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Eco-bioremediation potential of mealworm gut microbiome for polystyrene degradation in nutrient-based media

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Abstract—Polystyrene is a petroleum-based polymer widely used in single-use packaging and thermal insulation. Its high molecular weight and structural stability make it highly resistant to degradation, leading to persistent environmental accumulation. Recent studies have highlighted the potential of insect gut microbiota, particularly from mealworms, to biodegrade plastic waste. In this study, gut extracts were inoculated into Nutrient Broth (NB) and Potato Dextrose Broth (PDB), each supplemented with polystyrene as emulsion, powder, or film. NB showed the highest reduction in polystyrene weight (0.93%) by day 28, compared to PDB (0.73%). Total plate count and yeast-mold count analyses revealed microbial proliferation in both media, with NB exhibiting a higher final bacterial count (7.562 log cfu/mL) than PDB (6.510 log cfu/mL), while fungal counts were comparable. Scanning Electron Microscopy confirmed structural damage to polystyrene films, including surface roughening and micro-pitting, especially in NB. These findings indicate that media composition significantly influences microbial growth and degradation efficiency. NB appears to favor bacterial communities with enhanced plastic-degrading capabilities, demonstrating superior biodegradation potential in liquid culture.

Keywords— **gut microbiome, microbial bioremediation, polystyrene degradation, *Tenebrio molitor***

INTRODUCTION

Polystyrene (PS) is a petroleum-based polymer commonly used in single-use food containers (Zhang *et al*., 2024). Unlike its monomer and oligomer forms, PS is non-degradable due to its high molecular weight and structural stability (Park *et al*., 2023). It exhibits an extremely low biodegradation rate, with an estimated half-life in terrestrial ecosystems of several hundred years. This persistence leads to environmental accumulation, posing a significant ecological threat and necessitating effective biodegradation strategies (Jiang *et al*., 2024).

Biodegradation is generally more economical and environmentally sustainable than physical or chemical methods. Several strains of *Xanthomonas sp*., *Sphingobacterium sp*., and *Bacillus sp*. With PS-degrading capabilities have been isolated (Quan *et al*., 2023). Since then, numerous bacterial and fungal species have been identified for their ability to degrade PS. However, their biodegradation rates remain notably slow (Ding *et al*., 2024; Yang *et al*., 2020).

Research interest in plastic-degrading insects has grown significantly, particularly focusing on larvae of *Lepidoptera and Coleoptera*, such as *Zophobas atratus*, *Tenebrio molitor* (mealworm), *Tenebrio obscurus*, *Tribolium castaneum*, *Plesiophthalmus davidis*, and *Galleria mellonella*, which can consume various plastics (Q. Wang *et al*., 2024). Among these, *T. molitor* has been widely studied due to its robust gut microbiota, ease of rearing, and ability to fragment and ingest polystyrene without significant physiological stress (Yang *et al*., 2015; Peng *et al*., 2021). However, most studies have focused on survival rates, weight changes, plastic characterization, gut microbiome analysis, and PS consumption (Quan *et al*., 2023). To date, no studies have specifically investigated PS degradation by mealworm gut microbiota in liquid media. Liquid media offer a controlled and reproducible system to study plastic biodegradation under varying nutritional conditions (Lokesh *et al*., 2023)

The culture media Nutrient Broth (NB) and Potato Dextrose Broth (PDB) possess distinct nutritional compositions that may influence both the growth and metabolic activity of mealworm gut microbiome community. This study investigates how variations in these culture media affect PS degradation efficiency and the resulting metabolite profiles. NB medium, enriched with nitrogen and peptides, is anticipated to foster a more diverse bacterial consortium with enhanced PS degradation activity. Conversely, PDB medium, with its carbohydrate-rich composition typically employed for fungal and yeast cultivation, is predicted to promote eukaryotic microorganisms (particularly fungi) as dominant contributors to PS degradation. These differential media properties provide critical insights into how environmental conditions shape microbial community dynamics and PS degradation activity, while simultaneously facilitating

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identification of the optimal medium for supporting PS biodegradation by the mealworm gut microbiome.

This study aims to analyze PS degradation by the mealworm gut microbiome in two culture media—Nutrient Broth and Potato Dextrose Broth—each containing plastic and mealworm gut extracts.

MATERIALS AND METHODS

A. Mealworm and Plastic

Mealworms were obtained from a local feed supplier in Bandung and identified as *Tenebrio molitor* Linn 1758 using established beetle identification guides (Calmont & Soldati, 2008; Robinson, 2005). Prior to gut extraction, fifty 11th-instar larvae (12-13 mm length, 60-80 mg weight) were housed in 1.5-l polypropylene containers under dark conditions at ambient temperature (25 ± 1°C) and controlled humidity (57.48 ± 0.09%).

PS pellets and film were used. PS film (Good Fellow GF93064585, Merck-Millipore) was cut into 5 × 5 mm pieces before sterilization. The films were immersed in 70% (v/v) ethanol for 30 minutes, placed in sterile Petri dishes, dried at 45–50°C overnight, and equilibrated to room temperature before use.

PS powder for gravimetric analysis was prepared by grinding PS pellets (Sigma Aldrich 331651-500G, Merck-Millipore) and sieving through a No. 50 mesh. The powder was weighed into pre-weighed sterile glass Petri dishes and irradiated with UV light (365 nm) for seven days at a fixed distance of 50 cm from the source.

B. Preparation of Suspension Gut Suspension Samples

Ten mealworms were surface-sterilized with 70% ethanol. Their intestinal tracts were dissected using sterile scissors and forceps, transferred to a centrifuge tube, and mixed with 200 µL of sterile physiological saline. The guts were homogenized with a mini pestle, then diluted with 800 µL saline to a final volume of 1 mL. The suspension was stored at 4°C for further use.

C. PS Biodegradation Assay

PS powder was added to NB and PDB at 1% (w/v) as the sole carbon source. For each medium, three replicate flasks (n=3) were prepared. Each flask received 1 mL of gut suspension in 100 mL of medium. Control flasks contained media and PS without inoculum.

All cultures were incubated in 250-mL Erlenmeyer flasks at 30°C with agitation at 150 rpm. Every four days over 28 days, each flask was weighed to determine remaining PS. Weight loss percentage was calculated as:

$$Weight\ loss\ (\%) = \left(\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}}\right) x\ 100$$

D. Characterization of PS Biodegradation via Surface Analysis Using Scanning Electron Microscopy (SEM)

A total of 1 mL of gut suspension was inoculated into 100 mL of NB or PDB containing 5 × 5 mm PS films at 1% (w/v). Controls contained media and PS films without gut suspension.

On day 28, films were retrieved, agitated in 1% SDS for four hours, and rinsed three times with distilled water (Zhang *et al*., 2024).

Surface morphology analysis of the PS films was performed using SEM. Prior to imaging, the PS film samples were coated with a gold layer under a current of 25 mA at a pressure of 0.3 MPa and subsequently examined under the scanning electron microscope.

E. Total Plate Count (TPC) Analysis

Total bacterial counts were determined by serial dilution. From each dilution, 100 µL was inoculated into 20 mL of Nutrient Agar (Oxoid) and incubated at 30°C for 24 hours. Duplicate plates were used for each dilution, and colonies were enumerated.

F. Yeast and Mold Count

Yeast and mold counts were determined similarly. From each dilution, 100 µL was inoculated into 20 mL of Sabouraud Dextrose Agar (Oxoid) and incubated at 30°C for 24 hours. Duplicate plates were used, and colonies were counted.

G. Statistical Analysis

Statistical analysis was performed using GraphPad Prism 10.4.0. Significant differences in weight change, total plate count, and yeast and mold count were evaluated using one-way ANOVA (n=3) with Tukey's and Tamhane's T2 post-hoc tests.

RESULTS AND DISCUSSIONS

The ability of microorganisms to degrade PS in liquid media was assessed by measuring PS weight reduction every four days. NB showed a higher rate of PS weight loss than PDB (Fig. 1). By day 28, PS weight loss in NB (0.93%) was greater than in PDB (0.73%). This correlated with a higher total plate count in NB (7.562 log CFU/mL) compared to PDB (6.510 log CFU/mL). The superior degradation in NB likely reflects the dominance of bacterial taxa secreting oxidative and hydrolytic enzymes, such as laccases and serine hydrolases, which initiate PS depolymerization (Liu *et al*., 2023; Mamtimin *et al*., 2023). These enzymes cleave the polymer backbone, promoting fragmentation and bio-assimilation. Thus, the higher TPC in NB indicates not only greater bacterial growth but also more efficient enzymatic PS turnover. NB's rich nutrient profile supports rapid microbial growth and enzyme production. These results suggest that bacteria play a more dominant role in PS degradation than fungi or yeasts from the mealworm gut.

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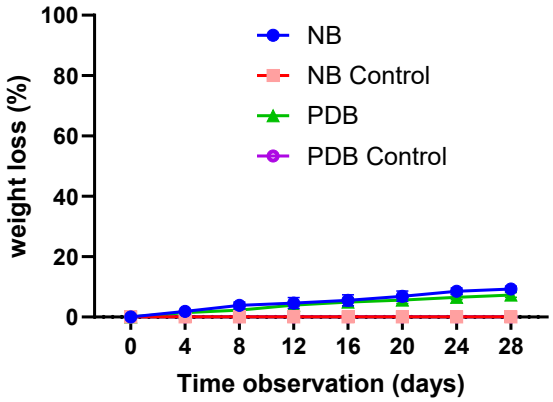
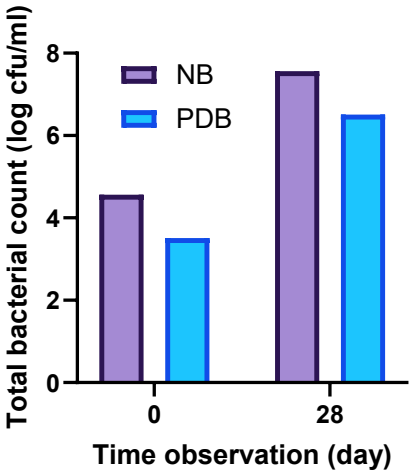


Figure 1. PS weight loss in liquid media NB and PDB during experiment.

To confirm microbial survival during the assay, total plate count and yeast-mold count were measured on days 0 and 28 (Fig. 2A). On day 0, NB had the highest colony count (4.562 log cfu/mL), followed by PDB (3.105 log cfu/mL). After 28 days, counts increased to 7.562 log cfu/mL in NB and 6.510 log cfu/mL in PDB. The TPC after 28 days positively correlated with PS weight loss, with NB showing greater degradation.

A



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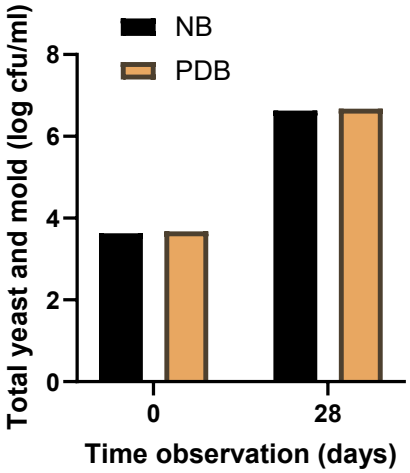

Figure 2. Growth of gut microorganisms in NB, and PDB media from day 0 to day 28. (A) Total Plate Count (TPC) (B) Yeast and Mold Count.

Yeast and mold counts were similar in both media on day 0 (~3.68 log cfu/mL) and increased to ~6.65 log cfu/mL by day 28 (Fig. 2B). Despite the high fungal count in PDB, PS weight loss was lower than in NB. This suggests that PS degradation in PDB is not directly proportional to microbial activity and that bacteria are more effective degraders.

The nutrient composition of NB and PDB significantly influenced PS biodegradation efficiency, particularly through the carbon/nitrogen (C/N) ratio. NB has a lower C/N ratio due to its higher nitrogen content, while PDB is carbon-rich. Nitrogen in NB, from peptone and yeast extract, supplies ammonia and amino acids for microbial protein synthesis, including depolymerases like laccase and peroxidase (Yang *et al*., 2020). In contrast, PDB's high carbohydrate content and limited nitrogen restrict enzyme synthesis despite abundant energy. This aligns with Ding *et al*. (2024), who identified nitrogen as a critical cofactor for enzymatic activity. Additionally, peptides in NB may induce enzyme expression via cross-feeding between bacteria (Quan *et al*., 2023), while nitrogen-deficient PDB promotes fungal fermentative metabolism, which is less effective for PS degradation. Thus, NB's balanced nutrients and optimal C/N ratio favor enzyme-producing bacteria, enhancing PS degradation.

The difference in degradation between NB and PDB highlights the need to optimize culture conditions for large-scale bioremediation. Although PDB supported fungal growth, its lower PS degradation suggests that fungi may need longer incubation or bacterial synergy for effective plastic breakdown (Liberto *et al*., 2024). Future studies could explore mixed-media formulations or sequential cultivation to leverage both bacterial and fungal communities.

These findings imply that nutrient composition in natural or engineered environments can significantly affect plastic-degrading microbiota. In applied settings, nitrogen-rich conditions could enhance bacterial-driven PS bioremediation, useful in wastewater or compost systems.

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Visualization of the PS film surface obtained through scanning electron microscopy (SEM) analysis is presented in Figure 3. SEM analysis of the control PS film revealed a uniformly smooth and intact surface, with no apparent signs of physical degradation or morphological alteration.

SEM analysis of PS films revealed clear morphological changes after 28 days of incubation with gut microbiota in both media (Fig. 3). Control films remained smooth and intact.

In NB, PS films showed localized damage with small, well-defined pits (arrow), suggesting enzymatic or oxidative attack by bacteria. The surrounding smooth areas indicate degradation at specific colonization sites.

In PDB, films exhibited broader, deeper erosion with irregular ridges and partial delamination, suggesting slower, possibly fungal-mediated degradation. The circular erosive patterns indicate polymer softening and biofilm formation.

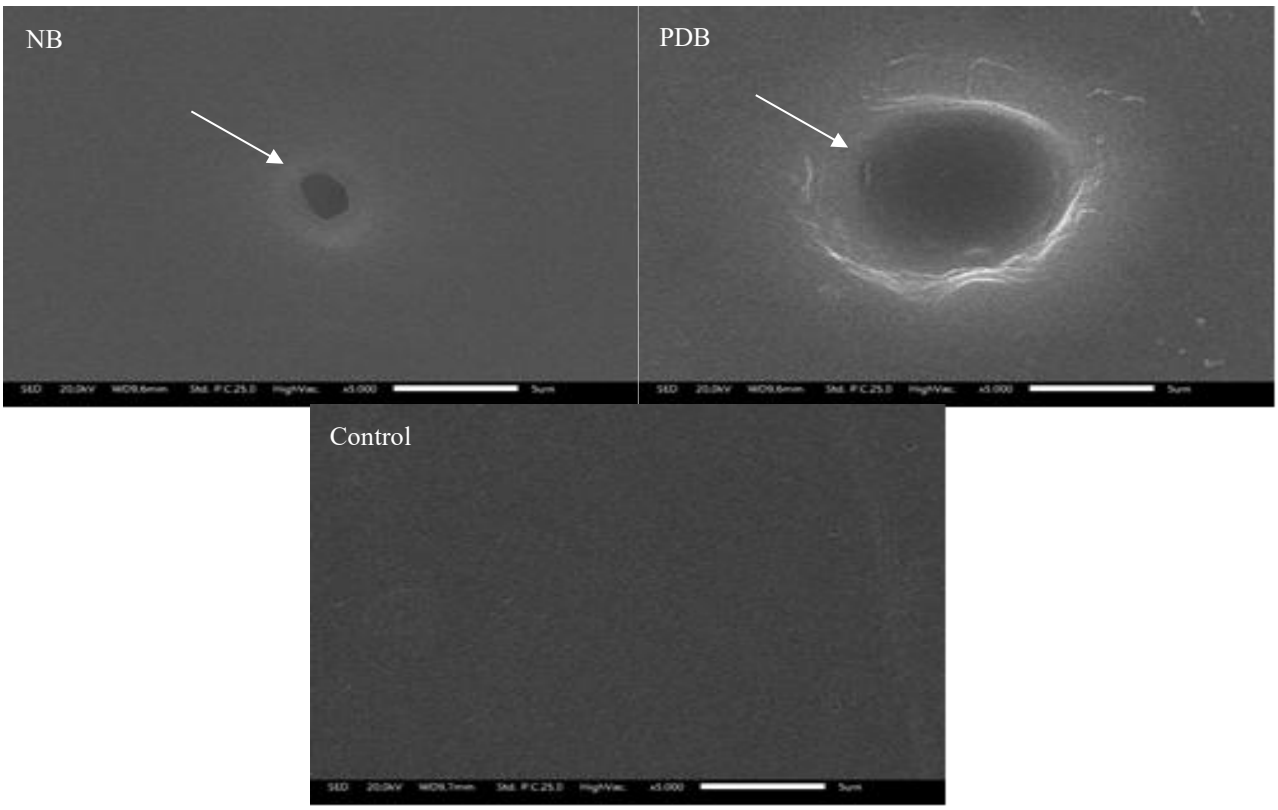

Figure 3. Surface Morphology Analysis of PS Film Using Scanning Electron Microscopy (SEM) at 5000× Magnification in NB and PDB Media. The arrows indicate morphological surface alterations on the PS film.

Gut microorganisms in both media caused morphological changes, transforming smooth surfaces into rough, perforated ones—a key indicator of microbial degradation (Liberto *et al*., 2024). Bacterial adhesion to PS films modifies surface chemistry, increasing hydrophilicity and reducing hydrophobicity (Quan *et al*., 2023). Microbes secrete depolymerizing enzymes, using plastic polymers as growth substrates. Surface roughness also provides adhesion sites and protection from shear forces, accelerating degradation (Lin *et al*., 2024). PS hydrophobicity further influences microbial colonization and biodegradation potential.

Overall, SEM results support gravimetric and microbial data, confirming that microbial activity physically degraded PS surfaces. The finer pits in NB suggest bacterial-dominated degradation, while rougher cavities in PDB imply mixed or fungal-associated degradation.

Together, the higher bacterial growth, significant PS weight loss, and surface erosion in NB indicate synergy between microbial growth and enzyme-mediated degradation. Integrating gravimetric, microbiological, and morphological evidence supports that bacterial-dominated consortia are more effective than fungal-dominated systems under nutrient-rich conditions. These results demonstrate the mealworm gut microbiome's potential for plastic waste management.

CONCLUSIONS

This study shows that the *T. molitor* gut microbiome effectively degrades PS in liquid media, with efficiency strongly influenced by culture medium. NB outperformed PDB, achieving higher PS mass reduction (0.93% vs. 0.73%) and more pronounced surface damage, as shown by SEM. NB's superiority aligns with its ability to enrich bacteria producing depolymerizing enzymes, while PDB's fungal-dominated consortium degraded PS more slowly.

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