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Efficiencies of Thermophilic Bacteria Species in Degrading Biodegradable Low-density Polyethylene Mixtures in Aquatic Ecosystems

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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

The study examined the efficiencies of bacteria thermophiles responsible for the depolymerization of biodegradable Low-Density Polyethylene (LDPE) blends in two aquatic environments to suggest model bacteria species that could be used for reducing the accumulation of single-use LDPE in both marine and freshwater ecosystems. Each of the biodegradable LDPE, polyethylene, and cellulose was placed in respirometry jars filled with 500 mls of the freshwater and marine water respectively in a randomized design of 4 by 2 by 3 following the American Standard Testing and

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Materials (ASTM) procedure. To identify the bacteria species, bacterial isolation was done using pour plate and streak methods. The bacteria species were identified by morphological, biochemical, and molecular methods. The thermophilic bacteria species were confirmed by sequencing to be *Bacillus cereus*, *Pseudomonas species* among others. The results revealed that the bacteria isolates on LDPE were responsible for the biodegradation processes of the LDPE. This study concluded that *Bacillus cereus* and *Pseudomonas species* have the bioremediation potentials to break down single-use biodegradable Low-Density Polyethylene (LDPE) in aquatic environments within six (6) months.

Keywords: Bioremediation; biodegradable LDPE; microorganisms; bioplastics; environment.

1. INTRODUCTION

Plastics comprised of non-biodegradable polymers are currently the principal issue of municipal solid garbage, raising several environmental challenges especially with low-density polyethylene (LD). One of the most well-known petroleum polymers used in packaging is LDPE. Low-density polyethylene packaging nylons are widely used in domestic and industrial applications as food, beverage, cosmetics, and pharmaceutical packaging materials.

Conversely, polyethylene (PE) and other petroleum-based plastics degrade slowly in aquatic and terrestrial ecosystems; resulting in a variety of harmful compounds. Currently, to solve the challenges, emphasis is being placed on producing polymer and polymer additives from renewable resources in order to encourage the production of nature-based plastics such as biodegradable polymers with high biodegradability traits [1,2]. Biodegradable polymers are emerging as novel packaging options, as they provide high performance throughout use without the release of toxic elements at the end of their useful life [3]. Environmentally friendly polymers can degrade biologically in soil, compost, sewage, and marine settings, thereby preventing the buildup of recalcitrant polymeric wastes. This biodegradation can be carried out efficiently by microbes that are suggested to secrete polymerase enzymes with evidence of carbon (iv) oxide evolution [4]. Therefore, biodegradation is an important step in the breakdown of polymeric substrates into organic components in the environment [5].

Thus, bioplastics can be suggested as feasible options to non-degradable synthetic plastics as the substitutions of traditional non-biodegradable plastic with real bioplastic is an accepted eco-innovation. Yet, there is need to identify microbial communities responsible for the breakdown processes as well as study their efficiencies in degrading polymers in aquatic ecosystems. Basically, plastics can biodegrade in the environment through four mechanisms which are photodegradation, thermo-oxidative, hydrolytic, and microbial degradation. However, microbial biodegradation otherwise known as biodegradation is cheaper and safer as plastics are subjected to total biodegradation by indigenous heterotrophic microbes with the production of water and carbon dioxide as end products [6] while the other methods may not lead to ultimate degradation. Microbial degraders and their metabolic enzymes are among environmental agents that participate in biodegradation process which embroils bio-deterioration, bio-fragmentation, biodegradation, and mineralization. Biodeterioration involves microbial activity that provokes plastic cracks and aggravates change in physical properties because of biofilm formation as well as bio-fragmentation process with break in the long polymer carbon chain [7]. The next stage which is the biodegradation of oligomers to monomers is involved. Thereafter, secondary degraders assimilate the monomers as carbon source to increase their cell biomass with production of H_2O, CO_2, N_2, and CH_4 as metabolites. Thus, mineralization involves assimilation of oligomers and excretion of completely oxidized metabolites [8,29]. In this way, the metabolites contribute to soil fertility and decrease plastic accumulation in the environment, thereby reducing the cost of waste management. The biodegradation rate is strongly influenced by several factors such as polymer properties, presence of branching and additional functional groups that promote higher hydrophilicity and environmental factors [9].

In this study, biodegradable LDPE mixtures was subjected to the activities of indigenous bacteria species colonizing the aquatic environment using the ASTM respirometry method. This aimed to identify suitable bacteria species responsible for

the biodegradation of the bioplastic packaging film in aquatic ecosystems.

2. MATERIALS AND METHODS

2.1 The Study Area

A freshwater was sampled from Isokun River, Ogo-Oluwa Street Ilara Mokin, Ondo State, Nigeria at latitude 5.1023^{o} E and longitude 7.3491^{o} N. There were minimal activities around the freshwater bodies; thus, it was unperturbed. The average temperature of the area ranges between 25°C and 30°C while the average relative humidity is below 65.

2.2 Sample Collection

Both freshwater and marine water were collected for the samples in this study. To collect samples of the freshwater, a sterile glass bottle was rinsed with 1N Nitric acid and later the glass bottle was rinsed thoroughly with distilled water, and later with the freshwater. Thereafter, a 200 µm mesh was used to filter the freshwater to remove the zooplanktonic organisms and floating debris. The freshwater samples were collected around 8:00 a.m. hours and later stored at 4˚C in a refrigerator.

2.3 Sources of the Test Polymers

Samples of cellulose were obtained from the Environmental Management and Toxicology (EMT) Laboratory of The Department of Biological Sciences, Elizade University, Ilara-Mokin, Ondo State, Nigeria. The biodegradable LDPE was obtained from the Advanced Polymer Composites Group, Material Science and Manufacturing Unit, Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa while the Polyethylene (PE) sample was purchased from a shopping mall in Akure Town, Ondo State, Nigeria.

2.4 Preparation of Water Samples

To prepare the simulated marine water; 500 mls of distilled water were obtained and carefully poured into sterilized conical flask of 1 litre. Following the method of Lake Products Company (10 LPC, 2021) for the preparation of marine water, 24.53 g/L of NaCl, 2 5.20 g/L of MgCl, 4.09 g/L of Na_2SO4, 1.16 g/L of $CaCl_2$, 0.695 g/L of KCl, 0.201 g/L of $NaHCO_3$, 0.101 g/L of KBr, 0.027 g/L of H_3BO_3, 0.025 g/L of $SrCl_2$, 0.003 g/L of NaF amount of salts were measured aseptically and poured into the round bottom flask containing distilled water and then filled up to the 1 litre mark on the round bottom flask. The flask was neatly corked and covered with aluminum foil. The pH of the marine water was adjusted to 8.2 using 0.1 N solution of hydrochloric acid.

2.5 Sterilization and Preparation of Test Polymer

The biodegradable LDPE and PE were sterilized by exposing the test polymers to ultraviolent light for four hours. However, the cellulose was in powdery form and was neatly packaged in sterile packaging nylon.

2.6 Experimental Design

The procedures of the American Standard Testing and Materials (ASTM) method for assessing polymer biodegradation in aquatic environments were followed. Twenty-four one-liter-respirometry glass jars were used to set up the experiments. Each of the jars used was approximately 15 cm in height and 7 cm in width. For the experimental setup, twenty-four respirometry glass jars were washed carefully, drained, and sterilized by drying at 170^{0}C for 15 minutes in an oven. In all, twelve respirometry glass jars were prepared for the freshwater and the marine water respectively. Then, five hundred mls of the freshwater and the marine water were added respectively to each set of 12 respirometry glass jars. Five hundred milligrams of the sterile test polymers (biodegradable LDPE, PE (nylon-6 films), and cellulose) were cut into sizes of 3 cm by 4 cm respectively. The test polymers were exposed to ultraviolet rays for four hours to sterilize them. The sterile nylon-6 films (negative control) and the biodegradable LDPE were aseptically introduced into the freshwater and marine water respectively using a sterile spatula. However, the cellulose powder was aseptically poured into fresh and marine water respectively as a positive control. Blank was set as control and prepared both for freshwater and marine water respectively.

Thereafter, for trapping the CO_2 evolved from the microbial depolymerization of the three test samples, 40 mls of 1 N KOH were poured aseptically into twenty-four (24) glass beakers of 50 mls by volume. Each of the sterile beakers was positioned appropriately in the respirometry glass jars to capture the evolved CO_2. The

experimental design was in triplicate and arranged in a randomized complete block design of 2 by 4 by 3. Each of the test jars was tightly closed and incubated at 35ºC for four months. Samples of the test polymers, freshwater and marine water were collected for microbial and molecular analyses.

3. LABORATORY ANALYSES

3.1 Physicochemical Properties of Water Samples

The temperature was measured on-site with the aid of a digital thermometer. Measurement of the water temperature was taken and values recorded in degree Celsius. The pH, total dissolved solids (TDS) and electrical conductivity (EC) of the water samples were measured with a HANNA HI 9810 pH-TDS meter while the Total Suspended Solids (TSS) were measured mg/L using the filtering technique according to the established protocols of [10]. For the dissolved oxygen (DO), a 300 ml glass-stoppered vial was filled with sample without trapped air. One ml each of manganese sulphate and alkaline iodide solution was added consecutively and the vial was tightly closed to avoid air bubbles. To mix the contents, the bottle was gently tilted numerous times. Allowing the precipitate to settle, 1.5 mls of H_2SO_4 were added, re-stoppered, and mixed by inversion several times. Twenty-five milliliters aliquot of $Na_2S_2O_3.5H_2O$ solution were titrated to a colorless endpoint using a starch indicator. The DO was calculated using the following formula:

$$DO\ (mg/L) = \frac{T\ cm^3 \times 100}{\text{Volumes of aliquot}}$$

Where,
T = Volume of titrant used.

However, Nephelometric method was used to determination the turbidity of the two aquatic environments. Following the method of [11], heavy metal analysis was carried out using atomic absorption spectrophotometry.

3.2 Isolation, Purification and Identification of Bacteria Species

Different agars were prepared to determine the presence of some microorganisms involved in the biodegradation of the cellulose, bioplastic and polyethylene samples.

One ml of each of the freshwater and marine water samples with their respective test polymers were measured into 10 mls by volume of the test tube to which 9 mls of distilled water was added. Using the serial dilution technique, 1ml of the mixture was added to 9 mls of distilled water and this was done consecutively until 10^{-4} was reached. Then, Nutrient Agar (NA), Salmonella Shigella Agar (SSA), Mannitol Salt Agar (MSA), Yeast Extract Agar (YEA), and De Man, Rogosa and Sharpe Agar (MRS) was used after preparing them according to their manufacturers' instructions. One ml of the serially diluted specimen was then used for the pour plate technique in enumerating the microbial load of the water samples as described by [12].

After satisfactory growth of microorganisms had been observed on each growth medium, visible colonies were counted in the nutrient agar plate with a colony counter. Thereafter, different colonies were picked and assessed for bacterial loads. The bacterial growths were sub-cultured on freshly prepared agar media until pure cultures were observed.

After the pure cultures were isolated, characterization of the pure culture were carried out according to their morphological and biochemical features using the methods of [12] for identification of the bacteria isolates.

3.3 Bacterial Isolates

Genomic DNA was extracted from the cultures received using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005). The 16S target region was amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486) with the primers presented in Table 1. The PCR product was run on a gel and cleaned up enzymatically using the EXOSAP method. The extracted fragments were sequenced in the forward and reversed directions (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-Up Kit™, Catalogue No. D4050).

The purified fragments were analyzed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction for every sample, as listed in Table 1. BioEdit Sequence Alignment Editor version 7.2.5 was used to analyze the ab1 files generated by the ABI 3500XL Genetic Analyzer and results were obtained by a BLAST search (NCBI) [13].

Table 1. Molecular Information about the Primers used for Identification of Bacteria Isolates

Name of Primer	Target	Sequence (5' to 3')
16S-27F	16S rDNA sequence	AGAGTTTGATCMTGGCTCAG
16S-1492R	16S rDNA sequence	CGGTTACCTTGTTACGACTT

4. RESULTS

4.1 Physicochemical Properties of the Aquatic Environments

Table 2, presents the qualities of the physicochemical parameters of the aquatic environments. The mean values of temperature for the freshwater were more than 20.00 °C while that of marine water was above 26.00°C. The pH of the freshwater and simulated marine water were 7.7 and 8.2 respectively. The values of the dissolved oxygen reported in the freshwater samples were above 2.60 while that of the marine water was about 0.29 less.

In addition, the electrical conductivity of the freshwater sample was about 306.00 µS/cm while that of the marine water was about 385.00 µS/cm. The turbidity values of the freshwater and marine water were 7.80 m and 6.30 m respectively. Moreover, the highest (155.00 ml/L) of total dissolved solids was reported in marine water while 153.00 ml/L was reported in freshwater. The mean values for total suspended solids were 1.0 ml/L for freshwater and 1.1 ml/L for marine water.

Moreover, the concentrations of cadmium (Cd), lead (Pb), zinc (Zn), and iron (Fe) for freshwater were 0.008 ppm, 0.032 ppm, 0.272 ppm, and 0.219 ppm respectively while in marine water, the mean concentrations for the heavy metals were in this order, zinc (Zn) (0.780 ppm) > iron (Fe) (0.5585 ppm) > lead (Pb) (0.0955 ppm) > cadmium (Cd) (0.0225 ppm).

4.2 Bacterial Load of Freshwater ($\times 10^4$ cfu/g) with the Biodegradable LDPE, Polyethylene, Cellulose and Control within Four Months

As shown in Fig. 1, the bacterial loads of freshwater samples with test materials for the period of four months were presented. The highest (1.83±0.03 cfu/g) bacterial load was recorded in the last month (Day 120) of the experiment in the freshwater with bioplastic. Although, there was a continuous increase in the bacteria loads in that same sample throughout the four months such that there was 1.33±0.07 cfu/g for the first month (30 days) < 1.37±0.03 cfu/g for the second month (60 days) < 1.53±0.03 cfu/g for the third month (90 days) < 1.83±0.03 cfu/g in the fourth month (120 days).

Furthermore, in freshwater with a nylon-6 PE sample, there was an increase in bacteria load of 3.13±0.12 cfu/g in the second month (60 days), followed by a gradual decrease in bacteria load to 1.33.03±0.12 cfu/g at 120 days. Similar results were recorded in the other samples across the four months.

Table 2. Physicochemical properties of freshwater and Simulated Marine Water

Analysis	Fresh water	Marine water	WHO/USEPA limits
Electrical conductivity (µS/cm)	306.00	385.00	NA
Temperature (°C)	20.30	26.20	NA
pH	7.70	8.20	6.50–8.50
Dissolved Oxygen (DO)	2.67	2.31	NA
Turbidity (m)	7.80	6.30	5.00
Total Dissolved Solid (mg/L)	153.00	155.00	1000
Total Suspended Solid (mg/L)	1.00	1.10	NA
Cadmium (Cd) (ppm)	0.008	0.0225	0.01 to 3
Lead (Pb) (ppm)	0.032	0.0955	0.065 to 10
Zinc (Zn) (ppm)	0.272	0.780	0.2 to 300
Iron (Fe) (ppm)	0.2185	0.5585	0.50 to 300

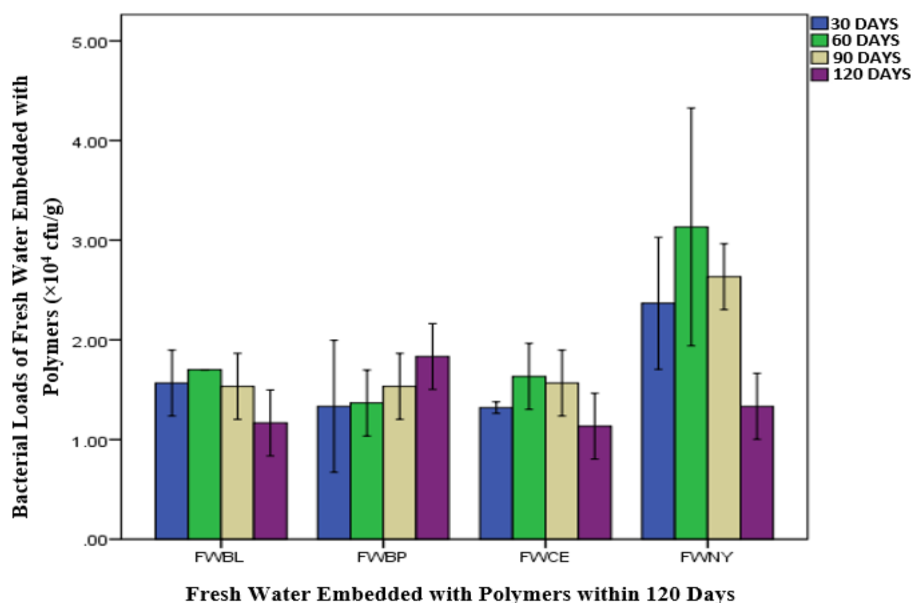

Fig. 1. Bacteria Load of Freshwater with Biodegradable Nylon, Cellulose and Synthetic Nylon ($\times 10^4$ cfu/g)

Key: FWBL = Freshwater Blank, FWCE = Freshwater Cellulose, FWBP = Freshwater Bioplastics, FWNY = Freshwater Nylon 6

Freshwater with cellulose had a bacterial load of 1.32±0.01 cfu/g in the first month (30 days), 1.63±0.03 cfu/g (second month (60 days), 1.57±0.03 cfu/g (third month (90 days), and 1.13±0.03 cfu/g in the fourth month (120 days).

In addition, a freshwater sample for control showed 1.57±0.03 cfu/g, in the first month (30 days), and an increase of 1.70±0.00 cfu/g in the second month (60 days) occurred. However, there was a gradual reduction (1.17±0.03 cfu/g) in the bacteria load in the last month of the study (120 days).

4.3 Bacteria Load of the Marine Water ($\times 10^4$ cfu/g) with the Biodegradable LDPE, Polyethylene, Cellulose and Control in 120 Days

In Fig. 2, the bacterial loads of simulated marine water samples with test materials across the four months of the experiment were revealed. Marine water with bioplastic had the highest bacterial loads of 4.50±0.06 cfu/g, 4.97±0.12 cfu/g in the first (30 days) and second (60 days) months, respectively, although it gradually decreased in the bacterial loads to 3.97±0.03 cfu/g, 2.47±0.03 cfu/g in the third (90 days) and fourth (120 days) months, respectively. Nevertheless, marine water with nylon 6 (the PE) samples reported a continuous increase in the bacteria loads of 1.17±0.03 cfu/g, 1.40±0.06 cfu/g, 2.10±0.06 cfu/g respectively, in the first (30 days), second (60 days), third (90 days), and fourth (120 days) months of the experiment.

Similar to marine water with cellulose samples, the bacteria load increased (1.23±0.07 cfu/g in the first month (30 days), 1.20±0.00 cfu/g in the second month (60 days), 1.40±0.00 cfu/g in the third month (90 days), 1.53±0.03 cfu/g in the fourth month (120 days)) across the period of the experiment.

However, there was a decrease in bacteria loads in the marine water sample for control across the four months (1.93±0.03 cfu/g in the first (30 days), 1.63±0.03 cfu/g in the second (60 days), 1.27±0.03 cfu/g in the third (90 days), and 1.00±0.00 cfu/g in the fourth month (120 days).

4.4 Morphological/Biochemical Characteristics of Bacterial Isolates

Table 3 showed the morphological and biochemical characteristics of bacterial isolates from the sampled aquatic environments. A total of eight (8) bacteria species were isolated throughout the experiment and their probable identities (subject to confirmation by molecular methods) were *Bacillus cereus, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgericus, Streptococcus agalactiae, Actinomyces bovis, and Staphylococcus epidermidis.*

Table 3. Morphological and Biochemical Parameters Used in Identification of Bacterial Isolates

Isolates	1	2	3	4	5	6	7	8
Shape in *plate*	Irregular	Irregular	Circular	Circular	Circular	Irregular	Irregular	Irregular
Size	Small	Small	Small	Large	Large	Large	Large	Average
Pigment	Creamy white	Creamy white	White	Creamy milk	Milk	Milk	Greyish white	Greyish white
Opacity	Opaque	Opaque	Transparent	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation	Flat	Raised	Low convex	Flat	Flat	Convex	Flat	Convex
Surface	Rough	Rough	Dull	Smooth	Smooth	Rough	Smooth	Rough
Edge	Tentate	Lobate	Entire	Entire	Tentate	Tentate	Tentate	Lobate
Consistency	Friable	Friable	Friable	Viscord	Friable	Viscord	Friable	Friable
Amount	Moderate	Moderate	Scanty	Moderate	Moderate	Scanty	Moderate	Moderate
Gram reaction	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
Gram shape	Rod	Cocci	Rod	Cocci	Short rod	Cocci	Long rod	Cocci
Arrangement of cell	Chain	Tetrad	Pair/cluster	Pair/cluster	Cluster	Chain	Pair/cluster	Cluster
Catalyst test	+	+	-	+	+	+	+	+
Coagulate test	-	-	-	-	+	-	-	+
Motility test	+	+	+	+	+	-	+	+
Indole test	+	+	+	+	+	+	+	+
Simmon's citrate test	-	-	-	-	-	-	+	+
Fructose	+	+	+	+	+	+	+	++
Lactose	-	-	-	-	+	+	+	++
Maltose	-	+	+	-	-	+	-	++
Glucose	+	+	-	+	+	+	+	++.
Sucrose	-	+	-	+	+	+	+	++
Galactose	++	++	++	++	+	+	+	++
Mannitol	++	-	++	++	-	-	+	++
D-xylose	++	-	++	++	-	-	+	++.

Isolates	1	2	3	4	5	6	7	8
Salicin	+	-	-	+	-	-	+	++
Arabinos	+	+	+	+	+	+	+	+
Probable Identity	*Bacillus cereus*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Proteus vulgericus*	*Streptococcus agalactiae*	*Actinomyces bovis*	*Staphylococcus epidermidis*

Keys: + = Positive, ++ = Positive and Gas production, - = Negative

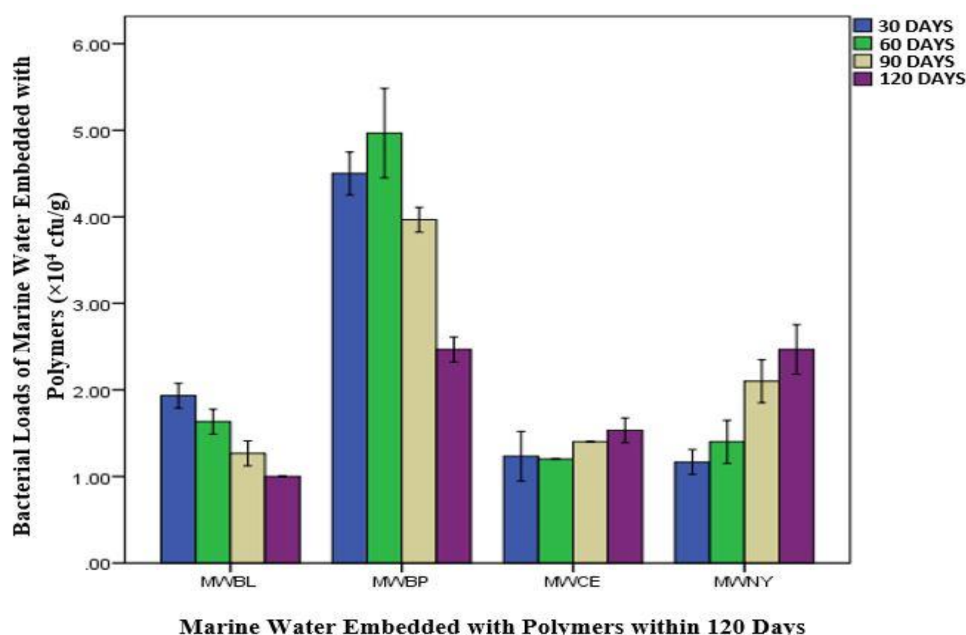

Fig. 2. Bacteria Loads of Marine Water with Bioplastics Nylon, Cellulose and Synthetic Nylon ($\times 10^4$ cfu/g)

Key: MWBL = Marine Water Blank, MWCE = Marine Water Cellulose, MWBP = Marine Water Bioplastics, MWNY = Marine Water Nylon 6

4.5 Distributions of Bacterial Isolates of Freshwater with the Biodegradable LDPE, Polyethylene, Cellulose and Control across Months

In Table 4, the distributions of bacteria isolated in freshwater samples with test materials across the four months were shown. Bacteria isolates such as *Bacillus cereus, Enterococcus faecalis, and Proteus vulgericus* were obtained and present throughout the four months across all the samples (water with cellulose (CE), water with bioplastic (PBS 1020), and water with nylon 6 samples), although *Proteus vulgericus* was absent in the third month in the water embedded with cellulose sample. However, *Staphylococcus epidermidis* was present in two (water with cellulose (CE), and water with nylon 6) samples across the four months, whereas *Staphylococcus epidermidis* was present in the first, third, and fourth month in the water with bioplastic (PBS 1020) samples and was found absent in the second month.

4.6 Distributions of Bacteria Isolates of Marine Water with the Biodegradable LDPE, Polyethylene, Cellulose and Control across Months

Table 5 showed the distributions of bacterial isolates reported in marine water samples with test materials in the four-month period of the experiment. *Enterococcus faecalis and Proteus vulgaricus* were present in marine water with cellulose (CE), and marine water with nylon 6 samples across the four months. Similarly, *Bacillus cereus* and *Proteus vulgericus* were present in the marine water with bioplastic (PBS 1020) across the four months while *Enterococcus faecalis* was isolated in the third and fourth months of the same samples. However, in marine water entrenched with cellulose (CE), *Pseudomonas aeruginosa* was present in the first and second months only while the presence of *Bacillus cereus* was observed in the first, third, and fourth months. Similarly, in marine water with nylon 6, *Bacillus cereus* was present throughout the months.

4.7 Molecular Analyses of The Bacteria Isolates

Plate 1 and Table 6 exhibited the agarose gel indicating the amplification of the 16S target region and the BLAST (Basic Local Alignment Search Tool) results, which correspond to the similarity between the sequences queried and the biological sequences in the NCBI database of bacterial isolates, respectively. The probable identity of isolates was selected for molecular confirmation because they were predominant in all the various sampled environments.

Thereafter, the bacterial isolates *Bacillus cereus (ISO 1), Staphylococcus aureus (ISO 4) and Actinomycetes bovis (ISO 7)* were sent out for confirmation by molecular methods (DNA Sequencing). Subsequently, further analysis of the sequence using the BLAST of the NCBI server showed that all the isolates were *Bacillus cereus* with 99.85% (ISO 1), 99.68% (ISO 4) and 99.68% (ISO 7) similarities, respectively.

Table 4. Distributions of Bacteria Isolates in Freshwater Across Months

Polymer	February	March	April	May
Cellulose (CE)	*Staphylococcus epidermidis, Bacillus cereus, Enterococcus faecalis, Proteus Vulgericus,*	*Enterococcus faecalis, Proteus Vulgericus, Staphylococcus epidermidis, Bacillus cereus,*	*Staphylococcus epidermidis, Bacillus cereus, Enterococcus faecalis,*	*Staphylococcus epidermidis, Bacillus cereus, Proteus Vulgericus, Enterococcus faecalis,*
Bioplastic (PBS 1020)	*Bacillus cereus, Enterococcus faecalis, Proteus Vulgericus, Staphylococcus epidermidis*	*Bacillus cereus, Enterococcus faecalis, Proteus Vulgericus,*	*Staphylococcus epidermidis, Bacillus cereus, Proteus Vulgericu, Enterococcus faecalis,*	*Bacillus cereus, Staphylococcus epidermidis, Proteus Vulgericus*
Nylon-6	*Enterococcus faecalis, Proteus Vulgericus, Bacillus cereus, Staphylococcus epidermidis*	*Proteus Vulgericus, Enterococcus faecalis, Bacillus cereus, Staphylococcus epidermidis*	*Bacillus cereus, Staphylococcus epidermidis, Proteus Vulgericu*	*Proteus Vulgericus, Bacillus cereus, Staphylococcus epidermidis, Enterococcus faecalis,*

Table 5. Distributions of Probable Bacteria Isolates of Marine Water Across Months

Polymer	February	March	April	May
Cellulose (CE)	*Bacillus cereus, Enterococcus faecalis, Proteus Vulgericus, Pseudomonas aeruginosa*	*Enterococcus faecalis, Proteus Vulgericus, Pseudomonas aeruginosa*	*Proteus Vulgericus, Bacillus cereus, Enterococcus faecalis*	*Enterococcus faecalis, Bacillus cereus, Proteus Vulgericus*
Bioplastic (PBS 1020)	*Bacillus cereus, Proteus Vulgericus*	*Proteus Vulgericus, Bacillus cereus*	*Enterococcus faecalis, Bacillus cereus, Proteus Vulgericus*	*Bacillus cereus, Enterococcus faecalis, Proteus Vulgericus, Pseudomonas aeruginosa*
Nylon-6	*Proteus Vulgericus, Bacillus cereus, Enterococcus faecalis*	*Bacillus cereus, Enterococcus faecalis, Proteus Vulgericus, Pseudomonas aeruginosa*	*Proteus Vulgericus, Bacillus cereus*	*Enterococcus faecalis, Proteus Vulgericus, Bacillus cereus*

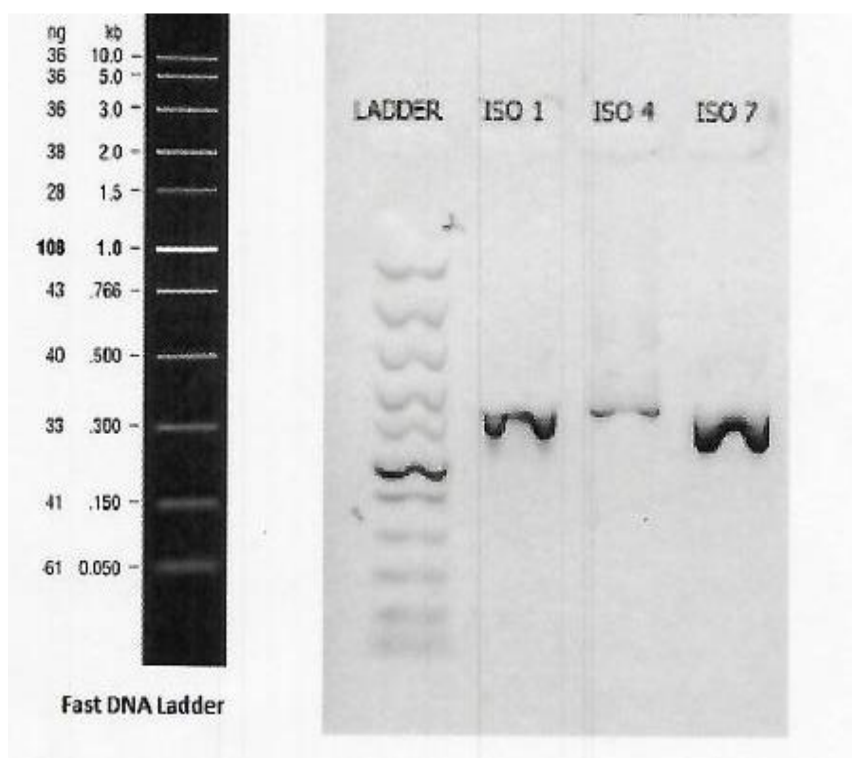

Plate 1. Photographic Image of an Agarose Gel Indicating the Amplification of the 16S Target Region for Bacteria Isolates

Table 6. Blast Results of Sequence Queried for Bacteria Isolates

Name of Sample	**ISO 1**
Percentage ID	99.85%
Predicted Organism	*Bacillus cereus*
NCBI Accession	MK202350.1
Name of Sample	ISO 4
Percentage ID	99.68%
Predicted Organism	*Bacillus cereus*
NCBI Accession	FJ790330.1
Name of Sample	ISO 7
Percentage ID	99.68%
Predicted Organism	*Bacillus cereus*
NCBI Accession	ON763278.1

4.8 Experimental Setup for Assessing Biodegradation Test of Aquatic Environments

Plate 2 showed the biodegradation setup with the freshwater and marine water containing the test samples with a beaker containing KOH placed on top of another beaker.

4.9 Physical Observation of Biodegradation Process of Polymers by Bacteria Species across Months

Plates 3 and 4 showed the physical observations of the biodegradation processes of bioplastic (PBS 1020) and nylon 6 material samples observed across the four months of the experiment before microbial attack up to the final degradation of the samples in the different aquatic environments.

The plates showed the degradation processes of bioplastic, which was visibly observed in the first and second months. However, the bioplastic could barely be visibly observed in the sampled environments in the third month and was completely not visible in the fourth month of the experiment due to the microbial activities; whereas for nylon 6, there was no change that was observed throughout the four months.

Plate 2. Experimental Setup for Aquatic Environments

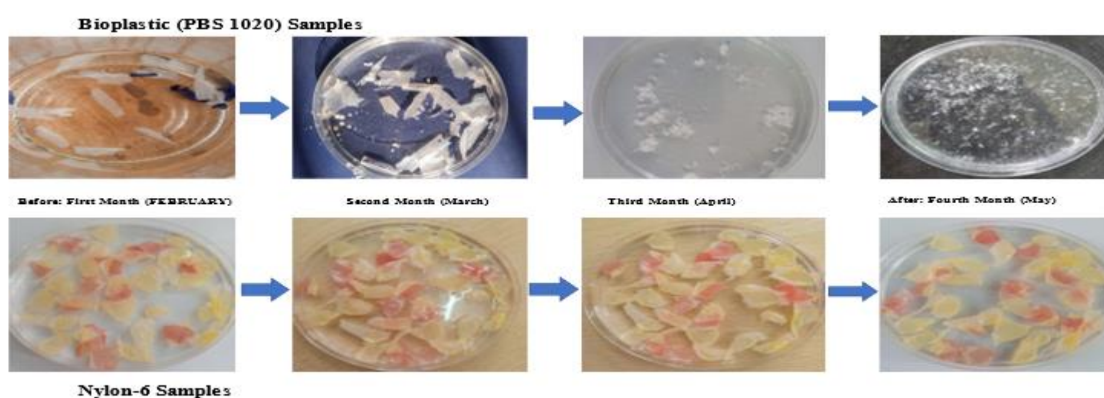

Plate 3. Stages in the Biodegradation Processes of Bioplastics and Nylon 6 per Month in Freshwater

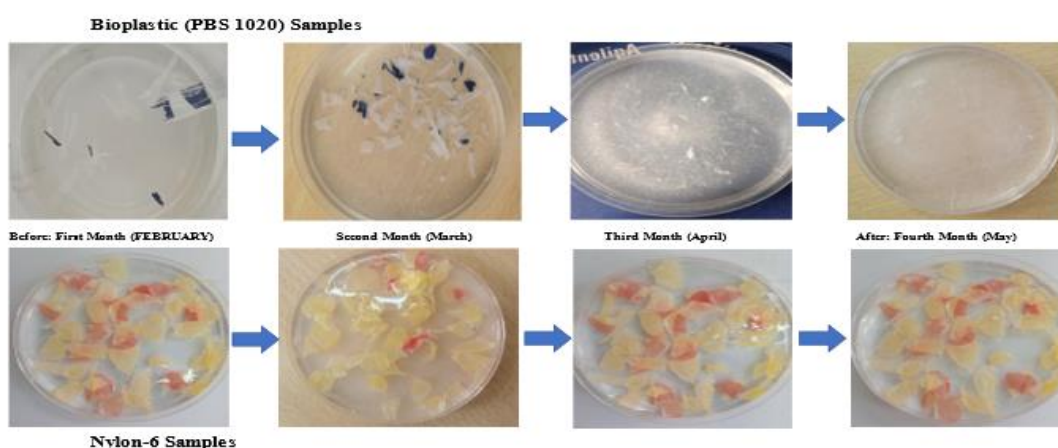

Plate 4. Stages of Biodegradation Processes of Bioplastics and Nylon 6 per Month in Marine Water

5. DISCUSSION

Bioremediation of biodegradable single use LDPE bioplastics by indigenous microbes has been identified as a green method for resolving plastic pollution problems in the environment [14; 15]. In this study, bioplastic single use LDPE nylon (PBS 1020), conventional nylon 6 (PE) and cellulose (CE) were subjected to microbial degradation in fresh water and marine water. In

the two aquatic environments, cellulose, being a natural plastic, biodegraded faster than the bioplastic LDPE [16] while Nylon 6 was found to be recalcitrant because there was no change in the weights of the Nylon 6 after being subjected to microbial activity for four months. This showed that the biodegradability of polymers dependents on the nature of the polymer and other environmental factors. This outcome supports the findings of [17] and [18] who concluded that the biodegradation of bioplastics materials depends on the environment and the nature of the bioplastic material. Among several test methods for evaluating polymer biodegradation, respirometry methods are reliable procedures for determining the final biodegradation (mineralization) of polymeric carbon mineralized as CO_2 under aerobic environments [19] Other major biodegradation tests, such as weight loss, and microbial monitoring, among others, are not established methodologies for making the scientific assertion that polymeric materials are biodegradable and compostable [20]. Conversely, evidence of carbon dioxide and water released as end products of the aerobic biodegradation of polymeric materials is necessary to establish biodegradability claim. The test samples were cut into 2 cm x 2 cm pieces to ensure easy microbial degradation. [21] confirmed that the thickness of a material can affects the biodegradability of the material. Besides, [22] reiterated that factors affecting the process of biodegradation include humidity, temperature, oxygen content, pH, nutrient availability, and the presence of microorganisms. The results of the physicochemical properties of the fresh water and marine water samples, which were necessary for optimum growth of the microorganisms responsible for the biodegradation as well as increased microbial activity. The physicochemical properties of the freshwater and marine water recorded amounts of the various variables with slight differences in their values and could be due to the time of assessment and differences in temperature between the sites, as well as other environmental factors. This is supported by the findings from [23] and [24] who obtained similar results, although parameters obtained from the freshwater and marine water differ slightly. This may be attributed to other environmental factors and anthropogenic influence around the source of the sampled water.

However, the concentrations of lead and cadmium in freshwater sample were within the WHO recommended standard, while in the marine water sample it exceeded the limit but was within the recommended standard for the United States Environmental Protection Agency (USEPA). This may be due to the composition of the test polymeric materials. The high bacterial loads in the water were often due to putrefactive activities going on the bioplastics and cellulose which are primarily carried out by these bacteria. Initially, there was always a steady increase in the bacteria loads which gradationally decreased towards the end due to the loss of nutrients which the microbes are acting on for survival in the biodegradation processes in all the sampled (terrestrial and aquatic) environments. This is supported by research carried out by [25]; although in marine water and freshwater with the synthetic nylon 6 there were constant increase in the bacterial load and thereafter sudden decrease, which could be because of the recalcitrant nature of the non-biodegradable Nylon 6. The bacteria known to have very high activity in terms of plastic degradation in the freshwater samples and marine water were *Bacillus cereus, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgericus, Streptococcus agalactiae, Actinomyces bovis, and Staphylococcus* epidermidis.

According to [26] these are mostly involved in the degradation of different polymeric substances, including thermoplastic degradation. Moreover, [27] reiterated that *Bacillus sp., Pseudomonas sp., Staphylococcus sp., and Salmonella* have been tested to be used as starter cultures in the disintegration of different forms of polymeric materials. The plastics serve as carbon source as bioplastic's hydrolytic disintegration involved enzymatic hydrolysis followed by microbial absorption of tiny molecules of hydrolysable bioplastic monomers [28; 29]. However, molecular analysis of the most predominant microbes isolated from all the sampled environments showed that *Bacillus cereus* was confirmed to be the microbes responsible for the biodegradation processes. These strains isolated in this study could have potential for use in the management of bioplastic wastes.

6. CONCLUSION

All the *bacteria species* isolated and identified in this research are known to degrade heteropolymers of different types efficiently. A combination of these microorganisms in the samples analyzed in this research has

showed that they can synergistically be used to degrade heteropolymers. Thus, it is concluded that the biodegradation of real biodegradable bioplastic materials can occur in aquatic environment with evidence of total biodegradation which also depends on both the environmental conditions in which they are placed and the chemical nature of the polymeric material.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yunus M, Fauzan R. Mechanical properties of bioplastics cassava starch film with Zinc Oxide nanofiller as reinforcement. In IOP conference series: Materials Science and Engineering. 2017;210(1): 012015-012023.
2. Dada OE, Bada AA, Okorodo, EI. Natural biodegradation rates of single-use blended bioplastic packaging nylon entrenched in freshwater and marine water environments of the tropics. Pollution. 2023;9(3):11-20.
3. Nazareth M, Marques MR, Leite MC, Castro IB. Commercial plastics claiming biodegradable status: is this also accurate for marine environments? Journal of Hazard Materials. 2019;366:714–722.
4. American Society for Testing and Materials (ASTM) Standard. Standard Test Method for Crimp Frequency of Manufactured Staple Fibers, ASTM D. 2012;3937-12:256-259.
5. Akinmulewo AB, Nwinyi OC. Polyhydroxyalkanoate: A biodegradable polymer (a mini review). Journal of Physical and Conferenc Series. 2019 ;1378:042007.
6. Ghosh K, Jones BH. Association of Chemical Scientist. Sustainable Chemistry and Engineering. 2021;9(18):6170-6187.
7. Schmidt C, Krauth T, Wagner S. Export of plastic debris by rivers into the sea. Environmental Science and Technology. 2017;51:12246–12253
8. Dada OE. Land-based plastic pollution and biocontrol in developing countries : Issues challenges and directions. Journal of Engineering. 2020;25(1):1–10.
9. Alsafadi D, Ibrahim MI, Alamry KA, Hussein MA, Mansour A. Utilizing the crop waste of date palm fruit to biosynthesize polyhydroxyalkanoate bioplastics with favorable properties. Science of Total Environment. 2020;737:139716.
10. APHA, AWWA, WPCF. Standard methods for the examination of water and waste water 20th Edition. APHA, NY, USA; 1998.
11. Jeffries TC, Rayu S, Nielsen UN, Lai K, Ijaz A, Nazaries L, Singh BK. Metagenomic functional potential predicts degradation rates of a model organophosphorus xenobiotic in pesticide contaminated soils. Frontier Microbiology. 2018;9:147.
12. Aurora DR, Aurora BB. Textbook of Microbiology. CBS Publishers and Distributors Private Limited, India. 2017;696.
13. Karan H, Funk C, Grabert M, Oey M, Hankamer B. Green bioplastics as part of a circular bioeconomy. Trends Plant Science. 2019;24:237–249
14. Muniyasamy S, Dada OE. Recycling of plastics and composites materials and degradation technologies for bioplastics and biocomposites. In: Waste Management in fashion and textile industry. Nayak K, Patnaik I. (eds.). Book Chapter 17. Elsevier Woodhead Publishing, United Kingdom. 2021;346-357.
15. Nomadolo N, Dada OE, Swanepoel A, Mokhena T, Mniyasamy S. A comparative study on the aerobic biodegradation of the biopolymer blends of poly(butylene succinate), Poly(butylene adipate terephthalate) and Poly(lactic acid). Polymers. 2022;14:1894-1899.
16. Hauenstein O, Reiter M, Agarwal S, Rieger B, Greiner A. Bio-based polycarbonate from limonene oxide and CO_2 with high molecular weight, excellent thermal resistance, hardness and transparency. Green Chemistry. 2016; 18:760–770
17. Pellis A, Malinconico M, Guarneri A, Gardossi L. Renewable polymers and plastics: Performance beyond the green. New Biotechnology. 2021;60: 146–158.

18. Yasin S, Bakr ZH, Ali GAM, Saeed I. Recycling nanofibers from polyethylene terephthalate waste using electrospinning technique. In:Waste recycling technologies for nanomaterials manufacturing. Makhlouf ASH, Ali GAM. (eds.) Springer International Publishing: Champere, UK. 2021;701–727.
19. Muniyasamy S, Ofosu OB, Thulasinathan B, Swetha A, Rajan T, Ramu SM, Soorangkattan S, Muthuramalingam JB, Alagarsamy A. Thermal-chemical and biodegradation behaviour of alginic acid treated flax fibres/ poly(hydroxybutyrate-co-valerate) PHBV green composites in compost medium. Biocatalysis and Agricultural Biotechnology. 2019;22: 1-8.
20. Gandini A, Lacerda TM, Carvalho AJF, Trovatti E. Progress of polymers from renewable resources: furans, vegetable oils, and polysaccharides. Chemical Review. 2016;11:1637–1669.
21. Wieczorek AM, Croot PL, Lombard F, Sheahan JN, Doyle TK. Microplastic ingestion by gelatinous zooplankton may lower efficiency of the biological pump. Environmental Science Technology. 2019;53:5387–5395
22. Neves A, Moyne M, Eyre C, Casey B. Acceptability and societal impact of the introduction of bioplastics as novel environmentally friendly packaging materials in Ireland. Clean Technology. 2020;2:127–143.
23. Kleij E, AW, Luinstra GA. Fully bio-derived CO_2 polymers for non-isocyanate based polyurethane synthesis. Green Chemistry. 2020;22:969–978.
24. Shen M, Song B, Zeng G, Zhang Y, Huang W, Wen X, and Tang W. Are biodegradable plastics a promising solution to solve the global plastic pollution? Environmental Pollution. 2020;263(Pt A):114469.
25. Moshood TD, Nawanir G, Mahmud F, Mohamad F, Ahmad MH, AbdulGhani A. Sustainability of bioplastics: New problem or solution to solve the global plastic pollution? Current Research in Green and Sustainable Chemistry. 2022;5:100273-100291.
26. Venkatesh S, Mahboob S, Govindarajan M, Al-Ghanim KA, Ahmed Z, Al-Mulhm N, Gayathri R, Vijayalakshmi S. Microbial degradation of plastics: Sustainable approach to tackling environmental threats facing big cities of the future. Journal of King Saud University – Science. 2021; 33:101362-101368.
27. Havstad MR. Biodegradable plastics. Plastic Waste Recycling. Elsevier. 2020; 97-129.
28. Ragas AMJ. Wear and tear of tyres: a stealthy source of microplastics in the environment. International Journal of Environmental Resources and Public Health. 2017;14:1265.
29. Dada OE, Adeola AA. Carbon Emission Pathways of Biodegradable Thermoplastic-Based Species in Natural and Simulated Aqueous Conditions. Asian Journal of Ecology and Environment. 2024;23(7): 49-63.

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