

UTILIZATION OF *Pseudomonas* sp. FROM LIVESTOCK SLAUGHTERHOUSE WASTE FOR BIODEGRADATION OF POLYETHYLENE PLASTIC WASTE

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ABSTRACT

Indonesia faces increasing plastic pollution, particularly from polyethylene (PE), which is persistent and highly resistant to natural biodegradation due to its stable carbon-carbon backbone. This study evaluated the potential of *Pseudomonas* sp. isolated from slaughterhouse rumen fluid at Segoroyoso Slaughterhouse, Yogyakarta, to interact with and modify PE plastic surfaces. Rumen fluid was collected from three cattle, cultured on selective *Pseudomonas* Agar Base medium, and purified through repeated streaking. The isolate exhibiting characteristics consistent with *Pseudomonas* sp. was revitalized, and its interaction with PE was evaluated through five treatments: P₀ (bacteria without plastic), P₁ (plastic without bacteria), P₂ (bacteria + 1% PE), P₃ (bacteria + 3% PE), and P₄ (bacteria + 5% PE), incubated for 24–48 hours and assessed descriptively using scanning electron microscopy (SEM). Bacterial colonies were observed attaching to the PE surface after four days of incubation. Compared with P₁, PE surfaces in P₂–P₄ exhibited increasing irregularity, roughness, groove formation, and erosion corresponding to higher PE concentrations, along with reduced diameter in some PE beads. These findings indicate that *Pseudomonas* sp. from slaughterhouse rumen waste has the potential to attach to and modify PE surfaces. However, since results were based primarily on descriptive morphological observations without quantitative esterase activity data, replicate numbers, or inferential statistical analysis, the most effective treatment condition could not be determined quantitatively. Further research is recommended to measure PE mass loss, bacterial population, esterase activity, chemical changes via FTIR, and degradation products to obtain more robust quantitative evidence of PE biodegradation and establish the optimal treatment condition.

Keywords: Degradation, Esterase, PE, *Pseudomonas* sp., Utilization.

ABSTRAK

Indonesia menghadapi peningkatan pencemaran plastik, khususnya polietilena (PE) yang bersifat persisten dan sulit terdegradasi secara alami karena rantai karbon-karbonnya yang stabil. Penelitian ini bertujuan mengevaluasi potensi *Pseudomonas* sp. yang diisolasi dari limbah cairan rumen rumah potong hewan (RPH) Segoroyoso, Yogyakarta, dalam berinteraksi dan memodifikasi permukaan plastik PE. Cairan rumen dikumpulkan dari tiga ekor sapi, diisolasi pada media selektif *Pseudomonas* Agar Base, dan dimurnikan melalui penggoresan berulang. Isolat yang menunjukkan karakteristik *Pseudomonas* sp. direvitalisasi dan diuji interaksinya dengan PE melalui lima perlakuan: P₀ (bakteri tanpa plastik), P₁ (plastik tanpa bakteri), P₂ (bakteri + 1% PE), P₃ (bakteri + 3% PE), dan P₄ (bakteri + 5% PE), diinkubasi

selama 24–48 jam dan dianalisis secara deskriptif menggunakan *scanning electron microscopy* (SEM). Koloni bakteri mulai menempel pada permukaan PE setelah empat hari inkubasi. Dibandingkan dengan P₁, permukaan PE pada P₂–P₄ menunjukkan ketidakteraturan, peningkatan kekasaran, alur, dan erosi yang makin nyata seiring naiknya konsentrasi PE, disertai penurunan diameter pada sebagian butiran PE. Temuan ini mengindikasikan bahwa *Pseudomonas* sp. dari limbah rumen RPH berpotensi melekat dan memodifikasi permukaan PE. Namun, karena hasil bersifat deskriptif-morfologis tanpa data aktivitas esterase, jumlah ulangan, maupun uji statistik inferensial, kondisi perlakuan paling optimum belum dapat ditentukan secara kuantitatif. Penelitian lanjutan disarankan untuk mengukur kehilangan massa PE, populasi bakteri, aktivitas esterase, perubahan gugus kimia melalui FTIR, serta produk degradasi guna memperoleh bukti biodegradasi PE yang lebih kuantitatif dan menentukan kondisi optimal.

Kata kunci: Degradasi, Esterase, PE, *Pseudomonas* sp., Pemanfaatan.

INTRODUCTION

Plastic was initially developed as an alternative material to reduce dependence on natural resources such as wood. However, its widespread use has subsequently become a major environmental concern because of its persistence and accumulation in terrestrial and aquatic ecosystems. In Indonesia, plastic waste continues to represent a substantial and growing component of municipal solid waste. According to the most recent data from the National Waste Management Information System (SIPSN) under the Ministry of Environment and Forestry (KLHK), covering 249 regencies/cities, national waste generation in 2025 reached approximately 25 million tons, of which plastic accounted for around 20.45%, an increase from the 19.59% plastic share recorded in 2024 out of roughly 36 million tons of total waste generation that year (KLHK/SIPSN, 2025). This upward trend was further corroborated by an official statement from the Minister of Environment during World Environment Day 2025, themed "Ending Plastic Pollution," which noted that approximately 10.8 million tons, or nearly 20% of national waste, consists of plastic, while the national recycling rate stands at only 22%, with Java recording the highest recycling rate at 31%,

followed by Bali-Nusa Tenggara at 22.5% and Sumatra at 12%, while Eastern Indonesia still faces significant management challenges (Ministry of Environment, 2025).

It is worth noting that data collection for 2025 remains ongoing and these figures are likely to increase further as reporting is completed, yet the composition of plastic waste this year has already surpassed that of the previous year, reinforcing concerns that plastic pollution in Indonesia is intensifying rather than easing despite existing regulatory efforts. Plastic leakage into aquatic environments remains a significant concern as well; earlier nationwide assessments by the World Bank estimated that Indonesia generates approximately 7.8 million tons of plastic waste annually, of which about 4.9 million tons is mismanaged, with approximately 346.5 thousand tons of plastic waste per year entering the marine environment from land-based sources, with rivers accounting for approximately 83% of this leakage (World Bank, 2021).

The accumulation of plastic waste in aquatic environments can threaten aquatic organisms through ingestion, entanglement, and physical alteration of habitats. Therefore, reducing plastic

pollution requires not only improved waste management but also the development of environmentally sustainable approaches for plastic degradation. Polyethylene (PE) is one of the widely used plastic polymers and is characterized by a stable carbon-carbon backbone that makes it highly resistant to natural degradation. Its persistence in the environment has consequently encouraged research into biological approaches involving microorganisms capable of colonizing and modifying plastic surfaces. In this context, *Pseudomonas* sp. represents a potential candidate because members of this genus have been reported to possess metabolic and enzymatic capabilities associated with the transformation of persistent organic polymers. Accordingly, investigating *Pseudomonas* sp. isolated from slaughterhouse rumen waste as a potential biological agent for PE degradation may provide an alternative approach for addressing persistent plastic pollution.

Plastic was initially developed as an alternative material to reduce dependence on natural resources such as wood, valued for its light weight, durability, and low production cost. However, its widespread and excessive use has since become one of the major environmental concerns worldwide due to its persistence and continued accumulation in terrestrial and aquatic ecosystems. Among the various types of plastic polymers, PE is one of the most common, widely used as the base material for packaging, including single-use containers, bags, and films (Geyer et al., 2017). PE is characterized by a stable carbon-carbon backbone, which grants it durability but also makes it highly resistant to natural biodegradation. As a result, if not properly managed, PE waste accumulates in large quantities in the environment, posing severe ecological risks such as climate change, soil and water pollution, and disruption of natural ecosystems (Wei & Zimmermann, 2017). Furthermore, the production, consumption, and disposal

processes of PE generate substantial carbon emissions, contributing to global warming and the increasing occurrence of extreme weather conditions (Budiarti et al., 2019).

Given these persistent problems, biological control approaches have become increasingly important, particularly through the search for bacterial candidates capable of degrading PE. Moradali et al. (2017) state that *Pseudomonas* sp. can produce esterase enzymes capable of hydrolyzing plastic polymer compounds, including PE, through enzymatic action on the polymer's chemical bonds. These esterase enzymes work by breaking down the long PE polymer chains into smaller intermediate compounds, which are subsequently degraded further by microbial metabolic processes into simpler, non-toxic byproducts. This enzymatic degradation pathway offers a promising, environmentally sustainable alternative to conventional plastic waste management methods, which often rely on incineration or landfilling that carry their own environmental drawbacks. Accordingly, further exploration of *Pseudomonas* sp. as a potential biological agent for PE degradation may provide a valuable contribution toward addressing the persistent problem of plastic pollution.

Pseudomonas sp. bacteria can be found in slaughterhouse (RPH) rumen waste, which consists of digested feed materials that become waste after slaughter and can lead to putrefaction and environmental pollution if improperly managed (Quartinello et al., 2021; Guo et al., 2025). Meanwhile, the growing accumulation and persistence of polyethylene (PE) plastic necessitate sustainable biodegradation strategies, making the utilization of rumen waste as a microbial source for PE degradation a promising waste-valorization approach. Although previous studies have examined rumen microbes primarily for feed digestion and focused on polyester-degrading bacteria from soil, compost, or

marine sediments, the potential of slaughterhouse rumen waste specifically as a source of PE-degrading *Pseudomonas* sp. remains largely unexplored. In particular, it is not yet established whether *Pseudomonas* strains adapted to the rumen's anaerobic, high-organic-load environment retain or develop esterase-producing capacity relevant to PE hydrolysis when cultured under aerobic, plastic-exposed conditions.

Addressing this gap, the specific contribution of this study is twofold: first, it

identifies and characterizes a *Pseudomonas* sp. isolate from underutilized slaughterhouse rumen content; second, it evaluates the isolate's esterase production and PE degradation performance. Ultimately, by bridging rumen waste valorization with PE biodegradation and evaluating performance under simulated aquatic conditions, this study aims to provide new evidence toward an environmentally sustainable, waste-derived strategy for PE pollution management.

MATERIALS AND METHODS

Materials. The bacterial isolate used in this study was obtained from rumen fluid collected from three cattle at the Segoroyoso Slaughterhouse (RPH), Yogyakarta, Indonesia. A volume of 0.1 mL of rumen fluid was collected from each individual cattle and subsequently used for bacterial isolation. The samples were cultured on *Pseudomonas* Agar Base for *Pseudomonas* spp. to facilitate the selection of bacteria belonging to the genus *Pseudomonas*. After incubation, bacterial colonies showing growth on the selective medium were isolated and purified by repeated streaking until pure colonies were obtained. The resulting isolates were evaluated based on their colony morphology and growth characteristics on the selective medium. An isolate showing characteristics consistent with *Pseudomonas* sp. was selected and subsequently cultured for the plastic degradation experiment.

The selected *Pseudomonas* sp. isolate was then inoculated into *Pseudomonas* Agar Base for bacterial propagation prior to the plastic degradation assay. The bacterial culture was subsequently exposed to polyethylene (PE) substrates at different concentrations. Changes in the PE surface before and after bacterial treatment were evaluated using Scanning Electron Microscopy (SEM).

Sample Fluid Collection. Fresh rumen fluid samples were collected from cattle slaughtered at the Segoroyoso Slaughterhouse, Yogyakarta, Indonesia. Approximately 50 mL of rumen fluid was collected at around 23:00 WIB, approximately 2 h after slaughter. The samples were transferred into sterile, tightly closed containers and processed as soon as possible to minimize changes in the bacterial population. For bacterial isolation, 10 mL of rumen fluid was transferred into 90 mL of sterile 0.85% saline solution and homogenized using a shaker incubator at 37°C for 30 min. The resulting bacterial suspension was serially diluted up to 10⁻⁶. Aliquots from the appropriate dilution levels were inoculated onto *Pseudomonas* Agar Base, a selective medium used for the isolation and selection of *Pseudomonas* sp. The inoculated plates were incubated at 37°C for 48 h. Individual colonies showing growth on the selective medium and distinct colony morphology were selected and transferred to fresh *Pseudomonas* Agar Base plates for purification. Repeated streaking was performed until morphologically uniform colonies were obtained. The purified isolate showing characteristics consistent with *Pseudomonas* sp. on the selective medium was subsequently selected for bacterial

culture refreshment and used in the polyethylene (PE) degradation experiment.

Bacterial Culture Refreshment.

The purified bacterial isolate selected as *Pseudomonas* sp. was subjected to culture refreshment before being used in the plastic degradation experiment. The bacterial isolate was transferred aseptically to *Pseudomonas* Agar Base and incubated under the appropriate incubation conditions until sufficient bacterial growth was obtained. The refreshed bacterial culture was subsequently used as the inoculum for the plastic treatment experiment. Bacterial growth was monitored during the refreshment process to ensure that the isolate was viable and suitable for subsequent application. Therefore, the results were interpreted based on the observed bacterial growth and morphological changes of the PE substrate (Quartinello et al., 2021).

Enzyme Production Test. The plastic degradation experiment consisted of five treatments (P₀–P₄), as presented in Table 1. *Pseudomonas* Agar Base was used as the medium for bacterial cultivation and treatment preparation. P₀ served as the bacterial control and consisted of *Pseudomonas* sp. without a plastic substrate. P₁ consisted of the plastic substrate without bacterial inoculation and served as the plastic control. Treatments P₂, P₃, and P₄ consisted of *Pseudomonas* sp. with polyethylene (PE) substrate at concentrations of 1%, 3%, and 5%,

respectively. All treatments were incubated under the same experimental conditions for 24–48 h. Following incubation, the PE samples were collected for morphological evaluation using scanning electron microscopy (SEM) (Guo et al., 2025).

PE Degradation. Polymer plastic with a diameter of +/- 6 mm was placed in an Eppendorf tube containing 50 nM esterase enzyme and 50 mM phosphate buffer solution at pH 7.2 with a volume of 500 µL. The mixture was incubated at 30°C for 96 hours, and polymer plastic degradation by the esterase enzyme was analyzed (Austin et al., 2018).

Scanning Electron Microscope (SEM) Visualization. Polymer plastic with a diameter of +/- 6 mm was visualized using SEM before and after treatment with esterase enzyme for 96 hours. The sample was treated with esterase enzyme, rinsed with 1% SDS, followed by rinsing with d-H₂O and ethanol. The sample was coated with an 8 nm iridium layer, attached to an aluminum section with a carbon binder, and silver paint, a conductor, was applied around the sample to reduce charge. SEM visualization of rumen fluid samples was conducted under high vacuum and high-tension conditions (Quartinello et al., 2021).

Data Analysis. The data analysis used was descriptive by observing SEM results as a marker for biodegradation. There are five treatments, which can be seen in Table 1.

Table 1. Treatment of esterase enzyme testing by *Pseudomonas* sp. bacteria.

Treatment	Composition
P ₀	Control (<i>Pseudomonas</i> Agar Base + <i>Pseudomonas</i> sp. without plastic substrate)
P ₁	<i>Pseudomonas</i> Agar Base + plastic substrate
P ₂	<i>Pseudomonas</i> Agar Base + bacteria + 1% plastic substrate
P ₃	<i>Pseudomonas</i> Agar Base + bacteria + 3% plastic substrate
P ₄	<i>Pseudomonas</i> Agar Base + bacteria + 5% plastic substrate

RESULTS AND DISCUSSION

Inoculum Source and Revitalization of *Pseudomonas* sp. Bacteria. This research utilizes *Pseudomonas* sp. bacteria collected from the Segoroyoso Slaughterhouse around 11:00 PM and conducted approximately 2 hours after the slaughter, amounting to 50 mL, promptly stored at a temperature of 20°C. The revitalization of bacterial cultures aims to determine whether *Pseudomonas* sp. colonies can thrive in a

medium blended with PE. If growth occurs, it signifies that *Pseudomonas* sp. bacteria can indeed utilize PE polymer plastic for their growth, either as a nutrient or to facilitate metabolism. The revitalization of *Pseudomonas* sp. bacterial cultures involves using *Pseudomonas* Agar Base media + PE. This revitalization process takes approximately 7 days, yielding *Pseudomonas* bacteria that grow exceptionally well, as observed in Figure 1.



Figure 1. Results of rejuvenation of bacterial culture *Pseudomonas* sp.

Pseudomonas spp. are Gram-negative, rod-shaped bacteria that generally exhibit aerobic metabolism, although several species are capable of anaerobic respiration using nitrate or nitrite as alternative terminal electron acceptors. In addition, *Pseudomonas* sp. has been reported to produce extracellular oxidative and hydrolytic enzymes, including esterases, lipases, and cutinases, which contribute to the transformation and degradation of synthetic polymers (Wilkes & Aristilde, 2017). The process of plastic waste degradation by these enzymes occurs optimally in an environment without inhibitors that can impede enzyme activity.

Biodegradation of PE Beads by *Pseudomonas* sp. The biodegradation potential of PE beads by *Pseudomonas* sp. was evaluated based on bacterial growth and changes in the morphology of the plastic surface. *Pseudomonas* sp. was observed on the PE film after incubation in 10% NB medium supplemented with PE. Bacterial colonies began to appear on the PE surface after four days of incubation. In

addition, the diameter of several PE beads decreased after incubation with *Pseudomonas* sp. compared with the control group, indicating that bacterial treatment was associated with changes in the physical characteristics of the PE substrate.

The effect of bacterial application was further evaluated through treatments P₀–P₄. P₀ consisted of *Pseudomonas* Agar Base + *Pseudomonas* sp. without plastic substrate and served as the bacterial control, while P₁ consisted of *Pseudomonas* Agar Base + plastic substrate without bacterial inoculation and served as the plastic control. P₂, P₃, and P₄ consisted of 10% NB + *Pseudomonas* sp. with 1%, 3%, and 5% plastic substrate, respectively. This treatment arrangement enabled the effect of *Pseudomonas* sp. on PE morphology to be compared with the plastic condition without bacterial treatment.

SEM observations (Figure 2) revealed differences in PE surface morphology among treatments. The P₁ surface appeared relatively homogeneous,

whereas P₂ exhibited morphological alterations characterized by irregular structures and grooves. These changes may be associated with bacterial attachment and interaction of *Pseudomonas* sp. with the PE surface. More pronounced morphological changes were observed in P₃, where the PE surface appeared rougher, with more visible grooves and irregular structures compared with P₁ and P₂. In P₄, the surface also exhibited a rough and irregular structure with relatively dense longitudinal grooves. The progressive morphological alterations observed across P₂–P₄ indicate that exposure to *Pseudomonas* sp. was associated with increasing modification of the PE surface. These alterations may reflect bacterial attachment and interaction with the polymer surface during incubation, which can contribute to changes in surface structure and integrity. The differences in surface morphology among the treatments provide visual evidence of the interaction between *Pseudomonas* sp. and PE and support the potential role of microbial activity in modifying the polymer surface.

Changes in plastic surfaces following microbial exposure can be effectively visualized using scanning electron microscopy (SEM). According to Choudhary (2017), SEM is a microscopy technique used to generate high-resolution images of microscopic regions of a sample surface. Unlike conventional light microscopy, SEM uses a focused beam of energetic electrons to scan the sample surface, producing detailed images of its morphological characteristics. This technique enables the visualization of surface features at the microscale, including changes in surface roughness, grooves, irregular structures, and erosion. Therefore, SEM provides an appropriate approach for

characterizing morphological alterations in PE surfaces following exposure to microbial cultures. In the present study, the differences observed between the untreated plastic surface (P₁) and the bacterial treatments (P₂–P₄) demonstrate distinct morphological alterations associated with *Pseudomonas* sp. exposure, with the changes becoming more pronounced across the bacterial treatments.

Previous research on microbial plastic degradation using *Bacillus* and *Pseudomonas* sp. demonstrated that bacterial treatment could induce observable changes in the physical structure of plastic surfaces. SEM has been widely applied to characterize these changes, including surface erosion, increased roughness, cracking, and bacterial attachment or biofilm formation. Such morphological alterations provide evidence of microbial interaction with and deterioration of polymer surfaces (Ghatge et al., 2020). The present results are consistent with these findings, as the PE surfaces treated with *Pseudomonas* sp. (P₂–P₄) exhibited greater irregularity, roughness, and surface grooves than the untreated plastic control (P₁).

Overall, the SEM observations indicate that *Pseudomonas* sp. application was associated with morphological changes in the PE substrate. P₁ maintained a relatively homogeneous surface, whereas P₂, P₃, and P₄ showed progressively more apparent surface irregularities and erosion. These findings suggest that *Pseudomonas* sp. interacted with and modified the PE surface under the tested conditions. However, SEM observations primarily provide morphological evidence; therefore, complementary analyses are required to quantitatively confirm the extent of PE biodegradation.

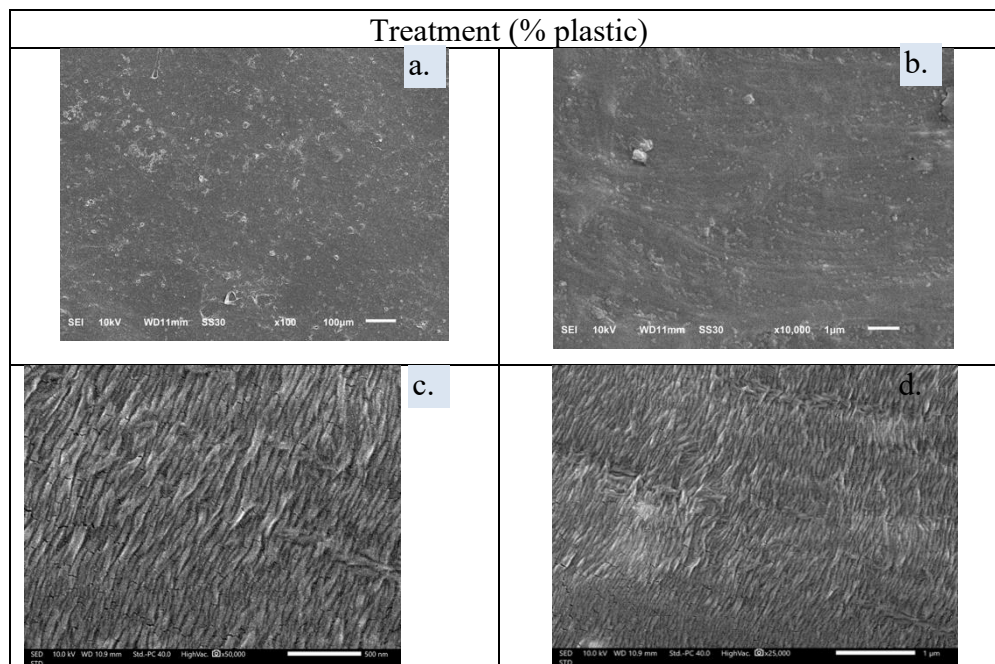


Figure 2. SEM images showing the morphology of plastic substrates before and after bacterial treatment: (a) *Pseudomonas* Agar Base + plastic substrate (P₁), (b) *Pseudomonas* Agar Base + bacteria (P₂) + 1% plastic substrate, (c) *Pseudomonas* Agar Base + bacteria + 3% plastic substrate (P₃), and (d) *Pseudomonas* Agar Base + bacteria + 5% plastic substrate (P₄).

Note: Compared with P₁ (a), the PE surfaces exposed to *Pseudomonas* sp. (b–d) showed progressively more pronounced morphological alterations, including increased roughness, grooves, and irregular structures. These features represent surface modification associated with bacterial exposure. In microbial–plastic interactions, such morphological changes may appear as grooves, pits, cracks, roughness, and localized erosion on the polymer surface. The differences observed in P₂–P₄ therefore indicate changes in the PE surface following bacterial treatment.

Mechanism of Action of PE surface Modification by *Pseudomonas* sp. Polyethylene (PE) is a highly persistent polymer characterized by a hydrophobic carbon–carbon backbone, which makes its natural degradation relatively difficult. The interaction between microorganisms and PE generally begins with microbial attachment to the polymer surface, followed by biofilm formation and interactions between microbial cells,

extracellular substances, and the polymer surface. These processes can alter the physical characteristics of the polymer surface, including its roughness, structural irregularity, and surface integrity. The extent of microbial interaction with plastic is influenced by several abiotic and biotic factors, including temperature, pH, oxygen availability, microbial growth and activity, polymer structure, and surface characteristics (Khan et al., 2022).

In the present study, the SEM observations provide evidence of morphological differences between the PE substrate without bacterial exposure (P₁) and those exposed to *Pseudomonas* sp. (P₂–P₄). The P₁ surface appeared relatively homogeneous, whereas the bacterial treatments exhibited progressively more pronounced surface alterations, including roughness, grooves, and irregular structures. These morphological changes indicate that *Pseudomonas* sp. was able to interact with and colonize the PE surface during incubation. Microbial attachment to hydrophobic PE surfaces may facilitate the

development of a biofilm and increase localized interactions between bacterial cells and the polymer surface. Such interactions can contribute to physical modification of the polymer surface and may increase its accessibility to microbial enzymes (Park & Kim, 2019).

Pseudomonas sp. are metabolically versatile microorganisms that are widely distributed in various environments, including soil, freshwater, marine environments, wastewater, and contaminated sites (Crone et al., 2020). Their metabolic versatility and ability to colonize different surfaces make members of this genus potential candidates for investigating microbial interactions with persistent polymers. In this study, the presence of *Pseudomonas* sp. on PE was accompanied by visible morphological alterations, particularly in P₂–P₄. The increasing prominence of surface roughness and grooves across these treatments suggests an association between bacterial exposure and PE surface modification. However, the morphological response should be interpreted primarily as evidence of changes in the PE surface following bacterial exposure rather than as direct evidence of complete polymer biodegradation.

A proposed mechanism for microbial transformation of PE described in the literature involves an initial alteration of

the polymer structure, followed by oxidation and subsequent utilization of lower-molecular-weight compounds by microorganisms. For example, Liu et al. (2023) described a pathway in which oxidized PE containing carbonyl groups may undergo further transformation involving monooxygenase, alcohol dehydrogenase, and aldehyde dehydrogenase, followed by fatty-acid metabolism through the β -oxidation pathway. This pathway represents a proposed mechanism based on previous literature and was not directly evaluated in the present study because photooxidation and the associated chemical transformations were not experimentally measured. Nevertheless, the SEM observations obtained in this study are consistent with the initial stage of microbial–polymer interaction, particularly bacterial attachment and modification of the PE surface.

Overall, the observed morphological alterations in P₂–P₄ support the potential of *Pseudomonas* sp. isolated from slaughterhouse rumen waste to interact with and modify PE surfaces. Further characterization of the chemical and structural changes in PE would help elucidate whether the observed surface modification is accompanied by polymer oxidation, molecular-weight reduction, or formation of degradation products.

CONCLUSION

SEM observations showed morphological changes on the PE surface across the bacterial treatments, indicating that *Pseudomonas* sp. isolated from slaughterhouse rumen waste has the potential to interact with and modify the PE surface. However, since SEM analysis is only qualitative and descriptive in nature, these observations alone are insufficient to determine which treatment represents the most effective biodegradation condition or

to establish the optimal percentage of *Pseudomonas* sp. application. Further research is therefore recommended, incorporating additional quantitative parameters such as plastic weight loss, bacterial population or concentration, enzyme activity, FTIR analysis, and degradation-product analysis, to obtain more robust and conclusive evidence of PE biodegradation and to accurately determine the optimal treatment condition.

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