

# The good, the bad and the ugly: Critical insights on the applications of microbes in microplastic degradation

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## Overview Review

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## Abstract

Microplastic pollution has become a global environmental challenge, with significant impacts on ecosystems and human health. Microbes have emerged as a promising tool in the combating against microplastic contamination. However, the complex relationship between microbes and microplastics presents both opportunities and challenges, leading to a nuanced understanding of their applications in degradation. This paper provides critical insights into the multifaceted roles of different microorganisms in microplastic degradation. It begins by highlighting the ‘good’ aspects, where several strains of microorganisms show the potential to break down microplastics through enzymatic activities and the formation of biofilms. Conversely, the ‘bad’ aspects of microbial involvement in microplastic degradation are examined. Microorganisms can facilitate the transport and bioaccumulation of microplastics in various ecosystems, potentially exacerbating their harmful effects. The ‘ugly’ side of microplastic degradation includes the production of harmful byproducts during microbial breakdown, raising concerns about secondary pollution and toxicity. The concept of plasticsphere is discussed in this context, focusing on the phototrophs, photoheterotrophs and heterotrophs. Novel technologies involving microbial degradation of microplastics are also explained. The work emphasises the need for a comprehensive and balanced approach regarding the application of microorganisms in microplastic degradation and remediation.

## Impact statement

This review summarises current available knowledge on microplastic degradation by microbes and explores the novel and emerging techniques for microplastic remediation using microbes. The paper incorporates in depth discussion on microbial (bacteria, fungi and enzymatic degradation) action on microplastics and discusses why the ‘plasticsphere’ is considered to be the new delicacy for the microbial communities. The paper further explores how microplastics can develop antibiotic resistance in bacteria. The effect of environmental factors and biofilms on microbial degradation of microplastics is discussed. The key knowledge gaps and future research directions were identified regarding the use of microbes for microplastic bioremediation.

## Introduction

Plastics are durable, lightweight, cost-effective, and have become the most widely and frequently used synthetic materials. Plastics are almost indispensable in many aspects of human life in modern society (Chae and An, 2018). Various items like shopping bags, plastic bottles, food containers, disposable cups, plumbing pipes, microwavable containers, plastic films, automotive parts, hookup wire, coaxial cables, and so on are made using plastic additives like polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), polypropylene (PP), polyethylene terephthalate (PET), nylon, polycarbonate, polytetrafluoroethylene (PTFE) (Amobonye et al., 2021). Between 1950 and 2019, the annual global production of plastics had increased dramatically from 2 million tons to 368 million tons, of this around 40% were for single-use plastics (Plastic Europe, 2020) which resulted in the rapid accumulation of plastics in the environment (Nielsen et al., 2019). Around 2050, the global volume of plastic wastes will reach 26 billion tonnes, of which around 50% will eventually enter various compartments of terrestrial as well as aquatic ecosystems, causing serious environmental impacts (Jambeck et al., 2015).

Microplastics (MPs) are defined as plastic particles having size less than 5 mm and can be categorised based on primary and secondary types, sizes, shapes and polymer compositions. They are widespread in the biosphere and are potential contaminants of grave concern (Klein et al.,

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2018a; Blair Espinoza, 2019) and are ubiquitously found in sediments (Zamprogno *et al.*, 2021), water, sea salt (Kosuth *et al.*, 2018), food (Barboza *et al.*, 2018), the atmosphere (Brahney *et al.*, 2021) and sewage sludge. The total amount of microplastics in the aquatic ecosystems is expected to reach 12,000 metric tons (Mt) by 2050 (Geyer *et al.*, 2017). MPs are persistent (Lambert and Wagner, 2018) and can bioaccumulate in the food chain and subsequently can affect plants and animals, including humans. MPs accumulation in the environment has adverse effects on the organisms including molecular stress, reduced growth rate and reproductive complications (Batel *et al.*, 2016). MPs also affect plant growth and fish reproduction (Guo and Wang, 2019). Due to the wide range of adverse effects of MPs reducing the levels of MPs pollution is the need of the hour (Vince and Hardesty, 2017). MPs contain various toxic substances added during manufacturing or collected from the environment (Andrady, 2011). Plastic pollution has long been regarded to be an irreversible problem (because of poor degradation and the inability to collect all plastic particles) that will affect Earth system processes. As a result, certain techniques to limit micro (plastics) loss to the environment during manufacture, use, and disposal have been proposed (Lambert and Wagner, 2018). However, isolating MPs in environmental matrices might be difficult (Lambert and Wagner, 2018). In recent times, bio-plastics have been developed but their biodegradation also produces MPs (Wei *et al.*, 2022). Physical treatment methods like sedimentation, filtration, and so on do separate MPs but retain them in the sludge. Current practices for handling plastic wastes (e.g., recycling, land-filling, incineration) have drawbacks that potentially exacerbate existing environmental problems. For example, incinerating synthetic plastics releases volatile and hazardous waste products, such as dioxins, heavy metals, sulphides, nitrogen oxides, and furans, all of which are thought to have the potential to cause cancer (Verma *et al.*, 2016). Furthermore, downcycling (Rahimi and Garcia, 2017) and cost-ineffectiveness (Gradus *et al.*, 2017) are also associated with synthetic plastic recycling. Additionally, landfilling is also not a good option since it takes a lot of space and has chances for leakage into the environment. Due to these drawbacks efforts are directed towards finding more environmental friendly approaches for managing plastic wastes. In this context, microorganisms have emerged as a likely alternative with numerous researches highlighting the capability of various microbial species for degrading MPs including PET (Taniguchi *et al.*, 2019), PE (Restrepo-Flórez *et al.*, 2014), PU (Magnin *et al.*, 2020), PS (Ho *et al.*, 2018) and PP (Arutchelvi *et al.*, 2008). In this context, bioremediation of MPs has emerged as an attractive and alternate method to remove MPs from the environment. The use of microbes for MPs degradation can help in the remediation of MPs without any environmental damage (Restrepo-Flórez *et al.*, 2014; Kumar Sen and Raut, 2015; Qi *et al.*, 2017), thus making it a promising and safe avenue for cleaning our natural ecosystems (Shah *et al.*, 2008). Various efforts have also been made to screen potential plastic-degrading microbes (Hidalgo-Ruz *et al.*, 2012) and develop biodegradable polymers. However, presently only a few microorganisms are known to be able to degrade MPs and there is a dearth of knowledge in the context of microbial degradation of MPs (Gu, 2003).

MP are mainly considered as persistent compounds offering a large ecological niche for the colonisation of microbial communities (Kooi *et al.*, 2017; Rummel *et al.*, 2017). Numerous investigations have been carried out to examine the interaction between MPs and the MP colonising microorganisms, which could likely impact the behaviour of MP in the environment by altering their chemical or physical properties (Rummel *et al.*, 2017; Roager and

Sonnenschein, 2019; Oberbeckmann and Labrenz, 2020). However, there was no consensus on whether these microorganisms specifically chose plastics to colonise. According to some research, the microbial communities on plastics do not seem to be substrate-specific (Oberbeckmann *et al.*, 2016). For instance, the lifetime and relative mobility of microplastics in the water column were shown to account for the differences in the microbial community compositions between floating microplastics and other substrates, such as stones and sediments (Zettler *et al.*, 2013; Fazey and Ryan, 2016). However, multiple investigations showed differences in microbial community between MP surface and the surrounding, possibly due to the limitation of nutrients or the type of MPs (Kirstein *et al.*, 2019). Research in other areas revealed that the types of carbon sources largely drive the turnovers of microbial community compositions (Goldford *et al.*, 2018). While microplastics are not commonly used by microbes, some specific bacteria that break down plastic may be able to obtain carbon from them (Aravinthan *et al.*, 2016; Green *et al.*, 2016). This suggests that some microorganisms have a selection advantage when it comes to breaking down microplastics. Furthermore, certain microbes also favour adhering to the hydrophobic surface of microplastics and interacting with them (Krasowska and Sigler, 2014).

Therefore, a deeper understanding of the properties of the microbial communities colonising microplastics – such as their structure, stability, and mechanism of community assembly – based on stochastic and deterministic models was required in order to gain insight into the relationship between microplastics and the attached microorganism (Stegen *et al.*, 2012; De Vries *et al.*, 2018). Nevertheless, there is still a dearth of pertinent data regarding microbial communities that colonise microplastics in different environments. MP degradation in nature mainly occurs via physical, chemical and biological means (Shah *et al.*, 2008; Ter Halle *et al.*, 2017; Ariza-Tarazona *et al.*, 2018). Light and oxygen can cause the degradation of MPs through abiotically and enhance the microbial availability through photodegradation, thermooxidative degradation and hydrolysis (Andrady, 2011). In deep sediments, light and oxygen are limited and redox conditions are the vital environmental factor for MP degradation, which requires further study (Rogers *et al.*, 2020). Under anaerobic conditions, microorganisms found on MPs utilise either plastics or other organic matters surrounding MPs as electron donors (Rogers *et al.*, 2020). To date, various anaerobic and facultative anaerobic plastic-degrading bacterial strains have been successfully isolated from plastics (Kathiresan, 2003; Auta *et al.*, 2017), suggesting possible biodegradation of microplastics in anoxic sediments.

Multi-disciplinary approaches are required in order to address complex issues regarding MP bioremediation such as screening of efficient microbes and their characterisation, evaluation of in situ toxicity, and so on. MP assessment is necessary for the preparation of appropriate feedstock for the biotransformation of MPs which are recalcitrant.

### **Microplastics in the environment: Distribution, accumulation, toxicity and health effects**

MPs are known to occur ubiquitously in marine environment, in surface water and sediments. The presence of MP has been extensively reported in deep sea water, sea surface, on shorelines, and in aquatic organisms in several countries around the world (Gray *et al.*, 2018; Khalik *et al.*, 2018). The existence of MPs has been reported in diverse types of ecosystems, including permafrost,

both in Arctic (Zhang et al., 2023), Antarctica (Aves et al., 2022); Tibetan plateau (Wang et al., 2023) and European alpine regions (Materić et al., 2020, 2021). The occurrence and accumulation of MPs were also evident in several mangrove ecosystems and coral reefs (John et al., 2022). Occurrence of MP is also reported in different fresh water systems, including Ottawa River (Vermaire et al., 2017), Antuã River (Rodrigues et al., 2018), Yangtze River (Hu et al., 2018), Vembanand Lake (Sruthy and Ramasamy, 2017) in North America, Portugal, China and India. These MP particles have negative effects on diverse marine organisms (Wright et al., 2013) and mainly act as potential vectors for Persistent organic pollutants (POP) and other toxic pollutants (Hermabessiere et al., 2017). The presence of MP was also reported in air using different types of sampling methods such as atmospheric sampling (Abbasi et al., 2019), dust collection (Zhang et al., 2019) and wet and dry deposition (Klein and Fischer, 2019). The size of the MP fibres ranges from 100 to 5000 µm (Cai et al., 2017). In most cases, population density and proximity to urban areas are the main factors which influence the abundance of MP. The presence of MP was mostly reported in various touristic centres of China (Jiang et al., 2019), Rhine and Main Rivers, China's Qinghai Lake (Xiong et al., 2018), the Lagoon of Venice (Klein et al., 2015), Jakarta Bay (Manalu et al., 2017) and the Ottawa river (Vermaire et al., 2017). Both domestic and industrial sewage spillage are significant sources of MPs. According to study made by Carr et al. (2016), no MP was found in effluents of a tertiary waste water treatment plant, whereas, one plastic particle per 1.14 litre of effluent was reported from secondary waste water treatment plant. The density of the MP particles affects the vertical distribution and buoyancy of the particles. Usually, low-density MP occur more in surface zone whereas, while high-density MPs accumulate in deep seas and benthic organisms (Eerkes-Medrano et al., 2015).

Most MP are toxic and known to disrupts endocrine activity. On exposure to MPs, the catalytic activity of the acetylcholinesterase (AChE) (which is essential for neurotransmission in neuromuscular junctions and brain synapses) in zebrafish larva can lead to death (Chen et al., 2017). Similar AChE activity inhibition was also reported in *Dicentrarchus labrax*, *Artemia franciscana* and *Oreochromis niloticus*. Moreover, body size, age influences the impact of MP on AChE activity. The presence of MP can also increase antioxidant defence response, cellular oxidative stress and lead to peroxidation of lipid which can induce disruption of membranes of presynaptic vesicles and damages the gill, muscles and liver in several fish species (Wen et al., 2018). According to Lei et al. (2018a, 2018b) MP caused neurodegeneration on *Caenorhabditis elegans*. The MP can accumulate in the gastrointestinal tract of fishes which affect the health and growth of fishes (Yin et al., 2018). However, there is a lack of adequate data related to the impact of MP in the GI tract of marine organisms. It was also reported that both chemical composition and shape may influence the ecotoxicology of MP particles. MP present in sediments are mostly fibres followed by fragments, beads, films and foam (Kooi and Koelmans, 2019); fibres are found to induce more toxic effects compared to fragments and beads. Polystyrene (PS) MP was reported to upregulate *nfa*, *il1b* and *ifng1–2* gene expressions, resulting in inflammatory responses on livers, guts and gills of zebrafish, induce ROS production and decrease GSH and SOD levels (Lu et al., 2018). MPs are considered to be potentially toxic to human health as it may enter the gastrointestinal (GI) tract by endocytosis of M cells and translocate to different parts of the body (Cox et al., 2019). According to study made by Forte et al. (2016), it was reported

that PS nanoplastics can up-regulate IL-6 and IL-8 gene expression, resulting in inflammatory responses and morphological alterations. Moreover, high concentration of PS nanoparticles can lead to cell death through apoptosis by activating Caspase 3, 7 and 9 (Bexiga et al., 2011). The cytotoxicity of MP particles can also lead to DNA double-strand breaks and/or the high depletion of GSH (Paget et al., 2015). The study made by Xu et al. (2019) reported PS can affect the human lung cells and inhibit cell viability. The presence of excess MP particles can cause abnormal behaviour, produce ROS and impacts the immune system of fish. Further research is needed to understand the underlying mechanisms of MP toxicity.

### Plastisphere: A new delicacy for the microbes?

Plastisphere describes a novel microbial community attached to plastics and distinct from the surroundings. Marine plastic debris provides a selective hydrophobic environment that stimulates the growth of early colonisers accelerating biofilm formation and further microbial succession (ZoBell and Anderson, 1936). Stimulation of microbial growth and respiration by inert surfaces is a well-documented phenomenon, which creates a favourable environment for microbial colonisation through micronutrient concentration (ZoBell, 1943). This could play an important role for increasing microbial activity in the upper layer of ocean gyres owing to the abundant plastic debris in oligotrophic areas of the oceans (Zettler et al., 2013).

Early scanning electron micrographs of plastic surface biofilms (Sieburth, 1975) provided clues about microbial diversity within the plastisphere. The bacterial community found on the MP surface is found to be significantly different from that in surrounding middle and upper waters or other particle types (Oberbeckmann et al., 2018). Various factors like season, temperature, humidity, surrounding environment, polymer type, surface morphology and size of MPs influence the abundance and diversity of colonising microbial groups (Reisser et al., 2014; De Tender et al., 2015; Dussud et al., 2018a, 2018b). For example, studies have shown different microbial communities attached to MPs from two different oceans, and the diversity of bacteria living in water columns and bacteria attached to microplastic debris (Amaral-Zettler et al., 2015). Heterotrophic bacteria are capable of rapidly colonising plastic surfaces, which can survive longer than in the surrounding aquatic environments (Webb et al., 2009). The biodegradability of plastic is influenced not only by the capacity of microorganisms but also by surface texture, hydrophobicity, electrostatic interactions and free energy of the material (Falahudin et al., 2020). MPs provide a novel ecological niche for microbial growth and colonisation and serves as a carbon source. Recent studies on the basis of molecular data (Zettler et al., 2013; Bryant et al., 2016; Dussud et al., 2018a, 2018b; Kirstein et al., 2018) also confirmed that plastispheres are comprised of various organisms including primary producers (e.g., phototrophs), heterotrophs, predators, symbionts and decomposers. Members of genus *Vibrio* are reported to be enriched on microplastic surface (Frere et al., 2018; Zettler et al., 2013), however, other researchers have contradicted the claim (Schmidt et al., 2014; Bryant et al., 2016; Oberbeckmann et al., 2018). SEM photomicrographs confirmed the presence of varied eukaryotic and bacterial microbiota on both Polypropylene (PP) and Polyethylene (PE) samples. DNA analyses validated that the communities on plastics and the surrounding water differed consistently. For example, photosynthetic filamentous cyanobacteria including *Phormidium* and *Rivularia* OTUs

occurred on plastics but were absent from seawater samples where unicellular *Prochlorococcus* dominated the bacterial phototroph community (Zettler et al., 2013). Marine suctorian ciliates of the genus *Ephelota* was also present known to harbour ectosymbiotic rod-shaped bacteria (Chen et al., 2008). Also, sulphide-oxidising Gammaproteobacteria of the genus *Thiobios* was present in the samples and on the surface of stalked ciliates (Zettler et al., 2013). Moreover, polycystine colonial radiolaria were found on MP surface. The identification of the *Vibrio* sequence recovered in high abundance and showed similarity (100%) with various *Vibrio* species from the gene bank. From the Mediterranean Sea, harmful dinoflagellate species under genus *Alexandrium* were reported to be present on plastic marine debris (Maso et al., 2007) and also a few from Atlantic Ocean.

### Phototrophs

Diatoms are common and omnipresent residents of plastisphere and are one of the early and dominant colonisers (Oberbeckmann et al., 2014; Eich et al., 2015; Masó et al., 2016; Michels et al., 2018; Kettner et al., 2019). Diatoms belonging to number of bacillariophyte genera including *Navicula*, *Nitzschia*, *Sellaphora*, *Stauroneis* and *Chaetoceros* (Zettler et al., 2013). Studies from Sargasso Sea reported diatoms including *Mastogloia angulata*, *Mastogloia pusilla*, *Mastogloia hulburtii*, *Cyclotella meneghiniana* and *Pleurosigma* sp. (Carpenter and Smith, 1972), and amplicon reads belonging to genera *Sellaphora*, *Amphora* and *Nitzschia* (Zettler et al., 2013). Furthermore, the taxa *Mastogloia*, *Nitzschia*, and *Amphora* have been reported from the Arabian Gulf only on the basis of morphological traits (Muthukrishnan et al., 2019). Metagenomic surveys (Bryant et al., 2016) have placed diatom clades at less than 1% of the eukaryotic community suggesting their replacement as the community matures. Cyanobacteria are also present along with diatoms (Bryant et al., 2016). Various filamentous bacteria like *Phormidium*, *Rivularia* and *Leptolyngbya* are also continuously reported on microplastics (Amaral-Zettler et al., 2020). Additionally, other known photosynthetic representatives included *prasinophytes*, *rhodophytes*, *cryptophytes*, *haptophytes*, *dinoflagellates*, *chlorarachniophytes*, *chrysophytes*, *pelagophytes* and *phaeophytes*.

Fungi are able to form chemical bonds like carbonyl, carboxyl and ester bonds which decreased the hydrophobicity of the MP. Fungi also uses MPs as a carbon source and as a result are able to degrade them. Yamada-Onodera et al. (2001) demonstrated *Penicillium simplicissimum* YK to successfully grow on solid medium supplemented with 0.5% PE after UV irradiation for 500 h. *Aspergillus niger* and *Penicillium pinophilum* was able to degrade thermo-oxidised (80°C, 15 days) low-density polyethylene (TO-LDPE) by 0.57 and 0.37% after 31 months. Additionally, the TO-LDPE weight decreased by three crystallinity and crystalline lamellar thickness units (0.4–1.8 Å), and increased small-crystal content (up to 3.2%) and mean crystallite size (8.4–14 Å) were observed. Dantzer et al. showed that serine hydrolase secreted from *Pestalotiopsis microspora* isolates were responsible for biodegrading polyurethane (PUR) MP. *Aspergillus tubingensis* VRKPT1 and *Aspergillus flavus* VRKPT2 are able to degrade high-density polyethylene (HDPE) efficiently (Sangeetha Devi et al., 2015) having weight loss of HDPE around  $6.02 \pm 0.2$  and  $8.51 \pm 0.1\%$ , respectively. White-rot fungi IZU154, *Trametes versicolor* and *Phanerochaete chrysosporium* have also shown excellent capability to degrade MPs (Deguchiet al.).

### Photoheterotrophs and heterotrophs

In addition to phototrophs, potential photoheterotrophic bacteria of the genera *Erythrobacter*, *Roseobacter* and '*Candidatus Pelagibacter*' (REF) are also common residents of plastisphere. Heterotrophic bacteria in seawater samples were dominated by *Pelagibacter* along with other free-living picoplanktonic bacterial groups with different levels of abundance in PP and PE (Giovannoni et al., 1990). Experiments to culture bacteria with plastic as only carbon source have given various assortments, including members of Gammaproteobacteria (Nakamiya et al., 1997; Yoon et al., 2012) and Firmicutes (Harshvardhan and Jha, 2013), as well as Actinobacteria (Gilan and Sivan, 2013). Fungal sequences from plastic debris have also been reported by various studies (Zettler et al., 2013; Debroas et al., 2017; Kettner et al., 2017, 2019). Fungal diversity in the plastisphere is somewhat less known, however, recent studies highlighted that in brackish and freshwaters fungal assemblages are dominated by members of *Chytridiomycota*, *Cryptomycota* and *Ascomycota* (Kettner et al., 2019). From visual analysis of microbial population, members of fungal genus *Malassezia* were also reported (Amend et al., 2019).

Studies have shown that bacterial groups belonging to phyla *Bacteroidetes*, *Proteobacteria*, *Cyanobacteria* and *Firmicutes* are frequent colonisers of MPs (Zettler et al., 2013; Dussud et al., 2018a, 2018b).

Bacteria belonging to genus *Corynebacterium*, *Arthrobacter*, *Pseudomonas*, *Micrococcus*, *Streptomyces* and *Rhodococcus* are capable of biodegrading plastics and have demonstrated that they can use plastics as their carbon source under laboratory conditions (Shah et al., 2008). Interestingly, it was discovered that significant differences exist in the diversity, abundance and activity of bacterial and physiochemical characters of plastics between biodegradable and non-biodegradable plastics, indicating the presence of plastic-degrading microbes (Negoro, 2000).

Auta et al. (2018) described two pure bacterial cultures, *Rhodococcus* sp. strain 36 and *Bacillus* sp. strain 27 from mangrove sediments having capability for PP MP degradation. *Bacillus cereus* and *Bacillus gottheilii* were found to degrade PE (weight loss: 1.6%), polyethylene terephthalate (PET; 6.6%), and PS (7.4%) while, for *B. gottheilii* MP weight loss was 6.2%, 3.0%, 3.6% and 5.8% for PE, PET, PP and PS, respectively (Auta et al., 2018). *Stenotrophomonas maltophilia* LB 2-3 was found to decrease molecular weight and tensile properties of polylactic acid (PLA) (Jeon and Kim, 2013a). *E. coli* was able to degrade polyurethanes (1–2% after 72 hours) (Uscategui et al., 2016). Polypropylene films (PP) and Bioriented Polypropylene (BOPP) polymers were reported to be degraded by microorganisms to some extent (Longo et al., 2011). *Pseudomonas aeruginosa* was found to degrade PS-PLA nanocomposites (Shimpi et al., 2012). Mohan et al. (2016) found that newly isolated *Bacillus* strains were able to degrade brominated high-impact polystyrene (HIPS). Bacterial strains (*Enterobacter asburiae* YT1 and *Bacillus* sp. YP1) residing in gut of waxworms were able to degrade PE (Yang et al., 2014).

Park and Kim (2019) reported a bacterial consortium consisting mainly of *Bacillus* sp. and *Paenibacillus* sp. was able to reduce the dry weight of MP particles by 14.7% and the mean diameter of MP particles by 22.8% after 60 days. Tsiota et al. (2018) reported Agios and Souda bacterial community to decrease the weight of HDPE MPs by 8 and 18% after 2 months, respectively. The gut microbes of earthworm (*Lumbricus terrestris*) containing members of *Actinobacteria* and *Firmicutes* genera were able to degrade LDPE MPs upon ingestion by the earthworm (Huerta Lwanga et al., 2018).

Syranidou et al., (2017) demonstrated that bacterial consortium was capable of developing dense biofilm on the weathered surface of PE and induced alterations in the surface topography and rheological properties. *Exiguobacterium* sp. strain YT2 isolated from gut of mealworm showed potential signs of degrading and mineralising PS MP over a time of 28 days (Yang et al., 2015a; Brandon et al., 2018).

### Predators

Predatory ciliate *Ephelota* and sulphide-oxidising ectosymbiotic bacteria were reported to exist in a symbiotic relationship on the plastisphere (Zettler et al., 2013; Kettner et al., 2019). Positive associations between *Amoebophrya* and *Suessiaceae* on polyethylene were reported as well (Kettner et al., 2019). SEM and molecular data have also confirmed that choanoflagellates, radiolaria and small flagellates such as *Micromonas* also constitute the predatory guild in the plastisphere that devours bacteria and other organisms (Amaral-Zettler et al., 2020).

### Pathogens

Various potentially pathogenic microorganisms are reported to be attached with plastic debris (Zettler et al., 2013) including the members of the *Campylobacteraceae*, *Aeromonas salmonicida* or *Arcobacter* spp. from all over the world (McCormick et al., 2014; Kirstein et al., 2016; Oberbeckmann et al., 2016; Jiang et al., 2018; Curren and Leong, 2019). Various phototrophic species able to cause harmful algal blooms are also reported from plastic debris (Masó et al., 2016; Casabianca et al., 2019). Plastisphere communities can be dominated by genus *Vibrio* during the summer

months. Moreover, they can transport potential protistan coral pathogens (Goldstein et al., 2014) and a known fish pathogen (Virsek et al., 2017). A recent study has reported the presence of *Campylobacteraceae* in microplastic particles which can cause gastrointestinal infections in humans (McCormick et al., 2014).

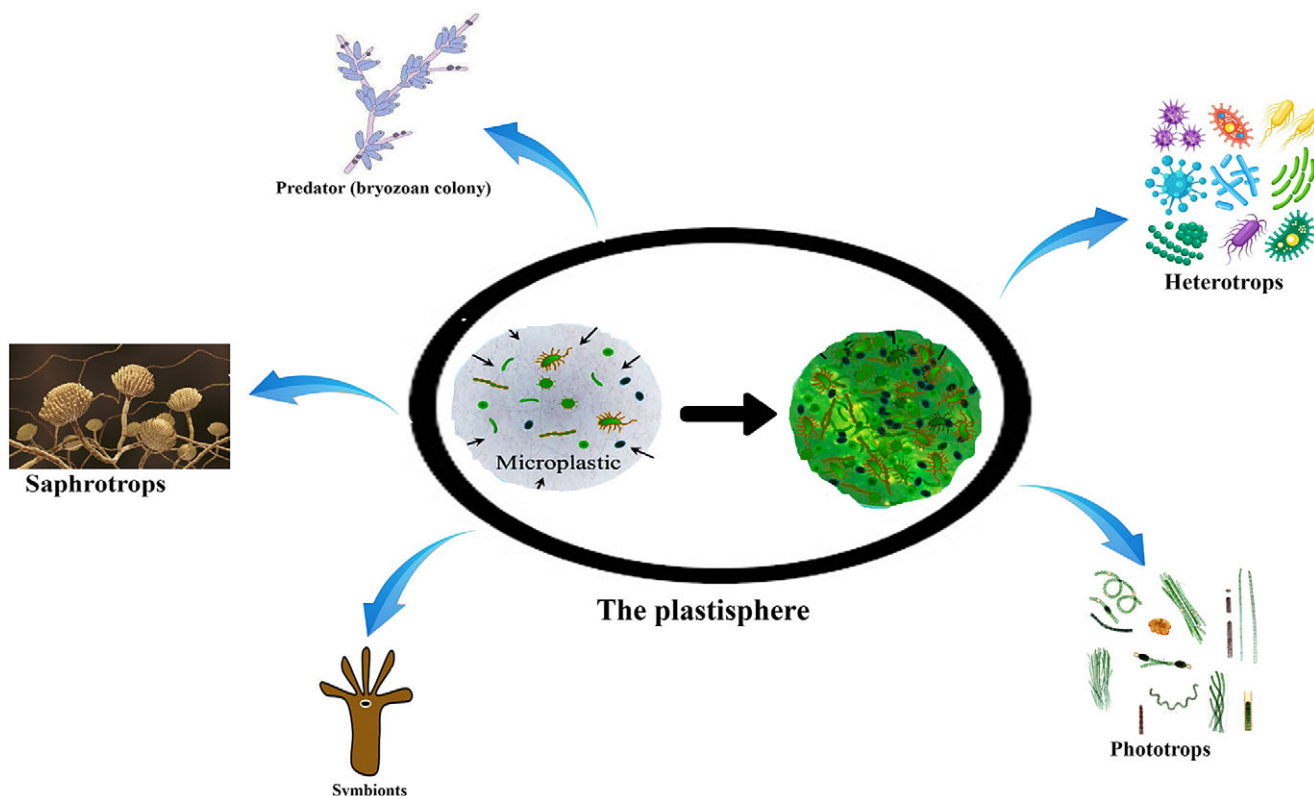
The knowledge regarding the microbial composition of MP-associated biofilm has revolutionised in the last half of the decade with the advent of metagenomics (Ivar do Sul et al., 2018). The biofilm communities differ significantly from their surrounding environment (Zettler et al. 2013; Oberbeckmann et al. 2014; Amaral-Zettler et al. 2015; Bryant et al. 2016; Debroas et al. 2017; Frere et al. 2018), which is obvious since microbial community compositions on natural particles usually differ from free-living microorganisms (Crespo et al. 2013; Rieck et al., 2015).

The outline of plastisphere is shown in Figure 1. Different strains of bacteria and fungi capable of degrading plastics, along with the types of microplastics degraded are summarised in Table 1.

### Action of microorganisms on microplastics

Microbial degradation of MPs occurs through various steps: (1) Initial degradation of polymers to small-size particles from large polymeric structures, (2) Degradation of polymers to their oligomer, dimer and monomers and, (3) Mineralisation of MPs by microbial biomass (Blair Espinoza, 2019). Upon complete mineralisation, microplastics breakdown forming carbon dioxide by various enzymes, and the transformation of produced intermediates to use as a source of energy and biomass production.

The majority of synthetic polymers such as polyethylene (PE), polypropylene (PP) and polystyrene (PS) are degraded very slowly (Weinstein et al., 2016). Even the novel consortia like *Enterobacter*



**Figure 1.** Plastisphere, the novel microbial community colonising and thriving on the plastic debris.

**Table 1.** Different strains of bacteria and fungi capable of degrading plastics, along with the types of microplastics degraded

Source of microbes	Isolate	Type of MP degraded	Incubation period	% of degradation	References
Bacterial isolates					
Polluted soil samples	<i>Lysinibacillus</i> sp.	Polypropylene and polyethylene	26 days	4 and 9%,	Jeon et al., 2021
Compost	<i>Bacillus cereus</i> , <i>Bacillus thuringensis</i> , <i>Bacillus licheniformis</i>	Polypropylene (PP) and poly-L-lactide (PLLA)	6 months		Jain et al., 2021
Cow dung sample	<i>Enterobacter</i> sp nov. bt DSCE01, <i>Enterobacter cloacae</i> nov. bt DSCE02, <i>Pseudomonas aeruginosa</i> nov. bt DSCE-CD03	Low-density polyethylene (LDPE) and polypropylene (PP)	160 days	64.25 ± 2% and 63.00 ± 2%	Skariyachan et al., 2021
Antarctic soil	<i>Pseudomonas</i> sp. ADL15, <i>Rhodococcus</i> sp. ADL36	Polypropylene (PP) microplastics	40 days	17.3% and 7.3%	Habib et al., 2020
Municipal landfill sediment	<i>Bacillus</i> sp., <i>Paenibacillus</i> sp.	PP	60 days	14.7%	Park and Kim, 2019
Mediterranean Sea	<i>Alcanivorax borkumensis</i>	HDPE	80 days	3.5%	Delacuvellerie et al., 2019
Landfill site	<i>Cupriavidus necator</i>	LDPE	21 days	33.7%	Montazer et al., 2019
Landfill site	<i>Micrococcus luteus</i>	LDPE	21 days	18.9%	Montazer et al., 2019
Landfill sites and waste treatment facilities	<i>Aneurinibacillus</i> sp., <i>Brevibacillus</i> spp.	LDPE and HDPE strips and pellets	140 days	LDPE and HDPE strips: 58.21 and 46.6% weight loss LDPE and HDPE pellets: 45.7 and 37.2% weight loss	Skariyachan et al., 2018
Dumpsite (Africa)	<i>Bacillus cereus</i>	LDPE film	16 weeks	35.72%	Muhonja et al., 2018
Mangrove sediments	<i>Bacillus gotthelii</i> , <i>Rhodococcus</i>	PE, PET, PP, PS	40 days	6.2, 3.0, 3.6, 5.8 and 6.4	Auta et al., 2018
Compost	<i>Bacillus thuringiensis</i> , <i>Bacillus licheniformis</i>	PP and poly-L-lactide (PLLA)	15 days	12%, 10%	Jain et al., 2018
Laboratory isolate	<i>Klebsiella pneumoniae</i>	LDPE film pretreated with hot dry air (70° C, 10 days)	60 days	18.4%	Awasthi et al., 2017
Plastic waste in soil	<i>Delftia</i> sp., <i>Stenotrophomonas</i> sp.	Untreated LDPE	90 days	Change of chemical properties	Peixoto et al., 2017
Mangrove sediments in Peninsular Malaysia	<i>Bacillus cereus</i> , <i>Sporosarcina</i> , <i>Globispora</i>	Polypropylene	40 days	12 and 11 %	Helen et al., 2017
Marine	<i>Lysinibacillus</i> sp., <i>Salinibacterium</i> sp.	PE	6 months	19%	Syranidou et al., 2017
Municipal solid waste	<i>Stenotrophomonas panacihumi</i> PA3–2	polypropylene (PP)	90 days	20.3 ± 1.39%	Jeon and Kim, 2016
	<i>Nitrosomonas</i> sp., <i>Nitrobacter</i> sp., <i>Burkholderia</i> sp., <i>Pseudomonas</i> sp.	HDPE, LDPE and PP	90 days	20%, 5% and 9%	Muenmee et al., 2016
Source of microbes	Isolate	Type of MP degraded	Incubation period	% of degradation	Reference
Fungal isolates					
	<i>Aspergillus</i> sp., <i>Penicillium</i> sp.	PP/PBAT	30 days		Oliveira et al., 2020
Guts of wax moth <i>Galleria mellonella</i>	<i>Aspergillus flavus</i>	HDPE	28 days		Zhang et al., 2020
Marine sediments	<i>Zalerion maritimum</i>	PE pellets	28 days		Paco et al., 2017
	<i>Bjerkandera adusta</i>	Gamma irradiated polypropylene and biomass			Butnaru et al., 2016

and *Pseudomonas* developed from cow dung showed faster biodegradation of PE and PP, demonstrated up to 15% weight loss after 120 days (Skariyachan et al., 2021). No information is available till date about the actions of depolymerases enzymes and the microbial degradation mechanisms of these microplastics and plastic debris (Ru et al., 2020). Bacteria, fungi and enzymes are identified based on their bioremediation capacity for PP, PE and PVC has been demonstrated based on their capacity of biofilm formation on plastic films, surface deterioration, thermal and mechanical properties alteration of plastics (Yang et al., 2015b; Auta et al., 2018; Giacomucci et al., 2019; Ru et al., 2020). Though most of the studies are based on macro-plastic films, however the same can be applied to MPs as well.

### Bacterial metabolism and degradation of microplastics

Biodegradation of plastics requires the action of microbial enzymes (both bacterial and fungal) for converting them into easily metabolizable fractions (dimers, oligomers and monomers) for example lipase hydroxylase, depolymerase and protease (Haider et al., 2019). After disintegration, microorganisms feed on those products as carbon source for their growth and reproduction (Haider et al., 2019; Zuo et al., 2019a). Apart from that, the potential microbial degrading organisms must possess appropriate enzymatic and metabolic pathways. Physical characteristics of microplastics should facilitate attachment of microorganisms to the surface and biological reactions should not be affected by the branching pattern.

The complex interaction between the available surfaces for the colonisation of microorganisms forming biofilm was studied by Fleming et al. (2017). The attachment processes that act on MP biofilm include (1) biofouling, (2) plasticiser breakdown, (3) assault on the polymer backbone, (4) hydration and (5) organism penetration into the polymer structure.

Various bacterial taxa are renowned as plastic colonisers, for example, *Arcobacter* spp., *Vibrio* spp., *Colwellia* spp., *Escherichia* spp. and *Pseudomonas* spp. (Oberbeckmann and Labrenz, 2020). In research conducted by Oberbeckmann et al. (2016), it was reported that assemblages of microbes on the surface of polymers are not substrate-specific. Curren and Leong (2019) found colonisation of diverse epiplastic bacteria including *Erthrobacter*, *Vibrio* and *Pseudomonas* species. While another research reported rapid colonisation of low-density polyethylene (LDPE) microplastic by coastal marine sediments bacteria including *Arcobacter* and *Colwellia* spp. (Harrison et al., 2014). Members of bacterial families like *Rhodobacteraceae* and *Flavobacteriaceae* and the genera *Albirhodobacter*, *Methylotenera*, and *Hydrogenophaga* have been found to be widespread on the surface of MPs (Oberbeckmann and Labrenz, 2020). Rosato et al. (2020) used a PCB-dechlorinating microbial culture to study microbial colonisation of various MPs (polyethylene, polyethylene terephthalate, polystyrene, polypropylene and polyvinyl chloride) and found all the MP to be colonised by microbes and also the microbes performed dechlorination proving their potential to remediate toxicity related to PCB polluted MPs (Rosato et al., 2020).

Research indicates that using bacterial consortia in MP biodegradation is more efficient rather than using single bacterial culture. Recent research conducted on MP degradation by using microbial consortia demonstrated maximum mineralisation in around 15 days (MeyerCifuentes et al., 2020).

Bacterial and fungal strains, including *Phaeosphaeria spartiniicola*, *P. halima*, *Mycosphaerella* sp. assemblages can decompose complex polymers and may potentially form biofilms on the surface

and ingest MP particles (Kawai et al., 2019). Various marine hydrocarbonoclastic bacteria (e.g., *Alcanivorax borkumensis*) demonstrated capacity to degrade alkanes, branched aliphatic, as well as isoprenoid hydrocarbons, and alkyl cycloalkanes (Davoodi et al., 2020). Researchers have also found possible potential of various marine bacteria for MP biodegradation due to their ability to form biofilms (Salvador et al., 2019). Evidence of LDPE degradation by *A. borkumensis* was observed while investigating biofilm formation on LDPE surface (Delacuveellerie et al., 2019). *Alcanivorax* sp. also interacts with plastic surface by adjusting its hydrophilicity of the cell membrane (Delacuveellerie et al., 2019). Recently, putative laccase isolated from actinomycete *Rhodococcus ruber* was found to be involved in PE biodegradation. Some bacterial strains obtained from sewage treatment plants, mulch films and landfills waste demonstrated the ability to utilise unpretreated PE, and PP strips as a carbon source (Jeyakumar et al., 2013). In the presence of starch, microorganisms showed accelerated hydrolytic biodegradation of PP and PE (Cacciari et al., 1993) also the enzymatic reactions make the polymers more vulnerable to biodegradation (Ru et al., 2020).

### Fungal metabolism and degradation of microplastics

Fungi are excellent candidates for potential plastic degradation owing to their variety of metabolic potential and ability to degrade complex chemical structures (Lacerda et al., 2020). Marine fungus *Zalerion maritimumis* was reported to be successfully biodegrade PE (Paço et al., 2017). A wide diversity of epiplastic fungal species was reported from various geographic locations indicating their abundance in aquatic environments (Lacerda et al., 2020). Different fungal genera such as *Aspergillus*, *Cladosporium*, and *Wallemia* as well as a wide variety of taxa such as, *Chytridiomycota* and *Aphelidomycota* were reported from various locations (Lacerda et al., 2020). The fungal diversity thriving in the biofilm of microplastics found dominance of members from *Chytridiomycota*, *Cryptomycota* and *Ascomycota* (Kettner et al., 2017). Brunner et al. (2018) reported around 100 fungal species able to degrade MP debris highlighting the potential of marine epiplastic fungal communities. The isolated fungal strains were found successful to degrade Polyurethane (PU) but not PE polymers (Brunner et al., 2018). Lignin-biodegrading fungi catalyses the oxidation of aromatic and non-aromatic substrates like chorophenolic or nonphenolic compounds (polymethylmethacrylate [PMMA] and polyhydroxybutyrate [PHB]) by producing laccase (Straub et al., 2017).

### Enzymatic degradation of microplastic: Microbial gene action and metabolism

Enzymes play a vital role in cellular functions regulation; microbes tend to modify their enzyme activity in response to changing environmental conditions. Inside the biofilm, the microbial enzymes build up a microenvironment interacting with the MP causing its degradation. Extracellular enzymes of microbes (esterase, lipase, lignin peroxidases, laccase and manganese peroxidases) are indispensable for increasing the hydrophilicity of plastic polymers by converting to functional carbonyl or alcohol groups, which can enhance microbial attachment and promotes biodegradation (Shahnawaz et al., 2019; Taniguchi et al., 2019). Extracellular enzymes such as hydrolases (such as lipases, esterase, poly(3-hydroxybutyrate) depolymerases and cutinases) operate on the plastic surface to break it down into smaller molecules (Sol et al.,

2020). Integration of monomers transported into the cytoplasm of microbes and finally their metabolism results in their assimilation (Zettler et al., 2013). There are two types of MP degrading enzymes according to their mechanisms namely, surface modification mechanisms and degradation mechanisms (Vertommen et al., 2005). The first category of enzymes consists of a group of hydrolases (lipases, carboxylesterases, cutinases and proteases), while the polymer degrading enzymes includes oxidases, amidases, laccases, hydrolases and peroxidases (Álvarez-Barragán et al., 2016; Gómez-Méndez et al., 2018). Large polymers are broken down into smaller fragments with the help of enzymes, the fragment then gets ingested by the microbes and finally gets incorporated into the metabolic cell cycle. The extracellular polymeric substance (EPS) regulates the microenvironment and maintains the microbial community (Lucas et al., 2008) also governing the characteristics of the biofilm determining microbial penetration rate (Lucas et al., 2008) and ultimately control the fate of the plastic particles.

Despite ample evidence that EPS and enzymes have a significant influence on the biodeterioration of MPs, there is currently a dearth of technical options for the total destruction of these MPs (Kumar et al., 2020). Thus, it can be said that although a variety of microplastic (PE, PP and PVC) degrading microbes has been identified but microbial enzymes known to successfully degrade PE (laccase, soybean peroxidase, manganese peroxidase) and PS (hydroquinone peroxidase) are very few (Zhang et al., 2004; Santos et al., 2013) and none for PP degradation (Arutchelvi et al., 2008).

Microorganisms are able to produce enzymes that can breakdown complex polymer of plastics using them as carbon source for their growth and metabolism. Their ubiquity in different environments makes them the most promising natural and sustainable approach of MPs degradation. MPs degradation by microbes is influenced by various factors, including the type and structure of the plastic, environmental conditions (e.g., temperature, pH, oxygen availability) and microbial diversity and biochemical activities (Syranidou et al., 2017a). Among the reported potential MP degraders, bacteria play a significant role through various mechanisms. Bacterial strains in the genera *Pseudomonas*, *Bacillus* and *Rhodococcus* have the ability to breakdown and utilise different types of MPs (Yakimov et al., 2007; Syranidou et al., 2017a). Various types of extracellular enzymes like lipases and esterases are produced by the bacteria which help in MPs degradation (Auta et al., 2018). MPs degradation potential have also been shown by fungi, especially by the species of *Aspergillus*, *Penicillium* and *Fusarium* (Tournier et al., 2020; Solanki et al., 2022). Enzymes like cellulases and ligninases are secreted by fungi which are able to degrade MPs polymers (Yang et al., 2022). Algae, especially diatoms, can also potentially degrade MPs with the help of their extracellular enzymes like lipases and proteases, which are able to degrade and modify MPs surface properties (Sun et al., 2020a; Zhu et al., 2021). Algae such as *Scenedesmus dimorphus* (a green alga), *Anabaena spiroides* (a blue-green alga), *Navicula pupula* (a diatom), and various species of *Oscillatoria*, have been observed to thrive on polythene surfaces in sewage water (Chia et al., 2020). The adhesion of these colonising algae on to the MPs surface marks the start of biodegradation, and various ligninolytic and exopolysaccharide enzymes produced by them facilitates plastic degradation (Priya et al., 2022). Additionally, with biofilm formation on the plastic surface, enhanced degradation of MPs takes place due to the secretion of a range of microbial enzymes, which also facilitates the establishment of a diverse microbial community, promoting efficient microplastics degradation (Oberbeckmann et al., 2018;

Ogonowski et al., 2018). Within the biofilm, synergistic interactions among various microorganisms creates a microenvironment favourable for enzymatic activity which further enhances the degradation potential (Wei and Zimmermann, 2017; Tournier et al., 2020).

Addressing the burning issue of plastic pollution requires detailed knowledge of the MPs degradation mechanism for implementation of effective remediation (Lopez-Pedrouso et al., 2020; Othman et al., 2021). A wide range of enzymatic processes including hydrolysis, oxidation, reduction and esterification, can breakdown polymer bonds or modify polymer functional groups (Lopez-Pedrouso et al., 2020; Othman et al., 2021). These enzymatic processes are crucial for breakdown of plastic polymer chains into smaller molecules that can serve as microorganisms' source of carbon and energy. One of the most common plastics in our daily life polyethylene terephthalate (PET) can be degraded by PET hydrolases (Carniel et al., 2017), which can be obtained from microorganisms like *Ideonella sakaiensis* and *Rhodococcus* sp. (Yoshida et al., 2016; Wei and Zimmermann, 2017) and offers a promising avenue for PET plastic waste management. Also, cutinases have shown excellent ability in PET degradation (O'Neill et al., 2007; Furukawa et al., 2019) especially, microorganisms *Thermobifida alba* and *Thermobifida fusca* can achieve this. Additionally, Microbes like *Trametes versicolor* and *Pycnoporus cinnabarinus* with the help of laccases oxidise phenolic compounds, influencing the fate of PS and PU (Bilal et al., 2019; Ghatge et al., 2020; Yao et al., 2022). Notably, in plastic degradation lignin peroxidases and manganese peroxidases, obtained from microorganisms such as *Phanerochaete chrysosporium* and *Pleurotus ostreatus*, have shown promise (Mukherjee and Kundu, 2014; Ojha et al., 2017; Rovaletti et al., 2023). Excellent plastic degrading potential is also shown by Cellulases, particularly against PS (Koeck et al., 2014; Pathak, 2017; Yan et al., 2021c). The likes of *Clostridium thermocellum* and *Cellulomonas fimi* contribute to our understanding of this multifaceted enzymatic approach to plastic waste management.

### Can microplastic develop antibiotic resistance in bacteria?

In the context of antibiotic resistance, two freshwater studies demonstrated microplastic-associated assemblages having an increased transfer frequency of a plasmid coding for trimethoprim resistance (Arias-Andres et al., 2018) and higher abundance of the gene *int1*, a proxy for anthropogenic pollution (Eckert et al., 2018).

MPs can selectively enrich both antibiotics and antibiotic-resistant bacteria on their surfaces in various environments (Wu et al., 2019; Su et al., 2020; Sun et al., 2020b; Wang et al., 2020). Zhang et al. (2020) isolated and characterised antibiotic-resistant marine bacteria from MP particles which showed the presence of several multidrug-resistant marine bacteria including pathogenic *Vibrio* species (Zhang et al., 2020). Other studies showed the presence of multidrug-resistant pathogens from *Vibrio* sp. (Laverty et al., 2020) and *E. coli* (Song et al., 2020) on marine microplastics. A study published recently described whole genome sequencing (WGS) of antibiotic-resistant microorganisms isolated from marine plastics (Radisic et al., 2020).

Enrichment of various Antibiotic Resistant Genes (ARGs) like *sul1*, *tetA*, *tetC*, *tetX* and *ermE* was reported from plastic particles in both sea and freshwater (Wang et al., 2020) and selective enrichment of *strB*, *blaTEM*, *ermB*, *tetM* and *tetQ* was seen on MP particles from



landfill leachates (Shi et al., 2020). Lu et al. (2020) reported the presence of upto 43 different ARGs on MP surface from vegetable soil using advanced high-throughput qPCR screening.

Using Shotgun metagenomics 64 different ARG subtypes providing resistance against 13 different antibiotics on macroplastics and microplastics were collected from North Pacific Gyre (Yang et al., 2019). This research and numerous others have discovered clinically relevant ARGs on MP particles, such as *sul1*, *tetA*, *tetC*, *tetX*, *ermE*, *aac(3)*, *macB* and *blaTEM*, which are typically present in human infections (Alcock et al., 2020), suggesting that MPs serves as reservoirs of clinically important antibiotic resistance genes. The numerous antibiotics and active metabolites present in various chemicals and heavy metals (Godoy et al., 2019; Chen et al., 2020; Mammo et al., 2020; Wang et al., 2020) adsorbed to MPs leads to multidrug resistance among different bacteria within microbial biofilm resulting in active selection of antibiotic resistance on MP surfaces.

Imran et al. (2019) have advocated that the development and spread of multiple drug-resistant human pathogens occur by co-contamination of MPs and heavy metals through co-selection mechanisms.

### Role of environmental factors in microbial degradation of microplastics

Various environmental factors like pH, moisture, salinity and temperature play vital roles in bioplastic degradation (Gong et al., 2012). While, factors like temperature and ionic strength facilitate the colonisation of microbes and biofilm formation (Rummel et al., 2017), the presence of nutrients and other pollutants, as well as the availability of light and pressure are known to affect the nature and extent of microbial attachment (Harrison et al., 2018). The surrounding environment is a major player for biofilm structuring and low nutrient and high salinity forms substrate-specific assemblages (Oberbeckmann et al., 2018). An optimum temperature is required which favours enzymatic activity and microbial growth (Shruti and Kutralam-Muniasamy, 2019). Moisture content and salinity are other important factors for biodegradation (Gong et al., 2012). Bioplastic degradation is kick-started by water uptake followed by the breakage of ester bonds. pH of the medium also affects the rate of reaction, alkaline condition favours hydrolysis of PLA (Elsawy et al., 2017). Comparative analysis revealed that the plastic material is a minor factor determining MP-associated biofilms while the most important factor was geographical region (Amaral-Zettler et al., 2015).

Keeping the above facts in mind, it can be inferred that finding a natural environment for complete bioplastic degradation is a difficult task. A potential solution to this situation is the use of engineered microorganisms which can degrade bioplastics with highest efficiency while withstanding extreme environmental conditions (Danso et al., 2018b).

### Role of microbial biofilms in microplastic degradation

Biofilms are diverse microbial communities consisting of bacteria, fungi, algae, and so on thriving on any surface submerged and in spatial proximity (Donal, 2002). The microorganisms are collectively benefited by having a stable consortia, availability of nutrients, and protection from desiccation (Zettler et al., 2013). Biofilm can affect microplastic structure and function in various ways, with the help of various enzymes. These enzymes can transform surface

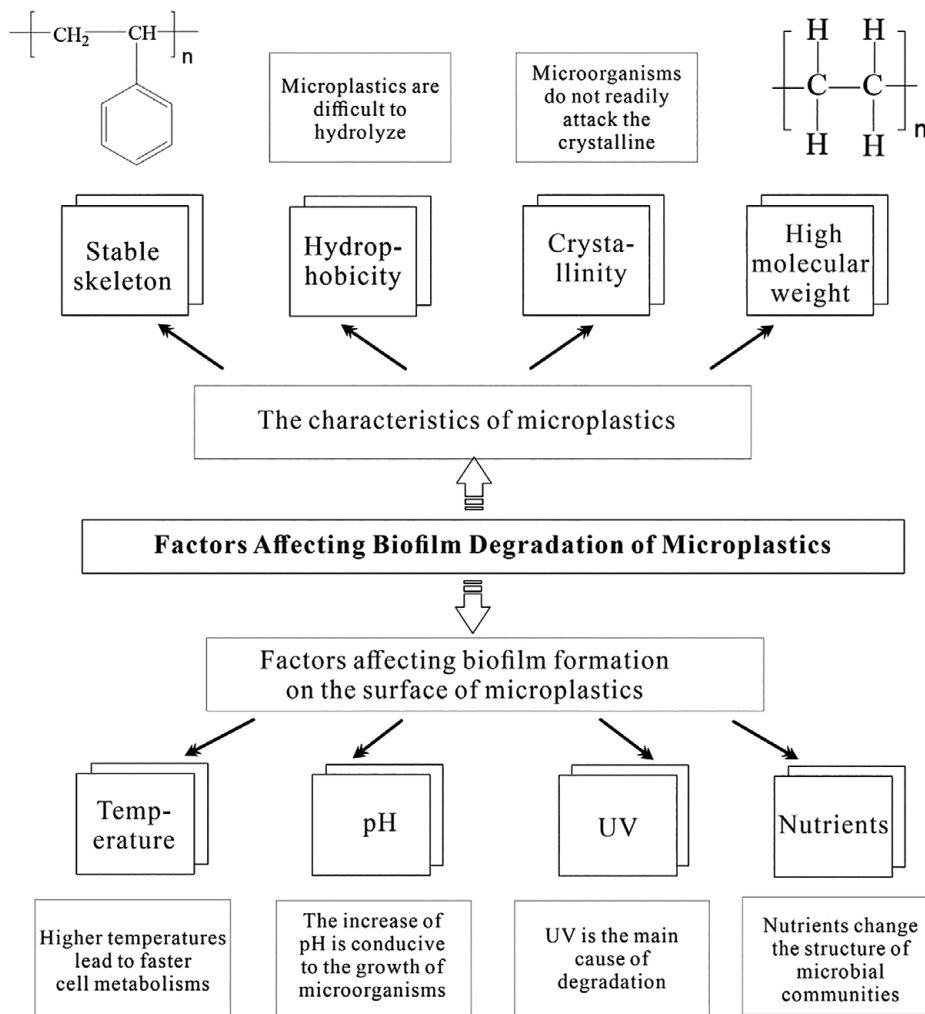
properties, additive degradation and metabolic by-product release, thus can determine the fate of MPs in marine environments (Miao et al., 2019).

MP biodegradation starts off after the attachment of first microbes and development of biofilm on the plastic surface (plastisphere). The plastisphere comprises of various microbial communities developing into a biofilm (Urbanek et al., 2018). The plastic surface encourages microbial colonisation and biofilm formation leading to a reduction in polymer buoyancy and hydrophobicity (Lobelle and Cunliffe, 2011). Various polymer additives being easily metabolised, encourages microorganisms' initial attachments and biofilm formation (Ru et al., 2020). Microbes ensure surface attachment by various mechanisms like, cell surface charge changes and hydrophobicity and modifications in EPS production, pioneer microbes then modify surface morphology for additional microbial colonisation. Various substratum property like crystallinity, melting temperature, roughness, and so on might influence microbial community assemblages during colonisation stages (Rummel et al., 2017). Various factors affecting biofilm degradation of MPs are summarised in Figure 2; biofilm formation process and microplastic degradation are shown in Figure 3.

Biofilm maturation into complex structures is apparently achieved through quorum sensing (QS) processes among cells. QS Signalling gene gets accumulated in the external environment and regulates neighbouring cells specific gene expression. A few bacterial species use QS to coordinate and regulate the disassembly of the biofilm (Sharma et al., 2019). Various researches have suggested active QS involvement in the organisation and development of multispecies biofilms in the marine environment (Hmelo, 2017).

After colonisation, biodeterioration of the polymer follows leading to loss in physical integrity and loss of polymer's mechanical properties (Kumar et al., 2020). The process is primarily achieved by exoenzymes secreted by microbes. At this point, the microbe's EPS offers strong adherence to the polymer surface, and the enzymes' catalytic activity commences the dissolution of the polymeric structure. EPS and enzymes are thought to have significant influences on the biodeterioration of MPs (Kumar et al., 2020). The deteriorated polymers are then converted to oligomers, dimers and monomers through biofragmentation and finally get assimilated (Amobonye et al., 2020). The catalytic activities of microbial enzymes control the biodegradation of MPs mediated by biofilm promoting fragmentation of the polymer. The fragments formed through enzymatic depolymerisation can be assimilated by microbes resulting in increase in their biomass (Degli-Innocenti, 2014). These enzymes hydrolyze polymers by a nucleophilic attack on the carbonyl carbon, resulting in either readily assimilable oligomeric or monomeric elements or compounds that require more processing before they can be digested by the microorganisms (Kumar et al., 2020).

During assimilation, the fragmented polymers gets integrated in the microbial cells (Lucas et al., 2008). Enzymes and carrier proteins are involved in assimilation of monomers with catabolic cycles for energy production (Hosaka et al., 2013; Durairaj et al., 2016). The assimilation step is mostly followed by mineralisation, in which the polymers get completely degraded and final products like CO<sub>2</sub>, N<sub>2</sub>, H<sub>2</sub>O and CH<sub>4</sub> are released. Complete mineralisation of PET has been reported to produce acetic acid which gets used in the Krebs cycle or integrated into lipid synthesis (Wilkes and Aristilde, 2017). Polymer physiochemical properties such as hydrophobicity, surface energies and functional groups influence the formation of biofilm (Bhagwat et al., 2021). For biological sedimentation, the



**Figure 2.** Various factors affecting biofilm degradation of microplastics. Reprinted after Sun et al. (2023), under a creative commons licence, open access.

conditioning layer is very important, which actually depends on the roughness, hydrophobicity and chemical nature of the initial matrix surface (Tu et al., 2020).

Different biochemical processes in microbe-mediated microplastic degradation and related biotechnological interventions are shown in Figure 4.

### Microbial remediation of microplastic: Novel and emerging techniques

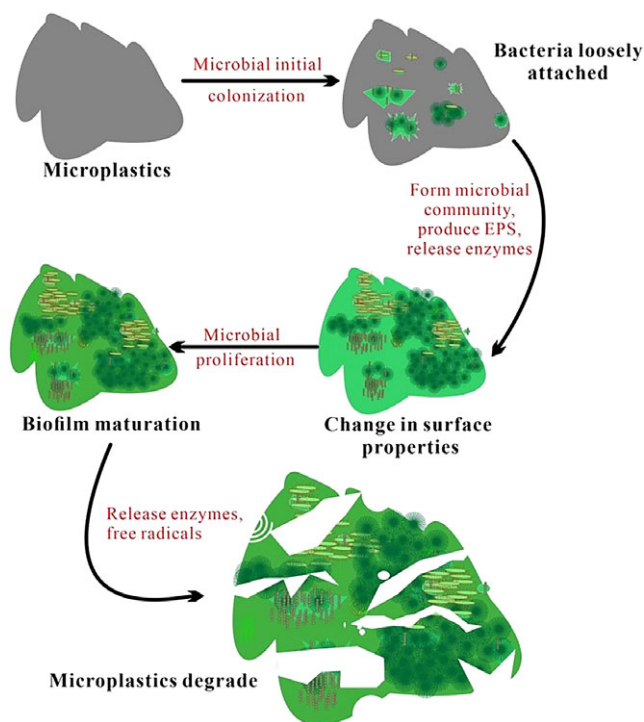
#### Absorption of microplastics by green algae

Algal cells can be effectively used for the breakdown of complex polymeric materials (Manzi et al., 2022). These algal cells may interact with MP particles and alter their properties which may determine the adsorption rate and fate of these MP particles (Kershaw et al., 2015). Algal consortium can be used in degradation of MP polymers as they do not require carbon source in the growth media and can easily adapt to different environmental conditions. Microalgae are known to produce biofilms on the surface of MP particles and help in the degradation process by producing ligninolytic and exopolysaccharide enzymes, this polymers on the other hand act as a source of carbon which promotes the growth of algal

cells. The crucial processes that promote the degradation of MP particles includes corrosion, hydrolysis penetration and fouling (Chia et al., 2020). Green algae such as *Oscillatoria subbrevis* and *Phormidium lucidum* have been reported to colonise the surface of LDPE and degrade it without any pretreatment (Sarmah and Rout, 2018). Recent advancement in biotechnology have led to the production of genetically modified microalgal cell factories capable of producing hydrolytic enzymes having the ability to degrade plastics. 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). These microalgae affect the vertical flux of the polymers by varying the density of the polymers which can be incorporated into hetero-aggregates promoting adsorption. However, this process is poorly understood (Tadsuwan et al., 2021).

#### Application of whole cell biocatalysis and microplastic immobilisation

Biocatalysis uses whole cells of bacteria, fungi, microalgae and plants to catalyse organic reaction. This provides high enantioselectivity and is considered as low or non-toxic, ecofriendly and green alternative to treat pollutant. Recent publications on



**Figure 3.** Bacterial colonisation, biofilm formation and degradation of microplastics. Reprinted after Sun et al. (2023), under a creative commons licence, open access.

biocatalysis concentrates on the use of enzymes and overexpression of enzymes in genetically engineered microbes but they are considered to be expensive as it requires resources to renew cofactors required for enzyme activities (Monti et al., 2011; Sheldon and Pereira, 2017). Biocatalysis may result in chemical transformation which may help in preparation of chiral compounds and organic compounds. They can effectively reduce carbon–carbon double bonds using highly selective processes and mild reactions. Thus, whole cell biocatalysis can be a sustainable technique in organic synthesis (Iqbal et al., 2012). Biocatalytic hydrogenation of alkanes can also serve as a crucial strategy for metal-assisted hydrogenation reactions. MP contains complex structures which can be effectively degraded and immobilised using whole cell biocatalysis.

### Microplastic biodegradation by hyperthermophilic composting technology

Hyperthermophilic composting (*hTC*) is done at high temperature above 90°C using hyperthermophilic bacteria (Yu et al., 2018). As this process is performed at a very high temperature it leads to more efficient bioconversion within short period. Moreover, *hTC* is also effective for the reduction of antibiotic resistance genes and mobile genetic elements in sewage sludge due to the involvement of high temperature (Liao et al., 2019). Sewage sludge is one of the major sources of MP particles and removal of these MP particles by conventional treatment procedures can be ineffective. *hTC* can be effectively used for *in situ* biodegradation of sludge-based MPs. In a study made by Chen et al. (2019) *hTC* reported removal of 43.7% of MP from sewage sludge. *hTC* was also capable of degradation of 7.3 % of the PS-MPs at 70°C in 56 days through bio-oxidation. High-throughput sequencing showed the presence of *Bacillus*, *Geobacillus* and *Thermus* was directly linked with *hTC* which accelerate MP degradation and associated –C–C– bonds cleavage at high

temperature (Manzur et al., 2004). In addition, *hTC* leads to the killing of pathogens associated with MP and lacks nitrification and denitrification processes, preventing the excess loss of nitrogen (Kanazawa et al., 2008).

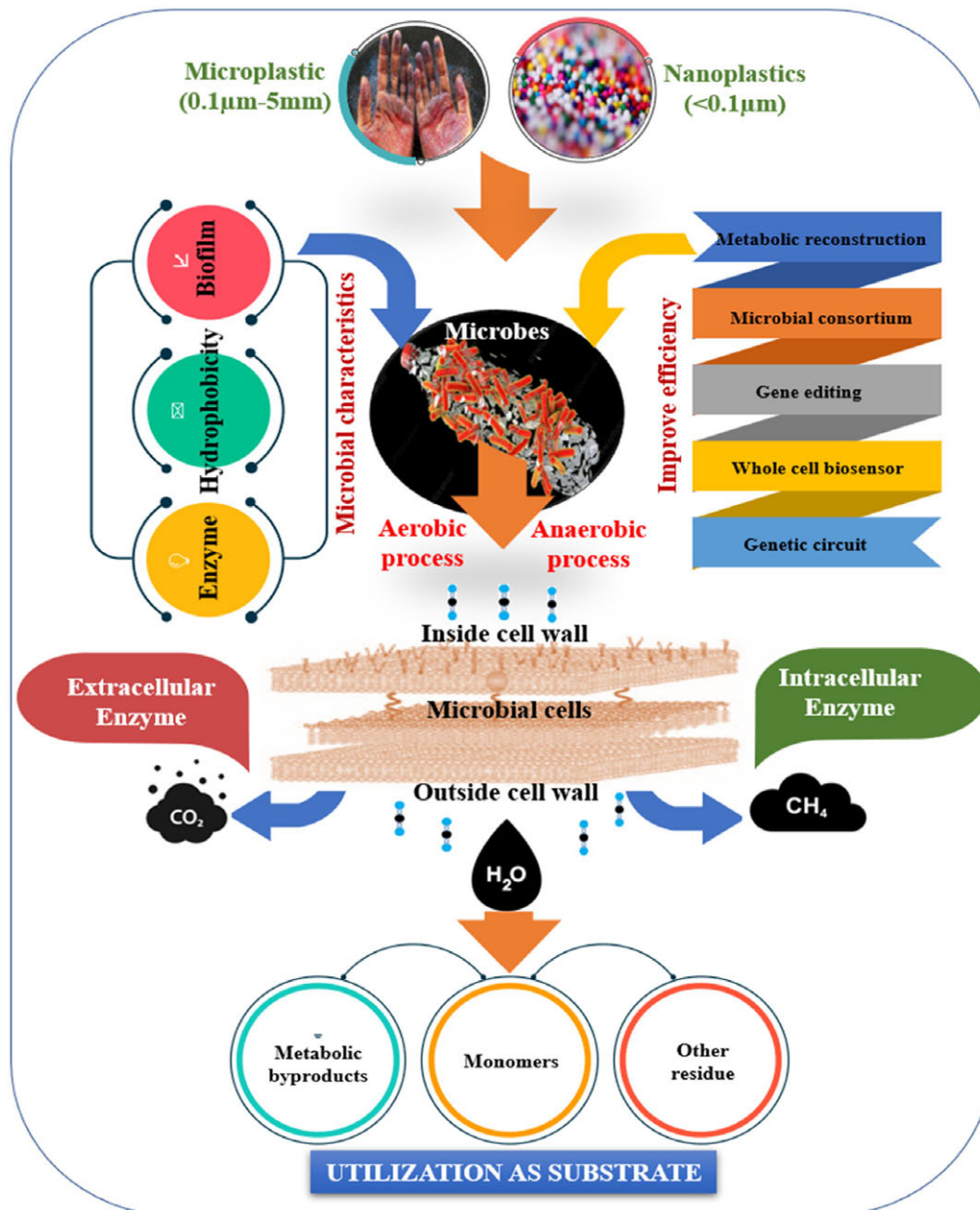
### Future research directions in microplastic bioremediation

Studies regarding accumulation, characterisation, identification of microplastics are numerous, however, studies regarding their mitigation are still lagging. Biodegradation process of microplastics can be understood following four common approaches namely, (1) the depletion of substrates, (2) accumulation of biomass, (3) reaction products and (4) changes in substrate properties. Additionally, standard processes for plastic biodegradation involves producing microbial films on the polymeric surfaces followed by their breakdown into smaller pieces (<20 µm). However, the methods are not economical and also not widely applicable. The micro-Fourier transform infrared (FTIR) can facilitate the mapping of samples, multiple polymer characterisation and identification of irregular shaped MPs and is widely accepted (Gong et al., 2018), however, the method is quite expensive and time-consuming. Other analytical methods employed for MP identification includes scanning electron microscope-energy dispersive spectroscopy (SEM-EDS), pyrolysis-gas chromatography-mass spectrometry and ESEM-EDS (Horton et al., 2017). MPs are classified as an emerging pollutants due to a paucity of MPs data from soil and water, which limits knowledge of their environmental impact. It is difficult to show the ecotoxicological risks of microplastics on the surrounding ecosystems due to a lack of quantitative data (Harrison et al., 2012; Goel, 2017; Gong et al., 2018). In a nutshell, microplastic research is still in its infancy and more research is needed to address links between plastics and microplastics generation.

MPs have become a potential substrate for colonisation in the oceans, with members of the family *Sphingomonadaceae* in particular selectively colonising microplastic polymers. Based on our reanalysis of available literature and critical review, available information and evidences of MPs degradation by microbes are not adequate. According to Tagg and Labrenz (2018) for ensuring environmental compatibility and sustainability, collective actions are needed in order to better understand microbial degradation of plastics focused research on understanding the microbial pathways that can potentially degrade plastic are needed.

Future studies should consider genomics and proteomics approach in order to amplify the rate of microbe-mediated degradation of MP. The use of fungi for degrading MP effectively is now receiving increased attention. Fungi that are able to withstand oxidation and corrosion will be identified and screened for investigating their degradation properties on MPs that are difficult to degrade paving the way towards new and innovative strategies focused at mitigating the environmental impacts of MPs (Paco et al., 2017). Since MP biodegradation is a complex issue testing the efficacy of the process under real-world conditions is of prime importance.

Although advanced and innovative methods have been developed for isolating strains capable of degrading MPs, still the number of bacteria selected for screening is mostly limited to taxa like *Bacillus*, *Pseudomonas*, *Chelatococcus* and *Lysinibacillus fusiformis*. However, the bacterial degradation efficiency of MPs is quite low (0–15%) and takes a long time (usually 0–3 months) showing that microbial degradation of MP is a slow and time consuming process. Thus, future studies should look into improving the bacteria-mediated degradation potential of MPs with the help of modern



**Figure 4.** Different biochemical processes in microbe-mediated microplastic degradation and related biotechnological interventions. Reprinted after Zhou et al. (2022) (Elsevier), licence number 5647011139377.

molecular techniques like *in vitro* transcription, *in situ* hybridization, high-throughput sequencing and PCR. Also, multiple bacterial consortia have been identified from various environments (Sangwan and Wu, 2008) which have potential for MP degradation. However, these are complex processes due to the involvement of various microorganisms and multiple enzymes. Therefore, in depth studies are needed to get a clear idea about the factors influencing and mechanisms involved in the degradation process.

Also, the progression of the whole process of biofilm formation needs to be studied in detail, especially the microorganisms initiating the colonisation of a novel MP particle in different environments and the successional process leading to further colonisation by other microorganisms. This will help us in getting an idea about the whole process of MP biodegradation.

Future research should be oriented towards microbial remediation of plastic, especially understanding the microbial degradation mechanism and factors affecting it under real-world conditions so that it can be applied in real-world situations and can perform optimally and especially in developing innovative approaches towards microbial remediation through novel methods which are environmentally sound and sustainable.

### Conclusions

Human activities have long continued to modify planet earth, but now it is time to assess our impacts on the planet. Plastics have provided many benefits and have made our life easier. Perhaps it is time to reconsider the importance we place on contemporary

conveniences. Planet Earth, with its many ecosystems and over 1030 microbial inhabitants (Flemming and Wuertz, 2019), has discovered a mechanism to biotransform and even to some extent degrade plastic materials. MPs are an emerging class of contaminants having ubiquitous presence in every environment and affecting them negatively. Currently, MPs are a serious issue not only in the aquatic ecosystem but also in terrestrial ecosystems. In this scenario, there is an urgent need to develop strategies for mitigating this problem. Very little knowledge is available on key depolymerase and the mechanisms of biodegradation of Low biodegradable MPs (e.g., PP, PE, PS, PVC) and the most common depolymerising method is pyrolysis. Thus, more research efforts are needed to identify depolymerases from the plastic-degrading strains. Surface-modifying enzymes and esterase's (e.g., cutinases, lipase, PETase) have been tested for MPs and their catalytic efficiency towards initial PET hydrolysis varies depending on crystallinity and environment conditions. Thus, to protect enzymes within an engineered cellular environment application of whole-cell biodegradation has been recommended. Nevertheless, degradation of biodegradable MPs takes place under assisted conditions. Regarding the highly crystalline MPs sustained thermal hydrolysis has been recommended to make the polymer bioavailable to microbes. For composting biodegradation, uncertainty remains in the requirement for specific collection and composting facilities and various contaminated and toxic MPs residues. Thus, the primary focus should be to reduce MP footprint through the use of natural alternatives (e.g., jojoba beads, pumice, ground nutshells) and MPs isolation in environmental matrices in order to reduce MPs release into the environment. Future studies should aim at multi-omics approaches to decipher biochemical transformation mediated by microbes. Furthermore, biotransformation of plastic debris by microbes may potentially have an important role in the generation of micron- and sub-micron-scale polymer particles, which could impact human health and food security.

In marine environments, biofilms induce transformation of surface properties, the degradation of additives, and the release of metabolic by-products with the help of modifying/hydrolysing microbial enzymes. Thus, biofilms can be potential candidates for mitigation of microplastics. However, we have limited knowledge about microbial communities on MPs coated by biofilm, factors affecting their colonisation and their interaction with plastic substrate. There is an urgent need for studies regarding episodic marine microbial communities and their ability to remediate MPs from aquatic environment. Bioengineered microbes and modified enzyme systems can provide exciting avenues of research in this regard.

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