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A review on bioremediation of microplastics and its legal management

Leela Kaur*, Neeraja Godara

Department of Environmental Science, Maharaja Ganga Singh University, Bikaner, Rajasthan. India

* Corresponding author Email: leela.kaur@gmail.com

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Abstract
Microplastics are plastic fragments of 5 or fewer mm in diameter, becoming an environmental nuisance. It threatens ecosystem health and functioning and impacts biotic and abiotic components. Microbial bioremediation is a promising and ecologically sound method for removing microplastics waste. This review focuses on the various classifications followed for microplastics, their sources and the methods of bioremediation and the legal management of the microplastics. We discuss the role of fungi, bacteria, algae in the biodegradation of microplastics. It is shown that bacteria and fungi have efficiency in breaking down microplastics either aerobically or anaerobically on both land and water ecosystems. The review also focused on the enzymes utilized in the biodegradation of different types of plastic polymers secreted by a variety of bacterial, fungal and algal species. Enzymes such as polyethylene terephthalate hydrolase, mono 2-hydroxyethyl terephthalic acid hydrolase, poly 3-hydroxyoctanoate depolymerase and cutinase like enzyme have potential to degrade microplastics and can be helpful to sustainable bioremediation approaches. It discusses the legal action taken by countries and organizations to lessen plastic garbage entering the environment and encourage sustainable plastic usage patterns by stringently implementing the laws and rules and drafting policies. The concomitant implication of bioremediation approaches and legal actions can prevent microplastics contamination in the environment.

Keywords
Microplastics, Toxicity, Bioremediation, Biodegradation, Legal action.

Introduction

The presence of anthropogenic litter in both aquatic and land-based ecosystems has seen a significant surge in recent decades, with approximately 60-80% of this waste being composed of plastic materials. The era of mass plastic production com-menced in the 1950s and has now reached a global output of over 390 million tonnes. It is estimated that established widespread uses of plastic include packa-ging materials (44% of total plastic production), buil-ding materials (18%), automotive components (8%), electronic appliances (7%) and agricultural materials (4%), household appliances and sporting equipment (7%) and others (12%). Out of all this, 90% plastic production is fossil based and remaining is post-consumer recycled plastic and biobased plastic (Plastics Europe, 2022). Microplastics are minuscule plastic fragments having diameter less than 5 mm in size, can be traced back to both primary and secondary sources (Horton et al., 2017a). Primary source microplastics encompass particles like polyethylene (PE), polypropylene (PP), and polystyrene (PS) found in beauty products and pharmaceuticals

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(Horton et al., 2017a). As a result of their detrimental effect on the environment, many nations such as Canada and the United States, have prohibited the selling of microplastics contained beauty products (Ballent et al., 2016). Secondary microplastics result from various physical, chemical, and biological methods that lead to the disintegration of plastic waste. Exposure to UV radiation accelerates the photo oxidation of plastic which breaks it into plastic fragments forming microplastics. Warm and well-aerated conditions are conducive for disintegration that generate microplastics, but cold and oxygen-deprived aquatic systems can result in the extremely slow disintegration of plastic particles, spanning centuries (Zhang, 2017). Microplastics from different sources manifest in various forms, such as pellets, fibres, and fragments, in environmental samples. Primary microplastics are most likely finding their way into aquatic environments through various means, including the discharge of household sewage or accidental spills (Horton et al., 2017a). Another notable origin of primary microplastics involves the utility of sewage sludge that contains synthetic fibres or sediment from toiletry products when applied to land (Horton et al., 2017a). Fibres are highly observed form of primary microplastics, primarily because of the continuous decomposition of clothing made from synthetic textiles and the release of microfibers during washing machine cycles (Napper and Thompson, 2016). Although synthetic fibres like polyester, acrylic, and polyamide are typically considered secondary microplastics, they are often released into the environment alongside primary microplastics (Horton et al., 2017a). A single item of clothing can release up to 1900 fibres during a wash, which can enter the environment through wastewater and sewage sludge; textile mills and plastic manufacturing facilities are potential sources of microplastics pollution. Microplastics originating from secondary sources play a significant role in contributing to microplastics pollution, primarily due to the substantial amount of larger plastic waste entering the environment (Duis and Coors, 2016). These secondary microplastics originate from the collection and disposal of municipal solid waste (Horton et al., 2017a). The decomposed fragments and large plastic pieces can find their way into aquatic environments through processes like wind dispersion, erosion of soil, or runoff discharging in aquatic system. Additionally, lightweight plastics possibly be carried everywhere on the land by the wind, whereas heavier plastics will probably get buried within the soil. (Horton et al., 2017a). The contemporary research indicates that agriculture is a major contributor to microplastics pollution in soil, stemming from practices like the use of waste sludge for soil improvement and the application of plastic as mulches to enhance crop yields (Rodríguez-Seijo and Pereira, 2019). Furthermore, proofs recommend that microplastics pollution can arise from tires and road markings, carried with runoff into surface water resources (Horton et al., 2017b; Kole et al., 2017). Also, recent studies have found that microplastics fibres are transported through atmospheric deposition, especially in densely populated areas, and potential sources of microplastics in the air are like artificial fibres and turf, landfills, and waste pyrolysis, which is possibly carried by wind into water resources or deposited onto land surfaces, with their distribution influenced by physical mechanisms and climatic factors. Microplastics are of a great concern in water bodies as they pose Eco toxicological threats to ecosystems. Microplastics can bio accumulate and bio magnify in the food chain. Because of hydrophobic nature and increased surface area, tends to adsorb heavy metals and other pollutants readily (Cole et al., 2011; Wang et al., 2017a), and some pollutants such as Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) get accumulated in fishes (Frias et al., 2010; Klein et al., 2015). The objective of the study is to analyse and provide an overview on the sources, bioremediation and legal management of microplastics (MP).

Classification of microplastics

The issue of large plastic debris has been a significant environmental concern for quite some time, it was not until the 21st century that the focus shifted towards minuscule plastic fragments, fibres, and particles collectively referred to as "microplastics". Researchers have employed various size ranges to define microplastics, including diameters of less than 10 mm, less than 5 mm, 2 to 6 mm, less than 2 mm, and less than 1 mm (Cole et al., 2011). This inconsistency poses a significant challenge when comparing data related to microplastics, underscoring the growing importance of establishing a standardized scientific terminology (Claessens et al., 2011). Proposed definition for Microplastics: "Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 µm to 5 mm, of either primary or secondary

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manufacturing origin, which are insoluble in water. (Frias & Nash, 2019). Andrady (2011) proposed introducing the term "Mesoplastics" into scientific nomenclature. This proposed terminology would distinguish between small plastics visible to the naked eye and those requiring microscopic observation for detection, addressing a notable distinction in plastic pollution research. Due to their minute size, microplastics are regarded as readily accessible to organisms across the entire food chain. Their structure and relatively extensive surface area increase their susceptibility to attracting waterborne organic pollutants and releasing potentially toxic plasticizers. Consequently, the ingestion of Microplastics could potentially introduce harmful toxins at the foundational levels of the food chain, creating the possibility for bioaccumulation (Cole et al., 2011). Lately, the production of microplastics has increased significantly, with its concentration reaching thousands of plastic fragments per cubic meter. In absence of proper interventions, this concentration is likely to get doubled in the coming years (Isobe et al. 2019). Furthermore, the issue is escalated by the lack of reliable and precise sampling methods, suggesting the possibility of discrepancy in the reported concentration of Microplastics, potentially leading to an underestimation of the problem. Various classifications of Microplastics have been given based on composition, shape, source and size as shown in Figure 1.

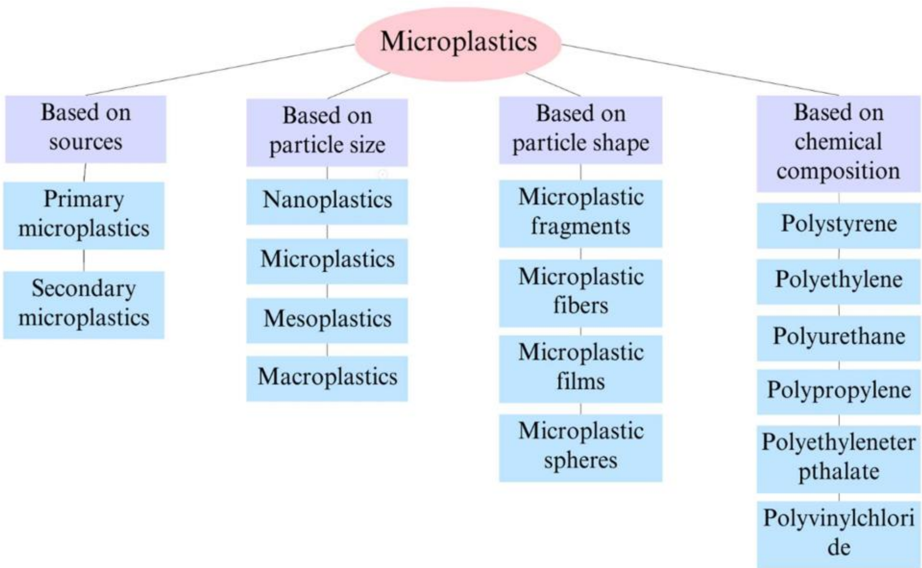

Figure 1
Different classifications of Microplastics (Adopted from Osman et al., 2023).

Classification of Microplastics based on particle size

Microplastics are classified into four types viz. Nanoplastics, Microplastics, Mesoplastics and Macroplastics (Table 1). Nanoplastics are extremely small plastic, size ranging from 1 nanometre to 1 micrometre, which are usually produced due to degradation of Microplastics. Microplastics size range from 1 micrometre to less than 5 millimetres, which are produced either intentionally or by degradation of plastics. The size of Mesoplastics range from 1 millimetre to 2 centimetres. While Macroplastics exceed the size of 1 centimetre.

Table 1. *Classification of Microplastics based on particle size (Adopted from Mendoza et al., 2020).*

Types plastics	Hartmann et al., 2015	Rocha Santos, 2015	EU Commission, 2011	Browe et al., 2007	Ryan et al., 2009	Des Forges, 2014	Claessen et al., 2013
Nanoplastics	1nm-1μm		1-100 nm	<1μm			
Microplastics	1μm-1mm	<5mm		1μm-1mm	<2mm	1μm-5mm	<1mm
Mesoplastics	1mm-1cm			>5mm	2mm-2cm		
Macroplastics	>1 cm				>2 cm		

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Classification of Microplastics based on sources

Primary microplastics. Primary microplastics are intentionally produced that have various shapes and microscopic sizes (Figure 2). Microplastics are used in beauty products face scrubbers and as carriers for drugs in the medical field (Sharma & Chatterjee, 2017). Within the cosmetics industry, primary microplastics serve as prevalent exfoliates, taking the place of natural substances like ground almonds and oat meal. They are promoted and sold under names such as micro-beads or micro-exfoliates. Other instances of primary microplastics employed in cosmetics encompass materials like polyethylene and polypropylene granules, polystyrene spheres, and microplastics with uneven shapes, all measuring less than 0.5 mm in diameter. Another application is in the air-blasting technology, this involves projecting acrylic, melamine, or polyester microplastics scrubbers onto machinery, engines, and boat hulls to remove rust and paint. But they shrink with repeated use and become contaminated with heavy metals like Cadmium, Chromium, and Lead (Cole et al., 2011).

Figure 2. *Primary sources of microplastics (Source: Leonardo, 2018)*

It is worth noting that, under the broader definition of microplastics, even virgin plastic production pellets (typically 2–5 mm in diameter) could be considered as primary microplastics. However, this classification has faced criticism.

Secondary microplastics. Secondary microplastics are tiny plastic fragments resulting from the degradation of larger plastic waste, reducing their size to less than 5 mm. This degradation can occur in both marine and terrestrial settings and is driven by various processes, including physical, biological, and chemical factors. These tiny particles present significant environmental and wildlife hazards as they can be consumed or absorbed by organisms, giving rise to health-related concerns (Cole et al., 2011). Secondary microplastics are major pollution causing plastics in water-based ecosystems with primary microplastics. The methods being utilized to produce secondary microplastics are represented in Figure 3.

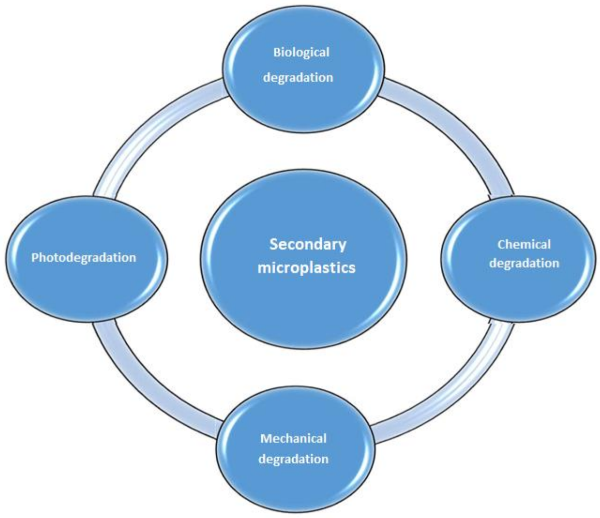

Figure 3. *Formation processes of secondary microplastics (Adopted from Bacha et al, 2021).*

(i) Photodegradation. Exposure to sunlight over extended periods can lead to the photodegradation of plastics, as ultraviolet (UV) radiation causes the polymer matrix to oxidize and chemical bonds to break. This degradation can result in the leaching of additives, which are designed to enhance durability and corrosion resi-stance, out of the plastics. In the marine environment, cold and saline conditions often limit the photo-oxidation of plastics, reducing the degradation process. However, plastics on beaches, with higher aerobic conditions and direct sunlight exposure, degrades more rapidly. Over time, these plastics become brittle, develop cracks, and turn yellow (Andrady, 2011).

(ii) ***Mechanical degradation.*** As plastics lose their structural integrity due to degradation, they become in-creasingly susceptible to fragmentation caused by abrasion, wave action, and turbulence. This process continues until these fragments reach microplastics size. It is speculated that microplastics may further degrade into nanoplastics, although the smallest microplastics reported in the oceans so far is 1.6 µm in diameter. The presence of nanoplastics in the aquatic ecosystem is expected to become more signi-ficant in the future, raising concerns about their po-tential impact on the marine food web (Andrady, 2011).

(iii) ***Chemical degradation.*** Plastics degrade by reactions with chemical substances. Oxidation reac-tions like direct photodegradation, electrochemical oxidation and photocatalytic oxidation play crucial role in plastic degradation. Adsorption process and

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catalysts (such as Pt, Mo, and other trace elements) degrade plastics.

(iv) ***Biological degradation.*** Biodegradable plastics are often considered an alternative to traditional plastics. However, they too can be a source of microplastics. These plastics are typically composed of synthetic polymers and starch, vegetable oils, or specialized chemicals (e.g., TDPA) designed to accelerate degradation under specific conditions, such as in industrial composting plants with high temperature, humidity, and aeration (O'Brine and Thompson, 2010). However, the decomposition is only partial. While the starch components decompose, a significant quantity of synthetic polymers remains (Andrady, 2011; Roy et al., 2011). In the colder marine environment and in the absence of terrestrial microbes, the decomposition of even the degradable components of bioplastics is prolonged. This increases the likelihood of plastics becoming fouled and reduces the UV exposure needed for the degradation process. Ultimately, microplastics are released into the marine environ-ment when decomposition occurs (Roy et al., 2011). Tin significantly impact the environment, as they can accumulate and leach toxic organic and inorganic pollutants, such as persistent organic pollutants and heavy metals. Microplastics are also known for their stability and inability to degrade, meaning they can persist in the environment for decades (Xiang et al. 2022). Microplastics have stability and inability to degrade and can persist in the ecosystems for decades (Xiang et al. 2022).

Classification of microplastics based on shape

Microplastics are divided into four shape profiles which are described as follows:

Microplastics fibres. These are secondary microplastics that mainly originate from clothing during washing or degraded ropes and fishing gear. They are believed to constitute approximately 90% of marine microplastics pollution and often have elongated shapes. Microplastics fragments: Irregularly shaped secondary microplastics that form due to the breakdown or abrasion of larger plastic objects, such as containers or bottles. Their shapes and sizes vary based on the source material and degradation process.

Microplastics films. Secondary particles that derive from thinner plastic items like bags and wrappers. These films break down into smaller pieces through environmental processes, contributing to microplastics pollution.

Microplastics spheres. Uniformly shaped, intentionnally manufactured primary particles added to products like facial cleansers and toothpaste for their abrasive properties. These spheres have a spherical shape and are considered primary microplastics because they are deliberately produced and incorporated into products. This categorization helps in classifying and comprehending the diverse forms in which microplastics exist in the environment, facilitating efforts to assess and address their impact on ecosystems and marine life while developing strategies to mitigate their presence. The most common microplastics polymers found in marine systems are polyethylene (PE), polyethylene terephthalate (PET), polyacrylamide (PA), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyurethane (PU) (Cunningham, 2018). Because of their minute size, Microplastics are regarded as readily accessible to organisms across the entire food chain. Their structure and relatively extensive surface area increase their susceptibility to attracting waterborne pollutants and releasing potentially toxic plasticizers. Consequently, the absorption of microplastics could potentially introduce harmful toxins at the foundational levels of the trophic level, creating the possibility for bioaccumulation (Cole et al., 2011).

<u>Sources of microplastics in the environment</u>

Microplastics pollution prevail across marine, freshwater, and terrestrial habitats globally (Castañeda et al., 2014), and were first observed in studies from the Sargasso Sea as plastic pellets found in the surface waters ranging in size from 2.5 – 5 mm, and in abundances of ~3500 items (Carpenter & Smith, 1972). A follow up study from the coastal waters of Southern New England first described the ingestion of similar sized plastic particles by eight of 14 examined fish species (Carpenter et al., 1972). However, a more extensive study covering a larger area of the North Western Atlantic confirmed that plastic fragments in similar sizes to those found by Carpenter et al. (1972) were indeed as widely distributed in the area as previously thought (Colton et al., 1974). These studies from the 1970s set the stage for what is now a global research topic, but microplastics pollution had little attention over the next 30 years, and the term microplastics was not coined until the publication of a science article in 2004 (Thompson et al., 2004). Today, studies have re-

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reported the presence and high abundances of microplastics particles globally from the densely human populated ecosystems such as tropical coasts (Nguyen et al., 2020), to the most remote ecosystems on Earth, including the Antarctic and Southern Ocean deep sea, the Arctic Ocean (Tekmen et al., 2020), deep-sea trenches (Welden & Lusher, 2017), and even the peak of Mt. Everest (Napper et al., 2020). As well as being geographically ubiquitous, microplastics are also vertically distributed throughout the water column, from the surface waters to the benthos (Van Cauwenberghe et al., 2013). Most of the plastic produced globally is buoyant, although, there are both physical and biological mechanisms that cause micro-pla-stics to sink in the marine environment (Kaiser et al., 2017). The sinking velocity of microplastics is dependent on particle shape and density, however, both these factors can be altered by UV degradation, exposure to physical processes such as wave action, tides, and currents, and bio fouling (Kowalski et al., 2016). Bio fouling is the process in which microplastics accumulate a layer or film of organic matter from the water column due to their hydrophobic properties (Kaiser et al., 2017). This layer of organic matter increases the density of the particle and helps it to sink to the benthic environment (Van Cauwenberghe et al., 2013). Larger plastics are more likely to encounter organic matter in the water column, and therefore become bio fouled quickly, whereas smaller plastic particles have a greater relative surface area and will begin to sink more quickly than larger plastics when bio fouling begins (Lobelle et al., 2021). Other biological means of microplastics transport throughout the water column include aggregates such as marine snow and fecal matter (Kvale et al., 2020). Marine aggregates consisting of oxygen-rich organic matter, microbes, faecal pellets, and phytoplankton are abundant in the global oceans (5300 per litre; Porter et al., 2018). These aggregates that form an integral part of organic and non-organic matter, transport from the upper layer of the oceans to the benthos, influence the sinking behaviour and bioavailability of microplastics (Coyle et al., 2020), and have been found to contain microplastics pollution in high abundances (1290 ± 1510 particles/m3; Zhao et al., 2018). Marine litter results from the improper disposal of various waste items that find their way into the oceans. In this section, we explore the origins of plastic litter and discuss the pathways, both direct and indirect, through which plastic can contaminate the marine environment. While the primary focus of this review is on microplastics, we also examine the indiscriminate disposal of macroplastics, as they can ultimately degrade into secondary microplastics over time. Land-based sources are responsible for a substantial majority, estimated at 80-90%, of the microplastics found in aquatic ecosystems (Duis and Coors, 2016). This category encompasses primary microplastics used in cosmetics and air-blasting, as well as plastics improperly discarded by individuals and plastic leachates originating from landfill sites. These plastics pose a substantial risk of infiltrating the marine environment, whether through rivers, wastewater systems, or transportation by offshore winds. Although wastewater treatment plants can capture larger plastic items and some smaller debris, a significant portion of microplastics can elude these filtration systems. Consequently, plastics that find their way into river systems, whether through direct disposal, wastewater discharge, or runoff from landfills, ultimately make their journey to the open sea. Numerous scientific studies have underscored the critical role of unidirectional freshwater systems in facilitating the transport of plastic waste from land to the ocean (Yang et al., 2021). Some of the most prominent contributors of plastic debris on land include construction, household material, packaging waste (including beverages and food packaging) (Čulin and Bielić, 2016; Alomar et al., 2016). Additionally, sewage sludge and industrial processes, are suspected origin of microplastics discharge into aquatic environments (Rolsky et al., 2020; Hale et al., 2020). Certain medications and construction materials, beauty and toiletry products, may also serve as potential origin of plastic pollution due to the presence of microplastics used as carriers or ingredients (Rochman, 2018). Personal care products such as face washes, soaps, sanitizers, laundry detergents, toothpaste, creams, lipsticks, sunscreens, and shower gels may contain microplastics (Guerranti et al., 2019). Synthetic fibres like polyester, nylon, and acrylics are known to shed from clothing and enter water bodies through wastewater discharge (Carney Almroth et al., 2018). The abrasion of vehicle tires is another origin of microplastics (Kole et al., 2017). Hence, it is evident that multiple sources of microplastics need effective control and minimization efforts. In recent times, single-use plastic products such as packaged water bottles, cutlery, and bags have emerged as major contributors to plastic pollution (Fadare et al., 2020). The widespread utility of single-use face masks of plastic polymers during the COVID

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-19 pandemic has further exacerbated the issue, causing a significant rise in microplastics waste (Fadare and Okoffo, 2020). So, there is a growing need to replace plastic face masks and other products with sustainable, eco-friendly alternatives that can be degraded. Aquatic based sources account for approximately 10-20% of the microplastics released into aquatic environments, and they originate from various activities related to the oceans (Li, 2018; Karbalaei et al., 2019). These sources include tourism, commercial fishing, vessels, and offshore industries. One major contributor is discarded or lost fishing gear, which release significant amounts of microplastics in the ocean (Naji et al., 2017). Alarmingly, over 600,000 tons of fishing gear are discarded in the ocean every year, significantly exacerbating the microplastics issue. Shipping-related activities too add to ocean-based microplastics pollution (Peng et al., 2018). Additionally, a substantial quantity of plastic waste from offshore industries, particularly the petrochemical sector, is finding its way into aquatic environment (Calero et al., 2021). The contribution of marine based origin points to microplastics pollution is relatively lower than that of land-based points of origin. Therefore, it is essential to implement control methods to reduce and mitigate the impact of these ocean-based sources on microplastics pollution in our marine environments. Another source of plastic debris arises from the production of plastic products that use granules and small resin pellets, known as nibs, as raw materials. Accidental spillage during transport, both on land and at sea, improper use as packing materials, and direct discharge from processing plants can introduce these raw materials into aquatic ecosystems. Notably, resin pellets are not confined to specific areas and have been detected in marine systems worldwide, including mid-ocean islands with no local plastic production facilities (Cole et al., 2011).

Microplastics in fresh water ecosystem. Microplastics are increasingly being discovered in the surface water environments such as rivers, lakes, estuaries, wetlands, and in the water stored in aquifer. Although the accumulation of minuscule plastics in these ecosystems is less compared to oceanic settings. Wetlands, particularly, serve as major recipients of microplastics from sewage system, irrigated crop lands, and industrial contaminated discharge, making them microplastics sinks. Lakes, being closed-water bodies with slower current rates, tend to accumulate microplastics more than rivers, where transport is more dynamic. With various sources contributing to microplastics pollution in freshwater, it is essential to implement inventive, productive, and environmentally sound mitigation means to safeguard these sources, particularly in the face of population boom and water scarcity concerns worldwide.

Microplastics in the Soil. Most of the plastic waste ends up in our oceans or gets disposed of on land. Approximately annually 125 to 850 tons of microplastics per million residents is added to irrigating soils of European countries as means to apply sewage solid sludge (Nizzetto et al., 2016b).
The photo-oxidative degradation process for microplastics buried in the soil is extremely soil due to the reduced oxygen, low temperature, and low exposure to UV radiations, leading to soils being regarded as a sink for microplastics (Duis and Coors, 2016). Also, Microplastics have the competency to influence the geochemistry of soils and interact with soil organisms (Machado et al., 2018), their precise impact on terrestrial environments remains poorly understood. Many studies have researched on the decomposition of microplastics by soil-dwelling organisms and their role in transporting microplastics within the soil, as well as investigating the survival, fitness, and interactions of these organisms when exposed to microplastics, revealing potential ecological impacts (Huerta Lwanga et al., 2018).

Microplastics in the Air. Atmospheric deposition is a potential cause of microplastics, with studies showing that synthetic fibers can become airborne and be deposited in urban areas, contributing to microplastics entering freshwater and marine environments (Dris et al., 2016). The health implications of inhaling or ingesting microplastics are not fully understood, but there is concern that they could toxicity by stimulating an immune response. (Wright and Kelly, 2017). Microplastics have been found in both outdoor and indoor air, with natural and synthetic fibers contributing to contamination; new methodologies have been developed to minimize airborne microplastics contamination and improve scientific accuracy. Indoor air contains fibers with 33% polypropylene, which are unlikely to be inhaled but can be ingested through dust, especially by young children, emphasizing the importance of considering indoor environments as potential sources of microplastics exposure (Dris et al., 2017).

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Bioremediation of microplastics

The existing strategies for managing plastic waste are deemed insufficient, prompting a search for new approaches that align with contemporary needs. One prominent strategy under exploration, given its environmentally friendly and commercial potential, is bioremediation. Bioremediation is characterized as a natural process occasionally harnessed to address environmental contamination, employing degrading microbes, predominantly fungi and bacteria. In this method, microorganisms serve as key agents to efficiently and cost-effectively cleanse or restore polluted environments through eco-friendly means. These microorganisms exhibit unique mechanisms enabling their survival in challenging conditions. Two crucial implications arise from this approach: the utilization of highly specific organisms tailored for distinct toxic compounds/elements and the preference for autochthonous microorganisms, ideally sourced from the same polluted environment targeted for recovery. Various remediation strategies can be employed within bioremediation approaches.

Mechanism and enzymes of microplastics biodegradation. The process of microbial degradation of microplastics (MPs) involves multiple biochemical reactions, including colonization, depolymerization, assimilation, and mineralization (Figure 4). Hydrolysis and oxidative degradation are the two main processes involved in the degradation of both hydrolysable and non-hydrolysable plastics. Hydrolysis involves the breaking down of hydrolysable plastics into smaller units called oligomers, which then permeates in the cells and aids as a source of carbon for microbe development.

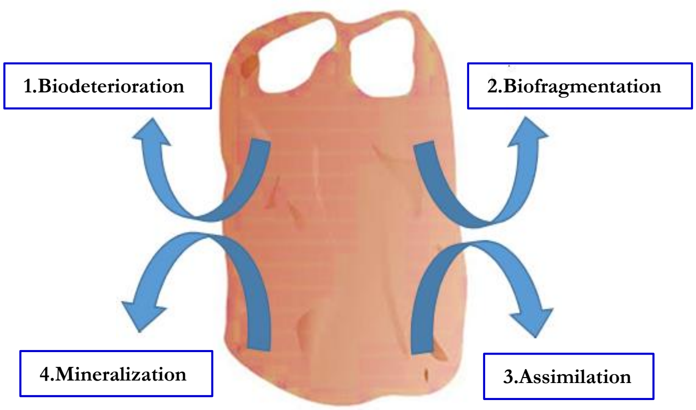

Figure 4. *Processes involved in biodegradation of MPs (Adopted from Pathak & Navneet, 2021).*

Oxidative degradation, on the other hand, involves the breakdown of both hydrolysable and non-hydrolysable plastics through the action of oxidoreductases, which are enzymes that catalyse oxidation-reduction reactions. While microplastics (MPs) exhibit long-term stability in natural settings, they are susceptible to decomposition by a particular microorganism. Microbes, owing to their robust adaptability across diverse environments, possess the capability to break down various organic pollutants, including MPs. The process of MPs degradation involves the utilization of MPs as substrates for the growth of biofilm. As the biofilm develops, it causes pitting and cracking, thereby weakening the structure of MPs. Bacterial enzymes play a crucial role by targeting the weakened MP fragments in both specific and nonspecific manners. Colonization occurs when microorganisms attach to the surface of MPs and release extracellular enzymes to cleave the polymers. Depolymerization is the breakdown of the polymers into low molecular weight oligomers, dimers, and monomers through hydrolysis or oxidative degradation. After depolymerization, the oligomers aids as a source of carbon for microbe development, regarded as assimilation. Mineralization is the degradation of microplastics into molecules, such as CO2, CH4, and H2O, through the TCA cycle. Plastic degrading enzymes include hydrolases, such as esterase, lipase, keratinase, and cutinase, and oxidoreductases, such as laccase, manganese peroxidase, hydroxylase, and lignin peroxidases. However, the mechanisms and enzymes of MP Biodegradation are not fully understood, and more research is needed to identify relevant enzymes and metabolisms. Understanding the process of MP biodegradation is crucial for resolving the growing problem of marine microplastics pollution and promoting the degradation of microplastics in the ocean (Zhai et al., 2023). Table 2 illustrates major microplastics and their biodegrading microbes. PE degrading biocatalysts are enzymes that can break down polyethylene (PE) into smaller components. Hydroxylase, laccase, peroxidase, and reductase are some of the biocatalysts that have been identified to degrade PE. A manganese peroxidase found in fungi has also been shown to degrade PE. Laccase enzyme produced by Actinomycetes, *Rhodococcus*, *Aspergillus flavus*, and *Pleurotus ostreatus* has been found to significantly degrade PE. Alkane hydroxylase enzyme produced by *Pseudomonas aeruginosa* and *Pseudomonas sp.* E4 have been involved

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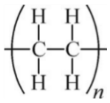
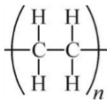
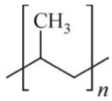
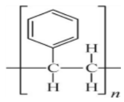
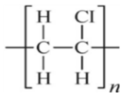
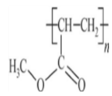
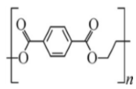
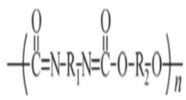
Table 2. *Major microplastics and biodegrading microbes (adopted from Wu et al., 2017).*

Polymer type	Formula	Chemical Density (g/cm^3)	Biodegradation	Molecular structure	Recycle ID code
High density polyethylene (HDPE)	$(C_2H_4)n$	0.917–0.965	Not reported		02 PE-HD
Low density polyethylene (LDPE)	$(C_2H_4)n$		Bacteria, fungi, wax worms, mealworms		04 PE-LD
Polypropylene (PP)	$(C_3H_6)n$	0.90–0.91	Not reported		05 PP
Polystyrene (PS)	$(C_8H_8)n$	1.04–1.10	Bacteria, mealworms		06 PS
Polyvinylchloride (PVC)	$(C_2H_3Cl)_n$	1.16 – 1.58	Fungi		03 PVC
Polymethylacrylate (PMA)	$(C_4H_6O_2)n$	1.17–1.20	Cyanobacteria		07 O
Polyethylene terephthalate (PET)	$(C_{10}H_8O_4)n$	1.37 – 1.45	Bacteria		01 PET
Polyurethane (PUR)	$(R(N=C=O)_n$	1.20	Fungi		07 O
Polyester (like Nylon, Acrylic)	–	1.24–2.30	Bacteria	–	07 O

in the direct oxidation of low molecular weight PE (LMWPE) and the main chain of PE, respectively. While biocatalysts have been identified, more research is needed to identify relevant enzymes and metabolisms for effective decomposition of microplastics in the ocean. PET, a type of plastic commonly used in packaging, is degraded by enzymes such as lipase esterase, and keratinase. The study mentions *Ideonella sakaiensis*, a bacterium that produces two enzymes, PETase and MHETase, which are capable of efficiently breaking down PET into two monomers, terephthalic acid (TPA) and ethylene glycol (EG). PETase is encoded by the gene ISF6_4831, while MHETase is encoded by ISF6_0224. The discovery of these enzymes is significant as it provides a potential solution to the plastic pollution, particularly in the case of PET. However, further research is needed to identify other relevant enzymes and metabolisms that can aid in the degradation of plastics in the environment (Zhai et al., 2023). Microbial decomposition of PS (polystyrene) has been demonstrated by bacteria and fungi, but the enzymes used in the primary depolymerization are not identified yet. The study by Amobonye et al. (2021) emphasizes a significant gap in knowledge regarding the degradation of polystyrene (PS). Despite the limited understanding, certain enzymes have been identified with the capability to degrade PS, shedding light on potential pathways for plastic degradation. Notably, an extracellular esterase from *Lentinus tigrinus* and specific polymerases from *Bacillus* and *Pseudomonas* have demonstrated PS-degrading activity (Zhai et al., 2023). Enzymes such as styrene monooxygenases (SMO) play a role in the breakdown of PS into styre-

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ne oxide. Styrene Oxide Isomerase (SOI) contributes to the conversion of styrene oxide into other intermediate products. Phenylacetaldehyde Dehydrogenase (PAD) is involved in the conversion of phenylacetaldehyde from styrene oxide. Multiple enzymes associated with phenylacetate degradation contribute to the further breakdown of PS. The degradation of PS results in the formation of styrene oxide, phenylacetaldehyde, phenylacetate, and phenylacetic acid. These intermediate products are smaller molecules that can be metabolized by microorganisms. The breakdown of PS into phenylacetic acid is particularly intriguing as it serves as an intermediate product of the tricarboxylic acid (TCA) cycle. Phenylacetic acid, being a TCA cycle intermediate, is a key compound in central metabolism and is involved in energy production and carbon utilization in many organisms. The enzymatic breakdown of PS into metabolizable derivatives aligns with the ability of microorganisms to utilize these smaller molecules as carbon sources. The connection to the TCA cycle suggests a potential biological relevance in the utilization of PS breakdown products by microorganisms for energy and carbon metabolism. Understanding PS degradation pathways and the involvement of specific enzymes has implications for addressing environmental plastic pollution. Enzymes identified in PS degradation pathways could be harnessed for biotechnological solutions in plastic waste management. While the knowledge gap in PS degradation is acknowledged, recent studies have identified specific enzymes and pathways involved in breaking down PS into metabolizable intermediates. The connection of PS breakdown products to the TCA cycle highlights potential biological relevance and opens avenues for further research in both environmental and biotechnological contexts. (Zhai et al., 2023).

Enzymes utilized in bioremediation

Ideonella sakaiensis 201-F6 is a bacterial strain capable of degrading polyethylene terephthalate (PET), popularly consumed as a synthetic plastic. The strain produces cutinase-like serine hydrolases called IsPET ase and IsMHETase, which are involved in the degradation process.

1. Cutinises for PET degradation: Cutinises, present in fungi and bacteria, such as *Fusarium solani pisi* and *Thermobifida fusca*, are known to degrade PET and po lyester etc (Hu et al., 2016). Various cutinases have demonstrated their capability to degrade PET. Cutinase enzymes from both fungal and bacterial sources are from the α/ß hydrolase family. However, there is no sequence homology between fungal and bacterial cutinases. This versatility makes cutinases valuable enzymes for potential applications in the biodegradation of different plastic materials.

2. Laccases for PE, PA degradation: Laccases are copper dependent enzymes majorly found in fungal lignin biodecomposition. These enzymes have shown degradation capabilities for various plastics such as PA, PE, and PP. Fungal species like *Cochliobolus sp., Phlebia spp., Podospora anserina*, and *Yarrowia lipolytica* have been reported to produce laccases and are involved in the breakdown of lignin. Bacterial laccases are stable at extreme conditions,

Table 3. List of plastic-degrading enzymes from microbial strains against various polymer types (Source: Jesus & Alkendi, 2023).

Microbial strain	Source or sample type	Identified enzyme	Polymer type	Size (mm)
Theilavia terrestris	Soil	Cutinase	PET	5
Thermobifida fusca	Culture collection	Hydrolase and carboxyl esterase	PET	0.1 – 0.6
Synechocaccus sp.	Culture collection	Esterase and hydrolase	PE	0.002
Pseudomonas aestusnigri	Crude oil polluted marine sand	Hydrolase	PE	0.1
Humicola insolens	Commercial product	Cutinase	PET	5
Bacillus subtilis	Soil	Polyurethanase	Impranil DLN PU	0.002
Aspergillus flavus	Wax moth gut	Laccase like multi copper oxidase	LDPE	< 0.2

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such as pH and temperature, compared to fungal laccases, indicating their potential use in microplastics bioremediation. Soil bacteria *Azospirillum lipoferum* produces a thermostable laccase with an optimal pH of 6.0. Other bacterial strains like *Bacillus subtilis* MTCC 2414, *Microbulbifer hydrolyticus* IRE-3, *Pseudomonas extremorientalis* BU118, and *Serratia marcescens* MTCC 4822 have been reported to produce laccases with efficient degradation process against plastics. However, the large-scale utilization of laccases is limited by certain difficulties such as low yield and high-cost production (Akpinar and Ozturk Urek, 2017).

3. Peroxidases in lignin degradation: Peroxidases are oxidoreductases that catalyse the oxidation of various inorganic and organic molecules using hydrogen peroxide. Most peroxidases have been reported in fungal species and are used in lignin decomposition along with laccases. The addition of manganese peroxidase has been shown to enhance the degradation of polyethylene (PE) by lignin-degrading fungi (Li et al., 2020).

Trace elements like manganese and copper play a crucial role in protecting cells from oxidative stress, and this protection, in turn, contributes to the retention of polymer-degrading activities. The marine fungus *Alternaria alternata* FB1 has demonstrated remarkable efficiency in degrading polyethylene (PE) polymers through the production of a substantial number of exoenzymes. This fungus produces 153 exoenzymes, which include significant contributors like peroxidase and laccase. Among the exoenzymes produced by *Alternaria alternata* FB1, peroxidase and laccase are specifically highlighted. These enzymes are known for their oxidative activities, contributing to the breakdown of polymer structures. A study by Gao et al. (2022) highlights the significance of this fungal activity, showcasing a 95% reduction in the molar mass of PE polymer. The reduction in molecular weight indicates effective polymer fragmentation which is a critical step in biodegradation. Considering the diversity of microbial enzymes, including those from bacteria, provides a comprehensive approach to understand and harness plastic biodegradation potential. Insights from studies on fungal and bacterial peroxidases have biotechnological implications for developing strategies to manage synthetic plastic waste (Gao et al., 2022). Studies on bacterial peroxidases are limited compared to fungal peroxidases. Investigating bacterial peroxidases for their role in plastic biodegradation could open new avenues for research and application. Future biodegradation studies focusing on bacterial peroxidases are suggested as a potential avenue for developing new strategies to break down synthetic plastics (Jesus & Alkendi, 2023).

Fungal bioremediation

Polyethylene (PE) is a widely produced polymer globally, primarily used in packaging applications due to its versatility. PE is derived from ethylene and is available in various forms, including high-density PE (HDPE), low-density PE (LDPE), and linear low-density PE (LLDPE). These different forms of PE vary in their degree of branching, molecular packing, crystallinity, and material density. The crystallinity percentage (%cry) of PE directly influences its properties. In the case of PE and other plastics, those with a higher degree of crystallinity tend to be more rigid. This increased rigidity results from stronger intermolecular forces that occur due to closer chain packing in the crystalline structure. This property affects the overall performance and suitability of PE for various applications (European Parliament, 2020; Geyer et al., 2017). The extensive study of fungi in terrestrial ecosystems, with a focus on species like *Aspergillus, Penicillium*, and *Trichoderma,* has revealed their remarkable ability to degrade plastic, particularly in isolation-based assays.

Trichoderma viride and *Aspergillus nomius* were isolated from a landfill and both demonstrated the ability to degrade low-density polyethylene (LDPE). Plastic degradation was evidenced by deformations, weight reduction, and decreased tensile strength of LDPE. Other Aspergillus species like *Aspergillus clavatus, Aspergillus flavus*, and *Aspergillus terreus*, all isolated from landfills, were shown to degrade polyethylene (PE), suggesting a broader potential for Aspergillus genera in plastic degradation. *Penicillium citrinum* was isolated from a plastic dump yard in India. Exhibited capabilities for degrading low-density polyethylene (LDPE). The findings highlight the global distribution of plastic-degrading fungi, with contributing to the diversity of identified species. The plastic degradation capabilities of these fungi were assessed through isolation-based assays, providing controlled environments for studying their interactions with plastics. The identification of diverse fungi capable of plastic degradation suggests potential applications in bioremediation efforts to address plastic pollution. The presence of specific genes, such as alkB, in the genome of some fungi,

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indicates the genetic basis for their plastic degrading capabilities (Khan et al., 2022). The study of fungi in freshwater environments has unveiled fungal strains with the potential to colonize or degrade plastics. Notable strains such as *Cladosporium cladosporioides, Xepiculopsis graminea, Penicillium griseofulvin*, and *Leptosphaeria spp.*, isolated from plastic heaps, were investigated for their potential to decompose polyurethane (PU) and polyethylene (PE). The findings, including subsequent incubation experiments involving various plastic debris types (PE, PP, PS, and polybutylene terephthalate (PBT). Some strains exhibited the capacity to degrade polyurethane (PU), indicating their potential role in breaking down certain plastics. None of the tested strains demonstrated the ability to degrade polyethylene (PE), highlighting variability in fungal plastic degradation capabilities. *Cladosporium* and *Alternaria* were notably enriched on plastic debris compared to the surrounding water. This enrichment signifies a distinct fungal community structure on plastic surfaces. The fungal communities associated with plastic debris differ from those in the surrounding water, indicating a selective process for fungal colonization of plastics. The findings prompt further research to explore the mechanisms underlying fungal interactions with different plastics, informing the development of strategies for plastic waste management and bioremediation (Alomar et al., 2017). The investigation of micro biota colonizing plastic in marine environments has historically focused more on bacteria than fungi, creating an understudied aspect of microbial communities. In marine studies, particularly in contrast to terrestrial environments, the emphasis has been on understanding the natural fungal communities associated with plastic rather than isolating and characterizing individual fungal species. Fungi, especially in marine environments, have been relatively understudied in comparison, creating a gap in our understanding of their roles in plastic-associated microbial communities. In marine ecosystems, there has been a shift towards examining the natural fungal communities present on plastic surfaces. Rather than isolating and characterizing individual fungal species, the focus is on understanding the broader dynamics of fungal communities that naturally colonize plastic. Specific attention is given to plastic types like polyethylene terephthalate (PET), commonly used in drinking bottles. Fungi have been identified as components of biofilms forming on PET surfaces exposed to marine environments. Studies often involve in-situ incubition, where plastic samples are deployed in the marine environment to allow natural colonization. In the case mentioned, after approximately six weeks of in-situ incubation, fungal colonization was observed on PET surfaces. Ascomycota, Basidiomycota, Chytridiomycota, members of these fungal phyla were identified as colonizers on PET surfaces in marine environments. Alongside fungi, prokaryotic microorganisms, such as bacteria, were also identified as part of the biofilm on plastic surfaces. Understanding fun- gal contributions to biofilm formation on plastic surfaces is crucial for comprehending the stability and persistence of plastic pollution in marine ecosystems. (Lenz et al., 2016). Marine fungi engage in polymer degradation through a process akin to that of bacteria and algae, involving attachment, colonization, and subsequent polymer breakdown. Two marine fungi, *Aspergillus tubingensis* and *Aspergillus flavus*, were observed to degrade HDPE films, altering the polymer surface significantly with the development of cracks. Unaffected polymers maintained a smooth surface after 30 days, while fungal activity, facilitated by enzymes, and induced crack formation, indicating degradation. Like bacteria, fungi grow and utilize polymers as their primary carbon source for degradation. Laccases, multi-copper oxidases, play a crucial role in fungal attachment, oxidizing various substrates, including polymers like PE and PVC. Esterases, such as cutinases and lipases, contribute to PET and polyurethane (PUR) degradation, with proteases and ureases being effective on PUR. The realm of enzymatic plastic degradation has seen a wealth of discoveries, showcasing the diverse capabilities of various enzymes in breaking down polymeric substrates. Several key studies contribute to our understanding of enzymatic plastic degradation: Cutinases sourced from *Humilica insolent*, *Pseudomonas medicine*, and *Fusarium solani* have demonstrated proficiency in polyethylene terephthalate (PET) degradation. Cutinases are enzymes capable of hydrolysing ester bonds present in PET, facilitating the degradation of this widely used plastic. Wang et al. (2017a) conducted the cultivation of various fungi species. The study resulted in the extraction and isolation of hydrolytic enzymes, including amylases, glucanases, xylanases, pectinases, and lipases. These enzymes exhibit the capability to hydrolyse a broad range of polymers found in algae, showcasing the versatility of fungal

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enzymes in breaking down complex organic compounds. Zang et al. (2023) identified two laccase genes, AFLA_006190 and AFLA_053930, which displayed heightened expression during the degradation of high-density polyethylene (HDPE). Laccases play a crucial role in the oxidation of various substrates, including polymers like HDPE, contributing to the breakdown process. Recent evidence suggests that enzymes involved in plastic degradation operate synergistically, enhancing overall efficiency. The coordinated action of different enzymes can target various components of polymeric structures, accelerating the degradation process. *Peniophora sp.* has been shown to possess at least eight genes encoding ten different laccases. This diversity represents a valuable resource with considerable potential for exploiting *Peniophora sp.* in bioremediation efforts. The range of laccases suggests versatility in targeting different types of polymers. The diverse array of enzymes discovered in these studies has significant implications for bioremediation efforts. Understan-ding the enzymatic arsenal of different microor-ganisms provides insights into developing strategies for effective plastic waste management (Roccuzzo et al., 2021). *Aspergillus fumigatus* LAR 9 demonstrated effectiveness in degrading Mater-Bi, a bio-based and biodegradable plastic. The ability of fungi to degrade various types of plastics suggests their potential to address plastic pollution across different polymer categories. In a study, microbial consortium, including fungi such as *Fusarium oxysporium, Paecilomyces lilacinus*, and *Paecilomyces farinosus*, colonizing the surface of poly(3-hydro-xybutyrate-co-3hydroxyvalerate) (PHBV) films were identified. The consortium strongly indu-ced PHBV degradation, showcasing the collaborative action of multiple microorganisms in plastic break-down (Viel et al., 2023). Several fungal species, including *Asteromyces cruciatus, Candida guillermondii, Debaryomyces hansenii*, and *Nia vibri*, were identified on poly(3-hydroxybutyrate-co3hydroxyva lerate) (PHBV). The diversity of fungal species associated with PHBV suggests the potential for a range of enzymatic activities targeting this polymer. The identification of efficient fungal isolates and their capabilities in degrading different plastics opens opportunities for enzyme engineering. Tailo-ring enzymatic solutions based on specific fungal isolates can contribute to targeted plastic waste management strategies. The identified fungal Isola-tes, their proficiency in degrading various plastics, and the collaborative actions of microbial consortia underscore the potential of fungi in biotechnological solutions for mitigating plastic pollution.

Table 4. *Fungal species and their enzymes capable to biodegrade plastics (Modified from Temporiti et al., 2022).*

Fungal species	Enzyme	Polymer type
Trichoderma, Fusarium, Phanerochaete chrysosporium, Bjerkandera adusta, Trametes versicolor and Rhizopus oryzae	Laccases, Peroxidases	Polyethylene (PE)
Ascomycota Trichoderma	LFuaccase and Peroxidase	Polyethylene (PE)
Aspergillus flavus	Laccase	HDPE
Aspergillus Fusarium	Esterases, Cutinases, Lipases and Carboxylesterases	polyethylene terephthalate (PET)
Humicola insolens, Fusarium solani pisi and Fusarium oxysporum	Cutinases	Polyethylene terephthalate (PET)
Aspergillus oryzae	Lipases	Polyethylene terephthalate (PET)
Beauveria brongniartii and Penicillium citrinum	Extracellular Polyesterases	Polyethylene terephthalate (PET)
Chaetomium globosum and Aspergillus terreus	Esterase and Urethane hydrolase	Polyurethane (PUR)
Aspergillus tubingenesis	Esterases and Lipases	Polyurethane (PUR)
Phanerocheate chrysosporium	Lignin peroxidase	Polyvinyl chloride (PVC)
Cochliobolus sp.	Laccase	Polyvinyl chloride (PVC)
Lentinus tigrinus	Esterase	Polystyrene (PS)

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Microalgae bioremediation

Microalgae are widespread photosynthetic entities that are present in both oceanic and fresh water ecosystems. The process of decomposition of a few contaminants by microalgae is well researched but limited literature is present on the decomposition of plastic by microalgae. The hydrophobic nature of plastics may hinder the colonization process by algae. The degradation of plastics by algae involves two distinct pathways, throwing a light on the potential mechanisms for breaking down the plastic in environmentally sound manner (Moog et al., 2019).

i. Polymer Molecular Weight Reduction: This pathway involves the enzymatic degradation of large polymer molecules. Enzymes act as catalysts, initiating the breakdown of complex structures into smaller fragments. Process: Algae-produced enzymes interact with macromolecules, particularly targeting carbonyl groups on the surface of polyethylene (PE). Carbonyl groups are key chemical features associated with the susceptibility of plastics to degradation. Significance: Enzymatic action facilitates the cleavage of high molecular weight polymers, leading to the gradual reduction in the size of plastic molecules.

ii. Oxidation of Low-Molecular Weight Molecule: In this pathway, low-molecular-weight plastic molecules undergo oxidation, a process involving the addition of oxygen or removal of electrons. Process: Algae are implicated in triggering the oxidation of smaller plastic fragments. This oxidative process contributes to the fragmentation of plastic at the molecular level. Oxidation of low-molecular-weight plastic compounds represents an alternative mechanism for breaking down plastic structures, complementing enzymetic degradation pathways (Roccuzzo et al., 2021).

iii. Enzymatic Interaction with Polyethylene (PE): Enzymes, particularly those interacting with carbonyl groups on the surface of polyethylene, play a pivotal role in triggering the biodegradation of PE. The presence of enzymes facilitates a targeted attack on the molecular structure of PE, initiating the degradation process. Environmental Impact: Understanding enzymatic pathways sheds light on the potential of natural systems, such as algae, to contribute to the reduction of plastic waste in the environment.

It was demonstrated that *Chlamydomonas reinhardtii*, a species of algae, exhibits the functional expression of PETase, an enzyme known for its role in polyethylene terephthalate (PET) degradation. The direct involvement of PETase in the degradation of polyethylene suggests the adaptability of algae to metabolize various types of plastic. Biotechnological Potential: This finding opens avenues for biotechnological applications, where the genetic modifycation or enhancement of algae could be explored to optimize their plastic-degrading capabilities. The identification of these pathways and the role of specific enzymes in the degradation of plastics by algae offer valuable insights for developing strategies to address plastic pollution and promote environmentally sustainable practices. (Roccuzzo et al., 2021). The utilization of microalgae for biotechnological applications, particularly in the context of biofuel production and bioremediation, represents a burgeoning field of research with promising implications. Microalgae are being extensively studied for their potential as biofuel feedstock due to their high lipid content and rapid growth rates. Their ability to convert sunlight into energy through photosynthesis makes microalgae a sustainable and renewable resource for biofuel production. Researchers are actively exploring methods to optimize microalga strains for enhanced biofuel yield, contributing to the development of environmentally friendly alternatives to traditional fossil fuels.

Microalgae as microbial chassis

Microbial chassis are organisms that serve as platforms for sustaining and supporting genetic components, facilitating the engineering of cellular functions. Studies have identified microalgae, particularly eukaryotic microalgae, as potential microbial chassis due to their amenability to genetic manipulation and their ability to house engineered genetic components for desired functions (Kim et al., 2020). Functional expression studies involving green algae and diatoms have been conducted to demonstrate the feasibility of using eukaryotic microalgae as model systems for bioremediation. Functional expression studies involving green algae and diatoms have been conducted to demonstrate the feasibility of using eukaryotic microalgae as model systems for bioremediation. The utilization of

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eukaryotic microalgae for bioremediation offers a sustainable and ecofriendly approach to addressing microplastic contamination. Compared to traditional methods involving bacteria, microalgae present advantages such as genetic manipulability, rapid growth, and the potential for large-scale cultivation, making them a viable and efficient option for remediation efforts. The use of eukaryotic microalgae for bioremediation aligns with ecofriendly principles, as it minimizes the need for synthetic chemicals and leverages natural processes. The use of eukaryotic microalgae as microbial chassis and bioremediation agents represents a promising avenue for future developments in the field of environmental biotechnology (Kim et al., 2020). The research into the biodegradation of microplastics by algae is an evolving field, and while it is still in its early stages, some algal strains have shown promising capabilities in breaking down microplastics. A notable study by Sarmah and Rout (2018) puts light on the mechanisms involved. Despite the nascent stage of research, certain algal strains have exhibited the ability to degrade microplastics and plastic particles. Production of Lignin and Extracellular Polysaccharides: Algae are known to produce lignin and extracellular polysaccharides, which are compounds found on their surfaces. These biogenic compounds play a vital role in the degradation of plastic waste, acting as agents that contribute to the breakdown of microplastic polymers. Lignin and Polysaccharides are used in plastic degradation. Lignin is a complex organic polymer known for its robust structure. Its presence on algal surfaces may contribute to the enzymatic breakdown of certain types of plastics. Polysaccharides; composed of sugar units, can facilitate microbial adhesion and enzymatic activity, potentially aiding in plastic degradation. Extracellular polysaccharides can enhance the adhesion of microorganisms, including algae, to plastic surfaces. This adhesion facilitates microbial colonization and the secretion of enzymes, fostering the degradation of microplastic materials. while research in the field of algal-mediated microplastic degradation is limited, the findings so far suggest that certain algal strains, through the production of lignin and extracellular polysaccharides, hold the potential to contribute to the reduction of microplastic pollution. Further exploration and understanding of these mechanisms are crucial for developing effective and environmentally friendly strategies for mitigating the impact of microplastics on ecosystems. (Sarmah and Rout, 2018). The observed capabilities of certain algal sources, including *Scenedesmus dimorphus, Anabena spiroides*, and the diatom *Navicula pupula*, to colonize and break down plastic polymers provide valuable insights into the potential of these microorganisms in addressing plastic pollution. Here is an elaboration on their degradation capabilities: These algal species have been documented to colonize the surfaces of plastic materials. Their ability to adhere to the plastic surface allows for a close interaction between the algae and the plastic polymer. The presence of these algal strains on the plastic surface suggests a potential role in initiating the breakdown of the polymer. While specific mechanisms may vary, diatoms, known for their intricate silica cell walls, can potentially secrete enzymes or engage in other biochemical processes contributing to plastic degradation. Algae, including diatoms, often produce extracellular polymeric substances (EPS), which may include polysaccharides and other compounds. The production of EPS can enhance the adhesion of algae to plastic surfaces and create a microenvironment conducive to enzymatic activity, contributing to the degradation of plastic polymers (Ramachandran et al., 2017). The degradation of microplastics can vary depending on the type of plastic polymer and the specific algal species involved. For instance, in one study, a higher degradation rate was observed for polyethylene terephthalate (PET) followed by polyethylene (PE) (Khoironi et al., 2019). Biotechnological approaches involving algae as a source of engineered PETase represent a cutting-edge strategy in the quest for sustainable solutions to address plastic pollution, particularly the degradation of polyethylene terephthalate (PET) plastics. PETase is an enzyme that naturally occurs in certain bacteria, capable of hydrolysing PET plastics into smaller, more biodegradable compounds. Utilizing algae for PETase production aligns with sustainable and eco-friendly practices, offering a natural solution to the challenges posed by PET plastic waste. (Zurier et al., 2020). Additionally, during a 60-day period, *Bacillus*

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sp. and *Paenibacillus* sp. effectively reduced the size of polypropylene (PP) plastic particles (Park and Kim, 2019). Microalgae contribute to the biodegradation of plastic waste with its enzymes that weaken the chemical bonds of plastic polymers. The attempt of using microalgae to convert these plastics into metabolites such as carbon dioxide, water and new biomass is of great interest.

Legal limits on microplastics

In response to the growing issue of microplastics, several international actions have been taken. Global initiatives include the Basel Convention, the UN's Decade of Ocean Science for Sustainable Development, and the Geneva Beat Plastic Pollution Dialogues. An Intergovernmental Negotiating Committee (INC) has been established with the aim of creating a legally binding agreement to address plastic pollution by the end of 2024. As part of this effort, the "High Ambition Coalition to End Plastic Pollution" was launched in August 2022. This coalition comprises countries including Canada, Chile, Costa Rica, Denmark, Dominican Republic, Ecuador, Finland, France, Georgia, Germany, Iceland, Peru, Portugal, Republic of Korea, Senegal, Sweden, Switzerland, and the UK. Their collective goal is to eliminate plastic pollution by 2040. The coalition will convene to identify key priorities for the INC's negotiation sessions, establish strategic objectives for reducing plastic pollution, and organize awareness events. In the United States, the Microbead-Free Waters Act, established in 2005 and effective in 2017, primarily addresses the issue of microplastics pollution, including that caused by plastic bags. This Act prohibits the sale of personal care products with microbeads, promotes biodegradable alternatives, plastic recycling, and wastewater treatment to prevent microplastics in aquatic ecosystems. Some critics argue that the act's scope is limited and does not promote enough biodegradable options to effectively combat plastic pollution. Additionally, U.S. states have implemented measures to reduce plastic bag use, aiming to mitigate environmental impacts and improve waste management. California passed a law in 2020 banning the sale of personal care products containing certain types of microplastics, specifically microbeads commonly found in exfoliating products. It was also first state to enact legislation in 2014, which imposed a state-wide ban on single-use plastics at major retail stores. It also introduced a minimum charge of 10 cents for recycled paper bags. Hawaii implemented a state-wide ban on non-biodegradable plastic bags and required paper bags to contain at least 40% recycled material across all populous counties. New York banned plastic bags from March 2020. Connecticut, Delaware, Maine, Oregon, and Vermont states have also enacted legislation banning single-use plastic bags. Vermont went further by placing additional restrictions on single-use straws and polystyrene containers. These state-level measures aim to reduce plastic bag usage, encourage the use of reusable bags, and contribute to environmental sustainability. The UK had set a strategic goal to ensure that all plastic packaging in the market would be either recyclable, reusable, or compostable by the year 2025. This ambition was aligned with the broader commitment to leave a cleaner environment for future generations, emphasizing the aim of achieving zero avoidable waste by 2050 and eliminating avoidable plastic waste by 2042. These policies were outlined in the December 2018 Resource and Waste Strategy, which aimed to reduce plastic waste. Subsequently, a series of consultations were conducted in February 2019, yielding several proposals like Reforming the UK Packaging Producer Responsibility System, Plastic Packaging Tax Consultation, Introduction of a Deposit Return Scheme (DRS) and Consistency in Household and Business Recycling Collections in England. The UK, alongside its domestic regulations on plastics, has actively engaged in various international agreements with the goal of reducing plastic pollution in marine environments. These agreements include:

(i). Commonwealth Clean Oceans Alliance: The UK is a participant in this initiative, which aims to combat marine plastic pollution within the Commonwealth nations.

(ii). UN Sustainable Development Goals: The UK has committed to the UN Sustainable Development Goals, which include targets related to reducing marine pollution, including plastic waste. In addition to these international commitments, the UK has obligations stemming from agreements like the UN Basel Convention on the Control of Trans boundary Movements of Hazardous Waste and their Disposal (the Basel Convention). As of January 1, 2021, this convention requires prior informed consent for the shipment of specific types of plastic waste and applies uniformly across the UK.

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(iii). European Union: European countries have phased out plastic microbeads from various products, including cosmetics. In 2018, they adopted the European Strategy for Plastics in a Circular Economy and initiated initiatives like "Zero Plastics to Landfill" to protect the environment. The EU has taken actions to address the issue of microplastics by implementing a ban on microplastics in cosmetics and personal care products since July 2018.This ban prohibits the use of microplastics in rinse-off cosmetic and personal care products, such as toothpaste, exfoliating scrubs, and shower gels. The EU has also initiated studies and research to understand the sources and impacts of microplastics on the environment and human health. These studies aim to develop further regulations to reduce the release of microplastics into the environment and prevent their harmful effects. The EU's efforts to address microplastics align with its broader goals of promoting sustainable development and protecting the environment.

(iv). United Nations: At the fourth United Nations Environment Assembly in March 2019, over 150 nations' environment ministers pledged to substantially eliminate single-use plastic items by 2030. They also recognized the need for long-term microplastics removal from oceans. The UN has recognized marine plastics and MPs under 13 out of its 17 sustainable development goals (SDGs) due to the pollution of the water body and the resulting adverse effects on ecosystems and livelihoods. Notable among the 13 SDGs that specifically and directly address plastic pollution is SDG number 14, which is aimed at the conservation and sustainable use of the oceans, seas, and marine resources for sustainable development. SDG 14 focuses on plastic pollution under target 14.1, which aims to prevent and significantly reduce all types of marine pollution, particularly those caused by land-based activities, by 2025. The target is expected to be measured by indicator 14.1.1b and evaluated by an index of coastal eutrophication and floating plastic debris. Only a single indicator of SDG 14 out of 247 indicators of the SDGs is meant to address the plastics problem, with the rest having no specific targets or indicators to measure their success, thus making implementation, reliable reporting, and monitoring by governments and organizations a huge challenge.

(v). Basel Convention: In May 2019, governments agreed to amend the Basel Convention, requiring importing countries to consent to the import of contaminated plastic waste, reflecting global efforts to address plastic pollution. The Basel Convention is currently the sole legally binding global regulation addressing the international movement of plastic waste. In 2018, Norway proposed adding plastic waste to the Annexes of the Basel Convention. This was aimed at strengthening control over cross-border plastic waste movements, preventing the influx of plastic waste into countries lacking proper waste management infrastructure. The resulting amendments, known as the Plastic Waste Amendments, were adopted in 2019 and came into effect in January 2021.

(vi). Global Partnership on Marine Litter (GPML): In response to the Manila Declaration's call, the GPML was established during the 2012 Earth Summit. It aims to address the issue of marine litter on a global scale, emphasizing collaborative efforts to combat this environmental challenge.

(vii). London Convention, 1972: The 1972 Convention on the Prevention of Marine Pollution by Dumping Wastes and Other Matter, known as the London Convention, was enacted. It serves to regulate the disposal of waste into the ocean and control all sources of marine pollution.

(viii). In 2018, China implemented restrictions on the import of plastic waste, leading to increased shipments to alternative destinations with inadequate waste management practices, including Malaysia, Thailand, Vietnam, and Turkey. However, tighter regulations introduced in 2021 resulted in a 25% reduction in Germany's plastic waste exports compared to 2020. While this reduction may initially lead to higher landfill rates, it should ultimately encourage a shift toward adopting a more circular economy approach.

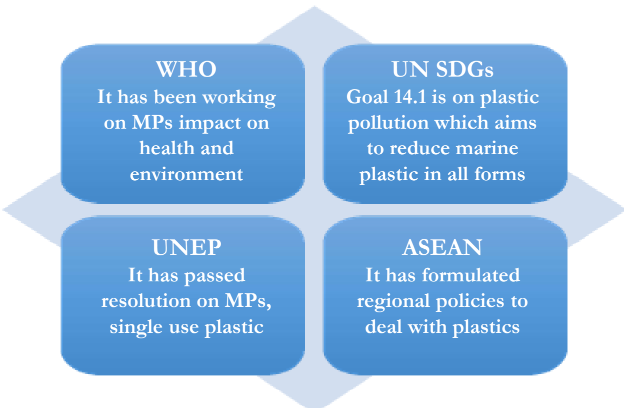

Figure 5. *Governance strategies by various international and regional organizations to combat microplastics and plastics (Adopted from Usman et al., 2022).*

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It's worth noting that, so far, only plastic wastes classified as "hazardous" are subject to the limitations outlined in the Plastic Waste Amendments. Procedures for non-hazardous plastic waste will undergo a review in 2024. These international actions demonstrate a global commit-ment to reducing plastic pollution, phasing out harmful plastics, and seeking sustainable alternatives to protect the environment and aquatic ecosystems (Osman et al., 2023).

Indian statute status of microplastics

A status report was submitted by the Central Pollution Control Board (CPCB) in response to Original Application No 99 of 2021 (SZ), initiated by the National Green Tribunal (NGT) based on a news item published in The Times of India Newspaper on April 5, 2021, highlighting the presence of microplastics in the air in Chennai. In its order on April 16, 2021, the NGT directed the CPCB to address the issue and provide guidelines on mitigating the impact of dangerous pollutants resulting from microplastics. The CPCB, in collaboration with the Tamil Nadu Pollution Control Board, was instructed to submit a report on the enforcement of notifications that ban single-use plastics in southern states like Tamil Nadu. Additionally, the NGT sought information on the implementation of Extended Producer Responsibility (EPR) fixed under the Plastic Waste Management Rules, 2016, and Rule (17) of the Solid Waste Management Rules, 2016. According to the CPCB's report, there is an ongoing revision of the National Ambient Air Quality Standards (NAAQS), which will include standards for monitoring atmospheric microplastics. The report emphasizes measures to control plastic pollution, thereby reducing microplastics in the environment. These measures encompass:

a) Strengthening infrastructure for the collection, segregation, channelization, and processing of plastic waste.

b) Imposing restrictions on the production and use of single-use plastic items.

c) Developing standardized protocols for the identification, characterization, and quantification of microplastics.

d) Ensuring effective implementation of Extended Producer Responsibility by producers and brand owners.

e) Conducting public awareness campaigns to prevent and control plastic pollution.

Additionally, the CPCB issued a letter on May 19, 2021, to southern states (Tamil Nadu, Andhra Pradesh, Karnataka, Kerala, Goa, Pondicherry, and Telangana), seeking information on the status of the ban on single-use plastics and the implementation status of provisions in the Plastic Waste Management Rules and Solid Waste Management Rules, particularly regarding the fulfilment of EPR liability by producers and brand owners.

Implementing a sin tax on plastic products is a crucial measure to reduce plastic usage, effective in Tamil Nadu since January 1, 2019, making Maharashtra the 26th state in India to adopt this approach. This tax system imposes a significant financial burden on both environmental polluters and manufacturers, categorizing pollution as a sinful act. Drawing parallels with the success of reduced cigarette usage globally due to similar taxation, the goal is to make plastics less affordable and convenient, enabling other materials to compete in the market (Indian Environment Portal. 2023).

i. Single-Use Plastic Ban in India: The Plastic Waste Management (Second Amendment) Rules of 2022 entail the following changes:

a. Ban on single-use plastics, effective from July 1, 2022. The ban specifically targets plastics with low utility and high potential for littering.

b. A requirement to increase the thickness of plastic carry bags to a minimum of 120 microns, effective from December 31.

c. Prohibition on the import of solid plastic waste, effective since March 2019.

ii. Infrastructure Responsibility: As per the 2016 Plastic Trash Management Rules, local bodies are mandated to establish infrastructure for the collection, processing, and disposal of plastic waste.

iii. Extended Producer Responsibility (EPR): The Plastic Waste Management (Amendment) Rules of 2018 introduced the concept of Extended Producer Responsibility (EPR). This requires producers to take responsibility for the post-consumer stage of their products, encouraging sustainable waste management practices.

iv. Un-Plastic Collective (UPC): The Un-Plastic Collective is a voluntary initiative jointly announced by the Confederation of Indian Industry, WWF-India, and UNEP-India. Its primary goal is to mitigate the adverse effects of plastic pollution on both ecological and social wellbeing globally. v. As per the Bureau of Indian Standards (BIS) 2017, plastic microbeads with a diameter of 5 mm or less, which are water-insoluble and constitute solid parti-

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cles used for exfoliation or cleansing in personal care products, have been included in the prohibited list.
Aligned with the Prime Minister's call to phase out single-use plastic by 2022, the Ministry of Environment, Forest and Climate Change in India has introduced the Plastic Waste Management Amendment Rules, in 2021. These rules, effective from July 1, 2022, prohibit the manufacture, import, and use of specific single-use plastic items known for their low utility and high littering potential. The initiative aims to address the environmental challenges posed by single-use plastics, with India leading efforts globally. At the 4th United Nations Environment Assembly in 2019, India championed a resolution on combating pollution from single-use plastic products, emphasizing the need for global attention to this issue. The resolution's adoption marked a significant milestone. The banned items include plastic sticks for various purposes, polystyrene for decoration, and a range of single use plastic commodities like plates, cups, cutlery, and banners. To combat plastic bag littering, the thickness of plastic carry bags has been increased, promoting reuse. Plastic packaging waste not covered by the phase-out will be managed under the Extended Producer Responsibility, with guidelines given legal force through the Plastic Waste Manage-ment Amendment Rules, 2021. The thickness of plastic carry bags increased from 50 to 75 microns from 30th September 2021 and to 120 microns with effect from 31 December 2022. The Swachh Bharat Mission is also strengthening waste management infrastructure. States and Union Territories are urged to form Special Task Forces to eliminate single-use plastics, while a National Level Taskforce coordinates nationwide efforts. Comprehensive action plans, backed by legal directions, are expected from state and central authorities to implement Plastic Waste Management Rules, 2016, and enforce regulations effectively. The government is actively promoting awareness through campaigns and initiatives, including essay writing competitions among school students. Furthermore, to foster innovation, the India Plastic Challenge – Hackathon 2021 has been launched, encouraging students and start-ups to develop alternatives to single-use plastics and digital solutions for plastic waste management. These collective efforts reflect India's commitment to combatting plastic pollution and promoting environmental sustainability (PIB, 2021). Currently, plastics are subject to a Goods and Services Tax (GST) rate of 18% or less. Proposing an increase in the GST on plastics to 28% could generate an additional revenue of approximately Rs 20,000 core annually. These funds could be instru-mental for the central and state governments to initiate a scalable program aimed at controlling plastic waste. This sin tax on polyethylene is envi-sioned to establish a fund dedicated to managing the significant volume of solid waste generated today. The proposed increase in the Other Cess (OC) tax, equivalent to GST or excise/VAT on plastics, is essential. Plastics used in daily life, such as those containing carbon, hydrogen, nitrogen, and oxygen (e.g., polyester, PET, and nylon), could be subject to the lowest tax levels due to their lesser environ-mental impact and potential for biodegradation. In contrast, polyethylene and isotactic polypropylene could face moderate taxation, as their source separation and controlled burning under oxygen-rich conditions can be harnessed for energy production, addressing 50% of the plastics' environ-mental impact during manufacturing and use (TOI, 2022).

Conclusions

Microplastic pollution is escalating as a grave concern in the environment, necessitating the urgent and comprehensive efforts towards the mitigation. The exploration of bioremediation as a potential solution provides a promising avenue for reducing microplastics contamination in diverse ecosystems. But there are a lot of hurdles and limitations in the application of microbes for the biodegradation of microplastics which can be overcome by different genetic manipulations. However, most of genetically modified microbes have only been validated under laboratory conditions and reports on their efficiency in field conditions are largely lacking. Also, the knowledge associated with different metabolic pathways and enzymes is largely lacking. The recent advances in metagenomic analysis and engineering of uncultivated microbial communities, sampled from contaminated sites, can assist in the development of novel processes of bioremediation and culture-independent techniques can open new avenues for the discovery of novel metabolic pathways and enzymes. Understanding the nuanced impacts on environmental health, the selection of reduction strategies should be a nuanced process, considering infrastructure, economic conditions, available alternatives, and societal readiness for a transition to a plastic independent economy.

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