Biofilms of Microplastics

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Abstract The occurrence of microplastics (MPs) in the terrestrial and marine environment has been gaining global attention. These microparticles carry biofilm

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communities that are distinct from the surrounding environment. MP-colonizing microorganisms are important links for the fate of MPs in different ecosystems. However, the influence of plastic-colonizing microorganisms on the fate of microplastics is largely unknown. Here we review the formation of biofilms and dynamic variation on the surfaces of microplastics together with the main research methodologies for biofilm analysis. The potential impacts of biofilm formation on the environmental fate of microplastics caused by MP-colonizing microorganisms such as weathering processes, vertical transport, sorption and release of contaminants, trophic transfer of MP particles, and potential environmental toxicity of MPs in the marine ecosystem are also reviewed. Future studies are needed on the processes and mechanisms of microplastic and biofilm interactions in the terrestrial environment.

Keywords Biodegradation, Biofilms, Extracellular polymeric substances, Microplastics, Toxicity, Vertical transport, Weathering

1 Introduction

According to the International Union of Pure and Applied Chemistry (IUPAC), biofilms are defined as aggregates of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substances (EPSs) adhere to each other and/or to a surface. Biofilms may form on living or nonliving surfaces and can be prevalent in the marine and terrestrial environments. Due to the large specific surface area of microplastics (MPs), many microorganisms including bacteria, fungi, algae, and protists can easily colonize the surfaces of microplastics in the form of biofilms. The formation and development of biofilms on the surfaces of microplastics may change the morphology and physicochemical properties of MPs in the environment, thus leading to diverse physical, chemical, and biological influences on the environmental fate of MPs such as weathering, vertical transportation, co-migration with chemical pollutants and pathogens, as well as biodegradation. In this chapter the methodologies and processes of biofilm formation and development on the surface of MPs are reviewed, and the different influences of biofilm formation on the properties of MPs are also investigated with the aim of better understanding the fate of MPs in the terrestrial environment.

2 Formation and Development of Biofilms on the Surfaces of Microplastics

2.1 Major Stages of Biofilm Formation

Biofilms are formed by EPS secreted by microorganisms including proteins, glycoproteins, and glycolipids which form a matrix around the microbes and enable them to attach to a variety of different biological and abiotic surfaces. Continuous changes in bacterial colonization of artificial surfaces (such as glass, stainless steel, and polycarbonate sheets) have been confirmed in seawater [1]. Different scholars divide the formation of biofilms into different stages from the core flora and time series.

Biofilm formation is divided into early stage (1–14 weeks), mid-stage (14–35 weeks), and late stage (35–45 weeks) based on changes in the core flora of the biofilm on the surface of plastic flakes exposed at the bottom of the harbor [2]. The formation process of biofilm on the surface of plastic flakes in the real marine environment is constructed. Wimpenny [3] gives a classic biofilm formation process in chronological order:

1. Rapid formation of organic molecular layers on clean solid surfaces.
2. Colonization by bacteria loosely attached to solid surfaces.
3. Colonization by bacteria more firmly attached, forming microbial communities and producing EPS.
4. Communities stretching outward to form regular and irregular structures.
5. Biofilms mature, new species enter the biofilm and grow, and organic or inorganic fragments are combined to form a solution gradient resulting in spatial heterogeneity of the biofilm.
6. Protozoa that phagocytose bacteria may prey on biofilms.
7. Mature biofilms may peel off and this cycle alternates or forms a top-level community.

Lennox [4] divides biofilm formation into five processes: (1) mucosal formation, (2) bacterial proximity and touching, (3) reversible and irreversible attachment, (4) exogenous species supplementation and growth, and (5) diffusion. Some researchers have also divided biofilm formation into four processes: (1) adsorption of dissolved organic molecules, (2) colonization by prokaryotes, (3) colonization by single-cell eukaryotes, and (4) colonization by invertebrate larvae and algal spores. These four processes may occur simultaneously or independently on the surface of the microplastic [5].

2.2 Factors Affecting Biofilm Formation on Microplastics

A conditioning layer comprising organic and inorganic materials is formed by adsorption within a few minutes of the first contact of the plastic surface with the

surrounding water. Microorganisms are in contact with the surface through repulsive or attractive interactions between cell walls and media surfaces. The initial condition layer may have the ability to control colonization by altering material-specific surface properties [6]. Biofilm formation is a multistage process mediated by a variety of factors including surface properties, nutrient solution, pH, and temperature [7]. The environment surrounding the matrix and the conditions of cell growth (such as temperature, carbon source, fluid flow, composition of nutrient media, and growth factors) are complex factors that affect the attachment of bacteria to the surfaces of MPs [8]. There are a variety of attachment mechanisms between microbes and matrices that increase the adhesion of the substrate surface through pili, bristles, flagella, and adjustment of EPS yield [9, 10]. The initial condition layer and the colonizer alter the surface properties of the material and promote the colonization of other organisms. Microbial cells can attach to the surface through specific and non-specific interactions, both depending on surface hydrophobicity/hydrophilicity, roughness, electric charge, and functional groups. The chemical properties of the condition layers are related to the roughness or hydrophobicity of the initial matrix surface and are important for biological sedimentation, indicating the importance of the first adsorption process [11]. Hook et al. [12] believe that surface hydrophobicity and polymer morphology do not affect the adhesion of bacteria to polymers. In contrast, Sanni et al. [13] propose a strong correlation between bacterial sedimentation and hydrophobicity, molecular flexibility parameters in the specific condition of poly(meth)acrylate.

3 Methodology of Microplastic-Associated Biofilm Research

3.1 Scanning Electronic Microscopy

Scanning electron microscopy (SEM) is a tool for observing the surface morphology of samples using secondary electron signal imaging [14] and is widely used in biological, medical, materials, geological, environmental, and other research fields. Energy-dispersive spectroscopy (EDS) combined with scanning electron microscopy (SEM/EDS) is a commonly used elemental microanalytical method that identifies and quantifies the target surface elements of a sample surface [15]. At present, SEM has become a common method for the study of morphology with MPs and their surface biofilms (Fig. 1). EDS is used to analyze the elemental composition of specific areas of MPs to characterize the aging and adsorption characteristics of MPs in the environment.

When observing the microplastic surface biofilm, the SEM sample preparation is usually subjected to cell fixation, dehydration, drying, and then sample analysis according to the SEM method [16, 17]. Cell fixation is an important step in sample preparation. During cell dehydration or drying, cells lose water and undergo

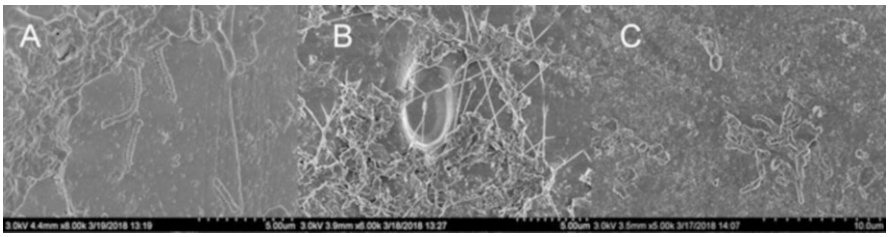

Fig. 1 Biofilm morphology on the surface of MPs at different seawater depths observed by SEM (**a**, 2 m; **b** 6 m; **c** 12 m)

structural changes, resulting in distortion of results [18]. Generally, glutaraldehyde or its combination with citric acid is used to fix microbial cells [19]. Sample drying methods generally include freeze-drying, room temperature or oven drying, and CO_2 critical point drying [17, 19, 20].

SEM can be used to visually identify microbial morphology and posture, characterizing the biodiversity on microplastic surfaces [21], or to analyze the surface morphology of MPs to understand the process of change for weathering and fragmentation of MPs in the environment [20] and helps to distinguish MPs from organic particles [22]. SEM coupled with EDS analysis can be used to identify microplastic samples, especially to distinguish carbon-dominated plastics from inorganic particles [23]. In addition, EDS is also a means of detecting harmful substances such as potentially toxic metals from the environment adsorbed on the surfaces of the MPs.

3.2 Crystal Violet Staining

Crystal violet is a staining solution commonly used in tissue or cell staining to stain the nucleus a deep purple color. Crystal violet is a basic dye that binds to DNA in the nucleus and binds to negatively charged surface molecules and polysaccharides in the extracellular matrix [24] while simultaneously allowing proteins to be stained. It is therefore often used as a biofilm semiquantitative method to characterize the biofilm formation process. Crystal violet staining is simple to operate, but it cannot distinguish the living status of cells. According to the mature state of the cells, potassium hydroxide is added to adjust the pH of the dye solution to 6.0–8.0. The lower pH dye solution is used for fresh cell staining, while the higher pH dye solution is suitable for matured cells. The pH can also be adjusted with aniline or pyridine to enhance the dyeing ability of the dye solution for old cells [25]. In addition, the combination of crystal violet and ammonium oxalate to view biofilms can improve the quality of protein-selective staining and enhance coloration and optical effects [26]. Cells stained with crystal violet can be decolorized with a solution such as sodium dodecyl sulfate solution, acetic acid, or ethanol [27–29], and the absorbance of the decolorizing solution is measured and can indirectly

represent the total amount of biofilm. Moreover, since the light could strongly interfere with the crystal violet staining effects, special care is needed to avoid light contamination during the preparation, storage and usage of crystal violet dye.

3.3 Laser Confocal Scanning Microscopy

Laser confocal scanning microscopy (LCSM) is a recent technique developed for the study of histomorphology. It can perform layered scanning on light-transmitting samples and is often used for the morphological study of the three-dimensional structure of bacterial biofilms [30]. LCSM is developed based on fluorescence microscopy technology and is mainly composed of a laser light source, a scanning device, a detector, a computer system, an image output device, an optical device, and a confocal system. The imaging principle is to use a laser scanning beam to form a point light source through a grating pinhole and scan the optical signal of the collecting point by point on the focal plane of the fluorescent marking specimen to reach the photomultiplier tube (PWT) through the detecting pinhole and then display the signal on the computer through signal processing. An image is formed on the screen. The term "confocal" refers to the LCSM having a pinhole light source in front of the illumination source and in front of the detector. After a series of lenses, it is finally focused on the pinhole of the light source and the pinhole [31].

LCSM can provide three-dimensional information about different cell and polymeric biofilm components such as phototrophic organisms, bacteria, and EPS [32]. In addition, the continuous development of fluorescent markers makes it possible for fluorescent dyes to target specific components of biofilms such as nucleic acids and protein residues and even to identify specific cellular physiological states, providing further description of the natural structure, composition, and cellular tissues of biofilms. According to the purpose of the research, the specific fluorescent dye to stain the sample can be selected [29], and the biofilm image along the *Z*-axis direction in 3D mode can be collected to obtain a complete series of stack format images. The three-dimensional structure of the biofilm can be calculated quantitatively using Imaris and ImageJ software [33]. It should be noted that the fluorescent dye should be stored at a suitable temperature according to the product description and should be protected from light during storage and use.

3.4 Flow Cytometry Combined with viSNE

Flow cytometry uses a device for automated analysis and sorting of cells. It can quickly measure, store, and display a series of important biophysical and biochemical parameters of dispersed cells suspended in a liquid. Flow cytometry and mass spectrometry flow cytometry are powerful analytical tools for simultaneously studying ten extrinsic markers in a single cell to identify rare subtypes and complex

cellular states in heterogeneous populations. These single-cell multiparametric extrinsic measurements have been used in many applications in biology and medicine [34]. Flow cytometry combined with microscopic observations reveal that micro(nano)plastics form agglomerates with mucus matter and associated microbial communities in seawater [35]. Dussud et al. [36] used 1 mmol L^{-1} pyrophosphate for cell detachment pretreatment and ultrasonication with an ultrasonic probe. The cell-separated sample was fixed with 1% (*v/v*) (final concentration) glutaraldehyde. The cells were then stained with a nucleic acid dye in the dark after which the cells were counted using a flow cytometer.

Visual stochastic network embedding (viSNE) is a tool for nonlinear dimensionality reduction and high-dimensional data visualization. It was originally used to visualize mass spectrometry flow cytometry data from healthy and leukemia blood samples, qualitatively distinguishing blood cell types and detecting abnormal phenotypic changes in blood cell populations. The optimized viSNE program can be used to distinguish species and different phenotypes present in biofilms. Flow cytometry is used in combination with viSNE, which quantifies the survival of large cells after cell decay and temperature stress, while in the field it detects changes in community structure driven by known environmental factors (flow conditions, dissolved organic carbon, calcium) and plastic contamination [37].

3.5 DNA Extraction and High-Throughput Sequencing

High-throughput sequencing (HTS), also known as next-generation sequencing (NGS), can sequence up to tens of millions of DNA strands in parallel at one time. It has become a common research tool in the life sciences and has been widely used in genomics, sequencing, epigenomics, and functional genomics. High-throughput sequencing can complete a variety of sequencing tasks including genome-wide, transcriptome, and macrogenome and bring new methods for functional genomics analysis.

DNA extraction is a preliminary step for high-throughput sequencing. In contrast to natural media such as water and soil, MPs are highly polymeric, and the microbial content on the surface is low. It was found that the particle size, quantity, type, and physicochemical properties of MPs affect DNA extraction [29]. Commercial kits can be selected to extract whole-genomic DNA from microplastic surfaces to increase productivity. The extracted product is subjected to purity evaluation by agarose gel electrophoresis, and its quality is evaluated by NanoDrop [38]. According to the research needs, the appropriate primer template is selected for PCR amplification, and the amplified product can be sequenced on the machine after passing the test.

Zettler et al. [46] used high-throughput sequencing technology for the first time to analyze the microbial community diversity of six microplastic surfaces, and they found that the average number of microbial species per surface exceeded 1,000. Since then, more studies have focused on the microbial community structure and

Table 1 Biofilms from the surfaces of different types of matrix

Matrix type	Environmental media	Analysis method	Reference
PE	Seawater	Stained with crystal violet	[41]
Copper, PE	Tap water	Lipid biomarkers	[42]
PS	Coastal water	–	[43]
PET	Seawater	CSLM	[44]
Stainless steel, PC	Seawater	16S rDNA, FISH, DGGE	[45]
Floating plastics	Seawater	SEM	[21]
Plastic marine debris (PMD)	Seawater	SEM and next-generation sequencing	[46]
Cylindrical pellets	Seawater	–	[47]
Glass	Lake water, Wetland sediment	DGGE	[48]
Acrylic, Glass, Steel	Seawater	T-RFLP, 16S rRNA	[49]

diversity of biofilms on MP surfaces and spatiotemporal variability of microbial community structures on biofilms on the surfaces of MPs [38–40].

Some typical biofilms formed on the surface of plastic and non-plastic materials are listed in Table 1.

4 Biofilms on Plastic Surfaces and Their Physicochemical Implications

4.1 Weathering

Plastic weathering is the process by which the physical integrity of a material is lost through the influence of abiotic and biological factors. Photooxidation is the most common non-biodegradable pathway and can be divided into three main steps: initiation (polymer chain breakage and radical formation induced by UV light), propagation (auto-oxidation), and termination (forming inert products). Weathered surfaces may exhibit changes in shape, increased surface roughness, and chemical changes (e.g., become more polar due to the formation of carbonyl groups) [6]. Over time the surface area of plastics which is available for microbial colonization increases [50], thus increasing the effects of microplastic biodegradation. On the other hand, the formation of biofilms alleviates the ultraviolet degradation by sunlight of plastics which hinders the physicochemical weathering process [51].

Biodegradation of polymers occurs in addition to physical weathering [52]. Flemming [53] reported a variety of patterns in which biofilms disrupt the structure and function of synthetic polymeric materials, namely, (1) fouling surfaces, altering surface properties, and contaminating adjacent media such as water by released microbes; (2) increased leaching of additives and monomers from the polymer matrix by microbial degradation; (3) attacking polymers and additives by

enzymes or biological groups, resulting in loss of embrittlement and mechanical stability; (4) hydration and fungal hyphal penetration of the polymer matrix, causing expansion and increasing conductivity; and (5) degradation of the polymer color by excretion of lipophilic microbial pigments. Gewert et al. [54] investigated the biodegradation pathways and products of six plastic polymers. The six plastics were divided into two categories according to the main chain components. One has a carbon chain as the main skeleton (PE, PP, PS, and PVC), and the other contains heterocyclic atoms (PET and PU). Ultraviolet radiation and oxygen are the main factors leading to the fracture of the C-C skeleton in the initial stages of microplastic degradation. The small molecular polymers after fracture may be further degraded by microbial intracellular or extracellular enzymes.

4.2 Vertical Transport

The vertical transport of MPs in the ocean is influenced by multiple physical, chemical, and biological processes [55]. Density is an important parameter to control the vertical migration of MPs. Plastic density is commonly 0.85–1.41 g cm^{-3}. Low-density plastics (density less than seawater) float on the surface of seawater for migration, medium-density plastics (density close to seawater) are suspended in seawater, and high-density plastics (density greater than seawater) migrate on the seabed by suspension or mass transfer [5]. Reisser et al. [56] analyzed the distribution of low-density plastic particles below 0–5 m depth in the sea. It was found that the concentration of plastic particles decreased exponentially with increasing water depth and the smaller the particles, the easier it was for them to migrate vertically. MPs are affected by physical and biological processes during migration and by density changes. A survey of the North Atlantic found that the density of oceanic MPs increased significantly compared to nearshore MPs, mainly due to biofouling [57]. On one hand, biofilms may increase the density of MPs causing them to sink. On the other hand, biofilms may increase the buoyancy of plastic particles with higher density than water, and they more readily float [6]. With the impacts from biofilms, physical and chemical processes such as flocculation occur between the microplastic particles and the agglomerates formed settle to the seabed. Some plankton ingest MPs coated with biofilms which in turn release plastic particles with altered physical and chemical properties, increasing their sinking rate [58]. The plastic particles that converge on the bottom layer are reduced in density due to the feeding of benthic organisms on their surface biofilms, thus regaining buoyancy [59].

Numerical simulation is the main research method for studying the vertical migration of MPs in the ocean. Kukulka et al. [60] used a turbulent mixing model to simulate the migration of plastic particles in the vertical direction under buoyancy and turbulence. Isobe et al. [61] established a vertical two-dimensional particle tracking model to simulate the migration of plastic particles in coastal waters. The sediment deposition model can be used for the simulation of high-density MPs.

Ballent et al. [62] used the Mohid model (a general three-dimensional numerical calculation model) and the experimentally obtained sedimentation-resuspension parameters to simulate the migration of high-density MPs in the Nazaré canyon and found the MPs moving up and down in the canyon under tidal currents. After the model is established in the actual research, the parameters of the MP migration process need to be obtained and verified to identify the rationality of the simulation results.

4.3 Transport of Plastic-Associated Pollutants Through Biofilms

MPs have a large specific surface area and readily adsorb different pollutants including persistent organic pollutants, potentially toxic metals, and pathogens. Additives are certain chemicals added to the molecular structure of plastics to improve their properties. They have hydrophilic groups and metabolic properties and are difficult to leach with weak solvents. Plastic additives may leach and migrate as the environment changes, for example, bisphenol A and nonylphenol, which are highly hydrophilic [5]. Jang et al. [63] found the brominated flame retardant HBCD and bisphenol A on PS foam collected on the Korean coast. Plastics can adsorb persistent organic pollutants (POPs) and can act as important carriers for the transportation and diffusion of organic pollutants. Bakir et al. [64] studied the potential of microplastic transport and removal of hydrophobic organic pollutants (HOCs) in estuarine environments and found that the potential for PE transport and removal of phenanthrene and 4,4′-DDT is much greater than that for PP and PVC. Potentially toxic metals are also common contaminants adsorbed on microplastic surfaces. For example, the detection rate for Cd and Pb in the biofilms of microplastic samples was 6.9% and 7.5%, respectively, from two beaches in south-west England [65]. In addition, chemical contaminants such as drugs and antibiotics were also detected on microplastic fragments.

The distribution and diffusion of the various abovementioned pollutants in MPs and the surrounding water environment may be affected by biofilms. On one hand, biofilms may enhance the adsorption capacity of pollutants on the surface of MPs. On the other hand, specific microbes in the biofilm can metabolize and degrade organic pollutants adsorbed on the MPs [6]. Biofilms are an organic phase composed of water, lipids, and proteins, and they can adsorb water, inorganic and organic solutes, and particles [66], representing a potential barrier to the adsorption, diffusion, and release of chemicals. The viscosity of EPS contributes to the ability of biofilm-coated MPs and heteropolymers to adsorb contaminants [67]. Biofilms can increase the mass transfer resistance of pollutants to the contact with and exit from the plastic polymers [68]. Kinetic laboratory study of HOCs adsorbed onto MPs shows that when microplastic surfaces are in the presence of biofilms, the diffusion coefficient is reduced by approximately four orders of magnitude [69]. A range of

bacteria, fungi, and algae in the biofilms can degrade HOCs [70], with the additives released from MPs being used as a nutrient source to promote microbial growth [71].

5 Biofilms on Plastic Surfaces and Their Biological Effects

5.1 Microbial Community Structure

MPs have become a popular topic in microbial colonization research because of their small particle size, wide distribution, and large specific surface area. Once released into the environment, MPs are rapidly colonized by microorganisms such as fungi and bacteria and by diatoms or that form biofilms on the plastic surface [2, 72]. Because of the unique surface properties of MPs, the microbial communities colonizing the surface are different from those in the surrounding environment. MPs provide a unique microhabitat that supports the growth of some microbial consortia [73]. Thus, Zettler et al. [46] introduced the term "plastisphere" to describe the environmental niche formed by these plastics.

Microplastic surfaces in aquatic ecosystems are novel ecological habitats for marine organisms, and the composition and diversity of biofilm communities have been investigated in numerous studies [21, 46, 74]. Different methods have been used to study the bacterial composition of the plastisphere. With the development of molecular biology technology, high-throughput sequencing technology has been widely used to reveal the composition and diversity of microbial communities on the surfaces of MPs. Some studies find that microbial abundance and diversity on the surface of MPs are lower than those in the surrounding water or sediments [74, 75]. The microbial community structure of the plastisphere is largely influenced by geographical factors, spatial location, and exposure time [2, 76–78]. In addition, different types of polymers and environmental factors also have a significant impact [79, 80]. Miao et al. [81] evaluated the effects of substrate type on microbial communities and found altered metabolic pathways in microbiomes colonizing MPs. Similar results have also been found in the study of the composition and function of PE MPs communities in soil ecosystems by Huang et al. [39]. Compared to natural matrices, microbial communities colonizing the surfaces of MPs exhibit different functions and may trigger different ecological effects on the environmental fates of MPs. Further investigations are therefore needed to illustrate the potential effects of the structure and function of microorganisms colonizing the surfaces of MPs, especially the ecological effects in aquatic systems and the soil environment.

5.2 Trophic Transfer

Due to their small size and widespread presence in the marine environment, MPs can be ingested by a series of marine organisms such as zooplankton, invertebrates,

crustaceans, and fish [82, 83] and can be transmitted along the food chain through predation [83–85]. Intake of MPs may interfere with the food chain as low-nutrient organisms are predated by high-nutrient organisms and then transmitted along the food chain [86, 87]. In contrast to marine microplastic contamination, the distribution and potential impact of MPs in soil ecosystems are poorly understood. Studies show that earthworms and collembolans can transport MPs in soils and increase their mobility [88–90]. Zhu et al. [91] found that predator-prey relationships among different trophic levels can increase the migration of MPs in soils. Moreover, the movement of MPs by soil fauna may affect the bioavailability of MPs to other soil organisms [92]. In addition, most studies have focused on virgin MPs ingested by organisms along the food chain, neglecting the fact that most of the surfaces of MPs in the environment are weathered and covered by biofilms [6]. There have been few studies on the bioaccumulation of MPs and MP particles attached to biofilms at the nutritional level. Microorganisms such as bacteria and algae attached to the surface of MPs may be taken up as food by predators such as fish, thus increasing the risk of ingesting MPs [93]. In addition, the buoyancy of MPs adhering to biofilms may change, allowing them to migrate from surface waters to the bottom of the water column, thereby increasing the chance of being ingested by benthic organisms [58, 79, 94]. In summary, the formation of biofilms on the surfaces of MPs may affect the feeding preference for MPs ingested by organisms through alteration of physical and chemical properties or increasing the bioavailability of MPs [6]. Considering the actual environment, future studies should focus on the role of microorganisms and surface biofilms in the effects of MPs on nutrient transfer.

5.3 Toxicity and Adverse Effects

MPs are usually made from highly hydrophobic materials and chemical additives and are thus susceptible to contamination by a number of chemical pollutants such as POPs, potentially toxic elements, antibiotic resistance genes (ARGs), and pathogens [46, 73, 95–97]. MPs are colonized by diverse and metabolically complex microbial consortia and can be regarded as a novel microbial niche and may serve as a vector for chemical pollutants which may increase the environmental risk from the adsorbed chemical pollutants [98–100]. Environmental MPs are available to every level of the food web from primary producers to higher trophic-level organisms [101]. After a long process from source to sink, MPs are colonized by microorganisms and wrapped by biofilms [102]. The migration of hydrophobic organic pollutants (HOCs) between plastic debris and water may be affected by biofilms which have the ability to metabolize HOCs [6]. MPs have been reported to exhibit concentrations of POPs up to six orders of magnitude greater than the background concentration in the surrounding seawater [103]. Gong et al. found potentially pathogenic bacteria on LDPE MPs exposed in lake water and considered that MPs could serve as transfer vectors for harmful microorganisms in water [104]. Similarly, Wu et al. [73] compared biofilms on MPs with two natural substrates (rocks and

leaves), finding that specific ARG subtypes and several pathogenic bacterial hosts were selectively enriched by MP biofilms. Diffusion of specific microorganisms (especially pathogenic microorganisms) in MP biofilms may increase the risk of disease to other organisms including humans. However, the link between the toxicity and adverse effects on MPs and biofilms is still not fully understood. In conclusion, MPs and their associated biofilms represent ecological risks and potentially adverse effects on the environmental safety and health. Future studies are required to clarify the mechanisms of interactions among MPs, biofilm-colonizing microorganisms, and chemical pollutants.

5.4 Biodegradation

Plastics exposed to the environment may undergo either weathering or biodegradation processes under the complex influences of physical, chemical, and biological factors. The biodegradation of plastics is driven mainly by multiple degradation pathways [55]. Biodegradation of long-chain polymers is usually limited due to their large molecular weight and lack of efficient microorganisms for degradation. The biodegradation process of petroleum-sourced plastics usually includes [105, 106] (1) biofilm formation on the plastic surface, (2) depolymerization, (3) catabolism of the depolymerization by-products, and (4) biomineralization of organic matter.

The biodegradation of plastics has been reported in several studies over the last 30 years. However, there is general agreement that the process is extremely slow under normal conditions [107–110]. Biodegradation requires a crucial initial step that is the formation and development of a microbial biofilm either at the surfaces or directly into the cracks in the MPs [111]. MPs act as a novel, functionally important microhabitat in aquatic and terrestrial ecosystems and exhibit a distinctive microbial community structure which is markedly different from the surrounding environment [75, 76, 78]. Compared with planktonic bacteria, plastic-related bacterial biofilms have stronger ability to degrade plastics [112]. Delacuvellerie et al. [72] found several genera of hydrocarbon-degrading bacteria enriched on several plastics, and these bacteria are potential players in plastic degradation. Yoshida et al. [113] screened a novel bacterium, *Ideonella sakaiensis* 201-F6, that is able to biodegrade poly(ethylene terephthalate). More plastic-degrading microorganisms have subsequently been found in the environment [111, 114–118]. Although several microorganisms are involved in the degradation of plastics, it remains a challenge to obtain a strain suitable for commercial exploitation. Moreover, efficient screening techniques are a prerequisite for the isolation of highly efficient MP-degrading bacterial strains or consortia. To date, few studies have focused on the degradation of MPs by microbial consortia.

Given the importance of biofilms in changing the physicochemical properties and environmental fate of MPs, further studies are needed to investigate the biofilm-mediated sorption of hazardous chemical contaminants, pathogens, and ARGs. Studies on mechanisms of interaction, combined biological toxicities, and ecological

risks between MPs and their associated biofilms are also needed. In addition, biofilm maturity (dynamic formation processes) may have a great influence on these aspects. Moreover, the screening, isolation, and characterization of high-efficiency plastic-degrading microorganisms from biofilms, together with their enzymatic and molecular mechanisms for plastic biodegradation, are needed toward a better understanding of microplastic pollution and bioremediation in the terrestrial environment.

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