and determine the weight loss of the plastic material.

2.5.1. Characterization and Identification of isolates

The isolates were characterized and identified based on their colony appearance, cellular morphology, and biochemical reactions, as described in Bergey's Manual of Systemic Bacteriology [9].

2.5.2. Biochemical tests

2.5.2.1. Catalase test

This test indicates the presence of catalase. Catalase is an enzyme that catalyzes the release of oxygen from hydrogen peroxide. It differentiates those bacteria that produce the enzyme catalase from non-catalase-producing bacteria. The slide method was used to perform this test. Using an inoculating loop, a small amount of colony was transferred to a clean, grease free slide; then, a drop of 3% hydrogen peroxide was placed on the slide. The presence of active bubbles indicates a positive result.

2.5.2.2. Mannitol test

The Mannitol test was done to see if the microorganism was capable of fermenting sugar or not. This test involves the use of Mannitol Salt Agar (MSA) (HiMedia, India). MSA was prepared according to the manufacturer's instructions. An inoculating loop was used to pick and streak the bacteria isolate on a mannitol salt agar plate. The plates were placed in the incubator for 24 hrs at 37°C. A change in color from red to yellow indicates a positive result [12].

2.5.2.3. Triple sugar iron (TSI) test

This microbiological test was done to check the ability of bacteria to ferment sugars and to produce hydrogen sulphide. Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow [9]. The slanted TSI agar (HiMedia, India) was stabbed with the picked bacteria isolate at the center of the agar, ensuring it touched the bottom of the tube. The test tubes were then incubated at 35°-37°C for 18 to 24 h. An alkaline/acid (red slant/yellow butt) reaction indicates positive for dextrose fermentation, and an acid/acid (yellow slant/yellow butt) reaction indicates positive for the fermentation of dextrose, lactose, and/or sucrose. An alkaline/alkaline (red slant, red butt) reaction indicates negative for carbohydrate fermentation, a black colour indicates the presence of H_2S, and the presence or formation of bubbles or cracks in the agar signifies the production of gas, that is, the formation of CO_2 and H_2 [9].

2.5.2.4. Citrate utilization test

This test is used to detect if the organism can utilize citrate as a source of energy. Citrate agar (HiMedia, India) was used in performing the test. The medium contains citrate as the main source of carbon and inorganic ammonium salts as the main source of nitrogen [9]. An inoculating loop was used to pick the isolate from the center of a colony and streaked it on a slant. The slant was incubated at 37°C for up to 4-7 days. A change in color from green to blue indicates a positive result.

2.5.2.5. Coagulase test

The slide test method was performed with the use of human blood plasma. A drop of 0.9% saline water was placed on a clean, grease-free slide, and a loop full of the bacteria isolate was smeared with the saline water; then, a drop of the plasma was added on the slide. The slide was swirled and observed closely for any visible clumping within 10 sec [9].

2.5.2.6. Oxidase test

The filter paper method was used to carry out this test. Freshly prepared Kovac's reagent was used to soak the filter paper. An inoculating loop was used to take a small amount of the colony and smear it on the filter paper. An appearance of deep purple to blue colour indicates a positive result [7].

2.6. Screening of plastic degrading isolates

The isolated microorganisms were purified by subsequent sub-culturing to achieve a single population and thereafter screened to check their ability to degrade plastics and polyethylene using MSM with the addition of agar-agar (HiMedia, India). Polyethylene and plastic powder were added to the MSM at a final concentration of 0.1%. The medium was placed in a shaker for 1h and autoclaved at 121°C for 20 min. The medium was poured into sterile plates and allowed to solidify. Wells were made in the agar using a good borer, and the isolated organisms were added to the well. The plates were then incubated at 37°C for 2-4 weeks, and growth around the well was observed [13]. The media on the Petri dish with the addition of polyethylene terephthalate (PET) powder and without the introduction of microorganisms were used as the control.

2.7. Determination of plastic film degradation

The pour plate technique after serial dilution was carried out to test the plastic degrading ability of the isolates. The cell suspension of the culture was added to a sterile Petri dish, followed by the addition of warm nutrient agar media. The plate was swirled to ensure homogenization of the culture. The polystyrene and polyethylene were then aseptically placed on each plate containing the bacterial samples. The plates were placed in the incubator at 37 °C for a few weeks. The plates were periodically removed, and the film was observed for any sign of microbial growth [7].

3. Results and Discussion

3.1. Total heterotrophic counts of the soil samples

Table 1 shows that sample 4 has the highest total viable count of 300.00±0.00 x 10^{-4}, and sample 3 has the lowest count of 20.25±24.90 x 10^{-4}. This shows that the population of microorganisms in soil sample 4 is higher and the least in soil sample 3.

Table 1. Mean bacteria count in CFU/g (mean and standard deviation).

Soil sample	10^{-4}
1	114.75±127.57
2	164.25±156.75
3	20.25±24.90
4	300.00±0.00

3.2. Characterization of bacteria isolates from the soil

The bacteria were identified using methods described by Bergey's Manual of Systemic Bacteriology. Gram staining reaction and biochemical tests such as citrate, mannitol test, oxidase, glucose fermentation test, coagulase test, etc., with reference obtained from PIBWIN, were used to identify the organisms as shown in Table 2.

Table 2. Biochemical and microscopic test result.

S/N	Ca	Ct	Ma	Coa	Oxi	Glu	Suc	Lac	Shape	Gram stain	Gas	H_2S	Probable organism
1	+	+	+	+	+	+	-	-	C	+	-	-	*Staphylococcus aureus*
2	-	+	+	-	-	+	+	+	C	+	-	-	*Streptococcus pneumonia*
3	-	-	+	-	-	+	-	-	C	+	+	-	*Streptococcus faecium*
4	-	+	+	+	+	+	-	-	C	+	-	-	*Staphylococcus hyicus*
5	+	-	-	+	+	+	-	-	C	+	-	-	*Micrococcus radiodurans*
6	-	-	+	+	+	+	-	-	C	+	-	-	*Staphylococcus sp*
7	+	+	-	+	-	-	-	-	R	+	-	-	*Bacillus sp*
8	+	-	-	-	+	+	-	-	C	-	+	-	*Neisseria sp*
9	-	-	-	-	+	+	-	-	C	-	-	+	*Neisseria sp*
10	-	+	-	+	+	+	+	+	C	+	-	-	*Micrococcus luteus*
11	+	+	-	-	+	+	-	-	C	+	-	-	*Micrococcus radiodurans*
12	+	+	-	-	+	+	-	-	C	-	-	-	*Neisseria sp*
13	-	-	-	+	-	+	-	-	C	+	-	-	*Streptococcus equi*
14	-	-	+	-	-	+	-	-	R	-	-	-	*Escherichia coli*
15	-	+	-	-	-	+	-	-	C	+	-	-	*Streptococcus mitior*
16	-	+	-	-	-	+	-	-	R	+	+	-	*Lactobacillus sp*
17	-	+	-	-	-	+	-	-	C	+	-	-	*Streptococcus sp*
18	-	-	-	+	-	+	-	-	C	+	-	-	Streptococcus agalactiae
19	+	-	-	-	-	+	-	-	R	+	-	-	*Listeria sp*
20	-	-	-	+	+	+	-	-	C	+	-	-	*Streptococcus equi*
21	-	-	-	-	+	+	-	-	C	-	-	-	*Neisseria sp*
22	-	-	-	-	+	+	-	-	C	-	-	-	*Neisseria sp*
23	-	-	+	-	+	+	-	-	C	+	-	-	*Micrococcus mucilaginosus*
24	-	-	+	+	-	+	-	-	R	+	-	-	*Bacillus sp*
25	+	-	+	+	+	-	-	-	C	+	-	-	*Staphylococcus aureus*
26	-	-	+			+	+	+	R	-	-	-	*Pseudomonas sp*
27	-	-	+	-	-	+	-	-	C	-	+	-	*Streptococcus sp*
28	-	-	+	-	+	+	+	+	C	+	-	-	*Micrococcus mucilaginosus*
29	-	+	+	+	+	+	-	-	C	+	-	-	*Staphylococcus aureus*
30	+	+	-	-	-	-	-	-	R	+	-	-	*Proteus sp*
31	+	-	+	+	+	+	-	-	C	+	-	-	*Staphylococcus xylosus*
32	+	+	+	-	+	+	-	-	C	+	-	-	*Staphylococcus varians*
33	-	-	-	-	-	+	-	-	C	+	-	-	*Micrococcus sp*
34	+	-	-	-	+	+	-	-	C	+	-	-	*Cellobiosococcus sp*
35	-	-	+	+	+	+	-	-	C	-	-	-	*Streptococcus sp*
36	+	-	-	-	-	+	+	+	C	-	-	-	*Neisseria sp*
37	-	+	-	-	+	+	-	-	C	+	-	-	*Streptococcus sp*
38	+	-	-	-	-	+	-	-	C	+	-	-	*Micrococcus sp*

KEY: + positive; - negative; C cocci, R rods; Ca catalase; Ct Citrate; Ma Mannitol; Coa Coagulase; Glu glucose, Oxi oxidase, Suc sucrose, Lac lactose.

3.3. Screening of plastic degrading microorganisms

The following isolates grew around the well on the MSM media with polyethylene terephthalate powder: *Staphylococcus aureus, Staphylococcus hyicus, Bacillus sp, Streptococcus mitior*, *Lactobacillus sp*, *Micrococcus sp*, *Proteus sp*, *Pseudomonas sp*, *Niesseria sp,* and *Micrococcus mucilaginosus*.

3.4. Physicochemical parameters of the soil samples

Table 3 shows the texture, color, and type of each sample of soil collected. Most of the samples are loamy soil with dark-brown to brown color with fine/coarse texture, while the minority is clay and sandy soils with brown color and coarse/ fine texture.

Table 3. Texture, colour, and type of each soil sample.

Soil sample	Texture	Type of soil	Color
1	Coarse	Sandy	Dark brown
1a	Coarse	Clay	Reddish-brown
1b	Fine	Sandy	Light brown
1c	Fine	Clay	Reddish-brown
2	Fine	Loamy	Dark brown
2a	Coarse	Sandy	Brown
2b	Coarse	Loamy	Brown
2c	Fine	Loamy	Dark
3	Coarse	Loamy	Brown
3a	Fine	Loamy	Brown
3b	Fine	Loamy	Dark brown
3c	Coarse	Loamy	Dark brown
4	Fine	Loamy	Dark
4a	Coarse	Loamy	Dark
4b	Fine	Loamy	Light brown
4c	Coarse	Sandy	Brown

3.5. Organic matter and moisture content.

The organic matter and moisture soil content for each soil sampled was obtained, and the result is presented in Table 4. Table 4 shows the percentage of organic matter and moisture content available in each soil sample. Sample 3c has the highest percentage organic matter of 0.88% and moisture content of 1.81%. Sample 1b has the lowest percentage organic matter of 0.04%; samples 3b and 4c have the lowest percentage moisture content of 0.04%.

Table 4. Organic matter and moisture content percentage of each soil sample from each dumpsite.

Soil sample	Organic Matter (%)	Moisture Content (%)
1	0.25	0.19
1a	0.40	0.43
1b	0.04	0.12
1c	0.42	0.48
2	0.37	0.53
2a	0.38	0.23
2b	0.57	0.52
2c	0.80	0.25
3	0.47	0.21
3a	0.17	0.07
3b	0.24	0.04
3c	0.88	1.81
4	0.13	0.26
4a	0.87	0.48
4b	0.13	0.09
4c	0.30	0.04

Table 5 shows the mean and standard deviation of the organic matter and moisture content available in each soil sample. Sample 2 has the highest mean and standard deviation of organic matter of 0.53±0.20 while sample 3 has the highest mean and standard deviation of moisture content of 0.53±0.86. Sample 1 has the lowest mean and standard deviation of organic matter of 0.28± 0.15, sample 4 have the lowest mean and standard deviation of moisture content of 0.22±0.20. The result in Table 5 shows that there is no significant difference in the organic matter of the various sampled soil, [F (3, 12) = 0.636; ρ> 0.05]. There is also no significant difference in the moisture content of the soils sampled [F (3, 12) = 0.350; ρ > 0.05].

Table 5. Mean difference of organic matter and moisture content.

		Mean±SD	Df	F	P
Organic Matter	1	0.28± 0.15	3, 12	0.636	0.606
	2	0.53±0.20			
	3	0.44±0.32			
	4	0.36±0.35			
Moisture Content	1	0.31±0.18	3,12	0.350	0.790
	2	0.38±0.16			
	3	0.53±0.86			
	4	0.22±0.20			

Table 6 shows the mean and standard deviation for pH, conductivity, temperature, and total dissolved solids performed on the different soil samples at different locations on the dumpsites.

Table 6. Mean and standard deviation of physicochemical parameters.

Soil sampled	pH	Electrical conductivity (µS/cm)	Temperature (°C)	TDS mg/l
1	7.51±0.24	357.75±145.27	26.13±0.37	178.70 ± 72.67
2	7.69±0.23	469.10±236.00	26.20±0.14	234.35 ±117.88
3	7.60±0.14	436.58 ± 373.01	26.05±0.29	225.90 ± 177.97
4	7.61±0.17	523.50±118.71	26.05±1.00	259.90 ±63.21

3.6. Polyethylene film

Organisms were also introduced to degrade polyethylene. The result of the difference in the degradation between four and six weeks is presented in Table 7. Table 7 shows that *Pseudomonas sp* and *Micrococcus radiodurans* are able to degrade the polyethylene film with weight loss percentages of 25% and 25%, respectively. *Staphylococcus hyicus and Bacillus sp* do not degrade the polyethylene during the space of four and six weeks. The difference in weight for four and six weeks of introducing organisms is presented graphically in Figure 2.

Table 7. Weight difference of polyethylene film.

S/N	Probable organisms	Initial weight	Final wt (g) after 4	Final wt (g) after 6	Difference in wt (g) after 4	Difference in wt (g) after 6
1	*Staphylococcus sp*	0.16	0.14	0.13	0.02	0.03
4	*S. hyicus*	0.18	0.18	0.18	0.00	0.00
5	*M. radiodurans*	0.16	0.12	0.11	0.04	0.05
7	*Bacillus sp*	0.14	0.12	0.10	0.02	0.04
9	*Niesseria sp*	0.18	0.17	0.15	0.01	0.03
13	*Strep.equi*	0.16	0.14	0.13	0.02	0.03
14	*E. coli*	0.16	0.14	0.14	0.02	0.03
15	*Strep.mitior*	0.16	0.15	0.15	0.01	0.01
16	*Lactobacillus sp*	0.14	0.14	0.13	0.00	0.01
17	*Streptococcus sp*	0.16	0.12	0.11	0.02	0.03
24	*Bacillus sp*	0.12	0.10	0.10	0.02	0.00
25	*Staphylococcus sp*	0.12	0.12	0.11	0.00	0.01
26	*Pseudomonas sp*	0.18	0.14	0.13	0.04	0.05
27	*Streptococcus sp*	0.14	0.13	0.13	0,01	0.01
28	*M.mucilaginosus*	0.12	0.10	0.10	0.02	0.01
29	*Staphylococcus aureus*	*0.16*	*0.14*	0.12	0.02	0.04
30	*Proteus sp*	0.18	0.18	0.17	0.00	0.01
35	*Streptococcus sp*	0.16	0.13	0.12	0.03	0.04
37	*Streptococcussp*	0.18	0.16	0.16	0.02	0.02
38	*Micrococcus sp*	0.16	0.16	0.15	0.00	0.01

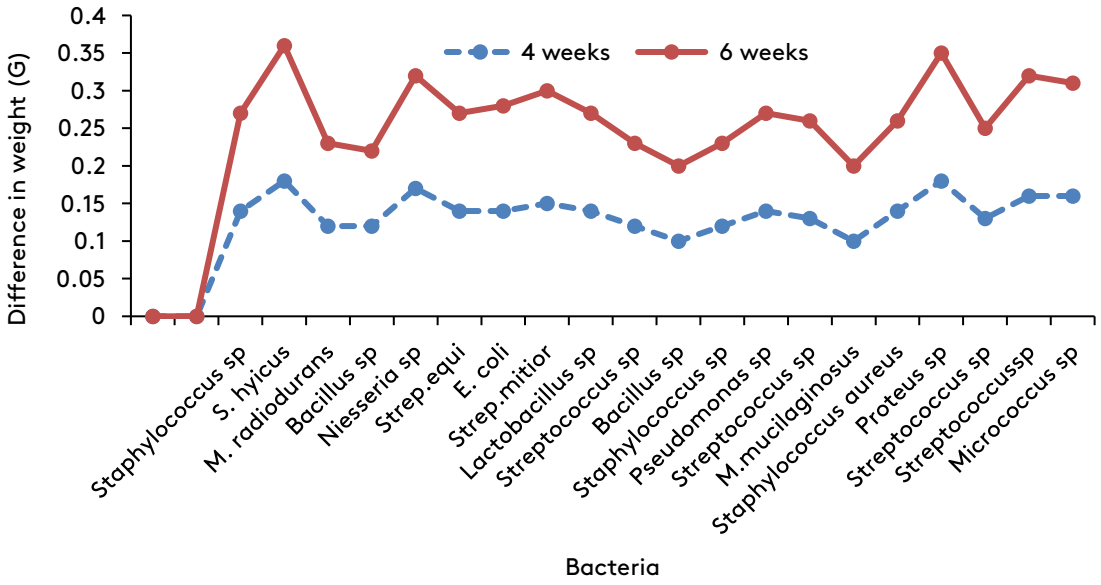

Fig.2. Difference in the weight of polyethylene film after four and six weeks.

3.7. Polystyrene film

Bacteria isolates were also introduced to polystyrene (disposable plastic cups) to determine the biodegrading ability of these organisms. The initial weight was recorded; also, the weights of the plastics were recorded at four and six weeks. The result of the various weights is presented in Table 8. Table 8 shows that *Staphylococcus sp* had the highest degrading ability on polystyrene film after six weeks. *Pseudomonas sp* and *Lactobacillus sp* did not degrade the polystyrene film at both four and six weeks. The differences in weight at four and six weeks are presented in Figure 3.

Table 8. Difference in the weight of polystyrene film after 4 and 6 weeks.

S/N	Probable organisms	Initial Weight (g)	4 weeks (g)	6 weeks (g)	Difference in Weight 4 weeks (g)	Difference in 6 weeks (g)
5	*M.radiodurans*	0.32	0.30	0.29	0.02	0.03
14	*E.coli*	0.30	0.28	0.27	0.02	0.03
15	*Streptococcus mitior*	0.30	0.28	0.26	0.02	0.04
16	*Lactobacillus sp*	0.30	0.30	0.30	0.00	0.00
25	*Staphylococcus sp*	0.30	0.25	0.24	0.05	0.06
26	*Pseudomonas sp*	0.30	0.30	0.30	0.00	0.00
27	*Streptococcus sp*	0.30	0.30	0.29	0.00	0.01
28	*M.mucilaginosus*	0.30	0.26	0.25	0.04	0.05
29	*Staphylococcus sp*	0.26	0.24	0.23	0.02	0.03
30	*Proteus sp*	0.40	0.38	0.37	0.02	0.03
35	*Streptococcus sp*	0.30	0.29	0.28	0.01	0.02
38	*Micrococcus sp*	0.30	0.30	0.29	0.00	0.01

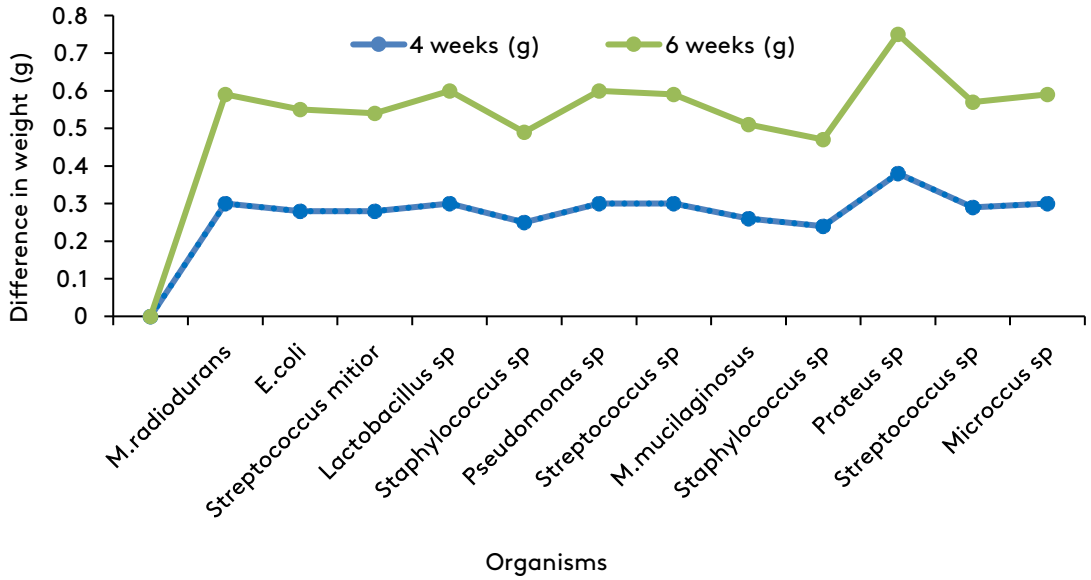

Fig.3. Difference in weight of polystyrene film at 4 and 6 weeks.

3.8. Degradation of polyethylene film

The weight difference in the degradation of polyethylene film is represented in Table 9. The results in Table 9 show that *Proteus sp*, *Streptococcus sp*, *Staphylococcus xylosus*, and *Bacillus sp* are able to degrade the polyethylene film with the highest weight loss difference within the six weeks. The difference in weight for four and six weeks is presented in Figure 4.

Table 9. Weight difference for polyethylene film in nutrient broth.

S/N	Probable organism	Initial weight (g)	Final weight (4wks) (g)	Final weight (6 wks) (g)	Difference in weight (4 wks) (g)	Difference in weight (6 wks) (g)
1	*S. aureus*	0.14	0.12	0.12	0.02	0.02
3	*Streptococcus faecium*	0.12	0.10	0.10	0.02	0.02
4	*S. hyicus*	0.12	0.10	0.09	0.02	0.03
5	*M. radiodurans*	0.16	0.14	0.13	0.02	0.03
7	*Bacillus sp*	0.16	0.14	0.13	0.02	0.03
9	*Niesseria sp*	0.18	0.14	0.14	0.04	0.04
15	*Streptococcus mitior*	0.14	0.14	0.13	0.00	0.01
16	*Lactobacillus sp*	0.12	0.10	0.09	0.02	0.03
17	*Streptococcus sp*	0.16	0.12	0.11	0.04	0.05
26	*Pseudomonas sp*	0.12	0.12	0.12	0.00	0.00
27	*Streptococcus sp*	0.16	0.14	0.12	0.02	0.04
28	*M. mucilaginosus*	0.12	0.10	0.10	0.02	0.02
24	*Bacillus sp*	0.14	0.12	0.10	0.02	0.04
30	*Proteus sp*	0.18	0.16	0.12	0.02	0.06
37	*Streptococcus sp*	0.18	0.16	0.12	0.02	0.06
31	*Staphylococcus xylosus*	0.16	0.12	0.10	0.04	0.06

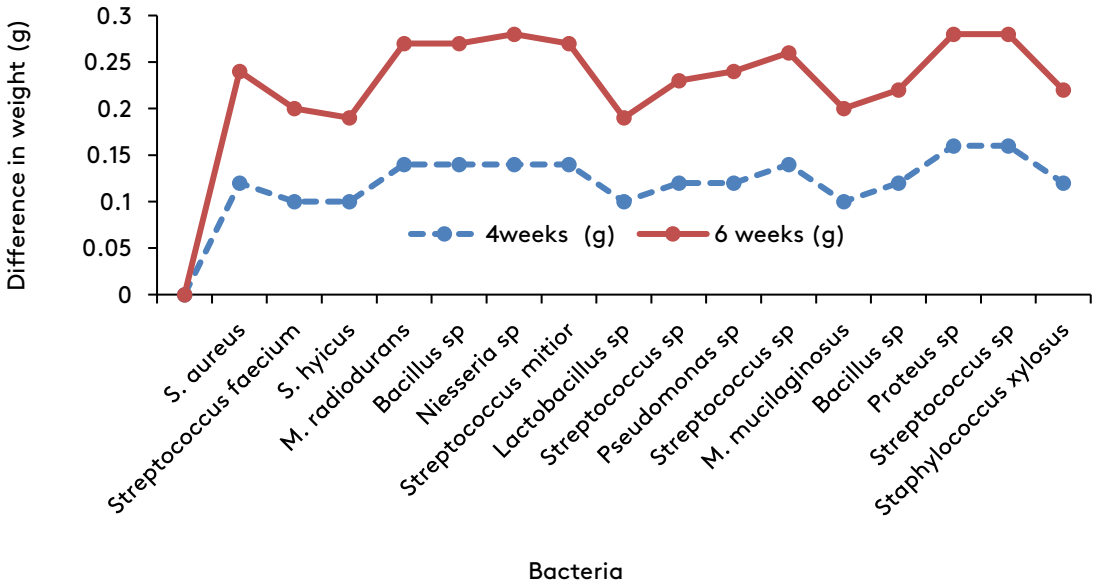

Fig. 4. Difference in weight of polyethylene film at 4 and 6 weeks.

This study describes the isolation, identification, and characterization of plastic degrading microorganisms from the soil. Microorganisms can degrade these synthetic plastics but take a longer period due to the structure of the plastics. Priyanka and Archana [14] tested the organic matter and moisture content of various soil samples collected from different sites. They observed a higher moisture content and organic matter percentage than the recorded moisture content and organic matter percentage in this study. The findings in this report show that sample 3c had the highest percentage of organic matter of 0.88% and moisture content of 1.81%. Sample 1b had the lowest percentage organic matter of 0.04%, while samples 3b and 4c had the lowest percentage moisture content of 0.04%. Sample 2 had the highest mean conductivity of 469.10±236.00, and sample 4 had the highest mean total dissolved solids (TDS) of 259.90 ±63.21. The total viable count for bacteria ranged from 11.8x 10^5 to 0.2x 10^{11} CFU/g, which showed that the soil was home to a vast quantity of microorganisms. Some of the isolated bacteria were *Staphylococcus sp*, *Streptococcus sp*, *Pseudomonas sp*, *Bacillus sp*, *Neisseria sp*, *Escherichia coli*, *Proteus sp.*, *Micrococcus sp.*, *Listeria sp.*, and *Lactobacillus sp*. The *Staphylococcus sp*, *Streptococcus sp*, *Pseudomonas sp*, *Bacillus sp*, *Neisseria sp*, *Escherichia coli*, *Proteus sp.*, *Micrococcus sp.*, and *Lactobacillus sp* were able to degrade polyethylene film. The *Staphylococcus sp* and *Micrococcus sp* showed significant degrading ability on the polystyrene film with a difference in weight of 0.06 g and 0.05 g, respectively, while *Staphylococcus sp, Proteus sp, Streptococcus sp,* and *Bacillus sp* showed significant degrading ability on the polyethylene film with a difference in weight of 0.06 g, 0.06 g, 0.05 g, and 0.04 g, respectively. The *Bacillus sp, Staphylococcus aureus, Pseudomonas sp, and Micrococcus radiodurans* had the highest plastic degrading weight difference for polyethylene with a percentage weight loss of 14.2%, 27.7%, 25%, and 25%, respectively. This result correlates with the findings of Afreen *et al*. [6] in their research on the characterization of plastic degrading bacteria isolated from landfill sites in which they successfully isolated *Pseudomonas sp* and *Bacillus sp* as polyethylene degrading bacteria. Priyanka and Archana, [14] in their study on the biodegradability of polythene and plastic with the help of microorganisms: a way for brighter future, tested the ability of *Staphylococcus aureus* and other species of bacteria to degrade polyethylene in the laboratory. This result also correlates with that of Sharma *et al*. [17] in their report on the impact of soil composting using municipal solid waste on the biodegradation of plastics. They were able to isolate *Staphylococcus aureus and Pseudomonas sp* as polyethylene degrading bacteria. This result is also in consonance with the report of Divyalakshmi and Subhashini [13]. They performed research on the screening and isolation of polyethylene degrading

bacteria from various soil environments and isolated *Staphylococcus aureus*. On the exposure of *Staphylococcus sp* to the polystyrene film, it actively degraded it with a percentage weight loss of 20% in six weeks. At six weeks, *Lactobacillus sp* and *Pseudomonas sp* did not degrade the polystyrene film. The *Bacillus sp, Proteus sp, Staphylococcus sp,* and *Streptococcus sp* had the highest weight loss percentage of polyethylene film in the broth medium of 33.3%, 33.3%, 33.3%, and 31.2%, respectively. *Lactobacillus sp and Pseudomonas sp* could not degrade the polyethylene film at four and six weeks. *Bacillus sp* is a Gram-positive, aerobic, non-spore-forming rod. *Bacillus sp* have been isolated from the soil in the Niger Delta with some related species, with the degradation of hydrocarbons related to raw petroleum [15]. The result of this work agrees with the report of Arkatkar *et al*. [16]; they recorded proof of microorganisms that could degrade hydrocarbon and suggested that they should also be able to degrade polyethylene since their degradation was comparable [17]. Both *Bacillus mycoides* and *Bacillus subtilis* displayed varying levels of capacity to degrade polyethylene. Also, it is expected that their separation from soil continually dirtied by oil slick may have conferred on them such degrading capacity.

5. Conclusions

Staphylococcus aureus, Pseudomonas sp, Bacillus sp Streptococcus sp, and *Micrococcus sp* were able to degrade the various plastic materials with significant weight loss percentage within six weeks. *Staphylococcus aureus* degraded all the different types of plastic materials with a high percentage weight loss at four and six weeks. This research has shown that bacteria can degrade and utilize plastics for their metabolic activity; therefore, the use of plastic degrading microorganisms to reduce the rate of plastic pollution should be adopted. These specific strains of microorganisms can be cultured in large quantities and used in bioremediation. The following recommendations are suggested. The production of plastics should be minimal, while the production of natural, easily degradable plastics should be encouraged. Plastic degrading microorganisms should be used in the bioremediation of the accumulation of plastic and the enzymes produced by plastic degrading microorganisms that enable them to degrade plastics. They should be extracted and produced in large quantities for use in bioremediation. Public awareness about plastic pollution and its negative impact on the planet and aquatic ecosystem should be made through the media as most people are unaware of the harm synthetic plastic have on the ecosystem. Proper waste disposal systems should be made available for people living in urban and rural areas and those living close to the aquatic environment.

References

[1] Sakhalkar, S. and Mishra R.L. (2011). Screening and identification of soil fungi with potential of plastic degrading ability. *Indian journal of applied research*, *3*(12), 62-64.

[2] Emmanuel-Akerele, H. A., Akinyemi, P. O. (2022). Physico-chemical and microbiological assessment of soils from dumpsites for plastic degrading microorganisms. *Pollution*, *8*(2), 501-512.

[3] Hossain, K. S., Das, S., Kundu, S., Afrin, S., Nurunnabi, T. R. and Rahman, S. M. M. (2019). Isolation and characterization of polythene degrading bacteria from garbage soil. *International journal of agriculture environment and bioresearch, 4*(5), 254-263.

[4] Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A and Law K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, 347(6223), 768-771.

[5] Patil, R. C. (2018). Screening and characterization of plastic degrading bacteria from garbage soil. *British Journal of environmental sciences, 6*(4), 33-36.

[6] Afreen, B., Nouman, N. R., and Saima, I. (2020). Characterization of plastic degrading bacteria isolated from landfill sites. *International journal of clinical microbiology and biochemical technology, 4*(1), 30-35.

[7] Sharma, J. G., Tapasuita U., Ashima N., Krittika A., Puja M., and Arup. (2014). Isolation and characterization of plastic degrading bacteria from soil collected from the dumping grounds of an industrial area. *International journal of advanced and innovative research*, *3*(3), 2278-7844.

[8] Adeolu, O. A. and Tope, C. F. (2012). Environmental and health concerns associated with the open dumping of municipal solid waste: A Lagos, Nigeria experience. *American journal of environmental engineering, 2*(6), 160-165.

[9] Wanger, A., Chavez, V., Huang, R., Wahed, A., Dasgupta, A., and, Jung. A. K. (2017). *Microbiology and Molecular Diagnosis in Pathology: A Comprehensive Review for Board Preparation, Certification and Clinical Practice. 1,*98- 101.

[10] Corwin, D. L., and Yemoto, K. (2020). Salinity: Electrical conductivity and total dissolved solids. *Soil science society of America journal, 84*(5), 1442-1461.

[11] Oyeyiola G.P, and Agbaje A. B. (2013). Determination of the soil microflora of a soil near microbiology laboratory at the university of Ilorin main campus. *Egyptian academic journal of biological sciences, 5*(1), 35-41.

[12] Libretexts. (2021a). Bacterial colony morphology. Retrieved september 1, 2021, *https://bio.libretexts.org/Learning_Objects/Laboratory_Experiments/Microbiology_Labs/Microbiology_Labs_I/08%3A_Bacterial_Colony_Morphology.*

[13] Divyalakshmi S and Subhashini A. (2016). Screening and isolation of polyethylene degrading bacteria from various soil environments. *Journal of environmental science, toxicology and food technology 10*(12), 01-07.

[14] Priyanka, N., and Archana, T. (2011). Biodegradability of polythene and plastic by the help of microorganism: A way for brighter future. *Journal of environmental and analytical toxicology*, *1*(2), 23-29.

[15] Eziuzor, C. S., and Okpokwasili G.C. (2009). Bioremediation of hydrocarbon contaminated mangrove soil in a bioreactor. *Nigerian journal of microbiology, 23*(1), 1777-1791.

[16] Arkatkar, A., Arutchelvi, J., Bhaduri, S., Uppara, P. V., and Doble, M. (2009). Degradation of un-pre-treated and thermally pre-treated polypropylene by soil consortia. *International biodeterioration and biodegradation,* 63(1), 106-111.

[17] Singh, V., Dubey, M., and Bhadauria, S. (2012). Microbial degradation of polyethylene (low density) by *aspergillus fumigatus* and *penicillium sp*. *Asian journal of experimental and biological sciences, 3*(3), 498-501.