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Tuning enzyme concentration and particle size for LDPE biodegradation using lipase and laccase systems

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Abstract

The persistent accumulation of low-density polyethylene (LDPE) waste in the environment has necessitated the exploration of eco-friendly degradation methods. This study aimed to degrade LDPE films using lipase (Lip) and laccase (Lac) enzymes obtained from *Aspergillus flavus*. The effects of enzyme concentration and LDPE particle size on the degradation rate were examined. LDPE samples were prepared in three particle sizes: 0.5, 1, and 2 cm. These samples were incubated with Lip, Lac, and a combination of both enzymes (Lip-Lac) at two concentration levels: 50% and 100% (v/v). The degradation process or extent of degradation was monitored over 10 and 30 days by analyzing percentage weight loss and observing surface morphology using scanning electron microscopy (SEM). Results indicated that the highest degradation occurred in the Lip-Lac system with 0.5 cm particles at 100% enzyme concentration, yielding a weight loss of 23.81% after 30 days, thereby suggesting that the blend performed better than the single enzyme system. SEM analysis confirmed extensive surface erosion and cracking in smaller particles treated at higher enzyme concentrations. This study also demonstrated that both enzyme concentration and LDPE particle size significantly influence biodegradation efficiency. Taken together, the bifunctional enzyme system is an efficient treatment method for enhancing the degradation process of plastics such as LDPE.

Keywords: LDPE plastic; Enzymatic degradation; *Aspergillus flavus*; Lipase; Laccase

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1. Introduction

Plastics are primarily made of synthetic polymers consisting of chains of carbon atoms with other elements—such as hydrogen, oxygen, nitrogen, and sulfur—attached to them.[1] Polymers are widely used due to their durability and stability. Common examples include polystyrene,[2] polyethylene (PE),[3] polyurethane,[4] nylon,[5] PE terephthalate (PET),[6] polypropylene,[7] and polyvinyl chloride, all of which are present in almost every aspect of our lives.[8] Since the discovery of plastics in the 1950s, there has been a significant increase in their production to meet the global demand.[9] It is estimated that more than 6.3 billion metric tons of plastic waste have been generated over the past 75 years,[10]

from a total of over 450 million tons produced annually worldwide.[11,12] This increase in production is mainly due to the manufacturing of single-use disposable plastics, which make up around 50% of all plastic products. The significant increase in plastic waste disposal, which consists of materials that do not easily break down and thus remain in the environment, has resulted in land[13] and ocean pollution.[14,15] Due to their practical physicochemical qualities (*e.g.*, crystallinity, molecular weight, hydrophobicity, and additive composition), some polymers, such as low-density PE (LDPE), are utilized in a variety of applications.[16] At present, PE is derived from thin polyolefin plastic sheets and films utilized in the packaging of products. Furthermore, plastic bags constitute 60% of the total plastic production, which includes LDPE, and are considered the most common form of solid waste.[17] LDPE is characterized by good strength, chemical resistance, flexibility, and transparency. Its hydrophobic nature prevents microorganisms from accessing it.[18] The resistance of LDPE to microbial degradation is largely due to its high molecular weight, which limits enzyme accessibility, and the absence of functional groups, which prevents effective microbial attachment or breakdown.[19] In addition, its low surface energy further hinders microbial colonization by reducing surface wettability and interaction with enzymes.[20] LDPE is characteristically inert, and its rate of degradation is extremely slow, taking several decades, which causes it to remain in nature for an extended period. Due to its complicated 3D structure and large molecular weight, it is recalcitrant to degradation.[21] Despite being widely used for managing LDPE waste, incineration and landfilling methods are linked to several environmental drawbacks. Thus, biodegradation has been demonstrated to be the optimal choice for managing plastic waste.[22]

Since it is virtually impossible to eliminate their manufacture, there is growing interest in developing more effective and rapid methods for reducing the accumulation of these widely used yet environmentally harmful plastic items.[23,24] It is highly likely that scientists, who are constantly engaged in researching and developing biologically safe methods, will identify a solution to address the rapid accumulation of plastic waste. Researchers have identified particular microorganisms that can generate enzymes capable of decomposing these polymers.[25-28] Enzymes that degrade the carbon backbone of polymers are classified under the hydrolase family, including esterases,[29] lipases (Lips),[30] depolymerases,[31] and PETases.[32] Buchholz *et al.*[33] enumerated and discussed enzymes that require water to function. These enzymes can break down the plastic polymer into simpler monomeric units that are easily decomposable within the environment and can serve as a carbon source for microorganisms.[34] The microorganisms then further metabolize these units into end products, such as CO_2, H_2O, CH_4, and N_2. Ehrhardt and Rothenberg[35] noted that green hydrogen is also a realizable outcome. The by-products of microbial plastic degradation offer significant potential for addressing plastic pollution by repurposing these products in other applications, thereby creating a recycling loop for these harmful materials that are otherwise difficult to eliminate and contribute to environmental pollution. Municipal and public waste management is currently the biggest environmental issue affecting emerging countries, especially Nigeria,[36-39] where some cities are stench-filled with tons of uncontrollable solid and plastic waste.[40-44] As a result of Nigeria's current economic state, LDPE sachets are commonly used for packaging water, popularly known as "pure water."[45] These pure water sachets (PWS) represent the most affordable packaging option.[46] While the use of LDPE has gained widespread acceptance across various communities, it has unfortunately resulted in the rise of a solid waste stream, as LDPE degrades at an extremely slow rate.

This study was initiated to investigate how Lip and laccase (Lac) enzymes impact the degradation rate of LDPE wastes (specifically PWS). While several studies have explored LDPE degradation using microbial enzymes, most have focused on either enzyme concentration or polymer structure in isolation. For instance, Liu *et al.*[47] examined LDPE degradation using *Yarrowia lipolytica*, but without optimizing for both enzyme load and particle size simultaneously. Mazaheri and Nazeri[48] reported the effectiveness of *Stenotrophomonas* spp. and *Alcaligenaceae* in degrading LDPE, demonstrating microbial potential, though their work did not incorporate enzymatic concentration gradients or particle size variations as interactive variables. Khandare *et al.*[49] used marine bacteria for LDPE biodegradation and highlighted particle preparation and treatment time, but lacked an integrative assessment of enzyme-specific parameters, such as those studied with *Aspergillus flavus* enzymes in this research. DSouza *et al.*[50] worked with an *Aspergillus* consortium to degrade LDPE under controlled conditions, yet did not combine enzyme concentration and plastic size optimization, a gap this study aims to bridge using a bifunctional Lip-Lac system. The novelty of the study is that it is the first to simultaneously optimize both enzyme concentration and plastic particle size to maximize LDPE degradation using enzymes from *A. flavus*. The study supports Sustainable Development Goal (SDG) 12 (Responsible Consumption and Production) and SDG 13 (Climate Action). By utilizing microbial enzymes from *A. flavus* (a naturally occurring fungus) for the degradation of LDPE plastic waste, our study promotes the development of green technologies aligned with the

United Nations Agenda 2030. Our proposed enzymatic approach holds promise for application in decentralized waste treatment facilities, bioreactors for plastic waste remediation, and as a pre-treatment step to accelerate subsequent biodegradation. More importantly, such eco-friendly strategies can reduce the environmental footprint of incineration and landfilling, which aligns with circular economy principles and global sustainability targets.

2. Materials and methods

2.1. Materials

Low-density PE (LDPE) samples (PWS) were collected from dumpsites within Gidan Kwano village (Nigeria). Enzymes used were produced at the Step B Laboratory, Centre for Genetic Engineering and Biotechnology, Federal University of Technology, Minna (FUTMinna), Bosso Campus, Nigeria. The incubation media used for the LDPE samples and all analytical chemicals were procured from the Department of Chemical Engineering, FUTMinna (Nigeria).

2.2. Preparation of PE films

The LDPE samples were cut into 0.5 × 0.5, 1 × 1, and 2 × 2 cm strips and weighed using a digital weighing balance (MAB220, Wensar, India), as previously reported.[49,51] The samples underwent a rigorous cleaning process, which involved immersion in a solution of 70% ethanol for 30 min, followed by washing with distilled water, and drying in an oven at 50°C for 20 min.

2.3. Production of degrading enzymes

Ab initio, the medium was prepared through the following steps. First, the Sabouraud Dextrose Agar was prepared by dissolving 20 g of the powdered non-synthetic medium in two separate 2000 mL conical flasks labeled "Lipase" and "Laccase," respectively, each containing distilled water.[52] For the Lip medium, 2 mL of olive oil (0.1% substrate concentration) was added. For the Lac medium, 2 mL of guaiacol (0.1% substrate concentration) was added.[53] Both media were sterilized in an autoclave at 121°C for 3 h under a pressure of 1.5 N/m. After autoclaving, the medium was removed from the autoclave and allowed to cool before inoculation. The 72-h-old broth culture of *A. flavus* (5 mL) was inoculated into 1 L of each medium and incubated for 5 days on a shaker incubator at ambient or room temperature (25–28°C ± 2°C). After 5 days, crude enzymes were readily produced. The crude enzyme solutions were centrifuged at 4000 rpm using a high-speed refrigerated centrifuge. The supernatants contained the crude enzymes, while the sediments consisted of fungal spores. The Bradford protein assay was used to quantify the total protein content in the crude extract, using bovine serum albumin as the standard.[54] The crude enzyme concentration was measured at 10 mg/mL (20% purity). To reduce the concentration for further assays, 10 mL of the extract was diluted with an equal volume of distilled water, resulting in a final protein concentration of 5 mg/mL, while maintaining the purity at 20%.

2.4. Incubation of PE samples

2.4.1. Isolation of LDPE film in the single-enzyme system

The pre-weighed LDPE films (0.5 × 0.5, 1 × 1, and 2 × 2 cm) were aseptically transferred into three pre-labeled 100 mL conical flasks, respectively. About 20 mL of the prepared degrading enzyme was added to each conical flask and thoroughly mixed with the LDPE samples. For assessing the effect of different enzyme concentrations, the prepared LDPE strips (1 × 1 cm each) were transferred into two pre-labeled 100 mL conical flasks, respectively.[55] Then, 100% pure Lip and a 50% diluted Lip solution were added separately to each flask and thoroughly mixed with the LDPE samples. To minimize heat dissipation and preserve a sanitized environment, a foil stopper was used to seal the conical flasks.[12] The procedure was repeated for Lac. After 10 days of incubation, samples were collected, and the LDPE films were cleaned with ethanol and distilled water, then allowed to air-dry naturally. Following that, the LDPE samples were evaluated to determine their level of biodegradability. After 30 days, the process was terminated. The 30-day incubation period was selected to allow sufficient time for measurable enzymatic degradation of LDPE by Lac and Lip.

2.4.2. Isolation of LDPE film in the two-enzyme system

The pre-weighed LDPE films (0.5 × 0.5 and 2 × 2 cm) were transferred into two pre-labeled 100 mL conical flasks, respectively. Lip and Lac enzymes (20 mL) were added to each conical flask and thoroughly mixed with the LDPE samples. For assessing the effect of different enzyme concentrations, the prepared LDPE strips (1 × 1 cm) were transferred into two pre-labeled 100 mL conical flasks, respectively.[55] The Lip-Lac enzyme mixture at 100% and 50% concentrations was added to the two conical flasks, respectively. To minimize heat dissipation and preserve a sanitized environment, a foil stopper was used to seal the conical flasks.[12] The solution was gently stirred to obtain a homogeneous mixture. The LDPE films were cleaned using ethanol and distilled water, then allowed to air-dry naturally. Biodegradation of the LDPE samples was analyzed after 10 days of incubation. Following that, the LDPE samples were evaluated to determine their level of biodegradability. The process was terminated after 30 days, in accordance with Yang *et al.*[56]

2.5. Physicochemical analysis of LDPE degradation

2.5.1. Determination of dry weight of residual LDPE

To obtain the dry weight of residual LDPE, the LDPE films were extracted from the degradation medium and cleaned using a 70% ethanol solution, followed by rinsing with distilled water. The purified LDPE films were allowed to air-dry to a constant weight. The percentage of weight reduction was calculated using Equation I.[51]

$$\text{Percent weight loss}\,(\%) = \frac{\text{Initial sample weight} - \text{Final sample weight}}{\text{Initial weight}} \times 100 \quad \text{(I)}$$

2.5.2. Scanning electron microscopy (SEM)

Following weight analysis, the LDPE samples were cut into 5 mm fragments. To observe the surface morphology of the LDPE, SEM (SU3800/SU3900, Hitachi High-Tech, Japan) was employed.[57] Each sample was carefully mounted onto a specimen holder (stub) to ensure proper placement and fit within the SEM chamber, with all fragments trimmed to suitable dimensions for imaging.

3. Results and discussion

3.1. Effect of enzyme concentration on LDPE degradation

After incubation of LDPE in 50% and 100% enzyme concentrations for 10 and 30 days, the weight of the residual samples was measured (Table 1). The initial weight and size of the material influence degradation. Smaller particles have a higher surface area-to-volume ratio, facilitating better enzyme interaction and, consequently, higher degradation rates. Therefore, reducing the initial size of the material can enhance the degradation process. As displayed in Table 1, Lip and Lac enzymes were incubated with the LDPE samples at 50% and 100% concentrations. At 50% Lip concentration, the weight reduced from 0.0036 to 0.0035 g (day 10) and 0.0034 g (day 30), corresponding to 2.78% and 5.56% LDPE weight loss, respectively, thereby indicating greater degradation over time. The 100% enzyme concentration observed a higher percentage weight loss compared to the 50% enzyme concentration; for instance, the Lac enzyme system reported 5.13% and 8.33% weight loss at 50% and 100% Lac concentration, respectively, after 30 days. Likewise, Liu *et al.*[47] recorded a weight loss of 8.9% and 11.8% for LDPE films at day 7.

Table 1. Percentage weight loss of LDPE samples at different enzyme concentrations

Enzyme	Concentration (%)	Initial weight (g)	Final weight (g)		Weight loss (%)	
			Day 10	Day 30	Day 10	Day 30
Lip	50	0.0036	0.0035	0.0034	2.78	5.56
	100	0.0036	0.0034	0.0032	5.56	11.11
Lip-Lac	50	0.0043	0.0041	0.0039	4.65	9.30
	100	0.0046	0.0043	0.0039	6.52	15.21
Lac	50	0.0039	0.0039	0.0037	0.00	5.13
	100	0.012	0.012	0.011	0.00	8.33

Abbreviations: Lac: Laccase; LDPE: Low-density polyethylene; Lip: Lipase.

In terms of degradation efficiency, LDPE incubation in 100% Lip, 100% Lip-Lac, and 100% Lac recorded 11.11%, 15.21%, and 8.33% weight loss, respectively. This observation is relatively consistent with the 10.15% loss reported by Mazaheri and Nazeri[48] with *Stenotrophomonas* spp. enzymes. This implies that LDPE was more effectively degraded by the Lip-Lac enzyme system, suggesting that while Lip alone exhibits notable degradation efficiency, the Lip-Lac enzyme system has much higher degradation efficiency. In addition, higher enzyme concentrations enhance the degradation efficiency, as observed from Table 1. Our findings corroborated that of Kunlere *et al.*,[58] who used *A. flavus* to degrade LDPE. Concentrations below 50% are likely to result in even lower degradation rates. Given that 50% concentration already yields modest weight loss (*e.g.*, 5.56% in the Lip system), reducing the concentration further would likely diminish the degradation efficiency, making it less effective for practical purposes. Increasing the incubation period beyond 30 days may further enhance degradation, as the enzymes have more time to act on the material. Conversely, reducing the incubation time might not allow for sufficient degradation, leading to lower weight loss percentages. Therefore, longer incubation periods are generally favorable for improved degradation. Yao *et al.*[59] reported a high weight loss on day 30 for their LDPE film samples. Similarly, in this study, optimal degradation was observed on day 30 across all systems. For instance, the Lip-Lac system at 100% concentration reported weight loss of 15.21% on day 30 compared to 6.52% on day 10. This indicates that prolonged exposure of LDPE to the enzyme system enhances the degradation process. In a previous study, untreated LDPE reported a weight loss of 38.82% using a combined system of Lip, Lac, esterase, and manganese peroxidase.[53] Figures 1-4 illustrate the relationship between enzyme concentration and LDPE weight loss, as derived from the data in Table 1.

A high weight loss denotes effective biodegradation, indicating that the material is efficiently broken down by the enzymes. Conversely, a low weight loss suggests limited degradation, which may be inadequate for practical

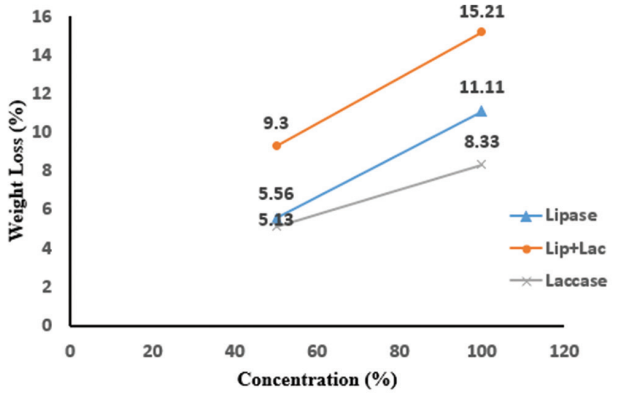

Figure 1. Weight loss (%) of low-density polyethylene as a function of enzyme concentration for all systems (lipase [Lip], laccase [Lac], and Lip-Lac)

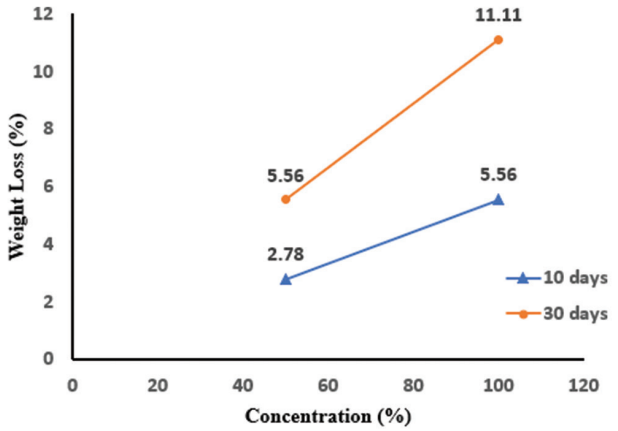

Figure 2. Weight loss (%) of low-density polyethylene as a function of enzyme concentration for the lipase enzyme system

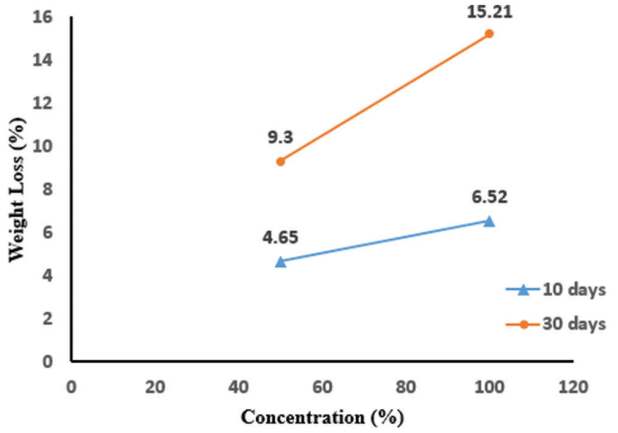

Figure 3. Weight loss (%) of low-density polyethylene as a function of enzyme concentration for the lipase-laccase enzyme system

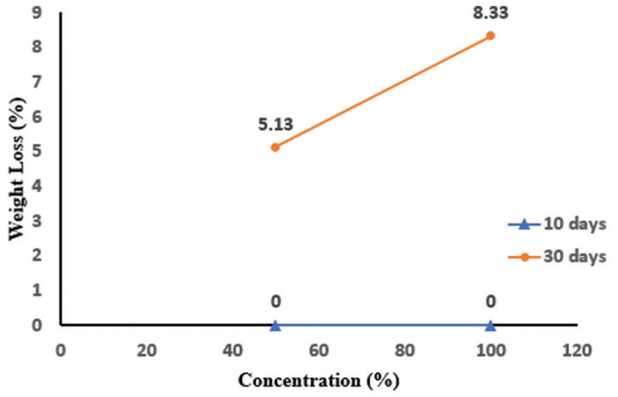

Figure 4. Weight loss (%) of low-density polyethylene as a function of enzyme concentration for the laccase enzyme system

applications. Therefore, achieving higher weight loss is desirable for effective waste management. The Lip-Lac system exhibited the most significant weight loss (Figure 3). At 100% concentration, the weight loss reaches 15.21%, indicating a high degradation rate. This suggests that the combined action of Lip and Lac enzymes synergistically enhanced LDPE degradation.

3.2. Effect of LDPE particle size on LDPE degradation

Harrat *et al.*[60] previously reported weight loss of 7.6% and 5.53% using *Candida tropicalis* SLNEA04 and *Rhodotorula mucilaginosa* SLNEA05, respectively. Their findings are consistent with our results for the Lip system on day 10 for 0.5–1 cm LDPE samples. Table 2 presents the initial and final weights of LDPE samples with varying particle sizes after enzymatic treatment. The data indicate that smaller particles (0.5 cm) experienced greater weight loss compared to larger ones (1.5 and 2.0 cm). This suggests that reducing particle size enhances the surface area available for enzymatic action, leading to more efficient degradation. The influence of incubation time is most pronounced in the 0.5 cm particle size across all enzyme systems, particularly in the Lip-Lac system. Weight loss percentages increased significantly from day 10 to day 30, indicating that longer exposure enhances degradation. If the incubation period were extended to 60 days, it would be reasonable to anticipate further degradation, potentially leading to higher weight loss percentages, assuming enzyme activity remains stable over time. Notably, no formal enzyme activity assay was conducted before the degradation experiments. This assumption is supported by DSouza *et al.*,[50] who reported a peak weight loss of 26.15% after 55 days of incubation. Similarly, Awasthi *et al.*[61] conducted experiments over a comparable timeframe (10–30 days) using *Rhizopus oryzae*, a fungus known to secrete Lip.

According to Table 2 and Figures 5-8, the smallest particle size of 0.5 cm consistently resulted in the highest

Table 2. Percentage weight loss of enzyme-treated LDPE samples with varying particle sizes

Enzyme	Sample size (cm)	Initial weight (g)	Final weight (g)		Weight loss (%)	
			Day 10	Day 30	Day 10	Day 30
Lipase	0.5	0.0015	0.0014	0.0012	6.67	20.00
	1.0	0.0036	0.0034	0.0032	5.56	11.11
	2.0	0.0117	0.0116	0.0114	0.85	2.56
Lip-Lac	0.5	0.0021	0.0019	0.0016	9.52	23.81
	1.0	0.0046	0.0043	0.0039	6.52	15.21
	2.0	0.0111	0.0108	0.0104	2.70	6.31
Laccase	0.5	0.0016	0.0016	0.0014	0.00	12.5
	1.0	0.0038	0.0037	0.0035	2.63	7.89
	2.0	0.0105	0.0105	0.0103	0.00	1.90

Abbreviations: Lac: Laccase; LDPE: Low-density polyethylene; Lip: Lipase.

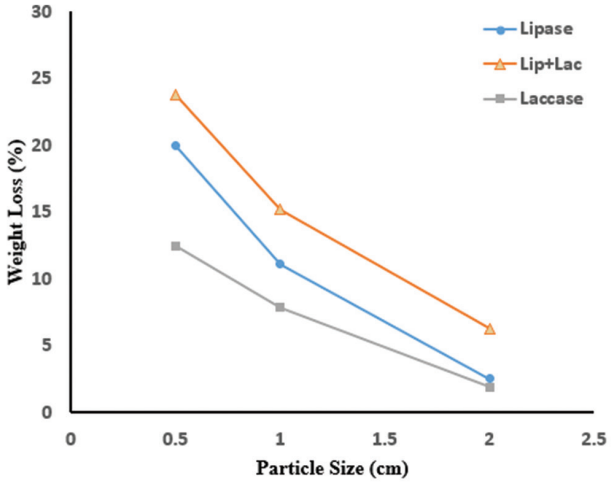

Figure 5. Relationship between low-density polyethylene weight loss (%) and particle size for all enzyme systems (lipase [Lip], laccase [Lac], and Lip-Lac).

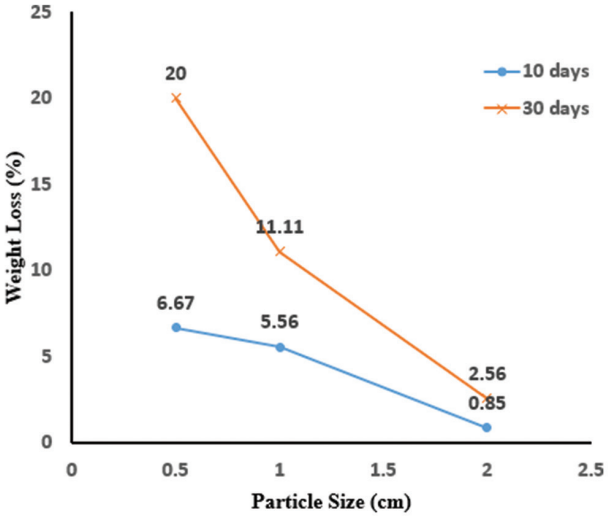

Figure 6. Relationship between low-density polyethylene weight loss (%) and particle size for the lipase enzyme system

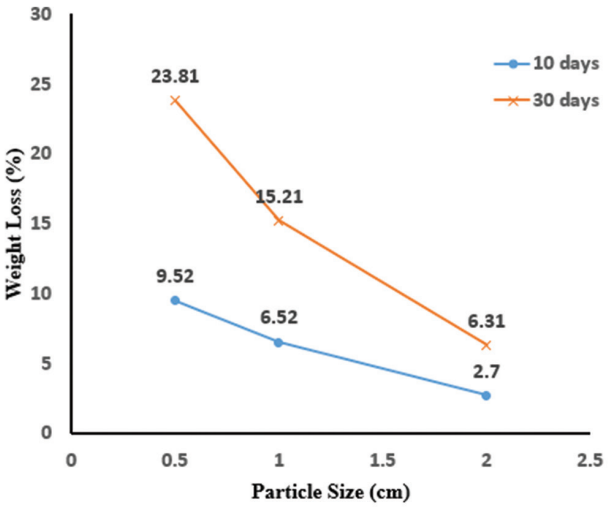

Figure 7. Relationship between low-density polyethylene weight loss (%) and particle size for the lipase-laccase enzyme system

percentage weight loss across all enzyme systems. However, on day 10, 0% weight loss was recorded for particle sizes of 0.5 and 2 cm in the Lac system, despite Lac being among the main fungal enzymes involved in PE biodegradation.[62] Smaller particle sizes provide a larger surface area-to-volume ratio, facilitating better enzyme-substrate interactions. This principle is well-established in biodegradation studies, where increased surface area enhances microbial and enzymatic activity, thereby resulting in higher degradation rates. During LDPE degradation, several physical and mechanical properties have been reported to decline, including tensile strength, tear strength, elongation at break, Young's Modulus, stiffness, hardness, and weight by 10.15%.[63]

Reducing the particle size below 0.5 cm could potentially increase degradation rates due to a greater surface area. However, practical limitations, such as handling difficulties and potential agglomeration, may arise. Conversely, increasing the particle size beyond 2 cm would likely decrease degradation efficiency, as the reduced surface area would limit enzyme access to the polymer chains. Optimal LDPE biodegradation is achieved by combining smaller particle sizes (0.5 cm) with higher enzyme concentrations (100%). Such a combination maximizes the surface area for enzyme interaction and ensures sufficient enzymatic activity to effectively break down the polymer chains. The Lip-Lac system demonstrates the most favorable results,

consistently achieving higher weight loss percentages across all particle sizes. This synergistic effect enhances LDPE breakdown, making it the preferred enzymatic treatment for effective biodegradation.

3.3. Microscopy imaging of enzyme-treated LDPE samples

3.3.1. Effect of different enzyme concentrations

Rough surfaces and pore structures are observed in all LDPE samples incubated with enzymes, and the features are more significant in samples incubated in 100% enzyme concentration, likely due to enzymatic activity on the LDPE samples.[64] Figures 9-12 display the SEM images of LDPE films (1 × 1 cm). Each image illustrates the surface morphology of LDPE under different treatment conditions: untreated/control (Figure 9), Lip-treated (Figure 10), Lip-Lac-treated (Figure 11), and Lac-treated (Figure 12). The consistent sample size and imaging technique across these figures facilitate a comparative analysis of the effects of enzymatic treatments on LDPE surface degradation. The SEM images in Figure 9 serve as a baseline, showcasing the pristine condition of the LDPE film without any enzymatic treatment. The smooth and defect-free surface confirms the material's resistance to degradation in the absence of enzymatic activity. These control images are crucial for comparing the extent of degradation observed in the enzyme-treated LDPE samples. It was previously established that the PE surface changes after 30 days of incubation with enzymes.[65]

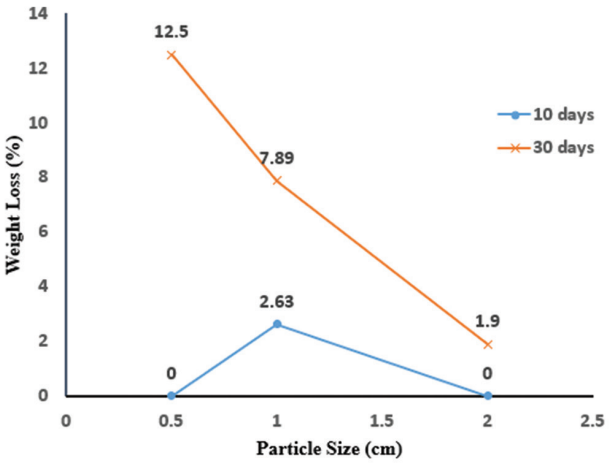

Figure 8. Relationship between low-density polyethylene weight loss (%) and particle size for the laccase enzyme system

Figure 9, representing the control LDPE film, exhibits a smooth and uniform surface, indicating no degradation. In contrast, Figure 10, depicting the LDPE film treated with Lip, displays noticeable surface alterations, such as pits, grooves, and rough textures, similar to a previous observation using *Bacillus* spp. YP1.[66] Das and Kumar[67] inferred that Lip treatment initiates the LDPE degradation process, leading to polymer surface breakdown. Figure 10 presents the SEM images of LDPE films treated with Lip at different magnifications. The images reveal the formation of surface irregularities, including pits and cracks, indicating the onset of enzymatic degradation.[68,69] Higher magnifications highlight more detailed degradation features, such as micro-cracks and increased surface roughness. The presence of white and dark areas in the images corresponds to differences in electron density, with white regions typically representing denser or elevated areas and dark regions indicating depressions or voids.[70] Figure 11 comprises six SEM images (at different magnifications) of LDPE films treated with a combination of Lip and Lac at various concentrations. The images demonstrate that higher enzyme concentrations lead to more pronounced surface degradation, evident through extensive cracking, pitting, and roughness.[71] At higher magnifications, the images reveal finer degradation details, such as micro-fissures and increased porosity. The similarities across the images include the presence of degradation features, while differences arise from the varying degrees of surface damage corresponding to enzyme concentration.

Figure 12 displays six SEM images of LDPE films treated with Lac at different concentrations. Sowmya *et al.*[72] assessed the degradation potential of crude Lac on PE using weight loss, SEM, and Fourier transform infrared (FTIR) analysis. Consistent with the present study, the

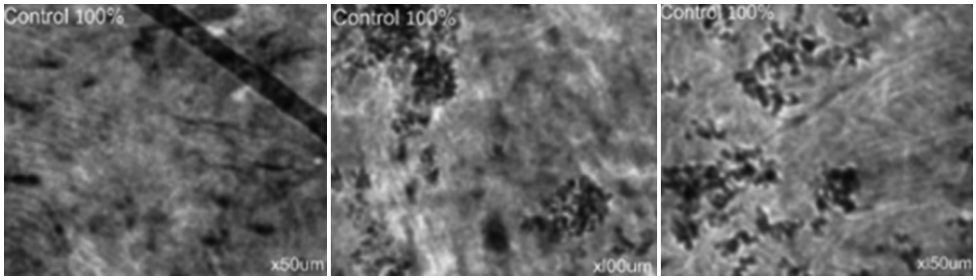

Figure 9. Microscopic images of untreated/control low-density polyethylene films (1 × 1 cm). Magnifications: ×50 (left); ×100 (middle); ×150 (right).

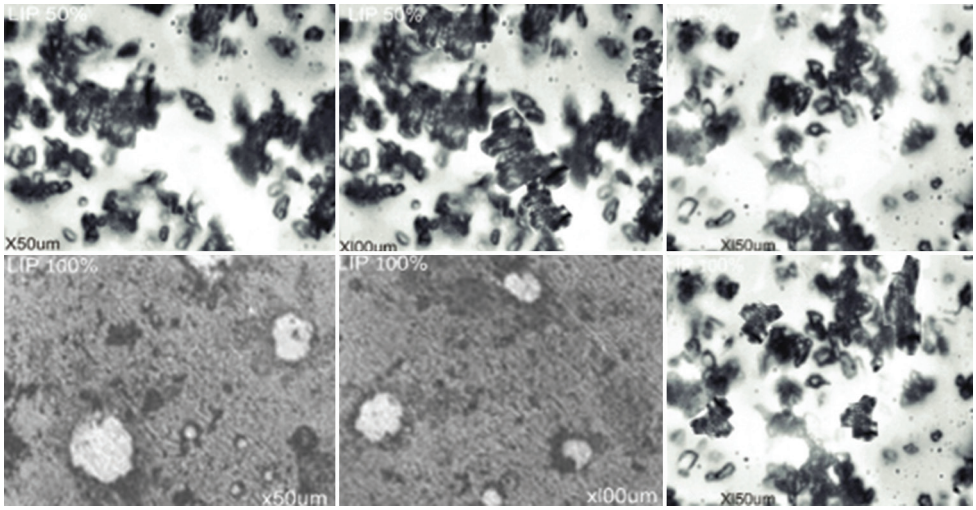

Figure 10. Microscopic images of low-density polyethylene films (1 × 1 cm) treated with lipase. Magnifications: ×50 (left); ×100 (middle); ×150 (right).

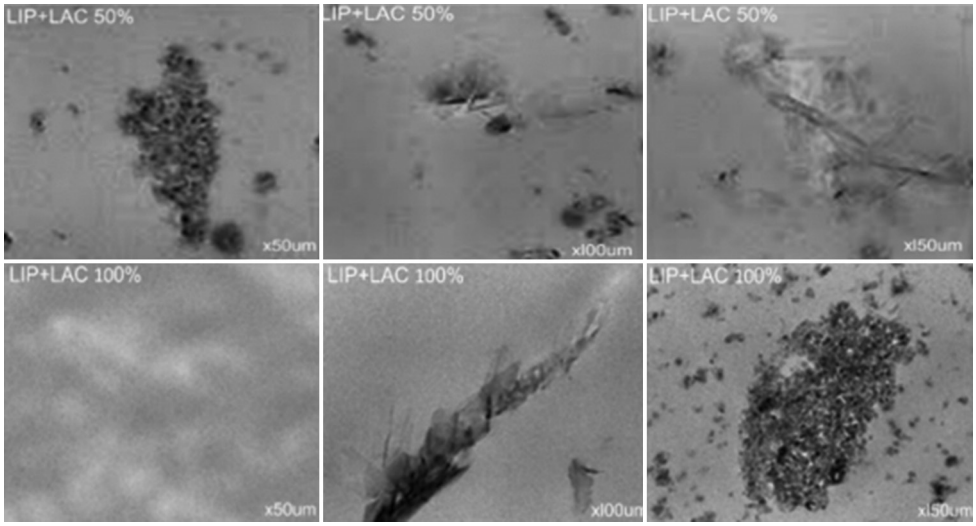

Figure 11. Microscopic images of low-density polyethylene films (1 × 1 cm) treated with lipase-laccase enzymes. Magnifications: ×50 (left); ×100 (middle); ×150 (right).

SEM images reveal that higher Lac concentrations induce more significant surface degradation, characterized by increased roughness, cracking, and pit formation. These degradation features align with observations reported by Mumtaz *et al.*[68] However, the severity and extent of surface damage differ across the images and correlate directly with the enzyme concentration used.

3.3.2. Effect of different LDPE particle sizes

Figure 13 presents SEM images of untreated LDPE films at three different particle sizes: 0.5, 1, and 2 cm. The first image (left; 0.5 cm) appears darker, indicating a smoother and more uniform surface with minimal electron scattering. The second image (middle; 1 cm) exhibits a gray tone, suggesting slight surface irregularities. The third image (right; 2 cm) is lighter, implying increased surface roughness or contamination. Variations in shading reflect differences in surface topography and electron density, with darker areas representing smoother surfaces and lighter areas indicating rougher textures.[73] The images displayed irregular/rough surfaces observed for LDPE samples with a particle size of 0.5 cm in all enzyme systems and less rough surfaces as the sizes increase. The surface erosion observed suggests high enzymatic activity on the films compared to the smooth surface observed in the control samples.[74]

The control SEM images in Figure 13 serve as a baseline, showcasing the pristine condition of LDPE films without enzymatic treatment. The smooth and uniform surfaces confirm the material's resistance to degradation in

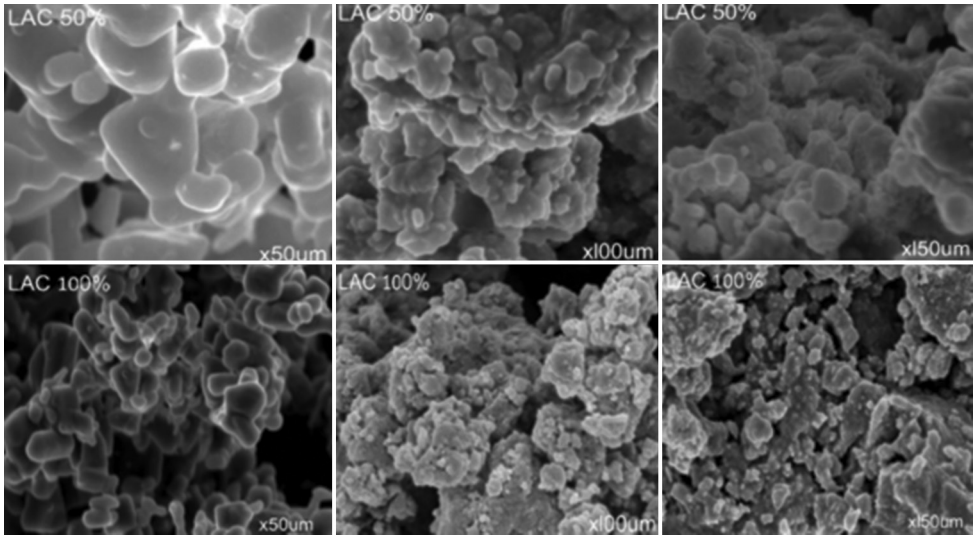

Figure 12. Microscopic images of low-density polyethylene films (1 × 1 cm) treated with laccase. Magnifications: ×50 (left); ×100 (middle); ×150 (right).

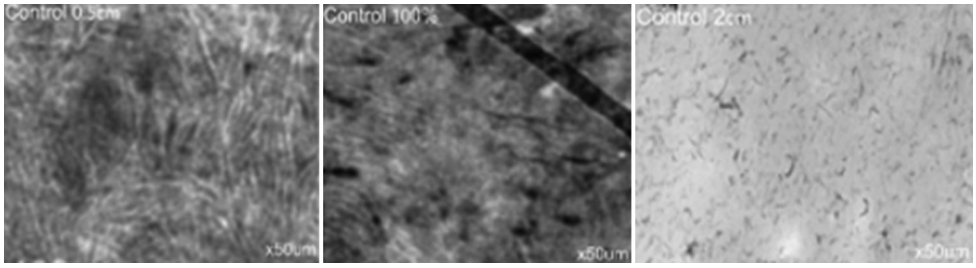

Figure 13. Microscopic images of untreated/control low-density polyethylene films with particle sizes of 0.5 (left), 1 (middle), and 2 cm (right). Magnifications: ×50.

the absence of enzymatic activity. These images are crucial for comparing the extent of degradation observed in the enzyme-treated samples. Figure 14 comprises nine SEM images arranged into three columns, with each column corresponding to a specific particle size (0.5 [left], 1 [middle], and 2 cm [right]) and each row corresponding to a specific magnification (50× [top], 100× [middle], and 150× [bottom]). When the particle size is 0.5 cm, the images (magnification: 50×) revealed significant surface degradation characterized by pits, cracks, and rough textures. Similar characteristics were observed by Khandare *et al.*[49] after 30 and 90 days of incubation for control and bacterial-degraded LDPE. These features indicate effective enzymatic action by Lip on smaller particles. The images for the 1 cm samples displayed moderate degradation with visible surface irregularities, though less pronounced than in the 0.5 cm samples. However, a minimal surface change was observed when the particle size is 2 cm, suggesting that larger particles are less susceptible to Lip-mediated degradation. Similarities across the images include the presence of degradation features, while the differences lie in the severity and extent of surface damage, which correlate with particle size and magnification levels, as also observed by Azeko *et al.*[75]

The SEM images in Figures 14-16 reveal degradation patterns consistent with established literature, such as surface roughness, cracks, and pits resulting from enzymatic action. However, unexpected features, such as excessive degradation in larger particles or minimal changes in smaller ones, could indicate anomalies in the experimental setup or enzyme activity. Such deviations warrant further investigation to ensure consistency with known biodegradation mechanisms. Figure 15 mirrors the LDPE structure observed in Figure 14, displaying SEM images for the same particle sizes and magnifications but treated with a combination of Lip and Lac enzymes. At 0.5 cm particle size, the structure exhibited extensive surface degradation, including deep cracks, pits, and erosion; according to Yang *et al.*,[56] it indicates a synergistic effect of the enzyme combination. Moderate degradation was observed when the particle size was 1 cm, with more pronounced features than in the Lip-only treatment. Surface changes are minimal at 2 cm particle size, similar to the Lip-only treatment. Compared to the structure in Figure 14, the structure presented in Figure 15 demonstrates enhanced

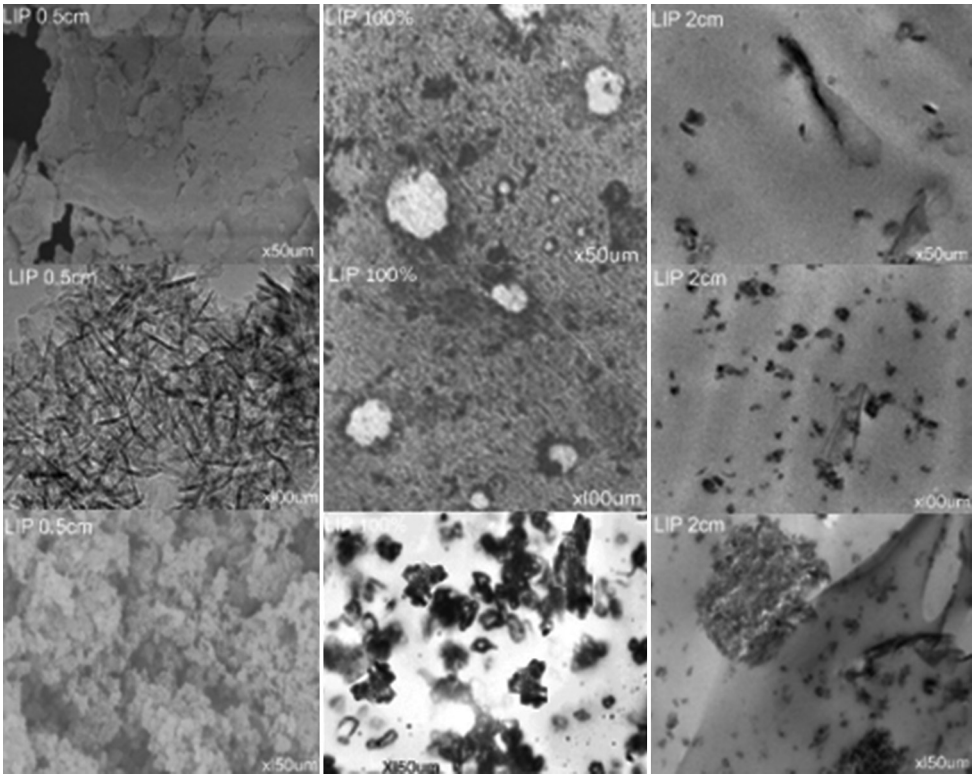

Figure 14. Microscopic images of lipase-treated low-density polyethylene films with particle sizes of 0.5 (left), 1 (middle), and 2 cm (right). Magnifications: ×50 (top); ×100 (middle); ×150 (bottom).

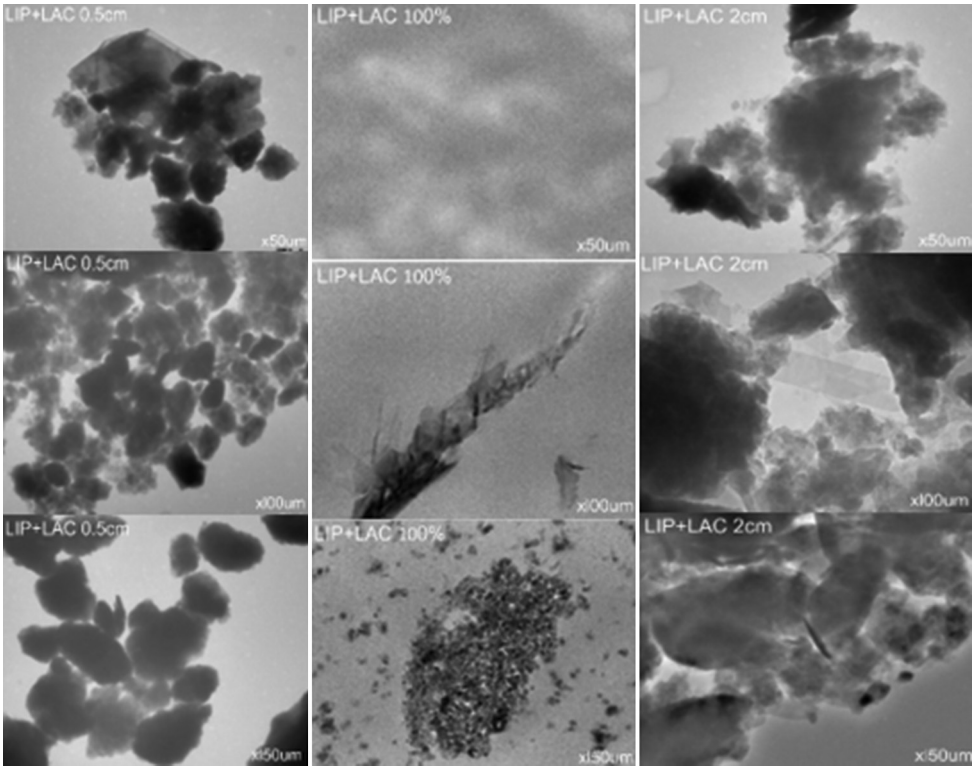

Figure 15. Microscopic images of lipase-laccase-treated low-density polyethylene films with particle sizes of 0.5 (left), 1 (middle), and 2 cm (right). Magnifications: ×50 (top); ×100 (middle); ×150 (bottom).

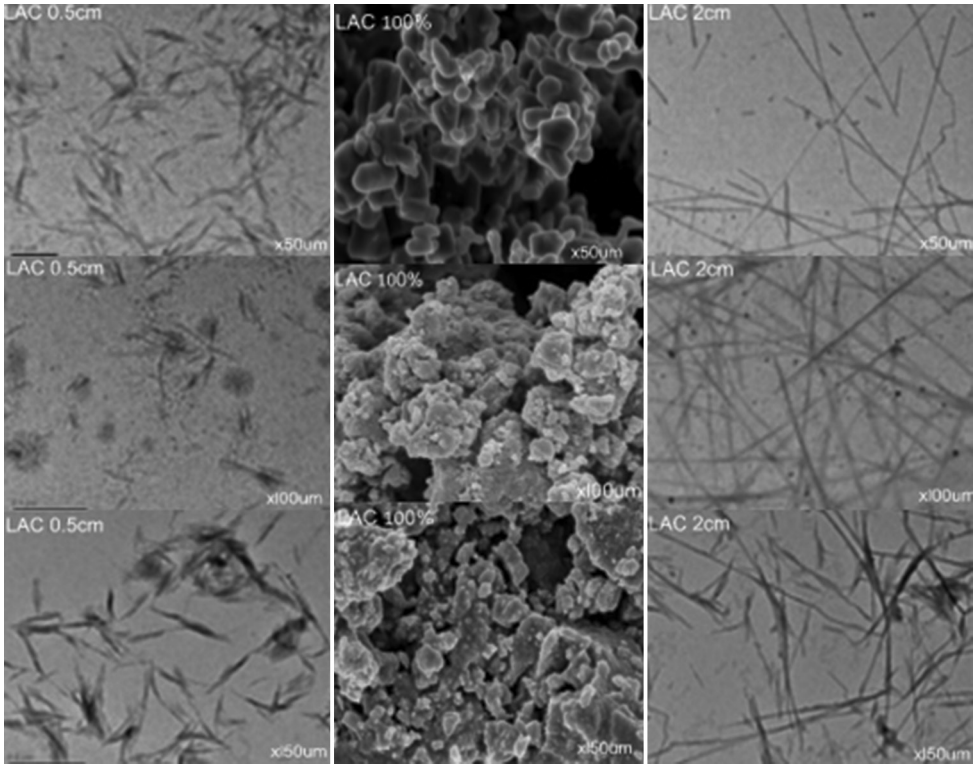

Figure 16. Microscopic images of laccase-treated low-density polyethylene films with particle sizes of 0.5 (left), 1 (middle), and 2 cm (right). Magnifications: ×50 (top); ×100 (middle); ×150 (bottom).

degradation, particularly in smaller particles, due to the effectiveness of the combined enzymatic treatment.

Figure 16 presents nine SEM images of LDPE films (with different particle sizes) treated with Lac. The images demonstrate significant surface degradation at 0.5 cm particle size, including cracks and pits, indicating effective enzymatic action. When the particle size is 1 cm, moderate degradation is observed, with visible surface irregularities. Finally, 2 cm particle size exhibited a minimal surface change due to the limited enzymatic impact on larger particles. The similarities across the images include the presence of degradation features, while the differences relate to the severity of surface damage, which correlates with particle size and enzyme concentration.

In the present study, the magnifications ranged from 50 to 150 µm, whereas Yao *et al.*[59] only analyzed their samples at 20 µm. Figures 9-12 emphasize the effect of enzymatic treatments on LDPE films without considering particle size variations, while Figures 13-16 examine the combined impact of enzyme treatments and particle sizes. The latter set provides a more comprehensive understanding of how particle size influences enzymatic degradation, highlighting the importance of optimizing both parameters for effective biodegradation. The proposed enzymatic treatment offers a low-cost, eco-friendly, and scalable method for the management of PE-based plastic waste, particularly sachet water LDPE films, which are a major contributor to solid waste in many developing countries, including Nigeria. The findings support potential integration of the enzyme system into municipal solid waste (MSW) management systems or decentralized waste treatment facilities, whereby enzymatic formulations may be used to accelerate LDPE degradation under controlled conditions.

4. Conclusion

Enzymes possess the ability to degrade PE materials, which constitute a significant portion of environmental pollutants that pose serious threats to humans, animals, and plants. After incubation periods of 10 and 30 days, both Lip and Lac enzymes demonstrated the ability to degrade LDPE material, with the 30-day incubation resulting in significantly higher LDPE degradation. The effects of enzyme concentration and LDPE particle size on degradation rate were investigated. Weight loss analysis revealed a higher degradation rate of 15.21% after the 30-day incubation period in 100% Lip-Lac. Surface imaging of the LDPE films treated with different enzyme concentrations indicated a higher surface change in films treated with higher enzyme concentrations due to higher enzymatic activity. The results demonstrate that higher enzyme concentrations and longer incubation

periods lead to greater LDPE degradation, establishing a proportional relationship between enzyme concentration, incubation time, and the extent of degradation. The effect of particle size on degradation rate was determined. A higher percentage degradation rate of 23.81% was observed after the 30-day incubation period for LDPE samples with a particle size of 0.5 cm. Surface imaging revealed that smaller particle sizes exhibited rougher surfaces compared to larger ones, indicating more extensive surface degradation. The degradation efficiency was significantly enhanced when a two-enzyme system (Lip-Lac) was employed, compared to single-enzyme systems. Furthermore, a decrease in particle size corresponded with an increase in the degree of degradation. This study supports previous findings that certain microbes and enzymes can be employed to degrade LDPE plastics. Future studies should explore enzyme immobilization and reusability strategies, building on the findings from this study.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

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Consent for publication

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Availability of data

Data are available from the corresponding author upon reasonable request.

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