

REVIEW

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# A critical examination of advanced approaches in green chemistry: microbial bioremediation strategies for sustainable mitigation of plastic pollution

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## Abstract

**Background** The escalating concern regarding the environmental impact of plastic waste necessitates the adoption of biodegradable methodologies to curtail its adverse effects. A profound comprehension of the intricate interplay between bacteria and polymers becomes imperative for devising effective solutions to address plastic-induced environmental challenges.

**Main body of the abstract** Numerous microorganisms have evolved specialized mechanisms for the degradation of plastics, rendering them amenable to application in green chemistry for the elimination of hazardous plastics from the ecosystem. This article offers a comprehensive survey of contemporary microbial bioremediation approaches geared towards augmenting plastic waste management and ameliorating plastic pollution. Emphasis is placed on elucidating the potential of microorganisms in mitigating the deleterious repercussions of plastics on ecosystems and human health, underscoring the significance of advanced strategies in green chemistry for sustainable plastic pollution mitigation.

**Short conclusion** Current research emphasizes the effectiveness of naturally occurring soil microorganisms, particularly fungi like *Aspergillus* and bacteria like *Bacillus*, in breaking down plastics. To harness this potential on a broader scale, optimization of microbial activity conditions and pre-treatment with environmentally beneficial compounds are essential.

**Keywords** Ecosystem health, Microplastics, Sustainable remediation, Biodegradation, Plastic waste

## Background

According to recent statistics, the world's industries produce roughly 140 million tonnes of plastic annually, with a more significant proportion of that amount being released into the environment as garbage [1–4].

The use of chemicals, detergents, cosmetics, medicines, and food packaging accounts for thirty percent of these tonnes [5]. Approximately 64% of synthetic plastics are made of polyethylene, which has a high molecular weight and hydrophobicity. A considerable amount of the 500 billion to 1 trillion polythene materials manufactured annually worldwide end up in the natural environment (land and water) [6–9]. This raises serious environmental concerns. Approximately 10% of municipal garbage produced worldwide is attributed to packaging materials like polythene [10–12]. However, only 5% of the trash is recycled, and the remainder is

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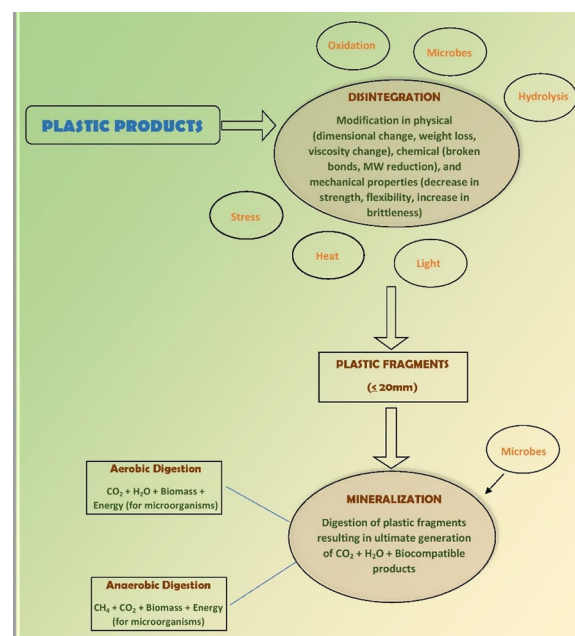
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buried underground, where it takes roughly 100 years for the material to decompose naturally without the aid of bacteria [13–15]. This results from their resistance, perseverance, and inability to degrade [16–18]. Humans may be subjected to severe health and ecological stresses as a result of this pollution. Particularly worrying microplastics have been linked to instantaneous mortality when ingested by aquatic creatures [19–22].

Due to the rise in environmental issues caused by plastics, the usage of physical [23, 24], and chemical [25–27] methods to break down plastic garbage has been condemned. The biological destruction (Biodegradation) of plastic using bacteria and fungus [28–30] has gained popularity recently owing to their efficacy, affordability, environmental friendliness, and sustainability. Multiple factors, including substrate accessibility, polymer surface area, shape, and molecular weight, all play a role in the biodegradation process [31, 32]. One may use a variety of metrics to assess this deterioration, including by the amount of carbon dioxide released into the atmosphere, the percentage change in the mechanical characteristics and/or chemical structure of the polymer, and the amount by which the substrate itself degrades. At the beginning of research into microbial biodegradation, scientists looked into how microbes may affect the physical qualities of plastics, such as water absorption, crystallinity, and tensile strength. Plastic waste may be assimilated into carbon sources or degraded into important alkane compounds using microbial biotechnology. This offers a promising possibility to increase plastic recycling and, by extension, to minimize environmental plastic pollution [33–35].

Microbes may break down plastics by first producing extracellular enzymes, then attaching those enzymes to the surface of the plastic, then hydrolyzing the plastic into short polymer intermediates, and finally ingesting those intermediates as a carbon source in order to produce carbon dioxide (Fig. 1). In recent years, several bacteria capable of degrading these polymers have been found, despite the synthetic nature of these polymers. Together with the bacterial consortia, abiotic factors facilitate the mineralization, assimilation, depolymerization, and fragmentation of environmental plastic wastes into carbon dioxide, nitrogen, methane, and water molecules, monomers, dimers, and oligomers [36–38]. Since the 1970s, certain strains of bacteria from the genera *Aspergillus* [39–41], *Penicillium* [42–44], *Streptomyces* [45–47], *Pseudomonas* [48–50], and *Bacillus* [51–53] have been utilized to break down plastic trash. Though the microorganisms responsible for plastic breakdown have been narrowed down, further study is required to confirm the identities of the specific causes.



**Fig. 1** Biodegradation process of plastic waste

Global concern has been raised about the enormous amount of poly(ethylene terephthalate) (PET) [54–56], polyvinyl chloride (PVC) [51, 57], polyamide (PA), polyethylene (PE), polypropylene (PP) that appears to take centuries to break down in the environment. Moreover, the COVID-19 pandemic has amplified the already alarming issue of plastic pollution, driving an unprecedented surge in the demand for single-use plastics such as personal protective equipment (PPE) [58]. This surge has further strained an already overwhelmed waste management system and exacerbated the pollution of our natural environments. In response to this, there is a growing emphasis on exploring innovative strategies to tackle plastic pollution. One such strategy gaining traction is plastic bioremediation. As the imperative for sustainable solutions intensifies, the focus on biodegradable methodologies gains prominence, necessitating a nuanced understanding of the intricate symbiosis between bacteria and polymers. This discourse delves into advanced microbial bioremediation strategies grounded in green chemistry, offering a comprehensive exploration of cutting-edge approaches to enhance plastic waste management and alleviate the escalating spectre of plastics pollution.

The primary contributions of this research article are as follows:

1. From the standpoint of developing plastic waste management, the study provides substantial

information regarding microbes capable of effectively digesting polymers.

2. The study describes several ways in which bacteria may be used to break down plastic.
3. The study's principal goal is to understand how bacteria are used in the control of trash plastics.
4. The study also seeks to uncover existing developments in the microbial breakdown biodegradation of plastic trash.

The remainder of the study is structured as follows: Section "Research approach" provides specifics on the methodology used, which was derived from best practises for critical literature reviews. In the section titled "Biodegradation of Plastic Waste by Microorganisms," we briefly address the bioremediation by various microbes. The section under "Limitations" describes the restrictions that this study had to operate within. The last section of the paper, "Conclusions," outlines the entire work.

### Research approach

The Web of Science, Scopus, PubMed, and Google Scholar were among the databases searched as criteria for including and excluding the study. The search was conducted using key terms related to the microbial bioremediation of plastic waste. Additionally, the "AND" and "OR" Boolean operators were used to construct relevant words. After data source evaluation, all filtered sources were collected and checked for duplication using Mendeley Desktop Version 2.61.1. Titles and abstracts served as the primary criteria for screening. Full-text screening was also applied to the remaining articles. Studies evaluating incomplete publications (In press) and papers on the auxiliary subject were disregarded. We further excluded correspondence, discussions, editorials, books, systematic reviews, book chapters, conference abstracts, doctoral dissertations, and brief communications. This study included papers that discussed the role of microbial bioremediation in the removal of plastic waste. Additionally, we manually looked through relevant and referenced papers from the research and reviews that were included.

### Main text

The process of plastic waste decomposition by microbes is closely linked to the chemical makeup of the polymers, environmental factors, and microbial behaviour. Microorganisms are essential in the process of decomposing plastic polymers into smaller pieces, which eventually results in the transformation of these pieces into innocuous chemicals such as carbon dioxide and water. The degradation of polymers is an intricate process that is affected by both inherent characteristics

of the polymer and external environmental influences. The chemical composition of a polymer, which includes its arrangement, presence of different atoms, and other substances, greatly affects its vulnerability to degradation [59]. Polymers consisting only of carbon chains, particularly those containing double bonds, exhibit greater inertness in comparison to polymers including heteroatoms or additives [60]. Their high level of purity reduces their susceptibility to external influences, hence decelerating the process of deterioration.

The length and content of the carbon backbone are significant factors. Polypropylene, which has longer chains, often demonstrates resistance to degradation [61]. However, the inclusion of heteroatoms might potentially undermine this resistance. Moreover, the degradation rates are influenced by the polarity of the polymer, with nonpolar molecules exhibiting lower susceptibility to degradation. The degree of crystallinity of a polymer also impacts its degradation. Crystalline polymers have a higher resistance to degradation compared to amorphous compounds [62]. They require less water and oxygen to start decomposing. The molecular weight of polymers is a significant factor that affects their degradation rate. Polymers with larger molecular weights have smaller relative surface areas, resulting in slower degradation [63].

The degradability of a polymer is further influenced by the production method and the additives employed. Within landfills, the combination of UV radiation and heat can trigger breakdown by auto-oxidation, causing polymers to break down into microplastics [64, 65]. These microplastics are then further degraded by microbes, resulting in the production of carbon dioxide and water. Polymers such as polyethylene, polypropylene, and polystyrene mostly break down in the presence of oxygen and exposure to UV radiation [66]. This process results in the formation of different end products, which vary depending on the kind of polymer.

The process of anaerobic degradation that occurs in landfills leads to the generation of methane and water, which is facilitated by microbial enzymes that aid in the breakdown of polymers [67]. During the process of degradation, petrochemicals undergo changes such as increased brittleness, discoloration, and the formation of new functional groups. Microorganisms have a tendency to attack the shapeless parts of plastics, whereas the structured sections break down at a slower rate.

### Bioremediation by *Achromobacter* sp.

*Achromobacter* (Alcaligenaceae family) is a bacterial genus belonging to the Burkholderiales order. The cells are straight rods motile by one to twenty percent of flagella. They are aerobic and may be found in

fresh and saltwater and soil. Also recognized as a contaminant in laboratory cell cultures [68, 69]. In a study published in 2022, to expedite the biodegradation of thermo-oxidatively pretreated PVC and Low-Density Polyethylene (LDPE), researchers have successfully identified *Achromobacter denitrificans* from compost [70]. In bacterial flasks made of PVC and LDPE, the percentage of dry weight lost was 12.3% and 6.5%, respectively, and the amount of extracellular protein was 326.4 and 112.32 mg/L, respectively. PVC underwent treatment that caused its pH to rise to 5.12, and its thermal stability was enhanced by 29 °C. Fourier Transform Infrared Spectroscopy (FTIR) results show that chain breakage in the major backbone, synthesis of new groups, and oxidation of antioxidants have all altered the chemical composition of LDPE. The carbonyl groups formed as a byproduct of LDPE breakdown are responsible for the appearance of peaks between 1700 and 1850  $\text{cm}^{-1}$ . Scanning Electron Microscopy (SEM) verified surface changes in LDPE and PVC.

Another research found that a novel bacterial *Achromobacter xylosoxidans* influences the structure of High-Density Polyethylene (HDPE) [71]. By studying the coding sequences of the 16S ribosomal subunit, a hitherto undiscovered strain of *A. xylosoxidans* known as PE-1 was extracted from the soil and identified. Degradation of the HDPE chemical structure was seen in analyses of foil samples performed using SEM and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR). As a consequence, HDPE foil was found to lose around 9% of its weight. On the basis of a comparison between the spectrum of the raw material before the bacterial treatment and the range from a database of spectra, it was anticipated that the microorganisms primarily depended on the HDPE for their carbon and energy needs.

#### Bioremediation by *Aspergillus* sp.

Fungi of the genus *Aspergillus* are often found living as saprophytes in the soil, where they consume dead plants and other organic matter, including seeds and grains. The individuals that belong to this genus can flourish in environments with high osmotic pressure. Because of the high oxygen tension, species of the genus *Aspergillus* may be found in almost all environments rich in oxygen. In these environments, they often take the form of moulds on the surface of the substrate [72–74]. An investigation was carried out on the biodegradation of black LDPE sheets by a fungus isolated from several Egyptian landfills [75]. For 16 weeks, minimum salt medium and LDPE sheets were heated to 30 °C and rotated at 120 rpm in a rotary shaker. The fungal strains *A. fumigatus* MF 276893 and *A. carbonarius* MH 856457.1 were found to

be promising LDPE biodegradation agents. The sheet weight loss percentage was much higher in a mixed culture of the two strains compared to a single isolate. Physical and chemical treatments were also used to increase the degradation capacity. By 5.89% (chemical treatment), 17.76% ( $\text{HNO}_3$  treatment), and 39.1% (heat treatment), biodegradation was found to be accelerated. New functional groups associated with hydrocarbon biodegradation were validated by FTIR, demonstrating the essential involvement of manganese peroxidase in the process. In addition to surface changes in biodegraded LDPE (as determined by SEM), differences in FTIR spectra of mixed culture biomass before and after biodegradation proved that LDPE was depolymerized. It has been reported that these strains are capable of complete biodegradation of plasticizers such as tributyl acetyl citrate, 1,2-benzenedicarboxylic acid diisooctyl ester, diisooctyl phthalate, and bis(2-ethylhexyl) phthalate, using Gas Chromatography-Mass Spectrometry (GC-MS). Another research determined five fungal isolates, including Brown rot, White rot, *A. flavus*, and *A. Niger* fungi isolated from various landfills in Peshawar, Pakistan [29]. Weight loss percentage analysis after 30 days of incubation was used to determine the biodegradation potential of these isolates against LDPE polymers. white rot, brown rot, *A. flavus*, and *A. niger* fungus all demonstrated biodegradation percentages of 22.7%, 18.4%, 16.1%, and 22.9%, respectively.

Further research used *Fusarium solani*, *A. versicolor*, and *A. flavus*, all of which were retrieved from a municipal waste yard in Chennai, India, to study the biodegradation of LDPE [76]. The polymers were tested for degradation by exposure to microbial cultures for 60 days in the lab. FTIR spectra verified the biodegradation of LDPE, whereas Field Emission Scanning Electron Microscopy (FESEM) micrographs demonstrated that the fungi had colonized the polythene matrix as a result of their metabolic activities. Sturm test results suggest *A. versicolor* strain is a more promising LDPE-degrading option than the *F. solani* and *A. flavus* strains. Under controlled laboratory conditions, the biodegradation rate of LDPE sheets was measured after being inoculated with bacteria and fungi collected from various locations around the Dandora dumpsite [77]. Researchers incubated the LDPE sheets for 16 weeks at 37 °C for bacteria and 28 °C for fungus. *A.s oryzae* strain A5 showed the greatest fungal degradation activity, decreasing the average weight by  $36.42 \pm 5.53\%$ . Findings suggest that *Aspergillus*, *Bacillus*, and *Brevibacillus* are promising candidates for biodegrading LDPE. Moreover, a group of researchers extracted fungal candidates from a nearby dumping site. Mushrooms were grown in a broth made of mineral salts and LDPE powder. In broth

medium supplemented with LDPE, only two (RH06 and RH03) of the nine isolates showed the maximum growth response. The findings showed that after 45 days of culture, there was a 5.13% drop in the weight of LDPE film when using isolate RH03, and there was a 6.63% decrease when using isolate RH06. In addition to this, the tensile strength of the treated film was found to be reduced by 58% over the board and 40% in each isolate. The LDPE film's surface developed a groove and a roughness, as shown by an electron microscopy analysis. Moreover, DNA sequencing and Polymerase Chain Reaction data confirmed that strains RH06 is *A. nomius* and RH03 is *Trichoderma viride*, with a 96% and 97% degree of similarity, respectively. The ability of *A. clavatus* to degrade LDPE in an aqueous medium was observed for 90 days [78]. PE mass loss, CO<sub>2</sub> evolution measured by the Strum test, FTIR, and SEM/Atomic Force Microscopy (AFM) morphological alterations all corroborated the deterioration. Researchers used enrichment culture and screening processes to identify two strains of *Lysinibacillus* sp. and *Aspergillus* sp. from waste soils in Tehran, which showed outstanding capacities to break down LDPE [79]. UV-irradiated and non-irradiated pure LDPE films without pro-oxidant additives underwent 126 days of biodegradation in soil with and without mixed cultures of selected microorganisms. As seen by carbon dioxide soil measurements taken after 126 days, biodegradation was moderate in the absence of microorganisms; UV-irradiated and non-UV-irradiated LDPE mineralization was only 8.6% and 7.6%, respectively. Biodegradation was much more effective when the targeted microorganisms were present, with biodegradation percentages for UV-irradiated and non-UV-irradiated films being 29.5% and 15.8%, respectively. When UV-irradiated LDPE was biodegraded in soil containing the designated microorganisms, the percentage decline in the carbonyl index was more pronounced. X-ray diffraction (XRD), FTIR, and SEM confirmed that the chosen microorganisms were able to alter and colonize both kinds of PE.

An *A. flavus* fungi PEDX3 was identified from the digestive tract of the wax moth, *Galleria mellonella* [41]. The results of a 28-day incubation period demonstrated that strain PEDX3 was capable of breaking down HDPE MPP (microplastic particles) into the MPP with reduced molecular weight. As measured by FTIR, the breakdown of PE was further confirmed by the presence of carbonyl and ether groups of MPP. Additionally, Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was used to look for possible degradation enzymes. At the end of the degradation process, two genes, AFLA 053930 and AFLA 006190, encoding laccase-like multicopper oxidases, were found to have had their expression

levels increase, indicating that they encode probable PE-degrading enzymes. In another investigation, *A. flavus* VRKPT2 and *A. tubingensis* VRKPT1 isolated from the PE trash deposited in marine coastal regions were tested under in vitro conditions to be efficient in HDPE breakdown [80]. The isolated fungus was identified based on internal transcribed space (ITS) homology sequence analysis. Even after 1 month of incubation, the biofilm development detected using an epifluorescent microscope revealed the vitality of fungal strains.

#### Bioremediation by *Bacillus* sp.

*Bacillus* species are rod-shaped, aerobic, sporulating bacteria abundant in nature. They may be either obligatory aerobes, which are oxygen-dependent, or facultative anaerobes and may thrive without oxygen [81]. A study was set out to determine how effective bacterial isolates were in degrading microplastics in the Vaigai River in Madurai, India [82]. After being properly processed, the isolates were included in the degradation of UV-treated PE and PP. Four bacterial isolates, including *Bacillus* sp. (BS-2), *Bacillus paramycooides* (BP), *Bacillus cereus* (BC), and *Bacillus* sp. (BS-1), passed the first screening and were evaluated for the 21-day degrading experiment. Bacterial isolates were stuffed into the microplastics, and a shake flask experiment was conducted using two different methods, each with a control. Degradation of the microplastics was demonstrated by a decrease in their weight, an increase in their fragmentation, and a shift in their surface area compared to control studies (microplastics without isolates). Although PP degradation was most significant with BP (78.99 ± 0.005%) and BC (63.08 ± 0.009%) when used separately, the greatest PP and PE degradation were achieved when BC and BP were used together (78.62 ± 2.16% and 72.50 ± 20.53%).

Activated sludge was studied as a potential biocatalyst for the degradation of microplastics in water [83]. It was initially tested for its ability to hydrolyze PET polymers pretreated at 100 °C for an hour. To assess degradation potential, the consortium undertook a typical CO<sub>2</sub> evolution test at pH 7–7.5, 30 °C, 168 days reactor residence time, and 2.63 g/L PET concentration. After being incubated, the group was able to break down 17% of the PET. Surface erosion was responsible for the unaltered molecular weight. Biodegradation was also noticeably accelerated in the presence of abundant oxygen. *Agromyces mediolanus* PNP3 and *B. cereus* SEHD031MH were discovered to be two of the consortium's isolated bacterial strains. Even though growth was optimal for both strains when grown on PET medium alone, only *B. cereus* showed enzyme activity in a clear-zone assay. The bacterial degradation of

polyhydroxybutyrate (PHB) was studied in a solid-media culture setting over a range of temperatures and salinities [53]. After 14 days of cultivation on PHB film, studies show that *Bacillus* sp. JY14 can destroy around 98% of PHB. This species was shown to be able to biodegrade P(3HB-co-3HV) and P(3HB-co-4HB).

In a study, sixty marine bacteria were tested for their capacity to digest LDPE [84]. When tested using polythene as the only carbon source for growth, only three were discovered to be effective. Positive isolates were identified by comparing their 16S rRNA gene sequences. The researcher determined that they belonged to the genus *Kocuria*, species M16; genus *Bacillus*, species M27; and genus *Bacillus subtilis*, species H1584. During a 30-day incubation period with H1584, M27, and M16 isolates, PE lost 1.7%, 1.5%, and 1% of its weight. Hydrophobicity on the cell surface was highest (32% in M16), then 15% in H1584, and finally 27% in M27. A triphenyltetrazolium chloride reduction assay was used to verify the vitality of the isolates grown on the PE surface. Calculations of the Keto Carbonyl Bond Index, Ester Carbonyl Bond Index, and Vinyl Bond Index from FTIR spectra showed increases consistent with PE biodegradation.

#### Bioremediation by *Collectotrichum* sp.

*Collectotrichum* (sexual stage: Glomerella) is a genus of endophytes or phytopathogens that are symbionts to plants. Some species in this genus may have a symbiotic relationship with their host plants [85]. Thirty fungi were tested for biodegradability of LDPE films in mineral salt medium agar [86]. *Stagonosporopsis citrulli*, *Collectotrichum fructicola*, *Thyrostroma jaczewskii*, and *Diaporthe italiana* grew much faster than *Aspergillus niger* when grown on LDPE film as the sole carbon source. For a further 90 days, they were grown in a broth made of mineral salts and LDPE film instead of any other carbon source. CO<sub>2</sub> emissions ranged from 0.45 to 1.45 g/L for *D. italiana*, 0.36 to 1.22 g/L for *T. jaczewskii*, 0.33 to 1.26 g/L for *C. fructicola*, 0.37 to 1.27 g/L for *S. citrulli*, and 0.33 to 1.27 g/L for *A. niger* when they were cultured on LDPE film. Compared to the levels of lignin peroxidase and manganese peroxidase secreted by the same fungus, the quantity of laccase enzyme produced was reported to be much higher. It was further investigated how these fungi degrade LDPE sheets when cultured. Weight loss was recorded as 28.78, 45.12, 48.78, 46.34, and 43.90%; tensile strength as 3.34, 1.86, 0.43, 1.78, and 1.56 MPa for LDPE films cultured with *A. niger*, *S. citrulli*, *C. fructicola*, *T. jaczewskii*, and *D. italiana*, respectively. After incubation with various fungi, especially *C. fructicola*, FTIR measurement revealed an increased carbonyl index in LDPE films.

The biodegradation of LDPE films was validated by SEM analysis, which revealed morphological changes on the film's surface, including cracks, scions, and holes. The Volatile Organic Compounds, 1,1-dimethoxydecane, 1,3-dimethoxy-5-(1-methylethyl)-benzene, and 1,3-dimethoxy-benzene were found in these fungi. In terms of biodegradation of LDPE, *C. fructicola* shows promise as a resource and may be incorporated in fungal-based plastic degrading systems.

#### Bioremediation by *Comamonas* sp.

Gram-negative, rod-shaped spirilla (often called "rods") are found in bacteria of the genus *Comamonas*. These microorganisms are chemoorganotrophic, meaning they feed off of organic matter rather than sugars, and they are aerobic [87]. The breakdown of dimethyl phthalate (DMP) by a *Comamonas testosterone* bacterial strain, DB-7, was investigated in a study [88]. The results indicate that DMP at varying doses was quickly destroyed, with over 99% degradation occurring within 14 h at 450 mg/L. The breakdown rate of DMP was found to be positively proportional to the inoculum volume of the bacteria, with the ideal degradation temperature being 30–35 °C and pH 9.0, respectively. According to HPLC (High-performance liquid chromatography) and LC/MS (Liquid Chromatography-Mass Spectrometry) studies of metabolic products, phthalic acid (PA) and mono-methyl phthalate (MMP) are the primary degrading intermediates formed by DB-7 during the breakdown of DMP.

#### Bioremediation by *Enterobacter* sp.

The genus *Enterobacter* includes rod-shaped, non spore-forming, gram-negative, facultatively anaerobic bacteria of the Enterobacteriaceae family. The type genus of the family Enterobacterales [89]. A group of researchers conducted research on the breakdown of LDPE by the recently discovered *Enterobacter cloacae* AKS7 [90]. A progressive rise in Extracellular Polymeric Substance (EPS) production by the organism (AKS7) was also identified, indicating the establishment of an effective biofilm on the LDPE surface. In addition, two AKS7 mutants with significantly reduced cell-surface hydrophobicity compared to their wild type were screened. The results of which contrasted to wild-type AKS7 cells, the mutants exhibited lower levels of LDPE breakdown. Further analysis showed that, in contrast to wild-type cells, AKS7 mutant cells lacked the ability to adhere to LDPE. The findings showed that AKS7's hydrophobic cell surface promotes the growth of microbial biofilm on LDPE, leading to more efficient breakdown of the plastic by the microbe. Given these results, the organism may be

evaluated as a bio-remediating agent for the long-term degradation of polythene-based toxic waste.

#### Bioremediation by *Halomonas* sp.

*Halomonas* is a genus of salt-tolerant (halophilic) bacteria. They are rod-shaped gram-negative bacteria and develop in the presence of oxygen. However, it has been reported that some may grow without oxygen [91]. Four bacterial strains with the ability to biodegrade LDPE were identified by a research group [92]. The 16S rRNA gene sequencing technique indicated that bacterial isolates H-265, H-256, H-255, and H-237 were closely related to *Alcanivorax* sp., *Exigobacterium* sp., *Halomonas* sp., and *Cobetia* sp., respectively. Researchers used the Bushnell-Haas medium to incubate these bacterial strains separately for 90 days while providing them with LDPE sheets as a carbon source. Bacterial isolates were able to develop a viable biofilm on the surface of LDPE during the biodegradation experiment, reducing the films' thermal stability. After the incubation research, the bacterial isolate H-255 was shown to have caused a maximum LDPE film weight decrease of 1.72%. FESEM and AFM demonstrated that bacterial adhesion to the film altered its physical structure (surface erosion, roughness, and deterioration). When compared to a control LDPE film, the spectra obtained using ATR-FTIR demonstrated a shift in the peaks associated with C–H stretching and C=C bond stretching and the development of additional peaks associated with C–O stretching and –C=C– bond stretching. Furthermore, carbon remineralization and enzymatic activity validated the biodegradation of LDPE film. This research demonstrated that some marine bacteria actively biodegrade LDPE film, and that these bacteria have the potential to lessen marine plastic pollution.

#### Bioremediation by *Klebsiella* sp.

The gram-negative, encapsulated, non-motile, facultatively anaerobic, lactose-fermenting, rod-shaped bacterium *Klebsiella pneumoniae* is characterized by its unique characteristics. It occurs naturally in the soil, and around 30% of strains are capable of fixing nitrogen under anaerobic environments [93]. *Klebsiella pneumoniae* CH001, a clinical isolate, was screened for bioremediation of HDPE [94]. After 60 days of growth in nutritional broth at 30 °C and 120 rpm, results indicated that this strain could develop a substantial biofilm on HDPE surfaces. The Universal testing machine (UTM) results indicated a considerable drop in HDPE film's tensile strength (60%) and weight (18.4%). In addition, SEM research revealed surface fractures in the HDPE, while AFM findings demonstrated an increase in surface roughness during bacterial incubation. Taken

together, findings suggest that *K. pneumoniae* CH001 is a promising option for the environmentally responsible breakdown of HDPE in natural settings.

#### Bioremediation by *Penicillium* Sp.

*Penicillium* is a genus of ascomycetous fungus that is an integral component of the mycobiome of several species. Certain species of the genus generate penicillin, an antibiotic chemical that kills or inhibits the development of certain types of bacteria. Other species are used in cheese production. According to the tenth edition of the Dictionary of the Fungi (2008), the broad genus has more than 300 species [95]. Because of its rapid colony development in the screening medium, the isolate *Penicillium citrinum* was chosen for biodegradation research. In a research, 16 plastic-degrading fungi were isolated from plastic-laden landfill soil in Bhopal, India [44]. Fungi capable of decomposing PE were screened for using a mineral salt agar medium spiked with 3% LDPE powder. Untreated LDPE fragments lost  $38.82 \pm 1.08\%$  of their weight when exposed to *P. citrinum*; however, after being pretreated with nitric acid, biodegradation increased by  $47.22 \pm 2.04\%$ . New functional groups ascribed to hydrocarbon biodegradation appeared in FTIR spectra, suggesting enzymatic participation in the process. Depolymerization of LDPE was validated by changes in the FTIR spectra and FE-SEM of LDPE samples (both untreated and pretreated) before and after biodegradation. Variations in the rates of thermal breakdown between biodegraded and control samples provide more evidence of biodegradation. The remarkable competence of *P. citrinum* in LDPE degrading without any pre-treatment has been reported for the first time in this work.

To effectively biodegrade polyvinyl alcohol (PVA) in vitro, researchers set out to discover and broadly screen endophytic fungi (from specified plants) [42]. Seventy-six endophytic fungi were cultured in total on a PVA screening agar medium. Using a combination of phenotypic traits, ITS ribosomal gene sequences, and phylogenetic analysis, 10 isolates were found to have a potential biodegrading effect and were subsequently identified. After 10 days of growth at 150 rpm and 28 °C, four strains showed maximal PVA-degradation in the liquid medium. *Penicillium brevicompactum* OVR-5 removed 81% of PVA, *Talaromyces verrucosus* PRL-2 removed 67%, *Penicillium polonicum* BJL-9 removed 52%, and *Aspergillus tubingensis* BJR-6 removed 41%. OVR-5 was found to be the most promising PVA biodegradation isolate, producing laccase, manganese peroxidase, and lipase enzymes at an ideal pH of 7.0 and an optimal temperature of 30 °C. This work hypothesized a possible PVA breakdown mechanism for OVR-5 in

light of investigations of its metabolic intermediates, which GC–MS discovered. Both SEM and FTIR verified the biodegradation findings.

The antarctic filamentous fungus was studied for its ability to degrade polyurethane (PU), polystyrene (PS), and PE samples in a liquid solution [96]. Plastic samples were either inoculated with Antarctic fungus (*Mortierella*, *Geomyces*, *Penicillium* species), treated, left untreated, or artificially aged in a UV chamber for 500 h per ASTM G155. All samples were kept in an incubator for 90 days at 18 °C. The rate of weight loss was examined as a function of time to evaluate the physical–chemical and biological degradation of plastics. In the artificial ageing chamber, polymers suffered an oxidative breakdown, which sped up their biodegradation (seen as morphological and structural alterations). *Penicillium* sp., of the three fungal strains, showed the most significant breakdown at 28.3% in PU, 8.39% in PS, and 3.5% in LDPE.

In a study, the researcher used garbage bags to isolate fungi and their ability to degrade LDPE. In this case, ethanol-treated LDPE was used alongside untreated LDPE [43]. F1 isolation demonstrated the most degradation out of the three fungal isolates, and this isolate damaged the untreated sheet similarly. Areas of degradation were seen in the surface morphology of F1-treated LDPE as analyzed by SEM. FTIR testing revealed that F1 affected the polymer's production of carbonyl and C=C groups. F1 fungus, when grown in the laboratory, was discovered to release the lipase enzyme. Molecular testing confirmed that isolate F1 was indeed *P. simplicissimum* strain Bar2. In another study, *P. simplicissimum* was discovered in a Shivamogga district landfill by a group of researchers [97]. Findings indicate that treated PE (38%) was more easily degraded by *P. simplicissimum* than autoclaved (16%) or surface-sterilized (7.7%) PE. *P. simplicissimum* was tested for enzymes that degrade PE. Laccase and manganese peroxidase were shown to be active enzymes. Based on these findings, *P. simplicissimum* was reported as a potential answer to the world's PE crisis.

A group of investigators evaluated "Bionolle®" polyester-modified PET films biodegradation in comparison with unmodified PET films in terms of time to decompose [98]. The films' weight was recorded before and after being incubated with the filamentous fungus *P. funiculosum* or their extracellular hydrolytic enzymes released by "Bionolle®" for 84 days. FTIR and X-ray Photoelectron Spectroscopy (XPS) studies revealed significant chemical alterations in polymeric chains. In addition to hydrolytic enzymes, oxidative ones were likely involved in the degradation of films by fungi, as shown by the significant decrease in the number of aromatic rings

formed from terephthalic acid. Additionally, "Bionolle®" did not accelerate modified film deterioration.

#### **Bioremediation by *Phanerochaete* sp.**

*Phanerochaete* is a crust fungus genus belonging to the Phanerochaetaceae family. It has historically been classified based on the fruit body's general shape and microscopic features, such as the spores, cystidia, and hyphal structure. According to molecular analyses, the genus is polyphyletic, with members scattered over the phlebioid clade of the Polyporales order [99, 100]. A study examined the biodegradability of starch-blended PVC films using controlled laboratory studies utilizing selected fungus isolates and *in-situ* burial in soil [101]. SEM revealed the surface anomalies such as colour change and mild disintegration in PVC films after 90 days. Isolation of fungal strains characterized by robust growth and adhesion to plastic sheets. *Phanerochaete chrysosporium* PV1 was chosen among the strains exhibiting the highest levels of activity and later confirmed to be this species by rDNA sequencing. FTIR and Nuclear Magnetic Resonance (NMR) studies revealed new peaks, suggesting substantial structural changes and transformation in the films. Gel permeation chromatography (GPC) backed this up by showing a considerable reduction in the molecular weight of polymer film from 80,275 to 78,866 Da (treated). The release of more carbon dioxide (7.85 g/l) than the control (2.32 g/l) in the respirometric technique provided further evidence of the biodegradation of starch-blended PVC films. Hence, suggesting *P. chrysosporium* PV1 is a fungal strain with excellent potential for bioremediation of plastic waste.

#### **Bioremediation by *Pseudomonas* sp.**

There are 191 different species of the genus *Pseudomonas*, which are all gram-negative gamma-proteobacteria in the family Pseudomonadaceae. Members of this genus exhibit a high degree of metabolic variability, allowing them to colonize a wide variety of habitats [102, 103]. It is suggested by research that the *Pseudomonas* sp. found in the digestive tracts of superworms might effectively biodegrade Polyphenylene sulphide (PPS) [49]. The biodegradation time of the bead form of plastic was drastically reduced due to its superior degradation efficiency compared to the standard film type of plastic. Therefore, this work employed plastic beads with a diameter of 300 μm to assess the *Pseudomonas* sp. mediated PPS biodegradation over 10 days instead of film-type plastics. This technology not only compares and verifies the biodegradation performance of different polymers in 10 days, but it also quickly identifies the best bacteria for plastic biodegradation.



As reported, another research set out to examine the biodegradation capabilities of five bacterial strains against PVC, PS, PP, and PE films under aerobic conditions [51]. A generalised aerobic breakdown mechanism for plastics is shown in Fig. 2. *B. flexus* and *P. citronellolis* were chosen as suitable PVC film degraders after preliminary screening. Biodegradation of PVC films was tested using the two strains in 2-L flasks. Fragmentation of the film was found after 45 days of incubation, indicating PVC biodegradation. PVC incubated with *P. citronellolis* had a 10% decrease in average molecular weight, as determined by GPC, suggesting that PVC polymer chains were attacked. These findings led to the selection of the *P. citronellolis* strain for biodegradation experiments. As determined by chemical evaluation of the films after 30 days of incubation, the waste PVC polymers had biodegraded, resulting in a gravimetric weight loss of up to 19%. In conclusion, this study documents *B. flexus* and *P. citronellolis* ability to biodegrade PVC sheets. Both strains were shown to have a negligible effect on PVC polymer, suggesting that they work primarily against PVC additives.

A soil-dwelling bacteria capable of degrading polyester PU was isolated and characterized as strain MZA-85 [104]. It was determined that the bacterium was *Pseudomonas aeruginosa* by 16S rRNA gene sequencing. The strain MZA-85 altered the surface morphology of PU film, as shown in SEM. The FTIR spectrum exhibited an augmentation of the organic acid functional group and a concomitant diminution of the ester functional group. Results from GPC showed a rise in polydispersity, suggesting that microorganisms break down PU polymer chains. After conducting a p-Nitrophenyl acetate hydrolysis experiment, it was discovered that the bacteria produced cell-associated esterases. GC-MS confirmed the synthesis of adipic acid and 1,4-butanediol monomers. Cell growth in the presence of breakdown products and the Sturm test showed that strain MZA-85 mineralized PU into H<sub>2</sub>O and CO<sub>2</sub>. These results suggest that strain MZA-85 and its enzymes may recycle pure monomers in biochemical monomerization. On the other hand, *Pseudomonas* sp. AKS2 can degrade 5 ± 1% LDPE in 45 days without pre-oxidation, which is much quicker than the degradation rates reported in previous investigations [105]. This could be attributable to agents modifying

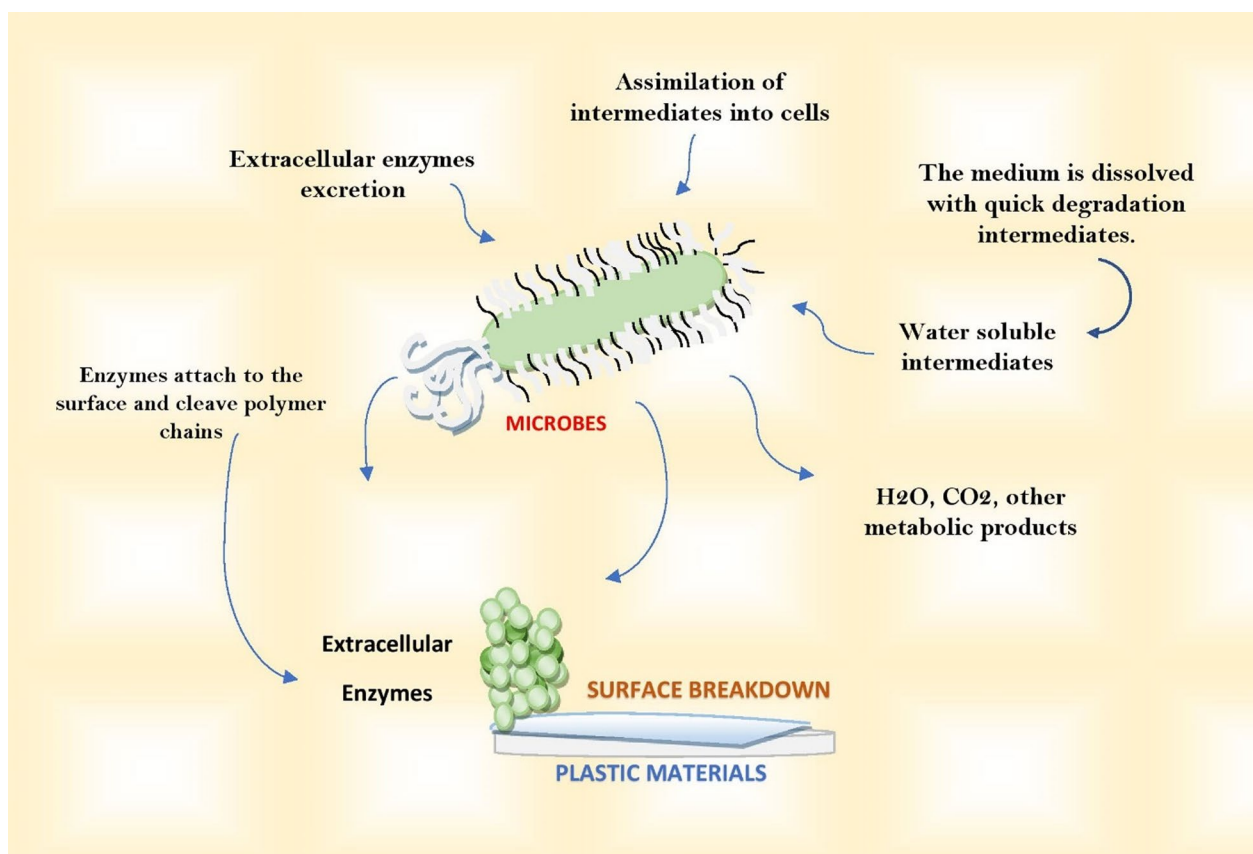


Fig. 2 A generalised aerobic breakdown mechanism for plastics

the hydrophobic contact between the polythene and the microbe, which may affect the breakdown rate. Accordingly, this research links the capacity for biofilm formation among bacteria to their ability to degrade polymers and shows a connection between hydrophobic contact and polymer breakdown.

#### Bioremediation by *Rhizopus* sp.

The fungus genus *Rhizopus* is well-known for its extensive plant saprophytic and its role as a specialist animal parasite. They are present in several organic things, including mature fruits and vegetables, tobacco, peanuts, bread, leather, syrups, and jellies [95]. A fungal lab isolate, *Rhizopus oryzae* NS5 was studied for the biodegradation of LDPE [106]. One month of incubation in a potato dextrose broth at 120 rpm and 30 °C resulted in the development of fungi on the surface of PE. Approximately  $8.4 \pm 3\%$  of the weight and 60% of the tensile strength of PE was shown to decrease gravimetrically. The SEM study of the PE surface revealed hyphal penetration and degradation. After fungal isolation, AFM showed increased surface roughness. Fungal hyphae formed a biofilm on PE fragments. This research demonstrates the potential of *R. oryzae* NS5 for eco-friendly and sustainable PE breakdown.

#### Bioremediation by *Streptomyces* sp.

*Streptomyces* is the most populous genus of Actinomycetota and the type genus of the Streptomycetaceae family. There are around 500 recognized species of *Streptomyces* bacteria. The genomes of streptomycetes are gram-positive. Most streptomycetes generate spores and have a unique “earthy” odour due to the synthesis of the volatile metabolite geosmin, primarily found in soil and decomposing plant matter [107]. As PET trash, drinking bottles were pulverized and categorized into four particle sizes in research work. In their work, they investigated the biodegradation of PET by *Streptomyces* species [47]. Extracted samples totaling 50 mg were divided into four groups based on particle size, each of which was then incubated with a different set of microorganisms in a culture medium at 28 °C for 18 days. Degradation values were then calculated on particular days. The biodegradation percentages for 500, 420, 300, and 212 m PET particle sizes were reported to be 49.2%, 57.4%, 62.4%, and 68.8%, respectively. To further verify the biodegradation process, the byproducts were analysed by GC–MS. Experimental results may be better predicted using the Michaelis–Menten activation or inhibition model, according to the kinetic modelling of biodegradation.

Researchers isolated microorganisms from Andhra Pradesh and Telangana waste soil to prevent plastic buildup and rid the environment of plastic [108]. The degrading activity of these microorganisms is determined using the clear zone approach and polythene powder. Changes in the granules’ physical and structural properties occurred over time after microbes had attached to polymer particles. To determine the effectiveness of biodegradation, the weight technique was used in the laboratory for 2, 4, and 6 months. Experimental results demonstrated that *Streptomyces* sp. had the greatest plastic degradation ability, degrading up to 46.7%; this was followed by *A. flavus* (16.45%), *Pseudomonas* sp. (24.22%), and *A. niger* (26.17%) during a 6-month period. The results of this study demonstrate the critical function that *Streptomyces* sp. plays in the breakdown of polythene powder and polymer granules.

#### Bioremediation by *Zalerion* sp.

The marine fungus, *Z. maritimum*, was discovered in the waters off the coast of Portugal [109]. The researcher assessed mass changes in the fungus *Zalerion maritimum* and PE pellets after different exposure times in a minimum growing medium [110]. Results indicated that *Z. maritimum* is able to use PE under test circumstances, resulting in a reduction in both pellet mass and size. These results point to a naturally occurring fungus that, with its low food requirements, might play an active role in the biodegradation of microplastics.

#### Bioremediation by the synergistic effect

Scientists evaluated the PET-associated lipase activity of bacteria isolated from petroleum-contaminated soils [48]. Bacterial strains and consortiums were cultivated on a liquid carbon-free basal medium (LCFBM) using PET as the only carbon source. Consistent with the ATR-FTIR findings, this work found hydrolysis byproducts of PET using  $^1\text{H}$  NMR analysis. Together, PET and its cleavage product bis(2-hydroxyethyl) terephthalic acid (BHET) supported the growth of five strains of *Bacillus* and *Pseudomonas* species. The consortium’s secreted enzymes could completely convert BHET to the biologically functional monomers ethylene glycol and terephthalic acid (TPA). Strains with different enzymatic abilities for the metabolic breakdown of ethylene glycol and TPA, the building components of PET polymers, were discovered in draught genomes, cooperating and cross-feeding in a nutrient-limited environment utilising PET as the primary carbon source.

Two distinctive cultures of *Arthrobacter* and *Streptomyces* sp. were extracted from farming soils and found to thrive solely on PE film [45]. The suspension phase of culture was very fruitful for the growth of

*Arthrobacter* sp. *Streptomyces* sp. produced huge biofilms on the PE film, showing that the two strains had distinct metabolic types and lived in different microenvironments with differing nutritional availability. CO<sub>2</sub> evolution, increased carbonyl index, reduced hydrophobicity, and the biofilm development on the film surface were all indicators that a 90-day inoculation experiment might deteriorate PE film. However, a combination of the two strains had a far greater effect on these negative characteristics.

Yet another study utilized a synergistic system consisting of *Thermobifida fusca cutinase* (TfC) and *Microbacterium oleivorans* JWG-G2 to decompose a high crystalline PET film and BHET oligomers [111]. Ethylene glycol terephthalate (EGT) has been discovered as the unique degradation product of *M. oleivorans* JWG-G2 alone. The synergy degrees for the degradation of PET film and BHET oligomers with the addition of TfC as a second biocatalyst were determined to be 2.26 and 2.79, respectively. After treating PET film with *M. oleivorans* JWG-G2 at  $5 \times 10^3 \mu\text{L}/\text{cm}^2$  and TfC at 120 g/cm<sup>2</sup>, the highest concentrations of TPA (47 nM) and mono(2-hydroxyethyl) terephthalate (MHET) (330 nM) were found. The degree of surface degradation of PET film was higher than that generated by each treatment on its own. Synergistic microbe-enzyme treatment is based on the occurrence of extracellular PET hydrolases, and a whole genome sequencing research of *M. oleivorans* JWG-G2 showed the presence of extracellular PET hydrolases, including three a lipase, an esterase, and carboxylesterases.

Microplastics composed of LDPE and PS were biodegraded using pure bacterial strains *Lysinibacillus massiliensis* and *Bacillus licheniformis*, as well as a mixed bacterial culture of *Bacillus* sp. and *Delftia acidovorans* [112]. The biodegradation of Microplastic-PS and Microplastic-LDPE with particle sizes between 300 and 500  $\mu\text{m}$  was evaluated for 22 days at  $25 \pm 2 \text{ }^\circ\text{C}$ , 7.15 pH, and 160 rpm. Microplastic-LDPE and Microplastic-PS were both more efficiently decomposed by mixed bacterial cultures than by pure bacterial cultures, and the biodegradation efficiency of Microplastic-LDPE was found to be larger than that of Microplastic-PS, as evidenced by a greater decrease in peak intensity and spectrum distortion, as well as higher inorganic carbon values and colony forming unit.

In another research breakdown of Linear Low-Density Polyethylene (LLDPE) plastic using a microbial culture comprising *Brevibacterium* sp. and *Pseudomonas aeruginosa* was studied [50]. Pieces of  $1 \times 1 \text{ cm}^2$  LLDPE plastic weighing 10 g were placed in containers containing Nutrient Broth growing material. Gravimetric test at pH 7.0, 25  $^\circ\text{C}$  for 30 days demonstrated that a

mixed bacterial culture could degrade LLDPE plastic by 2–7%. The results of this study show that LLDPE plastic may be degraded by mixed bacterial cultures by being used as a carbon source.

Also, novel thermophilic consortiums of *Aneurinibacillus* sp. and *Brevibacillus* sp. isolated from sewer treatment plants and waste management landfills were evaluated for their ability to degrade PP, HDPE, and LDPE films and pellets [113]. Over the course of 140 days, researchers tested the degradation ability of 36 plastic-degrading isolates. To test the efficacy of degradation, multiple combinations of the eight isolating factors that showed the highest percentage of degradation were tested. For the three types of plastic that were selected for further examination of degradation under varying temperature settings, the combination of IS1, IS3, ISA, and ISC revealed the best % weight loss. At 50  $^\circ\text{C}$ , the weight reduction percentages for PP, LDPE, and HDPE strips treated with the consortia of four isolates were  $56.3 \pm 2$ ,  $46.6 \pm 3$ , and  $58.21 \pm 2\%$  and, for pellets treated with the consortia, they were  $44.2 \pm 3$ ,  $37.2 \pm 3$ , and  $45.7 \pm 3\%$ , respectively ( $p \leq 0.05$ ). After 140 days, new adsorption bands could be seen by FTIR scanning of the plastic sheet. AFM and SEM showed biofilm formation and structural alterations on treated plastic strips, while Energy Dispersive X-ray Spectroscopy (EDS) showed a considerable drop in carbon content. NMR revealed methyl and aldehyde groups, whereas GC–MS showed fatty acid byproducts. Four strains—ISC, ISA, IS3, and IS1—identified as *Brevibacillus brevis* btDSCE04, *Brevibacillus* sp. btDSCE03, *Brevibacillus agri* btDSCE02, and *Aneurinibacillus aneurinilyticus* btDSCE01, respectively were found (Table 1).

## Recent advances and challenges

### Reengineering of microbes

New possibilities for developing game-changing biorecycling solutions have emerged as a result of recent developments in biology and biotechnology. First, cutting-edge synthetic biology techniques and metabolic engineering methods have created many potential for reengineering and enhancing bacteria that can successfully digest solid plastic wastes and directly utilise the degraded products for biomanufacturing. Enzyme engineering techniques have been used to improve a number of plastic-degrading enzymes. Also, emerging approaches to protein engineering, such as AI-guided protein design and mutation and direct evolution, may increase the likelihood of creating novel enzymes with resistance to inhibitors or contaminants, temperature tolerance, stability, specificity, and superior activity. Additional methods employ protein alignment data to compare plastic substrate architectures.

**Table 1** An overview of microbial bioremediation of plastic waste

Type of plastic	Microorganism	Place of isolation of microorganisms	Degradation temperature	Degradation time	Microorganism isolation media	Reported degradation	Researchers	References
PE	<i>Aspergillus flavus</i>	The gut of wax moth <i>Galleria mellonella</i>	24 ± 3 °C	14 d	SCS	Up-regulated expression	Zhang et al. (2020)	[41]
	<i>Streptomyces</i> sp. and <i>Arthrobacter</i> sp.	Agricultural soils	25 °C	90 d	CDM and LCFBM	The weight losses of plastic films ranged from 0.22 to 0% in CDM and from 0.49 to 0% in LCFBM medium	Han et al. (2020)	[45]
LDPE	<i>Penicillium simplicissimum</i>	Local dumpsite of Shivamogga district	-	3 months	NH <sub>4</sub> NO <sub>3</sub> + K <sub>2</sub> HPO <sub>4</sub> + NaCl + MgSO <sub>4</sub> · 7H <sub>2</sub> O + agar	Rather than autoclaved (16%) or surface (7.3%) polyethylene degradation is 38% more effective	Sowmya et al. (2015)	[97]
	<i>Zalerion maritimum</i>	Marine	25 °C	28 d	Glucose + malt extract + peptone	43% degradation	Paço et al. (2017)	[110]
	<i>Penicillium citrinum</i>	Municipal landfill soils of Bhopal, India			Mineral salt agar medium	38.82 ± 1.08% weight loss	Khan et al. (2022)	[44]
	<i>Alcanivorax</i> sp., <i>Exigobacterium</i> sp., <i>Cobetia</i> sp., and <i>Halomonas</i> sp.	Water column from Diu Island, India	30 °C	60 d	ZMB and ZMA	1.76% weight loss	Khandare et al. (2021)	[92]
	<i>A. fumigatus</i> MF 276893 and <i>Aspergillus carbonarius</i> MH 856457.1	Landfills sites in Sharqiyah Governorate, Egypt	30 °C	16 weeks	Minimal salt agar and CDM (without sucrose)	The biodegradation rates after thermal, HNO <sub>3</sub> and Gamma-irradiation treatment were 39.1%, 17.76%, and 5.79%, respectively	El-Sayed et al. (2021)	[75]
<i>Collectotrichum fructicola</i>	Thailand Institute of Scientific and Technological Research, Bangkok, Thailand	27.0 ± 2.0 °C	90 d	MSM	LDPE films cultured with <i>A. niger</i> , <i>S. citrulli</i> , <i>C. fructicola</i> , <i>T. jaczewskii</i> , and <i>D. italiana</i> showed weight loss of 28.78%, 45.12%, 48.78%, 46.34%, and 43.90%, respectively	Khruengsa et al. (2021)	[86]	

**Table 1** (continued)

Type of plastic	Microorganism	Place of isolation of microorganisms	Degradation temperature	Degradation time	Microorganism isolation media	Reported degradation	Researchers	References
	Brown rot, White rot, <i>Aspergillus flavus</i> , and <i>Aspergillus Niger</i>	Disposal sites at Peshawar, Pakistan	28 °C	30 d	PDA	Weight loss percentage showed that white rot, Brown rot, <i>Aspergillus flavus</i> , and <i>Aspergillus Niger</i> showed 22.7%, 18.4%, 16.1%, and 22.9% biodegradation, respectively	Hyder et al. (2021)	[29]
	<i>Penicillium simplicissimum</i>	Municipality garbage	28±2 °C	150 d	PDA and RBA (Rose Bengal Agar)	F1 and F2 lost 28.72±2.55 and 12.96±2.00 percent of their initial weight compared to untreated LDPE after 80 d	Ghosh and Pal (2021)	[43]
	<i>Enterobacter cloacae</i> AKS7	Agricultural land of South 24 Parganas, West Bengal, India	30 °C	45 d	Yeast extract + MgS O <sub>4</sub> ·7H <sub>2</sub> O + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + CaCl <sub>2</sub> ·2H <sub>2</sub> O + FeS O <sub>4</sub> ·7H <sub>2</sub> O + NaCl + Na <sub>2</sub> M oO <sub>4</sub> ·2H <sub>2</sub> O + K <sub>2</sub> HPO <sub>4</sub> + MnSO <sub>4</sub> + KH <sub>2</sub> PO <sub>4</sub>	Cell-surface hydrophobicity	Sarker et al. (2020)	[90]
	<i>Bacillus subtilis</i> H1584, <i>Bacillus pumilus</i> M27 and <i>Kocuria palustris</i> M16	Pelagic waters, Arabian Sea, India	-	30 d	Bushnell-Haas medium	M16 has a hydrophobicity of 32% on its cell surface	Harshvardhan and Jha (2013)	[84]
	<i>Fusarium solani</i> , <i>Aspergillus versicolor</i> , and <i>Aspergillus flavus</i> ,	Municipal dump yard in Chennai, India	25 °C	60 d	SDA	17% weight loss by <i>A. flavus</i> , 19% by <i>A. versicolor</i> and 13% by <i>F. solani</i>	Das et al. (2018)	[76]
	<i>Aspergillus Bacillus</i> and <i>Brevibacillus</i>	Dandora dumpsite Nairobi-Kenya	37 °C	16 weeks	Bacteria: 15% glycerol slants; Fungi: PDA	<i>Aspergillus oryzae</i> strain A5, 1 (MG779508) was responsible for a 36.4±5.53% decrease in average weight	Muhonja et al. (2018)	[77]

**Table 1** (continued)

Type of plastic	Microorganism	Place of isolation of microorganisms	Degradation temperature	Degradation time	Microorganism isolation media	Reported degradation	Researchers	References
	<i>Trichoderma viride</i> and <i>Aspergillus nomius</i>	Local landfill soil in Medan	26 ± 2 °C	45 d	SDA	LDPE film weight was decreased by 5.13% and 6.63%, respectively when treated with RH03 and RH06	Munir et al. (2018)	[40]
	<i>Rhizopus oryzae</i> NS 5	Lab isolated	30 °C	30 d	PDA	A reduction in weight of 8.4 ± 3.0% and a weakening in tensile strength of 60%	Awasthi et al. (2017a)	[106]
	<i>Aspergillus clavatus</i> JASK1	Landfill soil	25–30 °C	90 d	PDA	35% weight loss	Gajendiran et al. (2016)	[78]
	<i>A. flavus</i> , <i>Pseudomonas</i> sp., <i>Aspergillus niger</i> , and <i>Streptomyces</i> sp.	Andhra Pradesh and Telangana areas' garbage soil	30–35 °C	6 months	MSM	The plastic degradation capacity of <i>Streptomyces</i> sp. is the greatest, at 46.70%; this is followed by the plastic degradation capacities of <i>Aspergillus niger</i> (26.17%), <i>Pseudomonas</i> sp. (24.22%), and <i>A. flavus</i> (16.45%)	Deepika et al. (2015)	[108]
	<i>Pseudomonas</i> sp. AKS2	Kolkata municipal solid waste dumping ground soil	30 °C	45 d	Yeast + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + MgSO <sub>4</sub> ·7H <sub>2</sub> O + NaCl + CaCl <sub>2</sub> ·2H <sub>2</sub> O + Fe SO <sub>4</sub> ·7H <sub>2</sub> O + Na <sub>2</sub> Mo O <sub>4</sub> ·2H <sub>2</sub> O + Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O + MnSO <sub>4</sub> + K <sub>2</sub> HPO <sub>4</sub> + KH <sub>2</sub> PO <sub>4</sub>	5 ± 1% weight loss	Tribedi and Sil (2013)	[105]
	<i>Aspergillus niger</i> and <i>Lysinibacillus xylanilyticus</i>	Landfills	30 °C	126 d	Mineral medium-agar	29.5% weight loss for the UV-irradiated films and 15.8% for non-UV-irradiated films	Esmaili et al. (2013)	[79]
LDPE and PP	<i>Bacillus cereus</i> and <i>Bacillus paramycoides</i>	Vaigai River, Madurai, India	–	21 d	–	–	Nanthini Devi et al. (2021)	[82]

**Table 1** (continued)

Type of plastic	Microorganism	Place of isolation of microorganisms	Degradation temperature	Degradation time	Microorganism isolation media	Reported degradation	Researchers	References
LDPE and PS	<i>Lysinibacillus massiliensis</i> and <i>Bacillus licheniformis</i> , and a mixed bacterial culture of <i>Bacillus</i> sp. and <i>Delftia acidovorans</i>	Municipal wastewater treatment plant Vrgorac—Split—Dalmatia County, and the sediment from the river Kupa, Karlovac County	25 ± 2 °C	22 d	MSM	Microplastic-LDPE and Microplastic-PS were degraded more effectively by mixed bacterial cultures than by pure bacterial cultures	Kučić Grgić et al. (2021)	[112]
LDPE and PVC	<i>Achromobacter denitrificans</i> Ebl13	Compost	–	–	–	12.3% (PVC) and 6.5% (LDPE) weight loss	Maleki Rad et al. (2022)	[70]
LLDPE	<i>Pseudomonas aeruginosa</i> and <i>Brevibacterium</i> sp.	Laboratory isolate	25, 30, and 35 (°C)	30 d	Nutrient Broth	Weight reduction of 5.22% occurred at 25 °C	Dwicania et al. (2019)	[50]
HDPE	<i>Klebsiella pneumoniae</i> CH001	Plastic waste dumpsite, Diesel Locomotive Works (DLW), Varanasi, India	30 °C	60 d	Nutrient agar	60% reduction in tensile strength and an 18.4% decrease in weight	Awasthi et al. (2017b)	[94]
	<i>Aspergillus</i> sp.	Gulf of Mannar, India	30 °C	30 d	PDA	In fungi isolates, VRKPT1 caused a weight loss of 6.02 ± 0.2%, whereas VRKPT2 caused a loss of 8.51 ± 0.1%	Devi et al. (2015)	[80]
	<i>Achromobacter xylosoxidans</i>	Landfill along the Mleczna River in Radom, Poland	27 °C	50 d	CDM and the modified Davis Minimal Broth medium	Mass loss percentages varied from 3.64 to 9.38%, with an average loss of 6.10 ± 0.13%	Kowalczyk et al. (2016)	[71]
PVA	<i>P. brevicompactum</i>		30 °C		PVA screening agar medium	–	Mohamed et al. (2022)	[42]

**Table 1** (continued)

Type of plastic	Microorganism	Place of isolation of microorganisms	Degradation temperature	Degradation time	Microorganism isolation media	Reported degradation	Researchers	References
PET	<i>Agromyces mediolianus</i> PNP3 and <i>Bacillus cereus</i> SEHD031MH	Activated sludge	30 °C	168 d	Yeast extract + trace elements	17% of PET degradation	Torena et al. (2021)	[83]
	<i>Pseudomonas</i> and <i>Bacillus</i> Species	Petroleum-polluted soils	30 °C	40 d	Rhodamine B agar containing olive oil	3% weight loss	Roberts et al. (2020)	[48]
	<i>Streptomyces</i> species		28 °C	18 d		Sizes 500, 420, 300, and 212 µm in PET particles showed biodegradation percentages of 49.2%, 57.4%, 62.4%, and 68.8%, respectively	Farzi et al. (2019)	[47]
PET modified with polyester "Bionolle"	<i>Penicillium funiculosum</i>	Dump in Sosnowiec	30 °C	84 d	CDM	30-fold higher weight loss	Nowak et al. (2011)	[98]
BHET and PET	<i>Thermobifida fusca cutinase</i> and <i>Oleivorans</i> JWG-G2	Laboratory isolate	35 °C	16 d	MSM	BHET oligomers had 2.79 synergy and PET film degradation 2.26	Yan et al. (2021)	[111]
PU, PS, and PE	<i>Penicillium</i> , <i>Geomyces</i> , <i>Mortierella</i> species	Antarctic soils	18 °C	90 d	PDA	A breakdown analysis of plastics over time reveals that PU degrades at a rate of 28.3 percent, PS at 8.39 percent, and LDPE at a meagre 3.53 percent as they age	Oviedo-Anchundia et al. (2021)	[96]
Polyhydroxyalkanoates (PHAs)	<i>Bacillus</i> sp. JY14	Marine soil of Korea	30 °C	14 d	Sodium dodecyl sulfate	98% PHB degradation	Cho et al. (2021)	[53]
2,6-Dimethylphenol (2,6-DMP)	<i>Mycobacterium neoaurum</i> B5-4	Topsoil from a dimethylphenols-contaminated area in Suqian city, Jiangsu province, China	30 °C	12 h	MSM	More than 90% degradation	Ji et al. (2020)	[114]
polyphenylene sulfide	<i>Pseudomonas</i> sp.	The gut of superworms ( <i>Zophobas morio</i> )	–	10 d	LCFBM	15 times smaller size beads	Li et al. (2020)	[49]



**Table 1** (continued)

Type of plastic	Microorganism	Place of isolation of microorganisms	Degradation temperature	Degradation time	Microorganism isolation media	Reported degradation	Researchers	References
PVC	<i>Pseudomonas citronellolis</i> and <i>Bacillus flexus</i>	Laboratory isolate	-	45 d	MSM	After being exposed to <i>P. citronellolis</i> , the average molecular weight of PVC decreased by 10%	Giacomucci et al. (2019)	[51]
Starch blended PVC	<i>Phanerochaete chrysosporium</i> PV1	Soil	30 °C	90 d	SDA	Polymer film molecular weight dropped from 80,275 to 78,866 Da	Hameed et al. (2014)	[101]
PE and PP	<i>Brevibacillus</i> sp. and <i>Aneurinibacillus</i> sp.	Cow dung	55 °C	120 d	Ammonium sulfate, di-potassium phosphate, potassium phosphate, magnesium sulfate	Treatment with a consortium of four isolates was shown to reduce the weight of LDPE, HDPE, and PP strips by 58.21 ± 2, 46.6 ± 3, and 56.3 ± 2%, respectively, while the same treatment reduced the weight of PP pellets by 45.7 ± 3, 37.2 ± 3%, and 44.2 ± 3%	Skariyachan et al. (2018)	[113]
Polyester PU	<i>Pseudomonas aeruginosa</i> MZA-85	Dumping area of Gujranwala	37 °C	4 weeks	MSM	Degradation of the polyester diol portion	Shah et al. (2013)	[104]
Dimethyl phthalate	<i>Comamonas testosterone</i>	Soil from agricultural fields in Chongqing, China	30–35 °C	2 d	MSM	Within 14 h, 99% of 450 mg L <sup>-1</sup> DMP is degraded	Li et al. (2017)	[88]

ZMB Zobell Marine Broth, ZMA Zobell Marine Agar, PDA Potato Dextrose Agar, CDM Czapek-Dox medium, SDA Sabouraud Dextrose Agar, MSM Mineral Salt Medium, SCS Sole Carbon Source, PE Polyethylene, LCFBM liquid carbon-free basal medium, LDPE low-density polyethylene, PP polypropylene, PS polystyrene, PVC polyvinyl chloride, LLDPE linear low-density polyethylene, HDPE high-density polyethylene, PVA polyvinyl alcohol, PET poly (ethylene terephthalate), BHET acid bis(2-hydroxyethyl) terephthalic acid, PU polyurethane, PHB polyhydroxybutyrate, PHAs polyhydroxyalkanoates, d days

Modifying cellulosome structure to create a multi-enzyme complex that can effectively degrade PET is an interesting area of research. Large cellulosomes may be created using modern synthetic biology methods, allowing for increased action toward stubborn cellulose [115]. Similarly, high-crystalline PET might be degraded by microbial cell factories if PETsome were developed. Identifying the substance that might serve as a PET-binding domain is crucial, similar to the cellulose-binding domain [116]. An effective bacterial cell surface expression system has recently been established [117].

### Mutagenesis

In addition, site-directed mutagenesis has been widely used for enzyme redesign; nonetheless, the success of this approach is fundamentally tied to the accessibility of three-dimensional protein structures. A carboxylesterase from *Archaeoglobus fulgidus* was modified using in silico site-directed mutagenesis to produce a BTA-hydrolase from *Thermobifida fusca* [118]. Molecular docking analysis was then used to compare the interactions of PET with polypropylene following this study. The findings as a whole suggested that the binding affinity of the mutant carboxylesterase for PET was unaffected by the alterations.

Using the IsPETase crystal structure and computational modelling, researchers have performed site-directed mutagenesis on 15 amino acid domains in the enzyme's first contact shell [119]. The enzyme was able to depolymerize 90% of the supplied PET in 10 h after disulfide bridges were added to increase its thermostability, and residues crucial for substrate binding were mutated. When the strain was optimized, it could break down 16.7 g of PET per litre per hour. Enzymatic PET degradation, which takes 10 h and is 90% effective, is comparable to chemical PET degradation, which takes 8 h and is 98% effective [120].

Enzyme modelling and experimental results revealed *I. sakaiensis* PETase's binding pockets and a variety of cutinases [121]. Like IsPETase, a cutinase isolated from *Thermobifida* has its binding pocket residues involved in substrate interaction determined [122]. A study comparing the IsPETase enzyme to others, such as Thf42 Cut1, determined that the binding pocket structure of IsPETase is responsible for its efficacy. When compared to other cutinases, which can only hydrolyze linear PET molecules, this PETase has a shallower and broader surface, making it possible to attach to aggregated PET molecules [121]. The TfCut2 from *Thermobifida fusca* was analyzed in the same fashion. By using computational modelling to identify critical residues and site-directed mutagenesis to modify these residues on the selected

substrates, researchers expanded the disintegration rate of PET film by a factor of 12.7 [123].

### Adaptive Laboratory Evolution (ALE)

Initiating and promoting evolutionary adaptation processes, such as ALE, is a potent method for enhancing or creating certain phenotypes in microbial strains [124]. ALE is a powerful strain engineering method for introducing mutations to enhance metabolic pathways and enzymes for fast growth on a range of carbon sources and stress tolerances when combined with omics approaches to characterise the induced changes. Numerous ALE instances have arisen for the better usage of plastic monomers; these monomers are crucial for the construction of plastic-upcycling or -degrading cell factories.

After having its genome sequenced, scientists discovered that *Pseudomonas pseudoalcaligenes* CECT 5344 has the capacity to use furoic acid, furfuryl alcohol, and furfurals as carbon sources. Growth on furfurals, however, was discovered to have a significant delay of many days. The ALE-adapted strain grew better on furfurals and had shorter lag periods [125]. This strain improved due to a point mutation in an AraC family activator gene (BN5 2303) in the HTH protein region (L261R). This mutation regulates the upstream hmfABCDE gene cluster.

In addition, terephthalate-independent *P. putida* KT2440 mutants that were successfully isolated from ALE have been shown to use ethylene glycol, a monomeric component of PET [126]. These mutants have missense mutations and a 15 bp deletion in gclR, a transcriptional regulator of the glyoxylate carboligase pathway (PP 4283). PP 2046- and PP 2662-encoded transcriptional regulators and porins improved ethylene glycol growth in ALE-derived *P. putida* KT2440. Secondary mutations may stabilise flux balances during the first phase of ethylene glycol oxidation to glyoxylate.

Current ALE tactics focus mostly on optimising the use of plastic monomers, but there is enormous potential for ALE to be used in the creation and improvement of plastics depolymerization enzymes. A wide variety of enzymes, including esterase, lipase, and cutinase, have been found to depolymerize PET and PLA but with low selectivity and turnover [127]. Novel plastic depolymerizing enzymes might be developed via ALE or directed evolution, two methods that show promise for acquiring enzymatic activity from promiscuous enzyme families.

### Obstacles to overcome

The field of microbial bioremediation for plastic waste management encounters several formidable challenges

that necessitate concerted efforts to overcome. One prominent obstacle lies in the substrate specificity exhibited by microorganisms. While certain microbes demonstrate efficacy in degrading specific types of plastics, the vast array of plastic polymers presents a challenge in developing microbial solutions that universally address the diversity of plastic materials. Moreover, the rate of plastic degradation through microbial processes is often sluggish. Accelerating this degradation without compromising efficiency remains a significant research challenge, particularly as the volume of plastic waste continues to escalate. Environmental conditions further complicate matters, with factors such as temperature, pH, and the presence of other chemicals influencing the effectiveness of microbial bioremediation. Optimizing these conditions for widespread applicability and scalability across diverse environments poses a considerable hurdle.

The emergence of biodegradable plastics and biopolymers designed to mimic traditional plastics adds complexity to the field. Microorganisms may struggle to differentiate between these bioplastics and conventional plastics, potentially impacting their effectiveness in degrading target materials. The lack of standardized protocols for assessing and categorizing microbial biodegradation of plastics is another critical challenge. Establishing uniform methodologies and metrics is essential for meaningful comparisons and advancements in the field.

Scaling up microbial bioremediation from laboratory experiments to real-world, large-scale applications presents engineering and logistical challenges. Ensuring the viability of microbial processes on an industrial scale while maintaining cost-effectiveness requires innovative solutions and a thorough understanding of the complexities involved. Additionally, the ecological impact of introducing specific microorganisms into ecosystems needs careful assessment. While microbial bioremediation holds promise, unintended consequences on the environment must be thoroughly evaluated through comprehensive risk assessments.

Furthermore, navigating the evolving regulatory landscape surrounding the use of microorganisms for plastic bioremediation poses challenges. Compliance with regulatory standards and ensuring the safety of processes and end-products are critical considerations for the responsible development and deployment of microbial solutions in plastic waste management. Overcoming these multifaceted challenges demands interdisciplinary collaboration, continuous research and development, and a holistic approach that considers the intricacies of both microbial processes and the environmental contexts in which they are applied.

## Limitations

This study has a number of caveats, the first of which is that the search was conducted in a specific segment of the most regularly used libraries. Throughout our search, we skipped to check a few libraries. The decision was made to restrict attention to studies that appeared in reputable peer-reviewed publications. It was determined not to look through grey publications. Second, only relevant results were obtained since the search terms were restricted to just those most closely associated with the initial query. There is a risk that a manuscript could have been disregarded which may discuss microbial remediation of plastic biodegradation but not using the phrases sought for. By developing a methodology, the authors ensured they would have complete command over the search and selection of papers. Finally, the study only considers the most common microorganisms used in the treatment for plastic biodegradation. The authors have made an attempt to provide bibliographic information for all relevant and well regarded publications.

## Conclusion

The annual manufacturing of plastic has topped 300 million tonnes, and recycling has almost failed as a sustainable method for the disposal of plastic trash. With the accumulation of these materials in the environment, particularly in rivers and oceans as macro-, meso-, micro-, and nano-plastics, it is of the utmost significance to discover creative methods to reduce this environmental threat. There have been several efforts to identify and isolate microbes with the ability to use synthetic polymers. Using specific microbial strains for plastic biodegradation has recently been shown to be a viable option.

These findings give fresh insights into LDPE and HDPE biodegradation processes by a consortium of microorganisms with putative metabolic complementarities. Another research found novel bacterial strains that may alter the chemical composition of HDPE. Based on these results, it seems that synergistic microbe-enzyme treatment might be a promising future direction for plastic degradation research. The capability of the microbial strain to digest microplastic particles provides a potential application for the remediation of microplastics. The PET-degrading activity shown by the analyzed bacterial strains holds promise for further study and its application to the successful removal of microplastics from water and wastewater using novel and potent technological approaches. This research also uncovered a thermo- and halo-tolerant bacterium that can degrade PHB in both solid and liquid states. In light

of these findings, it seems that this strain of bacteria may be useful for degrading a wide range of PHAs. These methods, when combined, may be used to create a streamlined bioprocessing setup, a microbial system that can effectively break down plastics and upcycle them into high-value compounds. Technological and economic obstacles, such as the toxicity of waste products to degrading enzymes and high operational costs, should be addressed for the benefit of the industry as a whole.

Current research suggests that naturally occurring soil microorganisms, such as bacteria and fungi, are quite effective in breaking down plastic. More often than not, fungi are more potent degraders than bacteria. In the lab, however, fungi and bacteria demonstrated the ability to break down plastic. The maximum degradation capability was found for *Aspergillus* fungi and *Bacillus* bacteria among the studied taxa. For this notion to be utilized commercially and on a wider scale, more work is required to increase its degrading capability by evaluating optimal conditions for microbial activity. Another strategy that might be used to improve plastic biodegradation is the pre-treatment with compounds that are beneficial to the environment.

#### Abbreviations

ALE	Adaptive laboratory evolution
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
BC	<i>Bacillus cereus</i>
BHET	Bis(2-hydroxyethyl) terephthalic acid
BP	<i>Bacillus paramycoides</i>
CDM	Czapek-Dox medium
DMP	Dimethyl phthalate
EDS	Energy Dispersive X-ray Spectroscopy
EGT	Ethylene glycol terephthalate
EPS	Extracellular Polymeric Substance
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
GPC	Gel permeation chromatography
HDPE	High-Density Polyethylene
HPLC	High-performance liquid chromatography
ITS	Internal transcribed space (ITS)
LC/MS	Liquid Chromatography-Mass Spectrometry
LCFBM	Liquid carbon-free basal medium
LDPE	Low-Density Polyethylene
LLDPE	Linear Low-Density Polyethylene
MHET	Mono(2-hydroxyethyl) terephthalate
MMP	Mono-methyl phthalate
MSM	Mineral salt medium
NMR	Nuclear Magnetic Resonance
PA	Phthalic acid
PA	Polyamide
PDA	Potato Dextrose Agar
PE	Polyethylene
PET	Poly(ethylene terephthalate)
PHB	Polyhydroxybutyrate
PP	Polypropylene
PPS	Polyphenylene sulfide
PS	Polystyrene
PU	Polyurethane
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
RT-PCR	Reverse Transcription-Polymerase Chain Reaction

SCS	Sole carbon source
SDA	Sabouraud Dextrose Agar
SEM	Scanning Electron Microscopy
TfC	<i>Thermobifida fusca cutinase</i>
TPA	Terephthalic acid
UTM	Universal testing machine
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray diffraction
ZMA	Zobell Marine Agar
ZMB	Zobell Marine Broth

#### Acknowledgements

We thank the Department of Biotechnology, Delhi Technological University and CSIR-IIP, Dehradun for help during the course of this study.

#### Author contributions

Tushar Agarwal: investigation, writing-original draft preparation, editing; Neeraj Atray: reviewing, validation; Jai Gopal Sharma: supervision, conceptualization, methodology.

#### Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

#### Availability of data and materials

Not applicable.

#### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

None.

Received: 6 February 2024 Accepted: 14 May 2024

Published online: 18 June 2024

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