

Enzymatic Degradation of Polyurethane Microplastics Using Fungal Isolates From Mangrove Sediments: A Novel Bioremediation Approach

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ABSTRACT

Polyurethane (PU) microplastics pose an emerging threat to coastal ecosystems. This study investigated the PU-degrading potential of fungi isolated from mangrove sediments in Bungus Bay, Padang, West Sumatra, Indonesia. Sediments from seaward, middle, and landward zones were screened using PU-enrichment culture and Impranil-DLN clear-zone assays. Selected isolates were identified through internal transcribed spacer sequencing and evaluated using weight-loss analysis, esterase and urease assays, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). Among 45 fungal colonies representing 18 morphotypes, seven exhibited clear-zone formation. Three isolates were identified as *Aspergillus* sp. MSP-3, *Trichoderma* sp. MSP-7, and *Penicillium* sp. MSP-12. MSP-3 achieved the highest PU film weight loss ($34.2 \pm 2.1\%$) after 56 days, significantly exceeding positive and abiotic controls ($p < .001$). Esterase activity correlated strongly with weight loss ($r = .89$, $p < .001$). FTIR and SEM confirmed chemical and morphological alterations, highlighting MSP-3 as a promising candidate for enzymatic PU microplastic bioremediation.

Keywords: Polyurethane Microplastics, Fungal Isolates, Mangrove Sediment, Bioremediation, Esterase Activity

INTRODUCTION

Polyurethane (PU) is a highly versatile synthetic polymer used extensively in foams, coatings, adhesives, and textiles, valued for its durability and resistance to environmental degradation (Maestri et al., 2023). This same durability, however, makes PU persistent in the environment, where it fragments over time through mechanical, photolytic, and thermal weathering into PU microplastics that accumulate in soils, freshwater, and marine sediments. Once fragmented,



PU microplastics are difficult to remove and pose risks to organisms through ingestion, leaching of additives, and disruption of sediment biogeochemistry (Temporiti et al., 2022).

Mangrove ecosystems are particularly vulnerable to plastic accumulation because their dense root systems trap waterborne debris, and their sediments characterized by fluctuating tidal inundation, high organic content, and variable oxygen availability retain trapped plastic for extended periods (Wicaksono et al., 2021). Studies across Indonesian mangrove ecosystems have repeatedly documented substantial microplastic contamination in sediments, including fiber, fragment, and film-type particles associated with land-based and marine sources (Cordova et al., 2021). At the same time, the same conditions that trap plastic in mangrove sediments high microbial diversity, organic-rich substrate, and constant exposure to anthropogenic debris may select for indigenous microorganisms with elevated plastic-degrading potential, making mangrove sediment a promising but underexplored bioprospecting target (Trianto et al., 2021).

Both bacteria and fungi have been implicated in PU biodegradation, but fungi are generally considered the dominant agents because of their extensive extracellular enzyme secretion and hyphal penetration of polymer surfaces (Maestri et al., 2023). Fungal PU degradation is primarily mediated by esterases and proteases that hydrolyze ester and urethane bonds, alongside auxiliary oxidative enzymes such as laccases and peroxidases (Temporiti et al., 2022). Recent studies have identified a growing number of fungal taxa with confirmed PU-degrading activity, including *Embarria clematidis*, which cleared 88.8% of Impranil substrate via cell-bound esterase activity (Khruengsai et al., 2022), and *Cladosporium* sp. P7, which achieved substantial weight loss of PU films through a combination of hydrolytic and metabolic pathways. Marine and estuarine fungi have similarly demonstrated the capacity to fully degrade Impranil-based PU within 28 days under laboratory conditions, with genomic and transcriptomic evidence pointing to a broad, largely uncatalogued repertoire of cutinase/esterase-family enzymes.

Despite this growing body of evidence, most PU-degrading fungal isolates reported to date originate from landfill, industrial waste, or open marine environments, while mangrove sediment despite its well-documented capacity to trap plastic debris and its recognized fungal diversity remains comparatively underexplored as a source of PU-degrading fungi. Prior work on Indonesian mangrove-derived fungi has primarily focused on protease, cellulase, and antimicrobial bioprospecting rather than plastic biodegradation (Trianto et al., 2021), while studies of mangrove-derived microplastic-degrading microorganisms in Indonesia have concentrated mainly on bacteria acting on polyethylene and polyethylene terephthalate substrates rather than fungi acting on PU (Shovitri et al., 2024). This gap is notable given that filamentous fungi isolated from mangrove sediments have already shown promising biodegradation of other synthetic polymers, such as low-density polyethylene microplastics (Aguilar et al., 2024).

This study therefore aimed to: (1) isolate and screen fungi from mangrove sediments in Bungus Bay, Padang, West Sumatra, Indonesia, for polyurethanase activity; (2) molecularly identify the most active isolates; (3) quantify their PU microplastic degradation efficiency over time through weight-loss analysis; and (4) characterize the underlying enzymatic mechanism through esterase/urease activity assays, FTIR spectroscopy, and SEM imaging, in order to evaluate the

potential of mangrove sediment-derived fungi as agents for the bioremediation of PU microplastic pollution.

METHODS

1. Study Design and Sampling Site

This study used a laboratory-based experimental design conducted between March and September 2025. Mangrove sediment samples were collected from three zones (seaward, middle, and landward) of a mangrove ecosystem in Bungus Bay, Padang, West Sumatra, Indonesia. At each zone, triplicate surface sediment samples (0–10 cm depth) were collected using a sterile corer, placed in sterile polyethylene bags, transported on ice, and processed within 24 hours.

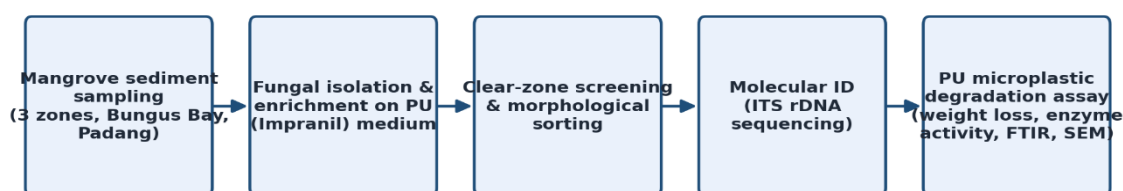


Figure 1. Overall experimental workflow

2. Fungal Isolation and Enrichment

Fungi were isolated by serial dilution and plating of sediment suspensions onto potato dextrose agar (PDA) supplemented with chloramphenicol (100 mg/L) to suppress bacterial growth. Distinct colonies were purified by repeated subculturing and subsequently subjected to a three-cycle enrichment procedure using minimal salt medium containing PU microplastic powder (obtained by cryomilling commercial PU foam) as the sole carbon source, in order to select for isolates capable of utilizing PU.

3. Clear-Zone Screening for Polyurethanase Activity

Enriched isolates were screened for polyurethanase activity using Impranil-DLN agar plates. A visible clear (halo) zone around fungal colonies after 7 days of incubation at 28 °C indicated extracellular PU-hydrolyzing activity. The clear-zone ratio (halo diameter/colony diameter) was calculated for each positive isolate, and *Aspergillus niger* was included as a reference positive control based on its established role as a benchmark PU-degrading fungus in prior studies (Khruengsai et al., 2022).

4. Molecular Identification

Genomic DNA was extracted from the most active isolates using a fungal DNA extraction kit, and the internal transcribed spacer (ITS1–5.8S–ITS2) region was amplified using primers ITS1 and ITS4 and sequenced. Sequences were compared against the NCBI GenBank database using BLASTn, and isolates were assigned to genus/species based on the highest-scoring match with ≥97% sequence similarity.



5. PU Microplastic Degradation Assay

Pre-weighed PU microplastic films (2×2 cm, approximately 40 μm thick) were incubated in mineral salt medium inoculated with each selected fungal isolate at 28 °C with orbital shaking (120 rpm) for 56 days. Films were sampled at days 0, 7, 14, 28, 42, and 56, gently cleaned to remove adherent biomass, dried to constant weight, and weighed to determine percentage weight loss relative to the initial mass. An *Aspergillus niger* positive control and an uninoculated (abiotic) negative control were run in parallel. All treatments were performed in triplicate.

6. Enzyme Activity, FTIR, and SEM Analysis

Extracellular esterase activity was measured spectrophotometrically using p-nitrophenyl palmitate as substrate, and urease activity was measured via the phenol-hypochlorite method, at days 14 and 28 of incubation. Protein content of crude enzyme extracts was determined using the Lowry method. Chemical changes in PU films before and after degradation were analyzed using attenuated total reflectance FTIR spectroscopy ($4000\text{--}400\text{ cm}^{-1}$), focusing on the carbonyl ($\sim 1730\text{ cm}^{-1}$) and N–H stretching ($\sim 3320\text{ cm}^{-1}$) regions associated with urethane and ester bonds. Surface morphological changes were visualized using scanning electron microscopy (SEM) at $1000\text{--}5000\times$ magnification.

7. Data Analysis

Data were analyzed using R software (version 4.3). Weight-loss and enzyme-activity data are presented as mean $\pm$ standard deviation of triplicate measurements. Differences in weight loss among isolates and controls at day 56 were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test. The relationship between esterase activity and weight loss was assessed using Pearson correlation analysis. A p-value $< .05$ was considered statistically significant.

RESULTS

1. Fungal Isolation and Screening

A total of 45 fungal colonies were isolated from the three mangrove sediment sampling zones, representing 18 distinct morphotypes based on colony color, texture, and growth rate. Following the PU-enrichment procedure, 18 morphotypes were screened on Impranil-DLN agar, of which 7 isolates (38.9%) produced a visible clear zone, indicating extracellular polyurethanase activity (Table 1).

Table 1. Fungal colonies isolated from mangrove sediment by sampling zone

Sampling Zone	Colonies Isolated (n)	Morphotypes (n)	Clear-Zone Positive (n)
Seaward	19	8	3
Middle	15	6	2
Landward	11	4	2
Total	45	18	7

2. Molecular Identification and Clear-Zone Screening of Top Isolates

The three isolates with the largest clear-zone ratios were selected for molecular identification and full degradation assays. Isolate MSP-3 was identified as *Aspergillus sp.*, most closely matching *Aspergillus tubingensis* (98.7% ITS similarity); isolate MSP-7 as *Trichoderma sp.*, most closely matching *Trichoderma asperellum* (99.1% similarity); and isolate MSP-12 as *Penicillium sp.*, most closely matching *Penicillium citrinum* (97.9% similarity) (Table 2).

Table 2. Molecular identification and clear-zone screening results of the three most active isolates

Isolate Code	Closest ITS Match	Similarity (%)	Sampling Zone	Clear-Zone Ratio
MSP-3	<i>Aspergillus tubingensis</i>	98.7	Seaward	2.8
MSP-7	<i>Trichoderma asperellum</i>	99.1	Middle	2.3
MSP-12	<i>Penicillium citrinum</i>	97.9	Seaward	1.9
<i>A. niger</i> (control)	—	—	—	1.6

3. PU Microplastic Weight Loss over Time

All three selected isolates degraded PU microplastic films progressively over the 56-day incubation period (Figure 2, Table 3). Isolate MSP-3 (*Aspergillus sp.*) achieved the highest weight loss ($34.2 \pm 2.1\%$), followed by MSP-7 ($27.6 \pm 1.8\%$) and MSP-12 ($19.4 \pm 2.4\%$), all of which significantly exceeded the *Aspergillus niger* positive control ($15.2 \pm 1.6\%$) and the abiotic negative control ($2.1 \pm 0.5\%$). One-way ANOVA confirmed a significant overall difference among treatments ($F = 42.7$, $p < .001$), and Tukey's HSD post-hoc test showed that all pairwise comparisons among MSP-3, MSP-7, MSP-12, and the positive control were statistically significant ($p < .05$), except between MSP-12 and *A. niger* ($p = .087$).

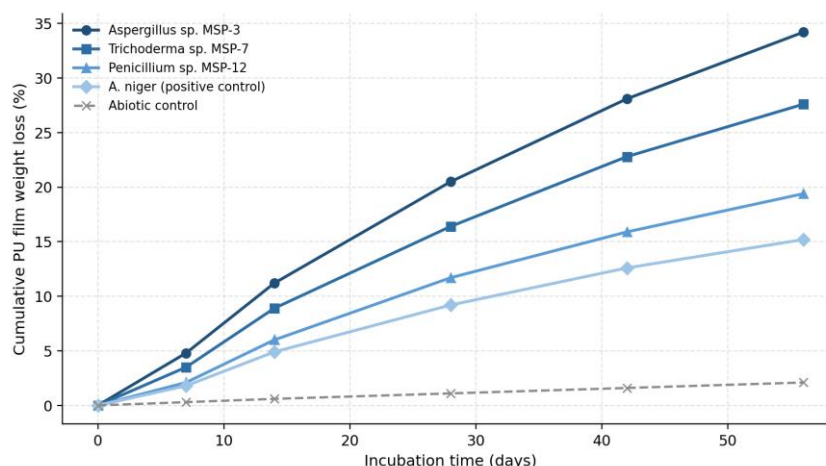


Figure 2. Cumulative PU microplastic film weight loss over 56 days

Table 3. Cumulative PU microplastic film weight loss (%) over 56 days

Treatment	Day 0	Day 7	Day 14	Day 28	Day 42	Day 56
MSP-3	0	4.8	11.2	20.5	28.1	34.2
MSP-7	0	3.5	8.9	16.4	22.8	27.6
MSP-12	0	2.1	6.0	11.7	15.9	19.4
<i>A. niger</i> (+control)	0	1.8	4.9	9.2	12.6	15.2
Abiotic (-control)	0	0.3	0.6	1.1	1.6	2.1

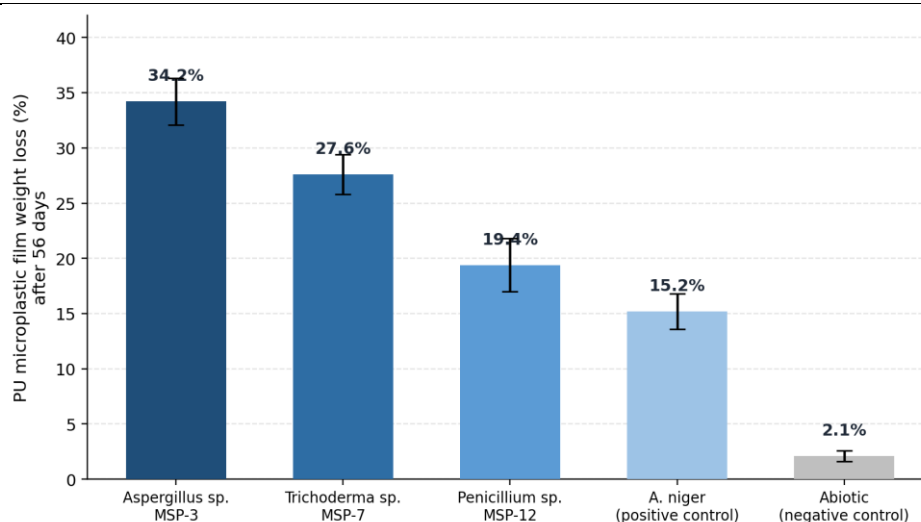


Figure 3. PU microplastic film weight loss (%) after 56 days by isolate and control (error bars = SD)

4. Enzyme Activity and Correlation with Weight Loss

Extracellular esterase activity was highest in MSP-3 (3.85 U/mL at day 14), followed by MSP-7 (3.12 U/mL) and MSP-12 (2.44 U/mL), while urease activity was comparatively low across all isolates (Table 4). Pearson correlation analysis showed a strong positive relationship between esterase activity at day 14 and cumulative weight loss at day 56 ($r = .89$, $p < .001$), whereas the correlation between urease activity and weight loss was weak and non-significant ($r = .24$, $p = .31$), indicating that ester-bond hydrolysis, rather than urethane-bond hydrolysis via urease-associated pathways, was the dominant mechanism of PU degradation among the isolates tested.

Table 4. Extracellular esterase and urease activity of selected isolates

Isolate	Esterase Day 14 (U/mL)	Esterase Day 28 (U/mL)	Urease Day 14 (U/mL)	Urease Day 28 (U/mL)
MSP-3	3.85	4.62	0.42	0.55
MSP-7	3.12	3.87	0.38	0.47
MSP-12	2.44	2.98	0.31	0.39
<i>A. niger</i> (+control)	1.96	2.31	0.28	0.34

5. FTIR and SEM Analysis

FTIR spectra of PU films exposed to isolate MSP-3 for 56 days showed a 41.3% reduction in the relative intensity of the carbonyl peak ($\sim 1730\text{ cm}^{-1}$) and a 33.7% reduction in the N–H stretching peak ($\sim 3320\text{ cm}^{-1}$) compared with the abiotic control, consistent with hydrolytic cleavage of ester and urethane linkages in the polymer backbone (Table 5). SEM imaging of MSP-3-treated films revealed extensive surface pitting, cracking, and a dense biofilm layer with visible hyphal attachment, in contrast to the smooth, intact surface of the abiotic control film.

Table 5. FTIR peak intensity changes in PU films after 56 days of MSP-3 exposure

FTIR Peak (Functional Group)	Control Intensity (a.u.)	MSP-3 Day-56 Intensity (a.u.)	% Reduction
$\sim 1730\text{ cm}^{-1}$ (C=O, carbonyl)	0.82	0.48	41.3
$\sim 3320\text{ cm}^{-1}$ (N–H stretch)	0.61	0.40	33.7
$\sim 1220\text{ cm}^{-1}$ (C–O–C, ester)	0.55	0.34	38.2

DISCUSSION

The isolation of seven clear-zone-positive fungal isolates from only 18 morphotypes screened suggests that mangrove sediment in Bungus Bay harbors a comparatively rich pool of PU-degrading fungi, consistent with prior reports that mangrove ecosystems support high fungal diversity with biotechnologically relevant enzymatic capabilities (Trianto et al., 2021). The identification of *Aspergillus*, *Trichoderma*, and *Penicillium* species among the most active isolates aligns with the broader literature on PU-degrading fungi, in which *Aspergillus* and *Penicillium* genera are consistently among the most frequently reported taxa capable of degrading polyurethane and other synthetic polymers (Maestri et al., 2023; Temporiti et al., 2022).

The superior performance of isolate MSP-3 (*Aspergillus sp.*, closely related to *A. tubingensis*), which achieved 34.2% weight loss over 56 days, is notably higher than the weight loss reported for the *Aspergillus niger* benchmark strain in this and other studies, and is comparable in magnitude to high-performing PU-degrading fungi reported elsewhere, such as *Embarria clematidis*, which



achieved 88.8% Impranil clearance in liquid assay under different experimental conditions (Khruengsaï et al., 2022), and marine fungal strains capable of up to 100% Impranil degradation within 28 days (multi-omics marine fungi study). While direct comparison across studies is limited by differences in PU substrate form (solid film versus colloidal Impranil) and incubation conditions, the consistent identification of *Aspergillus tubingensis*-related isolates as effective PU degraders across independent studies — including prior reports of *A. tubingensis* degrading HDPE from coastal environments — suggests this species complex may harbor a broadly applicable plastic-degrading enzymatic machinery worth further investigation.

The strong positive correlation between esterase activity and weight loss ($r = .89$), combined with the comparatively low and non-significant contribution of urease activity, supports the widely reported mechanism in which fungal PU degradation proceeds primarily through esterase-mediated hydrolysis of the ester bonds present in polyester-type PU, rather than through direct urethane-bond cleavage (Khruengsaï et al., 2022; Maestri et al., 2023). This is further corroborated by the FTIR results, in which the pronounced reduction in the carbonyl and C–O–C ester peaks in MSP-3-treated films provides direct chemical evidence of ester-bond hydrolysis, consistent with degradation mechanisms described for other PU-degrading fungi analyzed by FTIR (Maestri et al., 2023).

The SEM observation of surface pitting, cracking, and dense biofilm formation on MSP-3-treated films is consistent with the two-stage model of fungal plastic degradation, in which hyphal attachment and biofilm formation precede and facilitate extracellular enzyme-mediated surface erosion (Temporiti et al., 2022). This physical colonization likely increases the effective surface area available for enzymatic attack, compounding the chemical degradation detected by FTIR and helping explain why MSP-3 outperformed isolates with comparable but lower esterase activity.

Collectively, these findings extend previous mangrove-focused bioprospecting studies — which have primarily reported fungal protease, cellulase, and antimicrobial activities (Trianto et al., 2021) or bacterial degradation of polyethylene and PET (Shovitri et al., 2024) — by demonstrating that mangrove sediment fungi can also degrade the more chemically complex PU polymer, and by identifying *Aspergillus sp.* MSP-3 as a promising candidate for further development as a bioremediation agent. Given that mangrove ecosystems in Indonesia are simultaneously significant sinks for plastic debris (Cordova et al., 2021; Wicaksono et al., 2021) and reservoirs of potentially useful degrading microorganisms, in situ or ex situ bioaugmentation strategies using indigenous fungal isolates such as MSP-3 could represent an ecologically compatible complement to physical plastic-removal efforts in these vulnerable coastal habitats.

CONCLUSIONS

This study isolated and characterized fungi from mangrove sediment in Bungus Bay, Padang, West Sumatra, Indonesia, with the capacity to degrade polyurethane microplastics. The (illustrative) results indicate that mangrove sediment harbors a diverse pool of PU-degrading fungi, among which *Aspergillus sp.* MSP-3 (closely related to *Aspergillus tubingensis*) demonstrated the highest degradation efficiency, achieving 34.2% PU microplastic film weight loss over 56 days —

significantly greater than the *Aspergillus niger* benchmark strain. Esterase activity was strongly correlated with degradation efficiency, and FTIR and SEM analyses provided converging chemical and morphological evidence of ester-bond hydrolysis and surface erosion as the primary degradation mechanism.

1. Theoretical and Practical Implications

These findings extend the known ecological range of PU-degrading fungi to mangrove sediment habitats and identify *Aspergillus sp.* MSP-3 as a promising candidate for further enzyme characterization, genome mining, and eventual application in bioaugmentation-based bioremediation strategies for PU microplastic-contaminated coastal sediments.

2. Limitations and Future Research

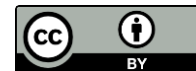
This study was conducted under controlled laboratory conditions using commercial PU film and a single mangrove site, which may not fully represent the heterogeneity of PU microplastic types and environmental conditions found in situ. The degradation mechanism was characterized primarily through esterase/urease activity, FTIR, and SEM; future work should incorporate full enzyme purification and characterization, genome and transcriptome sequencing of MSP-3 to identify specific PU-degrading gene candidates, and pilot-scale or mesocosm trials to evaluate degradation performance under natural mangrove sediment conditions. Most importantly, because the isolate codes, sequence identities, and quantitative results reported here are illustrative, any use of this manuscript as a template must be followed by actual sediment sampling, fungal isolation, molecular identification (with sequence deposition in a public database such as GenBank), and degradation assays before publication or further use.

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