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Critical Perspectives

Aquatic Biofilms—Sink or Source of Microplastics? A Critical Reflection on Current Knowledge

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Abstract: The scientific understanding regarding sources, occurrence, and effects of microplastics in the aquatic environment has advanced rapidly, leaving some meaningful knowledge gaps virtually untouched. One of them is the interactions of microplastics and biofilms, microbial communities ubiquitous in aquatic ecosystems and fundamental for a range of ecosystem-level processes. It is evident that biofilms can quickly develop on the microplastic surface and consequently change particle properties and, as such, its fate and ecotoxicity. Moreover, microplastics interact with ubiquitous biofilms that are developed on any surfaces in aquatic ecosystems. Although the knowledge about these interactions is at best limited, it is expected that microplastics attach to the water–biofilm interface or penetrate the biofilm matrix. Microplastics can accumulate and ab- or adsorb to those biofilms where they are subjected to transformation processes such as fragmentation. Thus, biofilms may function as a sink. Changes in environmental conditions may, however, stress biofilms initiating their dieback and microplastic release, which could turn biofilms into a source of microplastics. We argue that the accumulation and release dynamics are a largely overlooked but potentially important piece to the puzzle that is a comprehensive understanding of microplastic fate in the environment and thus under the influence of multiple interacting factors. *Environ Toxicol Chem* 2022;41:838–843. © 2021 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

The first scientific articles that identified microplastics in the environment are nearly 50 years old (Carpenter & Smith, 1972). In 1972, Carpenter and Smith accidently found many plastic pellets in a neuston net when they sampled the pelagic *Sargassum* community in the western Sargasso Sea. They further investigated those particles and described plastic pellets as a new habitat for diatoms, hydroids, and bacteria—a first indication of the importance of microplastic–microorganism interactions. However, the same year, when they found some microplastic pellets in a fish gut (Carpenter et al., 1972), the scientific attention shifted toward animal species. Although the ingestion of microplastics by (in)vertebrates can have many consequences for those organisms and higher trophic levels (Xu et al., 2020), the interaction of microplastics and microorganisms deserves attention. The latest research suggests that biofilm (i.e., communities of aquatic microorganisms attached to a surface) formation plays a crucial role in the fate, behavior, and bioavailability of microplastics in the aquatic environment (Miao et al., 2021; Qi et al., 2021).

In addition to biofilm developing rapidly on the surface of microplastics (defined in the present article as particle-associated biofilm), microplastics can interact with biofilms covering any surface in aquatic ecosystems (defined in the present article as substrate-associated biofilm). Although this issue has not yet been systematically studied, evidence suggests that aquatic biofilm in lotic systems could be important for the fate of microplastics (Huang et al., 2021). Biofilms may indeed function as a temporary sink for microplastics and, under certain conditions, become a source (i.e., remobilization of captured microplastics from biofilm).

Therefore, the aim of this critical perspective is to provide a brief overview of the current scientific knowledge on the interactions between microplastics and aquatic microorganisms

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in the form of biofilms. Specifically, we focused on two perspectives. First, we summarize and discuss changes in the properties, fate, and ecotoxicity of microplastics due to *particle-associated biofilms*. In the second part, we focus on *substrate-associated biofilms*, where interactions with microplastics may occur in lotic systems. These interactions are, according to our analyses, a current blind spot in our scientific knowledge regarding the fate of microplastics in aquatic ecosystems.

PARTICLE-ASSOCIATED BIOFILM

To date, most studies that investigated interactions between microplastics and biofilm have focused on the plastic particles serving as a surface for biofilm growth (Oberbeckmann et al., 2015; Rummel et al., 2017). In fact, as soon as microplastics enter aquatic ecosystems they attract a diverse microbial community composed of bacteria, fungi, and protozoa forming together with algae and diatoms a biofilm on their surface (Miao et al., 2021).

Mincer et al. (2019) suggested that plastics in the environment are instantaneously colonized by a microbial biofilm and estimated that between 1000 and 15,000 metric tons of microbial biomass are harbored on marine plastic debris. Although the development of biofilm on microplastics is not yet fully understood, it is expected that it follows the general process of biofilm formation on natural surfaces (Figure 1): in the aquatic environment, microplastics are first coated by a layer of organic and inorganic substances (called "ecocorona" [Galloway et al., 2017]). Then, the contact of microorganisms and microplastics begins with electrostatic attraction and repulsion between the cell wall and the coated surface. Attached microorganisms begin to secret extracellular polymeric substances (EPS) and thereby start forming a stable biofilm (Rummel et al., 2017). The development of the ecocorona and biofilm on microplastic surfaces can take from several hours to days. It is therefore plausible that pristine microplastics practically do not exist in the aquatic environment. In addition, the biofilm on microplastics has been shown to harbor microorganisms capable of degrading plastics at a rather high abundance (McCormick et al., 2014), potentially contributing to their degradation in the environment (Han et al., 2020).

Theoretically, only microplastics with a density higher than water should sink, whereas microplastics with a lower density are expected to float near the water surface. However, vertical transport of microplastics depends on many factors, such as weather conditions, the shape and size of the microplastic, and the presence of a biofilm (Karkanorachaki et al., 2021; Miao et al., 2021; Semcesen & Wells, 2021). The growth of biofilm on microplastics can increase particle size and density (Kalčíková et al., 2020), simulating the potential for microplastics to be transferred from the water surface through the water column to sediment (Jemec Kokalj et al., 2019; Kooi et al., 2017; Figure 2).

During this journey, microplastics become more easily available for animals within the water body. Although many organisms have the ability to distinguish between high- and low-quality food sources, developed biofilm "camouflages" plastic particles, stimulating their ingestion (Vroom et al., 2017). Indeed, the presence of algae on the surface of microplastics, for instance, increases their attractiveness to various organisms because algae are able to exude cues that "flavor" microplastics and are thus preferably eaten by zooplankton (Procter et al., 2019). Moreover, algae synthesize and thus provide highly unsaturated fatty acids which are considered essential for higher trophic levels, pointing to a high nutritious quality (Guschina & Harwood, 2009). Nonetheless, such overgrown microplastics have lower nutritional value in comparison to algae. Capturing and processing nutrient-poor particles can ultimately affect the energy budget of the organism, leading to a lower availability of resources for maintenance and growth (Korez et al., 2019; Sussarellu et al., 2016).

In addition, biofilms on microplastics have a much higher sorptive capacity for various pollutants in comparison to pristine microplastics (Kalčíková et al., 2020; Qi et al., 2021; Wang et al., 2020; Yu et al., 2019). This observation may be important for the assessment of microplastic toxicity as well as the fate and effect of co-occurring pollutants.

First, high adsorption of pollutants on the biofilm may increase the ecotoxicological profile of the microplastic particle (Kalčíková et al., 2020). Several studies have documented no effect of microplastics (with or without biofilm) on various organisms (Gambardella et al., 2019; Jemec Kokalj et al., 2019; Kalčíková et al., 2017), while studies using microplastics loaded

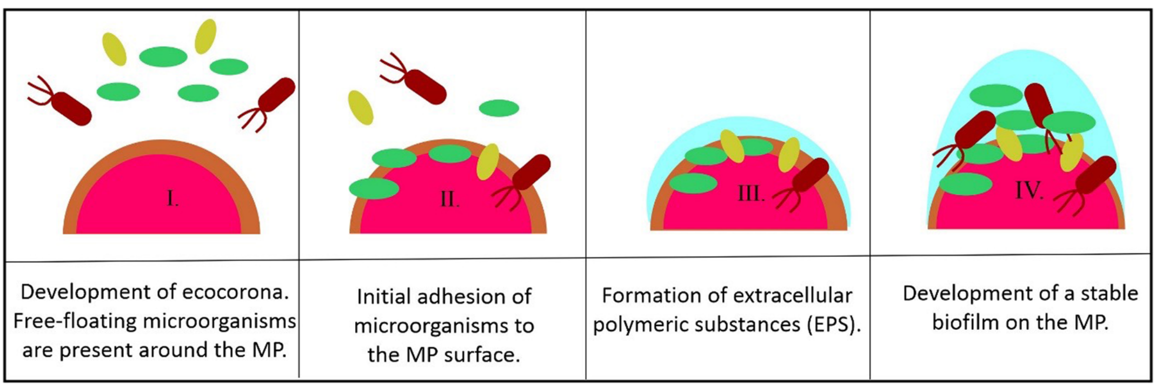

FIGURE 1: The general process of biofilm formation on a microplastic particle. MP = microplastic particle.

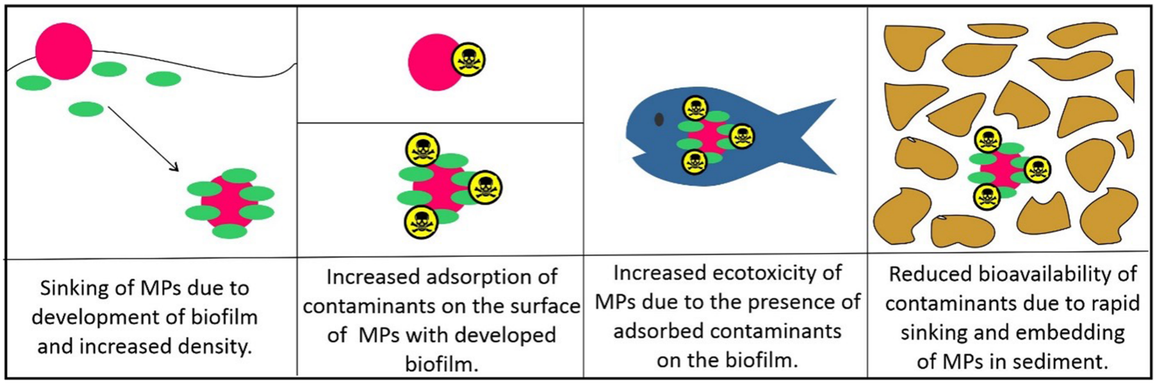

FIGURE 2: The effects of biofilm formation on the properties and ecotoxicity of microplastics. MPs = microplastic particles.

with pollutants have shown the opposite (Avio et al., 2015; Qi et al., 2021). Further studies indicated that the development of a biofilm on the microplastic surface may enhance the combined ecotoxicity of pollutants (e.g., metals) and the plastic itself. This is most likely due to the increased concentration of pollutants adsorbed on the biofilm associated with microplastics (Kalčíková et al., 2020; Qi et al., 2021). In addition, Kurniawan et al. (2012) found that metals were less tightly bound to a biofilm than to ion exchange polymers, indicating weaker binding of metals to biofilms. This could potentially contribute to the increase in toxicity because metals from biofilm-associated microplastics are likely more easily remobilized compared to pristine microplastics (71% of adsorbed Ag was leached from microplastics with biofilm compared to 30% from pristine microplastics; Kalčíková et al., 2020). It is also worth noting that microplastics with biofilm are much larger (Kalčíková et al., 2020) and can remain longer in the gut (e.g., in mussels [Kinjo et al., 2019]), which may contribute to the increased toxicity.

On the other hand, several studies have suggested that when organisms are exposed to microplastics in combination with pollutants, the overall ecotoxicity may be reduced (Wakkaf et al., 2020; Zhang et al., 2020). This is because some microplastics can act as a sink for pollutants, lowering bioavailability (Liu et al., 2019). This is even more true for microplastics with biofilm because they can absorb even more pollutants and can be quickly disposed in sediment due to their larger size and usually quicker sedimentation (Miao et al., 2021). In this case *the presence of biofilm on microplastics can potentially reduce the ecotoxicity of the pollutant* in the aquatic ecosystems.

Although these interactions involving the growth of biofilms on microplastic surfaces are rather well studied, it must be emphasized that the ecological consequences of these interactions are not clearly understood. The increases or decreases in the ecotoxicological potential of microplastics due to the presence of biofilm depends on many factors but mainly on 1) the actual sorption capacity of microplastic together with its biofilm, 2) the properties of the pollutant, and 3) the time that the loaded microplastics are available in the water phase. If the pollutant is rapidly adsorbed onto biofilm harboring microplastics but the overall density of the particle is still low, such toxic particles may be transported further downstream and behave like a Trojan horse—increasing the bioavailability of the pollutant. On the contrary, if the pollutant is rapidly adsorbed to the microplastic and embedded into the sediment, the bioavailability of the pollutant in the aquatic ecosystem may be reduced.

SUBSTRATE-ASSOCIATED BIOFILM

In contrast to the implications of biofilm growth on microplastics and related consequences on the fate and effect of the latter (Oberbeckmann et al., 2015; Rummel et al., 2017), there is only limited information on the interaction of microplastics with substrate-associated biofilms (e.g., periphyton [Battin et al., 2016]). These biofilms are complex microbial communities attached to submerged surfaces which include stones, sediments, and coarse particulate organic matter. Thus, biofilms are literally ubiquitous in aquatic ecosystems (Battin et al., 2016). Therefore, microplastics—just as a range of natural particles (Graham, 1990; Sansone et al., 2002)—are highly likely to encounter these biofilms. It should be also noted that when microplastics reach the substrate-associated biofilm, they may already be covered by organic and inorganic matter and/or biofilm, which may facilitate their integration into biofilms.

The lack of empirical evidence, however, makes any prediction on the mechanism and nature of interaction between microplastics and substrate-associated biofilms speculative. Nonetheless, the research targeting colloids and engineered nanoparticles can provide a reasonable basis for some preliminary hypothesis that needs further attention (Figure 3).

The immobilization of microplastics by biofilms

The interaction of any particle with biofilms is initiated by the particle's transport to the water–biofilm interface. This process is driven by hydrodynamics, leading to particle attachment to biofilms. The latter depends on the properties of microbial cells, the particle itself, and the water quality parameters (e.g., ionic strength) influencing the properties of both cells and particles (Boltz & La Motta, 2007; Li et al., 2018). Small microplastics ≤1 μm (which may also be considered nanoplastics [particles 1–1000 nm]) can readily penetrate the

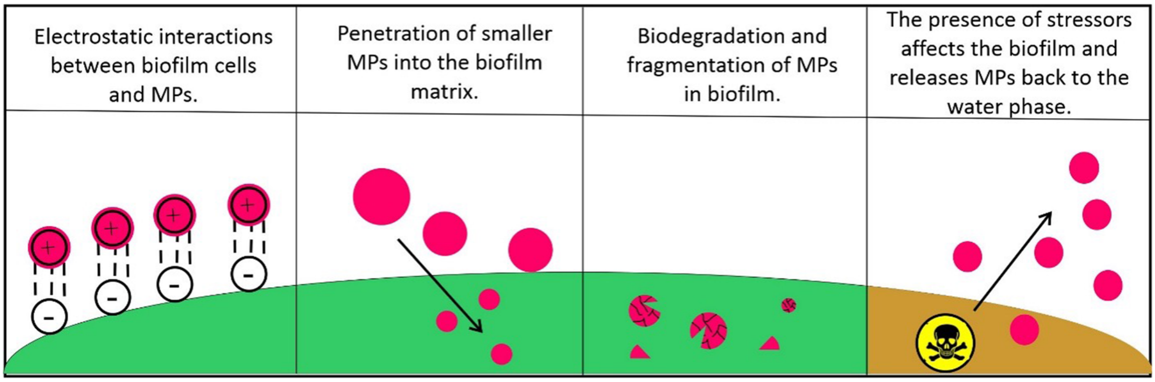

FIGURE 3: The possible interaction between microplastics and benthic biofilm. MPs = microplastic particles.

biofilm matrix (Drury et al., 1993), interfering with the biological activity of the biofilm through, for example, oxidative stress (study with polystyrene particles 100 nm, 100 mg/L; Miao et al., 2019). Larger microplastics, which are not able to penetrate the biofilm matrix, tend to accumulate at the water–biofilm interface. Because of this accumulation on the water–biofilm interface, microplastics may function as a physical barrier for fluxes of, for example, oxygen or nutrients. But such a scenario could only be expected under a continuous release of high numbers of microplastics, for example, at wastewater-treatment plant effluents (Murphy et al., 2016). Similar observations were made for particulates in wastewater (Li et al., 2018). Furthermore, the biofilm could, as shown on plant surfaces (Goss et al., 2018), overgrow attached microplastics, leading to an incorporation into its matrix and thus a strong bond. Similarly, recent findings suggest that substrate-associated biofilm developing on a concrete surface of open canals may retain high concentrations of microplastics (on average 20 items/kg of wet biofilm, with a ratio of microplastic abundance in biofilm to water of up to 164; Huang et al., 2021). The temporary retention and long-term retention of microplastics by natural biofilms have not yet been investigated, but some laboratory studies suggest that the retention of microplastics by biofilm may be substantial. For example, the presence of biofilms led to a complete retention of polystyrene microplastics (4.5 µm) by saturated porous media, whereas in the absence of biofilm only 40% were retained (Majumdar et al., 2014).

The degradation of microplastics by biofilms

Natural biofilms are considered efficient in the degradation of a wide range of pollutants (Edwards & Kjellerup, 2013) and, recently, microplastics (Faheem et al., 2020; Shabbir et al., 2020). It was suggested that the microorganisms within biofilms secrete enzymes, breaking covalent bonds linking carbon atoms within the polymer chain. This degradation process seems limited to the microplastic surface (Shabbir et al., 2020), and the degradation rate under natural conditions remains unclear. It is also possible that microplastics within biofilm could be further fragmented. This assumption is plausible because it is commonly reported for larger plastic particles covered by biofilms (Gerritse et al., 2020; Jacquin et al., 2019).

The remobilization of microplastics from biofilms

The development of any biofilm is characterized by the balance of growth, senescence (or dieback), and detachment. Detachment can occur naturally as erosion (i.e., continuous detachment of single cells) or a massive loss of biofilm (Stoodley et al., 2001; Telgmann et al., 2004). In this context, microplastics trapped in the biofilm matrix may be remobilized with the detached portion of the biofilm. This process was reported, for example, for colloids; but its relevance for microplastics remains unknown (Strathmann et al., 2007). Detachment can be caused by many factors, the most common being shear stress (Telgmann et al., 2004) and seasonal changes (Hao et al., 2020). In addition, stressors such as biocides (Arrhenius et al., 2014), antibiotics (Johansson et al., 2014), herbicides (Kish, 2006), nanoparticles (Ikuma et al., 2015), and salinity (Costello et al., 2018) may impair biofilm structure and functions and cause biofilm dieback. Under this scenario biofilms become weaker in their formation, which might facilitate further detachment and consequently the release of pollutants and microplastics. In fact, we have not been able to identify a single study addressing the possibility of biofilms functioning as a source of pollution (i.e., pollutants remobilization) as a result of biofilm dieback or detachment.

The immobilization, degradation/fragmentation, and release dynamics of microplastics remain unclear. These knowledge gaps may be a significant blind spot because related processes may be critical to fully understand the fate of microplastics in the aquatic environment and thus under the influence of multiple interacting factors.

CONCLUSIONS

Although the growth of aquatic microorganisms on microplastics has attracted substantial attention in recent years, we only scratched the surface of the processes involved in the interactions between microplastics and natural substrate–associated biofilms. Microplastics can attach to these biofilms and bind strongly but may be remobilized after other (environmental) stressors affect the biofilm. Therefore, these biofilms can play an important role—as both sinks and sources of microplastics. However, the limited research on these interactions makes any extrapolation of their role in the field impossible. It is consequently proposed to assess the ability of the biofilm to retain microplastics, the possible trophic transfer from the substrate-associated biofilms to higher trophic levels, and the effects of environmental and anthropogenic stressors on the release dynamics of trapped microplastics. All of this will inform science and ultimately policy and society on the fate of these contaminants and hopefully guide decision-making.

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