

## Identification and degradation potential of microplastics by indigenous bacteria isolated from Putri Cempo Landfill, Surakarta, Indonesia

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### Abstract

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Plastic waste on agricultural land can break down into microplastics (< 5 mm), which plants can absorb through their roots, potentially inhibiting plant growth. Utilizing microplastic-degrading bacteria isolated from landfills offers a potential solution to microplastic contamination in agriculture. This study aimed to isolate and identify bacteria from the Putri Cempo Landfill and evaluate their ability to degrade different types of plastic contaminants found in agricultural environments. Microorganisms were isolated from soil samples using Soil Extract Media (SEM), and pure cultures were established. Bacterial isolates were tested for their microplastic-degrading potential using polyethylene terephthalate (PET) plastic fragments. Molecular analysis was conducted to determine the taxonomy of the bacteria. Further degradation tests were performed on different types of microplastic contaminants (mulch, polybags, and sacks) to identify the most degradable material. Six bacterial isolates were obtained, with isolates CP1 and CP2 demonstrating microplastic degradation rates of 2.43% and 1.15%, respectively, over a 20-day incubation period. Molecular analysis identified CP1 as *Bacillus anthracis str.* and CP2 as *Bacillus cereus ATCC 14579*. Subsequent degradation tests on various agricultural microplastic contaminants revealed that sack materials treated with *Bacillus cereus* showed the highest degradation rate, with an 8.8% weight reduction, while polybag materials showed the lowest degradation rate, with a weight loss of only 0.59%.

**Keywords:** *Bacillus* sp., Bacterial Isolation, Microplastic Degradation, Molecular Analysis, Putri Cempo Landfill.

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### Introduction

The widespread use of plastics has resulted in significant pollution, affecting various environments, including agricultural land. Plastics are resistant to decomposition, leading to their accumulation in the soil. Through biological, chemical, and physical processes, plastics break down into smaller fragments, often less than 5 mm in size, known as microplastics (Hartmann et al., 2019; Wang et al., 2021). Agricultural practices rely extensively on plastic products like mulch, polybags, and sacks, making them a primary source of plastic contamination on farms. These plastics, once in the soil, can degrade further and contribute to microplastic pollution in agricultural fields (Zhang et al., 2020).

Microplastics dispersed in soil can negatively impact water flow, reduce soil aeration, and inhibit microbial activity (Khalid et al., 2023). They can also penetrate plants through cracks or breaks in young roots, spreading through the xylem or phloem systems (Li et al., 2020). The xylem system transports microplastics to the leaves, where they accumulate, impairing physiological processes, stressing plants, and reducing fertility (Lian et al., 2020). The presence of microplastics in plants poses further risks to human health, as they can enter the food chain and potentially cause cancer over time due to dioxin emissions. Dioxins, which are known carcinogens, are produced when plastics are exposed to heat or radiation (Alabi et al., 2019).

Remediation of microplastic-contaminated soil can be achieved through bioremediation, which utilizes living organisms to break down and eliminate microplastics. This process accelerates natural degradation by leveraging the metabolic capabilities of microorganisms to convert harmful substances into harmless ones (Dash et al., 2013). The use of isolated microorganisms, such as bacteria and fungi, as biodegradation agents for polymer-based materials has been widely explored. Previous studies have identified several bacterial species, including *Pseudomonas aeruginosa* (Jeon, 2015), *Bacillus* spp. (Auta et al., 2018; Harshvardhan, 2013), and *Rhodococcus* spp. (Auta et al., 2018), as effective bioremediation agents that can utilize polymers as their sole carbon source, thus reducing the polymer's mass. For instance, *Pseudomonas* sp. strains isolated from landfill environments have been shown to degrade polyethylene (PE) plastic mulch (Hou et al., 2022).

This study aims to identify bioremediation agents capable of degrading microplastic contaminants originating from agricultural plastic waste. Microorganisms were isolated from the Putri Cempo Landfill in Surakarta City, Indonesia, which serves as the largest site for plastic waste accumulation in the area (Prasenja et al., 2022). Despite the abundant availability of plastic waste at this site, no prior research has focused on isolating microplastic-degrading bacteria from this landfill or evaluating their potential for reducing microplastic contamination in agricultural soils.

## Material and Methods

### Sample collection

Sampling was conducted in May 2023 at the Putri Cempo Landfill, Surakarta City, Central Java, Indonesia (coordinates: 7°32'11.6309 "S - 110°51'18.52550 "E). Soil samples were randomly collected from a depth of 10 cm in three different locations where plastic waste was prevalent. The first sample was taken by the roadside, where large waste transportation machinery frequently passes. The second sample was collected from a mixed pile of garbage and soil, while the third sample was obtained from an area with significant vegetation surrounding the landfill. The soil samples were placed in sterile containers, stored in a cool box, and transported to the laboratory for analysis. Following the protocol of Rosariastuti et al. (2023), soil samples were handled with gloves and stored in a cool, sterile environment.

### Material and equipment

The materials used included soil samples from the Putri Cempo Landfill and various growth media: Nutrient Agar (NA) composed of 10 g/L beef extract, 10 g/L peptone, 5 g/L NaCl, 1,000 mL distilled water, and 15 g/L agar; Nutrient Broth (NB) with 3 g/L beef extract and 10 g/L peptone; and Soil Extract Media (SEM) prepared with 100 g of landfill soil, 900 mL distilled water, and 40 g NA. Essential equipment used in the experiments included a spectrophotometer, vortex mixer, incubator, autoclave, pH meter, Erlenmeyer flasks, refrigerator, hot plate, shaker, micropipette, analytical balance, Petri dishes, and stirrer.

### Analysis of collected soil samples

Soil quality parameters, including Cation Exchange Capacity (CEC), pH, and organic carbon content, were measured to evaluate the soil's suitability for supporting bacterial growth and activity, factors that influence the abundance of indigenous bacteria at the Putri Cempo Landfill.

### Isolation and purification of bacteria

Five grams of soil sample were added to 45 mL of physiological saline solution and diluted up to  $10^{-7}$ . Aliquots of 0.1 mL from the  $10^{-3}$ ,  $10^{-5}$ , and  $10^{-7}$  dilutions were inoculated onto Soil Extract Agar Media (SEAM) using the spread plate method, followed by incubation at 27°C for 48 hours. Bacterial colonies were isolated using sterilized inoculation loops and transferred to Petri dishes via the streak plate method. This purification process was repeated 10 times to obtain pure bacterial isolates, which were then cultured on Nutrient Agar plates and stored at 4°C for further use. The purification aimed to enhance the bacteria's activity, stability, and shelf life (Javed et al., 2018).

### Initial screening

Pure bacterial isolates were subjected to initial screening by inoculating them on agar media amended with 1x1 cm pieces of microplastic (Yang et al., 2014). The microplastic pieces were surface sterilized by soaking in 70% alcohol overnight. The bacterial inoculation was conducted using the point inoculation method, and plates were incubated for five days. Based on the microbial growth area, six isolates with the highest potential for microplastic degradation were selected for further testing.

### Analysis of bacterial growth curve

To evaluate bacterial growth dynamics, isolates were inoculated in NB media and observed periodically for turbidity at 600 nm wavelength using a spectrophotometer, every six hours. The bacterial growth curve,

reflecting cell growth dynamics, was categorized into five phases: lag, log (exponential), stationary, death, and long-term stationary (Park et al., 2023).

### Microplastic degradation test

The degradation test aimed to evaluate the capacity of bacterial isolates from the Putri Cempo Landfill to break down microplastics. Polyethylene terephthalate (PET) microplastic fragments, 5 mm in diameter, were pre-weighed and surface sterilized by soaking in 70% alcohol overnight. The degradation medium was sterile NB in 50 mL glass bottles. Bacteria were inoculated into the medium and allowed to reach the stationary phase. At this point, the sterilized microplastic was added to the medium and incubated for 20 days on a shaker at 60 rpm (Vianti and Purwiyanto, 2020). After incubation, the microplastic was filtered and weighed to assess weight loss due to bacterial degradation.

### Molecular identification

Genomic DNA of bacterial isolates was extracted using the Quick-DNA Magbead Plus kit (D4082; Zymo Research, Irvine, CA, USA). PCR amplification was performed using the MyTag HS Red Mix (Bioline, BIO-25048), with primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACG-3'), targeting the 16S rRNA gene. PCR involved 9.5 µL ddH<sub>2</sub>O, 12.5 µL MyTaq Red Mix, 1 µL of 20 pmol 27F primer, 1 µL of 20 pmol 1492R primer, and 1 µL DNA extract. Sequencing was performed using the bi-directional Sanger method, and DNA sequences were analyzed for similarities using the BLAST program in the NCBI database, followed by phylogenetic tree construction.

### Contamination degradation test

The microplastic degradation test involved two selected bacterial isolates, with a control setup lacking bacteria. The experiment followed a factorial design based on a Completely Randomized Design (CRD) with two factors: type of contamination (A1: mulch, A2: polybag, A3: sack) and bacterial treatment (B0: no bacteria, B1: isolate CP1, B2: isolate CP2). Microplastic contaminants (5 mm in size) were pre-weighed and surface sterilized. Bacteria (1 loop) were inoculated into NB media and incubated for 24 hours before the medium was amended with microplastic. Incubation continued for 20 days at 60 rpm under room temperature, after which the microplastics were filtered and weighed to measure weight loss due to degradation.

## Results and Discussion

### Soil chemical analysis

The chemical analysis of soil from Putri Cempo Landfill (Table 1) revealed favorable conditions for bacterial growth, with results showing a neutral pH, high Cation Exchange Capacity (CEC), and high C-organic content. Bacteria thrive in neutral pH environments, as they facilitate optimal metabolic activities. The high CEC indicates the soil's capacity to retain and exchange essential nutrients, such as calcium, magnesium, and potassium, which are critical for bacterial growth. These findings align with Ding et al. (2017), who observed that a high CEC enhances nutrient availability for soil bacteria. Similarly, the high C-organic content provides the necessary energy and carbon source for bacteria, supporting overall microbial activity, as noted by Lee et al. (2020).

Table 1. Results of Soil Chemical Analysis at Putri Cempo Landfill

Location	Analysis	Result	Class
TP	pH	7.12	Neutral
	CEC (me/100g)	26.62	High
	C-Organic (%)	3.60	High

### Soil bacteria isolation, purification, and initial screening

The suitable soil conditions facilitated robust bacterial growth during isolation on SEM media, resulting in 27 bacterial isolates. The purification process, as described by Figueroa-Bossi et al. (2022), aimed to isolate single colonies for pure culture growth. It involved four stages of dilution, using varying ratios of soil extract and distilled water, followed by the use of pure NA media. This approach enhanced the growth of single bacterial colonies, making subsequent molecular analyses more efficient.

Initial screening of the 27 isolates revealed that six of them were capable of forming colonies in the presence of plastic. Consistent with Park and Kim (2019), the selection criteria focused on the isolates' ability to utilize PE microplastics as the sole carbon source. The screening process eliminated bacteria that could not tolerate plastic-contaminated environments, supporting findings by Dussud et al. (2018), who demonstrated that bacteria could colonize both biodegradable and non-biodegradable plastics.

## Microplastic degradation test

The microplastic degradation test was conducted during the log phase, where bacterial activity is at its peak, ensuring optimal conditions for degradation (Wijanarka et al., 2016). As shown in Figure 1, bacterial isolates reached their highest growth rate by the 12th hour, marking the best time for the microplastic degradation test. Among the six isolates tested, only three showed measurable weight reduction of PET microplastics during the incubation period (Table 3). PET was chosen for this test due to its classification as a biodegradable plastic by microorganisms and enzymes (Ru et al., 2020).

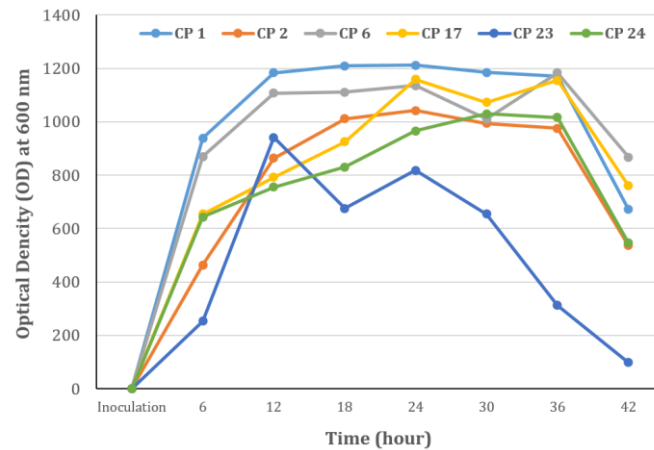


Figure 1. Growth curve of bacterial isolates

Results indicate that only a few isolates demonstrated significant degradation ability, suggesting that factors such as microplastic type, size, pH, salinity, and temperature play crucial roles (Chen et al., 2017). The reduction in microplastic weight was attributed to bacterial metabolic processes that break down the polymer structure into simpler compounds. Mukherjee et al. (2016) observed that bacteria like *Bacillus licheniformis* and *Lysinibacillus fusiformis* initiate microplastic degradation by forming surface cracks, leading to loose chemical bonds such as carbonyl groups, ketones, and aldehydes. Auta et al. (2018) described the degradation process as a two-step mechanism: bacteria first attach to the polymer's surface, form colonies, and then secrete extracellular enzymes that cleave the polymer chains into metabolizable monomers.

Table 2. Optical Density (OD) at 600 nm

Code	Inoculation	6	12	18	24	30	36	42
CP 1	0	939	1184	1210	1212	1185	1170	672
CP 2	0	464	864	1012	1043	994	976	537
CP 6	0	870	1108	1111	1136	1011	1184	867
CP 17	0	655	793	926	1159	1073	1155	760
CP 23	0	253	942	676	818	655	312	98
CP 24	0	644	756	831	966	1030	1017	546
P 2 5	0	925	1125	1147	1202	1238	1195	600

Table 3. Result of Microplastic Degradation Test

Code	Initial weight (mg)	Final weight (mg)	Difference (mg)	% Reduction
CP1	8.34	8.24	0.10	1.15
CP2	8.20	8.00	0.20	2.43
CP6	8.40	8.36	0.04	0.41
CP17	8.27	8.27	0.00	0
CP23	8.76	8.76	0.00	0
CP24	8.60	8.60	0.00	0

## Molecular identification of bacteria

The molecular identification of bacterial isolates CP1 and CP2 revealed DNA sequence lengths of 1421 bp and 1424 bp, respectively. BLAST (Basic Local Alignment Search Tool) analysis was used to compare these sequences with entries in the NCBI GenBank database. Results for isolate CP1 (Table 4) indicated a high percentage identity with several *Bacillus* species, including *Bacillus cereus* strain CM 2010 (16S ribosomal RNA, partial sequence), *Bacillus paramycooides* strain MLCCC 1A04089 (16S ribosomal RNA, partial sequence), and *Bacillus anthracis* strain Vollum (complete genome). Phylogenetic analysis (Figure 2) demonstrated that CP1 is closely related to *Bacillus anthracis* str. Collum, confirming its classification within the genus *Bacillus*. As described by Horiike (2016), phylogenetic analysis helps explain evolutionary

relationships among organisms, with the phylogenetic tree serving as a visual representation of these relationships. *Bacillus anthracis* is one of seven species closely related to *Bacillus cereus*.

Similarly, BLAST analysis for isolate CP2 (Table 5) revealed matches with *Bacillus cereus* ATCC 14579 (complete genome) and several other *Bacillus cereus* strains, confirming that CP2 belongs to the genus *Bacillus*. The phylogenetic tree (Figure 3) further identified isolate CP2 as closely related to *Bacillus cereus* ATCC 14579, supporting its classification within the *Bacillus* genus.

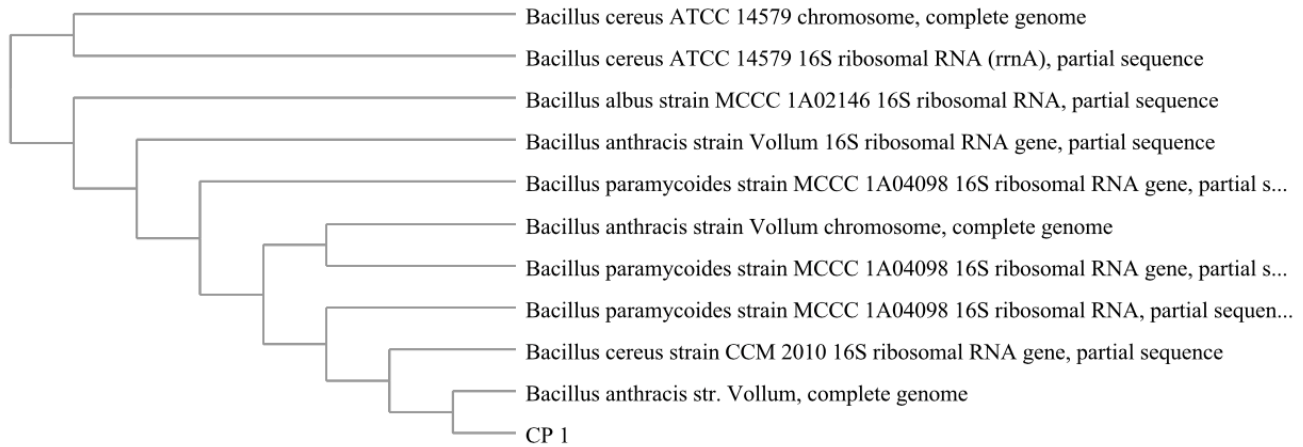


Figure 2. Phylogenetic tree of CP1, based on 16S rRNA gene sequence

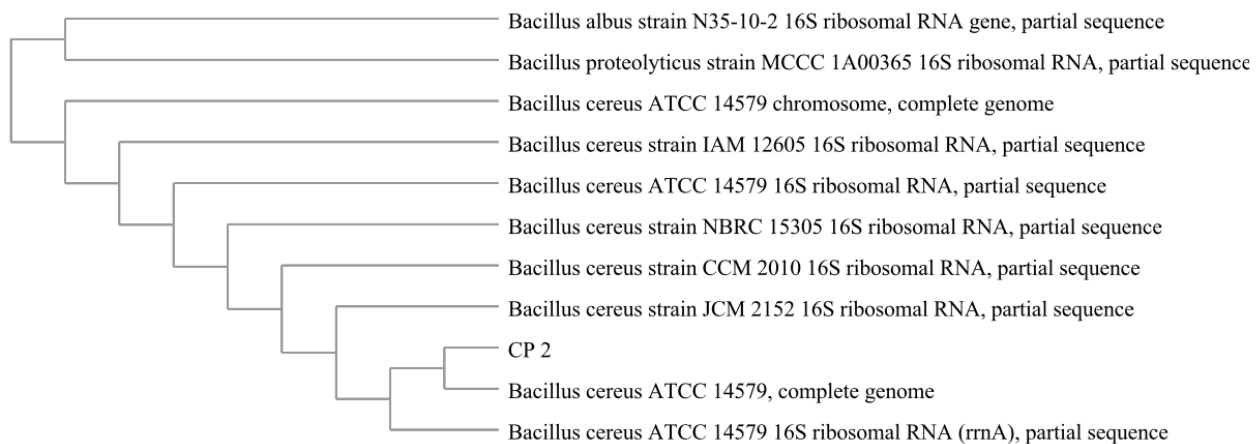


Figure 3. Phylogenetic tree of CP2, based on 16S rRNA gene sequence

Table 4. BLAST result from CP 1 bacterial isolates

Description	Max score	Total score	Query cover	E value	Per. ident	Accession
<i>Bacillus cereus</i> strain CM 2010 16S ribosomal RNA gene, partial sequence	2615	2615	100%	0.0	99.86%	<a href="#">KY628813.1</a>
<i>Bacillus paramycoides</i> strain MCCC 1A04098 16S ribosomal RNA, partial sequence	2615	2615	100%	0.0	99.86%	<a href="#">NR_157734.1</a>
<i>Bacillus anthracis</i> strain Vollum chromosome, complete genome	2615	28703	100%	0.0	99.86%	<a href="#">CP076225.1</a>
<i>Bacillus anthracis</i> str. Vollum, complete genome	2615	28663	100%	0.0	99.86%	<a href="#">CP007666.1</a>
<i>Bacillus paramycoides</i> strain MCCC 1A04098 16S ribosomal RNA gene _partial sequence	2614	2614	100%	0.0	99.86%	<a href="#">MW065486.1</a>
<i>Bacillus anthracis</i> strain Vollum 16S ribosomal RNA gene, partial sequence	2612	2612	100%	0.0	99.79%	<a href="#">AF290553.1</a>
<i>Bacillus albus</i> strain MCCC 1A02146 16S ribosomal RNA, partial sequence	2610	2610	100%	0.0	99.79%	<a href="#">NR_157729.1</a>
<i>Bacillus paramycoides</i> strain MCCC 1A04098 16S ribosomal RNA gene, partial sequence	2606	2606	99%	0.0	99.79%	<a href="#">MK183820.1</a>
<i>Bacillus cereus</i> ATCC 14579 chromosome, complete genome	2604	33858	100%	0.0	99.72%	<a href="#">CP034551.1</a>
<i>Bacillus cereus</i> ATCC 14579 16S ribosomal RNA (1mA), partial sequence	2604	2604	100%	0.0	99.72%	<a href="#">NR_074540.1</a>

Table 5. BLAST results from CP2 bacterial isolates

Description	Max score	Total score	Query cover	E value	Per. ident	Accession
<i>Bacillus cereus</i> ATCC 14579 chromosome, complete genome	2614	33983	100%	0.0	99.79%	CP034551.1
<i>Bacillus cereus</i> ATCC 14579 16S ribosomal RNA (rRNA), partial sequence	2614	2614	100%	0.0	99.79%	NR_074540.1
<i>Bacillus cereus</i> strain JCM 2152 16S ribosomal RNA, partial sequence	2614	2614	100%	0.0	99.79%	NR_113266.1
<i>Bacillus cereus</i> strain CM 2010 16S ribosomal RNA, partial sequence	2614	2614	100%	0.0	99.79%	NR_115714.1
<i>Bacillus cereus</i> strain NBC 15305 16S ribosomal RNA, partial sequence	2614	2614	100%	0.0	99.79%	NR_112630.1
<i>Bacillus cereus</i> ATCC 14579 16S ribosomal RNA, partial sequence	2614	2614	100%	0.0	99.79%	NR_114582.1
<i>Bacillus cereus</i> ATCC 14579 complete genome	2614	33950	100%	0.0	99.79%	AE016877.1
<i>Bacillus cereus</i> strain AM 12605 16S ribosomal RNA, partial sequence	2614	2614	100%	0.0	99.79%	NR_115526.1
<i>Bacillus albus</i> strain N35-10-2 16S ribosomal RNA gene, partial sequence	2612	2612	99%	0.0	99.79%	OQ876685.1
<i>Bacillus proteolyticus</i> strain MCCC 1A00365 16S ribosomal RNA, partial sequence	2608	2608	100%	0.0	99.72%	NR_157735.1

### Contamination degradation test

The results of microplastic degradation testing for various contaminants on agricultural land, as shown in Figure 4, indicated that all treatments resulted in increased degradation compared to the control group. ANOVA analysis, performed with a 95% confidence level, revealed that both the type of plastic contamination and the bacterial isolate significantly influenced the percentage of microplastic degradation, with a notable interaction between the two factors. Figure 4 illustrates the results of the Duncan's Multiple Range Test (DMRT), which assessed the degradation rates of different combinations of bacterial isolates and plastic types. The highest degradation rate was observed in the A3B2 treatment (sack + *Bacillus cereus*), which showed a 100% increase over the control treatment (A3B0: sack + no bacteria) and a 23.7% higher degradation than the second-best treatment (A3B1: sack + *Bacillus anthracis*). The lowest degradation was recorded in the A2B2 treatment (polybag + *Bacillus cereus*), indicating that polybags are more resistant to bacterial degradation. However, even this treatment demonstrated a significant improvement, achieving a 100% degradation rate compared to the control (A2B0: polybag + no bacteria).

*Bacillus cereus* has been identified as an effective microplastic degrader, commonly found in soil, plants, and food. Research by [Auta et al. \(2018\)](#) demonstrated that *Bacillus cereus* isolated from mangrove sediments in Peninsular Malaysia could degrade polypropylene (PP) microplastics by 6.6% over 40 days. The higher degradation rates for sack materials can be attributed to their thin sheets and the elemental composition of biodegradable PP, making them more accessible for microbial action. In contrast, thicker polybags and HDPE (High-Density Polyethylene) plastics are more challenging to degrade due to their dense, non-porous structures. [Mohanani et al. \(2020\)](#) found that PP degrades more readily by microorganisms compared to HDPE and polyethylene (PE). The lean plastic mulch and silver-black mulch, made from PE fabric, are more difficult to degrade, as their outer layer often contains a thin metallic film (e.g., aluminum) that adds to the complexity of the degradation process.

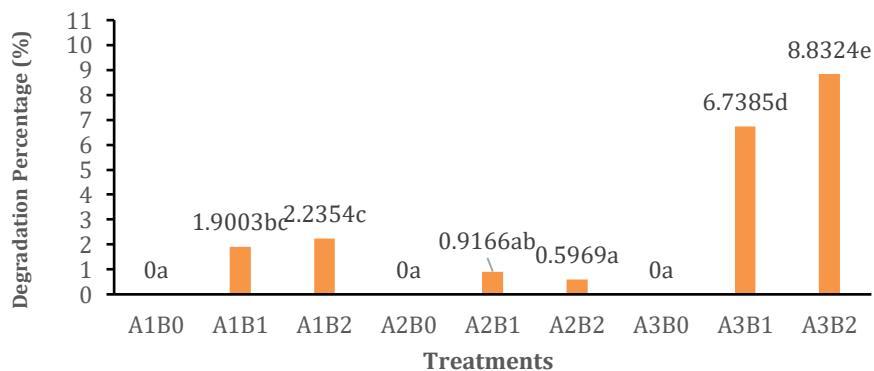


Figure 4. The influence of the type of bacteria and type of plastic on the percentage of plastic weight reduction

## Conclusion

The study identified *Bacillus anthracis* and *Bacillus cereus* as microplastic-degrading bacteria in soil samples collected from the Putri Cempo Landfill. Degradation tests on various plastic contaminants commonly found in agricultural settings revealed that no degradation occurred in the control treatment, which lacked bacterial isolates. The highest degradation rate was observed with *Bacillus cereus* on sack material, achieving an 8.83% reduction, followed by *Bacillus anthracis* on the same material with a 6.73% reduction. The CP2 bacterial isolate also showed degradation potential on mulch, with a 2.23% reduction. These results indicate that sack materials are the most susceptible to microbial degradation, while polybag materials present the greatest resistance. The findings confirm that *Bacillus* species can effectively degrade microplastics, demonstrating their potential for bioremediation in agricultural soils.

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