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Microbe-Microplastic Axis:

Microplastics As Both Microbial Toxin and Tool

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Abstract

Microplastics (MPs) in the environment are becoming a growing concern worldwide. Produced by the weathering of plastic waste or through intentional manufacturing, these microscopic synthetic particles are associated with adverse health effects in humans, animals, and even microbes. Due to their small size, MPs have an exceptionally large surface area that, in combination with their intrinsic hydrophobicity and natural weathering, gives them an increased affinity for toxins. Indeed, MPs can contain up to a million times the concentration of heavy metals, organic pollutants, and toxic secondary metabolites compared to their environment. Internalisation of or adherence to MPs by microorganisms has been linked to a decreased growth rate, altered gene expression, lower photosynthetic efficiency, and even cell death. However, despite their toxicity, the microbial utilisation of MPs as tools for protection, carbon and nutrient acquirement, and gene transfer through biofilm formation has now been recognised. Furthermore, microbe-mediated depolymerisation and mineralisation of MPs through the emergence of plastic-degrading enzymes and oxygen radicals highlights the employment of microorganisms as potential solution for the removal of MPs from the biosphere. Yet, it is unlikely that efficient degradation pathways for different types of plastic will evolve naturally in the near future, meaning that alternative approaches must be considered to clean-up the planet. Ultimately, this literature review presents the state of the art on MPs as microbial toxins and tools while exposing the MP-degradation potential of microorganisms and their role in decontaminating the environment.

Layman's summary

Whenever plastic waste in the oceans or landfills slowly erodes, small microplastics (MPs) are produced. This is becoming a major problem as these tiny particles spread everywhere on the planet and are causing people and animals to experience negative health effects. Even at the smallest level, microbes that are subjected to MPs suffer a negative influence on their overall functioning. Studies suggest that the toxicity of MPs is due to their affinity for toxins because of the particle's large surface area, rough exterior, and tendency to repel water. Indeed, MPs can contain up to a million times the concentration of heavy metals, organic pollutants, and microbe-produced toxic by-products compared to their environment. When interacting with microorganisms, MPs can slow down cellular growth, alter the cell's genetic performance, decrease the rate of photosynthesis, and even cause cell death. Nevertheless, microbes have found ways to use MPs for protection, increased food availability, and facilitate the exchange of genes by creating a complex slime layer attached to the surface of the MP. Moreover, some microbial species have been observed to break down plastics using specialised enzymes and subsequently use the products of the enzymatic breakdown process for energy and growth. Because of this ability, researchers believe that microbes could play a central role in cleaning up the planet. However, the current rate at which plastics are broken down is too slow to be useful and it will likely take centuries before the speed of these enzymes is sufficiently increased as a result of evolution which calls for the exploration of alternative ideas. Ultimately, this literature review looks at what we currently know about MPs as microbial toxins and tools, while also delving into how microorganisms can break down MPs and help clean up the environment.

Contents

1. Introduction

Ever since humans first learned to use tools, the leaps of development have been marked by the dominant material of that time. History tells us that the first brick cities were built in the stone age, the making of weapons in the bronze age, and the forging of steel in the iron age. By continuing this trend and looking at the most ubiquitous material today, it could be argued that we currently find ourselves in the plastic age. Indeed, the unique properties of synthetic polymers allowed them to be incorporated into virtually every aspect of our society through their use in packaging, construction, health care, electronics, machinery, textiles, and single-use disposables [1]. Because of their light weight, low cost and malleability, production after the second world war soared and continues to see exponential growth to this day, making plastic one of the most abundantly produced man-made materials on the planet rivalled only by steel and cement [1], [2]. However, it is their exceptional durability and resistance to chemical and mechanical stress that poses a major environmental threat [3]. Currently, none of the commonly used plastics such as polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), polypropylene (PP) and polyethylene terephthalate (PET) are biodegradable [3]. Furthermore, the majority of monomers that are used to make plastics, such as ethylene and propylene, are derived from fossil hydrocarbons, making both the generation and natural recycling an unsustainable and non-circular process [4]. While biodegradable and biobased plastics are an upcoming growing sector, this industry currently only accounts for a mere 0.5% of global plastic production, which is limited by the available agricultural feedstock and therefore remains an unviable large-scale replacement for petroleum-based plastics [5], [6]. Consequently, 79% of all produced plastics accumulate in the terrestrial (landfills) or aquatic (rivers, lakes, and oceans) environment, 12% is incinerated (promoting the release of toxic compounds in the air), and only 9% is fully recycled [2]. In fact, plastics are now so widespread that they are predicted to serve as geological indicator for the modern (Anthropocene) era for future geologists [7].

1.1 Formation and Distribution of Microplastics

Despite their strength and durability, most of the discarded plastics end up as microplastics (MPs), which are defined as fragments smaller than 5 mm in diameter [8]. These often microscopic polymers can be derived from any type of plastic and are subcategorised into primary and secondary MPs. Primary MPs are purposely manufactured for their small size and are mainly used in cosmetics as abrasive microbeads, and in textiles as (nylon) microfibers [8], [9]. Secondary MPs are formed when larger plastic pieces are fragmented by mechanical stressors (e.g. wind, rain, ocean tides, etc.) [9]. Alternatively, UV radiation from the sun can promote photo-oxidation and breakage of the C-C and C-H bonds, leading to physical separation of the polymer subunits [5], [8], [9]. Owing to their immense abundance in combination with poor capturing and recycling efforts, the entire planet's surface is now contaminated with MPs. From the depths of the Mariana trench [10], to the top of mount Everest [11], to the untouched snow of the arctic [12], MPs are found everywhere. While a small fraction of these particles exhibits non-uniform global distribution through atmospheric fallout originating from incineration plants, the current data highlights water as main transporter of MPs into the environment [6], [13], [14]. Initial contamination typically starts at bodies of surface water (rivers, lakes, swamps, etc.) where untreated wastewater is deposited [15]. Surprisingly, even treated wastewater still contains elevated levels of MPs as the decontamination process does not filter out these small hydrophobic particles [15]. In fact, evidence suggests that wastewater treatment plants contribute to the formation of MPs through physical abrasion and chemical oxidation of larger plastic fragments [16]. It is predicted that by 2025, 250 million tonnes of MPs supplied by the contaminated freshwater will have accumulated into the ocean where they are directly exposed to marine life [17], [18]. Adding

to that, more recent studies indicate that rainwater enables the transport of significant quantities of MPs from landfills to groundwater reservoirs [19]. Farmers in many parts of the world rely on groundwater and effluent/sludge from water treatment plants to irrigate and fertilise their crops which inadvertently contributes to the spread of MPs into the arable soil and terrestrial ecosystem [20], [21].

1.2 In Vivo Accumulation and Effects of Microplastics

The increasing accumulation of MPs in the environment poses a potential threat to wildlife and human health. Mounting evidence suggests that small MPs (5 - 0.001 mm) or nanoplastics (NPs, <0.001 mm) are able to penetrate the root system of edible plants and collect in the stem, leaves and fruits [22], [23]. While it appears that the effects of small MPs and NPs are negligible in the investigated plants, they do act as a starting point for bioaccumulation of these polymers to higher trophic levels [24]. As a result, in terrestrial organisms, the vast majority of MPs enter through ingestion. Contrary to land fauna, aquatic animals have the added burden of being significantly affected by MP exposure through respiration and skin contact which can even lead to death by clogging the gills [25], [26]. When contaminated plants (or their fruits), fish, or animals are consumed, the MPs and NPs typically collect in the gastrointestinal tract, particularly the intestine [27]. Large quantities of MPs in the intestine of smaller animals (fish, rodents, insects, etc.) has shown to significantly inhibit the uptake of nutrients by physically blocking the intestinal epithelial, leading to malnourishment, metabolic complications, and death [28]. Larger animals and humans are less affected by the direct effects of MPs but still experience negative effects that MPs and NPs can elicit [28]. Indeed, recent studies show that these small plastics can pass into the endocrine and lymphatic system and even cross the highly selective blood-brain barrier [29]. One study identified depositions of PS-MPs in microglial cells (brain-specific macrophages) after oral supplementation, causing altered gene expression, inflammation, and apoptosis in the surrounding murine and human brain tissue [30]. Presumably, it is not the inert MPs or NPs themselves that cause the adverse health effects, but rather the toxins and heavy metals that they can harbour. The large surface area together with the increased affinity for organic pollutants and heavy metals make MPs and NPs ideal vectors for the efficient transport and release of toxins into sensitive tissue/cells [31]. Unsurprisingly, the uptake of MPs and their effects are not exclusive to multicellular organisms and can have a significant impact on single-celled microbes too. Yet, recent discoveries also point out the possible positive effects MPs can have on microbial growth and survival.

1.3 Research Question and Approach

This literature review reports on the relationship between MPs and microbes while interrogating existing and potential future advantages of MPs for microbes and the environment they reside in. First, the noxious impact and associated molecular mechanisms of MPs will be addressed, which will further emphasise the harm that widespread introduction of MPs can cause and the urgent need for a solution. Next, an unconventional perspective will be provided by exploring MPs as potential tools for microbes to aid in their protection, carbon and nutrient uptake, and gene transfer. Finally, established enzymatic and oxidative degradation pathways will be highlighted while contemplating prospective novel (hypothetic) pathways and enzymes as a potential solution to decontaminate the planet.

2. Microplastics as Toxins

2.1 Adsorption Mechanisms of Toxins in Microplastics

MPs have been described to contain up to a million times the concentration of toxic compounds compared to their environment [9]. These compounds include heavy metals (e.g., Pb, Hg, Cd, As), persistent organic pollutants (POPs, often synthetic chlorinated compounds), and naturally occurring toxic secondary metabolites. The MP's high affinity for these compounds is the result of a combination of factors (environmental salinity, pH, temperature, etc.) but is predominantly dictated by three intrinsic properties. First, their extraordinarily high surface area [32]. As a law of nature, the relative surface area of an object is inversely correlated to its size/volume, meaning that the fragmented MPs originating from a larger plastic object retain a far greater surface area and thus an increased sorption capacity. The introduction of cracks and ensuing increase in porosity further raises the surface area which can reach a value as high as 4.37 m^2/g [32], [33]. Furthermore, the adsorption capacity of MPs is exacerbated through UV radiation exposure that promotes a more reactive surface. Indeed, sun-mediated photo-oxidation was found to induce the formation of reactive ketone and carboxyl groups on the surface of MPs, creating polar regions [34]. Holmes *et al*. showed that significantly more (toxic) trace metal ions were able to adhere to UV-bleached MPs by the presence of these newly-formed polar regions which were absent in virgin (unbleached) MPs [35], [36].

The second intrinsic property is the MP's hydrophobicity. Typically, synthetic POPs have a very low affinity for water which, depending on their density, causes them to either accumulate in the sediment or ocean surface microlayer (a subsurface layer between the ocean water and atmosphere) [37], [38]. Because MPs are also hydrophobic and tend to concentrate in these same aquatic regions, a natural affinity exists where MPs are approximately one hundred times better at sorbing POPs compared to naturally occurring compounds [39]. Furthermore, Pestana *et al*. presented for the first time that toxic hydrophobic microcystins (secondary metabolites) from aquatic cyanobacteria are able to accumulate up to 40-fold in MPs compared to the environment which, in a worst-case scenario, constitutes a lethal concentration when internalised by daphnids or smaller organisms [40]. Normally, if a compound (like a MP) is very hydrophobic, this is characterised by its non-polarity. However, because MPs are in the seemingly unique position of retaining a hydrophobic core and a polar surface, they are able to sorb hydrophobic POPs and secondary metabolites as well as charged heavy metal ions, thus increasing their toxicity.

Finally, the type of MP has a significant impact on their sorption capacity. The degree of polymer crystallinity, which is associated with the molecular chain arrangement of the polymer, is one of the most important features that determines how long toxins are retained [38]. PE, PP and PET are semi-crystalline, meaning that they are composed of both regular (crystalline/glassy) and non-regular (amorphous/rubbery) structures which dramatically enhances their toxicity [38]. The flexible amorphous regions exhibit a larger amount of free volume between polymer subunits, allowing for rapid absorption of toxins [38], [41]. Nevertheless, because absorption is a non-competitive and reversible process, amorphous regions perform poorly in retaining the captured toxins [38]. However, their relatively short-lived accumulation of toxins promotes intra-MP diffusion to the glassy regions that contain little void space but present higher cohesive forces where toxins are able to adsorb (usually through hydrophobic or electrostatic interactions) [38], [42]. The strong adsorption interaction allows the toxins to be retained for an extended period of time and is responsible for the slow but long-lasting release when consumed/internalised by organisms [43]. The described mechanisms for the concentration of toxins in MPs predominantly occur in aquatic environments due to the ability of MPs and toxins to flow (and thus interact) freely but also take place in the terrestrial environment in a slower fashion [38]. The combination of increased MP quantity in the environment together with a

growing heavy metal [44], POP [45] and secondary metabolite concentration [40] highlights MP's current and future toxic potential.

2.2 Microplastic-Mediated Toxicity in Microbes

The global prevalence of MPs and NPs in the environment have dramatically increased their bioaccumulation in all living organisms. While the gradual increase of intracellular plastic fragments has been recorded in both prokaryotes as well as eukaryotes, research has primarily focussed on elucidating mechanisms of MP/NP-mediated toxicity in eukaryotic organisms. Scientists found that, as a rule of thumb, the smaller the plastic fragment, the more severe its toxic effect on micro-organisms [46]. This can be attributed to small MPs/NPs crossing the cell membrane more easily and becoming internalised where the adhered toxins can directly interact with proteins, DNA, cell membrane and organelles [46]. For heterotrophic microbes like zooplankton, internalisation mainly occurs through ingestion [47]. Despite having no nutritional value, the abundance of ingested MPs/NPs remains high in these organisms because they are unable to discriminate between plastic particles and phytoplankton (their main food source) when engaging in filter/suspension feeding [48], [49]. In autotrophic microbes, the internalisation of small MPs/NPs occurs through both energy-dependent and independent mechanisms, which both pose distinct toxicity patterns to the cell. Energy-independent passive diffusion into the cell is heavily influenced by the size and charge of the MP/NP [46]. Liu *et al.* showed that PS-MPs of 5 µm were incapable of entering the cell due to their large diameter and weak Brownian motion, but that fragments of 500 and 50 nm were able to be internalised (Figure 1) [50]. Similarly, UV-exposed charged MPs/NPs of any size were unable to cross the negatively charged phospholipid bilayer [46]. Passive diffusion of MPs/NPs can be particularly harmful to the cell because MPs/NPs are directly deposited into the cytosol [46]. Due to the change in abiotic factors (pH, osmotic pressure, etc.), the adhered toxins can exhibit decreased affinity for the plastic particles and start to leach into the intracellular environment where they interact with cellular components [51]. Studies show that intracellular MPs/NPs increase the abundance of reactive oxygen species [52], [53] which have been found to depolarise the mitochondrial cell membrane [54] and damage the DNA [55].

Energy-dependent endocytosis of MPs/NPs is enabled by phago/pinocytosis and receptor-mediated cytosis (Figure 1). The former describes the non-selective bulk endocytosis of solid particles (phagocytosis) or in-fluid suspended/dissolved particles (pinocytosis). Using this mechanism, the MPs/NPs are enclosed in endosomes without any direct interaction with the cell membrane. Alternatively, recent studies suggest that NPs are able to interact with membrane receptors responsible for the formation of clatherin and caveolin-coated endosomal vesicles [56]. The functionality of these mechanisms to internalise NPs has been observed in both single-celled eukaryotes and zooplankton but has been reported to exclusively target particles with a size <120 nm, meaning that they can only facilitate the internalisation of NPs [48], [57]. Following endocytosis, the caveolar-coated vesicles transport the enclosed material to the endoplasmic reticulum (ER) or Golgi [58] where their exact toxic effect remains unknown, while the clatherin-coated endosomes are fused to lysosomes in an attempt to extract nutrients and neutralise potentially harmful compounds [56], [58]. However, because the MPs/NPs and most associated toxins (POPs and metal ions but not secondary metabolites) are resistant to (nearly all) lysosome-orchestrated hydrolytic degradation, their toxicity remains [50]. In fact, studies show that, depending on the concentration, MP/NP-adhered toxins are able to decrease the lysosomal membrane integrity, which can cause the lysosomes to burst and spill their digestive enzymes into the cytosol, leading to cellular damage and even cell death [50]. Fluorescent imaging of the intracellular MPs/NPs indicates that the majority of plastic particles reside in lysosomal bodies, suggesting that active transport constitutes the main pathway of entry [50]. Removal of the MPs/NPs occurs via energy-dependent lysosomal exocytosis (Figure 1) [50].

While MPs larger than 5 µm appear to be unable to enter single-celled organisms, they can still provoke adverse effects. Indeed, larger positively charged MPs are able to attach to the cell membrane/wall through electrostatic interactions where close proximity to the high concentrations of adhered toxins still harms the cell, albeit to a lesser degree than MP/NP internalisation would [59]. Furthermore, MP attachment reveals a new kind of toxicity, which is specific to phytoplankton. Attachment of larger MPs to the cell wall of phytoplankton has been shown to negatively affect growth and metabolism of these organisms, which is particularly worrisome as phytoplankton arguably comprise the most essential organisms in the aquatic ecosystem as primary food source [46]. Early evidence suggests that their hampered development is a result of a decrease in their photosynthetic efficiency. Lagarde *et al.* revealed that PP-MP attachment had a significant effect on the expression of genes related to chloroplast formation, oxidative stress and extracellular polysaccharide (EPS) biosynthesis in the microalgae *Chlamydomas reinhardtii* [46], [60]. In another example, Wu *et al.* identified a 55% decrease in chlorophyll-*a* concentration in the algae *Chlorella pyrenoidosa* when exposed to PVC and PP-MPs [61]. Most likely, the adsorption of MPs on the cell walls of phytoplankton causes a shading effect that prevents light from reaching the chloroplasts, leading to the observed transcriptional changes and subsequent decrease in chlorophyll production (Figure 1) [46]. Surprisingly, the affected organisms appear to combat the adverse shading effect that the MPs elicit by upregulating their EPS production in an attempt to prevent electrostatic MP attachment through the creation of a non-charged barrier [60], [62]–[65]. This notion is supported by the observation that diatoms (a type of phytoplankton) showed increased susceptibility to PS-MPs during their logarithmic phase, which is associated with low EPS production, as opposed to their stationary phase where EPS production was high [66]. The increase in EPS production following MP/NP exposure is not exclusive to phytoplankton and has been observed in many species of microbes. Remarkably, the formation of an EPS-based biofilm also allows for the colonisation of MPs, which provides the microbes with a range of potential benefits and reveals a new type of mini ecosystem.

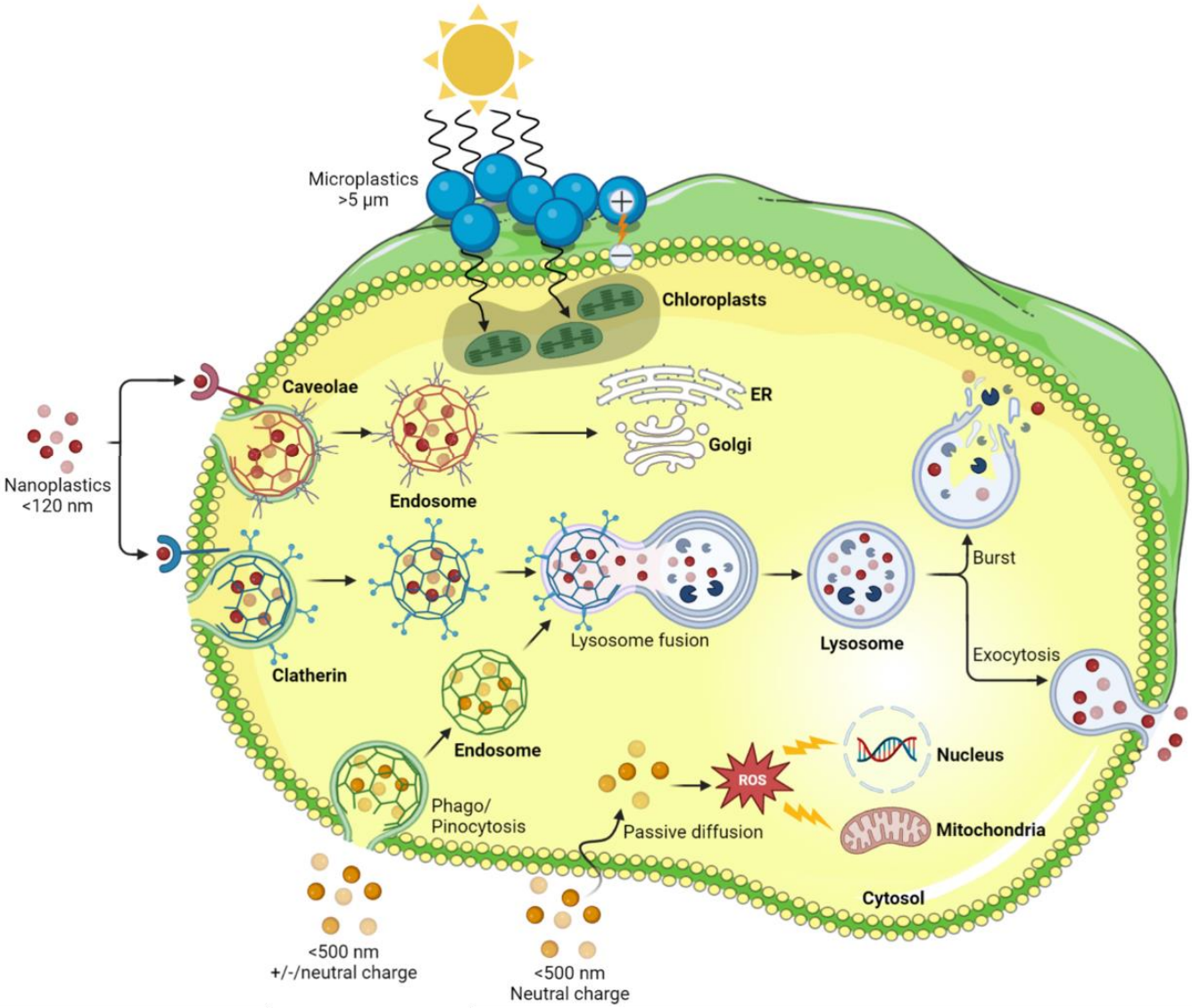

Figure 1. Micro/Nanoplastics interactions and transport. Nanoplastics (<120 nm) enter the cell through energy-dependent caveolae or clatherin coated vesicles following membrane receptor interaction. Caveolae coated endosomes are transported to the endoplasmic reticulum (ER) and Golgi apparatus. Clatherin coated endosomes fuse to lysosomes after which they burst the lysosome or undergo exocytosis. Nanoplastics (<500 nm) undergo phago/pinocytosis irrespective of charge and fuse to a lysosome. Uncharged polymer particles (<500 nm) enter the cell through passive diffusion which generates reactive oxygen species (ROS) that damage the DNA and depolarise the mitochondrial cell membrane. Positively charged microplastics (>5 µm) attach to the cell membrane/wall through electrostatic interaction where they cause a shading effect which decreases the photosynthetic efficiency in the chloroplasts. Cellular components are represented in bold. *Created with Biorender*

3. Microplastics as Microbial Tools

The ability to adapt to a changing environment is a defining feature of all living things. To this end, despite their widespread negative effects on biota, microbes have found ways of exploiting MPs to benefit their survival. The observed microbial colonisation of MP particles is not unexpected as these microbes appear to experience a greater protection from the environment and a higher frequency of horizontal gene transfer (HGT) while also enjoying an increased abundance of nutrients.

3.1 The Plastisphere

Recent studies show that MPs provide an emergent microhabitat niche for microbes [67], [68]. Recently coined by Zettler *et al.*, this so-called 'plastisphere' is able to support a large and diverse microbial community through the development of a biofilm in conjunction with a MP particle [69]. At first glance, it might appear that the plastisphere is nothing more than a MP that serves as colonisation landing pad. However, the plastisphere is comprised of multiple organic layers that facilitate its complex environment. In addition to the aforementioned toxins, the aquatic ecosystem contains a broad range of non-toxic natural organic matter (NOM) that is able to interact with MPs [70]. These heterogeneous compounds include an assembly of organically produced biomolecules (proteins, lipids, polysaccharides, and nucleic acids), which vary widely in molecular weight and composition. The diluted EPS floating in the environment that is produced in response to stress or to accommodate a more hospitable habitat constitutes the largest part of the NOM and accounts for a significant fraction of the total organic macromolecules found in the oceans [71], [72]. As a result, the NOM is able to encapsulate a MP particle through electrostatic/hydrophobic interactions, which forms the first layer of the plastisphere known as the eco-corona (Figure 2) [72], [73]. The strong affinity between the MP and NOM gives rise to the 'hard' eco-corona, which is characterised by the macromolecule's tight adsorption, slow exchange rate and long retention time [72]. Depending on the abundance and composition of NOM in the environment, the hard eco-corona can significantly alter the MP particle's chemical affinity and typically increases its ability to interact with its surroundings. Consequently, an expanded layer of fast exchanging and loosely attached NOM known as the 'soft' eco-corona is formed on top of the hard eco-corona (Figure 2) [72]. The NOM-to-NOM interactions in this layer can lead to the aggregation of biomolecules, which act like a net that can capture food particles, while also drastically increasing the probability of cell adherence to the soft eco-corona [72]. The increase in nutrient and cell concentration promotes the rapid colonisation of the MP particle and initiates the formation of an EPS-based biofilm, which constitutes the bio-corona, the final layer in the plastisphere (Figure 2) [72], [74]. Surprisingly, studies show that the biofilm that is formed on MPs retains a distinct composition that differs from biofilm created on other substrates. Indeed, Ledeboer *et al.* observed an increase in cellulose and O-antigens in the biofilm of *Salmonella enterica* when incubated in the presence of different types of MPs [75], [76]. Additionally, knocking out of the putative cellulose biosynthetic gene, yhjN, disrupted biofilm formation and decreased attachment to MPs compared to other organic substrates [75], [76]. Similar changes in biofilm composition have been observed in other prokaryotic organisms, which suggests that bacteria are able to specifically sense MPs and adjust their biofilm make-up to actively facilitate attachment [74]. It is important to note, however, that laboratory studies that focus on a single species do not represent naturally occurring biofilms, which typically harbour a diverse and dynamic microbial consortium. In their study, Seeley *et al.* highlighted these dynamics when they showed that the phyla *Gammaproteobacteria* and *Deltaproteobacteria* are typical early colonisers (first 24 h) of MPs after eco-corona formation, followed by *Bacteriodetes* which, after 24h, gradually claim an increasingly larger fraction of colonised PVC and PE-MPs [77], [78]. While the type of MP and its (acquired) properties as well as the arrangement of the attached NOM do play a

role in determining the taxonomic composition of the bio-corona, recent *in situ* studies point to spatial (geographical location) and temporal (seasonal changes) factors as main predictors of MP colonisers [79]–[83]. To illustrate the density of MP-residing microbes, it is estimated that on average, 1 gram of MP particles contains the equivalent microbial biomass of 10,000 litres of open ocean water [68], which prompts the question: why do microbes actively favour colonisation of MPs and what are the benefits that outweigh the known toxic effects?

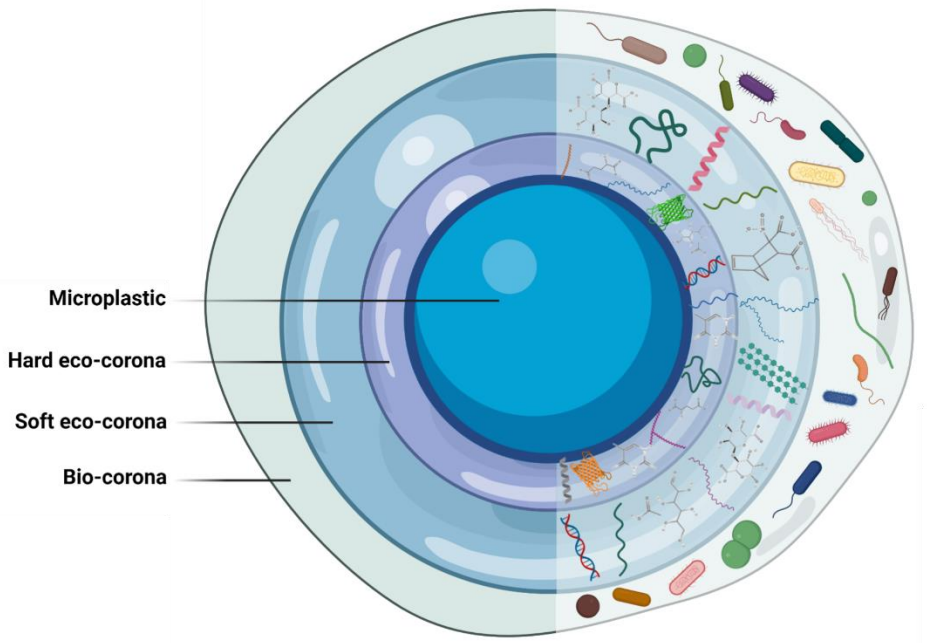

Figure 2. The plastisphere. The plastisphere consists of three layers that encapsulate a microplastic particle. The first layer is comprised of the hard eco-corona which contains tightly adhered natural organic matter (NOM). The soft eco-corona is made of loosely attached NOM bound to the hard eco-corona. Finally, the bio-corona consists of an extracellular polysaccharide-based biofilm that holds a wide variety of microbial species. *Created with Biorender*

3.2 Environmental and Chemical Protection

While the research on this domain is still in its infant stage, recent evidence suggests that bacteria that have formed a biofilm on the surface of different types of MPs are significantly more resistant to the damaging effects of UV radiation compared to free-flowing bacteria [84]. Researchers from different studies propose two explanations for this effect. First, the shielding effect of the biofilm. The microbial utilisation of a biofilm dates back approximately to 3.4 billion years when the earth was exposed to much higher levels of solar radiation caused by a lower abundance of ozone in the atmosphere [85], [86]. In the same time period (~3.5 billion years ago), a primitive form of non-oxygenic photosynthesis evolved [87]. It is thought that the organisms that required visible light to engage in this kind of photosynthesis used their biofilm as primary protection against the harmful short-wavelength solar radiation. Fossil records reveal that the 3D-structure of the biofilm, and thus their photo-protective properties, have been largely preserved to this day [85]. This provides microbes embedded in MP-adhered biofilms increased protection from the damage caused by UV light. Furthermore, the biofilm shields the eco-corona, which prevents degradation and subsequent detachment of the NOM, thereby preserving the plastisphere. Adding to that, the photoprotective ability of a biofilm was highlighted in a recent study that focussed on the UV-mediated decontamination process in wastewater treatment plants and found that MP-associated biofilm was able to block 95% of UV light [88]. Besides UV light, the biofilm also appeared to enable an elevated tolerance to chlorine exposure. Schwering *et al*. found that while non-pathogenic microbes without biofilm were killed at 5 mg/l chlorine (the global water

decontamination standard), non-pathogenic microbes in a biofilm required up to 40-fold more chlorine to be killed [89]. Interestingly, they found that multi-species biofilms were significantly more resistant to chlorine exposure, requiring up to 300-fold more chlorine compared to single-species biofilms [89]. Even though the exact cause for this strong change in chlorine tolerance remains elusive, the researchers propose the distinct 3D-structure and increased diameter of the multi-species biofilm as contributing factors. Furthermore, this suggests that the diverse microbial community on MP particles might not solely be the result of competition but could also arise from a symbiotic mutualism.

Another reason why MP-adhered microbes experience reduced UV damage is biofilm-induced sinking. Individual MPs are less dense than water, which causes them to float. However, the colonisation of a polymer particle and subsequent biofilm formation is able to increase the density of the MP and cause it to sink. Fazey *et al.* found that after 66 days, nearly all buoyant MPs had sunk to the bottom due to the formation of a biofilm [90]. The translocation from the aquatic euphotic (sunlight) zone to the sediment presents several benefits to the microbes residing on the MP particle. Primarily, they are no longer exposed to UV radiation, while the sediment typically provides elevated concentrations of nutrients and minerals that enhance microbial growth [83]. Additionally, it has been proposed that the perpetual sinking and resurfacing (when the biofilm eventually degrades) of MPs has given rise to a completely new and potentially beneficial cycle. Early evidence suggests that there exists a link between the sinking of nutrient-rich MP-associated biofilm, which rains down as so-called marine snow, and the boost in microbial growth in the deep ocean [91]. The current notion holds that the increased abundance in downward flowing concentrated organic matter can be easily ingested by zooplankton that excrete nutritious faecal pellets which serve as microbial food source. The ensuing enhanced microbial growth at these depths emphasises that the wide-spread introduction of MPs has the potential to globally alter ecological cycles in yet unforeseen ways. [91].

3.3 Increased Carbon Abundance

The aquatic dissolved organic carbon (DOC) reservoir is one of the largest reduced carbon pools on the planet, roughly equal in size to the atmospheric CO_2 pool [92], and accounts for 90% of the naturally occurring marine carbon [93]–[95]. In the aquatic environment, microbes use DOC as their main source of carbon. Recent studies identified a correlation between marine plastic pollution and an increase in DOC in the ocean surface microlayer [91], [96], [97]. Romera-Castillo *et al*. showed that a significant quantity of DOC was leaching directly from MPs as a result of photo-dissolution. Indeed, UV-mediated oxidation of the MP hydrocarbon polymer causes small (micron-scale) carbon-chain fragments to break off and dissolve in the water where they are readily taken up and metabolised by microbes [96], [97]. Surprisingly, microbial growth in the presence of different types of MPs was lower when exposed to artificial light, which is when DOC concentration increased as a result of photo-dissolution, than in the dark. The researchers attributed this to the concomitant release of oxygen radicals when MPs underwent photo-dissolution, which temporarily inhibited microbial growth [96]. It is estimated that MPs annually contribute approximately 236,000 metric tons of dissolved bioavailable DOC to the ambient oceanwater. Moreover, in heavily plastic-polluted areas, the plastic-derived DOC content in the ocean surface microlayer can constitute up to 10% of the total DOC [96], [97]. With the exponentially increasing flux of plastics entering the oceans, DOC leaching will likely play an expanding role in the carbon cycling and microbial growth.

In addition to carbon, MP exposure to sunlight also prompted the release of methane, ethylene, and other hydrocarbon gasses [98]. While not much is known about this, bacteria and archaea have been reported to use MP-produced methane as electron donor. In an aerobic environment, they can utilise methane monooxygenase to reduce NAD(P)H [99], while in anaerobic conditions some archaeal species are able to use reverse methanogenesis to oxidise methane and facilitate respiration [100]. Although never directly observed, it is conceivable that some microbial species are also able to use the

MP-adhered metal ions like iron or non-metals like sulphur as electron donor/acceptors [46], [101]. These recent findings, in combination with the drafted hypotheses, encourage further investigation as to the exact role of MPs and their adhered compounds in cellular respiration.

3.4 Horizontal Gene Transfer

Antimicrobial compounds find their way into the aquatic ecosystem through various routes including (treated) wastewater, surface and agricultural runoff, and the increased usage of antibiotics in aquaculture [102]–[105]. Resistance to these compounds can be enabled through the exchange of genetic material between microbes through horizontal gene transfer (HGT) [102]–[105]. Transmissible genetic material such as plasmids, transposons, integrons and even bacteriophages allow phylogenetically-diverse organisms that are in close proximity to one another to transfer resistance genes [103]. While the formation of a biofilm itself can protect organisms from antimicrobial compounds, it also facilitates the transfer of antimicrobial resistance genes (ARGs). Because MP-associated biofilms serve as microbial hotspots, they have been found to provide an ideal environment for HGT [106]. In fact, it has been observed that biofilm aggregates can capture free-flowing ARGs that are liberated when cells die. Indeed, studies show that wastewater effluent contains a significant increase in ARGs that are released during the decontamination process, while also harbouring an elevated abundance of MPs through the mechanical stress that is exerted on larger plastic fragments [107]. This combination generates a breeding ground for the propagation of multi-resistant 'super-bugs' in the area where treated wastewater meets the ocean [105]. MPs are recognised as emerging reservoirs of multidrug, multi-metal, sulfonamide, and aminoglycoside-resistance genes, which have been found up to 3 orders of magnitude higher on MPs than in the surrounding water [106], [108]–[110].

Furthermore, in addition to acquiring ARGs from their surroundings, MPs also appear to facilitate the development of ARGs through the ad/absorption of antibiotics [111]. Driven by the long-term but low concentration exposure to antibiotics, the MP-adhered microbes are able to acquire ARGs through selective pressure [111]. Usually, spontaneous beneficial mutations that give rise to the formation of ARGs reside in the non-motile genome (chromosomes), meaning that they can only be shared through vertical gene transfer [112]. However, sections of chromosome-embedded DNA have been reported to jump to genetically motile elements (plasmids, transposons, etc.) and engage in HGT [113]. If MPs continue to accumulate in the oceans at the predicted rate, they may severely influence the ecology of aquatic microbial communities and further exacerbate the development and frequency of antimicrobial resistance [114].

It is clear that microbes can use MPs to increase their odds of survival. What's more, some microorganisms have been found to degrade MPs and by doing so, inadvertently contribute to a more circular environment in the context of plastic pollution.

4. Microbes as Solution

Microbes have played a crucial role in the degradation of naturally occurring polymers for millions of years. Marked by a sudden decrease in coal depositions in the earth, it is estimated that approximately 300 million years ago, white rot fungi were the first organisms to evolve the ability to degrade the plant-derived polymers lignin and cellulose [115]. Since then, microbes from various domains have created enzymes to degrade other durable naturally occurring polymers like chitin, rubber, pectin, and even crude fossil oil. While synthetic plastics have only been introduced into the biosphere for a few decades, already, some microbial species have discovered ways of catalysing their degradation.

4.1 Microplastic Degradation

Realisation that MPs constitute a growing environmental problem has prompted the development of new ideas to prevent further contamination. For example, the use of innovative membrane filters in wastewater treatment plants has been proposed. These filters are highly effective at removing MPs from effluent, with some capable of capturing up to 99% of these particles [116]. In another example, photo-activated micromotors (small Au–Ni–TiO_2 particles that adhere to the MPs under light excitation) cause the agglomeration and subsequent easy filtering of MPs [117]. Remarkably, even the intentional ingestion by clams in large water basins that deposit the MPs in their shells has been proposed as a method to capture the plastic particles before they are released into the environment [118]. However, these strategies are often costly, and time-consuming and can only be implemented in advanced wastewater treatment plants, making them unfeasible solutions in most parts of the world [119]. Moreover, these strategies only prevent the release of new MPs and do not address the issue of already-released MPs that are present in the oceans and soil. As a result, researchers are turning to microbes as a potential large-scale and cost-effective solution for removing MPs from the environment. Indeed, since the turn of the century, it has been well-established that microbes can significantly accelerate the degradation of plastics, with some studies showing that the half-life of these materials can be reduced from millions of years to just decades or centuries in the presence of microbial activity [7]. The studied microbe-mediated degradation processes can be divided into three stages: biodeterioration/depolymerisation, assimilation, and mineralisation.

4.1.1 Biodeterioration/Depolymerisation

The biodeterioration of a MP particle starts with the establishment of a microbially-rich biofilm on the surface of the MP (Chapter 3.1) [120]. Biodeterioration is facilitated by the constant expansion and contraction of the MP in response to changing abiotic factors such as temperature, salinity, and mechanical stress [120], [121]. This, in combination with the invasiveness of the EPS, results in the formation of a biofilm that is able to penetrate the surface and exacerbate the formation of cracks within the MP [70]. Due to the continued growth of the EPS within these new-formed cracks, this process can become self-amplifying and facilitate the colonisation of the MP particle's inner layers and expose more of the synthetic polymer to potential microbial depolymerisation [70]. The mechanism of plastic depolymerisation, or the breakdown of a polymer into smaller molecules by microbially produced exoenzymes, is highly dependent on the MP's ability to be hydrolysed [122]. In order to function, enzyme-facilitated hydrolysis (e.g. lipases, cutinases, esterases, etc.) requires cleavable groups such as ester, carboxyl, or amide bonds. Because PET and polyamide plastics contain such molecular structural elements in their backbone, hydrolase-producing microbes are able to cleave these into lower molecular weight assimilable oligomers or monomers [122]. Moreover, it has been suggested that the same enzymes that are used to depolymerise cellulose are also responsible for the depolymerisation of these hydrolysable synthetic polymers [122], [123]. Nevertheless, in contrast to naturally occurring polymers, synthetic plastics retain a far higher crystallinity that prevents access to

the cleavable molecular groups and results in a decreased degradation rate in the order of years to decades [122]. Alternatively, plastics such as PE and PP contain a C-C backbone that lacks any ester, amide or other molecular functional groups and is therefore seemingly nonhydrolyzable [122], [124]. As a result, these plastics cannot be depolymerised through enzyme-facilitated hydrolysis alone and require an additional step to cleave these materials through oxidative reactions. Kang *et al*. were the first to identify laccase, a copper-containing oxidase enzyme involved in wood degradation, to enable the breakdown of PE by oxidising its amorphous regions [125]. Scanning electron microscopy revealed that incubation of PE-MPs together with isolated laccase induced the formation of cracks and reactive carbonyl groups on the surface of the MP polymer [125]. In another study, Ghatge *et al*. found manganese peroxidase from *Phanerochaete chrysosporium* to decrease the molecular weight of PE in the presence of hydrogen peroxide, indicating depolymerisation [126]. These groups of enzymes work by introducing oxygen atoms into the polymeric backbone, creating oxygenated products such as aldehydes or carboxylic acids. Consequently, these groups become susceptible to hydrolysis (by hydrolases), which enables further depolymerisation. Using a different molecular degradation approach, brown-rot fungi have been shown to excrete hydroxyl radicals that are produced through the intracellular redox cycling of Fe(III)-reducing oxidants [124]. Due to the highly reactive nature of these radicals, they indiscriminately attack the molecular structure of the synthetic polymers, causing non-enzymatic oxidation and generating hydrolysable groups [124]. Together, these processes represent known pathways for the depolymerisation of non-hydrolysable synthetic polymers.

Microbial species within the MP-associated biofilm evidently play different roles in the depolymerisation process by producing a variety of exoenzymes (hydrolases, oxidases, and peroxidases) and other complementary compounds (such as hydrogen peroxide and hydroxyl radicals). Furthermore, the affinity of MPs for trace metals like copper, manganese and iron can increase the bioavailability of these elements in the biofilm and enhance the function of degrading enzymes by acting as cofactors. This highlights the complex and multifaceted nature of the depolymerisation process, with different microbes and enzymes working together to break down the synthetic polymer.

4.1.2 Assimilation

On average, synthetic plastic monomers are 0.1-1 nm in diameter [127]. As described in Chapter 2.2, only particles with a size <500 nm are able to be assimilated by microorganisms. This means that in order for polymers to enter the cell through phago/pinocytosis or passive diffusion, they must first be (enzymatically) reduced to oligomers containing a maximum of 500-5000 monomer units (Figure 1). The reason why microbes expend energy to internalise these NPs is that, in addition to serving as a supply of carbon, they may potentially be used as an energy source. In fact, the maximum usable energy from the oxidation of PE is roughly equivalent to that of glucose, with PE yielding 425 kJ per mole of oxygen and glucose yielding 479 kJ [122]. Following assimilation, the oligomers are further metabolised intracellularly in an effort to mineralise them into completely oxidized metabolites.

4.1.3 Mineralisation

The newly identified bacterial species *Ideonella sakaiensis* serves as an ideal example of how microorganisms can adapt to a plastic-contaminated environment. Isolated from a Japanese PET recycling facility, *I. sakaiensis* evolved to efficiently degrade PET and its constituents as a source of energy and carbon [128]. While other microbial species have also been found to degrade PET with known (non-substrate specific) hydrolases [129], Yoshida *et al*. were the first to observe that *I. sakaiensis* was able to produce a novel enzyme that was able to depolymerise PET plastic at unprecedented speed [128]. Indeed, this so-called 'PETase' was able to degrade PET film at a rate of 8.2 mg per $\mu mol \cdot L^{-1}$ per day (under optimal conditions), which means it can break down a standard PET bottle in a just few months [130]. Subsequent research ascribed the enzyme's ability to rapidly

depolymerase PET to the occurrence of two disulphide bridges whereas homologous enzymes have only one. It is thought that this modification changes the flexibility of the active site and increases the enzyme's affinity for PET [131], [132]. These results placed PETase at the centre of research and caused the microbial PET degradation pathway to be the first (and currently only) to be fully elucidated. The degradation of PET-MPs starts when extracellular PETase cleaves the PET polymer into its subunit, mono(2-hydroxyethyl) terephthalic acid (MHET) (Figure 3). Despite their similar structure, PETase is unable to further hydrolyse MHET. To utilise the potential energy and carbon stored in MHET, *I. sakaiensis* produces the exoenzyme MHETase, which catalysis the formation of terephthalic acid (TPA) and ethylene glycol (EG). Following the assimilation of these compounds, EG is incorporated into the citric acid cycle through its conversion to acetate by acetyl CoA. Finally, TPA enters the β-ketoadipate pathway where it first undergoes the conversion to protocatechuate before it is transformed into various aromatic molecules [128], [131], [133], [134]. Ultimately, the products of both the citric acid cycle and β-ketoadipate will be excreted as H_2O, CO_2, N_2 or CH_4, which concludes the mineralisation of the PET polymer. The current literature does propose degradation/mineralisation mechanisms for PVC and PS in other species that emphasises the role of serine hydrolase as major player in their depolymerisation and subsequent uptake in various pathways, however, these studies are based on predictions and have not been verified through wet-lab experiments [134]–[136].

Currently, it appears that microbes are slowly developing ways to benefit from MPs and in doing so, contribute to decontaminating the environment and making synthetic polymers part of the circular

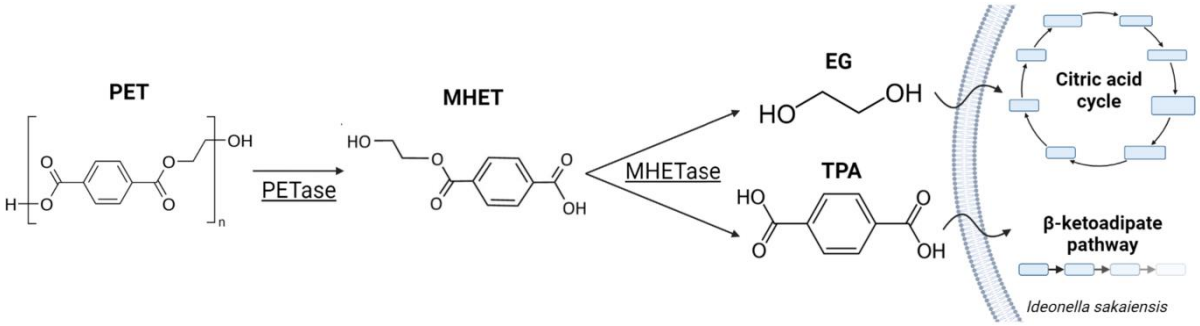

Figure 3. PET degradation by *Ideonella sakaiensis*. Polyethylene terephthalate (PET) is converted to mono(2-hydroxyethyl) terephthalic acid (MHET) by the enzyme PETase. MHETase transforms MHET into ethylene glycol (EG) and terephthalic acid (TPA) which are assimilated and incorporated into the citric acid cycle and β-ketoadipate pathway, respectively. Enzymes are underlined. *Created with BioRender.*

process. Nevertheless, the increasing rate of plastic pollution in combination with the slow evolution of degradation pathways calls for yet unexplored ways to accelerate the breakdown of MPs.

5. Discussion and conclusion

The ubiquity of MPs in the environment is having an increasingly negative impact on all living organisms. MPs have been found to bioaccumulate in the lungs/gills, tissues, and gastrointestinal tract of both terrestrial and aquatic animals, leading to a variety of health issues including digestive problems, immune system dysfunction, and hormonal imbalance. It is believed that the MP's affinity for toxins such as heavy metals, organic pollutants, and secondary metabolites as a result of its intrinsic properties and natural weathering, is the main cause of the MP's harmful effects on biota. While microbes are not excluded from the toxic potential that MPs harbour, their exploitation of MPs as platforms for protection, gene transfer and carbon/energy reserves is gradually recognised and constitutes an emerging field of research. Furthermore, the recently observed microbe-mediated degradation of MPs has prompted researchers to explore the potential of employing these microorganisms as effective strategies for reducing the abundance of MPs in the environment.

At the current rate, biological degradation is unable to counteract the growing environmental pollution with plastic materials. Even though several microbial species display (novel) enzymes that allow for the complete breakdown of synthetic polymers, this process remains too slow. If left to nature, it could take centuries before evolution catches up and generates efficient degradation pathways that match the present speed of MP emissions. Therefore, it is paramount that existing yet undiscovered MP-degrading microbes and their enzymes/pathways are exposed and subjected to biotechnological advancements.
As described in Chapter 4.1.1, the complete degradation of MPs often relies on a varied consortium of microbes that each occupy a different role in the breakdown process. To this end, further research into the development of a tailored and optimised composition of biofilm-associated microbes that promote rapid MP degradation is warranted. Depending on the potential observed increase in degradation speed, an optimised microbial consortium could be employed in wastewater treatment facilities to limit the abundance of MPs in the effluent. Implementation of such a strategy would be straightforward as all treatment plants already utilise activated microbial sludge as part of the standard water decontamination process. In practise, this would mean that a relatively simple modification to the existing activated microbial sludge composition that favours MP degradation would have to be established. Despite the average water decontamination process taking only 24h, the perpetual exposure to high concentrations of MPs in combination with ensured optimal conditions would likely elicit a high production of MP-degrading enzymes (or other degrading compounds). Moreover, even if this approach would decrease the number of MPs in the effluent by just 1%, this would still translate to an estimated reduction of 5,200 tonnes of annually released MPs in Europe alone [18]. One potential downside of this approach is that the increased biofilm formation may interfere with UV and chlorine-mediated microbial decontamination of wastewater, as described in Chapter 3.2. If applied on a global scale, this strategy would function as a cheap and reusable method to significantly reduce MP emissions. However, this approach would not aid in the clean-up of already released MPs which requires the exploration of different microbe-based solutions.

At the time of writing, apart from *I. sakaiensis*-derived PET degradation, comparatively few studies have been performed on uncovering other plastic-degrading microbes and their metabolic pathways involved in the mineralisation of MPs. By creating a better understanding of how these synthetic polymers are metabolised, new pathways may become available for biotechnological exploitation and employment for environmental MP decontamination. For this purpose, carbon isotopes can be used to trace the movement of plastic-derived carbon within microbial species that display polymer mineralisation. This approach provides two benefits. First, by taking an (e.g.) ocean water sample

supplemented with MPs that are manufactured with C^{14} in their backbone, microbial species that have assimilated these (depolymerised) plastics can be easily identified when checked for their radioactive signature. Previous studies have attempted to screen for MP-depolymerising microbes by simply observing a polymer's weight loss in the presence of specific microbial species, and report promisingly high degradation rates in relatively brief time frames [46]. However, this approach can paint a misleading picture because only measuring weight loss makes it impossible to discriminate between the degradation of polymers or their additives. Indeed, many commonly used plastics contain additives to modify their properties (flexibility, colour, strength, etc.), which are often easier to break down and mineralise, leading to a reduced polymer weight. Using C^{14} labelled MP polymers together with unlabelled additives would create a highly sensitive and high-throughput screening test for candidate species capable of MP-degradation, something (to the best of my knowledge) no study has attempted yet. Second, C^{14} labelling can provide valuable insights into MP-degradation pathways by reporting what fraction of the assimilated polymer is respired (as labelled CO_2) and what fraction is used for anabolism of carbon-containing compounds. Presently, 9 studies have been conducted using C^{14} to examine microbial mineralisation of MPs, 5 of which were published since 2021 [137]–[145]. This shows that carbon isotope labelling is an emerging and perhaps still underutilised technique for the exploration of MP-degrading microbes and the molecular mineralisation tools they use.

Presumably, in order to tackle the problem of global MP pollution, enzyme engineering is required. When isotope labelling (or similar technique) uncovers novel exoenzymes that allow for the depolymerisation of specific MP polymers, these enzymes can be optimised to increase their speed and robustness. As a prime example, despite the astonishing speed with which PETase can depolymerise PET plastic, the process is still too slow to be economically viable. Even using high concentrations of isolated PETase under optimal conditions, the process requires weeks to return a plastic bottle back into monomers. Furthermore, researchers found that PETase is very heat sensitive and loses activity at 37˚C [146]. A previous study attempted to increase the robustness of the enzyme by utilising a traditional enzyme engineering approach [146]. However, the researchers found a strong trade-off existed between enzyme stability and degradation speed when using rational enzyme engineering. In the end, they only achieved a marginal increase in thermo stability (maximum of 40°C) while maintaining high enzyme activity. Using a different approach, a paper published in Nature this year reported on the creation of FAST-PETase (Functional, Active, Stable and Tolerant PETase) that exhibited a 67-fold increase in enzyme activity at 50°C compared to wild-type PETase at ambient temperatures [147]. A combination of enzyme X-ray diffraction data and machine learning allowed them to construct a model that was able to predict the optimum in PETase robustness (heat, pH, salinity, etc.) and speed. This study not only demonstrated a proof of concept by creating optimised PETase using this approach, but it also opened the door to the optimisation of enzymes for the degradation of plastics other than PET. Furthermore, with the advent of increasingly more powerful and accessible computer algorithms, it is reasonable to assume that enzyme optimisation, or even the invention of entirely new *in silico* predicted enzymes will experience a boost in the coming years and significantly alter the way plastics are recycled. Indeed, the benefit of using isolated optimised depolymerases as opposed to living microbes, is that the liberated monomers can be recovered and used to create virgin plastics, thereby closing the plastic lifecycle loop. Lu *et al.* showed that 94.9% of PET depolymerised with FAST-PETase could be recycled back into consumer-grade plastic using chemical polymerization [147]. Overall, the main bottleneck in this approach appears to be the efficient transport of MPs from the environment (oceans and soil) to recycling facilities. One study proposes using buoyant nanoparticles coated with adhesive protein to facilitate the transport of MPs to the surface for easy mop-up, but the feasibility of this tactic is still unproven and might do more harm than good [148].

Finally, it might be theoretically possible to engineer and globally disperse microbial GMO's that are able to efficiently degrade and mineralise MPs. While this could significantly reduce the number of MPs in the environment, the risk of unanticipated effects by irreversibly releasing GMO's into the biosphere is too great to seriously consider this as a viable solution.

In summary, MPs and the toxins they contain display a clear harmful effect on humans, animals, and microbes, and have become a growing problem as plastic production continues to increase. Microbes belonging to various phyla have been found to use MPs to aid in their survival through the establishment of a MP-adhered biofilm that provides protection, increased carbon and nutrient abundance, and a platform for cross-species horizontal gene transfer. The recently observed microbial depolymerisation, assimilation, and mineralisation of synthetic polymers present microorganisms as candidates for the decontamination of MPs in the biosphere. Because it will take too long before efficient degradation pathways that target different plastic types are naturally evolved, ideas such as biofilm consortium optimisation, utilisation of carbon isotopes for the identification of new depolymerases, and machine-learning-assisted enzyme engineering have to be explored.

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