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THE ABILITY OF *Bacillus* sp. BACTERIA FROM THE ROKAN RIVER ESTUARINE WATERS TO DEGRADE MICROPLASTICS

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ABSTRACT

Microplastic pollution in aquatic environments has emerged as a critical global environmental concern. Its presence has been widely detected in various environmental compartments, including estuaries**.** The objective of this study was to assess the ability of *Bacillus* sp. isolated from the Rokan River estuary waters to degrade microplastics, namely polystyrene (PS), polyethylene (PE), and polypropylene (PP), and to observe changes in their surface morphology using Scanning Electron Microscopy (SEM). The method used was a one-factor Completely Randomized Design (CRD) experiment with three replications. The degradation test was conducted gravimetrically over 60 days of incubation. The results showed that the highest average degradation rate was found in PE (4.09%), followed by PS (1.86%) and PP (1.19%). The Kruskal-Wallis statistical test yielded a p-value of 0.361 ($p > 0.05$), indicating no significant difference among the plastic types. SEM analysis confirmed morphological changes, including cracks, pores, and holes, on the surfaces of the treated plastics. The highest average bacterial count was found in the PE treatment (20.8 x 10^{10} CFU/mL), followed by PS (18.8 x 10^{10} CFU/mL) and PP (13.5 x 10^{10} CFU/mL). Therefore, it is concluded that *Bacillus* sp. has potential as an early indicator of microplastic biodegradation, although the degradation rate remains relatively low.

Keywords: Degradation, microplastics, *Bacillus* sp., SEM

1. INTRODUCTION

Microplastic pollution is an increasingly concerning global environmental issue. In 2024, plastic waste in Indonesia was estimated to account for 13.98% of total national waste, with a volume of approximately 9.9 million metric tons[1]. Data from the Ministry of Environment and Forestry in 2023 recorded a total national waste generation of 28.015.503 tons/ year, with plastics accounting for 18.5%, or approximately 5.14 million tons/ year. Microplastics are defined as plastic particles smaller than 5 mm in size, which may occur as *fragments*, films, fibers, or foam[2]. Their presence has been widely detected in various environmental compartments, including estuarine waters. The Rokan River estuary is an estuarine area influenced by wastewater runoff from land, anthropogenic activities, transportation, and fishing activities, making it vulnerable to microplastic accumulation.

There are several methods that can be used to solve this problem, namely biological degradation through the use of microorganisms[3]. Several bacterial genera, such as *Bacillus, Pseudomonas, Rhodococcus, Ruminococcus, Mycobacterium, Micrococcus, Enterobacter, Staphylococcus,* and *Citrobacter,* have been reported to degrade various types of microplastics, albeit with varying degrees of efficiency[4]. A study conducted in the waters of Bagan siapi api showed that *Bacillus* sp. degraded LDPE by

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up to 30.38% over 30 days[5]. However, the ability of bacterial isolates from the Rokan River estuary to degrade PS, PE, and PP remains unknown. This study aims to examine the ability of Bacillus sp. isolated from the Rokan River estuary waters to degrade PS, PE, and PP microplastics, as well as to observe changes in the surface morphology of the plastics using the SEM method.

2. RESEARCH METHOD

Time and Place

This research was conducted from November 2025 to March 2026. The microplastic degradation test was carried out at the Marine Microbiology Laboratory, Department of Marine Science, Faculty of Fisheries and Marine Science, Universitas Riau. Morphological analysis was conducted using SEM at the Integrated Laboratory of the Universitas Riau.

Method

This study used a one-factor Completely Randomized Design (CRD) experimental method, with the factor being the type of microplastic (PS, PE, PP), each replicated three times and involving bacterial inoculation. In addition to the main treatments, negative controls were included for each plastic type without bacterial inoculation. The study was conducted in several stages, including sterilization of equipment and materials, rejuvenation of bacterial cultures, microplastic degradation testing, observation of surface morphology using Scanning Electron Microscopy (SEM), and analysis.

Procedures

Bacterial Culture Rejuvenation

The pure bacterial culture was rejuvenated by taking one loopful with an inoculating loop and streaking it onto fresh Zobell Marine Agar medium using the streak plate method. The plate was then incubated for 24 hours.

Preparation of Test Plastics

Samples of PS, PE, and PP plastics were cut into uniform 1 cm × 1 cm pieces and weighed to obtain their initial weights. The plastics were washed with distilled water, sterilized with 90% alcohol, and dried before use.

Microplastic Degradation Test

The biodegradation test was conducted using the gravimetric method, as described by Mardalisa et al.[6]. The plastic samples were placed in glass bottles containing 50 mL of Tryptic Soy Broth (TSB) medium, inoculated with 2 loops of the bacterial isolate, and incubated in a water bath at room temperature for 60 days. After incubation, the plastics were washed with sterile distilled water, sprayed with 70% alcohol, dried, and weighed to obtain the final weight. The degradation percentage was calculated using the following formula:

$$\%\ \text{degradation} = 1 - \frac{final\ weight}{initial\ weight} \times 100\%$$

Subsequently, inoculation was carried out on Plate Count Agar medium by taking 0.1 mL of the diluted sample and placing it into a petri dish containing solid Plate Count Agar medium, then spreading it using an L-shaped spreader. The plates were incubated upside down for 24–48 hours at 37°C. After 24–48 hours, bacterial enumeration was performed using the following formula[6]:

$$\text{number of bacteria}\left(\frac{cfu}{mL}\right) = \text{number of colonies} \times \frac{0{,}1}{Dilution\ Factor}$$

The plate-based plastic test was adapted from Azizah[4]. Selected bacterial isolates were inoculated into 9 mL of TSB medium (1–2 tubes) and incubated in a shaker incubator for ±48 hours at 30°C. A volume of 0.1 mL of the culture was then spread aseptically onto Zobell Marine Agar medium using an L-shaped spreader, after which the surface was covered with a piece of plastic, and the plate was incubated for 60 days.

Gram Staining

Bacterial isolates were taken using an inoculating loop. In the first step, the isolate was stained with crystal violet for 1 minute,

then rinsed with running water and air-dried. It was then stained with iodine solution for 1 minute, rinsed with running water, and air-dried again. In the third step, 95% alcohol was applied for 30 seconds, then rinsed with water and air-dried. In the fourth step, safranin was applied for 30 seconds and rinsed with running water, dried with absorbent paper, and air-dried before being observed under a microscope at 1000× magnification[7]. Gram-positive bacteria appear purple, whereas Gram-negative bacteria appear pink or reddish.

Microstructure Analysis

Microplastic samples that had been incubated with *Bacillus* sp. for 60 days were first dried, then coated with a thin layer of conductive metal (Au coating). The coated samples were placed on an SEM stub using carbon tape to keep the sample position stable during observation. The samples were then placed in the SEM vacuum chamber and observed using an electron beam emitted from the electron gun. SEM operates based on the principle of electron beam backscattering from the sample surface, which is then captured as a high-resolution image[8]. Images of the microplastic surface morphology were taken at several magnification levels and analyzed by comparing the surface conditions of the microplastics before and after bacterial treatment, as well as with the untreated control.

Data Analysis

Data were analyzed using the Shapiro-Wilk normality test. Since the data were not normally distributed, the non-parametric Kruskal-Wallis test was used. The results of the SEM analysis are described in detail based on changes in the morphology of the microplastic surfaces

3. RESULT AND DISCUSSION

Bacterial Culture Rejuvenation

Rejuvenation of the *Bacillus* sp. culture on Zobell Marine Agar produced well-grown colonies after 24 hours of incubation. The rejuvenation of the *Bacillus* sp. bacterial culture was performed as a preliminary step before the microplastic degradation test. The purpose of this rejuvenation was to ensure that the bacteria were in an active state so that their ability to degrade microplastics could be assessed, as well as to determine the degradation potential achieved during the testing period.

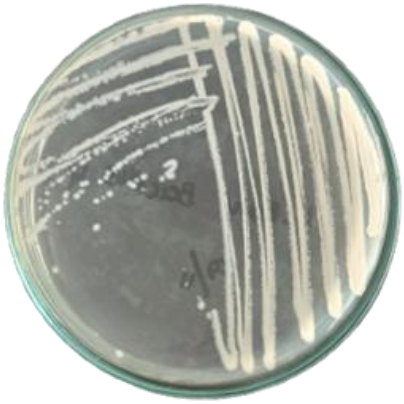


Figure 1. Pure culture of *Bacillus* sp.

Microplastic Degradation by Bacteria

The gravimetric test results showed a decrease in mass for all three types of plastic after 60 days of incubation, as presented in Table 1.

The highest average degradation percentage was obtained for PE (4.09%), followed by PS (1.86%) and PP (1.19%). The control plastics showed no decrease in mass (0%). The Kruskal-Wallis test produced an Asymp. Sig. Sig. value of 0.361 ($p>0.05$), indicating no significant difference among the treatments.

The higher degradation of PE compared to PS and PP is related to its chemical structure. PE has a linear carbon chain $(C_2H_4)n$. A previous study by Uwakwe et al.[9] showed that *Bacillus pumilus, B. siamensis, B. coagulans,* and *B. subtilis* degraded PE over a 60-day incubation period, with Bacillus siamensis exhibiting the highest degradation rate at 52%. These bacteria can degrade PE and utilize it as a carbon source. PS exhibited lower degradation due to the presence of the aromatic phenyl ring in its structure, which is rigid and thermodynamically stable, making it more resistant to enzymatic

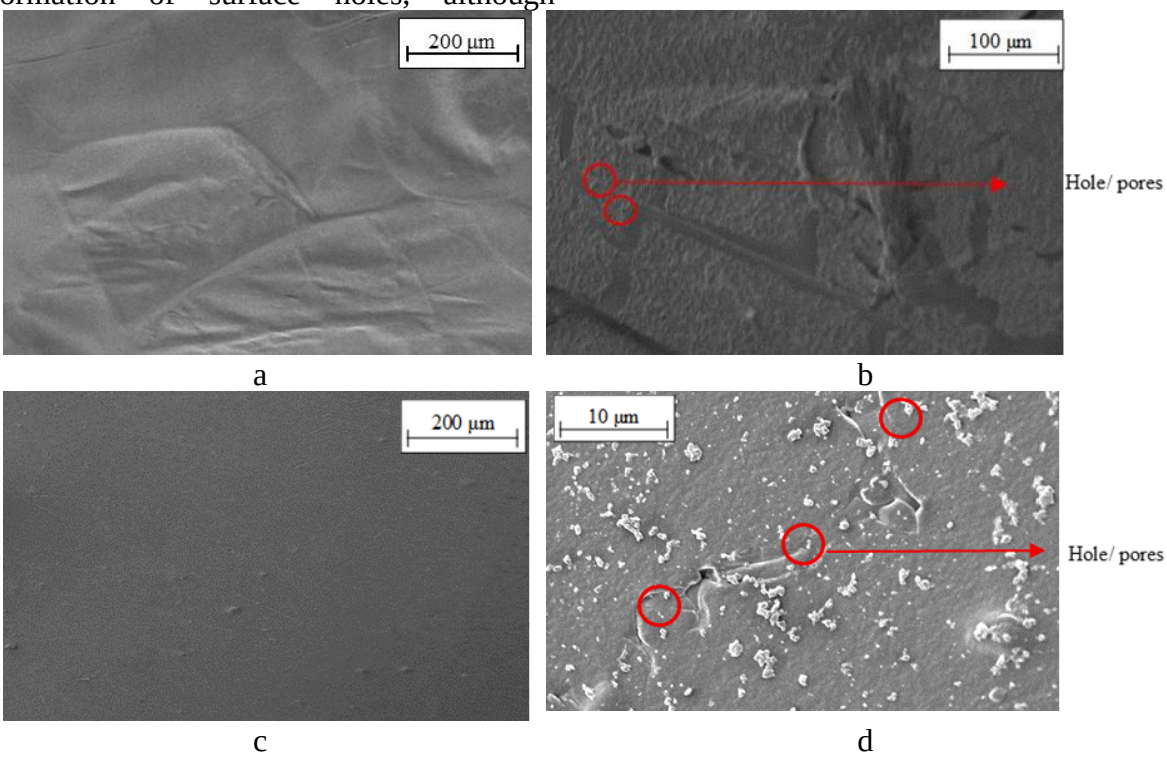
attack[10]. PP showed the lowest degradation because the additional methyl group (-CH_3) has the same chemical groups as PE polymers. However, it has additional methyl groups, making PP polymers stronger than PE polymers[4].

Table 1. Percentage of microplastic degradation by *Bacillus* sp

Plastics Type	Replication	Initial weight (g)	Final Weight (g)	Weight Reduction (g)	% Degradation	Avarage (%)
PS	1	0.0125	0.0118	0.0007	5.60%	1.86%
	2	0.0114	0.0114	-	-	
	3	0.0126	0.0126	-	-	
PE	1	0.0042	0.0041	0.0001	2.39%	4,09%
	2	0.0042	0.0040	0.0002	4.77%	
	3	0.0039	0.0037	0.0002	5.13%	
PP	1	0.0028	0.0027	0.0001	3.58%	1,19%
	2	0.0027	0.0027	-	-	
	3	0.0026	0.0026	-	-	
Control	1	0.0126	0.0126	-	-	0%
	2	0.0035	0.0035	-	-	
	3	0.0032	0.0032	-	-	

SEM Analysis

SEM results showed clear differences in surface morphology between the bacteria-treated samples and the controls. Slightly noticeable changes were observed in the PE plastic, appearing as evenly distributed holes and pores across the surface, accompanied by bacterial colonies forming a biofilm. The PS plastic exhibited cracking and the formation of surface holes, although degradation was limited. The PP plastic showed the least amount of change, with only initial signs of degradation, namely, cracks or uneven surface erosion forming. The control plastic sample appears to have an intact surface with no signs of bacterial degradation. The control plastic sample appears to have an intact surface with no signs of bacterial degradation.



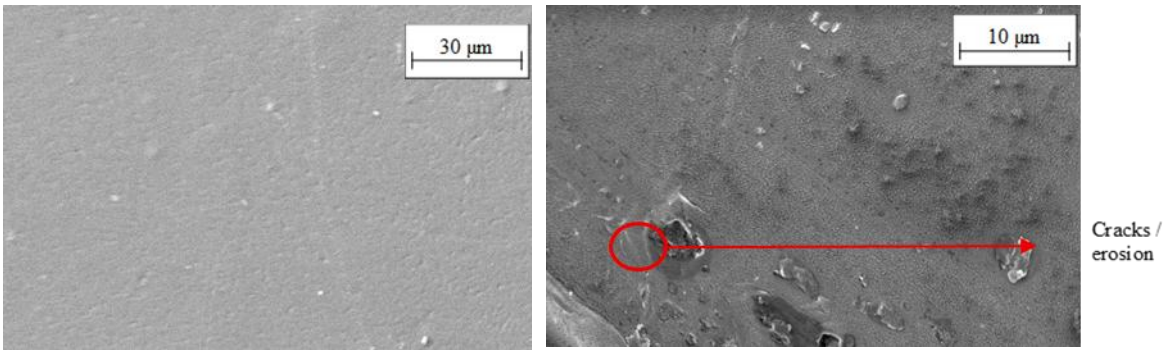


Figure 2. Scanning Electron Microscopy (SEM) Results. (a) The PS control plastic shows an intact surface; (b) the PS plastic shows the presence of holes or pores; (c) the PE control plastic shows an intact surface; (d) the PE plastic shows numerous holes or pores as well as the presence of bacteria; (e) the PP control plastic shows an intact surface; (f) the PP plastic shows cracks and erosion beginning to form

Based on the results of the SEM analysis, the PE polymer exhibited a higher degree of surface damage, characterized by the formation of cracks and small holes accompanied by the presence of biofilm-forming bacterial colonies in the surrounding area. PS polymers also underwent surface changes, but their pores were relatively smaller than those of PE. Meanwhile, PP polymers exhibited the least morphological changes. Surface changes resulting from enzymatic activity led to the formation of simpler compounds. These findings are consistent with those of Jeyavani et al.[11]; Hossain et al.[12], who stated that the degradation of microplastics by bacteria is characterized by the formation of cracks, holes, and increased roughness, as well as the formation of biofilms that play a role in enhancing contact between enzymes and the plastic substrate.

In addition to changes in surface morphology, the results of SEM analysis of the treated samples also revealed the presence of bacterial colonies, specifically bacterial cells adhering directly to the surface of the microplastics. It was observed that the bacteria adhering to the plastic surface were *Bacillus* sp. A Gram stain test was also conducted, which revealed that the bacterial culture exhibited morphological characteristics similar to those of the genus Bacillus, namely, it was Gram-positive, as indicated by a purple-stained cell wall following the staining process, suggesting that the isolate possessed the appropriate characteristics and exhibited a rod-shaped (bacillus) morphology, as shown in Figure 3.

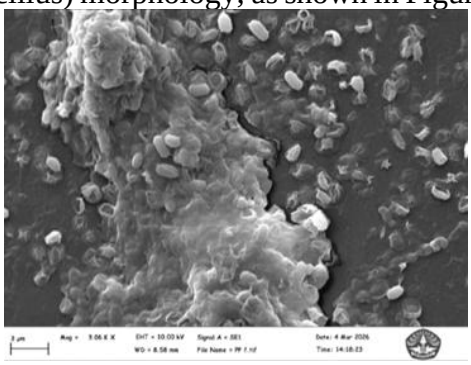


Figure 3. SEM image of bacterial morphology

Bacterial Count

The results of the Total Plate Count (TPC) after 60 days of incubation are presented in Table 2.

The highest average bacterial count was found in the PE treatment (20.8×10^{10} CFU/mL), followed by PS (18.8×10^{10} CFU/mL) and PP (13.5×10^{10} CFU/mL). Bacterial growth was consistent with the degradation percentages obtained, indicating that PE was more effective in supporting the growth and metabolic activity of *Bacillus* sp. as a substrate.

The uniformity of the initial inoculum, using a McFarland turbidity standard of 0.5 (equivalent to $\pm 1.5 \times 10^8$ CFU/mL), ensured that the differences in bacterial counts after 60 days reflect the bacteria's ability to adapt to each type of polymer

Table 2. Total Plate Count (TPC) *Bacillus* sp. at day 60

Plastics type	Replication	CFU/ mL	Average bacterial count (CFU/ mL)
PS	1	29.9×10^{10}	
	2	6.0×10^{10}	18.8×10^{10}
	3	20.6×10^{10}	
PE	1	11.3×10^{10}	
	2	23.3×10^{10}	20.8×10^{10}
	3	27.8×10^{10}	
PP	1	18.4×10^{10}	
	2	8.3×10^{10}	13.5×10^{10}
	3	13.7×10^{10}	

4. CONCLUSION

Bacillus sp. bacteria isolated from the Rokan River estuary waters have the potential to degrade PE, PS, and PP microplastics. The highest average degradation percentage was obtained for PE (4.09%), followed by PS (1.86%) and PP (1.19%). The Kruskal– Wallis test results ($p = 0.361$; $p > 0.05$) showed no significant difference among the plastic types. SEM observations confirmed morphological changes on the surfaces of the treated plastics, including cracks, pores, and holes, with PE showing the greatest changes. This study concludes that Bacillus sp. has potential as a locally sourced agent for microplastic biodegradation. However, further optimization of environmental conditions and incubation time is needed to improve degradation efficiency.

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