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REVIEW PAPER

Polyethylene-biodegrading Microbes and Their Future Directions

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Abstract Management of plastic waste is becoming a serious global environmental issue. Plastic pollution threatens a wide variety of ecosystems and brings damaging repercussions for many wildlife species. Polyethylene (PE) is a major petroleum-based plastic that has become indispensable in all aspects of modern life because of its many applications. PE is extremely resistant to natural biodegradation processes, resulting in its accumulation in the environment. Therefore, microorganism-mediated decomposition of PE is attracting attention as an ideal, sustainable method to reduce PE accumulation. In this review, we summarize capacities of various microbes (bacteria and fungi) to degrade PE, the physical products of PE degradation, and potential PE-degrading enzymes. Furthermore, we propose future directions for building PE-decomposition systems such as metabolons that use diverse enzymes to increase the activities and/or stabilities of potential PE degradable enzymes. Thus, this review article will contribute to developing PE-biodegradation systems using microbes and their biocatalysts.

Keywords: polyethylene, biodegradation, biocatalysis, multi-enzyme cascade reaction, scaffolds, synthetic biology

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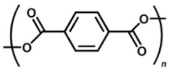
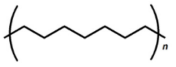
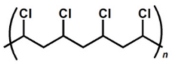
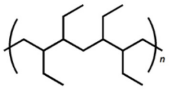
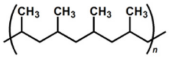
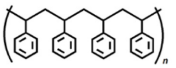
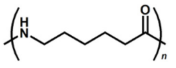
1. Introduction

Plastics are a synthetic polymer and have been widely used in industrial and household products because of their advantages, such as their low manufacturing cost, light-weight nature, waterproofness, flexibility, durability, and thermal properties. Conventional plastics are classified into one of seven categories: polyethylene terephthalate (PETE), polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), and other, which includes poly-carbonate and polyurethane (Fig. 1A). These plastics are used in many housewares as well as in everyday life. However, conventional plastics are significant contributors to environmental pollution because approximately seven billion tons of plastic waste has been generated so far, with > 70% of this waste remaining in landfills, dumps, or nature. If global annual plastic production and accumulation were to continue in line with its historical growth, plastic production would exceed 1 billion tons in 2050 [1]. Plastic waste can be degraded by chemical recycling processes such as photodegradation, thermo-oxidative degradation, and hydrolytic degradation [2-4]. However, chemical treatment of plastic waste cannot be a widespread practice for recycling plastics because of its energy costs and other limitations [5]. In this regard, plastic biodegradation by microorganisms is a notable alternative. In fact, biological process using various microbes have been attended to biodegradation of plastics [6-16].

PE is a petroleum-based plastic that can be divided into three types: high-density polyethylene (HDPE, linear form), linear low-density polyethylene (LLDPE, linear branch form), and low-density polyethylene (LDPE, branch form) (Fig. 1B). Each of these PE types differs in their density and degree of branching, which influences the availability of functional groups on their surface. Some microorganisms, including bacteria and fungi, have proven capable of

A

Recycling symbol	Name	Structure
1 PETE	Polyethylene terephthalate	
2 HDPE	High density polyethylene	
3 PVC	Polyvinyl chloride	
4 LDPE	Low density polyethylene	
5 PP	Polypropylene	
6 PS	Polystyrene	
7 Other	Other (often polycarbonate)	

B

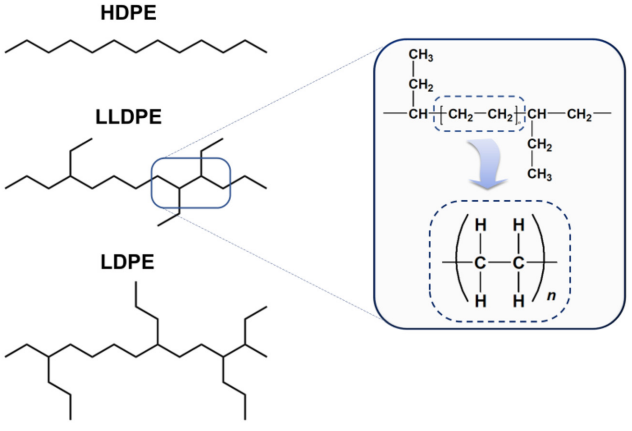


Fig. 1. Types of plastics. (A) Commonly used recycling symbols, names, and structures of plastics. (B) Structures of different types of polyethylene. HDPE: high-density polyethylene, LLDPE: linear low-density polyethylene, LDPE: low-density polyethylene.

decomposing certain PE film, sheets, discs, bags, and particles (pieces and powder) (Tables 1 and 2). In efforts to discover novel plastic-biodegrading microorganisms, environmental samples such as polymer waste are collected from dumps, landfills, and oceans and used to screen for plastic-biodegrading microbes. Moreover, some of the isolated microorganisms have been identified to species using their ribosomal RNA sequences. To observe physical or chemical change of PE during biodegradation, most PE-biodegradation studies quantify PE weight loss (WL) using thermogravimetric analysis (TGA), measure hydrophilic or hydrophobic characteristic on the PE surface using water contact angle analysis (WCA), evaluate modifications to the chemical structure of PE functional groups via Fourier transform infrared spectroscopy (FT-IR), visualize polymer structural changes via scanning electron microscopy (SEM), and identify metabolites released by PE biodegradation via gas chromatography-mass spectrometry (GC-MS) (Fig. 2) [17]. Furthermore, whole-genome sequencing and transcriptome analysis of isolated PE-biodegrading microorganisms have been also attempted to identify enzymes that may be involved in metabolic pathways related to PE degradation.

In this review, we summarize recent studies on PE biodegradation involving experimental techniques, conditions, and changes to PE chemical structures and metabolites created by microorganisms, including bacteria and fungi. Furthermore, we discuss microbial enzymes that might be involved in metabolic pathways related to PE degradation. Finally, we consider future directions, particularly synthetic biology-based approaches such as the development of scaffold systems for multi-enzyme cascade reactions for PE biodegradation with putative PE-biodegrading enzymes [18-20]. Techniques such as these hold potential to increase enzymatic activity and stability, overcome high costs associated with cofactors, and build metabolons.

2. Biodegradation of PE Using Bacteria

There are three types of PE-degradation methods: photodegradation, thermo-oxidative degradation, and biodegradation [21]. Photodegradation and thermo-oxidative degradation are classified as abiotic systems, whereas biodegradation is categorized as biotic degradation, which involves microbes [22]. In this review, we summarize microorganism-mediated biodegradation of several types of PE such as mulching film (MF), film (F), sheets (S), pieces (Pi), particles (Pa), bags (B), and powder (Po). We also summarize the bacterial genera, experimental methods and conditions, and weight-loss results for different types of PE (Table 1). These PE types have been used to isolate microorganisms that can decompose PE, and some of these microorganisms were isolated from different soil types [23]. Gram-negative and

Table 1. Bacteria used in PE-biodegradation studies

Genus	Analysis method	PE type	Experiment conditions		WL (%)	Reference
			Incubation temperature (°C)	Incubation period		
Individual culture						
Acinetobacter baumannii	SEM, FT-IR, GC-MS	PE (MF)	37	30 days	N/A	[24]
Alcanivorax sp. H265	WL, SEM, TGA, AFM	LDPE (F)	28	90 days	0.97	[25]
Bacillus amyloliquefaciens BSM-1	WL, SEM, FT-IR	LDPE (F)	33.3	60 days	11.0	[26]
Bacillus amyloliquefaciens BSM-2	WL, SEM, FT-IR	LDPE (F)	33.3	60 days	16.0	[26]
Bacillus mycoides	WL, FT-IR	LDPE (F)	30	60 days	11.4	[27]
Bacillus pumilus M2	SEM, FT-IR	LDPE (F)	N/A	30 days	1.50	[28]
Bacillus subtilis	WL, FT-IR	LDPE (F)	30	60 days	23.2	[27]
Bacillus subtilis	WL, FT-IR	LDPE (F)	32	30 days	9.26	[29]
Bacillus subtilis H1584	SEM, FT-IR	LDPE (F)	N/A	30 days	1.75	[28]
Bacillus sp. ISJ55	WL, FT-IR	LDPE (F)	35	60 days	1.50	[30]
Bacillus sp. strain A5	WL, FT-IR, GC-MS	LDPE (S)	37	16 weeks	35.7	[31]
Bacillus sp. strain SM1	WL, FT-IR	PE (Pi)	30	180 days	39.2	[32]
Bacillus sp. YP1	SEM, FT-IR, AFM, XPS, WCA	LDPE (F)	30	60 days	6.10	[33]
Bacillus tropicus (MK318648)	WL, FT-IR	LDPE (F)	37	40 days	10.2	[34]
Bacillus velezensis C5	WL, SEM, FT-IR, WCA	LDPE (F)	N/A	90 days	26.0	[35]
Brevibacillus borstelensis strain 707	WL, FT-IR	LDPE (F)	50	30 days	N/A	[36]
Brevibacillus sp. strain B2	WL, FT-IR, GC/MS	LDPE (S)	37	16 weeks	20.3	[31]
Chelatococcus daeguensis KCTC 12979	FT-IR	LDPE (P)	58	80 days	N/A	[37]
Cobetia sp. H237	WL, SEM, TGA, AFM	LDPE (F)	28	90 days	1.40	[25]
Comamonas sp.	SEM, AFM	PE (F)	28	90 days	N/A	[38]
Delftia sp.	SEM, AFM	PE (F)	28	90 days	N/A	[38]
Enterobacter asburiae YT1	SEM, AFM, XPS, FT-IR, WCA	LDPE (F)	30	60 days	10.7	[33]
Enterobacter sp. D1	SEM, AFM, FT-IR, LC-MS	PE (F)	37	31 days	N/A	[39]
Exiguobacterium sp. H256	WL, SEM, TGA, AFM	LDPE (F)	28	90 days	1.26	[25]
Halomonas sp. H255	WL, SEM, TGA, AFM	LDPE (F)	28	90 days	1.72	[25]
Klebsiella pneumoniae CH001	WL, SEM, FT-IR, GC-MS	HDPE (F)	30	60 days	18.4	[40]
Kocuria palustris M16	SEM, FT-IR	LDPE (F)	N/A	30 days	1	[28]
Lysinibacillus sp. JJY0216	WL, FT-IR, GC-MS, AFM	LDPE (F)	30	26 days	7.5	[41]
Microbulbifer hydrolyticus IRE-31	SEM, FT-IR	PE (Pa)	37	30 weeks	N/A	[42]
Micrococcus sp.	WL	PE (B)	N/A	1 month	6.61	[43]
Moraxella sp.	WL	PE (B)	N/A	1 month	7.75	[43]
Nocardia asteroides GK 911	SEM, FT-IR, Mineralization	PE (F)	N/A	6 months	N/A	[44]
Oscillatoria subbrevis	WL, SEM, FT-IR	PE (S)	32−34	42 days	≈30	[45]
Paenibacillus sp. DK1	WL, SEM, FT-IR	PE (B)	37	3 months	11.6	[46]
Phormidium lucidum	WL, SEM, FT-IR	PE (S)	32−34	42 days	≈30	[45]
Pseudomonas aeruginosa B2 (ATCC 15692)	WL, SEM, GC-MS	LDPE (F)	37	120 days	11	[47]
Pseudomonas aeruginosa E7	Apparent biodegradability	LDPE (Po)	37	80 days	N/A	[48]
Pseudomonas aeruginosa RD1-3	WL, SEM, FT-IR, AFM	PE (F)	28	8 weeks	3.62	[49]
Pseudomonas aeruginosa SKN1	WL, SEM	LDPE (Po)	37	60 days	10.3	[50]
Pseudomonas aeruginosa PAO1 B1 (ATCC 15729)	WL, SEM, GC-MS	PE (F)	37	120 days	20	[47]
Pseudomonas knackmussii N1-2	WL, SEM, FT-IR, AFM	PE (F)	28	8 weeks	5.95	[49]
Pseudomonas putida B3 (KT2440, ATCC 47054)	WL, SEM, GC-MS	PE (F)	37	120 days	9	[47]
Pseudomonas spp. P1	WL	PE (B)	40	8 weeks	31.4	[51]
Pseudomonas spp. P2	WL	PE (B)	40	8 weeks	39.7	[51]
Pseudomonas spp. P3	WL	PE (B)	40	8 weeks	46.2	[51]

Table 1. Continued

Genus	Analysis method	PE type	Experiment conditions		WL (%)	Reference
			Incubation temperature (°C)	Incubation period		
Pseudomonas sp.	WL	PE (B)	N/A	1 month	20.5	[43]
Pseudomonas syringae B4 (DC3000, ATCC 10862)	WL, SEM, GC-MS	PE (F)	37	120 days	11.3	[47]
Ralstonia sp. strain SKM2	WL, FT-IR	PE (Pi)	30	180 days	18.9	[32]
Rhodococcus rhodochrous ATCC 29672	SEM, FT-IR, Mineralization	PE (F)	N/A	6 months	N/A	[44]
Rhodococcus ruber	WL, FT-IR	LDPE (F)	30	4 weeks	8	[52]
Rhodococcus ruber C208	WL, SEM, Biofilm longevity	LDPE (F)	30	60 days	7.5	[53]
Serratia marcescens subsp.*marcescens*	SEM, FT-IR, DSC analysis	LLDPE (Po)	30	70 days	36	[54]
Staphylococcus arlettae (KX344032)	WL, FT-IR	PE (B)	37	4 weeks	13.6	[55]
Staphylococcus sp.	WL	PE (B)	N/A	1 month	16.4	[43]
Stenotrophomonas sp.	SEM, AFM	PE (F)	28	90 days	N/A	[38]
Streptococcus sp.	WL	PE (B)	N/A	1 month	2.19	[43]

The bacteria are listed alphabetically by genus.
PE: polyethylene, WL: water loss, SEM: scanning electron microscopy, FT-IR: Fourier transform infrared spectroscopy, GC-MS: gas chromatography–mass spectrometry, TGA: thermogravimetric analysis, AFM: atomic force microscopy, XPS: X-ray photoelectron spectroscopy, WCA: water contact angle, DSC: differential scanning calorimetry, LDPE: low-density polyethylene, HDPE: high-density polyethylene, LLDPE: linear low-density polyethylene, MF: mulching film, F: film, S: sheets, Pi: pieces, Pa: particles, B: bags, Po: powder, N/A: not available.

Table 2. Fungi used in studies of polyethylene (PE) biodegradation

Genus	Analysis method	PE type	Experiment condition		WL (%)	Reference
			Incubation temperature (°C)	Incubation period		
Individual culture						
Alternaria alternata	WL, SEM, FT-IR	PE (D)	N/A	3 months	0.8	[65]
Alternaria alternata FB1	SEM, FT-IR, GC-MS, XRD	PE (F)	≈25	120 days	95.0	[66]
Aspergillus clavatus JASK1	WL, SEM, FT-IR, AFM	PE (F)	N/A	90 days	35	[67]
Aspergillus flavus	WL, SEM	LDPE (Po, S)	28	30 days	16.2	[68]
Aspergillus flavus	WL, SEM	PE (Po)	28	90 days	2.12	[69]
Aspergillus fumigatus	WL, SEM	LDPE (Po, S)	28	30 days	20.5	[68]
Aspergillus fumigatus	WL, SEM	PE (Po)	28	90 days	1.38	[69]
Aspergillus fumigatus	SEM, FT-IR, pH level	LDPE (Pi)	28	12 weeks	N/A	[70]
Aspergillus glaucus	WL	PE (B)	N/A	3 months	28.8	[43]
Aspergillus niger	WL	PE (B)	N/A	3 months	17.4	[43]
Aspergillus niger	WL, SEM	LDPE (Po, S)	28	30 days	19.5	[68]
Aspergillus niger	WL, SEM, FT-IR, GC-MS	PE (F)	27	90 days	28.8	[71]
Aspergillus niger ATCC9642	Mineralization, SEM, FT-IR	PE (Po)	30	31 months	N/A	[72]
Aspergillus nomius	WL	LDPE (F)	26	45 days	6.3	[73]
Aspergillus sp.	WL, FT-IR, GC-MS	LDPE (S)	N/A	16 weeks	36.4	[31]
Aspergillus sp.	SEM	LDPE (S)	25	7−10 days	N/A	[74]
Aspergillus sp.	WL, SEM, FT-IR	LDPE (F)	N/A	90 days	≈3.8	[75]
Aspergillus sp. FSM-3	WL, SEM, FT-IR	LDPE (F)	33.3	60 days	8	[76]
Aspergillus sp. FSM-5	WL, SEM, FT-IR	LDPE (F)	33.3	60 days	5	[76]
Aspergillus sp. FSM-6	WL, SEM, FT-IR	LDPE (F)	33.3	60 days	7	[76]
Aspergillus sp. FSM-8	WL, SEM, FT-IR	LDPE (F)	33.3	60 days	7	[76]
Aspergillus terreus	WL, SEM	LDPE (Po, S)	28	30 days	21.8	[68]
Aspergillus terreus	SEM, FT-IR	LDPE (Pi)	28	3 months	N/A	[70]
Aspergillus terreus MF12	WL, FT-IR, GC-MS	HDPE (F)	30	30 days	9.4	[77]
Collectotrichum fructicola	WL, SEM, FT-IR, GC-MS	PE (F)	27	90 days	48.8	[71]

Table 2. Continued

Genus	Analysis method	PE type	Experiment condition		WL (%)	Reference
			Incubation temperature (°C)	Incubation period		
Curvularia lunata	WL, SEM, FT-IR	PE (D)	N/A	3 months	1.2	[65]
Diaporthe italiana	WL, SEM, FT-IR, GC-MS	PE (F)	27	90 days	43.9	[71]
Fusarium solani	SEM, FT-IR	LDPE (Pi)	28	3 months	N/A	[70]
Fusarium sp.	WL, SEM, FT-IR	PE (D)	N/A	3 months	0.7	[65]
Fusarium sp.	WL, SEM	PE (Po)	28	90 days	2.58	[69]
Fusarium sp. (FSM-10)	WL, SEM, FT-IR	LDPE (F)	33.3	60 days	10	[76]
Lasiodiplodia theobromae	WL, SEM, FT-IR	LDPE (F)	N/A	90 days	≈3.6%	[75]
Penicillium chrysogenum NS10 (KU559907)	WL, SEM, FT-IR, AFM	LDPE, HDPE (S)	28	90 days	LDPE 34.4%, HDPE 58.6%	[78]
Penicillium sp.	WL, SEM	LDPE (Po)	28	30 days	43.4	[68]
Penicillium oxalicum NS4 (KU559906)	WL, SEM, FT-IR, AFM	LDPE, HDPE (S)	28	90 days	LDPE 36.6%, HDPE 55.3%	[78]
Penicillium pinophilum ATCC11797	Mineralization, SEM, FT-IR	PE (Po)	30	31 months	N/A	[72]
Penicillium simplicissimum	WL, SEM, FT-IR	PE (D)	N/A	3 months	7.7	[65]
Penicillium simplicissimum YK	FT-IR, Growth assays	HDPE	N/A	3 months	N/A	[79]
Purpureocillium lilacinum	WL, SEM, FT-IR	LDPE (F)	N/A	90 days	≈2	[75]
Rhizopus oryzae NS5	WL, SEM, AFM, WCA	LDPE (F)	37	1 month	8.4	[80]
Stagonosporopsis citrulli	WL, SEM, FT-IR, GC-MS	PE (F)	27	90 days	45.1	[71]
Thyrostroma jaczewskii	WL, SEM, FT-IR, GC-MS	PE (F)	27	90 days	46.3	[71]
Trichoderma viride	WL, tensile strength	LDPE (F)	26	45 days	5.13	[73]
Zalerion maritimum	SEM, FT-IR, NMR	PE (Pa)	25	28 days	N/A	[81]

The fungi are listed alphabetically by genus.
WL: water loss, SEM: scanning electron microscopy, FT-IR: Fourier transform infrared spectroscopy, GC-MS: gas chromatography–mass spectrometry, XRD, X-ray diffraction analysis, AFM: atomic force microscopy, WCA: water contact angle, NMR: nuclear magnetic resonance, LDPE: low-density polyethylene, HDPE: high-density polyethylene, D: discs, F: film, S: sheets, Pi: pieces, Pa: particles, B: bags, Po: powder, N/A: not available.

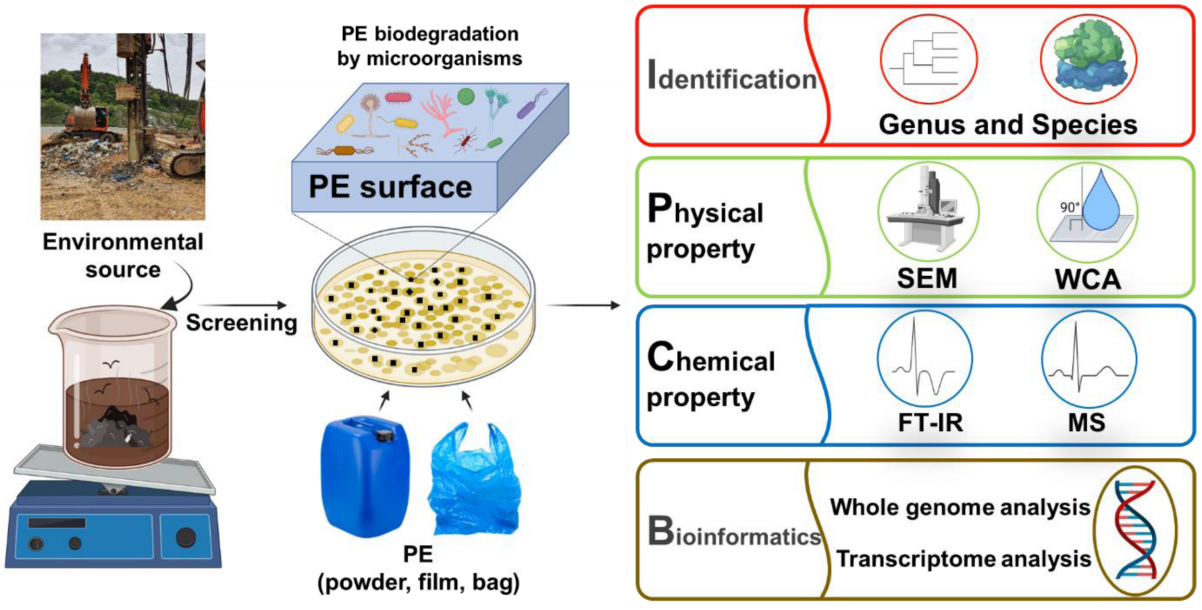


Fig. 2. Overview of techniques used for the analysis of PE-biodegrading microbes. PE: polyethylene, SEM: scanning electron microscope, WCA: water contact angle, FT-IR: Fourier transform infrared spectroscopy, MS: mass spectrometry.

gram-positive species belonging to the genera (alphabetically listed) *Acinetobacter* [24], *Alcanivorax* [25], *Bacillus* [26-35], *Brevibacillus* [31,36], *Chelatococcus* [37], *Cobetia* [25], *Comamonas* [38], *Delftia* [38], *Enterobacter* [33,39], *Exiguobacterium* [25], *Halomonas* [25], *Klebsiella* [40], *Kocuria* [28], *Lysinibacillus* [41], *Microbulbifer* [42], *Micrococcus* [43], *Moraxella* [43], *Nocardia* [44], *Oscillatoria* [45], *Paenibacillus* [46], *Phormidium* [45], *Pseudomonas* [43,47-51], *Ralstonia* [32], *Rhodococcus* [44,52,53], *Serratia* [54], *Stenotrophomonas* [43,55], and *Staphylococcus* [38,43] have been reported as potential PE-degrading microbes (Table 1). PE biodegradation often occurs several stages, specifically (I) biodeterioration, involving oxidative reactions that form carbonyl groups via enzymes released from microorganisms; (II) biofragmentation, which involves hydrolysis and/or fragmentation of PE carbon chains; (III) bioassimilation, in which small hydrocarbon fragments released from biofragmentation are metabolized by microorganisms; and (IV) mineralization, which involves intracellular conversion of hydrolysis products to microbial biomass with carbon dioxide and water released from the cell [56]. There are also several types of analytical methods used to characterize PE degradation; these methods include atomic force microscopy (AFM) and SEM, used to confirm structural changes on the PE surface; FT-IR, GC-MS, and X-ray diffraction (XRD), all used to identify changes to functional groups in PE materials; and TGA, WCA, and WL, which are used to measure the physically changed PE materials. Most isolated microorganisms are cultured at a mesothermal temperature (23 to 45°C), except *Brevibacillus borstelensis* [36] and *Chelatococcus daeguensis* KCTC 12979 [37], which are cultured at 50 and 58°C, respectively, because they can survive at high temperatures. The WL of PE, reflecting microorganismal decomposition rates of PE, are also summarized in Table 1. Among the evaluated microbes, *Bacillus subtilis*, *Bacillus* sp. strain A5, *Bacillus* sp. strain SM1, *Brevibacillus* sp. strain B2, *Oscillatoria subbrevis*, and *Pseudomonas* sp. showed the highest ability to degrade PE (over 20%) (Table 1). In descending order, their decomposition rates, as quantified with WL, are as follows: *Pseudomonas* sp. P3 (46.2%) [51], *Pseudomonas* sp. P2 (39.7%) [51], *Bacillus* sp. strain SM1 (39.2%) [32], *Bacillus* sp. strain A5 (35.7%), [31] *Pseudomonas* sp. P1 (31.4%) [51], *O. subbrevis* (≈ 30%) [45], *B. subtilis* (23.2%) [27], *Pseudomonas* sp. (20.5%) [43], and *Brevibacillus* sp. strain B2 (20.3%) [31]. A series of *Pseudomonas* spp., P1, P2, and P3, were isolated from a household garbage dump, a textile effluents drainage site, and a sewage sludge dump, respectively. They all showed an enhanced ability to degrade PE (B), which indicates that *Pseudomonas* spp. P1, P2, and P3 can used as potential PE-biodegradable bacteria. In Table 1, many *Bacillus* and *Pseudomonas* species, which can survive in extreme environments characterized by high temperatures, salinity, and alkalinity, demonstrate high potential for success in PE-biodegradation applications [57-59]. Thus, these microorganisms should be further studied to clarify certain characteristics of their metabolism, particularly the enzymes involved in PE biodegradation.

3. Biodegradation of PE Using Fungi

Fungi are attractive candidates for use in the decomposition of recalcitrant complex compounds including plastics, lignin, cellulose, and hemicellulose because they have high attaching ability to the hydrophobic surface of polymers [8,60-62]. Thus, they also have been used in PE degradation [63], particularly because their superior attachment to polymeric hydrophobic surfaces can cause swelling and rupture while penetrating polymer solids [61,63,64]. More than 10 fungal species have been isolated from various environments and evaluated to determine their ability to degrade different types of PE (Table 2). Specifically, the genera *Alternaria* [65,66], *Aspergillus* [31,43,67-77], *Collectotrichum* [71], *Curvularia* [65], *Diaporthe* [71], *Fusarium* [65,69,70,76], *Lasiodiplodia* [75], *Penicillium* [65,68,72,78,79], *Purpureocillium* [75], *Rhizopus* [80], *Stagonosporopsis* [71], *Thyrostroma* [71], *Trichoderma* [73], and *Zalerion* [81] have been reported to decompose several types of PE. Some researchers also assessed the ability of these fungi to degrade PE by using WL, SEM, FT-IR, GC-MS, XRD, and AFM. Among these fungi, *Alternaria alternata* FB1 showed 95% WL, representing the highest PE (F)-degrading ability reported so far. *A. alternata* FB1 is marine fungus that was isolated from plastic waste samples in a marine environment [66]. The isolate was grown on PDA medium with PE (F) for 120 days to observe its morphology and colonization. After 120 days, the morphology of PE (F) that was treated *A. alternata* FB1 was characterized by curling and shrinking, and the color of treated PE (F) changed from white to a dark yellow. Gao *et al*. [66] performed FT-IR and GC-MS to evaluate the changes in functional groups and products of PE biodegradation, respectively. In PE (F) treated with *A. alternata* FB1, new hydroxyl and carboxylic groups were observed, and transmittance of these functional groups increased as incubation time increased relative to untreated PE (F). Moreover, the C3 to C27 products were detected by GC-MS in the treated PE (F) sample after 60 days, and after 120 days, only one product (diglycolamine) was detected. These results indicate that *A. alternata* FB1 possesses the metabolic pathways necessary for PE degradation. Furthermore, Gao *et al*. [66] performed a transcriptome

analysis of this fungus cultured in a medium either with or without PE. According to this analysis, there were 153 potential enzymes including peroxidases, laccases, hydroxylases, dehydrogenases, oxidoreductases, oxidases, reductases, esterases, lipases, and cutinases that could be involved in biodegradation; additionally, glutathione peroxidase and laccase were identified as key enzymes for the *A. alternata* FB1 PE-degradation pathway. Other *Penicillium* spp., specifically *Penicillium chrysogenum* NS10 and *Penicillium oxalicum* NS4, that were isolated from the dumping plastic site also showed a superior ability to degrade PE [78]. Ojha *et al*. [78] evaluated the optimum conditions (temperature, pH, and medium sources) for increasing the mycelium weight of these two *Penicillium* spp. during PE biodegradation by response surface methodology. These fungi were grown in an optimized medium that included sucrose, $NaNO_3$, K_2HPO_4, $MgSO_4$, KCl, and $FeSO_4$ at 28°C for 90 days. At these optimized conditions, the WL values of *P. chrysogenum* and *P. oxalicum* were 36.6 and 58.6% for LDPE (S) and 36.6 and 55.3% for HDPE (S), respectively. Additionally, SEM and AFM analyses were performed to confirm these species' ability to degrade PE on LDPE and HDPE films, the surfaces of which showed morphological changes such as pits, cracks, biofilms, and erosion. The detailed mechanism of enzymatic PE degradation by these fungi has not yet been fully elucidated. However, the studies described in this review can support efforts to find biocatalysts for PE biodegradation under natural environmental conditions.

4. FT-IR and GC-MS Analysis of Microorganism-biodegraded PE

Structural changes in the functional groups in PE materials can be determined using FT-IR. PE contains strong single carbon–carbon and carbon–hydrogen bonds as long alkane chains, which can be changed to alkenes, alcohols, carboxylic acids, esters, ethers, amides, ketones, aldehydes, and hydroxyl groups by various microorganismal enzymes [17,82-84]. Changes to the functional groups in PE polymers can be analyzed through FT-IR, which measures the absorbance of functional groups at different wavelengths. We summarize some characteristic peaks in FT-IR [85] in Table 3. Long alkane chains in PE are modified into several types of functional groups. Peaks in FT-IR spectra are as follows: alkene (=C–H bond) at 650 to 1,000 cm^{-1} [46,54,65,79,86]; alcohol, carboxylic acid, ester, and ether (–C–O stretch) at 1,000 to 1,320 cm^{-1} [25,26,29,32-35,37-39,49,54,65,80,86,87]; amide groups (C–N or N–H stretches) at 1,200 to 1,680 cm^{-1} [35,38]; ketone and aldehyde (–C=O stretch) at 1,665 to 1,710 cm^{-1} [17,24,27-31,33,36,37,39,42,45,52,66,67,70-72,75,77,81,88,89]; aldehyde (H–C=O stretch) at 2,695 to 2,830 cm^{-1} [65]; alkane (–C–H stretch) at 2,850 to 3,000 cm^{-1} [46,55,65,86]; and hydroxyl group (O–H bond) at 3,100 to 3,500 cm^{-1} [31,34,40,42,55,66,71,75,80,81,86-88,90] (Table 3). According to the results of FT-IR, the physical properties of PE certainly change under treatment with biodegrading microbes, however because there are many cases in which the structures of PE alkane polymers can change, the exact products of PE treatment with biodegrading microbes have not yet been sufficiently identified.

In the previous investigations, the byproducts of PE degradation by cultured microbes have been isolated from culture supernatant and identified using GC-MS. Diverse compounds, including hydrocarbons, alkanols, carboxylic acids, and aromatic compounds have been detected in the analysis of microbe-treated PE [17,31,33,35,39-41,47,66,71,76,86]. These include 2-butene, 2-methyl, acetone, and ethene from *Acinetobacter baumannii* [24]; hexadecane, heptadecane, octadecane, tetracosane, hexacosane, heptacosane, eicosane, docosane, octacosane, nonacosane, triacontane, hexatriacontane, dotriacontane, tetradecanol, hexadecanol, and 1-hexacosene from *Bacillus* spp. [17]; 2-dodecanol, 1,8-nonanediol, and 1-decene from a mixture of a *Bacillus* sp. and a *Paenibacillus* sp. [86]; 6-methyl-5-hepten-2-ol, monobenzyl phthalate, *N*-acetylglutamic acid, and ethyldodecanoate from *Enterobacter* sp. D1 [39]; 2-

Table 3. Identification of FT-IR peaks from potential PE-biodegrading microbes

Wave number (cm^{-1})	Bond	Functional group	References
650–1,000	=C–H bond	Alkenes	[46,54,65,79,86]
1,000–1,320	–C–O stretch	Alcohol, carboxylic acid, esters, ethers	[25,26,29,31,32,34,35,37-39,49,54,65,80,86,87]
1,200–1,680	C–N or N–H stretch	Amide	[35,38]
1,665–1,710	–C = O stretch	Ketones, aldehyde	[17,24,27-31,33,36,37,39,42,45,52,66,67,70-72,75,77,81,88,89]
2,695–2,830	H–C = O	Aldehyde	[65]
2,850–3,000	–C–H stretch	Alkanes	[46,55,65,86]
3,100–3,500	O–H bond	Hydroxyl group	[32,34,40,42,55,66,71,75,78,80,81,86-88,90]

FT-IR: Fourier transform infrared spectroscopy, PE: polyethylene.

butene, 2-methyl, ethene-1,2-dichloro-, acetic acid methyl ester, methane, and trichloro- from *Klebsiella pneumoniae* CH001 [40]; decane, 2-methylundecane, 5,8-diethyldodecane, 2-methyltridecane, 2,6,11-trimethyldodecane, 2-methylpentadecane, 2-methylhexadecane, 2-methyloctadecane, docosane, tetracosane, hexacosane, heptacosane, isooctacosane, octacosane, nonacosane, 2-methylbenzaldehyde, 2,5-dimethylbenzaldehyde, 2,5-methyl-2,4-di-tert-butylphenol, benzenepropanoic acid, and decanedioic acid from *Lysinibacillus* sp. JJY0216 [41]; methylbenzene, tetrachloroethylene, 1,3-methylbenzene, octadecane, 7,9-di-tert-butyl-1-oxaspiro(4,5)-deca-6,9-diene-2,8-dione, hexadecenoic acid, hexadecenoic acid-ethyl ester, eicosane, octadenoic acid, docosane, 2-chloropropinic acid-heptadecyl ester, tricosane, octadecanoic acid-butyl ester, 1-nonadecene, tetracosane, pentacosane, 1,2-benzenedicarboxylic acid, diisooctyl ester, and hexacosane from *Pseudomonas aeruginosa* PAO1 [47]; diglycolamine from *A. alternata* FB1 [66]; and 1,3-dimethoxy-benzene, 1,3-dimethoxy-5-(1-methylethyl)-benzene, and 1,1-dimethoxy-decane with other compounds from *Diaporthe italiana*, *Thyrostroma jaczewskii*, *Collectotrichum fructicola*, and *Stagonosporopsis citrulli* [71]. The GC-MS analysis of PE-degraded byproducts may provide a better understanding of the biochemical hydrolyzing mechanisms by which microbes degrade PE. Future studies should aim to investigate the possible metabolic pathways for PE biodegradation by exploring microbial PE-degrading enzymes.

5. Putative Microbial PE-degrading Enzymes

Gao *et al*. [66] proposed a mechanism of PE biodegradation using the results of a transcriptome analysis of the marine fungus *A. alternata* FB1. In addition, our research group has also been proposed a mechanism of PE biodegradation via P450 monooxygenase and hydroxylase as trigger enzymes [91]. Alkane monooxygenase, hydroxylase, peroxidase, alcohol dehydrogenase, aldehyde dehydrogenase, Baeyer-Villiger monooxygenase, laccase, esterase, cutinase, and lipase are suggested to be involved in the decomposition of PE [8,66,91] (Fig. 3A). These enzymes have been reported as key enzymes in PE degradation in various microbes [25,45,46,48,49,65,66,71,88]. In general, breakdown of alkane chains occurs when hydroxyl groups are inserted into middle and terminal alkane chains by alkane monooxygenases and/or hydroxylases [92,93]. The modified alkane chains are then further converted to diverse products by the various enzymes mentioned above. Some

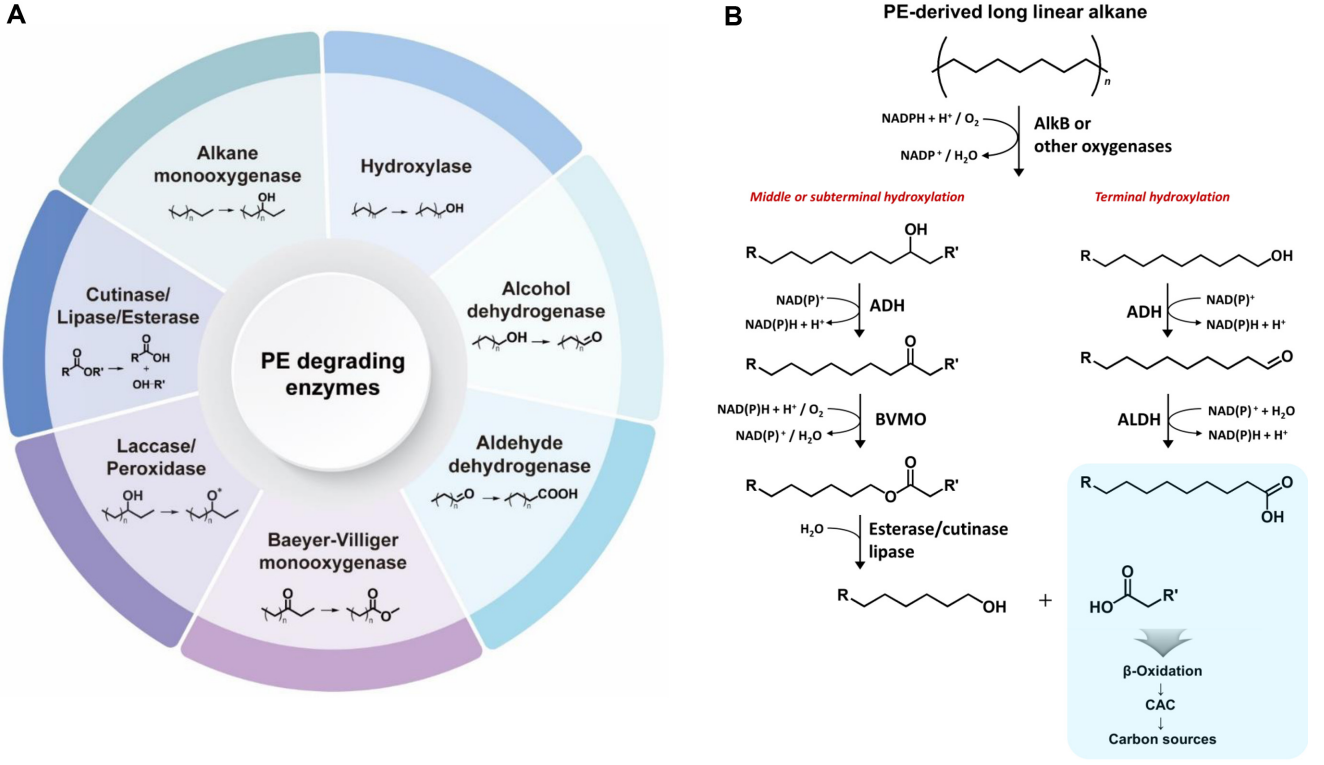


Fig. 3. (A) Potential PE-biodegrading enzymes. (B) The proposed degradation pathway of PE-derived long linear alkane by the potential biodegrading enzymes. PE: polyethylene, AlkB: alkane monooxygenase, ADH: alcohol dehydrogenase, BVMO: Baeyer-Villiger monooxygenase, ALDH: aldehyde dehydrogenase, CAC: citric acid cycle.

peroxidases, including catalase, manganese peroxidase, versatile peroxidase, oxygenase, and laccase are involved in PE degradation, which might lead to a reduction in PE molecular weight, and ultimately destroy the PE polymer after oxidation. Carbonyl groups can be introduced along the PE chain. The reduced PE molecules can be recognized as intermediate products and/or substrates for hydroxylase, monooxygenase, and oxygenase to produce alcohol compounds, which can be further oxidized to ketones by alcohol dehydrogenase, and then converted to esters by Baeyer-Villiger monooxygenase [22,66,94]. Esterase is a hydrolase that splits esters into alcohols and acids by using water molecules. Cutinase and lipase are subclasses of esterases that also catalyze the hydrolysis of high–molar mass polyesters and lipids, respectively [95]. Converted esters are subsequently cleaved by esterase, cutinase, and lipase [96], which can lead to the production and β-oxidation of fatty acids; the generated compounds can then be used as metabolites and carbon sources (as well as the CO_2 and H_2O) during mineralization [66] (Fig. 3B). Although several types of enzymes could be used to degrade PE, one enzyme alone may not be capable of fully degrading PE, as breaking or degrading consecutive alkane chains (C−C bonding) is difficult for a single enzyme. Thus, research efforts should focus on discovering and developing various enzymes for PE degradation.

6. Future Perspectives on PE Biodegradation Based on Synthetic Biology Approaches

Genetically engineering microbes to enhance their biodegradation capacities may be an unideal method to increase PE degradability due to poor genetic engineering techniques and variability in live cells. In synthetic biology, predictable pathways and genetic engineering accelerate development of new artificial bacteria, such as PE-biodegrading bacteria. To develop new artificial PE-biodegrading bacteria, PE's accessibility to bacteria should be considered, as PE is generally insoluble and large. Thus, biofilm formation using exopolysaccharides needs to be considered as the first step of PE degradation in which bacteria are able to adhere to the PE surface; after this step, depolymerization of the polymer chain could occur through the secretion of extracellular enzymes [97]. The depolymerized compounds (lower-molecular-weight compounds) could be transferred into the bacterial cytoplasm during the assimilation and mineralization stages, after which the bacteria could uptake the depolymerized PE compounds by releasing CO_2, CH_4, H_2O, and N_2. Additionally, the expression of heterologous enzymes in the flagella and formation of metabolons on the bacterial cell membrane could be considered to further investigate the development of PE-biodegrading bacteria [91,98,99].

The development of efficient PE-biodegrading bacteria should involve the adoption of various enzymes into cells for use in PE biodegradation. In this regard, the strategies for multi-enzyme cascade reactions have been covered in several comprehensive reviews [100-103]. Making scaffolds via enzyme immobilization represents a remarkable approach to increasing enzyme stability and overcoming the costs associated with expensive cofactors. The localization and clustering of multi-enzymes could be used for bottom-up design that improves their performance. Scaffold systems are synthetic biological methods in multi-enzyme assembly that can control spacing, orientation, types, and linkers, including the displayed tags for multi-enzyme complexes, which can also supply higher reducing powers using cofactor-coupled cascade reactions [104]. To assemble the multi-enzymes, nucleic acids and protein/protein- or protein/peptide-based scaffold systems can be used [104]. In this section, we discuss the protein/protein- or protein/peptide-based scaffold systems that can be used in the development of multi-enzyme cascade reactions in PE decomposition using potential PE-degrading enzymes. The assembly of multi-enzymes can be cross-linked by high-affinity interaction via binding domains or peptides (tags) placed at one or more enzyme termini. Assembly can then be achieved to control the spatial organization of enzymes, transfer or regenerate cofactors, and make substrate channeling [104-107]. RI anchoring disruptors (RIAD, 18 residues) and RI binding domains (RIDD, 44 residues) are strong affinity peptide pairs that are derived from the dock and lock peptide interacting family, which can be assembled using disulfide-bond interactions between cysteine residues. RIAD and RIDD enable multi-enzymes to make closer together than free enzymes (Fig. 4). Consequently, the catalytic efficiencies and/or stabilities of multi-enzyme complexes can be increased [108]. Metazoan peptide motifs (interacting domains) such as the GTPase-binding domain (GBD), Src homology 3 (SH3), and PSD95/Discs Large/ZO-1 (PDZ) are fused directly. These motifs were used in the mevalonate biosynthetic pathway with three enzymes, resulting in a 77-fold increase in metabolic flux [109]. The proliferating cellular nuclear antigen (PCNA) complex is a scaffolding system with a ring shape (Fig. 4). Multimeric PCNA was applied to connect three enzymes, specifically cytochrome P450 and its two redox partners, ferredoxin and ferredoxin reductase, to increase electron transfer efficiency [110]. This finding indicates that the PCNA system can be used for cofactor regeneration when multi-enzymes need cofactors. Co-localization of multi enzymes is driven by dockerin–cohesin interaction domains developed with cellulose binding domains (CBM), which

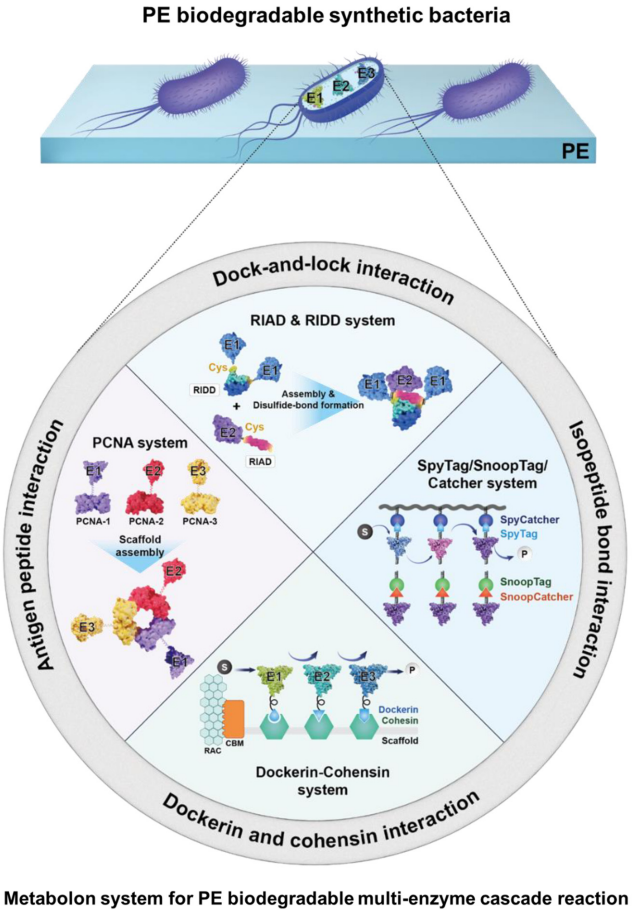


Fig. 4. Future application for PE decomposition using PE biodegradable synthetic bacteria. PE: polyethylene, RIAD: RI anchoring disruptor, RIDD: RI binding domain, PCNA: proliferating cellular nuclear antigen, RAC: regenerated amorphous cellulose, CBM: cellulose binding domain, S: substrate, P: product.

allow for the assembly of regenerated amorphous cellulose with the cellulosome and multi-enzymes (Fig. 4). Another well-known example of such a system is the SpyTag/SpyCatcher tagging system (Fig. 4), which has high affinity and has been used in several studies [111]. The SpyTag/SpyCatcher system and its derivatives are useful not only *in vitro* assembly of enzymes but also in *in vivo* systems. SpyTag and SpyCatcher contain 13 and 116 amino acids, respectively, which have high affinity via the formation of intermolecular isopeptide bonds under a wide range of temperatures and pH values. Another Tag/Catcher system, SnoopTag/SnoopCatcher, is also an orthogonal tagging system that can be combined with the SpyTag/SpyCatcher system to assemble polyprotein complexes [112]. Combined Spy/SnoopTag/Catcher systems can be further used to assemble and compartmentalize multi-enzymes to increase the activities of formed polyprotein complexes. To develop the enzymatic pathways for PE biodegradation, we need to discover and engineer the proper enzymes, which can be improved with multi-enzymatic scaffold platforms based on synthetic biological approaches. Additionally, we should consider increasing the reducing power and redox balance in multi-enzyme cascade reactions to create efficient PE-biodegradable biocatalysts systems in the future. Furthermore, these multi-enzymatic scaffolds would be involved not only in PE decomposition but also several types of biocatalytic processes in industrial fields.

7. Conclusion

The pollution of plastics including PETE, PE, PVC, PP, PS, polycarbonate, and polyurethane is increasing, and many researchers have been trying to remedy these environmental problems. We summarized studies of microorganism-mediated PE degradation. So far, evaluations of PE biodegradation have used FR-IR, SEM, GC-MS, AFM, WCA, XRD, and WL techniques, and considered various PE types (MF, F, S, Pi, Pa, B, and Po). We focused on comparing the PE-decomposing abilities of microbes quantified with WL, because WL can reflect the real biodegradation of PE. This review also summarized potential PE-degrading enzymes in various microorganisms. Furthermore, our future directions for the development of PE-biodegrading pathways using microbial enzymes and scaffold systems should be explored in future PE decomposition studies.

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Ethical Statements

The authors declare no conflict of interest.
Neither ethical approval nor informed consent was required for this study.

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