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Bioprospecting Evidence of Polyethylene Degrading Bacteria in the Mojo Pemalang Mangrove Rehabilitation Areas

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Abstract: Polyethylene is one of the most widely used microplastic polymers and causes environmental pollution. PE microplastic has durability and low biodegradability, which accumulates in water and sediment, which can inhibit mangrove growth. Damage to the mangrove ecosystem will impact the availability of carbon stocks, sediment stability, nutrient cycles, and shelter. Polyethylene-degrading bacteria are a solution developed to deal with plastic pollution. This research aims to investigate the polyethylene biodegradation potential of bacterial strains from the mangrove rehabilitation area in Pemalang. Sediment and water samples were taken at 15 locations within the rehabilitation area. The bacteria isolate was incubated for 20 days in a test bottle containing a polyethylene pellet and 100 ml of Bushnell Haas media. Degradation was assessed by measuring the decrease in residual mass and the degradation rate of the polyethylene polymer. Isolates A6, A10, SD5, and SD6 were found to degrade the polyethylene pellets with ranges of 1.52% to 5.02% and the removal constants up to 0.08% – 0.26% per day with the half-life of isolates needed around 277.7 – 1115.2 days. The formation of carbonyl groups and cracks in PE can be seen from FTIR and SEM analysis as parameters for degradation. Further research is needed to analyze the enzymatic reactions involved in microplastic degradation.

Keywords: polyethylene; mangrove; bacteria; biodegradation.

1. Introduction

Pemalang Regency is one of the regions in Central Java Province, which is located between 109°17'30“- 109°40'30” East and 6°52'30” - 7°20'11” LS with an area of 1,115.3 km2. Pemalang is known as an anthropogenic area that produces textiles, the batik industry, agriculture, tourism, capture fisheries, and aquaculture. The Environmental Agency states that the population of Pemalang Regency is 1.4 million people; each person produces 0.4 kg of plastic waste per day, and 80% of the waste enters the waters. Based on the geographical location, the length of the coastline of Pemalang Regency reaches +35 km with a sea area of 259.28 km^2 [1]. Pemalang has 813.8 ha of mangrove ecosystem area, and 40.18% (327 ha) is

located in Mojo Village, Ulujami District. The activities of residents in Mojo Village produce organic matter waste and microplastics that affect mangrove growth [2]. The waste settles on mangrove root sediments and is suspended in the sea, causing environmental pollution.

Plastic pollution causes a negative impact on ecological problems, especially in plant ecosystems and marine biota, which are transferred to the human body. Most plastic is found in marine environments and is fragmented into micro-sized particles that are unidentified by general methods. Microplastics are small particles that have a size of around 0.1µm – 5 mm [3]. As much as 92.4% of microplastic contamination from polymer types of polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride, nylon, polylactic acid, polyamide, and polyethylene terephthalate (PET) has been spread in the aquatic environment, sediments, rivers, seas, and terrestrial biota whose numbers are increasing every time [4,5]. About 54.5% of microplastics floating in the ocean are polyethylene [6]. Polyethylene plays an important role in the production of food packaging, thus dominating the amount of contamination in the waters. The abundance of microplastics in several regions of Indonesia reached 0.38 - 0.61 N/L on the north coast of the Surabaya Sea, 1.78 n/m^3 in Tallo River, 1.83 n/m^3 in Jenebarang River, 99.1 particles/L in Pekalongan waters, 9,729 - 89,164 n/m^3 in Jakarta Bay and 19.1 x 10^2 particles/m^3 which are divided into fiber, foam, fragments, and granules [7–11].

The additives in microplastics absorb heavy metals (Cd, Cr, and Cu) and persistent organic pollutants (polychlorinated biphenyls, pesticides, and polycyclic aromatic hydrocarbons), causing water and soil pollution [12–14]. Microplastic pollution in the aquatic environment will have an impact on the habitat of biota such as fish, shellfish, shrimp, and other marine biota that have the potential to be consumed by humans. The accumulation of microplastics in biota causes oxidative stress, impaired growth, decreased immune response, and reproductive complications [15,16]. Bagheri *et al*., [17] stated that in the gastrointestinal organs of *N. melanostomus* and *C. lamarcki*, microplastics were found as much as 39 MP/g and 19.8 MP/g with sizes of 1 - 5 mm. In addition, [18] reported that microplastics accumulated in human body tissues can cause decreased immunity, gut dysbiosis, impaired kidney function, and cancer. A total of 1 - 36 particles/g with a size of 20 - 800 µm were found in human feces [19].

Microplastic degradation in mangrove ecosystems can be carried out by bacteria by forming biofilms and secreting enzymes to damage plastic polymers [20]. Mangroves, as a carbon source, have the potential to be a place for superior bacterial growth and are able to adapt to the conditions of microplastic contamination in the area. [21] found that *B. siamensis* is able to degrade LDPE with a percentage of 8.46% within 90 days of incubation. Bacteria produce the extracellular enzyme to degrade PE by oxidation of the carbon backbone that increases carbonyl (C-O) and carbon-carbon (C-C) double bonds [22]. The ability of bacteria to degrade microplastics is based on the chemical structure of the polymer, molecular weight, plasticizers, and additives used in plastic manufacturing [23]. Therefore, this study was conducted to measure the effectiveness and characterize microplastic-degrading bacteria from the mangrove rehabilitation area, Mojo Pemalang, and extracellular enzymes that play a role in the degradation process of each microplastic polymer through FTIR analysis, SEM, and UV-Vis spectrophotometry.

2. Materials and Methods

2.1. Sampling.

Samples were taken as much as 1 L of water and 100 g of sediment at 15 points of the mangrove rehabilitation area of Mojo District, Pemalang Regency, at coordinates 109°30'0.11" - 109°30'53.50" east and 6°47'18.23" - 6°45'41.95" LS. Samples were put into bottles and stored at -4°C. The coordinates of sampling locations are presented in Table 1.

Table 1. The coordinates of sampling locations.

Stasiun	LONG	LAT	Consideration
1	109°30'0.11"E	6°47'18.23"S	Coastline in the western region
2	109°30'17.42"E	6°46'53.99"S	
3	109°30'38.27"E	6°46'36.35"S	
4	109°31'0.21"E	6°46'21.31"S	
5	109°31'26.01"E	6°46'11.80"S	Coastline in the eastern region
6	109°31'57.53"E	6°46'15.48"S	
7	109°32'18.39"E	6°46'42.33"S	
8	109°32'24.21"E	6°47'6.11"S	
9	109°30'48.47"E	6°47'13.17"S	Lagoon
10	109°31'3.77"E	6°47'0.74"S	
11	109°31'26.63"E	6°46'56.14"S	River and estuary
12	109°31'19.83"E	6°47'40.29"S	
13	109°31'38.83"E	6°47'57.17"S	
14	109°31'24.93"E	6°48'37.98"S	
15	109°30'53.50"E	6°45'41.95"S	

2.2. Screening for microplastic degrading bacteria.

A total of 100 µL of the sample was inoculated on Bushnell Haas (BH) medium in 1 liter of distilled water to which 2% polyethylene glycol (PEG) was added). The isolate was incubated for 7 days at room temperature [24,25].

2.3. Morphological characterization of microplastic degrading bacteria.

Bacteria that grew on Bushnell Haas media with the highest mass reduction of polyethylene granule residue were morphologically characterized, including gram staining, identification of colony shape, elevation, margin, and bacterial cells.

2.4. Microplastic degradation test by bacteria.

Bacteria that have been isolated and identified as being able to degrade microplastics are cultivated on nutrient broth (NB) media. Bacteria were incubated using a rotary shaker at 29°C with a speed of 120 rpm until they reached the log phase (wavelength of 600 nm). 1 ml of the log phase culture was inoculated into an Erlenmeyer containing 100 mL of Bushnell Haas (BH) medium with polyethylene granules added. Cell density was measured every 4 days during the 20-day incubation period. Tests were conducted with 3 repetitions [3].

2.5. Measurement of microplastic residue mass reduction and reduction rate.

Microplastic residues from Bushnell Haas media were filtered and washed using 70% ethanol. The initial mass of microplastic particles and the mass after incubation were measured using an analytical balance with an accuracy of 0.0001 g [26]. The percentage reduction in microplastic mass is measured using the equation:

$$(\%)\ \text{Weight loss} = \frac{(W0-W)}{W0} \times 100 \qquad (1)$$

Where W0 is the initial weight of polypropylene (g), and W is the final weight of polymer after treatment (g)

This data is processed to determine the microplastic reduction rate constant using a kinetic model based on initial and final mass in a certain interval (4 days) [27].

$$K = 1/2\left(\ln\frac{W}{W0}\right) \qquad (2)$$

Where K is where K is the first-order rate constant for polypropylene uptake per day, t is time in days, W is the weight of final polypropylene (g), and W0 is the initial weight of polypropylene (g)

The microplastic mass reduction rate constant, half-life (t1/2), is calculated based on the equation:

$$\left(t\frac{1}{2}\right) = \ln\frac{2}{K} \qquad (3)$$

Where t1/2 is the half-life, and K is the first-order rate constant for polypropylene uptake per day.

2.6. Fourier transform infrared (FTIR) analysis of polyethylene polymers.

Changes in microplastic polymer structure after degradation by bacteria were analyzed using FTIR spectroscopy with frequencies ranging from 4000-400 cm^{-1}. Analysis was carried out on microplastics that had been incubated with bacteria and on control samples that were not inoculated [28].

2.7. Scanning electron microscopy (SEM) analysis of polyethylene.

The morphology of the degraded microplastics after testing for 40 days was analyzed using SEM. Samples were coated with a gold layer at 25 mA under Argon (Ar) atmosphere at 0.3 MPa and visualized using SEM at 3.500 times magnification [26].

3. Results and Discussion

3.1. Morphology and growth analysis of bacteria.

Isolation of microplastic-degrading bacteria was carried out by adding 2% PEG to Bushnell-Haas Agar media. PEG is a plastic monomer that can be melted in the media when autoclaved. Bacterial isolates that grow on the media will use PEG as a carbon source for growth and metabolism. Bacterial isolates that grow are A6, A10, SD5, and SD6, with different morphological characteristics. Variations in colony and cell morphology are presented in Table 2.

Table 2. Bacteria isolation result.

Isolate	Shape	Colony morphology			Color	Cell morphology	
		Elevation	Margin	Texture		Shape	Gram
A6	Circular	Convex	Entire	Smooth	Yellow	Coccus	Positive
A10	Irregular	Flat	Undulate (wavy)	Smooth	White	Coccobacilli	Negative
SD5	Circular	Convex	Undulate (wavy)	Smooth	White	Bacil	Positive
SD6	Circular	Convex	Entire	Smooth	White	Coccobacilli	Negative

Based on Table 2, circular colonies with convex elevation and irregular colonies with flat elevation. In isolates, A6 and SD6 have a yellow and white margin, while A10 and SD5 have wavy margins with white colonies. The four isolates have a fine colony texture. Cell

morphology consists of two parameters, namely cell shape and Gram staining results. A6 and SD5 are gram-positive bacteria in the form of cocci and bacilli.

Meanwhile, A10 and SD6 are gram-negative bacteria in the form of coccobacilli. Gram-positive bacteria retain crystal violet staining because of their thick peptidoglycan layer, appearing purple, while gram-negative bacteria experience color loss and appear red in the presence of counterstain [29]. Gram-negative bacteria have a thin peptidoglycan layer and an additional outer membrane containing lipopolysaccharides, while Gram-positive bacteria have a thick peptidoglycan wall surrounding a single membrane [30].

Bacterial growth in the biodegradation test was used Bushnell-Haas media with the addition of PE pellets for 20 days of incubation. Measurement of bacterial growth was carried out using a spectrophotometer every 4 days. The growth measurement results of bacterial isolates A6, A10, SD5, and SD6 are presented in Figure 1.

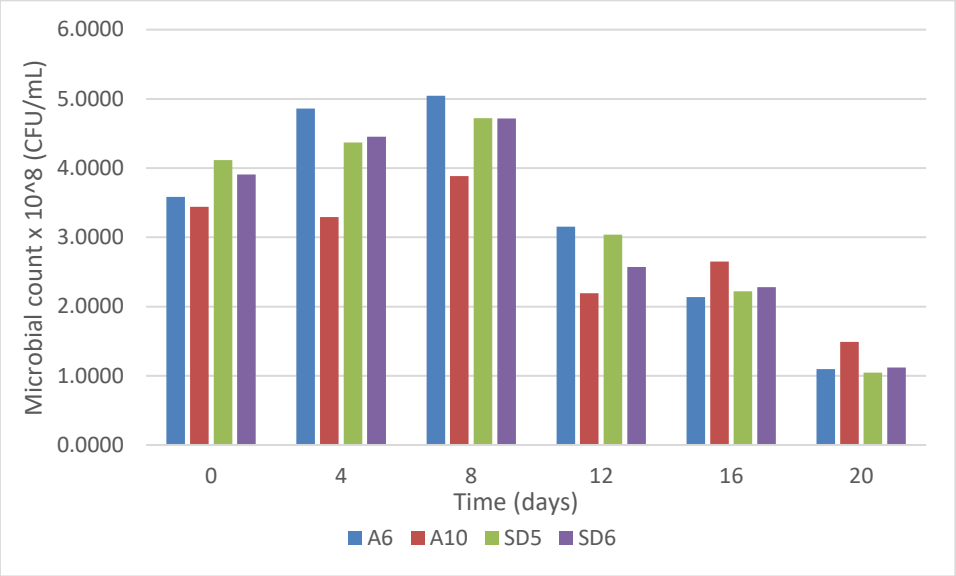
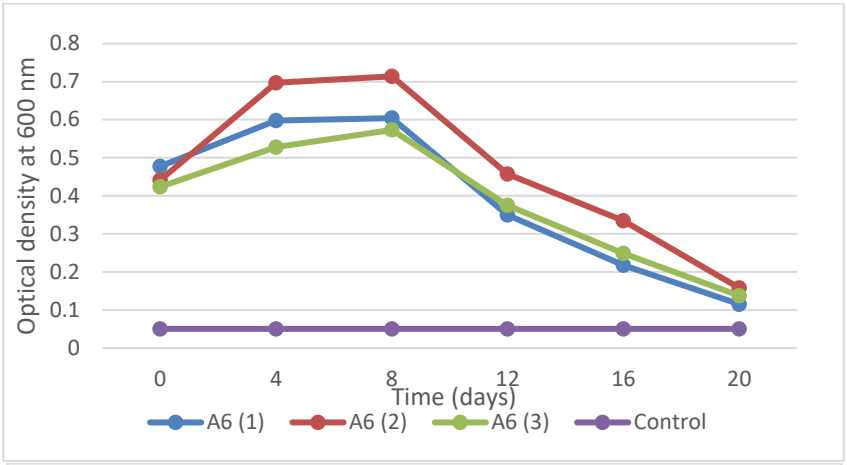
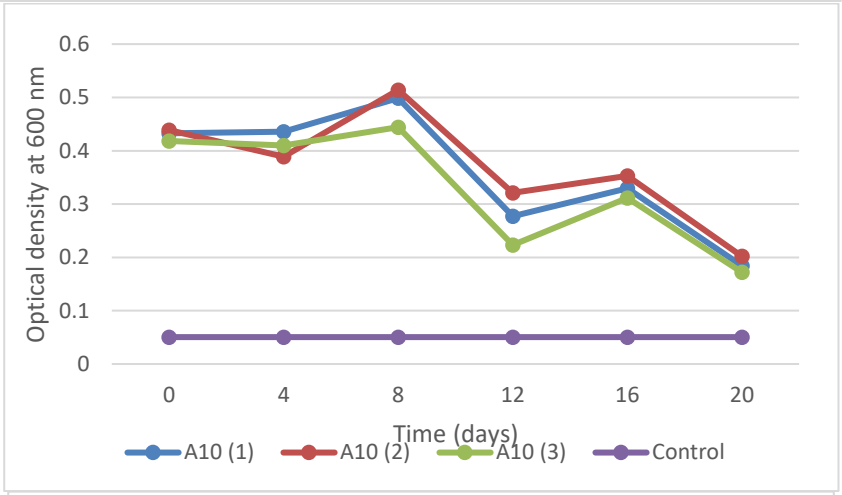


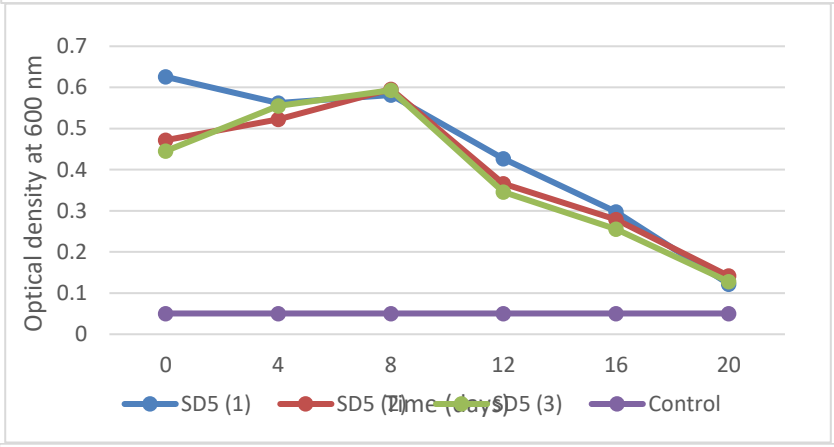
Figure 1. Counts of bacterial isolates in Bushnell Haas media with PE pellet during 20 days of biodegradation.

The variation in the number of bacteria based on Figure 2 shows that the highest bacterial growth was isolated A6 on day 8, which reached 5.04 x 10^8 CFU/mL, and on day 20, at 1.09 x 10^8 CFU/mL. On day 0, the bacteria were in the adaptation phase to go to the exponential phase until day 4. On day 8, the bacteria experienced a stationary phase and, after that, entered the death phase. Bacterial growth follows different phases: lag, exponential, stationary, and death [31,32]. During these phases, bacteria exhibit different metabolic conditions and growth rates. The exponential phase is characterized by rapid cell multiplication, while growth is suppressed in the stationary and death phases [33]. Bacteria require a carbon source for growth and metabolism. Polyethylene is added to Bushnell-Haas media to serve as a carbon source for bacterial growth. The ability of bacteria to form biofilms is based on colony formation as well as the secretion of extracellular enzymes to reduce polymer bonds in polyethylene functional groups. The polyethylene monomer formed from the enzymatic process will be absorbed through the semipermeable membrane and assimilated inside the bacterial cell to undergo several metabolic reactions, such as β-oxidation and the tricarboxylic acid cycle [34].

Microplastic degradation testing was carried out for 20 days of incubation, and it showed variations in bacterial growth. Each isolate has a different growth phase time.







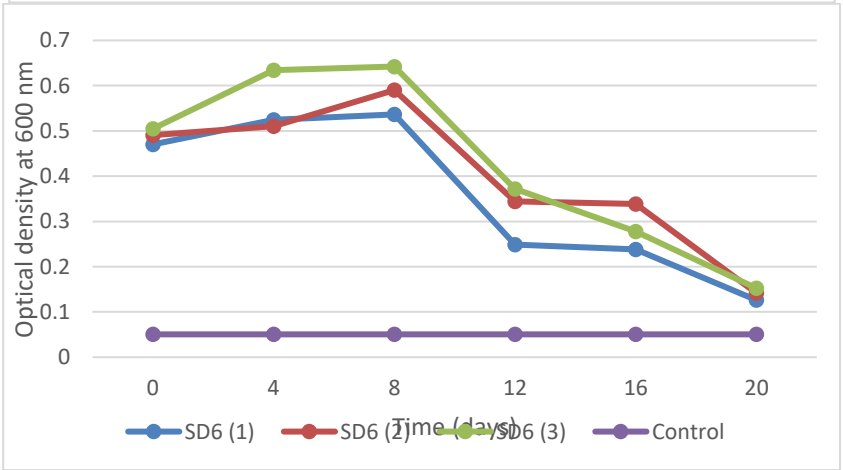


Figure 2. Growth curve of bacteria isolates during biodegradation assay in Bushnell Haas media with PE pellet.

The growth curves of isolates A6, A10, SD5, and SD6 with the addition of PE are presented in Figure 2.

Growth curves were obtained from optical density measurements using a UV-Vis spectrophotometer at a wavelength of 600 nm. A total of 10 mL of bacterial isolates was inoculated into 100 mL of Bushnell-Haas media with different initial optical densities. The highest initial optical density was isolated SD5 with 0.6257 for bacteria first inoculated on selective media. After 20 days, all bacterial isolates experienced a decrease in growth due to reduced nutrients, such as minerals and carbon sources, in the media. The adaptation phase and the rapid growth phase, namely the lag and exponential phases, began on day 0 to day 4, which were characterized by continuous cell division. The stationary phase is shown from day 4 to day 8, which means the bacterial growth rate is equal to the death rate, so the number of bacteria remains constant. The last phase is when the death rate is higher than the growth rate, which is the death phase. Several factors influence the growth of bacteria in various environments. *S. marcescens* and *B. albus* experienced increased growth during incubation. PE as a carbon source is used to the maximum by *S. marcescens* and sporulated by *B. albus* [35]. Intrinsic factors include nutrient content, pH level, water activity, and antimicrobial components, while extrinsic factors include temperature, relative humidity, and gas environment [36]. The lack of a carbon source is the main factor in isolates that affects the length of the bacterial growth phase. Bushnell Haas media only contains minerals and carbon sources in the form of PP granules. Bacteria must degrade the polymer into monomers, absorbed across the semipermeable membrane, and assimilated within the bacterial cell [34].

3.2. Polyethylene mass reduction by bacteria.

Measurement of polyethylene degradation by bacteria after incubation for 20 days showed varying results. The mass loss of polyethylene was measured using an analytical balance with an accuracy of d = 0.0001 gram. This value affects the reduction rate constant and degradation half-life. The percentage, constant, and half-life of polyethylene reduction are presented in Table 3.

Table 3. Percentage, constant, and half-life of mass reduction polyethylene.

Bacteria isolate	Mass reduction of polyethylene (%)	Removal constant (K) day^{-1}	Half-life (days)	F-value	Sig.
Control	0	0	∞		
A6	5.02	0.0026	277.7	2.173	.169
A10	4.48	0.0023	373.78		
SD5	4.78	0.0025	461.23		
SD6	1.52	0.0008	1115.2		

Biodegradation of polyethylene by bacteria resulted in a mass decrease of up to 5.02% for 20 days by isolating A6. [25] reported that *Pseudomonas marincola* from Muara Angke Bay, Jakarta has the ability to degrade polyethylene and polyethylene terephthalate by 4.5% and 6.5% after 14 days of incubation. *Serratia marcescens* from mangrove sediment in Southern China degrade PE up to 6.59% [35]. This decrease in polyethylene mass is due to the interaction of bacteria with microplastic surfaces through the mechanisms of biodeterioration, biofragmentation, assimilation, and mineralization [37,38]. Bacteria with the ability to degrade microplastics will attach to plastic surfaces to form biofilms [39]. Extracellular enzymes secreted by biofilm-forming bacteria will break polymer bonds into simpler structures, namely oligomers, dimers, and monomers [40]. The fragmentation results will be absorbed through the

semipermeable membrane and assimilated in the bacterial cell to undergo several metabolic reactions, such as β-oxidation and the tricarboxylic acid cycle [34]. The citric acid cycle or Krebs cycle, occurs in the bacterial cytoplasm by converting acetyl-CoA from the carbonyl and hydroxyl groups of microplastic monomers into energy in the form of ATP or GTP, NADH, and $FADH_2$. Furthermore, microplastics will be mineralized into the final products CO_2, H_2O, or CH_4 [41,42]. The ability of bacteria to form biofilms is based on the formation of colonies as well as the secretion of extracellular enzymes to reduce polymer bonds by dehydrogenation and C-C bond breaking in the functional groups of microplastics [43]. The extracellular enzymes produced include lipase, esterase, laccase, amidase, cutinase, hydrolase, and carboxylesterase [20, 44, 45] reported that the cell surfaces of *P. aeruginosa* and *S. haemolyticus* are hydrophobic, which indicates the presence of low acid or electron acceptor surfaces on the cell surface. The increase in hydrophobicity in bacterial species occurs due to hydrocarbons and biosurfacts on the surface of microplastics that transform the surface of hydrophobic polymers into hydrophilic. The high hydrophobicity of bacterial cells enhances the decomposition of PP, nylon, PE, and PS polymers [46]. In addition, bacteria will increase the hydrophilicity of the PE surface, making it more easily degraded [16].

Removal constant and half-life showed the ability of bacteria to degrade polyethylene in units of time (hours or days). Constant value became a parameter of the kinetic reaction to describe the degradation process of PE in a specific environment. A6 isolates have a removal contents value of up to 0.0026 $days^{-1}$, which means PE pellets are degraded at a rate of about 0.2% per day of the total mass of PE. A small constant value indicates slow degradation, so it takes a long time to break down. The half-life shows isolate A6 takes about 277.7 days for the PE granule to degrade by half. [21] identified *Bacillus siamensis* as capable of degrading 8.46% of LDPE within 90 days. Similarly, [47] reported LDPE degradation by *Bacillus* sp. strains BP4 and BP6, with degradation percentages of 7.23% and 8.19%, respectively, after 30 days. *P. saccharolyticum* strain ZRY and *B. cereus* strain ZRY from the mangrove ecosystem could degrade PE up to 0.6% and 1.0% for 60 days [48]. In addition, the reduction in PE mass by *Staphylococcus arletae* was 9.09% in 30 days [49]. A comparison of these data shows that isolated A6 degrades PE at a higher rate (Table 4).

Table 4. Microorganisms that can degrade microplastics.

Bacteria	Microplastic	Incubation period (d)	Mass reduction (%)	Location	References
Pseudomonas marincola	PE & PET	14	4.5 and 6.5	Muara Angke Bay Jakarta	[25]
Bacillus siamensis	LDPE	90	8.46	Waste disposal sites in Peshawar, KP, Pakistan	[21]
Bacillus wiedmannii	LDPE	90	5.39	Waste disposal sites in Peshawar, KP, Pakistan	[21]
Pseudomonas aeruginosa	LDPE	90	1.15	Waste disposal sites in Peshawar, KP, Pakistan	[21]
Bacillus sp. strains BP4	LDPE	30	7.23	Dump soil of Pekanbaru, Indonesia	[47]
Bacillus sp. strains BP6	LDPE	30	8.19	Dump soil of Pekanbaru, Indonesia	[47]
Pseudochrobactrum saccharolyticum strain ZRY	PE	60	0.6	Mangrove forest on Seagull Island, near Guangzhou City, China	[48]
Bacillus cereus strain ZRY	PE	60	1.0	Mangrove forest on Seagull Island, near Guangzhou City, China	[48]
Staphylococcus arletae	PE	30	9.09	Garden soil	[49]

Bacteria	Microplastic	Incubation period (d)	Mass reduction (%)	Location	References
Bacillus gottheilii	PE, PET, PP, PS	40	6.2; 3.0; 3.6; 5.8	Mangrove ecosystems in Peninsular Malaysia	[28]
Bacillus cereus	PE, PET, PS	40	1.6; 6.6; 7.4	Mangrove ecosystems in Peninsular Malaysia	[28]
Bacillus sp. YP1	PE	60	10.7	Waxworms gut	[50]
Enterobacter asburiae YT1	PE	60	6.1	Waxworms gut	[50]
Bacillus pumilus M27	PE	30	1.5	Pelagic waters of the Arabian Sea coast, India	[24]
Kocuaria palustris M16,	PE	30	1.0%	Pelagic waters of the Arabian Sea coast, India	[24]
B.acillus subtilis H1584	PE	30	1.75%	Pelagic waters of the Arabian Sea coast, India	[24]

Each bacterial species has different effectiveness in degrading microplastics. Extracellular enzymes produced by bacteria, such as lipase, protease, esterase, and laccase, have different polymer targets. *Bacillus siamensis* and *Bacillus wiedmannii* degrade LDPE by producing laccase and alkane hydrolase enzymes to hydrolyze and oxidize the polymer into monomers [21]. Bacteria can degrade microplastics into CO_2 and H_2O, but this process is slow, and only a few microorganisms can degrade polymers [51].

The significance value obtained is 0.169 or smaller compared to $\alpha = 0.05$. This shows that there are significant differences between the four bacterial isolates, namely A6, A10, SD5, and SD6, in polyethylene degradation. In contrast to research conducted by [28], *B. cereus* ($F = 1.927$; $p = 0.226$) showed no significant variation in statistical analysis results with a percentage reduction in microplastic mass of PS (7.4%) > PET (6.6%) > PE (1.6%). To determine differences in specific polyethylene degradation of bacterial isolates, a post hoc LSD test was carried out. The results of the post hoc LSD polyethylene degradation test are presented in Table 5.

Table 5. The LSD post hoc test of polyethylene degradation by bacteria.

	(I) Isolate	(J) isolat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
LSD	Isolate A6	Isolate A10	.54000	1.56966	.740	-3.0796	4.1596
		Isolate SD5	.23333	1.56966	.886	-3.3863	3.8530
		Isolate SD6	3.50000	1.56966	.056	-.1196	7.1196
	Isolate A10	Isolate A6	-.54000	1.56966	.740	-4.1596	3.0796
		Isolate SD5	-.30667	1.56966	.850	-3.9263	3.3130
		Isolate SD6	2.96000	1.56966	.096	-.6596	6.5796
	Isolate SD5	Isolate A6	-.23333	1.56966	.886	-3.8530	3.3863
		Isolate A10	.30667	1.56966	.850	-3.3130	3.9263
		Isolate SD6	3.26667	1.56966	.071	-.3530	6.8863
	Isolate SD6	Isolate A6	-3.50000	1.56966	.056	-7.1196	.1196
		Isolate A10	-2.96000	1.56966	.096	-6.5796	.6596
		Isolate SD5	-3.26667	1.56966	.071	-6.8863	.3530

* The mean difference is significant at the 0.05 level.

The LSD post hoc test showed that each bacterial isolate tested did not have a significant difference from other isolates. In detail, isolates A6 against A10 (p – value = 0.74), A6 against SD5 (p – value = 0.886) and A6 against SD6 (p – value = 0.056), A10 against SD5 (p – value = 0.85), A10 against SD6 (p – value = 0.096), and SD5 against SD6 (p – value = 0.071) did not have significant differences because the p-value > 0.05. Differences in the effectiveness of microplastic biodegradation are influenced by the polymer composition. [23] stated that several factors that distinguish the effectiveness of biodegradation are particle shape, exposure time, functional groups, surface charge, and the type of polymer. The ability of these bacteria is based on the level of tolerance and adaptability to use microplastic polymers as a

carbon source. Each bacteria has different interactions when attaching to the polymer surface and secreting types of extracellular enzymes.

3.3. Biodegradation analysis.

Fourier transform infrared spectroscopy (FTIR) is a widely used technique to identify and quantify microplastics in environmental samples. The structure of microplastics that are naturally degraded in nature and the results of biodegradation testing by bacteria will produce structural differences with control microplastics. The results of FTIR analysis of PE degradation by bacterial isolates are presented in Figure 3.

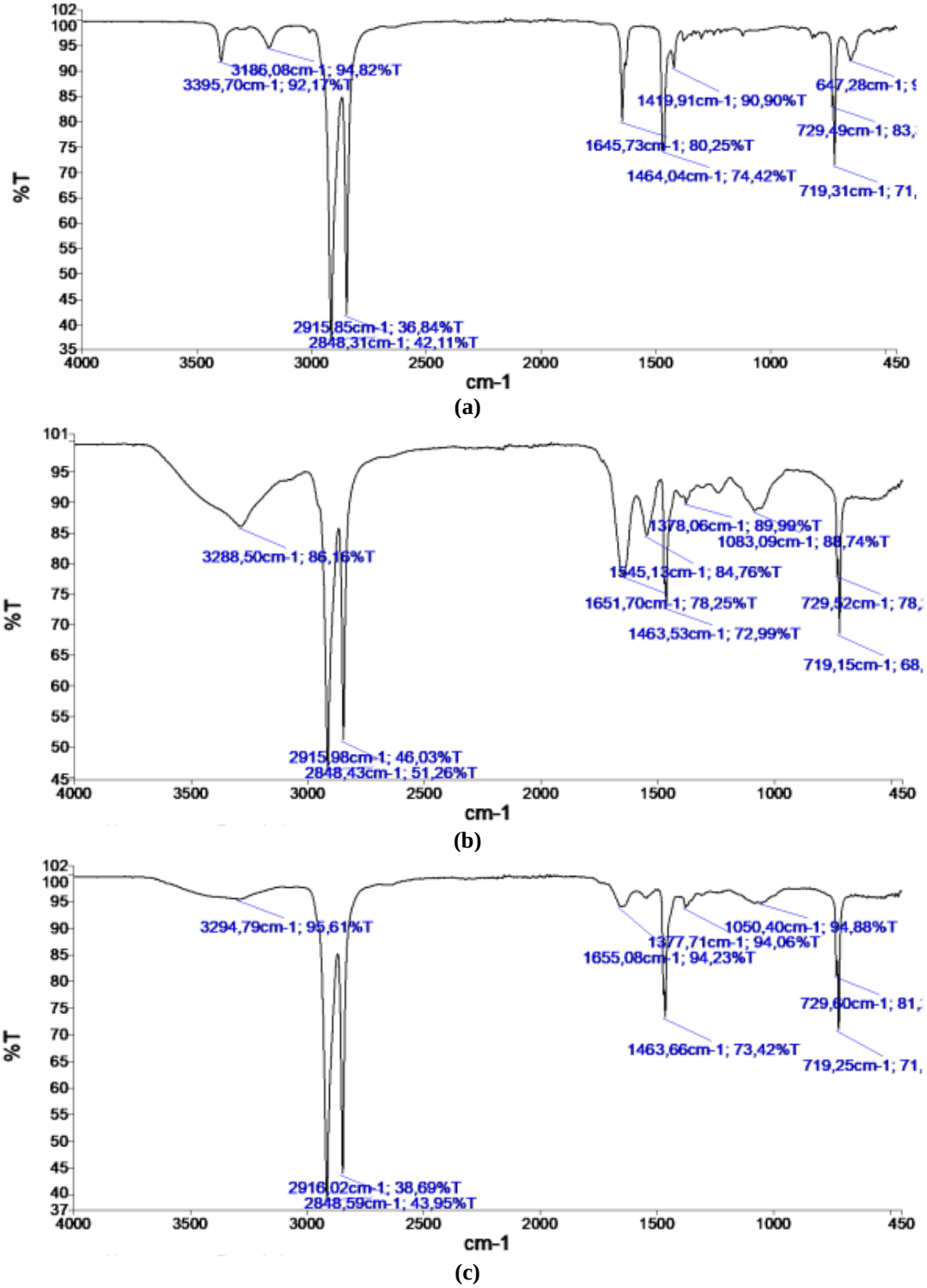
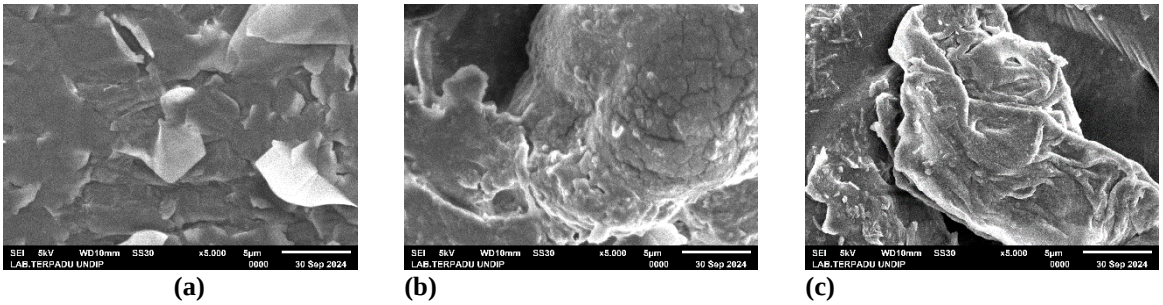


Figure 3. FTIR spectrum of **(a)** PE control (un-inoculated bacteria isolate); **(b)** PP microplastic inoculated with A6 isolate; **(c)** PP microplastic inoculated with SD5 isolate.

The polyethylene structure changes after the biodegradation test using bacteria as biodegradation agents. The FTIR analysis in the frequency range of 4000-450 cm^{-1} showed that PE polymer with inoculated bacteria has a different structure compared to the control (Figure 3). In PE control, absorption peaks were present at 647,28 – 729,29 cm^{-1} were attributed to the C-Cl stretching (halo compound), the peaks at 1419,91 cm^{-1} were assigned to the O-H bending (carboxylic acid), 1464,04 cm^{-1} C-H bending (alkane), 1645,73 cm^{-1} C=C stretching (cyclic alkene), 2848,31-2915,85 cm^{-1} C-H stretching (alkane), 3186,08 cm^{-1} O-H stretching (carboxylic acid), and 3395.70 cm^{-1} O-H stretching (alcohol). The main groups of polyethylene include the C-H stretch of the CH_2 group (alkyl chain), which was detected at the absorption peaks of 2915 and 2848 cm^{-1}, CH_2 bending (1464 cm^{-1}), and CH_2 rocking (729 and 719 cm^{-1}). In PE granules inoculated with isolates A6 and SD5, absorption peaks were present 719,15 – 729,52 cm^{-1}, and 719,25 – 729,60 cm^{-1} were assigned to the C=C bending (alkene). In polyethylene treated with A6 bacterial isolate, peaks at 2915 and 2848 cm^{-1} decreased by 46.03% and 51.26%. Additional absorption peaks at 1545 and 1083 cm^{-1} indicated the formation of nitro (NO_2) or ester groups. Peaks 729 and 719 are still there, indicating that the PE structure has not completely disappeared. Modifications of functional groups, such as oxidation and the formation of new groups, occur due to the degradation process. In polyethylene incubated with SD5 bacterial isolates, there was a drastic decrease in transmittance at 2916 cm^{-1} (38.69%) and 2848 cm^{-1} (43.95%) compared to the control, which indicated degradation. The absorption peaks at 729 and 719 cm^{-1} indicate a still-recognized PE structure. In addition, the absorption peaks at 1655 and 1463 cm^{-1} may be due to the presence of carbonyl groups (C=O), which occurs due to oxidation. This indicates initial degradation of PE due to bacterial activity, as seen from the decrease in %T and the appearance of carbonyl groups. Fourier-transform infrared (FTIR) spectroscopy has been widely used to analyze structural changes in PE during biodegradation, revealing the formation of new bonds and breakage of existing ones. The various functional group formations, such as hydroxyl, carbonyl, ester, and primary alcohol, indicated the degradation of microplastics [20]. The carbonyl index is used as a parameter of polymer biodegradability [52]. Determination of the carbonyl index makes it easy to quantify and identify the extent of degradation; therefore, it shows a strong correlation with the average molecular weight of the polymer [53]. Measurement using FTIR showed that carbonyls were detected at peaks 1395-1440 (O-H bending), 1706-1720 (C=O stretching), 1760 (C=O stretching), and 2500-3300 (O-H stretching).

PE surface morphology is a parameter assessing the degradation of polymer molecules. SEM scans of PE granules of control and bacterial isolate treatment for 20 days showed changes in particles of various sizes and shapes, as shown in Figure 4.



(a) **(b)** **(c)**

Figure 4. Surface changes of microbial degraded PE pellet at magnifications of 5000x. **(a)** control; **(b)** A6; **(c)** SD5.

The cracks and indentations formed on the PE surface are evidence of PE granule damage due to bacterial activity (Figure 4). Bacterial biodeterioration by forming biofilm and enzyme secretion affects the surface condition of PE compared to the control. Bacterial colonization will increase degradation and damage that causes the formation of erosion, holes, and pores in the PE microplastic film. PE control has a smooth and uniform surface without cracks and holes. The PE surface treated with A6 isolate had an uneven texture and formed depressions or light deformation, indicating that initial degradation had occurred as a result of enzyme interactions or oxidation. SD5-treated PE showed high levels of breakage, large cracks, holes, and fragmentation as evidence of continued degradation. The PE surface is not intact, which proves that the SD5 isolate has greater potential in decomposing PE. Research conducted by [52] states that irregular folds in the polyethylene polymer structure are caused by the formation of biofilms with the attachment of microorganisms on the surface. Based on this, the secretion of extracellular enzymes by the isolate results in the formation of biofilm on the cell surface. As a result, the enzymes entering into the pores of the polymer weaken the polymer properties [54]. These observations provide a reduction of weight and deterioration of PE by microbial activity [45].

4. Conclusions

Bacteria isolated from water and sediment in mangrove rehabilitation areas have the ability to degrade polyethylene with high degradation rates. A6, as an isolate with a degradation rate of up to 5.02% with a removal constant of 0.2% day^{-1} and a half-life of 277.7 days, can be developed to reduce the amount of polyethylene in the waters. In addition, the identification of enzymes secreted by A6 also needs to be done to improve the development of microplastic-degrading enzymes.

Author Contributions

Conceptualization, C.W and M.Z.; methodology,C.W and H.P.K.; data analysis, A.I and A.I.; resources, N.S. and A.P.H; writing—original draft preparation, C.W.; writing—review and editing, M.Z and A.I.; funding acquisition, M.Z. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The data of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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