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Biostrategies for the removal of microplastics: A Review

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ABSTRACT
Recent studies on plastic pollution have shown that microscopic plastic particles or microplastics are ubiquitous. Both abiotic and biotic components are affected by microplastics. There are several ways to get rid of microplastics, that include recycling, landfilling, incineration, and biodegradation. Biodegradation is still a widely used remediation technology due to its significant economic and environmental benefits. One or more bio-cultures, such as bacteria, mould, yeast, and algae, can be used for biodegradation. In this review, we look through the contributions of microorganisms in biodegradation and other biotechnological techniques to speed up the process.

Keywords-Microplastics, incineration, biotreatment strategies, microplastic degrading microorganisms, biotechnological interventions

1.Introduction

One of the most pressing issues of this century is the amount of plastic waste that is being generated in our planet (Barnes et al., 2009). The most common plastic polymers are polyethylene, polypropylene, polyvinyl chloride, polyethylene terephthalate, and polystyrene (Brandelli et al. 2017; Hardin 2021), and China is the world's top producer with 29.4% of worldwide production. Plastics are produced cheaply, and the resulting goods have superior qualities. Plastics are therefore used in a plethora of daily activities, and their significance in contemporary society is enormous. One of the biggest threats to the world's ecosystems is plastic pollution, which is known to have an effect on both the abiotic and biotic components. Worldwide plastic output surged to more than 360 million tonnes in 2018 and is projected to triple by 2050. Asia is the world's largest producer and consumer of plastic goods, with China accounting for the lion's share (32%) of this "white pollution" while the rest of Asia contributes only about 19% (Gumel et al. 2013).

Large amounts of plastic trash are produced as a result of the widespread use of plastics. Unfortunately, plastic garbage is often handled extremely poorly. For instance, just 9% of plastic garbage generated worldwide in 2015 was recycled, 12% was burned, and 79% was dumped in landfills or disposed off incorrectly (Geyer et al. 2017). Given this, it is not unexpected that numerous nations are attempting to control or lower plastic production. However, it appears that the current efforts are still insufficient and unable to produce noticeable changes on a worldwide scale. Global plastic output specifically climbed from 359 to 368 million tonnes from 2018 to 2019, an increase of 2.51%.

Numerous research have recently examined the dispersion, absorption, destiny, behaviour, impacts, and removal methods of microplastics. However, it is still uncertain whether the techniques created for microplastic cleanup are effective. Research on the breakdown of microplastics has advanced, concentrating on biological and non-biological methods. Microplastic treatments made possible by the activity of microorganisms like bacteria, fungi, and algae are regarded as attractive tools for economical and environmentally benign degrading techniques. Few articles have addressed plastic degradation, focusing on the use of contemporary biotechnological methods in the enhancement of microplastic degradation. There is still a lack of knowledge with regard to biotechnological interventions for microplastic removal, despite the recent publication of research papers and reviews on microorganism-mediated degradation and remediation strategies (Danso et al. 2019).

2. Sources of microplastics (Mps)

There are two distinct sources of microplastics. The main sources of microplastics are cosmetics, home goods, drug delivery devices, and polymeric raw materials (pellets, flakes, and powders) made of, among other things, polyethylene, polystyrene, polyvinyl chloride, polyamide nylon-6, and polypropylene. It is well known that personal care items including toothpaste, scrubs, cleaning supplies, and cosmetics include atypically shaped microplastics with diameters between 0.5 and 0.1 mm, which are primarily marketed as "micro-beads" or "micro-exfoliates" and contribute to primary microplastics. Since these pollutants are successfully eliminated by the skimming and settling treatment procedures, the existing wastewater treatment plants have demonstrated that tertiary treatment of water is not a source of microplastic pollution (Patel et al. 2009; 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. 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(Tiwari et al. 2020; John et al. 2021).

3. Impacts of microplastics

Most microplastics can unintentionally enter the food chain because their size range is close to that of the foods that zooplankton typically consumes. This preference for microplastics over food particles may result in a depletion of energy resources and have sublethal implications on the reproductive strategy of the species. Additionally, the intestinal blockage brought on by the plastic consumption can impair nutritional absorption and alter hormone balance. Inadequate nutrient uptake may cause a reduction in energy reserves and a shortfall in food assimilation, which in turn affects growth and reproduction and lowers an organism's capacity to survive in unfavourable environmental conditions (Besseling et al. 2013)

As was previously mentioned, microplastics can also be inhaled, and although the concentration of microplastics indoors is 0.4-56.5 particles/m^3, it ranges from 0.3 to 1.5 particles/m^3 outdoors. The size and density of the particles have a significant impact on the deposition of microplastics. Smaller, less dense particles have a tendency to accumulate deepest in the lungs, where they release chemotactic chemicals that lead to persistent inflammation. Only lately have microplastics been found in human blood and lung tissue (Jenner et al. 2022; Leslie et al. 2022). Additionally, it was hypothesised that nanoparticles could pass through the skin barrier, resulting in fibrous encapsulation and mild inflammatory reactions. 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).

In general, organisms at all trophic levels are susceptible to harmful effects from microplastics and nanoplastics that come from a variety of sources, including the environmental degradation of waste plastics. They have the potential to enter the food chain of aquatic fauna, resulting in intestinal blockage, altered nutrition absorption, endocrine disruption, immunological and neurological consequences, and loss of reproductive capabilities. Micro- and nanoplastics, toxic leachate, and metabolic abnormalities can all harm the cell walls of microalgae and limit photosynthesis as a result of shading effects. Micro- and nanoplastics can enter the body of a person or animal through the gastrointestinal tract, lungs, or skin, resulting in inflammatory and immunological responses (Kay et al. 2018; Wang et al. 2019b; Chia et al. 2021).

4. Methods of removal of microplastics

The majority of the conventional techniques for recycling microplastic degradation involve reintroducing plastic scrap into the processing unit's heating cycle as a primary technique, then converting waste into new plastic products by blending it with a virgin polymer, which can significantly lower production costs. Plastic wastes may occasionally undergo chemical or thermochemical modification in order to be recycled in the industrial loop. However, these microplastic particles are typically not effectively disposed off or segregated due to poor management practises. In a landfill used for composting or anaerobic digestion, the majority of them mix with the organic materials, causing excessive pollution and the production of toxic substances like dioxins, phthalates, tetrabromobisphenol A, polybrominated diphenyl ethers, and toxic metals like cadmium and lead (Verma et al. 2016).

Treatment methods

PHYSICAL	CHEMICAL	BIOLOGICAL
•Filtration •Sedimentation	•Coagulation •Flocculation	•Biodegradtion

Basic classification of mps removal methods

4.1 Physical methods of removal of MPs

Physical approaches generally used are adsorption, filtration, sedimentation, etc. The majority of these techniques have been tested in laboratories. Biochar, adsorbent magnetic polyoxometalate-supported ionic liquid phases, magnetic carbon nanotubes, electrocoagulation, rapid sand filter and dissolved air flotation, sponge made of chitin and graphene oxide, zirconium metal organic framework-based foam, a non-fluorinated superhydrophobic aluminium surface method, and coagulative colloidal gas aphrons were high efficiency methods used for the removal of MPs.

4.2 Chemical methods of removal of MPs

Chemical approaches use substances that either react to change or break down MPs into simpler forms, produce floc or exhibit adhesion, and then are removed from water through filtering or other means. Chemical methods classify those methods where MPs were treated or removed using chemicals. The fundamental idea of chemical addition involves floc development, aggregation, and agglomeration, which qualifies MPs for sedimentation or filtering.

5. Biodegradation of MPs

For decomposing MPs found in the environment, microorganisms are used to address the problem of MP pollution. For their ability to break down MPs found in environment and wastewater, a number of species have been tested. The majority of biological entities researched in the context of MPs degradation potential are bacteria. Microbes are able to break down complicated plastic polymers into simpler monomer forms. CO2 and water are the products of aerobic degradation, whereas CO2, water, methane, and H2S are the products of anaerobic degradation. This technique has been successfully tried with a number of algae, fungus, and bacteria.

5.1 Role of algae in the degradation of microplastics

The biological degradation of polymeric material can be successfully carried out using microalgae, their enzymes, and their toxins. The fundamental benefit is that, in contrast to the bacterial system, they do not require a rich carbon supply for growth and are adaptable to a wide range of habitats, where the majority of the microplastics are found. In wastewater streams, microalgae are known to colonise plastic surfaces, and this adherence causes the ligninolytic and exopolysaccharide enzymes to start breaking down the plastic. These polymers primarily act as a supply of carbon, increasing cellular proteins and carbohydrates while also speeding up growth. Scanning electron microscopy has recently been used to identify the surface disintegration of low-density polyethylene sheet caused by algal colonisation (Sanniyasi et al. 2021).

The biodegradation by algae are through processes lioke corrosion, hydrolysis, penetration, fouling, etc. The ability of *Oscillatoria subbrevis* and *Phormidium lucidum* to colonise and break down low-density polyethylene without the need of pro-oxidative chemicals or pretreatment was also discovered. A mixture of bacteria and algae, including *Chlorella fusca var. vacuolate, Chlamydomonas mexicana, Stephanodiscus hantzschii,* and *Chlorella vulgaris*, decomposed bisphenol A, an additive with estrogenic action frequently present in the polymers (Hirooka et al. 2005; Li et al. 2009; Ji et al. 2014).

The production of biofilms on the surface of polymers is typically linked to the breakdown of microplastics. The genus *Microcystis, Rivularia, Pleurocapsa, Synechococcus, Prochlorothrix, Leptolyngbya Calothrix, and Scytonema* were among the cyanobacterial strains that could also form biofilms on the microplastic polymers. In the biofilms that aid in photosynthesis, diatoms are also present in addition to cyanobacterial species (Bryant et al. 2016; Debroas et al. 2017; Dussud et al. 2018; Muthukrishnan et al. 2019).

The capacity of microalgae to use plastic monomers as a carbon source while creating enzymes that dissolve plastic and their simplicity of cultivation make them potential candidates for use as efficient microplastic degraders. Synthetic biology has produced a promising environmentally benign method for employing microalgae to biologically breakdown polyethylene terephthalate. This method involves the possibility of genetically altering algal strains to boost degradation capability.

5.2 Fungal degradation of microplastics

The varied range of organisms that make up the fungi are mostly saprotrophs, opportunistic parasites, or obligate parasites. They are extremely adaptable and may flourish in a variety of aquatic and terrestrial ecosystems and in a wide range of climatic conditions. They produce a wide variety of extracellular enzymes and natural biosurfactants like hydrophobins that can break down complex polymers into simple monomers, making them a source of electrons and carbons for microorganisms, facilitating the degradation and mineralization of complex pollutants. In addition to being able to tolerate several toxic chemicals and metals. (Olicón-Hernández et al. 2017).

Zalerion maritimum, Aspergillus niger, Cladosporium, and Penicillium simplicissimum are the main genera involved in the breakdown of various kinds of polymers, including polyethylene, polypropylene and polyethylene terephthalate, which use microplastics as their sole carbon source after being broken down by extracellular enzymes. They lessen their hydrophobicity and encourage the formation of various chemical linkages, including those with carboxyl, ester, and carbonyl functional groups. *Aspergillus fumigatus, Aspergillus tubingensis, Cladosporium pseudocladosporioides, Fusarium solani, Penicillium chrysogenum,* and isolates of *Pestalotiopsis microspora* were among the fungi that showed similar degradation of polyurethane. (Khan et al. 2017; Álvarez-Barragán et al. 2016).

It is known that pretreating microplastics, such as polyethylene, with substances like nitric acid and sodium hydroxide would hasten Aspergillus niger's rate of biodegradation of the material. *Aspergillus niger* and *Penicillium pinophilum*, acting through physical pretreatment procedures such as thermo-oxidization at 80 °C for 15 days, were responsible for the low-density polyethylene degradation, which manifested as 0.57 and 0.37% following incubation for 30 months. Similar to this, Aspergillus spp. and Lysinibacillus spp. demonstrated 15.8% and 29.5%, respectively, biodegradation of non-UV-irradiated polymer films and UV-irradiated polymer films, respectively.

5.3 Fungal enzymes associated with the degradation of microplastics

Fungi produce a wide variety of intracellular and extracellular enzymes that can catalyse a variety of processes and breakdown polymers made of petroleum. The processes of detoxification and fungal adaption heavily rely on intracellular enzymes. The epoxidase and transferase enzyme systems connected to the cytochrome P450 family are involved in oxidation and conjugation reactions and support the metabolism of aliphatic, alicyclic, and aromatic compounds. Epoxidation, sulfoxidation, desulfuration, dehalogenation, deamination, and other reactions are just a few of the many processes they carry out. The cytochrome P450 family of enzymes use cofactors such heme, NADPH + H+, and FAD to create the spore wall and maintain the integrity of the hyphal wall.

Hydrolases, on the other hand, are extracellular enzymes that help break down complex polymers and make pollutants more soluble, which in turn prevents bioaccumulation. They are engaged in this process. Enzymes from the class II peroxidases, which may oxidise a variety of substrates, can be effective cleaning agents for the environment. Examples include manganese peroxidase, lignin peroxidase, laccases, and dye-decolorizing peroxidases. Fungi that break down lignin create laccase, which catalyses the oxidation of aromatic and non-aromatic substrates such polymethylmethacrylate and polyhydroxybutyrate, which are chlorophenolic or nonphenolic molecules. The employment of these enzymes in large-scale reactors where polypropylene can be broken down at high temperatures and with high kinetics reactions may be encouraged by the thermostability of these enzymes. (Schwartz et al. 2018 (Straub et al. 2017)

Overall, the creation of several intracellular and external enzymes, such as oxidases and hydrolases, and natural biosurfactants like hydrophobins enable a wide range of fungal strains to degrade plastics into more ecologically friendly molecules.

Table 1: Microplastic degradation by fungi

Strain of Microbes	**Type of micro plastic degradation**	**Incubation period**	**Percentage of degradation**	**Enzyme**
Aspergillus sp.	Polypropylene/butylene-	30 days		Ligninase
Penicillium sp.	adipate-co-terephthalate	30 days		Ligninase
Zalerion maritimum	Polyethylene pellets	28 days		Ligninase
Bjerkandera adusta	Polypropylene and biomass	30 days		Ligninase
Aspergillus flavus	High-density	30 days		Laccase
Aspergillus niger	Low-density	28 °C and relative humidity of > 90% for 84 days	24%, 60% and 58% of its initial mass	
Aspergillus terreus	polyethylene	28 °C and relative humidity of > 90% for 84 days	24%, 60% and 58% of its initial mass	
Fusarium solani	Polyester polyurethane		100%	
Penicillium	Low-density	31 months		

5.4 Bacterial degradation of microplastics

Numerous research have been carried out employing microorganisms to break down microplastics. Microplastics-degrading bacteria have been found in a variety of habitats, including contaminated sediments, wastewater, sludge, compost, municipal landfills, and extreme climatic environments like Antarctic soils, mangroves, and marine sediments. Additionally, microorganisms that break down microplastic have been identified from the earthworms' stomach flora. According to general reports, microorganisms found in contaminated environments frequently learn how to activate the enzyme machinery that breaks down microplastics.

For the breakdown of microplastics, bacterial consortiums and pure cultures can both be employed. However, pure cultures have a number of benefits in the degradation process, providing an easy way to research the metabolic pathways involved. Furthermore, it is now possible to more readily track how environmental parameters like temperature, pH, substrate properties, and surfactants affect the degradation process (Janssen et al. 2002). A very slow rate of degradation is, however, the principal drawback. In order to accelerate the degradation process, more creative approaches are needed to improve the degrading bacterial isolates and optimise environmental conditions.

The primary form of degradation is physicochemical degradation, which shortens the polymer chains and modifies the functional groups of microplastics to make them more vulnerable to microbial enzyme activity. Enzymes that are used in biodegradation include hydrolases, carboxylesterases, amidases, laccases, amidases, cutinases, and lipases. Therefore, to perform a successful biodegradation process, in-depth knowledge of the metabolic pathways and associated enzymes is required. (Barth et al. 2016; Chen et al. 2020; Gómez-Méndez et al. 2018)

In terms of the various bacterial genera connected to the decomposition of microplastics, 21% belonged to Pseudomonas, 15% to Bacillus, and 17% came from hybrids of these two genera. Other bacteria linked to the biodegradation of microplastics included *Enterobacter asburiae, Bacillus sp., Nocardia asteroids, Rhodococcus rhodochrous, Streptomyces badius, Rhodococcus ruber, Comamonas acidovorans, and Clostridium thermocellum, as well as Exiguobacterium sp., Ideonella sakaiensis , Pseudomonas chlororaphis, Pseudomonas putida AJ*, and *Thermomonospora fusca*

In conclusion, microbiota, contaminated sediments, wastewater, sludge, compost, municipal landfills, severe conditions, and bacteria capable of digesting microplastics have all been isolated. Both pure cultures and microbial consortiums have been used to evaluate bacteria for their ability to break down microplastics. In particular, bacterial consortium exhibit higher effectiveness and community stability.

Table 02: Microplastic degradation by bacteria

Bacterial strains	**Type of Microplastic degraded**	**Incubation period**	**% of degradation**	**Biodegradation detection method/techniques**
Lysinibacillus sp.	Polypropylene, polyethylene	26 days	4 and 9%	Gas chromatography – mass spectrometry, Scanning electron microscopy
Enterobacter sp	Low-density	160 days	64.25 ± 2% and 63.00 ± 2%	Weight loss
Bacillus cereus	Polypropylene and poly-L-lactide	6 months		Fourier-transform infrared spectroscopy; Thermogravimetric analysis
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
Bacillus sp. strain	Polypropylene	40 days	4.0%	Weight loss; Fourier-transform infrared spectroscopy; Scanning electron microscopy
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
Bacillus cereus	Polypropylene	40 days	12%	Weight loss

Table 03: Microplastic degradation by actinomycetes

Actinomycetes strain	Type of Microplastic degrade	Incubation period	% of degradation	Biodegradation detection Method/technique
Pseudomonas sp . ADL15 and *Rhodococcus* sp. ADL3	Polypropylen	40 day	17.3% and 7.3	Weight loss; Fourier-transform infrared spectroscope
Rhodococcus ruber strain C208	Polyethylen	2 months	7.5%	Weight loss; Scanning electron microscope
Bacterial consorti	Low-density polyethylen	225 day	17.03%	Weight loss; Fourier-transform infrared spectroscopy; Scanning electron microscopy; elongation at bark
Microbacterium paraoxydan	Polyethylene (pre-treated with nitric acid	2 month	61.0%	Weight loss; Fourier-transform infrared spectroscope
Rhodococcus	Polypropylen	40 days	6.4%	Weight loss; Fourier-transform infrared spectroscopy; Scanning electron microscope
Actinomadura sp. T16-1 (Enzyme production	Polylactic acid (production of polylactic acid-degrading enzyme	96 hours	Not available	Enzyme activity
Rhodococcus ruber	Polystyrene	2 month	0.8%	Weight loss

Rhodococcus rhodochrous ATCC 29,67	Two polypropylene films (Statistical copolymer and block copolyme	6 month	Not available	Fourier-transform infrared spectroscopy; Proton nuclear magnetic resonance

5.5 Biodegradation by mixed consortia

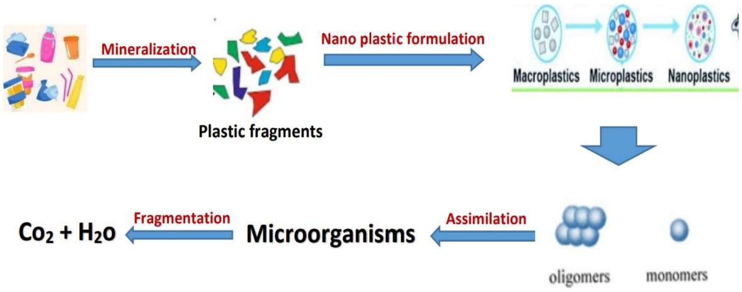
Due to the variety of enzymes produced by a mixed consortium, which consists of several microorganisms, the biodegradation of microplastics can be accelerated. During 40 days of exposure, one such consortium made up of *Staphylococcus sp., Pseudomonas sp., and Bacillus sp.* resulted in weight losses of 5.0 and 20.0% for 40 and 10 m polyethylene films, respectively.The reason why 10-m films degraded more quickly is probably because thinner films are more bioavailable to microbes.

A group of mesophilic bacteria were used by Park and Kim (2019) to degrade polyethylene microgranules. Mostly Bacillus sp. and Paenibacillus sp., isolated from municipal solid-waste disposal, made up the consortium. After 60 days of exposure, the microgranules' mean diameter shrank from 231 to 176 m, losing 14.7% of their weight in the process. Low-density polyethylene was biodegraded using a consortium of the same strains that were isolated from the crater of an extinct volcano. 75 and 150 days of exposure resulted in weight decreases of 7.5 and 13.5%, respectively. The comparison with Park and Kim's findings (2019) revealed that one of the key elements impeding the biodegradability of the microplastics was the environment from which microorganisms were separated.

6. Process of degradation of plastics

Microplastic particles can currently be disposed off using a variety of physical and chemical ways, such as landfilling, recycling, and burning. Commercially, methods for recycling chemicals like pyrolysis are quite well-liked. The slow pyrolysis processes involve treating the plastic trash at three distinct temperatures, including 300, 425, and 550 °C, to produce a mixture of char and tarry compounds. There are numerous studies that concentrate on recovering thermal energy from the pyrolysis of polypropylene, polystyrene, and polypropylene. For waste-to-energy methods like pyrolysis (an endothermic cracking process without oxidation) and incineration, the polluted, mixed, or degraded leftovers that are not appropriate for recycling can be used as feedstocks (Prata et al. 2020).

Microplastics can be degraded physically, chemically, or biologically, and the latter technique is linked to a variety of enzymes (Padervand et al. 2020; Bacha et al. 2021; Fig. 1). The fundamental procedure comprises actions like breaking down larger polymers into smaller particles, then breaking down the smaller polymers into oligomers, dimers, and monomers. Following this breakdown, bacteria help with the mineralization processes (see Fig. 2 for an example of a polyethylene mineralization process).



- **Abiotic factors** : UV radiation, Oxidation, temperature variations
- **Biotic factors :** Microbial colonization, Biodegradation, Biofilm formation

Fig1: Microplastic breakdown processes involving a combination of abiotic and biotic agents. The fragmentation of plastic waste results in microplastics, further broken down into smaller plastic particles at the nanoscale (nanoplastics). Nanoplastics are broken down into oligomers and monomers as a result of abiotic factors and extracellular enzymes, which are then ingested by microorganisms and used as a carbon source, leading to the total mineralization of plastic.

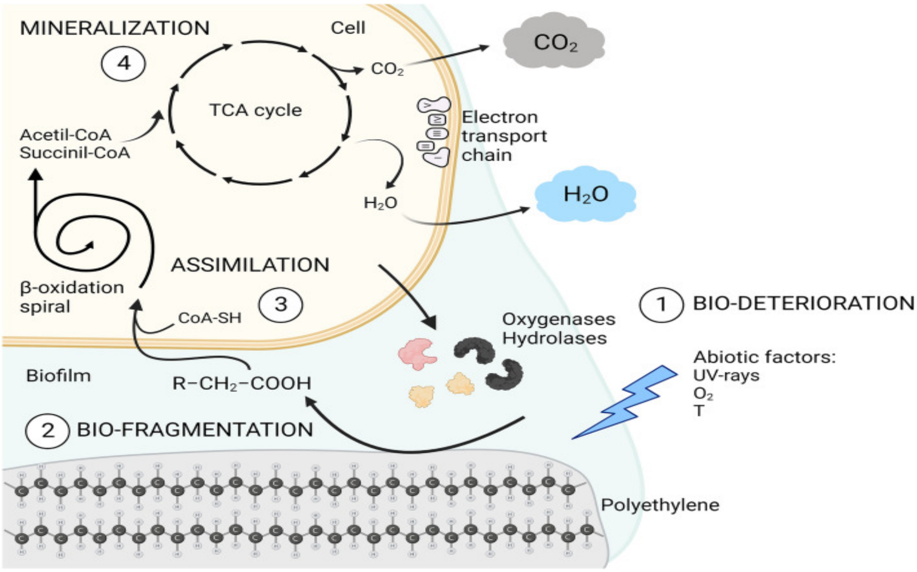


Fig. 2 Process of mineralization for polyethylene. The release of oligomers and monomers from plastic occurs as a result of bio-deterioration and bio-fragmentation processes caused by the combined action of abiotic agents and extracellular enzymes. Monomers are internalised by bacteria and enter the catabolic pathways as a source of carbon thanks to certain cell transport mechanisms. Carbon dioxide and water are the byproducts of aerobic metabolism in cells, which causes plastic to mineralize. Created with BioRender.com

Following full mineralization, carbon dioxide is released along with the creation of a number of intermediary molecules that serve as a fuel source for microbial development. Esterases, lipases, lignin peroxides, laccases, and manganese peroxides are a few extracellular enzymes that are crucial in the breakdown of microplastics because they make them more hydrophilic and turn them into carbonyl or alcohol residues (Taniguchi et al. 2019). Lipases, esterase, and cutinase are examples of hydrolase enzymes that work on plastic surfaces to break down microplastics by speeding up chain cleavage events. These enzymes don't permeate into the polymer; instead, they act on the surface, causing fissures to appear. The produced monomers eventually enter various metabolic pathways after being ingested by bacteria in their cytoplasm.

Although much research has been done on the biodegradation of microplastics using extracellular enzymes, little is known about the role of intracellular enzymes in the degradation of microplastics. In addition, it is still unclear which pathways are involved in the uptake of monomers. Following the breakdown of the microplastics, the tricarboxylic acid cycle and -oxidation pathway are often used by the cell to metabolise the metabolic intermediates with carbonyl and hydroxyl groups. The entire mineralization of plastic waste into H2O, CO2, N2, and CH4 occurs next. The process of surface colonisation of microplastics by degrading consortia and building a biofilm on the particles has been thoroughly studied by researchers. The process of microbe attachment involves a number of mechanisms, such as biofouling, the breakdown of plasticizers, and an attack on the polymer's backbone, which is then linked to hydration and microbe penetration in the polymer structure (Taniguchi et al. 2019) (Zettler et al. 2013).

The presence of prospective microbial degrading organisms with the appropriate enzymes and metabolic pathways, as well as other environmental parameters including temperature, pH, salinity, and moisture content, are also necessary for successful biodegradation. The surface and the structure of the polymer, amorphous and crystalline areas, crystal size, and lamellar thickness of polymers are additional factors that affect the biodegradation of microplastics. According to Shabbir et al. (Shabbir et al. 2020), polyhydroxyalkanoates depolymerase enzymes hydrolyze amorphous chain structures on the surface of fragmentation films and then erode crystalline chain structures.

In a nutshell, abiotic and biotic variables work together to contribute to microbial microplastic breakdown. The fragmentation of plastic waste results in microplastics, which can further break down into smaller plastic particles at the nanoscale (nanoplastics). The release of oligomers and monomers from plastic occurs as a result of bio-deterioration and bio-fragmentation processes caused by the combined action of abiotic agents and extracellular enzymes. Monomers are internalised by bacteria and enter the catabolic pathways as a source of carbon thanks to certain cell transport mechanisms. Carbon dioxide and water are the byproducts of aerobic metabolism in cells, which causes plastic to mineralize.

7. Modern biotechnology techniques to accelerate the breakdown of microplastics

7.1 Genetic engineering methods

Genetic alterations have been introduced to encourage the bacterial biofilm's ability to trap polyvinyl chloride (Liu et al., 2021). By genetically modifying *Pseudomonas aeruginosa* and removing the wspF gene, it has been made more capable of accumulating microplastics in its biofilm by increasing the production of sticky exopolymeric compounds. Additionally, the yhjH gene was added to the bacterium under the direction of an arabinose-induced promoter. Induced expression of the gene decreased the biofilm formation sufficient to release trapped microplastics because the function of yhjH was to lower levels of cyclic dimeric guanosine monophosphate. The synthetic "capture and release" technique would make it possible to develop effective microplastics scavengers for aquatic ecosystem bioremediation.

We can now modify microorganisms' genetic make-up to increase their capacity for biodegradation thanks to the development of various genetic engineering techniques. To increase the microorganisms' capacity for bioremediation in the presence of various hydrocarbons and heavy metals, several processes including recombinant DNA technology, gene cloning, and genetic modification have been carried out (Kumar et al. 2020). However, up to this point, very few studies have been done on the use of genetic engineering to develop a strain that is better at degrading plastics.

These methods are used to build novel pathways and can change the selectivity and affinity of an enzyme for various microplastics. Finding appropriate genes needed for metabolising and degrading microplastics as well as appropriate host organisms is crucial for successful gene editing.

Recent developments in various biotechnological techniques have made it possible to develop a number of genetically altered microalgal cell factories that can produce and secrete the enzymes needed for the breakdown of plastic. *Chlamydomonas reinhardtii*, a green microalga, was genetically altered to create polyethylene terephthalate hydrolase, which can break down terephthalic acid and polyethylene terephthalate films. *P. tricornutum*, which produced polyethylene terephthalate hydrolase and demonstrated catalytic activity against polyethylene terephthalate and the copolymer polyethylene terephthalate glycol, underwent a similar alteration with success(Moong et al. 2019).

7.2 Gene editing tools

Tools for gene editing have been used to modify the genomes of plants, animals, and microbes to express particular genes. The manipulation of organisms has been simpler with the development of many kinds of gene editing tools, including zinc finger proteins, transcription activator-like effector nucleases, and more recently, clustered regularly interspaced palindromic repeats (CRISPR)/Cas9.

The alteration of a gene of interest through genome editing also aids in the loss and gain of function studies that change the expression of several genes. (Jiang et al. 2013; Gaj et al. 2013)

Using this method, it is possible to effectively insert genes that code for microplastic degradation-related enzymes such polyethylene terephthalate hydrolase, dehalogenase, esterase, depolymerase, and laccase. Streptomyces albogriseolus LBX-2 has three distinct CRISPR sequences, which makes it a viable candidate for genetic engineering. Oxygenase is the primary enzyme involved in the breakdown of polyethylene.

7.3 Bioinformatics tools

A powerful technique for accelerating the biodegradation of plastic waste, particularly microplastic particles, is bioinformatics. Databases of different kinds, including The University of Minnesota Biocatalysis/Biodegradation Database, The Environmental Contaminant Biotransformation Pathway Resource, MetaCyc database, and BioCyc database related to biodegradation pathways, have been created to assess the biodegradation process by providing details on the metabolic pathways, the microbial enzymes, and the genes related to the process. In order to forecast the biodegradation pathways of harmful chemicals and identify the enzymes participating in a metabolic pathway of interest, these databases and computational approaches provide a foundation on which a novel strategy for the biodegradation of plastic can be developed (Ali et al. 2021).

Despite all these benefits, the main drawback of bioinformatics is the scarcity of experimental data and the validation of that data, both of which are essential for future research. Additionally, there is a significant information gap between the relevant enzymes of different groups of bacteria that breakdown synthetic polymers. Therefore, a thorough examination is needed to determine the best metabolic routes for the degradation of polymers and the enzymes that go along with them. In the near future, a mix of methods utilising metabolic engineering, genetics, molecular, and system biology, as well as bioinformatic tools, may aid in our search for an appropriate and long-term solution for the biodegradation of microplastics.

CONCLUSION

Recent studies on the biodegradation of microplastics by microorganisms demonstrate the urgent need for additional research into significant process variables and the identification of ideal conditions. Because of its effectiveness, biodegradation by bacteria, mould, and occasionally yeast has been examined as a potential technique for eliminating microplastics. All of the studied methods failed to entirely eliminate microplastics, especially not in a reasonable amount of time. Combining biodegradation with other methods of microplastic removal or degradation is a practical option. However, the complimentary methods are typically applied at the pretreatment stage to accelerate subsequent biodegradation.

Finally, it appears that there is still a lack of information regarding the existence of microplastics in the environment and their negative effects, especially in light of the effective remediation methods. The results of the published data show the inconsistent characterization processes and sample compositions, different matrices, variations in the size and form of the microplastics. It is difficult to define a roadmap for the encouraging advancement in biodegradation. To remove microplastics, it appears that combinations of biodegradation with chemical or physical treatments or the synergistic actions of different microorganisms, i.e., the application of microbial consortia, have the most potential in biodegradation of micro and nano-plastics which are considered to be environmental hazard affecting biotic life.

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