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REVIEW PAPER

Biotechnological methods to remove microplastics: a review

Uttpal Anand[1] · **Satarupa Dey**[2] · **Elza Bontempi**[3] · **Serena Ducoli**[3] · **A. Dick Vethaak**[4,5] · **Abhijit Dey**[6] · **Stefania Federici**[3]

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Abstract

Microplastics pollution is major threat to ecosystems and is impacting abiotic and biotic components. Microplastics are diverse and highly complex contaminants that transport other contaminants and microbes. Current methods to remove microplastics include biodegradation, incineration, landfilling, and recycling. Here we review microplastics with focus on sources, toxicity, and biodegradation. We discuss the role of algae, fungi, bacteria in the biodegradation, and we present biotechnological methods to enhance degradation, e.g., gene editing tools and bioinformatics.

Keywords Microplastics · Incineration · Microplastic degrading microorganisms · Biodegradation · Synthetic biology · Biotechnological interventions · CRISPR

Introduction

Plastic pollution is considered to be one of the most significant threats to global ecosystems and is known to impact both the abiotic and biotic components (Ogonowski et al. 2018; Everaert et al. 2020; Lusher et al. 2021; Liu et al. 2022; Su et al. 2022; Federici et al. 2022). In 2018, global plastic production increased to more than 360 million tons, and it is expected to triple by the year 2050 (Gumel et al. 2013; Plastics Europe 2020). According to a survey made by Plastics Europe (the Association of Plastics Manufacturers in Europe) and the European Association of Plastics Recycling and Recovery Organisations, Asia is the largest producer and consumer of plastic goods, with China contributing the lion's share (32%) to this "white pollution", while the rest of Asia produces nearly 19%.

Whereas, Europe, Canada, Mexico, and the USA produce fewer plastics than Asia (Tiwari et al. 2020; https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/).

Moreover, the coronavirus disease 2019 pandemic has increased the use of one-time useable plastic wares, gloves, masks, tissues, and other personal protective equipment which along with the generation of municipal waste has exacerbated the plastic pollution crisis (De-la-Torre and Aragaw 2021; Morgana et al. 2021; Patrício Silva et al. 2021; Yang et al. 2022). Most single-use masks and personal protective equipment are made up of various polymeric substances such as polypropylene, polyurethane, polyacrylonitrile, polyethylene, and polystyrene. Also, the imposition of lockdown has led to the surge in the use of different types of plastic largely composed of high-density polyethylene, low-density polyethylene, polypropylene, and polyethylene terephthalate. The incorrect disposal and poor waste management of plastic items have led to their ubiquitous presence in

✉ Satarupa Dey
dey1919@gmail.com

✉ Abhijit Dey
abhijit.dbs@presiuniv.ac.in

✉ Stefania Federici
stefania.federici@unibs.it

[1] Zuckerberg Institute for Water Research, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 8499000 Midreshet Ben Gurion, Israel

[2] Department of Botany, Shyampur Siddheswari Mahavidyalaya, University of Calcutta, Ajodhya, Shyampur, Howrah 711312, India

[3] Department of Mechanical and Industrial Engineering, INSTM Unit of Brescia, University of Brescia, Via Branze 38, 25123 Brescia, Italy

[4] Department of Environment and Health, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

[5] Institute for Risk Assessment Sciences, Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands

[6] Department of Life Sciences, Presidency University, 86/1 College Street, Kolkata, West Bengal 700073, India

all environments globally, and due to their persistent nature and low biodegradability, they will remain in these environments for prolonged periods of time (Andrady 2017; Aragaw 2020; Patrício Silva 2021).

It has been reported that around 4.8–12.8 million metric tons of plastic debris are disposed of in the ocean without a proper management strategy (Jambeck et al. 2015). This disposal of plastic debris into the ocean has led to a series of impacts on marine life (Galloway et al. 2017; Mendoza et al. 2018; Peng et al. 2020) and human health (Keswani et al. 2016). They result in problems like ingestion, entanglement, and suffocation to different marine species leading to reduced life quality, and impairment of feeding and reproductive ability (Staffieri et al. 2019; Wilcox et al. 2018). In addition, most plastics are positively buoyant and can be transported over a long distance, acting as carriers of non-native and invasive species (De-la-Torre et al. 2021).

Wasted plastics, exposed to ultraviolet (UV) irradiation and other environmental degradation processes, can degrade and fragment into smaller pieces: large microplastics (1 mm–5 mm), microplastics (1 µm–1 mm) and nanoplastics (1 nm–1 µm) (Waller et al. 2017; De-la-Torre 2020; Atugoda et al. 2022). However, there is no scientific consensus on the definition of microplastics. Here we define microplastic as plastics that are ≤ 5000 µm (or ≤ 5 mm) in diameter (ISO/TR 21960:2020), with "large microplastic" referring to the larger portion (> 1000 µm or > 1 mm) (Frias and Nash 2019; GESAMP 2019; Hartmann et al. 2019; Hale et al. 2020; Koelmans et al. 2020). Microplastics are the major contributors to plastic pollution in the marine ecosystem, freshwater ecosystem, soil ecosystem, and agroecosystem (Nizzetto et al. 2016; Mendoza et al. 2019; Wang et al. 2019b; Wong et al. 2020; Chia et al. 2021; Razeghi et al. 2021) and are known to be globally ubiquitous (Jambeck et al. 2015). They also contaminate soil through sewage sludge and wastewater which are widely used as fertilizers. Moreover, plastics are also used in agriculture mainly in a greenhouse, low tunnels and mulching and also as a coating for fertilizers, hormones, pesticides, and packaging material (Nizzetto et al. 2016).

Recently, several studies have investigated the distribution, uptake, fate, behavior, effects, and removal strategies of microplastics (Bahtt et al. 2021; Wong et al. 2020; Anik et al. 2021). Nevertheless, the effectiveness of the methods developed for microplastic remediation still remains unclear. Research in microplastic degradation has progressed focusing on biological and non-biological approaches. Microplastic treatments enabled by the action of microorganisms such as algae, fungi, and bacteria are considered attracting tools for cost-effective and eco-friendly degradation approaches. While research papers and reviews have recently been published on the microorganism-mediated degradation and remediation strategies (Chen et al. 2022; Bahtt et al. 2021; Qin et al. 2021; Kotova et al. 2021; Cholewinski et al. 2022), only few articles have addressed plastic degradation focusing on the use of modern biotechnological methods in the enhancement of microplastic degradation and there remains a lack of knowledge with respect to biotechnological interventions for microplastic removal (Danso et al. 2019; Patrício Silva et al. 2021). Therefore, it is crucial to summarize and analyze the current state of knowledge to determine microplastic degradation by microorganism, as well as to promote a better understanding of how modern biotechnological methods can be enabled to manage and degrade microplastics.

This review provides some background information on the impact of microplastic sources, their effects on marine life including microalgae, and their potential impact on humans. Then it categorizes the different types of microorganisms and enzymes associated with the degradation of microplastics. Finally, different biotechnological methods to increase the efficiency of bacterial cell degradation of microplastics and their possible application in field studies are discussed at length.

Sources of microplastics

Microplastics derive from two different origins. The primary microplastics are generated from cosmetics, household products, drug delivery systems (Patel et al. 2009), and polymeric raw materials (pellets, flakes, powders) composed of polyethylene, polystyrene, polyvinyl chloride, polyamide nylon 6 and polypropylene, among others. Personal care products such as toothpaste, scrubs, cleaning materials and cosmetics are known to contain irregularly shaped microplastics of 0.5 to < 0.1 mm in diameter, which are mainly marketed as “micro-beads” or “micro-exfoliates” and contribute to primary microplastics (Fendall and Sewell 2009). However, the existing wastewater treatment plants have shown that tertiary treatment of water is not a source of microplastic pollution, as these pollutants are effectively removed by the skimming and settling treatment processes (Carr et al. 2016).

The secondary microplastics are generated due to extensive fragmentation of large plastic items or particles in presence of environmental factors such as high temperature and exposure to UV radiation, stress, reactive ozone, oxidation, and atmospheric pressure (Tiwari et al. 2020; John et al. 2021). Polymeric materials can withstand oxidative-thermal degradation only when antioxidants and stabilizers are added. Physical abrasion also generates secondary microplastics. Moreover, biological agents like bacteria, fungi and algae are known to produce a plethora of enzymes which play a crucial role in microplastic degradation (Chia et al. 2020; John et al. 2021; Othman et al. 2021; Chen et al. 2022; Manzi et al. 2022; Miri et al. 2022; Zhu et al. 2022).

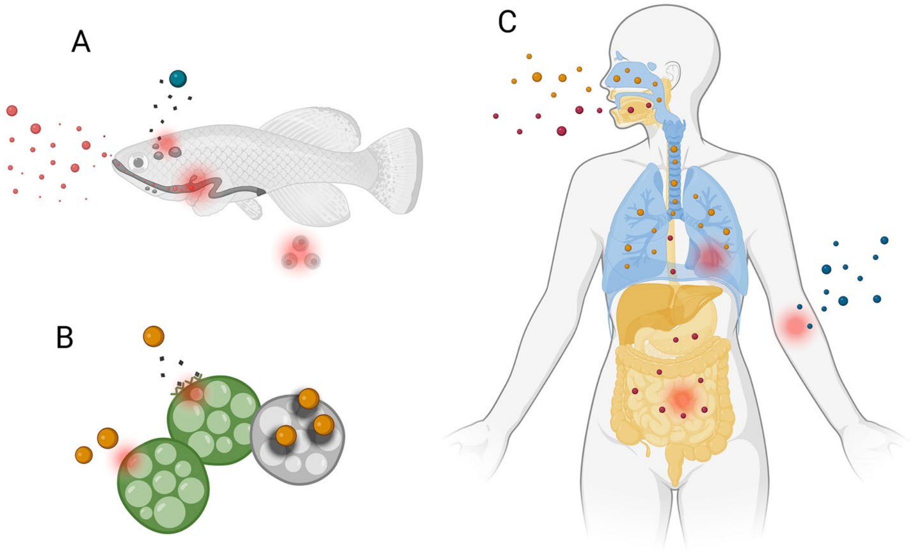


Fig. 1 Microplastics impact on living organisms. **A** Microplastics can enter aquatic fauna food chain, causing intestinal blockage and alteration in nutrients absorption, endocrine disruption, immunological and neurological effects, and loss of reproductive functionalities. **B** Microplastics and toxic leachate can damage microalgal cell walls, cause metabolic dysfunctions, and cause impairment of photosynthesis due to shading effects. **C** The three main routes of human microplastics exposures are identified as ingestion, inhalation, and dermal contact, triggering inflammatory and immune reactions. Created with BioRender.com

Secondary microplastics are mostly generated from a large plastic object made from the same polymers improperly disposed of in land and water systems, which undergoes physical abrasion leading to the weakening of the chemical bonds and subsequent oxidative-thermal degradation (Gerritse et al. 2020). Other secondary microplastic sources include the disintegration of synthetic fibers during the washing of clothes and commercial activities like thermal cutting of polystyrene. The increased use of single-use plastics has contributed to the overproduction of polyethylene, polypropylene, and polyethylene terephthalate products. Moreover, a wide range of electronic, automobile, textile, and paint industries also discharge microplastic products into the water bodies and river catchment areas which can lead to microplastic pollution (Kay et al. 2018; Wang et al. 2019b; Chia et al. 2021).

Impact of microplastics on marine organisms and humans

Microplastics could have a huge impact on the aquatic flora and fauna (Sathicq et al. 2021) (Fig. 1a) as they act as a vector for the transport of absorbed heavy metals, bacterial fish pathogens, multidrug resistant *E. coli*, persistent organic pollutants etc. (Enders et al. 2015; Viršek et al. 2017; Caruso, 2019; Song et al. 2020), and the possible leaching of chemical components added during their manufacturing process (Groh et al. 2019; Bacha et al. 2023). In addition, microplastics provide a novel habitat for the growth of microbial biofilms containing algae, bacteria and fungi and can potentially spread microbial pathogens and antimicrobial resistance (Zettler et al. 2013; Wu et al. 2019; Guo et al. 2020; Yuan et al. 2020).

As most microplastics have a size range similar to the foods that are normally consumed by the zooplankton, they can accidentally enter the food chain (Gregory 2009). This selection of microplastics instead of food particles can cause a loss of energy resources and also sublethal effects on the species reproductive pattern (Enders et al. 2015). Moreover, the ingested plastic can cause intestinal blockage, which subsequently reduces the absorption of nutrients and also causes a change in hormonal balance (Derraik 2002). The improper absorption of the nutrients may result in a decrease in the energy reserves and deficiency in food assimilation which in turn impacts growth and reproduction (Besseling et al. 2013; Wright et al. 2013a) and also decreases the ability of the organisms to survive in adverse environmental conditions (Bugoni et al. 2001).

Microplastic exposure can also cause other sublethal effects in marine organisms, including oxidative stress, altered gene expression, inflammation, and effects on the immune system and central nervous system (for review see Wright et al. 2013b; Vethaak and Martinez 2020). These adverse effects may be caused by residual monomers and additives release from plastic particles rather than by the particles themselves. Smaller microplastics below 10 µm were found to be more toxic to aquatic organisms than larger plastic particles.

The fishing and aquaculture industry is strongly impacted by microplastic pollution, and its viability and productivity are affected by the presence of plastic waste in water bodies (Rochman et al. 2015) and microplastic contaminated seafood products (Bråte et al. 2016; Smith et al. 2018; Curren et al. 2020; Li et al. 2021; Pan et al. 2022). The ubiquitous, persistent, and anticipated increase in microplastics pollution could in the long run also have a significant impact on marine biodiversity and ecological processes, such as primary producers at the basis of the food chain (Guzzetti et al. 2018; Vethaak and Martinez 2020).

The impact of microplastics on the growth and diversity of the microalgal population usually varied (Liu et al. 2019) (Fig. 1b). According to Sjollema et al. (2016), uncharged polystyrene particles negatively affected microalgae growth of *Dunaliella tertiolecta* at high concentrations (250 mg/l) and with decreasing particle size. The study made by Khoironi et al. (2019) reported that the growth of *Spirulina sp.* was severely impacted by the presence of high concentrations of microplastics, especially due to shading effects and reduced light intensity, with subsequent impairment of photosynthesis, and the damage of microalgal cell walls (Khoironi et al. 2019). Li et al. (2018) reported that both polyethylene and polypropylene gradually degrade and generate microsized plastics and release potentially toxic additives including plasticizers, polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and heavy metals such as cadmium, chromium, bromium, copper, and titanium which cause cell membrane damage and growth inhibition. Capolupo et al. (2020) reported that in the case of *Raphidocelis subcapitata* and *Skeletonema costatum* the cell growth is inhibited due to the leaching of additives.

Raphidocelis subcapitata, on the other hand, showed a higher growth rate in presence of plastic microbeads (Canniff and Hoang 2018), and similar enhancement of cell growth and photosynthetic activity was evident in *Dunaliella salina* in presence of larger microplastics (Chae et al. 2019). This enhancement of growth seemed related to the trace concentration of additive chemicals such as stabilizers, phthalates, and endocrine disruptors, which are leached out of microplastics (Chae et al. 2019).

The potential effect of microplastics on humans is far from understood and requires further research (Vethaak and Legler 2021). Microplastics have been reported in different foods such as mussels, commercial fish, and table salt (Li et al. 2018) and three different pathways of exposure to microplastics are identified, including ingestion of food containing microplastics, inhalation of microplastics in the air, and dermal contact with these particles (Revel et al. 2018) (Fig. 1c). According to a study made by Cox et al. (2019), it was estimated that each person usually intakes around 39,000–52,000 microplastics each year. These microplastics cause intestinal blockage and result in the inflammatory response and changes in gut microbe composition and metabolism.

As stated earlier, microplastics can also be inhaled and the outdoor microplastic concentration is between 0.3 and 1.5 particles per m^3, whereas the indoor concentration is 0.4–56.5 particles per m^3 (Dris et al. 2017). The deposition of microplastics is largely dependent on the size and density of the particles. The less-dense smaller particles tend to deposit deepest in the lungs, causing the release of chemotactic factors and resulting in chronic inflammation (Oliveira et al. 2020a; b). The presence of microplastics in human lung tissue and human blood was very recently confirmed (Jenner et al. 2022; Leslie et al. 2022).

It was also speculated that nanoparticles can transverse the dermal barrier (Revel et al. 2018) causing low inflammatory reactions and fibrous encapsulation (Oliveira et al. 2020a; b). Once in contact with mucous membranes or absorbed by the body, microplastics generate oxidative stress and cytotoxicity, mainly due to their persistent nature in the body and the leaching of toxic additives, which may result in inflammation, immune reactions, neurological damage, metabolic disruptions, deoxyribonucleic acid (DNA) damage, and even cancer (Wright and Kelly 2017; Revel et al. 2018; Rahman et al. 2021; Vethaak and Legler 2021; Gruber et al. 2022).

Overall, microplastics and nanoplastics derived from several sources, in particular from the environmental degradation of waste plastics, can exert toxic effects on organisms in all trophic levels. They can enter aquatic fauna food chain, causing intestinal blockage and alteration in nutrients adsorption, endocrine disruption, immunological and neurological effects, and loss of reproductive functionalities. Micro- and nanoplastics and toxic leachate can damage microalgal cell walls and metabolic dysfunctions, and impairment of photosynthesis due to shading effects. Micro- and nanoplastics can enter the human body through ingestion, inhalation, and dermal contact, triggering inflammatory, and immune reactions.

Processes of microplastic degradation

Most of the conventional methods discussed for the reuse of microplastic degradation include a primary method where the plastic scrap is re-introduced in the heating cycle of the processing unit, followed by the conversion of waste to new plastic products by blending it with a virgin polymer which can considerably reduce the cost of production. Sometimes, plastic wastes are chemically or thermochemically altered to be recycled in the industrial loop. However, in most cases due to poor management strategies, these microplastic

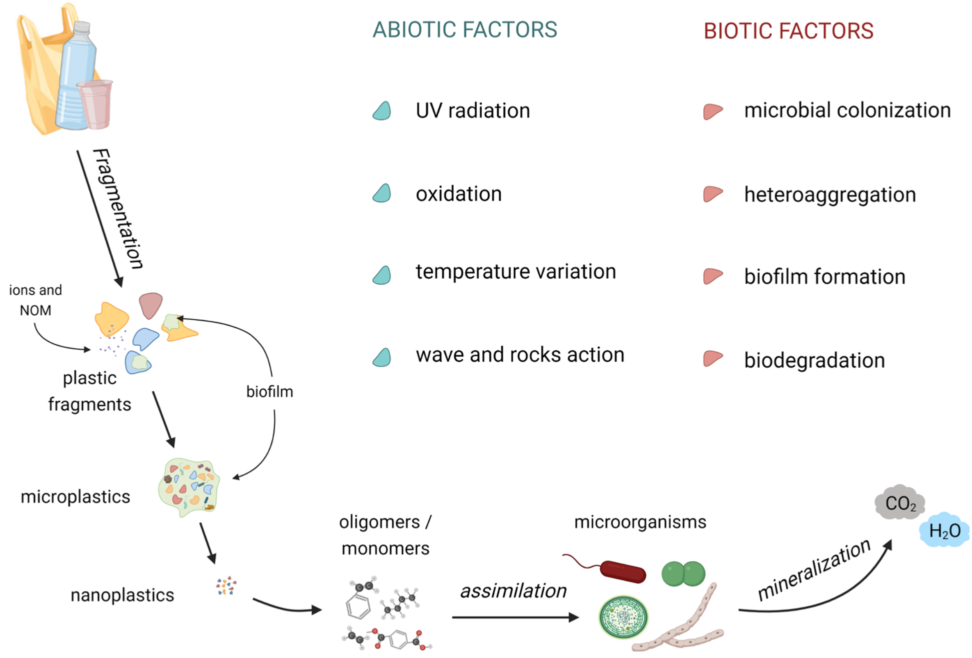


Fig. 2 Processes of microplastic degradation through abiotic and biotic combined factors. Microplastics derive from the fragmentation of plastic debris and can further degrade into smaller plastic particles at the nanoscale (nanoplastics). Due to abiotic factors and extracellular enzymes, nanoplastics are degraded into oligomers and monomers, then internalized by microorganisms and used as carbon source, resulting in the complete mineralization of plastic. Created with BioRender.com

particles are not disposed of properly or segregated properly. Most of them get mixed up with the organic components in a landfill, which is used for composting or anaerobic digestion, leading to excessive pollution and to the production of toxic compounds such as dioxins, phthalates, tetrabromobisphenol A, polybrominated diphenyl ethers, and toxic metals such as cadmium and lead (Verma et al. 2016).

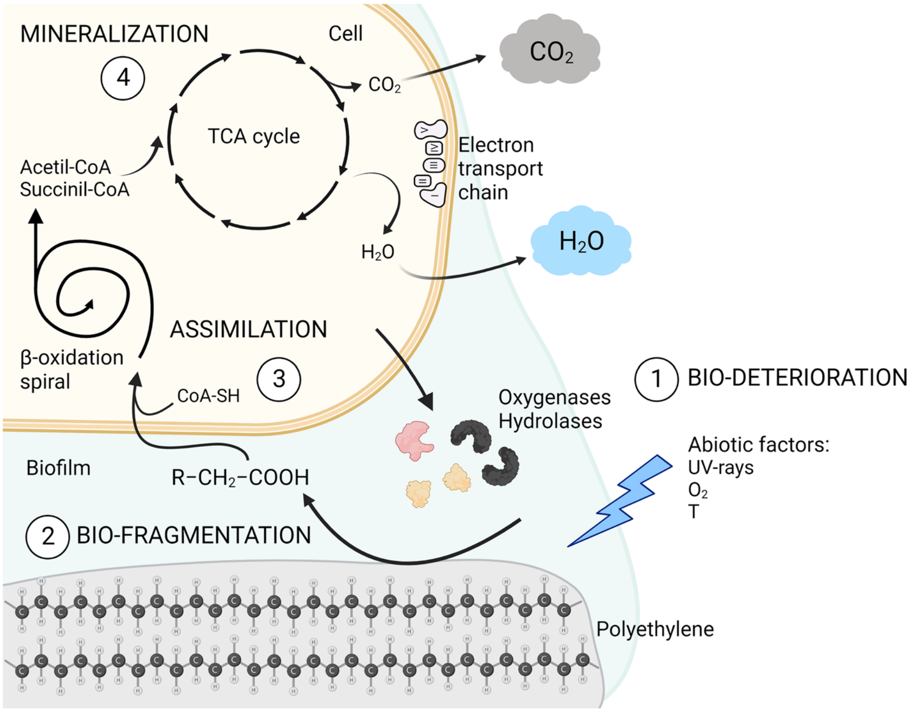
Currently, several physical, as well as chemical methods are popularly used for disposing of microplastic particles including incineration, landfilling, and recycling. Chemical recycling processes such as pyrolysis are extremely popular at the commercial level (Thiounn et al. 2020). In the slow pyrolysis methods, the plastic waste is converted to a mixture of char and tarry products which are treated at three different temperatures including 300, 425 and 550 °C (Dussud et al. 2018). There exist several pieces of research which focus on the pyrolysis of polypropylene, polystyrene, and polypropylene from where heat energy can be recovered. The contaminated, mixed, or degraded residues which are not suitable for recycling can be used as feedstocks for waste-to-energy strategies such as pyrolysis (an endothermic cracking process without oxidation), and incineration (oxidation of plastics) (Prata et al. 2020).

Degradation of microplastics can occur by physical, chemical, and biological methods and the biological degradation process is associated with a plethora of enzymes (Padervand et al. 2020; Bacha et al. 2021; Fig. 2). The basic process includes steps like the degradation of polymers to smaller particles, followed by the degradation of the smaller polymers to oligomers, dimers, and monomers. This degradation is followed by mineralization steps which are aided by microbes (see Fig. 3 for a representative scheme of polyethylene mineralization).

On complete mineralization, carbon dioxide is evolved along with the formation of several intermediate compounds which are used as a source of energy to promote the growth of microbes. The different extracellular enzymes that play a pivotal role in microplastics degradation include esterases, lipases, lignins peroxides, laccases, and manganese peroxides, which increase the hydrophilicity of microplastics and convert them to carbonyl or alcohol residues (Taniguchi et al. 2019). Hydrolases enzymes, such as lipases, esterase, and cutinase, act on plastic surfaces and degrade microplastics by enhancing the chain cleavage reactions. These enzymes fail to diffuse into the polymer, but they act on the surface resulting in the formation of cracks. The monomers generated are assimilated into the cytoplasm of microbes and finally enter into different metabolic pathways.

Although extensive research has already been done using extracellular enzymes in microplastics biodegradation, very little information is still now available on the role of intracellular enzymes in the degradation of microplastics; moreover, the pathways involved in the uptake of monomers are still not clear. Normally, after the fragmentation of the microplastics, the metabolic intermediates with carbonyl and hydroxyl groups are metabolized within the cell using the tricarboxylic acid cycle and β -oxidation pathway (Taniguchi et al. 2019). This process is followed by the complete mineralization of plastic debris into H_2O, CO_2, N_2, and CH_4 (Zettler et al. 2013). Researchers have made a thorough research on the process of surface colonization of the microplastics by degrading consortium forming a biofilm on the particles.

Fig. 3 Mineralization process for polyethylene. Due to a combined effect of abiotic factors and extracellular enzymes, plastic undergoes bio-deterioration and bio-fragmentation processes, resulting in the release of oligomers and monomers. Thanks to specific cell transport mechanisms, monomers are internalized by microorganisms and enter the catabolic pathways as carbon source. The final products of cells' aerobic metabolism, which results in the mineralization of plastic, are carbon dioxide and water. Created with BioRender.com



The attachment process of the microbes occurs through several mechanisms including biofouling, and degradation of plasticizers followed by the attack on the backbone of the polymer which is subsequently associated with hydration and penetration of microbes in the polymer structure.

Moreover, for efficient biodegradation, several factors are required which include the availability of potential microbial degrading organisms which possess suitable enzymes and metabolic pathways and other environmental factors such as temperature, pH, salinity, and moisture content (Raddadi and Fava 2019; Syranidou et al. 2019; Matjašič et al. 2021; Miri et al. 2022; Lin et al. 2022). The biodegradation of microplastics is also influenced by the surface and the structure of the polymer, amorphous and crystalline regions, crystal size, and lamellar thickness of polymers. Shabbir et al. reported polyhydroxyalkanoates depolymerase enzymes to hydrolyze the chains structures in the amorphous state on the surface of fragmentation films followed by erosion of chains in the crystalline state (Shabbir et al. 2020).

In brief, microbial microplastic degradation involves abiotic and biotic combined factors. Microplastics derive from the fragmentation of plastic debris and can further degrade into smaller plastic particles at the nanoscale (nanoplastics). Due to a combined effect of abiotic factors and extracellular enzymes, plastic undergoes bio-deterioration and bio-fragmentation processes, resulting in the release of oligomers and monomers. Thanks to specific cell transport mechanisms, monomers are internalized by microorganisms and enter the catabolic pathways as carbon source. The final products of cells' aerobic metabolism, which results in the mineralization of plastic, are carbon dioxide and water.

Techniques to monitor microplastic biodegradation

Different techniques have been applied to study microbial degradation of microplastics, which includes weight loss measurement due to leaching, CO_2 production due to degradation of low molecular weight polymers and loss of additives which affect the strength of microplastics (Baldera-Moreno et al. 2022). To get direct proof of the degradation process, morphological, chemical, thermal, and structural properties are investigated using various techniques/methods such as scanning electron microscopy, laser diffraction particle, differential scanning calorimetry, dynamic light scattering, X-ray diffraction, etc. (Huang et al. 2022). Chemical changes are usually tracked by vibrational spectroscopy techniques, such as Fourier transform infrared spectroscopy, nuclear magnetic resonance, mass spectrometry, and gas chromatography (Donelli et al. 2009; Chamas et al. 2020; Ivleva, 2021; La Nasa et al. 2021; Miao et al. 2020; Du et al. 2021).

The gravimetric weight loss method is another widely used method for the determination of the biodegradation of microplastics. However, this method should be used carefully as biodegradation of microplastics is an extremely slow process (Raddadi and Fava 2019), and depends on the incubation time and the assay conditions. Spectroscopy techniques can be used to determine the microplastics biodegradation efficiency of microbes, by monitoring the changes in the chemical functional groups of the polymer due to microbial activity (Singh and Sharma 2008). These changes may occur in hydrogen bonding, end-group modification, cross-linking and copolymer compositions. Fourier-transform infrared spectroscopy is considered one of the most efficient methods for the detection of chemical changes in the polymer and reference spectra of a wide variety of polymers are available in libraries for comparison (e.g., Celina et al. 1997).

Scanning electron microscopy allows the detection of microbial biofilm formation and surface degradation by monitoring the physical aspects of the polymer surface. The evaluation of polymer biodegradation can be done by checking the formation of cracks and holes in the polymer (Raddadi and Fava 2019).

Modification of microplastics tensile strength and elongation at break are the signals of microbial biodegradation. Indeed, microbial degradation leads to significant changes in mechanical properties and biochemical modification of polymers due to the formation of cross-linking bonds or film disintegration and shortening of the polymer chain (Nowak et al. 2011; Othman et al. 2021).

Role of algae in the degradation of microplastics

Microalgae and their enzymes and toxins can be effectively used in the biological breakdown of polymeric material (Moog et al. 2019; Chia et al. 2020; Manzi et al. 2022). The main advantage is that they do not require a rich carbon source for growth when compared to the bacterial system and are adapted to a wide variety of habitats where most of the microplastics occur (Yan et al. 2016). Microalgae are known to colonize the plastic surfaces in wastewater streams and this adhesion initiates plastic degradation by the production of ligninolytic and exopolysaccharide enzymes. Mostly these polymers serve as a carbon source and increase the cellular proteins and carbohydrates and increase the growth rate. Very recently, surface degradation or breakdown of low-density polyethylene sheet through algal colonization has been identified using scanning electron microscopy (Sanniyasi et al. 2021).

Algal biodegradation occurs mainly in different processes such as corrosion, hydrolysis, penetration, fouling, etc. (Chia et al. 2020). Both *Oscillatoria subbrevis* and *Phormidium lucidum* were also found to be able to colonize the surface of low-density polyethylene and degrade it without any pro-oxidative additives or pretreatment (Sarmah and Rout 2018). Bisphenol A, an additive with estrogenic activity commonly found in the polymers, was degraded by a combination of bacteria and algae including *Chlorella fusca* var. *vacuolate*, *Chlamydomonas mexicana*, *Stephanodiscus hantzschii*, and *Chlorella vulgaris* (Hirooka et al. 2005; Li et al. 2009; Ji et al. 2014).

In most cases, the degradation of microplastics is associated with the formation of biofilms on the surface of polymers. Several cyanobacterial strains, including the genus *Microcystis*, *Rivularia*, *Pleurocapsa*, *Synechococcus*, *Prochlorothrix*, *Leptolyngbya Calothrix,* and *Scytonema*, were also able to form biofilms on the microplastic polymers (Bryant et al. 2016; Debroas et al. 2017; Dussud et al. 2018; Muthukrishnan et al. 2019). Besides cyanobacterial species, diatoms are also present in the biofilms which help in photosynthesis (AmaralZettler et al. 2020).

With the recent advances in different biotechnological processes, several genetically modified microalgal cell factories can be created which are capable of producing and secreting enzymes required for plastic degradation (Shen et al. 2019). Green microalgae *Chlamydomonas reinhardtii* was genetically modified to produce polyethylene terephthalate hydrolase, able to degrade polyethylene terephthalate films and terephthalic acid (Kim et al. 2020). A similar modification was also successfully done in *P. tricornutum* which produced polyethylene terephthalate hydrolase and showed catalytic activity against polyethylene terephthalate and the copolymer polyethylene terephthalate glycol (Moong et al. 2019).

In short, microalgae could serve as effective microplastic degraders, thanks to their capability of using plastic monomers as carbon source by producing degrading enzymes and the ease of culture. The possibility of genetically engineering algae strains to enhance degradation capability has provided a promising environmentally friendly solution to biologically degrade polyethylene terephthalate using microalgae via synthetic biology.

Fungal degradation of microplastics

The fungi largely consist of a diverse group of organisms which are largely saprotrophs, or opportunistic or obligate parasites. They have tremendous adaptivity and can grow in a wide range of habitats both aquatic and terrestrial ecosystems under diverse environmental conditions. As well as being able to tolerate several toxic chemicals and metals, they produce a diverse range of extracellular enzymes and natural biosurfactants such as hydrophobins that can degrade complex polymers into simple monomers, making them a source of electrons and carbons for microorganisms, thus facilitating the degradation and mineralization of complex pollutants (Olicón-Hernández et al. 2017).

The main genus associated with the degradation of different types of polymers such as polyethylene, polypropylene, and polyethylene terephthalate includes *Zalerion maritimum*, *Aspergillus niger*, *Cladosporium*, and *Penicillium simplicissimum* (Paço et al. 2017; de Oliveira et al., 2020a, b; Devi et al. 2015), which use microplastics as sole carbon source after degradation by extracellular enzymes. They promote the formation of different types of chemical bonds (having carbonyl, carboxyl, and ester functional groups) and decrease their hydrophobicity. Similar degradation of polyurethane was evident in fungal strains such as *Aspergillus fumigatus*, *Aspergillus tubingensis*, *Cladosporium pseudocladosporioides*, *Fusarium solani,* and *Penicillium chrysogenum* and strains of *Pestalotiopsis microspora* (Khan et al. 2017; Álvarez-Barragán et al. 2016; Magnin et al. 2020; 2015; Russell et al. 2011).

In most cases, serine hydrolase plays a pivotal role in polyurethane degradation. Degradation of high-density polyethylene from marine coastal areas by two fungal strains *Aspergillus tubingensis* VRKPT1 and *Aspergillus flavus* VRKPT2 was reported to be 6.02 ± 0.2 and $8.51 \pm 0.1\%$, respectively (Devi et al. 2015). Recently, Kunlere et al. reported the promising degradation of low-density polyethylene by *Mucor circinelloides* and *Aspergillus flavus* isolated from a municipal landfill (Kunlere et al. 2019).

Pretreatment of the microplastics, for example, polyethylene, with chemicals such as nitric acid and sodium hydroxide is known to accelerate the rate of biodegradation of polyethylene by *Aspergillus niger* (Nwachukwu et al. 2010). Physical pretreatment processes including thermo-oxidization at 80 °C for 15 days were required to cause the degradation in low-density polyethylene mediated by *Aspergillus niger* and *Penicillium pinophilum*, showing 0.57 and 0.37% after incubation over 30 months (Volke-Sepúlveda et al. 2002). Similarly, *Aspergillus* spp. and *Lysinibacillus* spp. showed 29.5% of biodegradation of UV-irradiated and 15.8% of biodegradation of non-UV-irradiated polymer films (Esmaeili 2013).

Fungal enzymes associated with the degradation of microplastics

Fungi produce a diverse range of intra and extracellular enzymes which can catalyze diverse reactions and have the ability to degrade petroleum-based polymers. The intracellular enzymes perform a major role in fungal adaptation and detoxification processes (Schwartz et al. 2018). The enzyme systems associated with cytochrome P450 family epoxidases and transferases are associated with oxidation and conjugation reactions and help in the metabolism of aliphatic, alicyclic, and aromatic molecules. They perform a wide range of reactions such as epoxidation, sulfoxidation, desulfuration, dehalogenation, deamination, and epoxidation (Shin et al. 2018). The cytochrome P450 families of enzymes help in the preservation of hyphal wall integrity and formation of spore wall and utilized cofactors like heme, $NADPH + H^+$, and FAD.

On the other hand, extracellular enzymes include hydrolases which are involved in the breakdown of complex polymers (Sánchez, 2009) and increase the solubility of the pollutants subsequently reducing bioaccumulation (Olicón-Hernández et al. 2017). Enzymes belonging to the class II peroxidases such as manganese peroxidase and lignin peroxidase, laccases, and dye-decolorizing peroxidases, which oxidize a wide range of substrates, can be used as efficient tools for environmental cleaning. Lignin degrading fungi produce laccase which catalyzes the oxidation of aromatic and non-aromatic substrates such as chlorophenolic or non-phenolic compounds (polymethylmethacrylate and polyhydroxybutyrate (Straub et al. 2017). The thermostability of these enzymes may promote their uses in large-scale reactors where the degradation of polypropylene can be carried out at a high temperature, facilitating high kinetics reactions. A detailed list of different fungi associated with microplastic degradation is reported in Table 1.

Overall, a wide variety of fungal strains are capable of degrading plastics into more environmentally acceptable compounds, thanks to the production of a plethora of intracellular and extracellular enzymes, including oxidases and hydrolases, and natural biosurfactants such as hydrophobins.

Bacterial degradation of microplastics

Diverse studies have been conducted using bacteria for the degradation of microplastics. Bacteria capable of degrading microplastics have been isolated from a wide range of habitats including contaminated sediments, wastewater, sludge, compost, municipal landfills (Mehmood et al. 2016; Awasthi et al. 2020), and also from extreme climatic conditions like the Antarctic soils, mangrove, and marine sediments. Moreover, microplastic degrading microbes have also been isolated from the gut microflora of earthworms. It is generally reported that microbes living in polluted sites often develop an ability to activate the enzymatic system responsible for microplastic degradation.

Both pure cultures and bacterial consortiums can be used for microplastic degradation. However, pure cultures present several advantages in the degradation process, offering a convenient way to study metabolic pathways involved in the process. Moreover, the impact of environmental factors such as temperature, pH, substrate characteristics, and surfactants affecting the degradation process can be more easily monitored (Janssen et al. 2002). However, the main disadvantage is an extremely slow rate of degradation. Thus, more innovative methods are required to optimize conditions

Table 1 Microplastic degradation by fungi

Source of microbes	Isolated fungal strains	Type of microplastic degraded	Pretreatment	Incubation period	% of degradation	Enzymes	References
Not reported	*Aspergillus* sp. *Penicillium* sp.	Polypropylene/butylene-adipate-co-terephthalate		30 days			De Oliveira et al. (2020a, b)
Marine sediments	*Zalerion maritimum*	Polyethylene pellets		28 days			Paço et al. (2017)
Not reported	*Bjerkandera adusta*	Polypropylene and biomass	Gamma irradiated			Ligninase	Butnaru et al. (2016)
Marine coastal area	*Aspergillus flavus* VRKPT2	High-density polyethylene		30 days			Devi et al. (2015)
Endophytes of *Humboldtia brunonis, Psychotria flavida*	*Aspergillus* sp. *Paecilomyces Lilacinus*, *Lasiodiplodia theobromae*	Polypropylene				Laccase	Sheik et al. (2015)
Waste dump	*Aspergillus niger, Aspergillus terreus, Aureobasidium pullulans, Paecilomyces varioti, Penicillium funiculosum, Penicillium ochrochloron, Scopulariopsis brevicaulis, Trichoderma viride*	Low-density polyethylene	UV-irradiated	28 °C and relative humidity of > 90% for 84 days	24%, 60% and 58% of its initial mass		Nowak et al. (2012)
Soil, wall paint coated with polyurethane	*Fusarium solani*, *Spicaria* spp., *Alternaria solani*, and *Aspergillus flavus*	Polyester polyurethane			100%		Ibrahim et al. (2011)
Culture collection	*Penicillium pinophilum* ATCC 11,797	Low-density polyethylene powder		31 months			Volke-Sepúlveda et al. (2002)
Not reported	*Aspergillus niger, Penicillium funiculosum, Chaetomium globosum, Gliocladium virens* and *Pullularia pullulans*	Low-density polyethylene		28 days			Chandra and Rustgi (1997)

and improve the degrading bacterial isolates to shorten the degradation process. The use of a consortium of bacteria is usually preferred as it has been shown that biodegradation by a single bacterium often results in the generation of toxic end products (Dobretsov et al. 2013), which can be successfully eliminated in a stable microbial community (Singh and Wahid 2015).

The main process of degradation is represented by physicochemical degradation which reduces the polymer length and alters the functional groups of microplastics, making them more susceptible to microbial enzyme activity. Biodegradation using enzymes involves the action of lipases, esterases, laccases, amidases, cutinases, hydrolases, and carboxylesterases (Barth et al. 2016; Chen et al. 2020; Amobonye et al. 2021; Inderthal et al. 2021; Gómez-Méndez et al. 2018). Thus, in-depth knowledge of the metabolic pathways and associated enzymes is necessary to perform an efficient biodegradation process.

Physiochemical pre-treatment, including chemical oxidizing agents, thermooxidation, and UV irradiation of microplastics, is recommended to promote plastic biodegradation. These pre-treatments include UV irradiation, nitric acid treatment and blending with polymers like starch derivatives, cellulosic esters, polyhydroxybutyrate, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), and polycaprolactone, which increase the biodegradability of polypropylene (Gironi and Piemonte 2011). The addition of prooxidative and biodegradable substances like starch to low-density polyethylene, high-density polyethylene, polyvinyl alcohol, and polystyrene has been reported to enhance their biodegradability, promoting amylase activity (Zadjelovic et al. 2020).

The earliest study of microplastics biodegrading microorganisms was conducted by Cacciari et al. (1993), using a consortium of *Pseudomonas chlororaphis*, *Pseudomonas stutzeri*, and *Vibrio* sp. to degrade polypropylene. In the same study, the addition of starch was also reported to increase the biodegradation ability.

Later on, both Arkatkar et al. and Fontanella et al. reported biodegradation of polypropylene using a consortium of *Bacillus subtilis*, *B. flexus*, *Pseudomonas stutzeri,* and *Rhodococcus rhodochrous*, respectively (Arkatkar et al. 2010; Fontanella et al. 2013). These microbial isolates were found to form a biofilm, as reported in the study of Kowalczyk et al. (2016), by isolating *Achromobacter xylosoxidans.*

In a study conducted by Auta et al. (2018), two bacterial strains, belonging to *Bacillus* and *Rhodococcus*, isolated from mangrove sediments, showed polypropylene degradation efficiency of 4.0 and 6.4% after 40 days of incubation, respectively. They also reported that *Bacillus cereus* and *Bacillus gottheilii* were able to degrade microplastics (Auta et al. 2017). *B. gottheilii* induced microplastics weight loss percentages of 6.2%, 3.0%, 3.6%, and 5.8% for polyethylene, polyethylene terephthalate, polypropylene, and polystyrene, respectively.

Few other bacteria associated with polypropylene degradation included *Bacillus*, *Pseudomonas*, *Chelatococcus*, and *Lysinibacillus fusiformis*, which were obtained from a wide variety of habitats including mangrove habitat, compost, cow dung, and land contaminated with plastic wastes.

Gut microflora of several arthropods like *Tenebrio molitor* (mealworms) (Yang et al. 2015), *Plodia interpunctella* (Indian meal moth) (Yang et al. 2014) and *Galleria mellonella* (wax moths) (Kong et al. 2019) have also been reported to harbor microbes having microplastics biodegradation properties. A study by Yang et al. (2014) isolated two bacteria, *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1, from the gut of waxworms capable of degrading polyethylene by decreasing the hydrophobicity and damaging the surface of polypropylene. A later study conducted by Yang et al. (2015) isolated a bacterial strain *Exiguobacterium* sp. from the guts of mealworms able to form biofilm and degrade polystyrene.

Efficient biodegradation of low-density polyethylene was obtained using strains like *Microbacterium paraoxydans* and *Pseudomonas aeruginosa*, which showed nearly 61.0% and 50.5% degradation, respectively, within 2 months of incubation (Rajandas et al. 2012). Similarly, the biofilm of *Pseudomonas* sp. AKS2 has been reported to degrade low-density polyethylene up to 5 ± 1% within an incubation period of 45 days (Tribedi and Sil 2013) without any pretreatment. Likewise, degradation of polyethylene was also reported by isolating *Rhodococcus ruber* C208 at the rate of 0.86% per week (Sivan et al. 2006).

A consortium of bacteria consisting of *Bacillus* sp. and *Paenibacillus* sp. was able to reduce the dry weight of microplastics by 14.7% in 60 days (Park and Kim 2019). Moreover, Huerta Lwanga et al. (2018) investigated the earthworm (*Lumbricus terrestris*)-mediated degradation of low-density polyethylene. The isolates from the gut included genera Actinobacteria and Firmicutes which were also studied separately and observed to be able to degrade low-density polyethylene microplastics and release volatile compounds like eicosane, docosane, and tricosane. A consortium of *Enterobacter* and *Pseudomonas* from cow dung enhanced weight loss up to 15% within 120 days (Skariyachan et al. 2021).

Several marine hydrocarbonoclastic bacteria such as *Alcanivorax borkumensis* showed the ability to degrade alkyl cycloalkanes, isoprenoid hydrocarbons, alkanes, and branched aliphatic compounds (Davoodi et al. 2020). The research was done on the same isolate that previously showed biofilm formation on low-density polyethylene in the presence of pyruvate, hexadecane and yeast extract and the low-density polyethylene films (Delacuvellerie et al. 2019).

It was also stated that the presence of alkanes modifies the cell membrane hydrophilicity and produces biosurfactants to interact with the plastic surface and the formation of COOH/OH and C=O functional groups. Several actinomycetes including *Rhodococcus ruber* and *Streptomyces* were also involved in polyethylene biodegradation (Sivan 2011).

Overall, among the different genera of bacteria associated with microplastic degradation, 21% belonged to Pseudomonas, about 15% to Bacillus and 17% derived from mixtures of these two genera (Matjašič et al. 2021). Other bacteria associated with microplastic biodegradation included *Enterobacter asburiae*, *Bacillus* sp., *Nocardia asteroids*, *Rhodococcus rhodochrous* (Bonhomme et al. 2003), *Streptomyces badius*, *Rhodococcus ruber*, *Comamonas acidovorans* and *Clostridium thermocellum* (Paço et al. 2019), *Exiguobacterium* sp., *Ideonella sakaiensis* (Tanasupawat et al. 2016), *Pseudomonas chlororaphis, Pseudomonas putida AJ,* and *Thermomonospora fusca* (Ghosh et al. 2013). A detailed list of different bacteria and actinomycetes associated with microplastic degradation is reported in Tables 2 and 3.

To summarize, bacteria capable of degrading microplastics have been isolated from a wide range of habitats including contaminated sediments, wastewater, sludge, compost, municipal landfills, extreme environments, and microbiota. Bacteria have been tested for microplastics degradation, both using pure cultures and microbial consortium. Bacterial consortium, in particular, show greater efficiency and community stability.

Modern biotechnological methods to enhance microplastic degradation

Microplastics are gradually gaining attention due to their ubiquitous presence and negative impact on human health and the ecosystem. Microplastic exposure has increased many folds after the coronavirus disease 2019 pandemic due to the excessive use of single-use personal protective equipment products (Anand et al. 2022; De-la-Torre and Aragaw 2021; Yang et al. 2022). Microplastic exposure may occur through inhalation, digestion, and dermal absorption and might cause health issues like neurotoxicity, disrupt endocrine system, carcinogenicity, and metabolic disruptions (Naqash et al. 2020; Rahman et al. 2021; see also Section "Processes of microplastic degradation"). Thus, there is a need for efficient microplastic bioremediation.

Polyethylene terephthalate is a widely used thermoplastic polymer used for packaging which can degrade and generate microplastics. Isolates like, *Ideonella sakaiensis* 201-F6 are reported to produce polyethylene terephthalate-hydrolyzing enzymes able to degrade polyethylene terephthalate to terephthalic acid, and ethylene glycol which is non-hazardous monomers. Other bacterial strains can be genetically engineered by cloning the encoding genes of *Ideonella sakaiensis* 201-F6, promoting the generation of modified strains able to degrade polyethylene terephthalate in non-hazardous monomers. Moog et al. (2019) introduced polyethylene terephthalate-hydrolyzing enzymes into photosynthetic microalga, *Phaeodactylum tricornutum* which showed efficient polyethylene terephthalate hydrolyzing activity.

Genetic modifications have also been made to promote the capture of polyvinyl chloride within the bacterial biofilm (Liu et al. 2021). *Pseudomonas aeruginosa* was genetically engineered by deleting the *wspF* gene to increase the formation of sticky exopolymeric substances which enhance its capacity to accumulate microplastics in its biofilm. Moreover, *yhjH* gene was designed under the control of an arabinose-induced promoter and was introduced into the bacterium. Since the function of *yhjH* was to decrease cyclic dimeric guanosine monophosphate levels, induced expression of the gene reduced the biofilm formation suitable enough to release captured microplastics. The synthetic 'capture and release' system would enable the creation of efficient microplastics scavengers for the bioremediation of aquatic ecosystems.

The advent of different genetic engineering methods has enabled us to manipulate the genetic materials of microbes and enhance their biodegrading efficiency. Several procedures involving recombinant DNA technology, gene cloning, and genetic modification have been done to improve the bioremediation ability of the microbes in presence of different hydrocarbons and heavy metals (Kumar et al. 2020). However, till now very few works have been conducted on the application of genetic engineering for creating a better strain for degradation of plastics.

These techniques are used for the construction of novel pathways and can alter enzyme specificity and their affinity toward different microplastics. For successful gene editing, it is necessary to find suitable genes required for metabolizing and degrading microplastics and suitable host organisms like *E. coli* in which these genes are expressed. The main processes involved are polymerase chain reaction, antisense ribonucleic acid (RNA) technology, and site-directed mutagenesis. Antisense RNA technology has emerged as a new tool for genetic editing as artificially synthesized antisense RNA can effectively regulate the expression of genes in host cells. On the other hand, site-directed mutagenesis is also used to alter the activity of genes associated with microplastic degradation. In a recent study, Lameh et al. (2022) reported mutation of carboxylesterase by in silico site-directed mutagenesis to produce BTA-hydrolase in *Archaeoglobus fulgidus* to enhance its ability to degrade polyethylene terephthalate.

The main enzymes associated with microplastic degradation, such as manganese-dependent peroxidase, were

Table 2 Microplastic degradation by bacterial isolates

Source of microbes	Isolated bacterial strains	Type of microplastic degraded	Incubation period	% of degradation	Biodegradation detection method/ techniques	References
Polluted soil samples	*Lysinibacillus sp.*	Polypropylene, polyethylene	26 days	4 and 9%	Gas chromatography – mass spectrometry, Scanning electron microscopy	Jeon et al. (2021)
Cow dung sample	*Enterobacter* sp nov. bt DSCE01, *Enterobacter cloacae* nov. bt DSCE02, and *Pseudomonas aeruginosa* nov. bt DSCE-CD03	Low-density polyethylene, polypropylene	160 days	64.25 ± 2% and 63.00 ± 2%	Weight loss	Skariyachan et al. (2021)
Compost	*Bacillus cereus, Bacillus thuringiensis, Bacillus licheniformis*	Polypropylene and poly-L-lactide	6 months		Fourier-transform infrared spectroscopy; Thermogravimetric analysis	Jain et al. (2022)
Municipal landfill sediment	*Bacillus* sp. and *Paenibacillus* sp.	Polyethylene	60 days	14.7 %	Field-emission scanning electron microscope, Fourier transform infrared spectrometer, Gas chromatography-mass spectrometer, Scanning electron microscopy, Thermogravimetric analyzer	Park and Kim (2019)
	Biofilm composed by Pirellulaceae, Phycisphaerales, Cyclobacteriaceae, and Roseococcus	Polyethylene, polypropylene		NA	DNA extraction, amplification and sequencing (evaluation of the effects of substrate type on microbial communities)	Miao et al. (2019)
Earthworm gut	*Bacillus simplex* and *Bacillus* sp.	Low-density polyethylene	21 days		Scanning electron microscopy	Huerta Lwanga et al. (2018)
Mangrove sediments	*Bacillus* sp. strain 27	Polypropylene	40 days	4.0%	Weight loss; Fourier-transform infrared spectroscopy; Scanning electron microscopy	Auta et al. (2018)
Mangrove sediment	*Bacillus gottheilii*	Polyethylene, polyethylene terephthalate, polypropylene, and polystyrene	40 days	6.2%, 3.0%, 3.6%, 5.8%	Weight loss; Fourier-transform infrared spectroscopy; Scanning electron microscopy	Auta et al. (2017)
	Bacillus cereus	Polyethylene, polyethylene terephthalate, polystyrene	40 days	1.6%, 6.6%, and 7.4%		
Compost	*Bacillus thuringiensis*	Polypropylene and poly-L-lactide	15 days	12%	Fourier-transform infrared spectroscopy; Scanning electron microscopy; Thermogravimetric analysis	Jain et al. (2018)
Compost	*Bacillus licheniformis*	Polypropylene and poly-L-lactide	15 days	10%	Fourier-transform infrared spectroscopy; Scanning electron microscopy; Thermogravimetric analysis	Jain et al. (2018)

Table 2 (continued)

Source of microbes	Isolated bacterial strains	Type of microplastic degraded	Incubation period	% of degradation	Biodegradation detection method/techniques	References
Sewage treatment plants (STP)	Microbial consortia (including *Aneurinibacillus* sp. and *Brevibacillus* sp.)	Low density polyethylene, high density polyethylene and polypropylene	140 days	47%, 58% and 56%	Fourier-transform infrared spectroscopy; Scanning electron microscopy; Atomic force microscopy; Energy dispersive spectroscopy; Nuclear magnetic resonance; Gas chromatography-mass spectrometry	Skariyachan et al. (2018)
Mangrove sediments in Peninsular Malaysia	*Bacillus cereus*	Polypropylene	40 days	12%	Weight loss	Helen et al. (2017)
Mangrove sediments in Peninsular Malaysia	*Sporosarcina globispora*	Polypropylene	40 days	11%	Weight loss	Helen et al. (2017)
Sandy beaches in Northern Crete, Chania, Greece	*Agios Onoufrios* and Kalathas	Polystyrene films	6 months	0.19%	Weight loss; Fourier-transform infrared spectroscopy; Scanning electron microscopy	Syranidou et al. (2017)
	Pseudomonas aeruginosa and *Escherichia coli*	Polyurethanes		2.5% and 2.4 %	Weight loss; Scanning electron microscopy; Tensile strength and elongation at break	Uscátegui et al. (2016)
Municipal solid waste	*Stenotrophomonas panacihumi* PA3-2	Polypropylene	90 days	20.3 ± 1.39%	Weight loss; Fourier-transform infrared spectroscopy	Jeon and Kim (2016)
	Nitrosomonas sp., *Nitrobacter* sp., *Burkholderia* sp. and *Pseudomonas* sp.	High density polyethylene, low density polyethylene, polypropylene	90 days	15%-20% (High density polyethylene), 5%-9% (polypropylene),	Weight loss	Muenmee et al. (2016)
Plastic-eating mealworms	*Exiguobacterium* sp. strain YT2	Polystyrene	60 days	7.4% ± 0.4%	Cross polarization—magic angle spinning nuclear magnetic resonance; Thermogravimetric analysis coupled with fourier-transform infrared spectroscopy	Yang et al. (2015)
National Environmental Engineering Research Institute, Nagpur India	*B. flexus* + *P. azotoformans*	UV treated polymers	12 months	22.7%	Weight loss; Fourier-transform infrared spectroscopy	Aravinthan et al. (2016)
Plastic-eating waxworms gut	*Bacillus* sp. YP_1 *Enterobacter asburiae* YT_1	Polyethylene films	28 days	10.7 ± 0.2% 6.1 ± 0.3%	Weight loss; Fourier-transform infrared spectroscopy	Yang et al. (2014)
Compost	*Chelatococcus* sp. E1	Low-molecular-weight polyethylene	80 days	44.5%	Gel permeation chromatography; Fourier-transform infrared spectroscopy; Nuclear magnetic resonance; Tensile strength	Jeon and Kim (2013)

Table 3 Microplastic degradation by actinomycetes

Source of microbes	Isolated actinomycetes strains	Type of microplastic degraded	Incubation period	% of degradation	Biodegradation detection Method/techniques	References
Antarctic soil	*Pseudomonas* sp. ADL15 and *Rhodococcus* sp. ADL36	Polypropylene	40 days	17.3% and 7.3%	Weight loss; Fourier-transform infrared spectroscopy	Habib et al. (2020)
Not reported	*Rhodococcus ruber* strain C208	Polyethylene	2 months	7.5%	Weight loss; Scanning electron microscopy	Sivan et al. (2006)
Soils from waste coal, a forest and an extinct volcano crater	Bacterial consortia *Arthrobacter viscosus, Micrococcus lylae, Micrococcus luteus, Bacillus mycoides, Bacillus cereus, Bacillus pumilus; Bacillus thuringiensis*	Low-density polyethylene	225 days	17.03%	Weight loss; Fourier-transform infrared spectroscopy; Scanning electron microscopy; elongation at brake	Nowak et al. (2011)
Not reported	*Microbacterium paraoxydans*	Polyethylene (pre-treated with nitric acid)	2 months	61.0%	Weight loss; Fourier-transform infrared spectroscopy	Rajandas et al. (2012)
Mangrove sediment	*Rhodococcus*	Polypropylene	40 days	6.4%	Weight loss; Fourier-transform infrared spectroscopy; Scanning electron microscopy	Auta et al. (2018)
	Actinomadura sp. T16-1 (Enzyme production)	Polylactic acid (production of polylactic acid-degrading enzyme)	96 h	Not available	Enzyme activity	Sukkhum et al. (2009)
	Rhodococcus ruber	Polystyrene	2 months	0.8%	Weight loss	Mor and Sivan (2008)
	Rhodococcus rhodochrous ATCC 29,672	Two polypropylene films (Statistical copolymer and block copolymer)	6 months	Not available	Fourier-transform infrared spectroscopy; Proton nuclear magnetic resonance; ADP/ATP ratio	Fontanella et al. (2013)

produced by a genetically engineered strain of *E. coli* and *S. cerevisiae* BY 4741, similarly, laccase enzymes were produced by a modified genetic strain of *E. coli* BL21 and *P. chrysosporium* (Sharma et al. 2018; Paço et al. 2019). These genetically modified enzymes are capable of better degradation of polyethylene terephthalate.

Enzyme cutinase produced by microbes for the breakdown of polyester linkage can also be used in polyethylene terephthalate degradation which acts at an optimal temperature of 75 °C. Genetic engineered yeast produces bacterial cutinase which prevents formation in strategic positions with sugars which helps in the degradation of polyethylene terephthalate (Shirke et al. 2018). Islam et al. (2019) reported that genetically engineered cutinase enzyme reduces the degradation time from 41.8 to 6.2 h when compared with wild strain.

A similar enhanced biodegradation ability was observed in a consortium of marine microbes (Syranidou et al. 2019). However, despite their better ability in laboratory conditions, most of these genetically modified organisms have displayed unsatisfactory results in field studies.

Gene editing tools

Gene editing tools have been applied for genome engineering of plants, animals, and microorganisms for the expression of specific genes (Paço et al. 2019; Tang et al. 2020; Nidhi et al. 2021; Ozyigit et al. 2021; Bhattacharyya et al. 2022; Biswas et al. 2022; Mandal et al. 2022; Jiang et al. 2022). With the advent of different types of gene editing tools such as zinc finger proteins, transcription activator-like effector nucleases, and more recently, the clustered regularly interspaced palindromic repeats (CRISPR)/Cas9, the manipulation of organisms has become easier (Jiang et al. 2013; Gaj et al. 2013). Genome editing also helps in the manipulation of a gene of interest which can perform the loss and gain of function experiments which alter the expression of different genes.

This strategy can be efficiently used to incorporate genes encoding enzymes like polyethylene terephthalate hydrolase, dehalogenase, esterase, depolymerase, and laccase which are associated with microplastic degradation. Three different CRISPR sequences were identified in *Streptomyces albogriseolus* LBX-2 which makes it a suitable organism for genetic engineering, where the main enzyme associated with polyethylene degradation is oxygenase (Shao et al. 2019).

Bioinformatics

Bioinformatics has also become an effective tool for enhancing the biodegradation of plastic debris including microplastic particles (Purohit et al. 2020). Various types of databases such as The University of Minnesota Biocatalysis/Biodegradation Database, The Environmental Contaminant Biotransformation Pathway Resource, MetaCyc database, and BioCyc database related to biodegradation pathways have been established to evaluate the process of biodegradation by providing information on the metabolic pathways, the microbial enzymes and genes associated with the process (Gao et al. 2010; Wicker et al. 2016; Karp et al. 2019; Caspi et al. 2020). These databases and computational methods help to recognize enzymes involved in a metabolic pathway of interest and help in forecasting the biodegradation routes of toxic chemicals, providing a platform in which a novel approach for the biodegradation of plastic can be designed (Ali et al. 2021).

Despite all these advantages, the major disadvantage associated with bioinformatics is the lack of experimental data and its validation which is required for future research. Moreover, there is a wide knowledge gap between diverse groups of synthetic polymer degrader microorganisms and their responsible enzymes. Hence, an extensive investigation is required to identify suitable metabolic pathways for the degradation of polymers and their associated enzymes. In the near future, a combination of approaches, using bioinformatic tools, metabolic engineering, genetics, molecular, and system biology may help us to find a suitable and sustainable option for the biodegradation of microplastics.

Biosafety issues associated with genetically modified organisms

The advent of different genetic engineering techniques, and synthetic biological and genetic tools has allowed the development of genetically modified microbial scavengers for the mitigation of diverse types of pollutants (Mohamed et al. 2020; Wang et al. 2019a, b). However, several regulatory hurdles hamper the use of genetically modified microorganisms in an onsite experiment. Also, these genetically modified microbes have shown their efficiency in laboratory conditions, but onsite experiments are required to validate their effectiveness.

A diverse range of genetic tools has been developed only to prevent the negative impacts of genetically modified organisms on the field, including antibiotic gene-free genetic engineering tools (Ji et al. 2019) and suicide genetic systems (Honjo et al. 2019; Marguet et al. 2010; Scott et al. 2017). By using synthetic biology and metabolic engineering, an attempt has been already made to engineer microorganisms, which can be efficiently used as self-eliminate microbial scavengers for the bioremediation of various toxic environmental pollutants (Moog et al. 2019; French et al. 2020).

These techniques look promising and can be applied in the case of microplastics bioremediation. For biomedical applications of synthetically engineered microorganisms, programmed cell death circuits have been developed using synthetic biology (Marguet et al. 2010; Sedlmayer et al. 2018; Tran et al. 2021). After bioremediation is finished, these programmed cell death circuits can be employed to eradicate the microbial scavengers by themselves.

In short, the development of genetic engineering techniques has opened the possibility of modifying bacteria introducing exogenous genes for specific enzymes involved in the degradation of plastic. Moreover, modern gene editing tools, such as CRISPR/Cas 9, make the manipulation of organisms easier and more precise. However, some concerns of biosafety still limit the use of genetically modified microorganisms in an onsite experiment, limiting the real evaluation of gene editing effectiveness in microplastic bioremediation.

Conclusion

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 (Schloss and Handelsman 2005; Shilpa et al. 2022) and culture-independent techniques can open up new avenues for the discovery of novel metabolic pathways and enzymes.

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