Nig. J. Biotech. Vol. 41(2): 1-20 (Dec. 2024)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and https://bsn.org.ng
DOI: https://dx.doi.org/10.4314/njb.v41i2.1

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Assessment of the Enzymatic and Biodegradation Potentials of Polyethylene Degrading Microbes Isolated from Two Composite Sites

***[1]Okanlawon, T.S., [1]Agbaje, I.S., [2]Okanlawon, K.P., [1]Okafor, C.A., [3]Olufemi, S.E. and [1,3]Adeyemo, S.M.**

[1]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[2]Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[3]Genetics and Molecular Genetics Laboratory, Biology Department, Texas Southern University, USA

Abstract

This study investigated the viability of composting for actively breaking down synthetic polymer nylon by fungal and bacteria species. A total of thirty (30) samples were taken over the course of three months from two composting sites that contained different coloured polyethylene bags. The identification and characterization of isolates was done using morphological and biochemical techniques while the Analytical Profile Index (Biomeuriex API kit) was used to confirm the identities of the isolates. The overall load of heterotrophic bacteria varied between 3.30 x 10^4 cfu/g to 1.50 x 10^6 cfu/g, while that of fungi ranged from 1.02×10^4sfu/g to 1.26×10^6sfu/g. Pseudomonas sp. showed the highest prevalence (30%), followed by Staphylococcus sp. (16%). The least frequently occurring bacteria were *Micrococcus varians* and *Proteus mirabilis* (4% each). The most common species of fungi were *Aspergillus niger* (25%) and Fusarium sp. (23.33%), while *Aspergillus candidus* exhibited the lowest prevalence (3.33%). There was an increase in the temperature of the compost from 24° C to 29 °C in the two sites and an increase in the pH from 6.7 to 7.7. The percentage reduction in weight of white nylon is 6.7% in site 1 and 6.4 % in site 2, the blue nylon reduced by 9% in site 1 and 8.5% in site 2. There was 8.1% loss in weight in yellow nylon in site 1 and 7.5% weight loss in site 2. The black nylon reduced in weight by 8.7% in site 1 and 8.1% in site 2. The enzyme activity showed Fusarium sp. (++) and *Aspergillus niger* (++) having the highest cutinase activity while the lowest activity was observed in the bacteria. It can be concluded that the combined activity of microorganisms and their enzymes play a major role in the biodegradation of polyethylene which is a major nuisance in the environment.

Keywords: Sustainable environment, Biodegradation, Polyethylene, Enzyme assay, Pollution

Corresponding author's email: taiwookanlawon26@gmail.com, sadeyemo@oauife.edu.ng

Introduction

The use of plastic products has led to a major growth in the importance of biodegradability of plastic in recent decades (Ayilara *et al.*, 2023). According to Montaz *et al.* (2018), plastics are designed polymers made of tiny monomer units joined together into an extended chain by covalent connections created during polymerization. The ecology and public health have been negatively impacted by the widespread usage of plastics. Plastics have an impact on about 700 different species, particularly vulnerable ones. Microbiological decomposition of plastic trash is a promising

method that has been used extensively for a long time. About 92% of synthetic plastics manufactured are polyethylene, which is used to make plastic bags, bottles, disposable containers, packing materials, etc. Approximately fifty billion to one trillion plastic containers used worldwide destabilize ecological balance, which in turn causes major environmental problems when these products are recycled (Ghatge *et al*., 2020). Approximately 350 million and 400 million tons of non-biodegradable plastic are produced annually worldwide; of that amount, 5 million to 13 million tons of recyclable plastics are dumped into the marine environment annually, having an adverse effect on the ecosystem (Jambeck *et al*., 2015). Since 1940, when researchers produced and sold nylon in large quantities, studies on various synthetic polymers have increased. About 50 billion plastic bags are used in Nigeria each year, and 200,000 of them are landfilled every hour. Biodegradation of polyethylene by bacteria or microorganisms use it as a source of energy, transforming and utilizing the polymer to change its physical and chemical properties. These changes include a reduction in weight, morphological breakdown, and ultimately fixing carbon as a biomass (Restrepo-Florez *et al*., 2014).

Due to its strong resistance to degradation, polyethylene can persist in the environment for extended periods of time, which presents substantial environmental challenges. This resistance adds to the build-up of plastic debris within ecosystems. Its breakdown results in the creation of tiny plastic particles that may pollute soil, water, and the surrounding environment. Microplastics are dangerous to the aquatic environment because they enter the food chain, wildlife, and possibly human health. (Sarra *et al.,* 2022). They produce toxic additives; these additives can affect ecosystems and organisms when introduced into the environment and disrupt ecological balance. Plastic contaminants can disrupt the quality of soil, marine environments, and the movement of nutrients, many of those having the potential to alter ecological systems. Ecological balance and biodiversity are threatened by the buildup of plastic waste in natural settings (Naga *et al.,* 2023). However, if plastics like polythene are convenient, they also pose a risk to the well-being of humans and the long-term stability of the planet. Many polyethylene contaminants build up annually in diverse environments because of low recycling efficiency

Numerous academics have studied the biodegradation of stubborn polyethylene. The biodegradation of polyethylene in the environment is said to be greatly influenced by a few biotic and abiotic variables (Wilkes and Aristilde, 2017). Both pure cultures that can break down polyethylene and complex microbial communities from a variety of terrestrial (landfill soil, composting) and marine settings have been used in biodegradation research. Other studies have been done on the breakdown of polyethylene using either physical-chemical, microbiological, or a mix of the two. Thermal and UV treatment, alone or in combination, are examples of physical-chemical processes that can be used to shrink polymer chains and create oxidized groups on the surface of polymers (Sen and Raut, 2015, Montaz *et al*., 2018, Sarra *et al.,* 2022, Naga *et al.,* 2023).

Cutinases are esterases from the α/β hydrolase family. According to the latest studies, cutinase is a multifunctional enzyme that operates on soluble esters, insoluble triglycerides, and polyethylene terephthalate (Nikolaivits *et al*., 2018; Liu *et al*., 2019). Although the first discovery indicating it may degrade cutin into fatty acid monomers, cutinases have proven to be a useful instrument for plastic decomposition, reuse, and enhancement. *Aspergillus, Cladosporium, Fusarium, Penicillium*, and *Phanerochaete* are only a few of the fungal taxa that have been linked to polyethylene degradation (Glaser, 2019). In general, it is believed that fungi are more effective than bacteria in degrading polyethylene since they can adhere to the polymers' hydrophobic surfaces, create external enzymes that target recalcitrant fibers, and withstand adverse proliferation conditions (Sen and Raut, 2015). It is crucial to understand how biodegradation interacts with various elements in the biotic and abiotic settings. Fungi are part of the microbes responsible for the polyethylene's biodegradation. By conducting growth assays on media for a certain duration, their biodegradation activity on the polymer was investigated. Measurements were also made of the modifications to a polyethylene molecular size,

form, crystal structure, thickness, reduction in weight, and mechanical, visual, and dielectric properties are only a few examples of its attributes etc. (Barry and Digregorio, 2009). In pristine environments, plastics break down under aerobic conditions, anaerobically in sediments and landfills, and partially with or without oxygen in compost and soil. In the case of aerobic biodegradation, carbon dioxide, and water are produced, while methane, carbon dioxide, and water are produced during anaerobic biodegradation. There are some simple composting techniques that have been created for plastic breakdown (Chonde *et al*., 2013). Bioremediation is a biological process that employs microorganisms to remove, degrade and immobilize contaminants in the environment. Environmental contaminants include excessive nutrients, organic contaminants such as petroleum hydrocarbons, organo-pesticides, plastics and heavy metals. The microorganisms widely used include bacteria, archaea, fungi, and microalgae. Biological remediation has several advantages as it is considered a low-cost, simple operation, and eco-friendly process (Cui *et al*., 2022). This study assessed the biodegradation and enzymatic potentials of polyethylene degrading microbes isolated from two composite sites.

Materials and Methods.

Sample Collection

Four polyethylene (white, blue, yellow and black) bags were weighed (0.2g each) and buried for three months at a depth of 2 to 10 cm in the soil. Each month, samples were taken for physicochemical and microbiological analyses. Using sterile containers, soil samples from the nylons were collected monthly over the course of 90 days and transferred to the laboratory for microbiological examination. Thirty samples each from each site were collected for this research project from two compost sites of Glorious Vision University campus, Edo State and two from the campus of Obafemi Awolowo University, Osun State and mixed to form two different soil consortium.

Microbiological Analysis

Isolation of bacterial and fungi Isolates

Soil samples were homogenized, serially diluted and poured onto agar plates for uniform colonization after which the agar medium was poured into the plates. Potatoes Dextrose Agar, Nutrient Agar and Mackonkey Agar were used for the isolation following manufacturer's instructions (Okanlawon *et al*., 2023).

Total Bacterial and Fungi Count

Different colonies were seen after the incubation period, and colonies were counted and recorded in CFU/g and SFU/g of bacterial and fungal colonies respectively (Arimah *et al*., 2014).

Identification and Characterization

Colonial structure, morphological, and biochemical characteristics were used to determine the identity of bacterial isolates, which were then classified using Bergey's Manual of Determinative Bacteriology and the Compendium of Soil Fungi. (Bergey's Manual of Determinative Bacteriology Ninth Edition,1994; Okafor *et al*., 2023).

Confirmation of the Identities of the Bacterial isolates

BioMérieux's API identification products test kits, an effective technique for manually identifying microorganisms to the species level, were utilized to validate the names of both Gram positive and Gram-negative bacteria. The tool provides a big and strong database structure, which is available via the online exam. The API test kits utilized are API 20 E, Rapid 20 E, API 50 CHE, API CHB, API 50 CHL, API STAPH, API 20 STREP, and API 20C AUX. API strips provide reliable identities based on huge databases and are standardized, user-friendly diagnostic devices. The kits feature strips that can hold up to 20 tiny biochemical tests. The strips may be installed quickly, safely, and easily. (https://apiweb.biomerieux.com).

Landfill Method.

The nylon samples were weighed and buried for degradation for three months in different holes in each location. The three holes were roughly 2cm to 10cm deep and 0.5m in diameter. Nylon bags were collected from each location on the same day after each month. A fresh weight in grams was determined for each nylon bag shortly after the bags were removed from the field. The nylons were cleaned, dried and weighed again after removal from the holes. The soil burial test was carried out by monitoring the nylons' weight reduction over time.

The weight loss will be calculated using the formula Weight loss =(Wi-Wf)/Wi x 100 at the conclusion of 90 days from the initial days, where Wi is the original dry weight of the nylon before soil burial and Wf is the weight of the nylon after soil burial.

Physicochemical Parameters

pH of the soil sample

Ten grams of soil sample were weighed, homogenized with 90 mL of sterile distilled water then filtered using Whatman Filter paper (No. 1). After standardization with phosphate buffer solution at pH 4.0 and 7.0, the pH of the resulting filtrate was then evaluated using a pH meter (H19107, Hanna), (Corwin and Yemota, 2020).

Temperature of the soil sample

A collection of curved tube thermometers was used to gauge the temperature of the soil in each plot. These thermometers were positioned on the ridge top. The three depths used for measurement were 5 cm, 15 cm, and 25 cm. The measurements were made at 7:00, 14:00, and 18:00 (Corwin and Yemota, 2020).

Screening Cutinase Assay

The methodology for the cutinase enzyme assay was based on Pio and Macedo (2008). Using this procedure, different mixtures of 3.43 mL of substrate solution and 0.070 mL of fungal or bacterial supernatant were made. The substrate's stock solution included 1.12 mM paranitrophenyl butyrate diluted in 50 mM phosphate buffer (pH 7.2), 0.2% Triton X-100, and 0.43 M tetrahydrofuran. To compare the hydrolysis of paraanitrophenyl butyrate into paranitrophenol with a control reaction, spectrophotometric measurements were made at 405 nm. One unit of cutinase activity was defined as the amount of cutinase required to release one micromole of p-nitrophenol per minute under the specified conditions.

Results and Discussion

Table 1: Microbial Load Count

Isolation	THBC (cfu/g)	THBF (sfu/g)
Site 1	$3.3x10^4$	$1.02 x10^6$
Site 2	$1.04x10^4$	$2.50 x10^6$

Keys: THBC- Total Heterotrophic Bacterial Count, THBF- Total Heterotrophic Fungal Count

TABLE 2: The Biochemical Characteristics of Isolates

Gram reaction	Cellular	Catalase	Oxidase	Methyl red	Voges Proskauer	Indole	Nitrate Reduction	6.5% NaCl	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Citrate utilization	Glucose	Fructose	Maltose	Lactose	Sucrose	API conf.	Probable identity
+	C	+	-	+	-	-	+	+	-	-	-	+	+	+	+	+	+	+	***Staphylococcus* sp.**
-	R	-	-	+	-	-	+	+	-	-	-	+	+	+	+	+	+	+	***Pseudomonas* sp.**
-	R	+	-	-	+	-	+	-	+	-	-	-	+	+	+	-	+	+	***Serratia* sp.**
+	R	+	+	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	***Bacillus subtlis***
+	R	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	+	+	***Proteus* sp.**
+	C	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	***Micrococcus varians***
+	R	+	+	+	-	-	+	+	-	+	+	-	+	+	+	+	+	+	***Bacillus licheniformis***
+	R	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	***Bacillus cereus***
+	R	+	+	+	+	-	-	-	-	+	+	+	+	-	+	+	+	+	***Bacillus polymyxa***
+	C	+	-	+	-	-	+	+	-	-	-	+	+	+	-	+	+	+	***Micrococcus luteus***

Keys: C = Cocci, R = Rod, + = positive, - = negative

TABLE 3: Colony Morphology of Fungal Isolates

Isolate Code	Colour of Spores	Reverse Side of Agar	Nature of Aerial Hyphae	Pigmentation	Type of Aerial Hyphae
L1	Black	Creamy	Embedded	None	None
L2	White	Creamy	Embedded	None	None
L3	White	Creamy	Embedded	Brown	Fluffy
L4	Reddish white	Creamy	Embedded	Black	Fluffy
L5	Green	Creamy	Embedded	Brown	Velvet
L6	White	Creamy	Embedded	None	Fluffy
L7	White	Creamy	Embedded	None	Fluffy
L8	Blackish-brown	Creamy	Embedded	None	Velvet

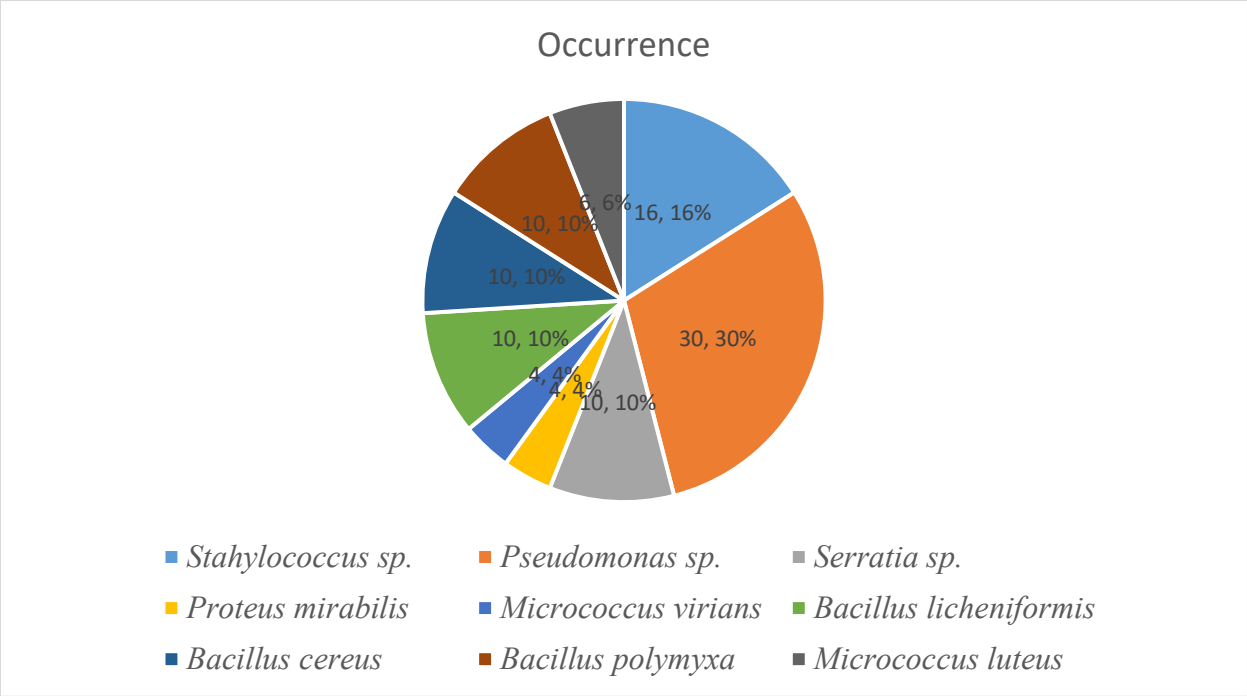

Fig. 1: Percentage occurrence and frequency of bacterial isolates

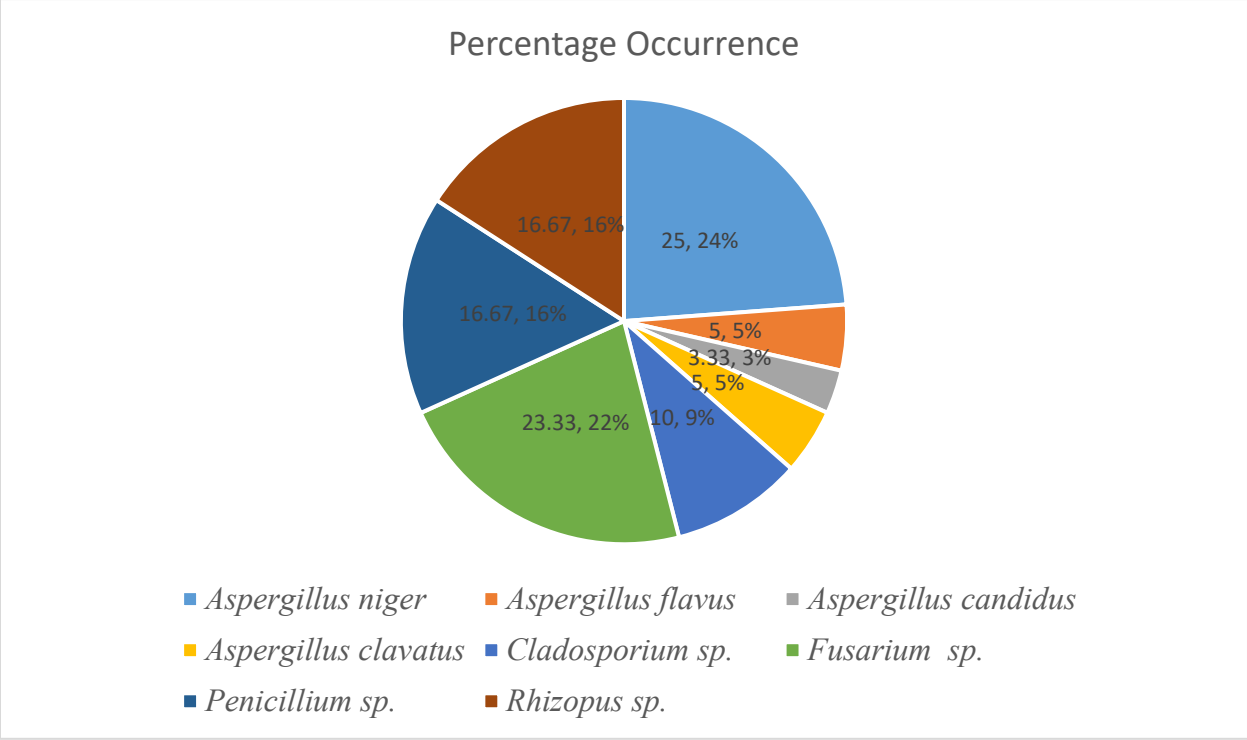

Fig 2: Percentage occurrence and frequency of fungal isolates

TABLE 4: Percentage (%) Weight Loss Observed in White Low-Density Polyethylene (LDPE) Nylon Samples during Degradation

Table 4a: Site 1: GVU 1

Duration (Month)	Initial weight of the nylon (g)	final weight of the nylon (g)	Difference in weight	% Weight reduction of sheets of nylon
0	1.00	1.00	0	0
1	1.04	1.02	0.03	2.4
2	1.02	0.97	0.05	4.9
3	0.97	0.91	0.06	6.7

Table 4b: Site 2: GVU 2

Duration (Month)	Initial weight of the nylon (g)	final weight of the nylon (g)	Difference in weight	% Weight reduction of sheets of nylon
0	1.08	1.08	0	0
1	1.08	1.06	0.02	2.2
2	1.06	1.01	0.05	4.7
3	1.01	0.95	0.15	6.4

TABLE 5: Percentage (%) Weight Loss Observed in Low Density Polyethylene (LDPE) Blue Nylon Sample during Degradation

Table 5a: Site 1: GVU 1

Duration (Month)	Initial weight of the nylon (g)	final weight of the nylons (g)	Difference in weight	% Weight reduction of sheets of nylon
0	1.08	1.08	0	0
1	1.08	1.06	0.02	2.2
2	1.06	1.00	0.06	5.7
3	1.00	0.91	0.09	9.0

Table 5b: Site 2: GVU 2

Duration (Month)	Initial weight of the nylon (g)	final weight of the nylon (g)	Difference in weight	% Weight reduction of sheets of nylon
0	1.10	1.10	0	0
1	1.10	1.079	0.021	1.90
2	1.079	1.020	0.059	5.50
3	1.020	0.933	0.087	8.50

TABLE 6: Percentage (%) Weight Loss Observed in Low Density Polyethylene (LDPE) Yellow Nylon Sample during Degradation

Table 6a: Site 1: OAU 1

Duration (Month)	Initial weight of the nylon (g)	final weight of the nylon (g)	Difference in weight	% Weight reduction of sheets of nylon
0	1.10	1.10	0	0
1	1.10	1.078	0.02	2.0
2	1.078	1.023	0.06	5.1
3	1.023	0.94	0.083	8.1

Table 6b: Site 2: OAU 2

Duration (Month)	Initial weight of the nylon (g)	final weight of the nylon (g)	Difference in weight	% Weight reduction of sheets of nylon
0	1.15	1.15	0	0
1	1.15	1.126	0.024	2.1
2	1.126	1.072	0.054	4.8
3	1.072	0.992	0.08	7.5

TABLE 7: Percentage (%) Weight Loss Observed in Low Density Polyethylene (LDPE). Black Nylon Sample during Degradation

Table 7a: Site 1: OAU 1

Duration (Month)	Initial weight of the nylon (g)	final weight of the nylons (g)	Difference in weight	% Weight reduction of sheets of nylon
0	1.17	1.17	0	0
1	1.17	1.14	0.028	2.4
2	1.14	1.077	0.063	5.5
3	1.077	0.983	0.094	8.7

Table 7b: Site 2: OAU 2

Duration (Month)	Initial weight of the nylon (g)	final weight of the nylons (g)	Difference in weight	% Weight reduction of sheets of nylon
0	1.21	1.21	0	0
1	1.21	1.18	0.03	2.3
2	1.18	1.12	0.06	5.0
3	1.12	1.03	0.09	8.1

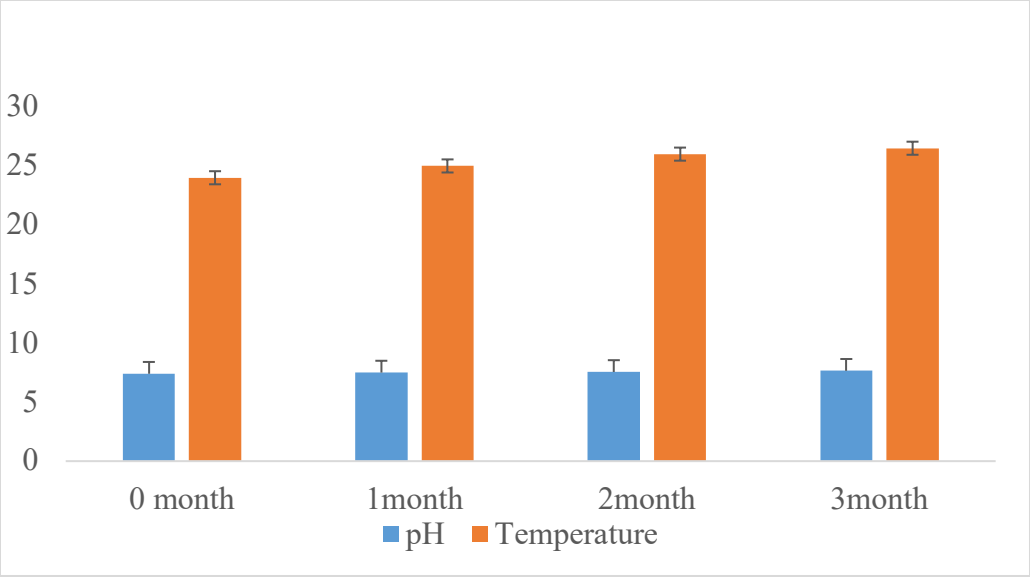

Fig. 3: Soil Parameters observed during three (3) months degradation of nylons for site 1

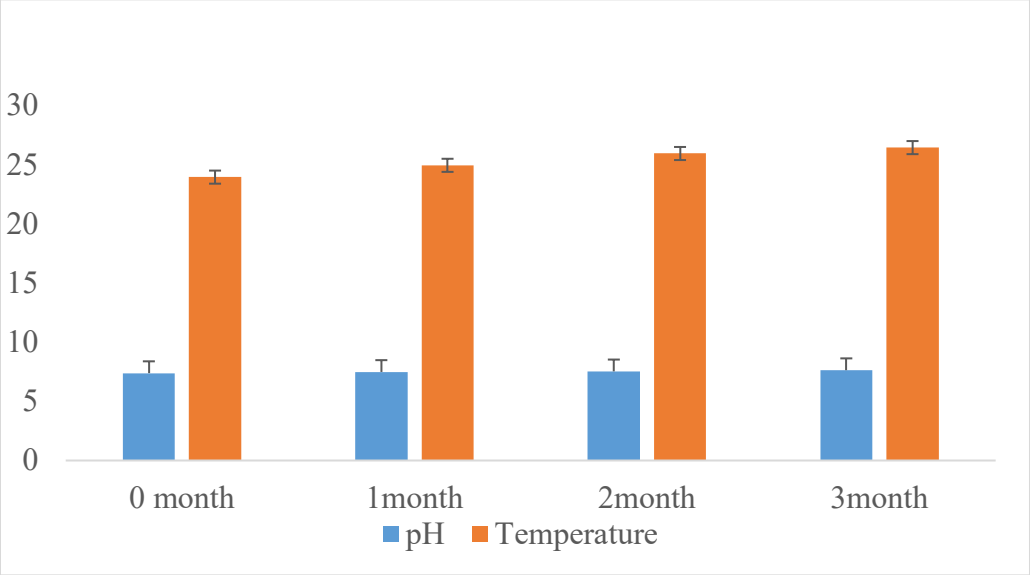

Fig. 4: Soil Parameters observed during three (3) months degradation of nylons for site 2

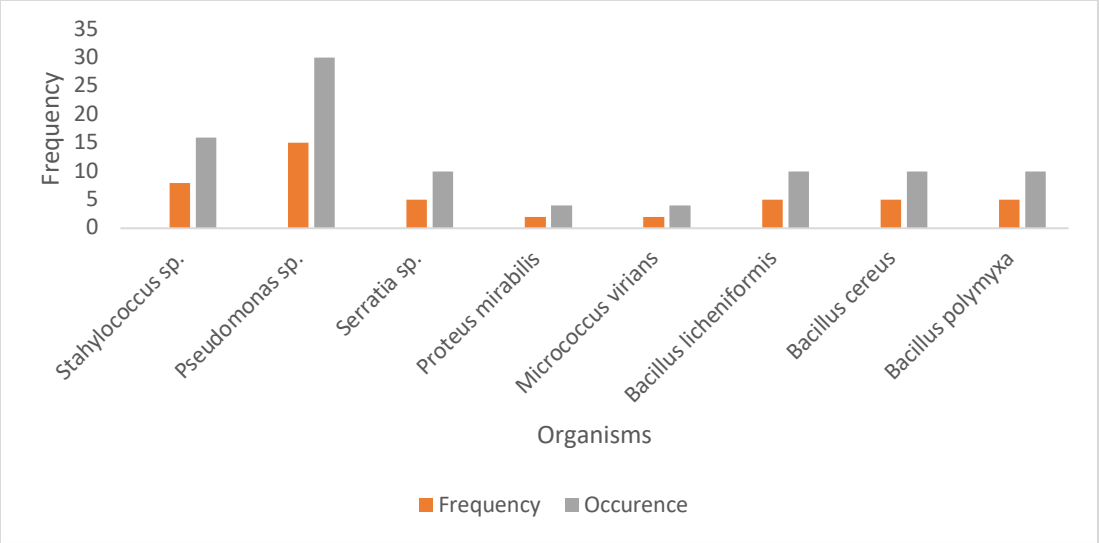

Fig. 5: Percentage occurrence and frequency of bacterial isolates

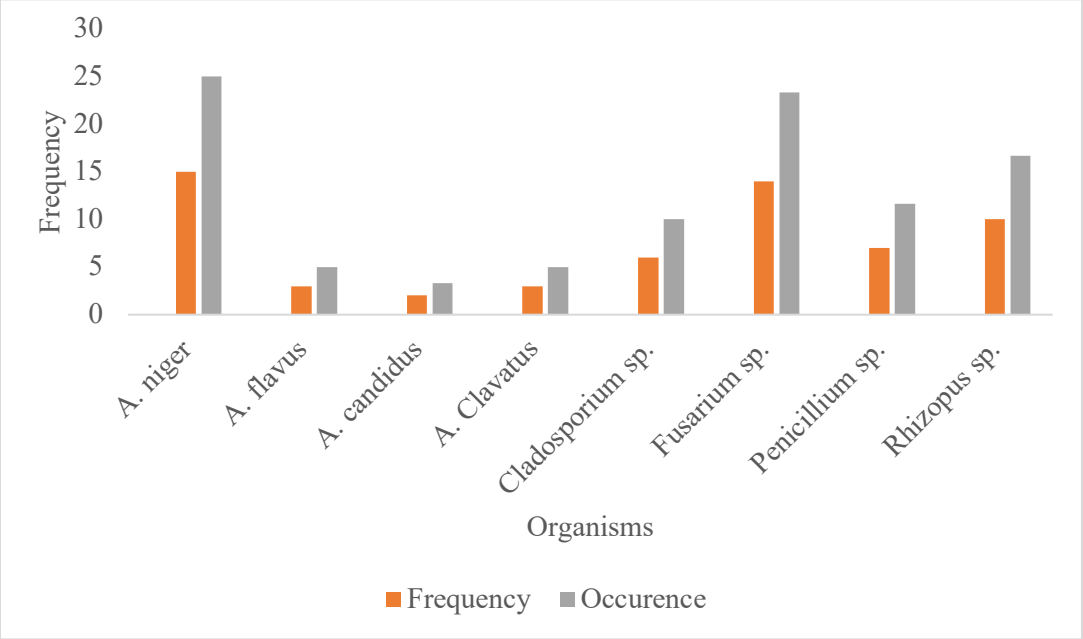

Fig. 6: Percentage occurrence and frequency of fungal isolates

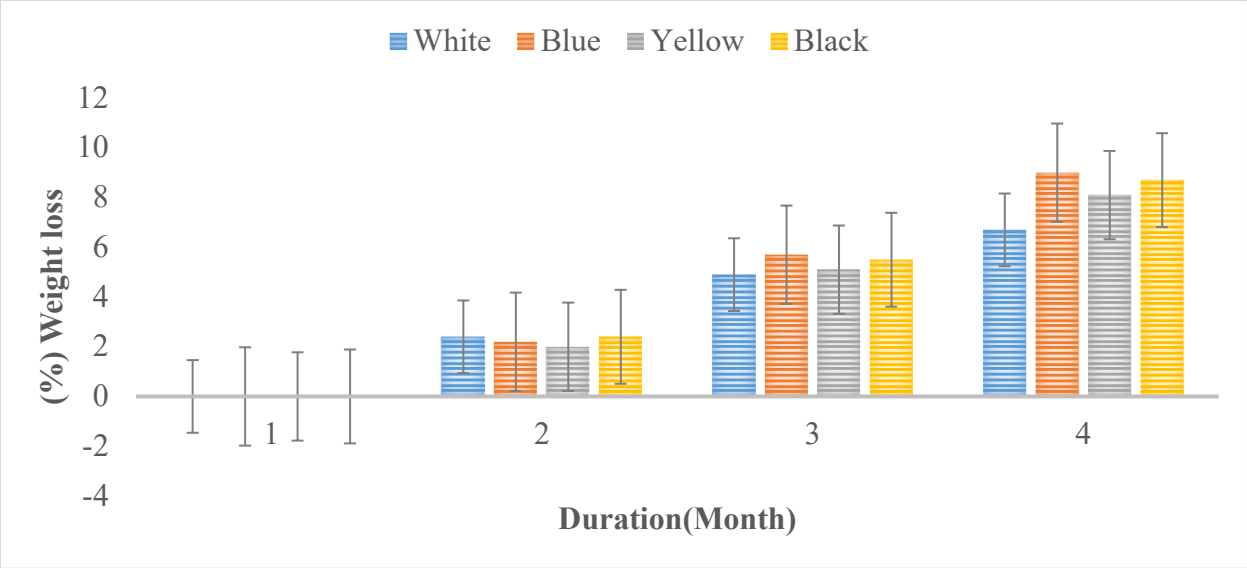

Fig. 7: Percentage (%) weight loss observed in all nylon samples during biodegradation for Site 1

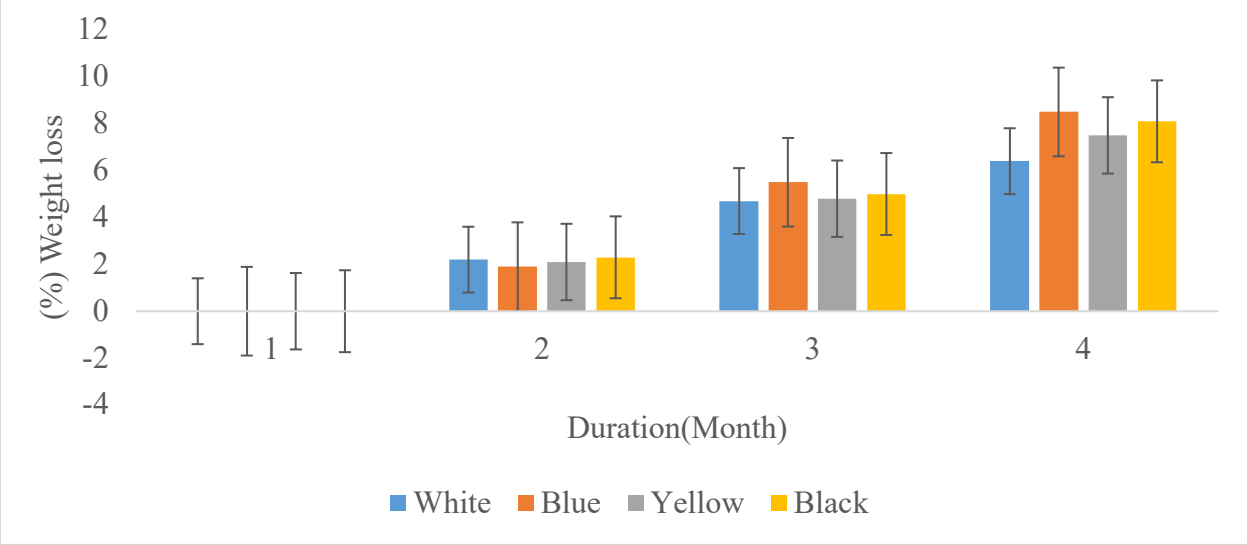

Fig. 8: Percentage (%) weight loss observed in all nylon samples during biodegradation for Site 2

Table 8: The cutinase activity of fungal isolates

Organisms	Result
Aspergillus niger	+ +
Aspergillus flavus	-
Aspergillus candidus	-

Aspergillus clavatus	-
Cladosporium sp.	-
Fusarium sp.	++
Penicillium sp.	-
Rhizopus sp.	-

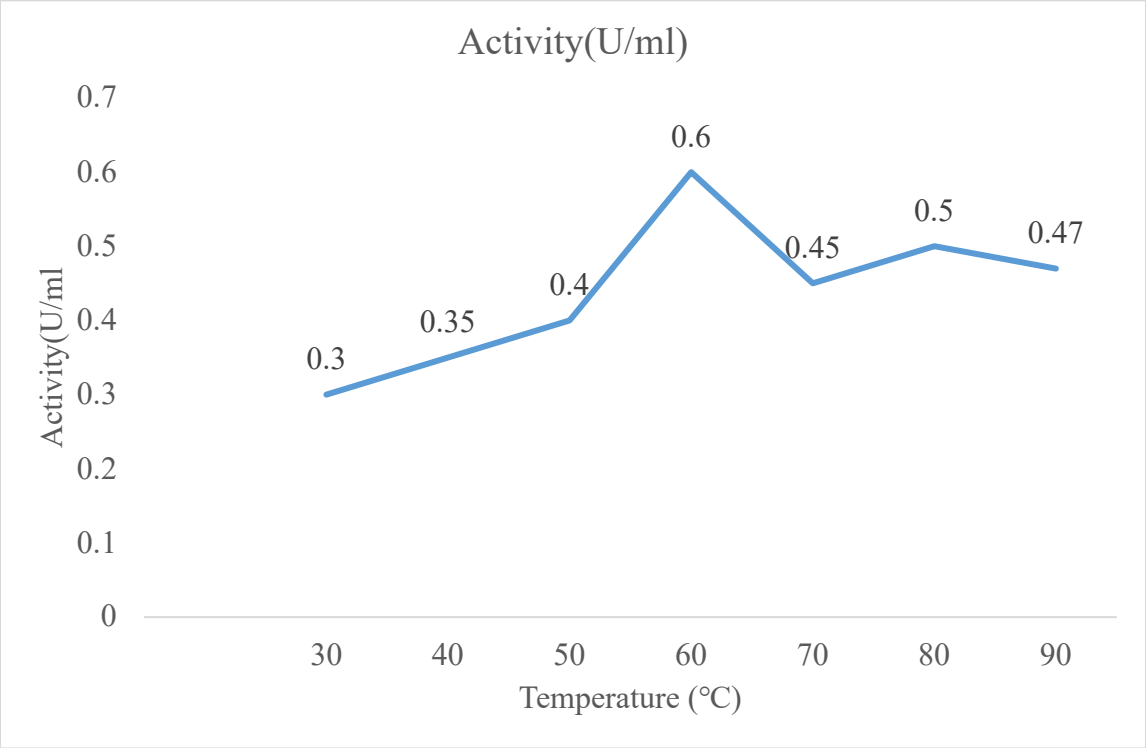

Fig. 9: Effect of Temperature on Cutinase enzyme

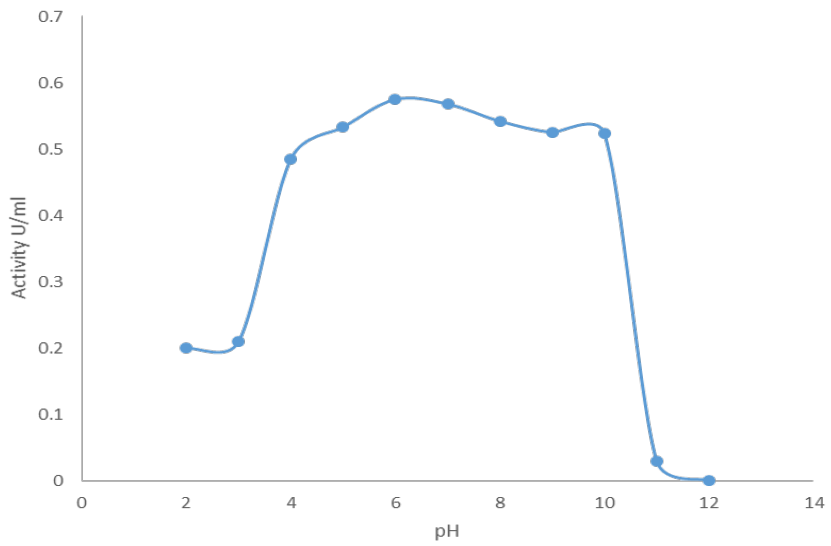

Fig. 10: Effect of pH on Cutinase enzyme

Statistical analysis of results

To compare the initial weight (untreated) and the final weight of each nylon in its respective site over the course of the experiment, statistical analysis, the results of the ANOVA test, and the DUNCAN multiple range of variables were used. These revealed that, while there was no significant difference in the weights of the various nylons, there was a significant difference between their initial and final weights after the experiment's 90 days, as all of the p values were less than p (0.05). The impact of different environmental conditions on the nylons is what led to these outcomes.

Discussion

Due to their resistance to deterioration, plastics like polythene and polypropylene are disposed of in landfills where they remain inert for an extended period. The improper disposal of plastics contributes significantly to the possibility of environmental pollution endangering life. Additionally, the burning of polyvinylchloride (PVC) plastics releases dioxins and furans, two organic pollutants that persist in the environment (Bari *et al.*, 2017).

The total viable count for bacteria ranged from 3.30 x 10^4 cfu/g to 1.50 x 10^6 cfu/g while 1.02×10^4cfu/g to 1.26×10^6cfu/g for fungi. Microbial diversity is a major component of biodegradation as observed in this study. The bacteria that were identified include *Pseudomonas* sp. had the highest occurrence (30%), while, *Staphylococcus* sp. (16%), *Bacillus cereus, Bacillus polymyxa, Bacillus licheniformis* and *Serratia* sp. have (10%) occurrence each. *Micrococcus luteus* has an occurrence of (6%). However, fungi isolated from the samples with their incidence rate include *Aspergilllus niger* had the highest occurrence (25%), *Fusarium* sp. (23.33%), *Rhizopus* sp. (16.67%) *Penicillium* sp. (11.67%), *Clasdosporium* sp. (10%), *Aspergilllus clavatus* and *Aspergilllus flavus have* (5% each) *and Aspergilllus candidus* had the lowest occurrence (3.33%) which is similar to that reported by Chonde *et al.* (2013). According to Asmita *et al*. (2015), the existence of microbes and their capacity to carry out diverse tasks, including clearing up oil leakage or decomposing garbage, are essential for preserving the integrity of the surrounding ecosystem.

It was recently discovered that there were variations in the relative propensity of actinomycetes and fungi to break down ground plastic bags (Nakei *et al.*, 2022). The decrease in low-density polyethylene by *Aspergillus niger* and *Aspergillus flavus* was reported by Deepika and

Jaya (2015) to have resulted in substantial variations in the weight loss of plastics relative to the original quantities. Different studies have also documented how certain fungal genera and species may break down various types of plastics. *Aspergillus flavus*, isolated from polyethylene-contaminated locations near Chennai, India, has been explored to biodegrade polymers, according to Raaman *et al*. (2012), according to other investigations, Penicillium sp. can break down polyethylene (Sowmya *et al*., 2014). The bacterial species that were used in the studies may potentially have an impact on the disparity of the findings for the degradation of polyethylene. Numerous bacterial species are connected to the biodegradation of polymers. Pseudomonas, Staphylococcus, Streptomyces, Bacillus, Acinetobacter, and Micrococcus are a few taxa whose ability to breakdown polyethylene has been experimentally demonstrated (Sangeetha *et al*., 2019, Sahan *et al*., 2023).

The results of the current study revealed that the blue, black, and yellow nylon samples for site one had high biodegradability indices of 9.0%, 8.7%, and 8.1%, respectively. The white nylon samples had the lowest biodegradation percentage 6.7% at the end of three months. This result correlates with the findings of Afreen *et al*. (2020). The greatest biodegradation percentages for the blue, black, and yellow nylon samples at site two were 8.5%, 8.1%, and 7.5%, respectively. The white nylon samples showed a 6.4% biodegradation percentage at the end of the three months. These results are comparable to those of Chonde *et al*. (2013) and Saira *et al*. (2022). Although, the post hoc tests (LSD and Duncan multiple range tests) conducted for the different nylon samples in the two compost sites showed that the means of the nylon weights are not statistically significant because all the p values are greater than p (0.005). These results might be a result of similar chemical materials that were used in the production of these nylon samples, which justified their biodegradation at almost the same rate. However, there was significance difference between the final weights of the nylons and their initial weights which show a relatively slow biodegradation ability of the microbes. The result indicated that LDPE can be degraded by the activities of microorganisms.

During the biodegradation processes, the compost soil's pH and temperature range from 6.7 to 7.45 and 25°C to 29°C for site one, respectively, and 7.4 to 7.65 and 24°C to 26°C for site two. Temperature, pH, contact duration, the type, the microbial load, the availability of nutrients, the composition of the microbial consortium, the properties of the pollutant, and salinity are the variables that affect this process. Al-Jailawi *et al*. (2014) has earlier demonstrated the effect of pH on Pseudomonas species and its effectiveness on biodegradation of LDPE plastic. Plastics only degrade at a faster pace in soil at a lower temperature of 25°C, which is likely what causes this. It is impossible to rule out the possibility of other environmental conditions contributing to the polymer's enhanced breakdown (Emadian *et al*., 2017). The pH of the soil is a significant element as well because it affects microbial activity (Emadian *et al*., 2017). Additionally, it has been proposed that some polymers, including polyethylene, can be degraded by bacteria other than those that can break down polychlorides (Ishii *et al*., 2008).

Fusarium sp. and *Aspergillus niger* strains with the highest cutinase activity were informally discovered and chosen for more research. A temperature of 60°C and pH 6.0 were the ideal conditions for cutinase activity, respectively. This demonstrates that the strains have cutinase activity, which is influenced by pH and temperature. Adiguzel *et al*. (2017) also reported similar outcomes. Extracellular and intracellular depolymerizers are enzymes involved in the significant biological breakdown of polymers, producing smaller, shorter molecules that can pass through microbes' membranes. (Singh and Rawat, 2020). Utilizing a variety of mechanisms, including metabolic, enzymatic, and microbiological organization, microbial biodegradation breaks down complex materials into simpler ones while emitting carbon dioxide. It is based on cometabolism and growth, respectively. As part of the biodegradation system, which includes fungi, bacteria, and yeasts, organic substances are destroyed at this stage (Saira *et al*., 2013).

The use of compost is a natural waste disposal method, but its effectiveness depends on a few conditions. For example, microbial growth during decomposing needs to be promoted by an

optimal ratio of carbon to nitrogen. Polyethylene is a carbon-rich substance, it has the potential to change the C/N balance, which could impact microbial breakdown (Namet *et al.,* 2021). Physical obstacles, restricted microbial access, and decreased surface area for microbial colonization are some of the ways that large or irregularly shaped polyethylene fragments might hinder the composting process. Polyethylene can obstruct the composting process and introduce hazardous elements into the compost if it is polluted with non-biodegradable compounds or toxic pollutants. A balanced moisture content is necessary for composting. Due to its hydrophobic nature, polyethylene may have an impact on the compost's ability to retain water, which could result in too little moisture for microbial activity (Namet *et al.,* 2021). Optimal temperatures promote microbial activity. Compost containing polyethylene has the potential to affect temperature regulation and microbial breakdown rate. Composting can be slowed down by cold weather, but problems might arise from too high temperatures. (Lin *et al.,* 2022). Aerobic microbial action in composting requires oxygen. Particularly in compacted forms, the physical characteristics of polyethylene can restrict oxygen transport, creating anaerobic conditions that impede effective biodegradation (Bher *et al.,* 2022). The pH level of the composting environment affects the decomposition of polyethylene. Elevated pH levels have the potential to impede microbial activity and impact the degradation of polyethylene (Lin *et al.,* 2022).

The combination and diversity of the microbes found in compost enhance its ability to decompose polyethylene effectively. It's possible that some microbes have the metabolic pathways required to break down plastics (Cai *et al.,* 2023). The breakdown of polyethylene happens in a time-dependent manner. Determined by variables like temperature, moisture content, and microbial activity, longer composting times might be necessary to accomplish meaningful biodegradation (Lin *et al.,* 2022).

The cutinase enzyme, which is linked to the enzymatic degradation of microplastics and plastic films, is released by these bacteria. However, there are many different types of microplastics in the environment, and their pollutants pose a serious obstacle to the degradation of microplastics. The review covers techniques for cutting down cutinases enzyme and possible solutions to deal with the waste polyethylene (PE), a type of microplastic that is being produced and is now in use. Cutinase enzymes found in a variety of microbes have the ability to significantly address the worldwide threat of microplastic contamination. (Sahu *et al*., 2023).

Enhancing polyethylene composting and encouraging efficient biodegradation by microbial communities require an understanding of and optimization of these factors. Innovations in plastic waste management and enhancing the sustainability of waste management techniques are still being researched and explored.

From the statistical analysis, the result of the ANOVA test for the comparison between the initial weight (untreated) and the final weight of all the nylons in their respectively sites within the duration showed that there is significant difference in their weights. This is because all the p values were less than p (0.05). These results is as a result of the effect of varying environmental conditions (pH and temperature) , other factors are moisture, soil composition etc. and the occurrence of the different types of microbes and their enzymatic activities at the different locations which justified their biodegradation at different rate. From the statistical analysis, the result of the ANOVA test for the percentage loss in weight in nylon samples used in sites 1 (GVU 1) and 2 (GVU 2) indicates that there is no significant difference between the weight loss of white and blue nylon samples. For site 2, the results of the ANOVA test for the percentage loss in weight in nylon samples used in sites 1 (OAU 1) and 2 (OAU 2) indicated that there is no significant difference between the weight loss of yellow and black nylon samples.

Conclusion

The outcomes of this research indicate that bacterial and fungal species constitute significant drivers for the breakdown of synthetic plastic and that bioreactors can be utilized in other researches on the biodegradation of complex plastics. Composting has been demonstrated to be a highly successful method for polymer biodegradation and a consortium of microorganisms play a very important role in this efficacy of biodegradation. The site 1 had the

highest occurrence of bacterial species while site 2 has the highest occurrence of fungal species. The presence of these microbes facilitated the high biodegradation index of the polyethylene used in the two sites. As observed during the study, the nutrient composition of the soil and environmental other factors also play a major role in the efficiency of composition as well as the presence of organic matter. The environmental conditions are also very important. We, however, suggest a longer period for compositing as this research work was time bound.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Afreen, B., Nouman, N. R. and Saima, I. (2020). Characterization of plastic degrading bacteria isolated from landfill sites. *International Journal of Clinical Microbiology and Biochemical Technology*, *4*(1): 30-35.

Arimah, M.A. and Ogunlowo, O.P. (2014). Identification of Lactic Acid Bacteria Isolated from Nigeria Foods: Medical importance and comparison of their Bacteriocin activities. *Journal of Natural Sciences Research, 4*: 23-37.

Asmita, K., Shubhamsingh, T. and Tejashree, S. (2015). Isolation of Plastic Degrading Micro-organisms from Soil Samples Collected at Various Locations in Mumbai, India. *International Research Journal of Environmental Sciences*, 4(3): 77-85.

Ayilara, M. S., Adeleke, B. S., Adebajo, M. T., Akinola, S. A., Fayose, C. A., Adeyemi, U. T., Gbadegesin, I. A., Omole, R. K., Johnson, R.M., Edhemuino, M., Ogundolie, F. A. and Babalola, O. O. (2023b). Remediation by enhanced natural attenuation; an environment-friendly remediation approach. *Frontiers in Environmental Science, 11.*

Bari, E., Sistani, A., Taghiyari, H.R., Morrell, J.J. and Cappellazzi, J. (2017) "Influence of test method on biodegradation of bamboo-plastic composites by fungi," *Maderas: Ciencia y Tecnolog´ıa*, *19*(4):455–462.

Barry, E. and Digregorio, G. (2009). Biobased Performance Bioplastic: Mirel. *Chemistry and Biology Innovations*, 34:10-19.

Bergey's Manual of Determinative Microbiology (1994). ISBN-10. 9780683006032 · ISBN-13. 978-0683006032 · Edition. Ninth · Publisher. LWW ·

Bher, A., Mayekar, P.C., Auras, R.A and Schvezov, C.E. (2022). Biodegradation of Biodegradable Polymers in Mesophilic Aerobic Environments. *International Journal of Molecular Sciences,* 23(20):12165.

Cai, Z., Li, M., Zhu, Z., Wang, X., Huang, Y., Li, T., Gong, H and Yan, M. (2023). Biological Degradation of Plastics and Microplastics: A Recent Perspective on Associated Mechanisms and Influencing Factors. *Microorganisms*, 11(7):1661-1671.

Cui, Q., Zhang, Z., Beiyuan, J., Cui, Y., Chen, L., Chen, H., and Fang, L. (2022). A critical review of uranium in the soil-plant system: Distribution, bioavailability, toxicity, and bioremediation strategies. *Critical Reviews in Environmental Science and Technology*, 53(3): 340–365.

Deepika, S. and Jaya, M. R. (2015). Biodegradation of low-density polyethylene by microorganisms from garbage soil. *Journal of Experimental Biology and Agricultural Sciences*, 3(1): 16-21.

Emadian, S.M., Onay, T.T. and Demirel, B. (2017). Biodegradation of bioplastics in 663 natural environments. *Waste Manage*, 59:526–536.

Ghatge, S., Yang, Y., Ahn, J-H. and Hurl, H-G. (2020). Biodegradation of polyethylene: a brief review. *Applied Biology and Chemistry,* 63:27-34

Ibrahim, N. I., Anwar, M., Khalid, M. H., Ismail, M. S. and Hamzah, M. M. (2011). Assessment of potential plastic-degrading fungi in Jordanian habitats. *Turkish Journal of Biology,* 35: 551-557.

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

Lin, C., Cheruiyot, N.K., Bui, X.T and Ngo, H.H. (2022). Composting and its application in

bioremediation of organic contaminants. *Bioengineered*, 13(1):1073-1089.

Liu, M, Zhang, T, Liangkun, Long, R., Zhang, and Shaojun, D. (2019). Polymer Degradation and Stability. *Bioresources Technology,*160: 120-125.

Montaz, Z, Habibi-Najaf,i H.B, Mohebbi M. and Oromiehe, L. (2018). Microbial degradation of UV-pretreated low-density polyethylene films by novel polyethylene-degrading bacteria isolated from plastic-dump soil. *Journal of Polymer and Environment*, 26: 3613-3625

Naga, R. M., Dhatri, K., Kadiyala, V and Mallavarapu, M. (2023). Additives of plastics: Entry into the environment and potential risks to human and ecological health. *Journal of Environmental Management*, 348: 119364.

Nakei, M., Misinzo, G., Tindwa, H. and Semu, E. (2022). Degradation of polyethylene plastic bgas and bottles using microorganisms isolated from soils of Morogoro, *Tanzania. Frontiers in Microbiology, 13*.

Nikolaivits, E., Kanelli, M., Dimarogona, M. and Topakas, E. A. (2018).A middle-aged enzyme still in its prime: Recent advances in the field of cutinases. *Catalysts, 8, 612*.

Okafor, C.F., Okanlawon, T.S., Olagunju, A. M., Isatoye, E. F., Oni, T. R. and Adeyemo, S. M. (2023). Invertase activities of lactic acid bacteria isolated from traditional fermented milk ("nono"), agadagidi and palm wine obtained from different locations in Ile-Ife, Osun State, Nigeria. *GSC Biological and Pharmaceutical Sciences*, *22*(01): 147–156

Okanlawon, T.S., Adeyemo, S. M. and Agbaje, I. S. (2023). Isolation and identification of microorganisms associated with Jollof rice sold at Bukateria in Obafemi Awolowo University, Ile - Ife, Osun State, Nigeria. *GSC Biological and Pharmaceutical Sciences*, *22*(01): 178–185

Pio, T.F. and Macedo, G.A. (2008). Cutinase production by *Fusarium oxysporum* in liquid medium using central composite design. *Journal of Industrial Microbiology and Biotechnology*, 35:59-67.

Raaman, N., Rajitha, N., Jayshree, A. and Jegadeesh, R. (2012). Biodegradation of plastic by Aspergillus spp. isolated from polythene polluted sites around Chennai. *Journal of Academia and Industrial Research*, 1(6): 313-316.

Restrepo-Florez, J.M., Bassi, A. and Thompson, M.R. (2014) Microbial degradation and deterioration of polyethylene-a review. *International Society for Biodeterioration and Biodegradation*, *88*:83–90

Restrepo-Florez, J.M., Bassi, A. and Thompson, M.R. (2014). Microbial degradation and deterioration of polyethylene—a review. *International Society for Biodeterioration and Biodegradation*, *88*, 83-90.

Sahu, S., Kaur,A. Khatri, M., Singh, G., Arya, S.K. (2023). A review on cutinases enzyme in degradation of microplastics. *Journal of Environmental Management*, 347:0301-0314.

Sammond, D.W., Yarbrough, J.M., Mansfeld, E., Bomble, Y.J., Hobdey, S.E., Decker, S.R., Taylor, L.E., Resch, M.G., Bozell, J.J., Himmel, M.E., Vinzant, T.B. and Crow- ley, M.F. (2014). Predicting enzyme adsorption to lignin films by calculating enzyme surface hydrophobicity. *Journal of Biology and Chemistry, 289*:20960–20969

Sangeetha Devi R., Ramya R, Kannan K., Robert Antony A., Rajesh Kannan V. (2019). Investigation of biodegradation potentials of high-density polyethylene degrading marine bacteria isolated from the coastal regions of Tamil Nadu. *India Maritime and Pollution Bulletin,* 138*:*549–560.

Sen, S.K. and Raut, S. (2015) Microbial degradation of low-density polyethylene (LDPE): a review. *Journal of Environmental and Chemical Engineering,* 3:462–473

Singh, S. and Rawat, P. S. (2020). "*Biodegradation of plastic," in Handbook of Research on Environmental and Human Health Impacts of Plastic Pollution*, pp. 435–461, IGI Global

Sneath , P.H. , Mair , N.S. , Sharpe , M.E. and Holt , J.G . (1986). *Bergey's Manual of Systematic Bacteriology*, Vol. 2 , Baltimore, MD : The Williams and Wilkins Co.

Sowmya, H.V., Ramalingappa, B., Nayanashree, G., Thippeswamy, B. and Krishnappa, M. (2014).

Polyethylene Degradation by Fungal Consortium. *International Journal of Environmental Research*, 9(3): 823-830.

Wilkes, R.A. and Aristilde, L. (2017) Degradation and metabolism of synthetic plastics and associated products by *Pseudomonas* sp. Capabilities and challenges. *Journal of Applied Microbiology,* 123:582–593