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Biodegradation of Plastics using Bacteria Isolated from Wastes Dumping Soil within Sokoto Metropolis

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Abstract

Plastic pollution, particularly polyethylene, has become a significant environmental concern worldwide due to its non-biodegradable nature. In urban areas, waste dumping sites serve as critical hotspots for the accumulation of plastic waste. The microbial degradation of polyethylene offers a promising solution to mitigate this environmental challenge. This study investigates the biodegradation potential of polyethylene plastics by bacteria isolated from the soil of waste dumping sites in Sokoto Metropolis, Nigeria. Soil samples were collected from three waste dumping sites within the metropolis, and bacteria were isolated using standard microbiological techniques. The isolates were screened for their ability to degrade polyethylene by assessing their growth and activity in minimal salt medium (MSM) supplemented with polyethylene strips as the sole carbon source. Key parameters, including pH and temperature, were analysed, and the degradation rate was monitored through weight loss of polyethylene discs, optical density readings, and changes in microbial growth at different time intervals (day 0 to day 28). The results indicated that isolates like *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Arthrobacter citreus* exhibited significant biodegradation activity, with weight loss of polyethylene ranging from 10% to 50% after 28 days of incubation. The bacterial isolates showed optimal growth at a temperature of 37°C and at a pH of 7.0. Isolates were identified using 16S rRNA gene sequencing, revealing a diverse range of bacteria, including species of *Bacillus* and *Pseudomonas*, known for their plastic-degrading capabilities. The findings contribute to the growing body of knowledge on sustainable waste management practices and underscore the importance of harnessing local microbial resources in the fight against plastic pollution. The biodegradation potential of these bacteria could be further explored for bioremediation strategies to mitigate plastic waste in urban environments.

Keywords: Polyethylene, Plastic, Biodegradation, Soil, *Bacillus subtilis*,

INTRODUCTION

Biodegradation is the process in which microorganisms like fungi and bacteria degrade the natural polymers (lignin, cellulose) and synthetic polymers (polyethylene, polystyrene) (Igior et al., 2024). It is defined as the process, which is capable of decomposition of materials into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms, that can be measured by standard tests, in a specified period of time, reflecting the available disposal condition (Katnic et al., 2024). Biological degradation is also chemical in nature, but the source of the attacking chemicals is from microorganisms. These chemicals are of a catalytic nature, e.g.,

enzymes (Katnic et al., 2024). The susceptibility of polymers to microbial attack generally depends on enzyme availability, availability of a site in the polymers for enzyme attack, enzyme specificity for that polymer, and the presence of a coenzyme if required (Katnic et al., 2024).

Global plastics production has increased remarkably since its large-scale manufacture began in the 1940s, reaching over 360 million tonnes (Mt) in 2021 (Nabi et al., 2024). Today, as much as 99 Mt of plastic waste is estimated to be improperly disposed of per annum (Parlak, 2024), causing significant environmental damage. For this reason, considerable efforts have been placed towards isolating novel microorganisms and enzymes with traits for plastic degradation. However, evidence for the

microbial degradation of a majority of even the globally most abundant plastic types remains very limited (Pavlov *et al.*, 2025). Biodegradable plastics are defined as those which can be degraded by biological, and principally microbial processes. Reliable reports of microbial plastic degradation are restricted mainly to polycaprolactone (PCL), polylactic acid (PLA), and polyurethane (PU), with a smaller number of studies on polyethylene terephthalate (PET) (Burelo *et al.*, 2025). These plastics, which contain C=O and C-O-C bonds within and along their polymer backbone, comprise only a minority of global plastics production (estimated as <1%, <1%, 5% and 7%, respectively (Primier *et al.*, 2020).

The accumulation of plastic waste in the environment, particularly in dumping sites, poses a significant ecological threat due to its non-biodegradable nature and its persistence over long periods (Dey *et al.*, 2024). Plastic waste is one of the most persistent and widespread pollutants in the environment, causing severe harm to the ecosystem, wildlife and human health (Islam, 2025). Traditional plastic disposal methods such as landfilling and incineration are inadequate and often contribute to environmental pollution, affecting soil, air and water quality (Dey *et al.*, 2024). Although some microorganisms have been identified with the potential to degrade plastics, the diversity and effectiveness of plastic-degrading bacteria in various dumping sites remain largely unexplored (Kumar *et al.*, 2024). There is a need to identify and screen bacteria that possess efficient plastic biodegradation abilities, which could provide a more sustainable solution for managing plastic waste (Mishra *et al.*, 2019).

Current plastic disposal methods, such as landfilling and incineration, are inadequate in addressing the scale of plastic pollution and often contribute to further environmental degradation (Singh *et al.*, 2024). Therefore, finding an alternative, eco-friendly solution is of paramount importance. The isolation and characterisation of plastic-degrading bacteria could lead to the development of biotechnological applications that offer a more sustainable and cost-effective solution to plastic pollution (Jayaseelan and Banerjee, 2024). Unlike chemical or physical methods, microbial degradation can be environmentally friendly, reducing the ecological footprint of waste management (Wang *et al.*, 2024). This study aimed to determine the potential of bacteria isolated from some

dumpsite soils and their potential in the biodegradation of plastics.

MATERIALS AND METHODS

Sampling Area and Sample Collection

Soil samples were collected in clean polyethylene bags from plastic waste disposal site in Mabera, Sokoto, located at 13.03046° N latitude and 5.25935° E longitude, as well as from two plastic waste disposal sites within Usman Danfodiyo University, Sokoto, with coordinates of 13.1204° N latitude and 5.2341° E longitude. Samples were collected from surface area (0-10) cm. The samples were transported to the microbiology laboratory for analysis.

Physico-chemical Analysis of Soil Samples

Determination of pH

The soil pH was determined according to the method of the International Institute of Tropical Agriculture (Oladoye, 2015). Twenty (20) grams of air-dried soil were sieved and placed in a 50 ml capacity beaker. Twenty (20) milliliters of distilled water were added and allowed to stand for 30 minutes, while stirring occasionally with a glass rod. The pH meter was calibrated with a buffer of pH 7.0 before use. The electrode of the pH meter was inserted into the partly settled suspension, and the reading on the pH meter was noted and recorded accordingly in triplicate, and the mean was taken.

Determination of Temperature

Temperature was determined at the point of sample collection by dipping the bulb of a mercury-in-glass thermometer into the soil, and readings were recorded using the procedure of AOAC.

Determination of Moisture Content

An empty crucible was weighed (W_0), and 2g of soil was added and weighed again (W_1). Samples were dried in a hot air oven at 105°C until constant weight was achieved (W_2). Both the container and the dried sample were weighed again (IITA). The moisture content was calculated as follows:

$$\% \text{ moisture} = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Determination of Nitrogen Content of the Soil

Total nitrogen was determined by the Macro-Kjeldahl digestion method of Juo (2021). Five

grams of soil sample were weighed into a 500ml Macro-Kjeldahl flask, and 20ml of distilled water was added. The content was swirled for five minutes and allowed to stand for 30 minutes. One tablet of mercury catalyst and 10g of K₂SO₄ were added, and 30ml of concentrated H₂SO₄ was added through an automated pipette. The content of the flask was heated gently in the digestion stand. After cooling, 100ml of distilled water was added and transferred into another clean Macro-Kjeldahl flask (750ml), and the sand residue was washed four times with 50ml of distilled water. All the washings were transferred into the same flask. Fifty milliliters (50ml) of H₃BO₃ indicator solution were added to a 500ml Erlenmeyer flask, which was placed under the condenser of the distillation apparatus, and 150ml of 10N NaOH were introduced. This was followed by distillation. For the condenser to remain cool (30°C) and prevent frothing, sufficient cold water was allowed to flow through the condenser. Ammonium was determined in the distillate by treating it with 0.01N standard H₂SO₄ using a 25ml burette graduated at 0.1ml intervals. The colour changed at the endpoint from green to pink (Premei *et al.*, 2020). The percentage nitrogen was calculated using the formula:

$$\% \text{Nitrogen} = \frac{N \times 0.014 \times V_d \times 10}{A \times \text{weight of sample}} \times 100$$

Where: N = Normality of acid,

V_d = Volume of the digest,

A = Aliquot of digest

Determination of Cation Exchange Capacity

Twenty milliliters of 0.1 M BaCl₂ saturating solution were added to 2 g of air-dried soil in a pre-weighed centrifuge tube (plastic) and continuously shaken for 2 hours in a Thermolyne shaker at 300rpm and at room temperature. After shaking, the solution was centrifuged (Marathon 3200R, Fisher Scientific, USA) at 3000 rpm for 10 minutes and decanted. This was followed by equilibrating the soil with three successive 20 ml increments of 0.002M BaCl₂. Each time, the solution was sonicated using a Vortex mixer (Model S8223, Genie, USA) for 30 seconds, followed by shaking on a Thermolyne shaker at 300rpm for 1 hour. The solution was centrifuged at 3000 rpm for 10 minutes, and the supernatant was discarded (Premei *et al.*, 2020).

The centrifuge tube plus soil and entrained 0.002M BaCl₂ solution were weighed following

the last decantation of the supernatant. Then, 10 ml of 0.005M MgSO₄ reactant solution was added to the soil and gently shaken at 200 rpm for 1 hour in a Thermolyne shaker. The exchange capacity of the reactant suspension was measured and adjusted to the exchange capacity of the 0.0015M MgSO₄ ionic strength reference solution by measuring the conductivity. After shaking the samples gently at 200rpm overnight, the conductivity of the reactant suspension was adjusted to that of the 0.0015 M MgSO₄ ionic strength reference solution using distilled water. The centrifuge tubes and contents were weighed to determine the volume of MgSO₄ or water that needed to be added to adjust the conductivity. This was followed by centrifugation at 3000rpm for 10 minutes, and the supernatant was decanted and retained for analysis. The solution was analysed for magnesium using the PerkinElmer Analyst 300 Atomic Absorption Spectrophotometer (Perkin Elmer, USA), and the pH was also measured using a pH meter (SympHony SB20, Mettler Toledo, USA). The CEC was calculated from the following equation:

$$\frac{\text{CEC in meq}}{100\text{g}} = \frac{100(0.01 - C_1)V_1}{\text{Oven dry weight of soil sample in gram}}$$

Where V₁ is the volume of final supernatant solution and C₁ is the concentration of Mg in the supernatant, meq= (milliequivalents/milliliter)

Determination of Soil Particle Size

The hydrometer method for the mechanical analysis of particle size distribution presented by Gee and Bauder and adopted by Ibrahim *et al.* (2014) was used. Air-dried soil was sieved using a 425µm pore size sieve. Fifty-one grams (51g) were transferred into 1 liter and shaken to mix. Fifty milliliters (50ml) of 5% sodium hexametaphosphate were added, followed by 100ml of distilled water. The soil suspension was stirred thoroughly for 15 minutes and transferred into a cylinder containing a hydrometer. Distilled water was added to the lower blue line of the cylinder. The volume changed to 1130ml, and the hydrometer was removed. The top of the cylinder was covered with a hand and inverted several times until all the soil was in suspension. The cylinder was placed on a flat surface, and the time was noted. The hydrometer was placed in the suspension, and the first reading (H1) was noted immediately after 40 seconds. Subsequently, the temperature (T1) was recorded after the hydrometer was removed. The suspension was allowed to stand for 3 hours, and the second

reading was taken for the hydrometer (H₂) and the temperature (T₂), respectively.

The percentage of sand, clay, and silt was calculated thus:

$$\begin{aligned}\text{Sand} &= 100 - (H_1 + 0.3(T_1 - 20) - 2.0)2 \\ \text{Clay} &= H_2 + 0.3(T_2 - 20) - 2.0 \\ \text{Silt} &= 100 - (\% \text{ sand} + \% \text{ clay})\end{aligned}$$

Enumeration of Bacterial Loads in the Soil Samples

A stock solution for serial dilution was made by dispensing 1 gram of soil into 100ml of distilled water, shaking thoroughly, and transferring one (1) mL into a test tube containing 9ml of sterile distilled water, subsequently making a serial dilution up to 10⁻⁵. Using the spread plate method technique, 0.1 ml of the suspension from dilutions of 10⁻³, 10⁻⁴, and 10⁻⁵ was plated aseptically on prepared Nutrient Agar (NA) and incubated at 30°C for 24 hours. The results were determined by multiplying the number of counts by the dilution used and expressed as colony-forming units per gram (cfu/g) of soil (Premi *et al.*, 2020).

Morphological and Biochemical Characterisation of the Bacterial Isolates

Gram's Staining

Gram staining was performed following Harley and Prescott (2002). The bacterial smear was stained with crystal violet, treated with iodine, decolourised with ethanol, and counterstained with safranin. Under oil immersion, Gram-positive bacteria appeared bluish-purple, while Gram-negative bacteria appeared red or pink.

Spore Staining Test

A bacterial smear was prepared on a slide, allowed to air-dry, and then the slide was heat-fixed, placed over a steaming water bath, covered with a piece of paper towel, and saturated with malachite green stain. The slide was then left to cool, rinsed thoroughly with water to remove excess stain, counterstained with safranin for 1-2 minutes, rinsed again with water, and blotted dry. Under a microscope, endospores appeared green, while vegetative cells appeared pink (Premi *et al.*, 2020).

Catalase Test

A drop of 3% hydrogen peroxide solution was placed on a glass slide onto which bacterial

colonies were added. The presence of catalase was observed by the formation of oxygen bubbles (Ochei and Kolhatkar, 2000).

Oxidase Test

An oxidase reagent (1% Tetramethyl paraphenylene diamine dihydrochloride) was placed on a Whatman paper filter, and a bacterial colony was smeared on the paper. The presence of the enzyme oxidase was observed by the appearance of a purple colour (Ochei and Kolhatkar, 2000).

Triple Sugar Ion Test (TSI)

The surface of a TSI agar slant was inoculated by streaking it with a sterile loop, and the butt of the agar was stabbed deeply to introduce the bacteria. The tube was incubated at 35-37°C for 18-24 hours. Observations were made for colour changes: yellow indicated acid production (sugar fermentation), red indicated an alkaline reaction (no fermentation), and black precipitate indicated H₂S production. Gas production was evidenced by cracks or bubbles in the medium (Ochei and Kolhatkar, 2000).

Methyl Red (MR) Test

The Methyl Red (MR) test detects the ability of bacteria to produce stable acids from glucose fermentation. It helps distinguish between bacteria that produce mixed acids and those that produce non-acidic end products during glucose fermentation. An MR-VP broth was inoculated with the bacterium. The broth was incubated. After incubation, Methyl Red reagent was added to the broth. The colour change was observed for some Bacterial isolates. A red color indicates a positive MR reaction, indicating the presence of mixed acids. Positive result, development of a red colour in the broth after the addition of Methyl Red reagent. Negative result, no colour change or development of a yellow colour (Ochei and Kolhatkar, 2000).

Voges-Proskauer (VP)

MR-VP broth was inoculated with the bacterium, and the broth was incubated. After incubation, α-naphthol and potassium hydroxide (KOH) reagents were added to the broth. The colour change was observed. A red colour indicates a positive VP reaction, indicating the presence of acetoin. Development of a red colour in the broth after the addition of α-naphthol and KOH reagents indicates a positive result. Negative

result, no colour change or development of a yellow colour (Ochei and Kolhatkar, 2000).

Indole Production Test

The indole test detects the ability of bacteria to produce indole from the hydrolysis of tryptophan by the tryptophanase enzyme. A tryptone broth was inoculated with the bacterium. The broth was incubated. After incubation, 1 ml of Kovac's reagent was added to the broth. The colour change was observed. A red colour in the organic layer indicates a positive indole reaction, indicating the presence of indole. Positive result, development of a red colour in the organic layer after the addition of Kovac's reagent. Negative result, no colour change or development of a yellow colour was observed (Ochei and Kolhatkar, 2000).

Molecular Characterisation of the Most Potent Bacterial Isolates

Genomic DNA Extraction

The single colony of each bacterial isolate was grown in Luria-Bertani (LB) broth overnight at 28°C. Two (2) ml of the culture were centrifuged at 5000 rpm for 5 minutes, and the pellet was suspended in 200 µl of TE buffer at pH 8, containing RNase (50 ng/ml), to prevent degradation. Then, 400 µl of lysis buffer was added, followed by mixing well and incubation for 15 minutes at 37°C with intermittent shaking every 5 minutes. Immediately, chloroform and isoamyl alcohol in a ratio of 24:1 were taken and mixed by inversion. Tubes were centrifuged at 10,000 rpm for 5 minutes, and the supernatant was transferred carefully to another microcentrifuge tube. To the supernatant, 0.1 volume of 3 M sodium acetate (pH = 5.2) and 0.6 volume of isopropanol were added, mixed well by inversion, and kept on ice for 10 minutes, followed by centrifugation at 10,000 rpm for 10 minutes. The pellet was washed with 70% ethanol with gentle shaking and centrifuged at 10,000 rpm for 3 minutes. The supernatant was removed, and the pellet was air-dried (Balakrishnan *et al.*, 2022). Extracted DNA was visualised using 0.8% agarose gel electrophoresis, and images were documented.

16S rRNA gene PCR amplification

Using 16S ribosomal RNA gene-specific universal primers 27F 50-AGA GTT TGA TCC TGG CTC AG-30 and 1492R 50-GGT TAC CTT GTT ACG ACT T-30 (Sigma), the 16S rRNA gene was amplified with a 50 µl reaction mixture containing 1X

reaction buffer (10 mM Tris [pH 8.3, 50 mM KCl, and 1.5 mM MgCl₂), 200 µl dNTPs, 0.05 U Taq DNA polymerase enzyme (Sigma, USA), 0.5 µM of each primer, and 1 ng template DNA. The thermal cycling conditions were: 5 minutes at 94°C for initial denaturation; 31 cycles of 30 seconds at 95°C, 1 minute at 54°C, 2 minutes at 72°C, and a final extension for 5 minutes at 72°C. The amplification reaction was performed with a thermal cycler (MyCycler, Bio-Rad, USA), and the PCR amplicons (approximately 1500 bp) were resolved by electrophoresis in 1% (w/v) agarose gel to confirm the expected size of the product (Balakrishnan *et al.*, 2022).

PCR Product Purification

Isolates capable of degrading plastic were purified by two procedures: Ammonium sulphate precipitation method and ZnCl₂ precipitation method. By the Ammonium sulphate precipitation method, it consisted of four steps: ammonium sulphate fractionation, chilled acetone, hexane treatment, and silica gel column chromatography. By ZnCl₂ precipitation method: 10 ml of the culture supernatants were concentrated by ZnCl₂ to a final concentration of 75 mM. The precipitated material was dissolved in 10 ml of sodium phosphate buffer (pH 6.5), extracted twice with equal volumes of diethyl ether. The pooled organic phase was evaporated to dryness, and the pellets were dissolved in 100 µl of methanol. Further purification was achieved by preparative TLC (Jiraporn and Niran, 2023).

DNA Sequencing of 16S rRNA Gene Fragment

By using the ABI DNA 3730 XL sequencing system (Applied Biosystems), the 16S rRNA purified PCR product was submitted. Sequencing of the bacterial isolate's 16S rRNA gene was carried out in both directions. The bacterial species was determined with the obtained sequence, which was searched for BLAST. The sequences were submitted to the NCBI GenBank after sequence matching percentages and accession numbers were obtained (Balakrishnan *et al.*, 2022).

Phylogenetic Analysis

The 16S rRNA gene sequence obtained in this study was aligned with the sequences published in the National Centre for Biotechnology Information (NCBI). Aligned sequences were edited, and a phylogenetic tree was constructed using MEGA (version 6) software. The phylogenetic tree for the relationship among strains was constructed by the Maximum

Likelihood Method with a bootstrap of 500 using the Kimura-2 parameter (Balakrishnan *et al.*, 2022).

Optimisation of pH and Temperature

A full-factorial experimental design was employed to study the effects of pH and temperature on the biodegradation activity of the bacterial isolates. pH levels of 6.0, 7.0, and 8.0 were adjusted using phosphate buffer for pH 6-7 and carbonate buffer for pH 8. Temperature levels of 25°C, 37°C, and 40°C were observed. Minimal salt medium (MSM) broth was prepared and supplemented with LDPE strips, adjusting the pH to the desired values. Each bacterial strain was inoculated into separate flasks containing the MSM broth with plastic strips and incubated at the designated temperatures in a shaker incubator for 30 days (Ochei and Kolhatkar, 2000).

Microbial Degradation of Plastic in Laboratory Conditions

Determination of Weight Loss

Discs of 1 cm were prepared from polythene bags and weighed, then aseptically transferred to conical flasks containing 50 ml of culture broth medium inoculated with different bacterial species. A control was maintained with a plastic disc in a microbe-free medium. Different flasks were maintained for each treatment and left in a shaker. After one month of shaking, the plastic discs were collected, washed using distilled water, and then weighed for the final weight. From the data collected, weight loss was calculated (Huang *et al.*, 2012).

Spectrophotometric Assay

The degradation ratio of bacteria for polyethene plastic was also estimated by measuring the cell growth by reading the optical density at 600 nm. Two millilitres from each incubated flask were taken, and the optical density was measured at 600 nm in a spectrophotometer at weekly intervals (i.e., day 0, day 7, day 14, day 21, and day 28) to account for the rate of biodegradation (Barlaz *et al.*, 2019).

RESULTS

Physical and Chemical Properties of Some Selected Plastic Waste Dump Sites Soil

Table 1 presents the physicochemical properties of soil samples from three plastic waste dump sites: Mabera (MBR), Usman Danfodiyo University

First Gate (UDFG), and Usman Danfodiyo University Mini Mart (UDMM). The pH values across the sites are slightly acidic, ranging from 6.71 ± 0.042 in MBR to 6.75 ± 0.07 in UDMM, with minimal differences. Magnesium (Mg) concentrations are stable at 0.83 ± 0.03 cmol/kg in MBR and UDFG but increase to 1.00 ± 0.00 cmol/kg in UDMM. Sodium (Na) levels remain the same in MBR and UDFG at 0.275 ± 0.035 cmol/kg, but increase to 0.345 ± 0.007 cmol/kg in UDMM. Potassium (K) content increases from 1.41 ± 0.01 cmol/kg in MBR to 1.79 ± 0.01 cmol/kg in UDMM. The cation exchange capacity (CEC) is highest in UDMM (3.35 ± 0.07 cmol/kg), followed by UDFG (2.85 ± 0.07 cmol/kg) and MBR (2.8 ± 0.141 cmol/kg). Calcium (Ca) is lower in UDFG and UDMM (0.24 ± 0.01 cmol/kg) compared to MBR (0.325 ± 0.035 cmol/kg). Electrical conductivity (EC) is higher in UDFG (194.9 ± 0.84 $\mu\text{S}/\text{cm}$) and UDMM (185.95 ± 0.77 $\mu\text{S}/\text{cm}$) compared to MBR (77.2 ± 0.98 $\mu\text{S}/\text{cm}$). Phosphate (PO_4) levels are similar across the sites, with a slight decrease in UDMM (0.15 ± 0.01 mg/kg). Organic carbon (OC) is highest in UDFG ($0.43 \pm 0.01\%$) and lowest in MBR ($0.13 \pm 0.01\%$). Nitrogen (N) content is slightly higher in UDFG ($0.050 \pm 0.001\%$) compared to MBR ($0.040 \pm 0.002\%$) and UDMM ($0.048 \pm 0.001\%$). Soil texture analysis shows an increase in sand content from MBR ($87.35 \pm 0.070\%$) to UDMM ($92.7 \pm 0.84\%$), with a corresponding decrease in clay and silt.

Bacteriological Counts of Soil Samples from the Sampling Sites

Table 2 shows the bacteriological counts of soil samples from three distinct sampling sites, Mabera MBR, UDUS First Gate, and UDUS Minimart, showing both the mean bacterial colony count (CFU) and standard deviation (S.D.) for each site. Mabera MBR exhibits a mean bacterial count of 5.3×10^{-6} CFU, accompanied by a high standard deviation (3.81×10^{-6} CFU). UDUS First Gate has the lowest bacterial count (2.005×10^{-6} CFU) and minimal variability (S.D. = 0.275×10^{-6} CFU). However, UDUS Minimart shows the highest bacterial count (7.60×10^{-6} CFU) with moderate variation (S.D. = 1.13×10^{-6} CFU).

Morphological and Biochemical Characterisation of the Isolates

Table 3 presents biochemical identification data of various bacterial isolates, showing several key characteristics essential for species differentiation. The shape of the bacteria (either rods or cocci) is indicated in the first

column, with the second column showing whether the organisms produce spores. The Table also includes a series of biochemical tests: Gram reaction (Gra Rn), catalase, oxidase, methyl red (M-R), Voges-Proskauer (V-P), indole production, fermentation of glucose, lactose, and sucrose, as well as hydrogen sulfide (H₂S) production and gas production. From the data, it can be seen that organisms like *Bacillus*

subtilis and *Bacillus cereus* show distinct biochemical reactions such as positive spore formation and certain fermentation patterns. In contrast, *Pseudomonas* species like *Pseudomonas aeruginosa* and *Pseudomonas putida* display negative results for spore formation and distinct biochemical characteristics, including a negative catalase test and specific fermentation profiles.

Table 1: Physico-chemical Analysis of some selected Plastic Waste Dump Sites Soil

Parameters	MBR ± S.D	UDFG ± S.D	UDMM ± S.D
pH (cmol/kg)	6.71 ± 0.042	6.73 ± 0.03	6.75 ± 0.07
Mg (cmol/kg)	0.83 ± 0.03	0.83 ± 0.02	1.00 ± 0.00
Na (cmol/kg)	0.275 ± 0.035	0.275 ± 0.035	0.345 ± 0.007
K (cmol/kg)	1.41 ± 0.01	1.43 ± 0.02	1.79 ± 0.01
CEC (cmol/kg)	2.8 ± 0.141	2.85 ± 0.07	3.35 ± 0.07
Ca (cmol/kg)	0.325 ± 0.035	0.24 ± 0.01	0.24 ± 0.01
EC (µs/cm)	77.2 ± 0.98	194.9 ± 0.84	185.95 ± 0.77
PO ₄ (mg/kg)	0.161 ± 0.701	0.161 ± 0.001	0.15 ± 0.01
OC (%)	0.13 ± 0.01	0.43 ± 0.01	0.305 ± 0.021
N (%)	0.040 ± 0.002	0.050 ± 0.001	0.048 ± 0.001
%Sand	87.35 ± 0.070	89.15 ± 0.35	92.7 ± 0.84
%Silt	5.15 ± 0.21	5.15 ± 0.21	3.30 ± 0.14
%Clay	7.1 ± 0.28	5.15 ± 0.21	3.25 ± 0.07

Keys: MBR = Mabera, UDFG = Usman Danfodiyo University First Gate, UDMM = Usman Danfodiyo University Mini Mart, OC = Organic Carbon, N = Nitrogen, PO₄ = Phosphate, Ca = Calcium, Mg = Magnesium, K = Potassium, Na = Sodium, CEC = Cation Exchange Capacity, EC = Electrical Conductivity and % = Percentage

Table 2 Bacteriological Counts of Soil Samples from the Sampling Sites

Sampling Sites	Mean Bacterial Colony Count (CFU/g) ± S.D
MBR	5.3 × 10 ⁶ ± 3.81
UDFG	2.05 × 10 ⁶ ± 0.275
UDMM	7.60 × 10 ⁶ ± 1.13

Keys: MBR = Mabera, UDFG = Usman Danfodiyo University First Gate, UDMM = Usman Danfodiyo University Mini Mart and S.D. = Standard Deviation.

Frequency of Occurrence of the Bacterial Isolates

Table 4 shows the frequency and percentage occurrence of six bacterial isolates from a sample set of 10, revealing that *Bacillus subtilis* and *Pseudomonas aeruginosa* are the most predominant species, each accounting for 30% of the isolates. Other species such as *Pseudomonas putida*, *Bacillus alvei*, *Staphylococcus aureus*, and *Arthrobacter citreus* are less frequent, each contributing 10%.

Polyethylene Plastic Biodegradation by Bacteria at 25 °C and pH 6.0

Table 5 presents data on the biodegradation of polyethylene plastic by various bacterial isolates at 25 °C and pH 6.0, showing both the initial and final weights of the plastic samples as well as the

percentage of weight lost. All isolates exhibit no change in the weight of the polyethylene, with each sample maintaining a final weight of 20g, indicating a 0% weight loss. This suggests that under the experimental conditions (25 °C and pH 6.0), none of the bacterial isolates tested demonstrated any significant biodegradation activity on polyethylene plastic.

Polyethylene Plastic Biodegradation by Bacteria at 37 °C and pH 7.0

Table 6 shows data on the biodegradation of polyethylene plastic by various bacterial isolates under conditions of 37 °C and pH 7.0. It includes the initial and final weights of polyethylene samples, along with the percentage of weight lost during the degradation process. The results show varying degrees of biodegradation among the isolates. *Bacillus subtilis*, *Arthrobacter*

citreus, and *Pseudomonas aurogenosa* exhibited the highest levels of biodegradation, with 50% weight loss. Other isolates, such as *Bacillus cereus* (40%) and *Pseudomonas aurogenosa* (35%), also showed notable biodegradation,

though to a lesser extent. On the other hand, *Bacillus alvei* and *S. aureus*, showed no degradation, as the final weight remained the same as the initial, corresponding to a 0% weight loss.

Table 3: Morphological and Biochemical Characterization of the Isolates

Code	Shape	Spore	Gra Rn	Catalase	Oxidase	M-R	V-P	Indole	Glucose	Lactose	Sucrose	H ₂ S	Gas	Identified Organisms
MBR 1	Rods	+	+	+	-	-	+	-	+	-	+	-	-	<i>Bacillus subtilis</i>
MBR 2	Rod	+	+	+	-	-	+	-	+	-	+	-	-	<i>Bacillus subtilis</i>
MBR 5	Rod	+	+	+	-	-	+	-	+	+	+	+	-	<i>Bacillus alvei</i>
UDFG 1	Rod	+	+	+	-	+	+	-	+	-	+	-	+	<i>Bacillus cereus</i>
UDFG 2	Rod	-	-	+	+	-	-	-	+	-	-	-	-	<i>P. aeruginosa</i>
UDFG 3	Rod	-	-	+	+	-	-	-	+	-	-	-	-	<i>P. putida</i>
UDFG 4	Rod	-	+	+	-	-	-	-	+	-	-	-	-	<i>A. citreus</i>
UDMM 1	Cocci	-	+	+	-	+	+	-	+	+	+	-	+	<i>S. aureus</i>
UDMM 2	Rod	+	+	+	-	-	+	-	+	-	+	-	-	<i>Bacillus subtilis</i>
UDMM 3	Rod	-	-	+	+	-	-	-	+	-	-	-	-	<i>P. aeruginosa</i>

Keys: MBR = Mabera, UDFG =Usman Danfodiyo University First Gate, UDMM = Usman Danfodiyo University Mini Mart, Gra Rn = Gram Reactions, M-R = Methyl Red, and V-P = Voges Proskauer.

Table 4: Frequency of Occurrence of Identified Bacterial Isolates

Identified Islates	Frequency of Occurrence	Percentage Frequency of Occurrence (%)
<i>Pseudomonas putida</i>	1	10
<i>Bacillus subtilis</i>	3	30
<i>Pseudomonas aeruginosa</i>	3	30
<i>Bacillus alvei</i>	1	10
<i>Staphylococcus aureus</i>	1	10
<i>Arthrobacter citreus</i>	1	10
Total	10	100

Table 5: Polyethylene Plastic Biodegradation by Bacteria at 25 °C and pH 6.0

Isolates	Initial Weight (g)	Final Weight (g)	Weight Lost (%)
<i>Bacillus subtilis</i>	20	20	0
<i>Bacillus subtilis</i>	20	20	0
<i>Bacillus alvei</i>	20	20	0
<i>Bacillus cereus</i>	20	20	0
<i>Pseudomonas aeruginosa</i>	20	20	0
<i>Pseudomonas putida</i>	20	20	0
<i>Arthrobacter citreus</i>	20	20	0
<i>S. aureus</i>	20	20	0
<i>Bacillus subtilis</i>	20	20	0
<i>Pseudomonas aeruginosa</i>	20	20	0

Polyethylene Plastic Biodegradation by Bacteria at 40 °C and pH 8.0

Table 7 shows data on the biodegradation of polyethylene plastic by various bacterial isolates at 40 °C and pH 8.0. The results indicate varied degradation capabilities among the isolates, as demonstrated by their percentage weight loss. *Bacillus subtilis* exhibited the highest

biodegradation activity, with both strains causing a 50% weight loss in the polyethylene samples, suggesting efficient degradation under the given conditions. Other strains, such as *Bacillus cereus* (40%) and *Pseudomonas aurogenosa* (45%), also showed significant but slightly lower degradation levels. In contrast, *Bacillus alvei* resulted in a 35% weight loss, while *Arthrobacter citreus* showed a lower

degradation rate of 30%. A number of strains, including *Pseudomonas aurogenosa*, *Pseudomonas putida*, and *S. aureus*, displayed

no significant degradation (0% weight loss). Additionally, *Bacillus subtilis* demonstrated a 20% weight loss.

Table 6 Polyethylene Plastic Biodegradation by Bacteria at 37 °C and pH 7.0

Isolates	Initial Weight (g)	Final Weight (g)	Weight Lost (%)
<i>Bacillus subtilis</i>	20	10	50
<i>Bacillus subtilis</i>	20	16	20
<i>Bacillus alvei</i>	20	20	0
<i>Bacillus cereus</i>	20	12	40
<i>Pseudomonas aeruginosa</i>	20	20	0
<i>Pseudomonas putida</i>	20	18	10
<i>Arthrobacter citreus</i>	20	10	50
<i>S. aureus</i>	20	13	35
<i>Bacillus subtilis</i>	20	16	20
<i>Pseudomonas aeruginosa</i>	20	11	45

Table 7: Polyethylene Plastic Biodegradation by Bacteria at 40 °C and pH 8.0

Isolates	Initial Weight (g)	Final Weight (g)	Weight Lost (%)
<i>Bacillus subtilis</i>	20	10	50
<i>Bacillus subtilis</i>	20	10	50
<i>Bacillus alvei</i>	20	13	35
<i>Bacillus cereus</i>	20	12	40
<i>Pseudomonas aeruginosa</i>	20	20	0
<i>Pseudomonas putida</i>	20	20	0
<i>Arthrobacter citreus</i>	20	14	30
<i>S. aureus</i>	20	20	0
<i>Bacillus subtilis</i>	20	16	20
<i>Pseudomonas aeruginosa</i>	20	11	45

Table 8: Cell Density (cells/mL) During Biodegradation of Polyethylene Plastic Over 28 Days at 600 nm

Isolate	0 Day (cells/mL)	7 Day (cells/mL)	14 Day (cells/mL)	21 Day (cells/mL)	28 Day (cells/mL)
<i>Bacillus subtilis</i>	4.464×10 ⁸	888	0.825×10 ⁸	6.600×10 ⁸	8.136×10 ⁸
<i>Bacillus subtilis</i>	2.840×10 ⁸	5.560×10 ⁸	5.920×10 ⁸	6.760×10 ⁸	7.312×10 ⁸
<i>Bacillus alvei</i>	2.568×10 ⁸	3.440×10 ⁸	4.240×10 ⁸	888×10 ⁸	4.968×10 ⁸
<i>Bacillus cereus</i>	2.392×10 ⁸	2.008×10 ⁸	2.424×10 ⁸	3.128×10 ⁸	4.600×10 ⁸
<i>Pseudomonas aeruginosa</i>	2.368×10 ⁸	2.496×10 ⁸	3.168×10 ⁸	3.552×10 ⁸	5.448×10 ⁸
<i>Pseudomonas putida</i>	1.480×10 ⁸	4.216×10 ⁸	4.568×10 ⁸	896×10 ⁸	5.928×10 ⁸
<i>Arthrobacter citreus</i>	3.968×10 ⁸	3.176×10 ⁸	1.704×10 ⁸	1.608×10 ⁸	9.920×10 ⁸
<i>S. aureus</i>	2.784×10 ⁸	3.328×10 ⁸	4.640×10 ⁸	808×10 ⁸	5.616×10 ⁸
<i>Bacillus subtilis</i>	8.960×10 ⁸	120×10 ⁸	312×10 ⁸	888×10 ⁸	5.144×10 ⁸
<i>Pseudomonas aeruginosa</i>	1.984×10 ⁸	1.784×10 ⁸	2.512×10 ⁸	3.048×10 ⁸	3.160×10 ⁸

Cell Density (cells/mL) During Biodegradation of Polyethylene Plastic Over 28 Days at 600 nm

Table 8 shows the cell density (cells/mL) of various bacterial isolates measured over a 28-day period during the biodegradation of polyethylene plastic, as assessed by spectrophotometric readings at 600 nm. Overall, most isolates exhibited a general increase in cell density over time, indicating active microbial growth likely associated with polyethylene degradation. *Bacillus subtilis*,

Pseudomonas putida, and *S. aureus* showed particularly strong increases, while *Arthrobacter citreus* demonstrated a declining trend in cell density.

Figure 1 Changes in cell density (cells/mL) over a 28-day period for different bacterial isolates involved in the biodegradation of polyethylene plastic, measured by spectrophotometric assay at 600 nm. Each curve represents the growth trend of a specific isolate, showing variation in biodegradation efficiency over time.

The BLAST of Similarity between the Sequence Queried and the Biological Sequences within the NCBI Database

Table 9 presents the results of a BLAST (Basic Local Alignment Search Tool) search, indicating the degree of similarity between the queried sequence from the sample and sequences in the NCBI (National Center for Biotechnology Information) database. In this case, the sample identified as "MBR1" has been compared to the

database, and it shows a similarity of 90.31% with the organism *Bacillus subtilis*. This percentage reflects the proportion of sequence identity between the queried sequence and the reference sequence in the database, signifying a high degree of genetic homology. A higher percentage of similarity, in this case, suggests that *Bacillus subtilis* is the most likely organism in the database that aligns with the queried sequence, confirming its potential biological identity.

Table 9: The BLAST Results showing Similarity between the Sequence Queried and the Biological Sequences within the NCBI Database

Sample ID	Predicted Organism	Percentage Similarity	Accession Number
MBR1	<i>Bacillus subtilis</i>	90.31%	CP103781

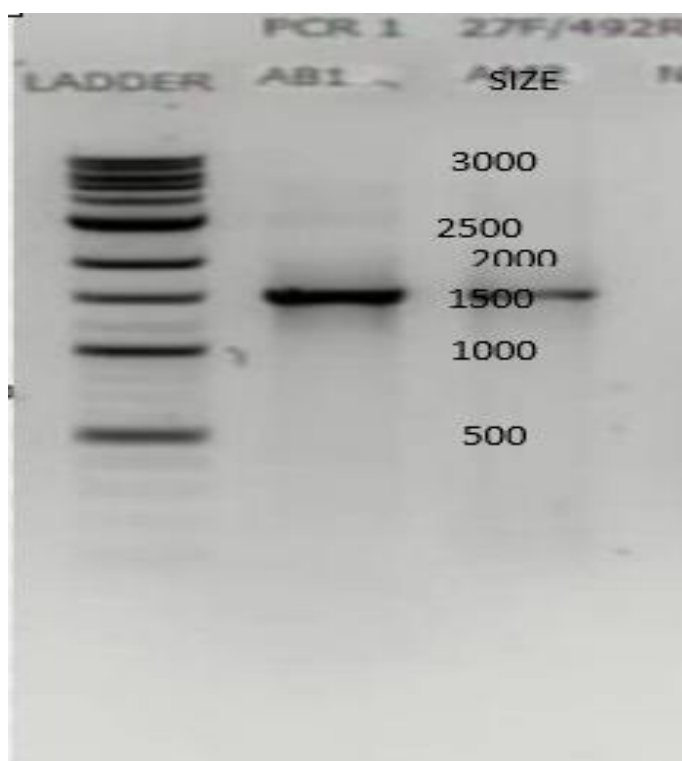


Plate 1: Gel Image of *Bacillus subtilis*

Phylogenetic Tree

The phylogenetic tree illustrates the evolutionary relationships among various strains of *Bacillus subtilis* and closely related species based on their 16S ribosomal RNA gene sequences. The clustering of strains such as *Bacillus subtilis* NBAII-PHD-Bs5, *Bacillus subtilis* strain FJAT-46179, and *Bacillus* sp. strain 23DM11 with a consistent bootstrap value of 26 suggests a high degree of genetic similarity, indicating they likely share a recent common ancestor. Similarly, *Bacillus tequilensis* strain

GD-4 and *Bacillus subtilis* strain SLB12 also form a closely related subgroup, reflecting their conserved genetic traits within the *Bacillus* genus. The relatively higher bootstrap support of 42 for the larger clade connecting all isolates underlines the overall genetic cohesion among these strains. Notably, *Bacillus subtilis* strain RO-NN-1, represented by a complete genome sequence, branches slightly apart from others, implying minor genetic divergence possibly due to complete genome sequencing providing a broader representation of its genetic material.

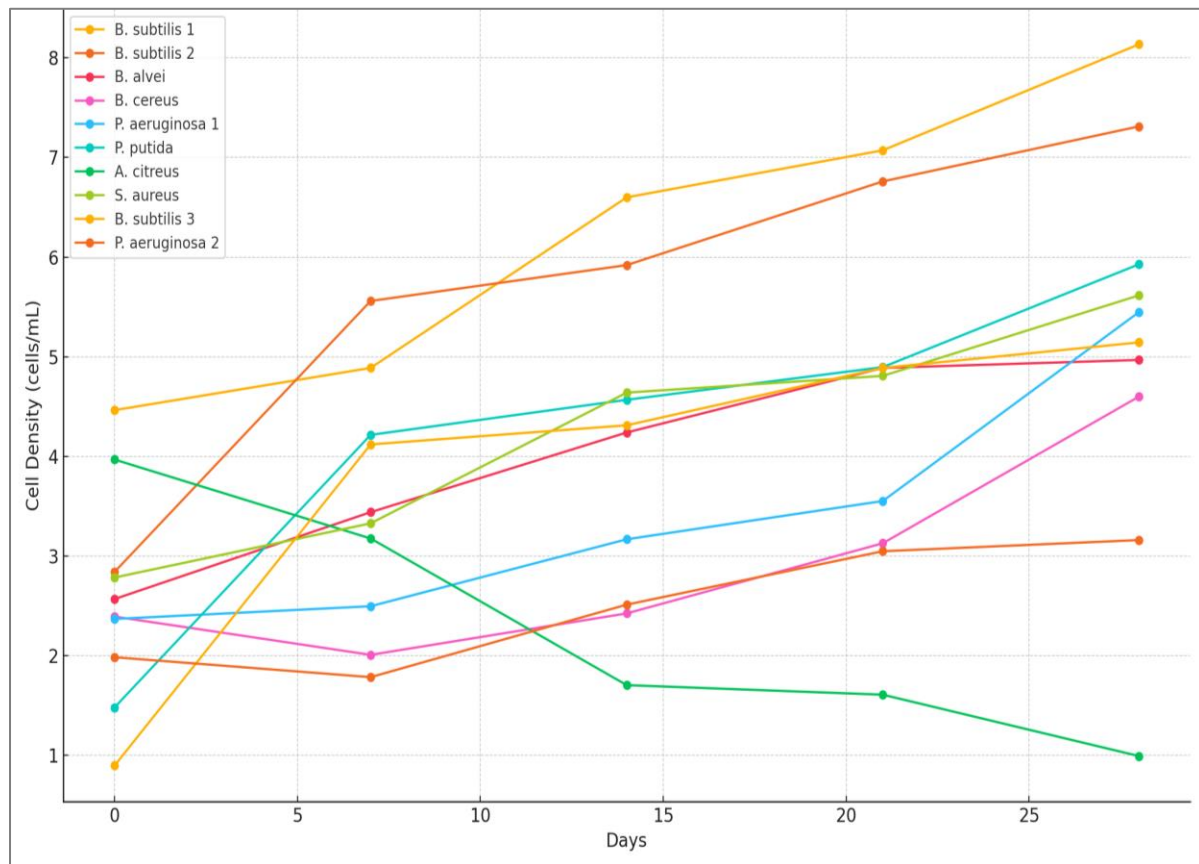


Figure 1: Changes in cell density (cells/mL) over a 28-day period measured by spectrophotometric assay at 600 nm.

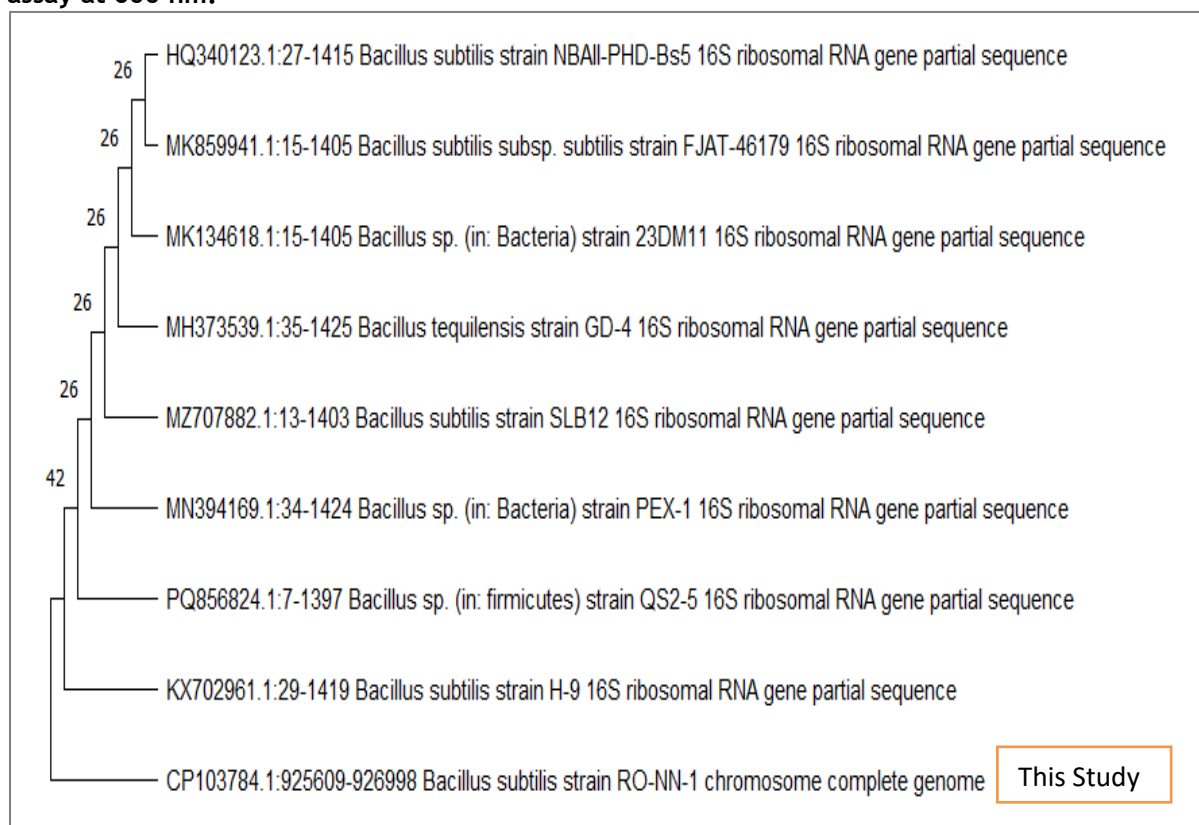


Figure 2: Phylogenetic Tree of *Bacillus subtilis* based 16S rRNA Sequence using Neighbor Joining Method

DISCUSSION

In this study, physico-chemical properties of soil samples collected from plastic waste dumpsites provide significant insights into the soil's capacity to support bacterial communities capable of biodegrading plastic waste. The pH range of 6.71 to 6.75 indicates that the soil is slightly acidic to neutral, which is typically conducive to a wide range of microbial activity, including the breakdown of complex organic compounds such as plastics (Hiltunen *et al.*, 2017). Soil pH influences microbial diversity, enzymatic activity, and the availability of nutrients, all of which play a role in the biodegradation process (Mondal *et al.*, 2024). A pH within this range suggests that the environment may favour bacteria that specialise in the degradation of synthetic polymers, as some microbial strains are particularly suited to thrive in slightly acidic or neutral conditions. This study is similar to the findings of Kumar *et al.* (2024), who reported that soil with a pH range of 6.5 to 7.0 promotes microbial communities capable of degrading plastics, particularly polyethylene.

However, Organic carbon (OC) content, ranging from 0.13 to 0.43, is another critical parameter in assessing the potential of the soil to support bacterial degradation of plastics. Organic carbon serves as an energy source for microorganisms, and its availability influences microbial community composition and metabolism (Scott, 2016). In the context of plastic waste biodegradation, the organic carbon content in the soil is relatively low, which could indicate limited natural nutrients for microbial growth (Udekwu *et al.*, 2024). However, the presence of plastic waste may supplement this deficiency by providing an alternative carbon source for bacteria capable of degrading plastic materials. The variation in organic carbon levels could also reflect the degree of plastic degradation occurring at different locations within the dumpsite, with areas of higher degradation potentially exhibiting a more significant microbial presence. This finding is similar to the study by Xie *et al.* (2022), which found that organic carbon content in soil, ranging from 0.10 to 0.40%, significantly influences the microbial community's ability to degrade plastics, with higher degradation rates observed in areas with more plastic waste, which serves as an alternative carbon source for plastic-degrading bacteria. This current study disagreed with the findings of Juliana and Cynthia. (2024) who worked on Bacterial Isolates of Soils of Different Waste Types in

Yenagoa Central Solid Waste Dumpsite, Nigeria. The findings show that the total organic carbon in plastic waste soil was 2.92.

Furthermore, nitrogen content in the soil, ranging from 0.04 to 0.05%, is another important factor influencing microbial activity, as nitrogen is a key element in protein synthesis and overall microbial growth. Although the nitrogen content is relatively low, it is still within a range that could support the growth of nitrogen-fixing bacteria, which may be present in the soil and play a role in enhancing the biodegradation of plastics (Xu *et al.*, 2023). Nitrogen fixation can contribute to the soil's nutrient pool, providing bacteria with essential compounds needed for the breakdown of plastics (Afsar *et al.*, 2024). The low nitrogen content, in combination with moderate organic carbon levels, suggests that the soil microbial community may be well-adapted to utilise a variety of resources for plastic biodegradation, including both organic and nitrogen sources. In a study by Ghosh *et al.* (2019), soil with moderate nitrogen levels, including nitrogen-fixing bacteria like *Azotobacter* and *Rhizobium*, showed enhanced biodegradation of polyethylene, highlighting the importance of nitrogen in supporting microbial growth for plastic degradation. Similarly, Singh *et al.* (2017) observed that adding nitrogen sources to soil led to increased microbial activity and improved plastic degradation, even when nitrogen levels were relatively low. These findings support the idea that moderate nitrogen content can effectively contribute to the growth of plastic-degrading microorganisms, promoting biodegradation.

The bacteriological analysis of soil samples collected from plastic waste dumpsites, with results ranging from 2.0×10^6 to 7.3×10^6 CFU/g (colony forming units per gram of soil), reveals a significant variation in bacterial population density across different sampling locations, which is crucial for understanding the potential of these bacteria to contribute to plastic biodegradation. The higher bacterial counts suggest an environment that supports a rich microbial community, capable of both degrading organic matter and potentially utilising plastic materials as a carbon source. These counts, ranging from 2.0×10^6 to 7.3×10^6 CFU/g, is indicative of a dynamic soil ecosystem where bacteria have adapted to the presence of plastic waste, enabling them to thrive in what is often considered a challenging substrate. Furthermore, the variation in bacterial density could reflect localised conditions such as moisture levels, temperature, and the degree of

plastic contamination, which may influence microbial growth and composition. This result disagrees with the findings of [Hilda et al. \(2022\)](#), who worked on the Isolation and identification of plastic-degrading bacteria from dumpsites in Lagos. The findings show that the bacteriological analysis of the plastic waste soil sample was 300.0×10^6 .

The biochemical analysis of soil samples collected from plastic waste dumpsites revealed diverse bacterial communities with potential for biodegradation of plastic waste. Each bacterium was characterised by specific biochemical reactions, providing insights into its metabolic capabilities and suitability for plastic degradation. *Bacillus species*, including *Bacillus subtilis* and *Bacillus alvei*, were prominent, exhibiting positive results for catalase, oxidase, and glucose fermentation, suggesting robust oxidative stress responses and carbohydrate utilisation. These traits are indicative of their ability to metabolise complex organic materials, including plastics, as a source of energy. The presence of *Bacillus subtilis* across multiple samples further indicates its potential adaptability to different waste environments, as this species is known for its resilience and versatile metabolic pathways ([Najar et al., 2024](#)).

In addition to *Bacillus species*, other bacteria such as *Pseudomonas aurogenosa* and *Pseudomonas putida* were identified in the soil samples. These Gram-negative rods are renowned for their ability to degrade various environmental pollutants, including plastic polymers, through extracellular enzymes, as highlighted by [Atanasova et al. \(2021\)](#). The biochemical reactions of these species, particularly the positive results for indole and hydrogen sulfide (H_2S) production, suggest their role in breaking down toxic compounds found in plastic waste. *Pseudomonas species*, being versatile in their metabolic capacity, are often utilised in bioremediation applications due to their ability to degrade hydrocarbons and synthetic polymers. Their presence in the soil samples implies an inherent potential for bioplastic degradation, making them candidates for further investigation in waste management strategies. This is in conformity with the research of [Hajjousef \(2022\)](#).

Moreover, other identified organisms, such as *Arthrobacter citreus* and *Staphylococcus aureus*, also demonstrated unique biochemical profiles that could contribute to the biodegradation process. *Arthrobacter citreus* exhibited positive

reactions for catalase and oxidase, as well as the ability to ferment glucose, while *Staphylococcus aureus* displayed similar biochemical characteristics, suggesting both species have potential in plastic waste decomposition through enzymatic and metabolic pathways. This result is related to the findings of [Hilda et al. \(2022\)](#), who worked on the Isolation and identification of plastic-degrading bacteria from dumpsites in Lagos. They isolated *Pseudomonas spp*, *Staphylococcus spp* and *Bacillus subtilis* strains from a plastic waste dumpsite in Lagos.

The weight loss data from the polyethylene plastic biodegradation study under different temperature and pH conditions reveal varying levels of biodegradation activity among the bacterial isolates. At 25°C and pH 6.0, no significant weight loss was observed across all isolates, with all remaining at 100% of their initial weight. This lack of degradation could suggest that, under these specific conditions, the bacterial strains tested are not equipped to initiate the biodegradation process of polyethylene plastics effectively ([Yang et al., 2024](#)). Polyethylene, being a highly stable synthetic polymer, generally requires specific environmental conditions or specialised enzymes for degradation ([Dhali et al., 2024](#)). The absence of any weight loss at this temperature and pH indicates that other factors, such as temperature, pH, or enzymatic activity, may play crucial roles in the biodegradation process.

In contrast, at 37°C and pH 7.0, a more dynamic pattern of biodegradation was observed. Several bacterial isolates, including *Bacillus subtilis* and *Pseudomonas aurogenosa*, showed a significant reduction in weight, losing 50% of their initial weight. This indicates a marked increase in the microbial activity under these more favourable conditions for bacterial growth, especially with the slightly neutral pH and the moderate temperature of 37°C. The higher temperature likely enhanced the metabolic rate of the bacteria, while pH 7.0 is generally more conducive for the activity of most plastic-degrading bacteria, which may have optimised their enzymatic pathways to target polyethylene degradation. The fact that some isolates, like *Pseudomonas aurogenosa* and *Pseudomonas putida*, exhibited minimal degradation suggests the specificity of bacterial strain capabilities, with certain species more proficient in utilising polyethylene as a carbon source.

At 40°C and pH 8.0, further variations in biodegradation performance were noted, with isolates like *Bacillus subtilis* achieving 50%

weight loss, suggesting a continued enhancement of bacterial degradation potential under even higher temperatures and slightly alkaline pH conditions. This is consistent with the notion that higher temperatures often stimulate increased enzymatic activity and microbial metabolism, which may facilitate greater polymer breakdown (Siddiqui *et al.*, 2024). However, not all isolates responded equally to these changes; for example, *Pseudomonas aurogenosa* and *Pseudomonas putida* still showed no weight loss, indicating that their enzymatic systems may not be activated efficiently at this elevated temperature or alkaline pH. This result is also similar to the findings of Soud (2019), who worked on Biodegradation of polyethylene plastic waste using locally isolated *Streptomyces* spp, which found *Bacillus subtilis* and *Pseudomonas* species to be the most potent plastic degraders. Omar (2017) also used the weight method for polyvinyl alcohol (PVA) plastic bags by using different isolates of *pseudomonas* and *Bacillus* (*Bacillus amylolyticus*, *Bacillus firmus*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas fluorescens*), microbes that cause the greatest degradation of polythene and plastics.

The observed increase in cell density for most bacterial isolates during polyethylene degradation is consistent with previous research highlighting the ability of certain microorganisms to utilise synthetic polymers as carbon sources (Das and Kumar, 2015). In particular, *Bacillus* spp. and *Pseudomonas* spp. have been widely reported as effective degraders of polyethylene due to their production of extracellular enzymes such as oxygenases and hydrolases. The strong growth trends exhibited by *Bacillus subtilis* and *Pseudomonas putida* in the present study corroborate these findings, suggesting their metabolic adaptability and capacity to exploit polyethylene under selective conditions. Conversely, the decline in *Arthrobacter citreus* cell density over time is consistent with previous observations that not all soil bacteria possess the enzymatic systems necessary for efficient plastic degradation or may experience inhibition due to the recalcitrant nature of the polymer (Shah *et al.*, 2008).

The most potent bacterial isolate in polyethylene plastic waste biodegradation was identified using molecular techniques. The isolate was identified as *Bacillus subtilis*. The molecular characterisation of the bacterial isolate MBR1 from the plastic waste dumpsites was conducted using DNA sequencing to identify

the potential organism responsible for biodegradation. The sequence was compared against the NCBI database using BLAST (Basic Local Alignment Search Tool), revealing that the isolate closely matched *Bacillus subtilis* with a percentage similarity of 90.31%. This high level of similarity suggests that *Bacillus subtilis* is the likely organism present in the sample, with its genetic profile aligning closely to known sequences of *Bacillus subtilis* present in the NCBI database, particularly with accession number CP103781. *Bacillus subtilis* is known for its versatile metabolic capacity, including the ability to degrade various organic compounds, making it a strong candidate for plastic biodegradation in the context of plastic waste management.

The identification of *Bacillus subtilis* based on the molecular data further corroborates its established role in bioremediation. This bacterium has been well-documented for its ability to degrade complex substrates, such as polymers, which are often resistant to natural degradation processes. The genetic similarity between the isolate and the NCBI reference strain indicates that *Bacillus subtilis* has the necessary genetic machinery to produce extracellular enzymes capable of breaking down the polymeric structure of plastics like polyethylene. These enzymes, including esterases, lipases, and hydrolases, are essential for the biodegradation of plastic polymers, facilitating the microbial assimilation of plastic components as carbon sources. Molecular identification of polyethylene plastic biodegradation by *Bacillus subtilis* was reported by several authors, including Ethiopia *et al.* (2024), who worked on the Identification and molecular characterisation of polyethylene-degrading bacteria from garbage dumpsites in Adama, Ethiopia.

CONCLUSION

Biodegradation of polyethylene is a promising eco-friendly method whereby plastic material waste is managed with minimum adverse effects on the environment. This study tested 10 isolates collected from waste dump sites for polyethylene-degrading ability. These isolates were identified by biochemical, weight loss and spectrophotometric analysis. The most potent bacterium species was characterised using 16S rRNA and belonged to *Bacillus* species. The biodegradation assay conducted demonstrated that *Bacillus subtilis* and *Pseudomonas* sp. have a great capacity to remove polyethylene from the environment, and their degradation ability

may improve plastic pollution removal. The effect of this degradation process was evident in the drastic weight loss of polyethylene films and the notable increase in the optical density. A pH of 7 and a temperature of 37°C were found as the optimal conditions for isolates in degrading polyethylene. This study showed that waste dumping sites could be a great source of polyethylene-degrading bacterial isolates.

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