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# Biodegradation of synthetic plastics by the extracellular lipase of *Aspergillus niger*

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## ABSTRACT

A rapid increase in plastic pollution is a major threat to the environment. One intriguing group of enzymes that can act as biocatalysts for the breakdown of polymers is lipase. This study reports the production of lipase from *Aspergillus niger* MG654699.1 utilizing agro-industrial residue (wheat bran) through solid-state fermentation. The produced lipase showed 176.55 U/mL of activity, 7.18 mg/mL of protein content, and 24.60 U/mg of specific activity under the optimal conditions of 37°C and pH 7.0. The biocatalytic activity of 30 kDa lipase resulted in 3.8%, 3.6%, and 5% weight loss of PE (polyethylene), PET (polyethylene terephthalate), and PS (polystyrene), respectively. Application of Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) confirmed the lipase-mediated deterioration of treated polymer samples. The alterations in functional groups and surface structures of the samples showed the chemical and physical impact of the applied enzyme. The findings of this study showed that lipase can be employed as an eco-friendly and green biocatalyst for effective depolymerization and deterioration of environmental plastic waste.

## 1. Introduction

Plastics are primarily the polymeric materials included in every aspect of life. In the past seventy years, it has been estimated that 8.3 billion tons of plastic have been produced (Rhodes, 2018). Plastics have been created at an increased rate since their manufacturing in order to fulfill the daily demand worldwide. According to a study carried out in 2019, it was estimated that global plastic manufacturing was around 400 million tons per year, with approximately 8 to 13 million tons discarded straight into the oceans each year (Dominik et al., 2019). Out of this, 12% of plastic is burned, 79% is dumped into landfills, and only 9% is recycled (Taghavi et al., 2021). Polyethylene (PE), polyethylene terephthalate (PET), and polystyrene (PS), with 30.3%, 8.4%, and 6.1% usage, respectively, are the most widely used polymers (Temporiti et al., 2022). The majority of existing waste management practices include recycling, burning, and dumping into landfills. Even some plastic is recycled, but a large portion still harms the environment by being burned or dumped into landfills (Safdar et al., 2024). Over the previous

few years, studies have concentrated on ways to break down PET by enhancing the characteristics of enzymes that hydrolyze PET, such as lipases, esterases, and cutinases (Patel and Lee, 2022). Enzyme-mediated hydroxylation can target C-H bonds within plastics, such as those in the main chain (-CH<sub>2</sub>) and at the terminals (-CH<sub>3</sub>), including the tertiary C-H bonds within branches of carbon (Jin et al., 2023). A study (Gao and Sun, 2021) has proposed many enzymes that degrade PET and PE, such as lipases, hydrolases, esterases, and cutinases. The most researched and widely used enzyme for aminolysis, alcoholysis, transesterification, esterification, and hydroxylation processes is lipase (Otari et al., 2020).

Lipases, which are triacylglycerol acyl hydrolases (EC: 3.1.1.3), are pervasive enzymes produced naturally by a wide range of plants, insects, animals, and microbes, but fungi, yeasts, and bacteria are the major sources of lipases (Reyes-Reyes et al., 2022). Yeast and fungi are the major and economical source of lipase production (Kumar et al., 2023). The enzyme is a member of the hydrolase class, which is recognized for breaking the carbon framework of several commonly utilized polymers.

**Abbreviations:** PE, Polyethylene; PS, Polystyrene; PET, Polyethylene terephthalate; A. *niger*, *Aspergillus niger*; SSF, Solid state fermentation; pNPP, p-Nitrophenyl palmitate; SDS-PAGE, Sodium dodecyl-sulfate polyacrylamide gel electrophoresis; kDa, kilodalton; SEM, Scanning Electron Microscopy; FTIR, Fourier Transform Infrared Spectroscopy.

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An environmentally acceptable substitute for conventional plastic recycling methods is the use of polymer-degrading enzymes (Temporiti et al., 2022). Lipases are gaining popularity because of their numerous applications in several industries like drug, detergent, pharmaceutical, and biodiesel (Kumar et al., 2019), dairy, animal feed, agrochemicals, food and beverage, flavor industry, fine chemical manufacture, perfumery (Safdar et al., 2023), textile, leather, paper and pulp, biosensors, cosmetics, amino acid and ester derivatives, cleaning, and bioremediation (Kumar et al., 2023).

Solid-state fermentation (SSF) is an economical method for the production of industrial enzymes since it uses cheaper substrates, requires less space and energy, requires less complicated equipment, produces more enzymes per unit of time, and is simpler to process (Demir and Tari, 2016). Additionally, there is a growing tendency towards utilizing agro-industrial byproducts effectively to produce the enzyme with additional value via SSF. Particularly, lipases from microbial sources are better due to their affordability, ease of manufacture, responsiveness to express in host systems, variety of specificity, broad pH, and thermal range (Melani et al., 2020). The lipase-producing fungi commonly include *A. niger*, *Mucor miehei*, *Candida cylindracea*, *Rhizopus arrhizus*, and *Humicola lanuginosa* (Ismail and Baek, 2020). *Aspergillus*, *Penicillium*, and *Fusarium* have the ability to degrade PE (Restrepo-Flórez et al., 2014), PET, and PS foam (Umamaheswari and Murali, 2013). Mishandled plastic waste has negative impacts on the surroundings, human health, and animal life. The primary objectives of the current work were to observe the ability of *A. niger* lipase to degrade commonly used plastics, including PE, PET, and PS. Wheat bran was used for the production of crude lipase under SSF. The molecular weight of the produced enzyme was determined by SDS-PAGE (sodium dodecyl-sulfate polyacrylamide gel electrophoresis) before its application in polymer degradation. Lipase-mediated degradation of selected polymers was analyzed by weight loss, FTIR (Fourier transform infrared spectroscopy), and SEM (scanning electron microscopy) analyses. Evaluating the use of fungal lipases for waste management will probably offer a novel and eco-friendly way to address the extensive plastic pollution detrimental to the environment and will reduce the negative consequences of plastic pollution on aquatic, animal, and human life, thereby enabling economic growth and improved living quality.

## 2. Materials and methods

### 2.1. Strain, chemicals, and samples

All the chemicals and a previously identified strain of *A. niger* (accession number MG654699.1) were obtained from the Department of Biochemistry, The Islamia University of Bahawalpur, Pakistan (Ismail et al., 2021) and cultured on SDA (Sabouraud dextrose agar) medium. SDA plates were made with 40, 10, 20, and 0.5 g/L of dextrose, peptone, agar, and chloramphenicol, respectively. The strain was cultured on these plates and incubated in an incubator (Velp Scientifica, model FTC 90E, Usmate, Italy) at 37°C. The substrate (wheat bran) and the plastic samples, including PE, PET, and PS, were obtained locally from Bahawalpur, Pakistan. The characteristics of selected samples are explained in Table 1. The primary sources of polymers included the polyethylene shopping bag, the polyethylene terephthalate water bottle, and the polystyrene food plate (Fig. 1a-c).

### 2.2. Inoculum and enzyme production

Spores were collected aseptically from one-week-old culture plates by using the method of (Mehmood et al., 2019) and were transferred into 100 mL of SDA (Sabouraud dextrose broth) media with 40 and 10 g/L of dextrose and peptone. The pH of the media was set at 7.0, and it was incubated for 48 hours at 37°C and 120 rpm on a shaking incubator (model SI4, SHEL LAB, Sheldon). Five grams of wheat bran and five milliliters of mineral growth medium (MGM) with the following

**Table 1**

Characteristics of polymeric samples selected for treatment with *A. niger* MG654699.1 lipase.

Polymer sample	Polyethylene	Polyethylene terephthalate	Polystyrene
<b>Abbreviation</b>	PE	PET	PS
<b>Sample source</b>	Shopping bag	Water bottle	Food plate
<b>Sample size</b>	2 × 2 cm	2 × 2 cm	2 × 2 cm
<b>Sample color</b>	Whitish grey	Transparent white	Pure white
<b>Sample density</b>	0.94 g/cm <sup>-3</sup>	1.35 g/cm <sup>-3</sup>	0.98 g/cm <sup>-3</sup>
<b>Sample shape</b>	Square pieces	Square pieces	Square pieces
<b>Sample physical properties</b>	Flexible, water proof, translucent, thin	Strong, lightweight, transparent	Soft, inert, good insulator

compositions were added to each of the three 250-mL Erlenmeyer flasks (g/L): 12 g of NaH<sub>2</sub>PO<sub>4</sub>; 2.0 g of KH<sub>2</sub>PO<sub>4</sub>; 0.3 g of MgSO<sub>4</sub>·7H<sub>2</sub>O; 0.25 g of CaCl<sub>2</sub>; 1% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; and 2% olive oil. The flasks were cooled after being autoclaved. Each flask with 5 mL of inoculum was then incubated at 30°C for a week. Following the incubation time, each flask with 100 mL of distilled water was shaken at 37°C for 30 minutes in order to extract the enzyme from the fermentation media. Filtration was done using two layers of muslin fabric. Following filtration, centrifugation was carried out for five minutes at 12000 rpm (HERMLE, model Z36HK, Germany). The enzyme source, which is the supernatant, was collected after centrifugation and was used for screening, activity, and protein content determination (Supplementary, Fig. S1).

### 2.3. Lipase assay and activity determination

The presence of lipase in the supernatant was confirmed with a qualitative screening assay. The media plates for this purpose were made with 0.01% indicator (methyl red), 2% agar, and 2% Tween 80. The media was allowed to solidify on autoclaved petri plates, and a circular well of 1 cm in diameter was produced. The supernatant containing the lipase (10 µL) was added into the wells, and the color change was observed after the incubation of 24, 48, and 72 hours. The main purpose of this analysis is the release of fatty acids after the lipolytic action of the added enzyme, causing the change in media color (Samad et al., 1989). The UV spectrophotometer (model LABINDIA UV-3000+) was used to assess lipase activity quantitatively in the presence of pNPP (*p*-Nitrophenyl palmitate) as a substrate (Paluzar et al., 2021). Under assay conditions, one unit of enzyme activity was measured as an amount of enzyme liberating one µmol of *p*-nitrophenol per minute. The protein content of crude enzyme was also determined using the Bradford assay (Bradford, 1976). Enzyme specific activity was also calculated by employing equation 1 (A'yuni and Ilmi, 2021):

$$\text{Enzyme specific activity} = \frac{\text{Lipase activity (U/mL)}}{\text{Protein content (mg/mL)}} \quad (1)$$

### 2.4. SDS-PAGE

After the confirmation of lipase from different assays, it was subjected to SDS-PAGE in order to find out the molecular weight. An electrophoretic method (LAEMMLI, 1970) was utilized for separating (12%) and stacking (5%) gels. The obtained lipase from *A. niger* was precipitated further using the method of (Ha et al., 2021). The processed sample was mixed with sample buffer and loaded into the gel along with 10-250 kDa of protein marker. The gel was run at a constant voltage (120 volts) and stained with Coomassie dye.

### 2.5. Biodegradation analysis

#### 2.5.1. Lipase-mediated degradation

The primary sources of selected polymer samples, including PE, PET, and PS, were converted to 2 × 2 cm pieces with an initial weight of 0.5 g



**Fig. 1.** Visual observation of polymer samples selected for enzyme treatment. (a) PE shopping bag, (b) PET water bottle, (c) PS food plate, (d)  $2 \times 2$  cm pieces of PE, (e)  $2 \times 2$  cm pieces of PET, (f)  $2 \times 2$  cm pieces of PS, (g) Lipase produced from *A. niger* MG654699.1, (h) Lipase-mediated degradation of PE, PET, and PS.

(Fig. 1d-f). All the samples were then sterilized for 1 hour with 70% ethanol, washed three times with distilled water, and finally dried overnight. The 25 mL of lipase extract along with 25 mL of 0.1 M phosphate buffer (pH 7.0) was used to individually inoculate PE, PET, and PS samples in separate 250 mL Erlenmeyer flasks at  $37^\circ\text{C}$  and 150 rpm for 30 days (Fig. 1g-h). After the completion of the incubation period, the treated samples were analyzed in terms of their biodegradation mechanisms, weight loss, and structural and physical variations.

#### 2.5.2. Weight loss

The weight-loss method can be used as a simple indicator of polymer biodegradation. Additionally, treatments lacking the enzyme were carried out as blanks. The weight of control and lipase-treated samples was determined using an analytical balance (model Shimadzu AUX-320). To determine the degradation by lipase, the weight of the treated polymer samples was deducted from the blank, and the overall weight loss was determined in terms of percentages (Li et al., 2022). Following a 30-day incubation period, the samples were removed from the flasks, rinsed with ethanol, and then washed thoroughly using distilled water in order to obtain results. The following equation 2 was used to calculate the degree of biodegradation as a percentage of sample weight loss:

$$\text{Weight loss (\%)} = (W_0 - W_t) \times 100 / W_0 \quad (2)$$

where  $W_0$  is the weight of the untreated samples and  $W_t$  is the weight of enzyme-treated samples after the degradation.

#### 2.5.3. FTIR

FTIR spectroscopy creates a chemical scan of the specimen under study and provides the chance to gather dynamic images. It is particularly helpful for researching heterogeneous and complex materials (Fan et al., 2021). To assure polymer linkage destruction, an Agilent Cary 630-FTIR spectrometer was used, and FTIR spectra of specimens were acquired before and after the degradation process in the frequency range of  $4000\text{--}400\text{ cm}^{-1}$ .

#### 2.5.4. SEM

Surface alterations in polymers were examined in most of the investigations through observable changes in control and treated SEM images and were mostly characterized as the emergence of pores, cracks, and holes (Matjašić et al., 2021). A Hitachi S2380N scanning electron microscope was used to study the changes in surface morphologies and biodegradation impacts of untreated and enzyme-treated polymers.



## 2.6. Instrumental

SDA plates with *A. niger* MG654699.1 culture was incubated in an incubator (Velp Scientifica, model FTC 90E, Usmate, Italy), and a shaking incubator (model SI4, SHEL LAB, Sheldon) was used to obtain the fungal inoculums. The fermentation media was centrifuged at 12000 rpm (HERMLE, model Z36HK, Germany), and the supernatant (crude lipase) was separated for assay determination through a UV spectrophotometer (model LABINDIA UV-3000+). The weight of plastic samples before and after the lipase treatment was determined using an analytical balance (model Shimadzu AUX-320). All these instruments were available at the Department of Biochemistry, The Islamia University of Bahawalpur, Pakistan. The structural alterations of plastic samples were observed through an Agilent Cary 630-FTIR spectrometer at the Department of Chemistry, The Islamia University of Bahawalpur, Pakistan, within the frequency range of 4000 to 400  $\text{cm}^{-1}$ . The background noise was eliminated by performing a blank scan in the same frequency range. The surface changes before and after the lipase treatment were examined with a scanning electron microscope (Hitachi S2380N SEM) at the Central Hi-Tech Laboratory, University of Agriculture, Faisalabad, Pakistan. The gold-coated dried samples and controls were examined at 1.8 kV using a Hitachi S2380N SEM. The micrographs of the control and treated samples were then obtained.

## 2.7. Statistical analysis

Three replications of each experiment were performed. The data was displayed as the mean  $\pm$  standard deviation (SD). Utilizing Statistics 8.1 software, the ANOVA was conducted. Tukey's HSD (honestly significant difference) test was utilized to ascertain the differences, and  $P < 0.05$  was determined to be statistically significant. The graphs were made with software programs, including OriginPro 9.0 and Microsoft Excel.

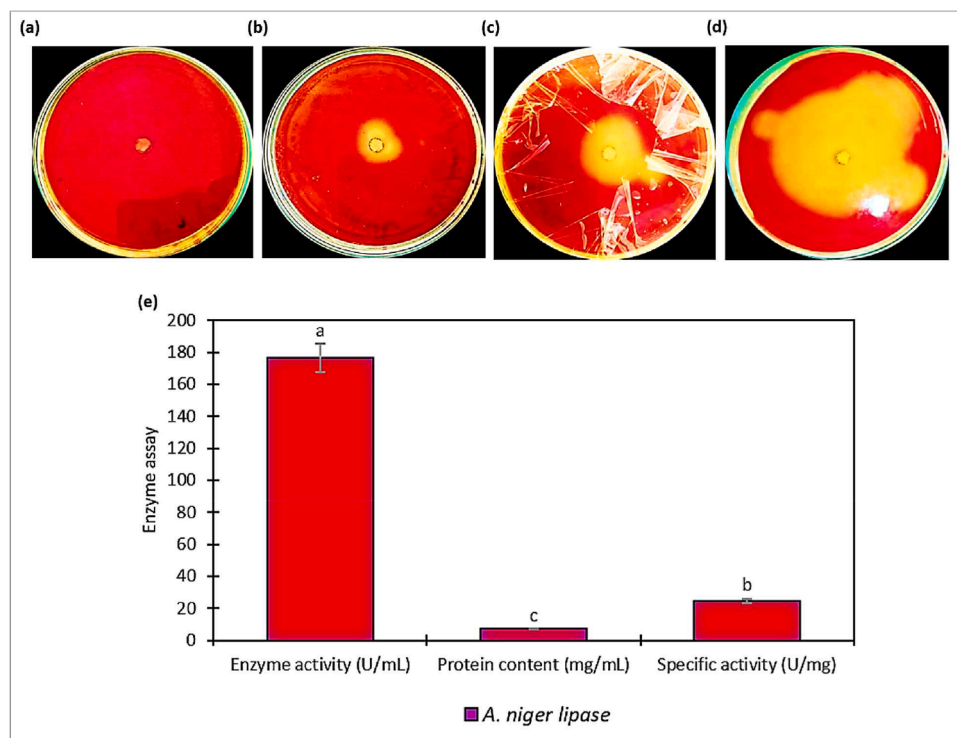
## 3. Results

### 3.1. Enzyme production and activity assays

The strain of *A. niger* with accession number MG654699.1 was previously identified by (Ismail et al., 2021) and used for the production of lipase. The crucial step in SSF is to choose an appropriate substrate since it serves as a support matrix, a source of nutrients, an inducer for the growth of microbes, and the generation of the required product. The lipase from *A. niger* was obtained after using the substrate of wheat bran in the fermentation process. The plate screening method was utilized to verify the production of lipase from the chosen strain. Indicator (methyl red) and substrate (Tween-80) were used to make the screening media plates. The findings showed that over an incubation period of 24 hours, the red color in the surroundings of wells containing it changed to yellow. As seen in Fig. 2a-d, these areas in the surroundings of wells became larger with time. The lipolytic action of the enzyme caused the release of fatty acids from triacylglycerols and resulted in color change. As a result, this technique for confirming the presence of lipase is thought to be simple, quick, and effective. The quantitative analysis of lipase obtained from *A. niger* MG654699.1 displayed 176.55 U/mL of enzyme activity, 7.18 mg/mL of protein content, and 24.60 U/mg of specific activity at the optimum conditions of pH 7.0 and 37°C (Fig. 2e). Each experiment was run in triplicate, and the results were found to be statistically significant at  $p < 0.05$  (Supplementary, Table S1).

### 3.2. SDS-PAGE

Lipases have been produced and studied by numerous scientists, and their size varies depending on the microorganisms that synthesize them. The size of the lipase produced from *A. niger* MG654699.1 was confirmed using the technique by (LAEMMLI, 1970). The bands of protein were seen using Coomassie dye, and their size was evaluated



**Fig. 2.** The assays for the confirmation and quantification of lipase produced from *A. niger* MG654699.1. (a) Screening assay control plate with no lipase added, (b) Screening assay plate with added lipase for 24 hours, (c) Screening assay plate with added lipase for 48 hours, (d) Screening assay plate with added lipase for 72 hours, (e) Graph showing the activity, protein content, and specific activity of the produced lipase. Each point of the graph, along with error bars, shows the mean value of experiments performed in triplicate. Means showing identical characters are at par, while those with different ones are statistically significant ( $p < 0.05$ ).

using the protein marker of 10–250 kDa. The lipase that was extracted from *A. niger* showed a size of 30 kDa, as shown in Fig. 3.

### 3.3. Enzymatic degradation of PE, PET, and PS

Lipases are members of the hydrolase group. They attack the ester bonds and facilitate the disintegration of triglycerides (Kaushal et al., 2021). Hydrolases play a crucial role in the breakdown of the carbon backbones of synthetic polymers. Although the degradative procedures are not fully known until now, the lipase produced from *A. niger* MG654699.1 can be a useful basis for more research. Polymers having C-C bonds can potentially be broken down by lipases in a way that is harmless for the surroundings. Techniques like analyzing the weight loss, FTIR spectra, and SEM images are used to assess how effective these enzymes are at breaking down plastics. These methods help to ascertain the advantageous impact of lipase on the disintegration of polymers. The produced lipase aided in the hydrolysis of chemical linkages, causing the degradation of polymeric materials. The hydrolases convert the substrates into products, as illustrated by equation 3:



Lipases degrade the lengthy carbon chains present in polymeric materials. The synthetic polymers found in our surroundings have a hydrophobic nature. The lipase produced by *A. niger* attaches hydrophobically to the surface of polymers. Most of the hydrolases have hydrophobic fissures near the site of action that can accommodate hydrophobic groups found in polymers. This property improves the ability of lipase to interact successfully with polymeric materials. The lengthy polymeric chains were hydrolytically split by the active site of

lipase and converted into smaller pieces. The schematic illustration in Fig. 4a-c displays the anticipated lipase degradation pathway for PE, PET, and PS.

### 3.4. Weight loss

The results obtained on the biodeterioration ability of lipase are shown in Table 2. After a 30-day incubation period, all the samples were removed from the flasks and observed for weight loss. Out of the three treatments, PS showed the best biodegradability of 5%, followed by PE and PET, which showed 3.8% and 3.6% of biodegradability, respectively. The system was kept intact for the incubation period of thirty days, and no nutrients were added or subtracted from the medium. The enzyme attacked the ester bonds within the polymers, causing them to break down. According to the results of this experiment, it is reasonable to believe that polymers served as the catalyst for the respective enzyme.

### 3.5. FTIR

The Agilent Cary 630-FTIR spectrometer was used to examine the polymer films before and after the biodegradation treatment. Changes in bond scission, chemical transformation, and the production of new functional groups have all been seen in polymer degradation. FTIR spectra of *A. niger* MG654699.1 lipase-treated samples of PE (polyethylene), PET (polyethylene terephthalate), and PS (polystyrene) confirmed the weight loss measurements (Fig. 5). As a result of identical but sharper peaks (reduced transmittance), it was determined that the associated functional groups were more present in that region of the spectrum. The results indicate the formation of new intense peaks attributed to alkoxy groups (C-O) at  $1204\text{ cm}^{-1}$  in PE samples (Fig. 5a),  $1016$  and  $1238\text{ cm}^{-1}$  in PET samples (Fig. 5b), and  $1025\text{ cm}^{-1}$  in PS samples (Fig. 5c). The attributing peaks to the carbonyl bonds (C=O) were not formed in the incubated PE and PS; however, these were formed in PET at  $1714\text{ cm}^{-1}$ . The alkene peaks (C=C) at  $1490$ ,  $1600$ , and  $1654\text{ cm}^{-1}$  were also formed (Fig. 5c). It was observed that C-H bonds were also formed at various points in Fig. 5a-c after enzyme treatment, which confirmed the structural changes due to enzymatic degradation. The alterations of bonds like C-O, C-H, C=O, and C=C and the introduction of hydroxyl groups at  $3300\text{ cm}^{-1}$  in PE and at  $3386\text{ cm}^{-1}$  in PS samples can be noticed. The development of hydrolyzing bonds (alkoxy, alkene, carbonyl, and hydroxyl) in polymeric structures was thought to be a critical phase in biodegradation procedures, which eased the biodegradation procedure by allowing fungal enzymes to target these chemical bonds and functional groups. There would not be any action between the fungal enzyme and the polymer samples if these functional groups were not formed.

### 3.6. SEM

SEM (scanning electron microscope) is a valuable technology that produces a detailed and vivid appearance of materials with excellent clarity and high accuracy. SEM is commonly used for the characterization of materials and to examine surface phenomena in materials. A Hitachi S2380N scanning electron microscope was used for the examination of all samples. Fig. 6 displays the micrographs of polymeric samples both before (Fig. 6a-c) and after (Fig. 6d-f) their treatment with enzyme. After deterioration, the original morphologies of polymeric films were altered. The surfaces of the polymeric films were smooth when not subjected to enzymatic treatment, but deformities, including various holes, cavities, cracks, and surface irregularities, appeared on the surfaces of the polymers after their treatment with lipase. This showed that the lipase had attacked the polymeric surfaces, thus confirming their degradation. The maximum surface deformities were shown by polystyrene with several pits and cavities on the surface. Polyethylene also showed surface cracks and holes, while polyethylene terephthalate showed the least surface changes.

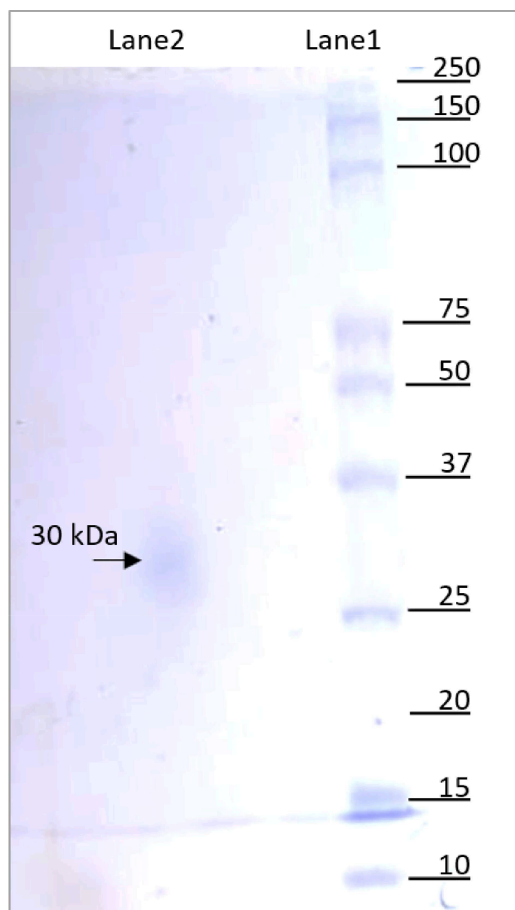
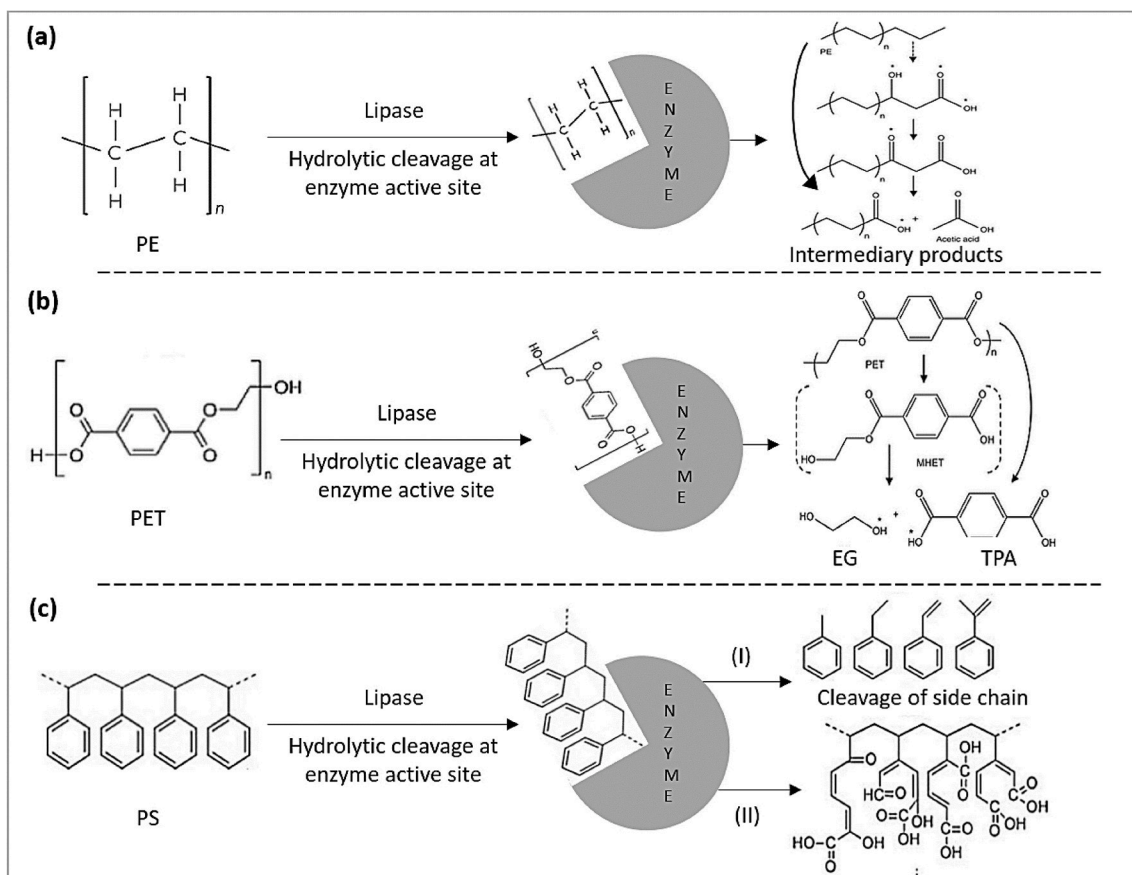


Fig. 3. Molecular size of lipase produced from *A. niger* MG654699.1. Lane 1 shows the protein marker, while Lane 2 shows the size of the produced lipase.



**Fig. 4.** Lipase-mediated degradative pathways. (a) Explained pathway for PE degradation. The enzyme attacked the C-C backbone along with dehydrogenation and oxidation. The large polymeric chains were converted into smaller units. An asterisk mark showed the location of additional functional groups being added to polymers. (b) Explained pathway for PET degradation. PET was hydrolyzed into ethylene glycol (EG) and terephthalic acid (TPA) after the action of the enzyme. The hydrolytic action of the enzyme produced the PET intermediate, including MHET [mono-(2-hydroxyethyl) terephthalate] and BHET [bis-(2-hydroxyethyl) terephthalate], which were further hydrolyzed into EG and TPA monomers. An asterisk mark showed the location of additional functional groups being added to polymers. (c) Explained pathway for PS degradation. The degradative process showed multiple steps, including (I) the breakdown of the side chains. A potential cleavage of the main and side chains caused the breakdown of PS.

**Table 2**

Comparative analysis of PE (polyethylene), PET (polyethylene terephthalate), and PS (polystyrene) weights before and after incubation.

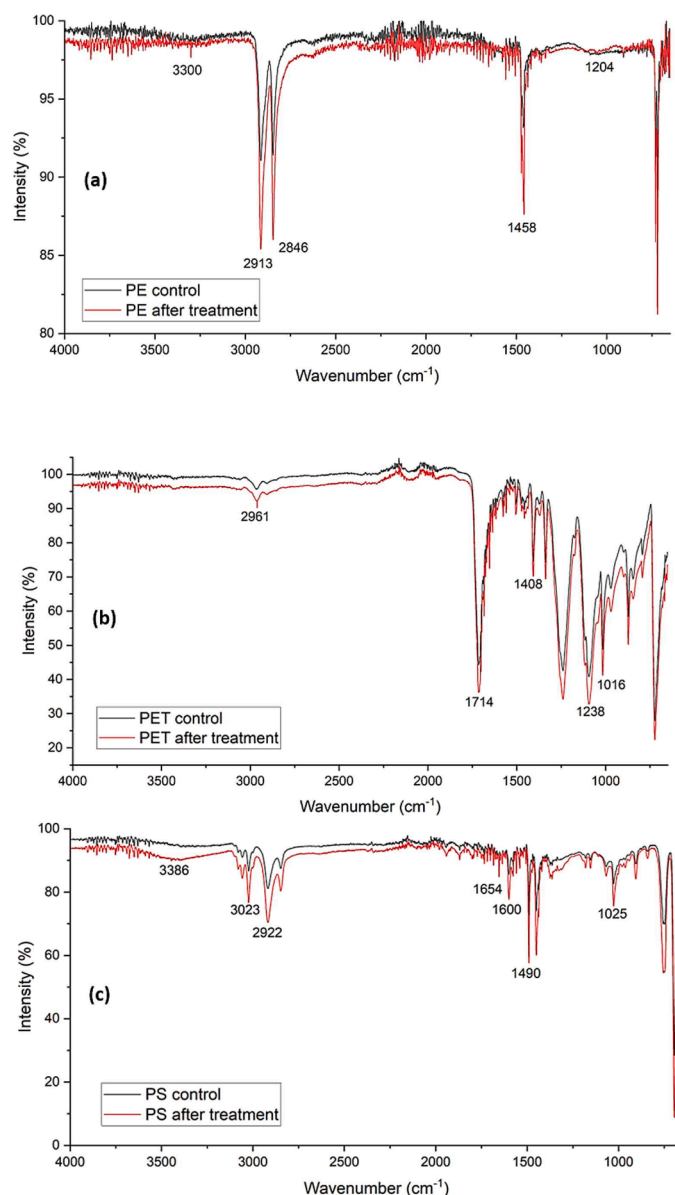
Plastic samples	Percentage weight loss mean $\pm$ standard deviation
<b>PE samples</b>	
Control	0 $\pm$ 0 <sup>c</sup>
<i>A. niger</i> MG654699.1 lipase	3.8 $\pm$ 0.15 <sup>b</sup>
<b>PET samples</b>	
Control	0 $\pm$ 0 <sup>c</sup>
<i>A. niger</i> MG654699.1 lipase	3.6 $\pm$ 0.17 <sup>b</sup>
<b>PS samples</b>	
Control	0 $\pm$ 0 <sup>c</sup>
<i>A. niger</i> MG654699.1 lipase	5.0 $\pm$ 0.26 <sup>a</sup>

Note: The mean values shown by differing letters represent the statistical difference at  $p < 0.05$  after performing all experiments in triplicate.

#### 4. Discussion

Overall, the results of the earlier investigation, which focused on the lipase from *A. niger*, were in line with the results of the present study. Solid-state fermentation has significant potential for the synthesis of enzymes. The selection of an appropriate substrate in SSF involves several factors, primarily related to the cost and availability of substrate; therefore, agricultural byproducts are seen as the most favorable option (Dwivedi et al., 2022). The nitrogenous source has a considerable impact on lipase synthesis. Wheat bran is comprised of  $13.1 \pm 1.3\%$  of protein,

and various publications have described its importance for the synthesis of fungal lipases (de Azevedo et al., 2020). One of the substrates for filamentous fungi producing lipases is olive oil, which serves as a carbon source and inducer of lipase synthesis (Putri et al., 2020). Indicators, along with substrates added to agar plates, can be used to identify lipolytic microbes. It is a quick and effective screening method for determining organisms that produce lipase. Surfactants known as tweens are commonly utilized as the substrate of screening tests as they facilitate optimal substrate-enzyme interaction and are easy to include in culture media (Geoffry and Achur, 2018). Suyanto et al. (2019) showed that the lipase produced by the SSF using *A. niger* had 10.83 U/mL of activity (Suyanto et al., 2019). Two fungi, including *A. niger* (13 F) and *F. solani* (7 F), produced crude lipase with an activity of 5.95 U/mL (Patel and Shah, 2020). A study by (Romero et al., 2012) found that 37°C was the best temperature for the lipase activity derived from *A. niger* ATCC MYA-135. *A. terreus* (AH-F2) isolated from oily samples and soil showed the production of lipase with 5.0 U/ml activity (Shabbir and Mukhtar, 2018). Two fungi, *Sordaria* sp. and *Stemphylium lycopersici*, isolated from *Tocoyena bullata* and *Humiria balsamifera* leaves, were capable of producing lipase with activities of 286 and 397 U/mL, respectively (Rocha et al., 2020). Our findings for the determination of lipase molecular weight are in agreement with the lipase produced by *A. niger* PTCC5010 which showed a band around 30 kDa after electrophoresis (ghamari, 2015). A 31 kDa lipase was extracted from *A. niger*, along with two other lipases of 43 and 65 kDa (Fernández-Lorente et al., 2005). There have been numerous reports of *A. niger* lipases with



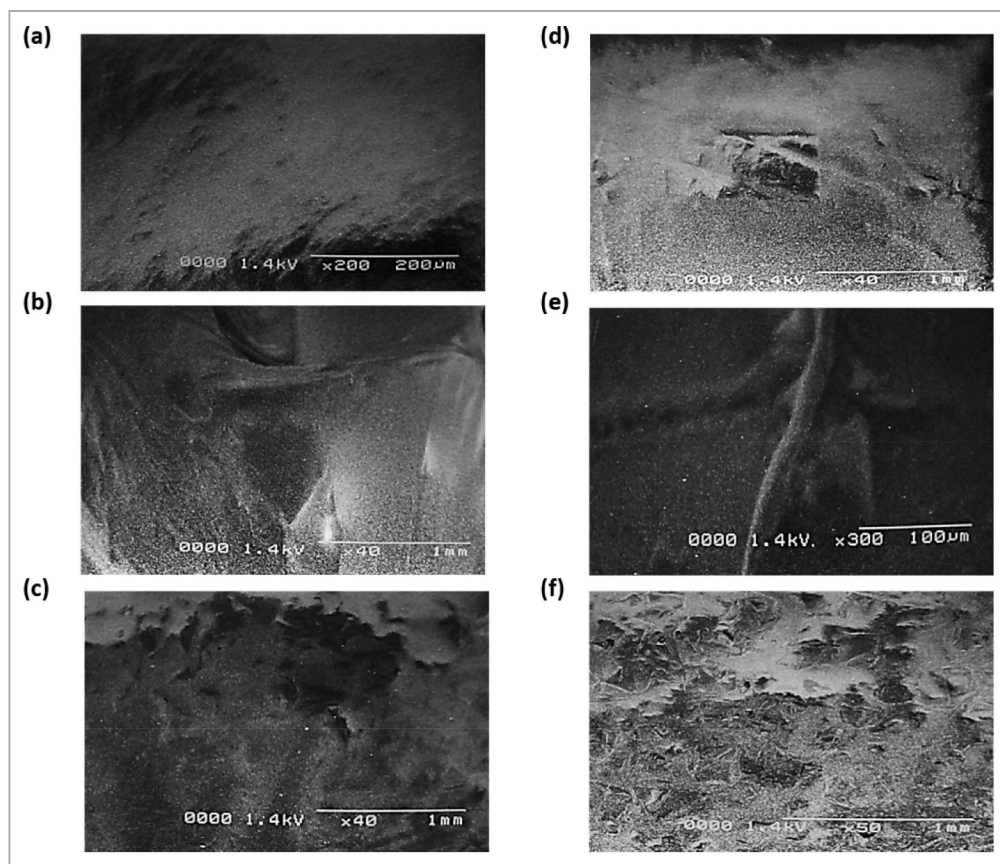
**Fig. 5.** FTIR analysis of (a) PE (polyethylene), (b) PET (polyethylene terephthalate), and (c) PS (polystyrene) plastic samples incubated with lipase from *A. niger* MG654699.1. Several functional groups were introduced in the plastic films after incubation in comparison to controls.

molecular weights between 11 and 67 kDa. The lipase of *A. niger* (NCIM 1207) showed 32.2 kDa of size on SDS-PAGE (Mhetras et al., 2009).

Many extracellular enzymes from fungi have been identified to degrade various types of plastics (Urbanek et al., 2020). Lipase, a multifunctional biocatalyst that is mostly utilized for the esterification and hydrolysis of oil and fat. As a potential substitute for conventional chemical catalysts, lipase can catalyze additional reactions, including acidolysis, alcoholysis, ammonolysis, and transesterification, with the benefits of significant energy efficacy and environmentally friendly (Facin et al., 2019). Lipases are widely used in biotransformation due to their commercial availability, vast substrate specificity, independence from coenzymes or cofactors, activity under minimal reaction conditions, and generally good chemical selectivity and stereomeric specificity. They exhibit significant catalytic activities in both aqueous and non-aqueous conditions (Vanleeuw et al., 2019). The action of *A. niger* MG654699.1 extracted lipase on PE, PET, and PS has been shown in Fig. 4, converting the PE long chain into smaller units (Lenz, 1993), PET into EG and TPA, MHET, and BHET, for conversion into EG and TPA monomers (Yoshida et al., 2016), (Świderek et al., 2023), and cleaving

the main and side chains of PS (Hou and Majumder, 2021). Various fungi, including *Aspergillus flavus*, *Aspergillus niger*, *Pullularia pullulans*, *Curvularia* sp., *Trichoderma* sp., and *Penicillium* sp., have demonstrated the ability to degrade PS using FTIR, weight loss, and microscopic analyses (Zhang et al., 2022). The enzymes displaying lipophilic activity (LiP) were discovered in *Streptomyces* sp. and were able to break down lignocellulose. Pometto et al. (1992) demonstrated the ability of these enzymes to break down PE (Pometto et al., 1992). The backbones of PET polymers usually have hydrolysable connections, making them vulnerable to lipase digestion. The lipase from *T. lanuginosus* has been shown to hydrolyze PET by interfacial activation (Eberl et al., 2009). As with PE and PET degradation, the researchers have found that PS degrading mechanisms also involve lipases, oxidoreductases, and laccases (Zhang et al., 2022). According to a study by (Hou and Majumder, 2021), alkane hydroxylases and P450 monooxygenases are most likely responsible for breaking C-C bonds present in the main chain of PS, while ring hydroxylating dioxygenases may cleave the side-chains. Although the whole biodegradation process and mechanisms are yet unknown, the lipase isolated from *A. niger* MG654699.1 provides a solid research basis





**Fig. 6.** Scanning electron micrographs of untreated plastic samples, including (a) PE, (b) PET, (c) PS, and *A. niger* MG654699.1 lipase-treated plastic sample, including (d) PE, (e) PET, and (f) PS. All the samples were completely dried before their analysis under SEM.

for future enzyme application and mechanism studies. The most widely used indicator to explain the rate of plastic degradation with several treatments is weight loss (Huang et al., 2021). The action of *A. niger* MG654699.1 lipase showed the degradation rate of 3.8%, 3.6%, and 5% for PE, PET, and PS, respectively (Supplementary, Fig. S2). The degradation of polyethylene for 28 days using *Aspergillus flavus* (PEDX3) resulted in a weight loss of 3.90% (Zhang et al., 2020). The lipase from *C. antarctica* hydrolyzed the PET and exhibited a weight loss of 0.4% after three weeks of treatment (Carniel et al., 2017). A study by Taghavi et al. (2021) stated that polyethylene and polystyrene showed 7.8% and 5.13% degradation, respectively, after 45 days of incubation.

The surface roughness of all plastic films indicated that selected strains played a beneficial role in biodegradation (Taghavi et al., 2021). The alterations at the molecular level cause a diminution of mechanical characteristics, which favors macroscopic degradation (Saliu et al., 2021). Oxidation products produced at various frequencies demonstrated the breakdown of the polymers (Sekhar et al., 2016). Previous FTIR studies showed that modifications to side chains and additions or deletions of functional groups are responsible for plastic degradation (Harshvardhan and Jha, 2013). The identified displacement of peaks and the production of hydrolyzing bonds and oxidation products in treated samples, such as hydroxyls, carbonyls, alcohols, and esters, represented the change in the chemical structure of plastics (Wilkes and Aristilde, 2017). A study by Singh et al. (2020) showed that the breakdown of polyethylene and polystyrene created more alkanes, whereas the degradation of polyethylene terephthalate produced only a small amount (Singh et al., 2020). SEM is an effective instrument for detecting surface defects, roughness, and the creation of grooves and cracks on plastic samples. Khandare et al. (2021) found that the surface roughness of treated polymers was caused by the action of lipase when observed with a scanning electron microscope (Khandare et al., 2021). The results

obtained from the current work showed that lipase can be produced from *A. niger* MG654699.1 under SSF. The produced lipase showed the degradation of selected polymers and, thus, can be used for the biodegradation of synthetic plastics.

## 5. Conclusion

The collected results indicated that *A. niger* MG654699.1 has the ability to produce extracellular lipase during SSF, and the application of wheat bran made the procedure economical. The obtained enzyme was observed to degrade the PE, PET, and PS samples within a 30-day incubation period. The molecular size of lipase was found to be 30 kDa after SDS-PAGE analysis. Among all the lipase-treated samples, PS showed the maximum weight loss (5%). Surface disintegration, biocatalytic degradation, and physical and chemical modification of treated polymers suggested that the application of lipase by *A. niger* should be considered to disintegrate the bulk amount of plastic waste at municipal levels.

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### Ethical approval

This article does not include any studies of human participants or animals by the authors of this investigation.

## Consent to publish

All authors have agreed to publish this article for the use of scientific communities and the general public at large.

## Declaration of generative Artificial Intelligence

There is no use of Artificial Intelligence tools to analyze and draw insights from data as part of the research process.

## CRedit authorship contribution statement

**Ayesha Safdar:** Writing – original draft, Investigation, Formal analysis. **Fatima Ismail:** Writing – review & editing, Supervision, Methodology. **Muhammad Imran:** Validation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the reported work.

## Data availability

No data was used for the research described in the article.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.envadv.2024.100563](https://doi.org/10.1016/j.envadv.2024.100563).

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