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Enzymatic Biodegradation of Polyethylene Terephthalate (PET) by Crude Lipase from *Geotrichum candidum*

Nurul Haviza[1], Armaini[1], Syafrizayanti[1]*

[1]*Department of Chemistry, Andalas University, Padang 25163, Indonesia*

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Abstract— The issue of plastic waste, particularly polyethylene terephthalate (PET), has become a major global environmental concern due to its resistance to natural degradation. One promising approach to addressing PET waste is the use of lipase enzymes produced by microorganisms. This study aims to explore the potential of the fungus *Geotrichum candidum* J1 to produce crude lipase enzymes capable of degrading PET plastic. The research involved stages including isolation and rejuvenation of the fungus, enzyme production through solid-state fermentation, and lipase activity assay using the cup-plate method. Subsequently, PET degradation testing was carried out using the crude enzyme extract under controlled conditions for 30 days. The results indicated that the crude lipase from *G. candidum* J1 exhibited lipolytic activity, as evidenced by clear zones formed on selective media. The average total protein yield reached 2.992 ± 0.05 mg/mL, indicating stable fermentation. Degradation tests showed a weight loss of PET between 5.9% and 6.4%. Characterization by Fourier Transform Infra Red (FTIR) confirmed the cleavage of ester bonds in PET structure, evidenced by spectral changes in carbonyl groups. Observations using a Scanning Electron Microscope (SEM) revealed morphological changes, including cracks and pores, on the PET surface post-treatment. In conclusion, the crude lipase from *G. candidum* J1 demonstrates significant potential as a biodegradation agent for reducing PET plastic pollution in an environmentally friendly manner.

Keywords— *Biodegradation; FTIR; Geotrichum candidum J1; Lipase enzyme; PET*

1. INTRODUCTION

Plastic packaging is a major contributor to environmental pollution due to its excessive use and poor waste management. Global plastic production reached over 400 million tons in 2019 and continues to rise annually [1]. Among various types of plastics, polyethylene terephthalate (PET) is widely used in beverage bottles and food containers, yet is resistant to natural degradation and highly persistent in the environment [2].

Conventional waste treatment methods such as incineration, landfilling, and mechanical recycling have limitations, including toxic emissions and low efficiency for certain plastics [3]. As a result, biodegradation a process where microorganisms or their enzymes break down polymers has emerged as an eco-friendly alternative [4,5].

The efficiency of plastic biodegradation largely depends on the tyspe of plastic, environmental conditions, and the metabolic capabilities of the degrading organisms. For instance, *Fusarium solani* produces cutinase, *Aspergillus oryzae* secretes lipase, and *Trichoderma harzianum* produces laccase, all of which have been reported to degrade synthetic polymers. Their natural adaptation to solid substrates and secretion of hydrolytic enzymes make them suitable candidates for solid-state fermentation systems designed for enzyme production. Solid-state fermentation using agro-industrial residues has been widely recognized as an efficient and cost-effective method for enzyme production.

Recent studies have investigated microbial degradation of plastic, including local isolates from landfill soil in Padang, Indonesia. In a study, the fungal isolate *Geotrichum candidum J1* was demonstrated to possess the ability to biodegrade plastics, specifically polyethylene and polystyrene [6]. Other fungi, such as *Fusarium solani* and *Aspergillus oryzae*, have also shown promising results in plastic degradation, aided by enzymes like cutinase, manganese peroxidase, and notably, lipase [7]. Lipase enzymes are capable of hydrolyzing ester bonds in synthetic polymers, making

***Corresponding author**.
Email address: syafrizayanti@sci.unand.ac.id
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them strong candidates for enzymatic plastic degradation [8].

This study aims to evaluate the potential of *Geotrichum candidum J1* as a source of crude lipase for the enzymatic degradation of PET. The research focuses on the production, characterization, and activity testing of the enzyme, with the goal of developing a more efficient and environmentally friendly method for reducing PET plastic waste.

2. EXPERIMENTAL SECTIONs

2.1. Materials

Geotrichum candidum J1 used in this study was isolated from landfill soil at Air Dingin, Padang, West Sumatra, Indonesia (0.9306° S, 100.4171° E). The strain used in this study was preliminarily identified as *Geotrichum candidum* isolate J1 based on a combination of morphological observations and molecular identification using the ITS rDNA region. A separate manuscript detailing the full taxonomic identification, phylogenetic analysis, and deposition of the strain in a microbial culture collection is currently under review. The sample of polyethylene terephthalate (PET) was obtained from commercial beverage bottles. The media used included Mineral Growth Medium (MGM), Potato Dextrose Agar (PDA, Merck, Germany), and Potato Dextrose Broth (PDB, Merck, Germany). Other chemicals used were NaH_2PO_4 (≥98%, Merck), KH_2PO_4 (≥98%, Merck), $MgSO_4{\cdot}7H_2O$ (≥98%, Merck), $CaCl_2$ (≥96%, Merck), $(NH_4)_2SO_4$ (≥98%, Merck), Tween 20 (Sigma-Aldrich), and Bradford reagent (Bio-Rad). All chemicals were of analytical grade.

2.2. Instrumentations

Glassware and general microbiological equipment were used including autoclave, laminar airflow cabinet, incubator, analytical balance (Shimadzu), optical microscope, rotary shaker, oven, and hotplate. Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed using a PerkinElmer FTIR Spectrometer. Surface morphology was observed using Scanning Electron Microscope (SEM, HITACHI FLEXSEM 100). UV-Vis spectrophotometric analysis was performed using a Shimadzu UV-1800 Spectrophotometer.

2.3. Fungal Rejuvenation

Rejuvenated on PDA medium and incubated at 37 °C for 72 hours. Fungal biomass was then transferred into PDB medium and incubated for use as an inoculum for enzyme production.

2.4. Crude Lipase Production via Solid-State Fermentation (SSF)

The Mineral Growth Medium (MGM) was prepared by dissolving 0.6 g NaH_2PO_4, 0.1 g KH_2PO_4, 0.015 g $MgSO_4{\cdot}7H_2O$, 0.0125 g $CaCl_2$, 0.025 g $(NH_4)_2SO_4$, and 1 mL vegetable oil in 50 mL distilled water. The solution was sterilized by autoclaving at 121 °C for 15 minutes. After cooling, 5 g of coconut bran and 5 mL of MGM were added to a 250 mL Erlenmeyer flask and autoclaved again. After cooling, 5 mL of *Geotrichum candidum* inoculum was added and incubated at 30 °C under static conditions for 7 days. After incubation, 100 mL distilled water was added and the mixture was shaken at 37 °C for 30 minutes. The extract was filtered and centrifuged at 128,794 rpm for 5 minutes. The supernatant was collected as crude enzyme extract.

2.5. Lipase Activity Assay (Cup-Plate Method)

Lipase activity was measured using the cup-plate method. The medium contained 10 g Difco peptone, 5 g NaCl, 1 g $CaCl_2{\cdot}2H_2O$, 20 g agar, and 10 mL Tween 20 in 1000 mL distilled water (pH 6.0). After sterilization and solidification in Petri dishes, 8 mm wells were made and filled with 0.1 mL of crude enzyme. Plates were incubated at 28 °C for 24 h. The clear zone diameter was measured, and activity index was calculated using the formula in Equation (1).

Enzyme activity index = (Total diameter of clear zone – Well diameter)/2 [9] (1)

2.6. Total Protein Determination by Bradford Method

A standard curve was prepared using Bovine Serum Albumin (BSA) at concentrations of 0.1–0.5 mg/mL. Each standard (0.5 mL) was mixed with 10 mL Bradford reagent and incubated at room temperature for 15 minutes. Absorbance was measured at 595 nm. Crude enzyme extract was diluted (1 mL in 10 mL), and 0.5 mL of the dilution was reacted with 10 mL Bradford reagent. After 15 minutes, absorbance was measured at 595 nm, and total protein was calculated based on the BSA standard curve.

2.7. PET Degradation Assay

PET samples were cut into 2 × 2 cm pieces weighing 0.5 g and sterilized with 70% ethanol, rinsed with distilled water, and dried overnight. The samples were incubated in 25 mL crude lipase and 25 mL phosphate buffer (0.1 M, pH 7.0) in 250 mL Erlenmeyer flasks at 37 °C and 150 rpm for 30 days. After incubation, the samples were analyzed for weight loss and structural changes.

2.8. PET Characterization by SEM and FTIR

After incubation, PET samples were washed with hot distilled water at 60 °C for 40 minutes, followed by sonication in warm deionized water for 30 minutes, and rinsed again in cold distilled water at 4 °C for 30 minutes. Samples were dried at room temperature and

analyzed using FTIR and SEM to observe functional group changes and surface morphology.

2.9. Weight Loss Determination

Weight loss (%) was used as an indicator of PET degradation. Control (untreated PET) and enzyme-treated samples were weighed using an analytical balance. After 30 days of incubation, the percentage weight loss was calculated by Equation (2).

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

where W_0 is the initial weight (control) and W_t is the final weight after treatment

3. RESULT AND DISCUSSION

3.1. Lipase Enzyme Production and Activity Assay

The production of lipase enzyme by *Geotrichum candidum* J1 was successfully carried out through solid-state fermentation using coconut meal as a substrate. The use of this agro-industrial residue provided a nutrient-rich matrix that supported fungal growth and enzyme secretion without any supplementation or optimization. The choice of substrate in SSF systems plays a crucial role in determining enzyme yield and quality. Previous studies have demonstrated that the use of protein and lipid rich substrates, such as wheat bran or olive oil residues, significantly enhances fungal lipaseproduction [10].

Coconut meal, with its balanced nutritional content, served as an effective medium, offering a cost-efficient and sustainable option for enzyme production [9]. The condition of the solid-state fermentation and the resulting crude lipase extract are illustrated in **Fig. 1**, where panel (a) shows fungal growth on the solid substrate, and panel (b) shows the crude enzyme obtained after extraction and centrifugation.

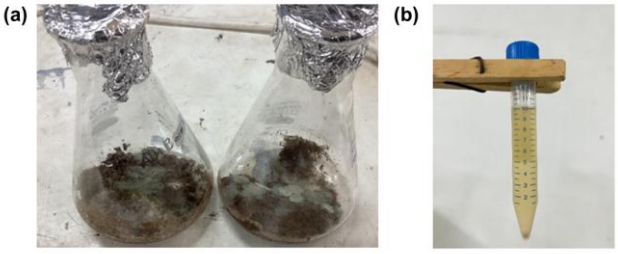

Fig. 1. Fermentation of *G. candidum J1* and crude enzyme. (a) Solid-state fermentation (SSF) of fungi on coconut meal substrate for lipase production (b) Supernatant containing crude lipase enzyme obtained after fermentation and centrifugation.

Following fermentation, the enzyme extract was subjected to a lipase activity assay using the cup-plate method. As shown in **Fig. 2**, a clear halo zone formed around the well indicates the presence of lipolytic activity. The treated sample exhibited a clear zone diameter of 19 mm, with a 14 mm well diameter, resulting in an Enzyme Activity Index (EAI) of 2.5 mm. Although the enzyme was in crude form, this activity level is comparable to other fungal lipase systems reported in prior research using optimized conditions [10]. The ability of *G. candidum J1* to secrete active lipase under simple fermentation conditions highlights its potential for practical applications.

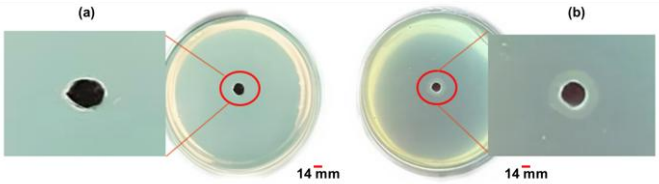

Fig. 2 Lipase activity assay results using the cup-plate method after 2 days of incubation. (a) Control without lipase enzyme addition. (b) Treatment with lipase enzyme filtrate.

The qualitative screening using Tween-20-enriched media has proven to be an efficient and reliable method to detect lipase activity. The surfactant facilitates substrate-enzyme interaction and visual identification of hydrolysis zones, making it suitable for rapid microbial screening[11]. The positive result observed in this study supports previous findings and reinforces the potential of *G. candidum J1* as a promising lipase producer.

3.2. Total Protein Content of Lipase Enzyme

Total extracellular protein content was determined using the Bradford method. A single batch of crude enzyme extract was analyzed in triplicate to ensure data reproducibility (**Table 1**). The supernatant appeared as a clear fluid following centrifugation and had a final volume of 10 mL.

Table 1. Initial characterization data of crude lipase enzyme extract

Characteristic	Value
Volume of crude lipase enzyme extract	10 mL
Average total protein of crude lipase enzyme extract	2.992 ± 0.05 mg/mL

Based on absorbance readings at 595 nm and a BSA standard curve, the average protein concentration was 2.992 ± 0.05 mg/mL. This value is relatively high for a crude fungal enzyme and reflects the strong secretion capability of the *G. candidum J1* isolate. Compared to the extracellular lipase of *Aspergillus niger*, which yielded 1.55 mg/mL [10], the crude lipase from *G. candidum J1* in this study exhibited a higher value. Reports on crude lipases of *Trichoderma* sp. have shown lower ranges of 0.5–1.5 mg/mL [12], further supporting the strength of this isolate. Although higher protein levels have been reported in optimized or recombinant systems (e.g., up to 4.8 mg/mL in optimized *Geotrichum* sp. [12], the result obtained here

is notable because it was achieved without any optimization or purification steps, highlighting the practicality and cost-effectiveness of this approach.

3.3. Toxicity Prediction of Pyrazoline Derivative

After 30 days of incubation, PET films treated with crude lipase showed a degradation percentage ranging between 5.9% and 6.4%, with the highest value recorded at 6.4%. This result confirms the enzyme's capability to hydrolyze ester bonds in PET polymers. Compared to previous reports, such as a 3.6% degradation using a 24.60 U/mg lipase. Lipase from *Aspergillus niger*, the degradation efficiency here is promising, especially considering the use of a crude enzyme [10].

While higher degradation rates have been reported using purified or recombinant enzymes (e.g., 75% by PETase from *Ideonella sakaiensis* and 60% by cutinase from *Thermobifida fusca*), those results involved highly optimized reaction conditions and engineered biocatalysts [12]. In contrast, the current findings reflect a more realistic and low-cost biodegradation approach, since the crude enzyme was directly obtained through solid-state fermentation using agro-industrial residue (coconut meal) without any purification or optimization steps, making it simple, inexpensive, and applicable for large-scale use [13].

3.4. FTIR and SEM Analysis of PET Functional Groups

FTIR spectroscopy was employed to detect changes in the functional groups of PET before and after enzymatic treatment. As shown in **Fig. 3**, the native PET spectra exhibited strong ester carbonyl (C=O) absorption around 1714 cm^{-1} and ester C–O stretching at 1246 cm^{-1}.

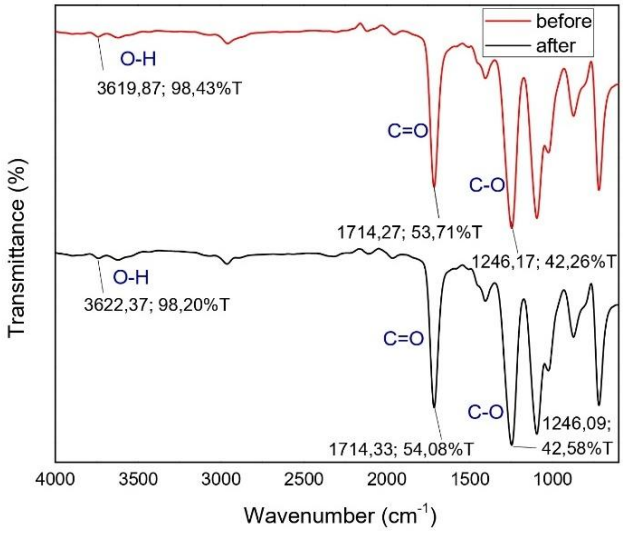

Fig. 3 FTIR spectrum of PET plastic before and after biodegradation

After treatment with crude lipase, a reduction in the intensity of the carbonyl band (1714 cm^{-1}) and a slight decrease in the ester C–O stretching (1246 cm^{-1}) were observed, indicating cleavage of ester linkages within the PET backbone. FTIR analysis showed changes in peak intensities rather than peak shifts, particularly in the carbonyl and C–O regions. Although the –OH region remained unchanged, the intensity reduction in ester-related bands may indicate partial cleavage of PET ester bonds. Although only minor variation was observed in the hydroxyl (O–H) region at 3622 cm^{-1}, the significant change in the carbonyl absorption is consistent with ester bond hydrolysis as reported in previous PET degradation studies [14].

Though the major ester peaks (C=O and C–O) remained strong, the observed spectral changes in the O–H region suggest the beginning of ester bond cleavage, indicative of early-stage depolymerization. [15]. Together, these results provide strong evidence for partial PET degradation. However, direct detection of degradation intermediates such as terephthalic acid (TPA) or MHET was not conducted in this study. Future work will employ chromatographic techniques (HPLC or LC–MS) to detect and quantify degradation products, thereby confirming the biodegradation pathway.

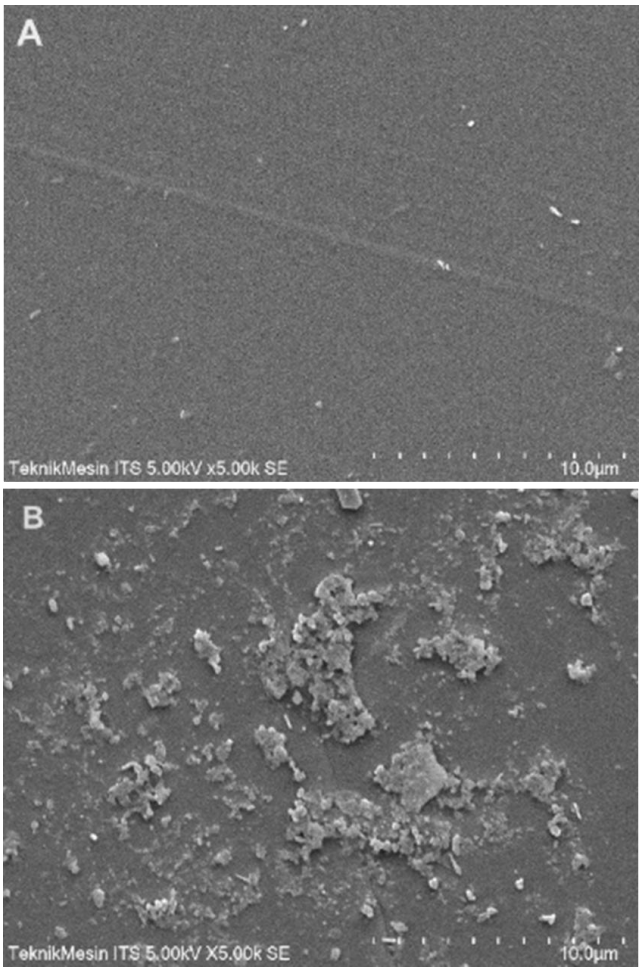

Fig. 4 SEM images of PET samples: (a) untreated control subjected to the same washing and drying procedure as the treated sample, and (b) PET treated with crude lipase from *G. candidum* J1.

SEM analysis was conducted to visualize physical changes in PET surface morphology before and after enzymatic degradation. SEM images (**Fig. 4**) confirm physical degradation of the PET surface as a result of enzymatic activity, supporting the FTIR findings. Untreated PET showed a smooth and intact surface. In contrast, PET treated with crude lipase showed roughened textures, small holes, and fragmented regions.

These morphological changes serve as direct visual evidence supporting FTIR data and weight loss measurements, confirming the occurrence of enzymatic PET degradation. The increased surface irregularity also enhances future enzyme-substrate interactions, promoting progressive degradation [16]. Although clear changes in PET morphology were observed, this study did not include the detection or quantification of PET degradation intermediates (e.g., TPA, BHET). Future work will include HPLC or GC-MS analysis to better elucidate the enzymatic depolymerization pathway.

CONCLUSION

The fungal isolate *Geotrichum candidum* J1 from landfill soil in Padang successfully produced crude lipase through solid-state fermentation using coconut meal as substrate. The crude enzyme showed clear lipolytic activity in cup-plate assays and a total protein yield of 2.992 ± 0.05 mg/mL, confirming stable production. When applied to PET films, the crude lipase induced a measurable degradation of 5.9–6.4% after 30 days, supported by FTIR detection of ester bond cleavage and SEM observation of surface cracks and pores. These findings confirm that crude lipase from *G. candidum* J1 was produced under simple fermentation conditions and was able to degrade PET films to a measurable extent under controlled laboratory conditions.

SUPPORTING INFORMATION

This article contains no supporting material. Supporting information can be provided by request to Corresponding Author (Syafrizayanti).

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CONFLICT OF INTEREST

There was no conflict of interest in this study.

AUTHOR CONTRIBUTIONS

Syafrizayanti: methodology, investigation, data analysis, writing manuscript, manuscript editing and discussion. NH & Armaini: Data analysis and writing manuscript.

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