



Review

Bioremediation of microplastics in freshwater environments: A systematic review of biofilm culture, degradation mechanisms, and analytical methods



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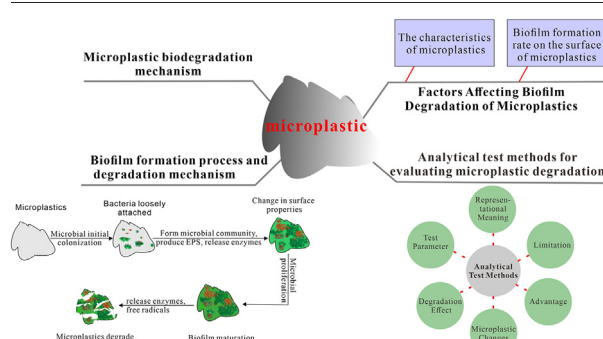
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HIGHLIGHTS

- The rate and quality of biofilm formation on the surface of microplastics affect the degradation of microplastics.
- Measuring changes in molecular weight distribution can provide a better understanding of biodegradation behavior.
- The strains in biofilms that play a role in the degradation of microplastics can be screened and optimized for commercial cultivation.
- Biofilms can be applied to the *in situ* treatment of microplastics in freshwater environments.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Damia Barcelo

Keywords:

Microplastics

Biofilm

Degradation

Analytical test methods

Enrichment

ABSTRACT

Microplastics, defined as particles <5 mm in diameter, are emerging environmental pollutants that pose a threat to ecosystems and human health. Biofilm degradation of microplastics may be an ecologically friendly approach. This review systematically summarises the factors affecting biofilm degradation of microplastics and proposes feasible methods to improve the efficiency of microplastic biofilm degradation. Environmentally insensitive microorganisms were screened, optimized, and commercially cultured to facilitate the practical application of this technology. For strain screening, technology should focus on microorganisms/strains that can modify the hydrophobicity of microplastics, degrade the crystalline zone of microplastics, and metabolise additives in microplastics. The biodegradation mechanism is also described; microorganisms secreting extracellular oxidases and hydrolases are key factors for degradation. Measuring the changes in molecular weight distribution (MWD) enables better analysis of the biodegradation behaviour of microplastics. Biofilm degradation of microplastics has relatively few applications because of its low efficiency; however, enrichment of microplastics in freshwater environments and wastewater treatment plant tailwater is currently the most effective method for treating microplastics with biofilms.

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1. Introduction

Plastic products are convenient, inexpensive, lightweight, and durable; thus, they are widely used in everyday life. However, the resistance of microplastics to degradation means that plastic waste remains in the environment for decades to centuries (Drummond et al., 2022; Du et al., 2021; Geyer et al., 2017; Kasmuri et al., 2022). Moreover, because of the relatively low density of plastics (Gewert et al., 2015; Jiang et al., 2018), many are buoyant in fresh and marine waters, allowing them to be easily transported by currents. As a result, plastic waste is distributed worldwide, even in polar regions that humans rarely visit (Peeken et al., 2018; Zarfl and Matthies, 2010). These plastic wastes may cause long-term harm to the environment, and their removal is difficult. However, owing to the huge demand for plastic products, global annual plastic production now exceeds 380 million tons (Paço et al., 2017), and the output is expected to increase to 2 billion tons by 2050 (Janaswamy et al., 2022). An increasingly large amount of plastic waste enters the aquatic environment and undergoes physical, chemical, and biological degradation by abrasion, ultraviolet radiation, hydrolysis, oxidation, and microbial decomposition, among other processes (Bhagwat et al., 2021; Jahnke et al., 2017). These processes ultimately break the leading polymer chains (Sorasan et al., 2022), thereby creating smaller plastic particles, including microplastics, which are defined as particles that are <5 mm in size (Law and Thompson, 2014; Thompson et al., 2004). It is generally believed that the degradation process is initiated by photo-oxidation and hydrolysis because of exposure to sunlight, air, and water (Gerritse et al., 2020), which, in combination, produce an immeasurable number of microplastics. After microplastics enter aqueous environments, owing to their chemical characteristics, microplastics in the water environment exist for a long time in various sizes (ranging up to 5 mm) and forms (e.g. fragments, fibres, film foams, and pellets), all of which may float on the water surface or attach to plants, rocks, and/or sediments.

Some microplastics are attached to plants and ingested by herbivores along with the plants (Goss et al., 2018). Microplastics suspended in the water column are often ingested by zooplankton (do Sul et al., 2013), some of which are subsequently excreted through physiological activities. Microplastics that cannot be excreted eventually enter higher trophic organisms, where their concentrations may be enriched through the food chain (Lin et al., 2022), and affect the health of high trophic level organisms (Fossi et al., 2016; Goss et al., 2018; Kim et al., 2018; Missawi et al., 2020), potentially affecting human health. Recent reports have detected the presence of microplastics in the human blood, faeces, and the placenta

(Ragusa et al., 2021). Microplastics have also been found in numerous animals, such as bivalves (Setälä et al., 2016), shrimps (Saborowski et al., 2022), deep-seabed invertebrates (Courtenne-Jones et al., 2019), and other macroinvertebrates (Sarkar et al., 2021), fish, and fin whales. The ingestion of microplastics may cause adverse and/or irreversible reactions in aquatic organisms, such as clogging of the digestive tract (Besseling et al., 2013), reduction of food intake (Sarkar et al., 2021), damage to the intestines (Yan et al., 2021), and causing oxidative stress (Saborowski et al., 2022). In addition, microplastics may depolymerise in water, releasing “extractable substances” into aquatic organisms, which directly affects the vigilance, predation, and survival rate of some aquatic biota (Capolupo et al., 2020; Klein et al., 2021; Seuront, 2018). In addition to their effects on animals, microplastics also affect plants. For example, plant interactions with microplastics can alter algal photosynthesis, growth, gene expression, colony size, and morphology (Prata et al., 2019), and the accumulation of microplastics can slow plant root growth (Kalčíková et al., 2020).

According to the different microplastic production methods, they can be divided into primary and secondary microplastics (Conkle et al., 2018). Primary microplastics include microbeads in cosmetic and personal care products, abrasives used in industrial blast cleaning, microfibres shed from synthetic textiles, and virgin resin particles that end up in wastewater pipes because of artificial flushing, rainfall, or in natural bodies of water. Secondary plastics are formed by the breaking up of larger plastics through natural weathering (environmental factors such as solar radiation, temperature, and ocean waves). This plastic waste can enter the aquatic environment from various forms of human activity (e.g., the discharge of treatment plant effluent, marine fisheries, and the atmospheric deposition of anthropogenically released plastic fibres). The migration and transformation pathways of microplastics in the environment are shown in Fig. 1. The smaller the microplastic particles, the higher the specific surface area, the greater the adsorption capacity, and the easier it is to interact with other pollutants in the water environment (Liu et al., 2022; Sorasan et al., 2022), including heavy metals (Bhagwat et al., 2021), persistent organic compounds (Liu et al., 2019; Yu et al., 2019), and pathogens (Wu et al., 2019), among others. Therefore, the removal of microplastics from water environments is a current research focus.

Numerous studies have explored methods for removing microplastics from water bodies, such as photocatalytic degradation (Shi et al., 2021; Uheida et al., 2021; Wang et al., 2020b), microbial decomposition (Auta et al., 2018; Hu et al., 2021), and ultra-high-temperature composting (HTC) technology (Chen et al., 2020b). Photocatalytic degradation requires

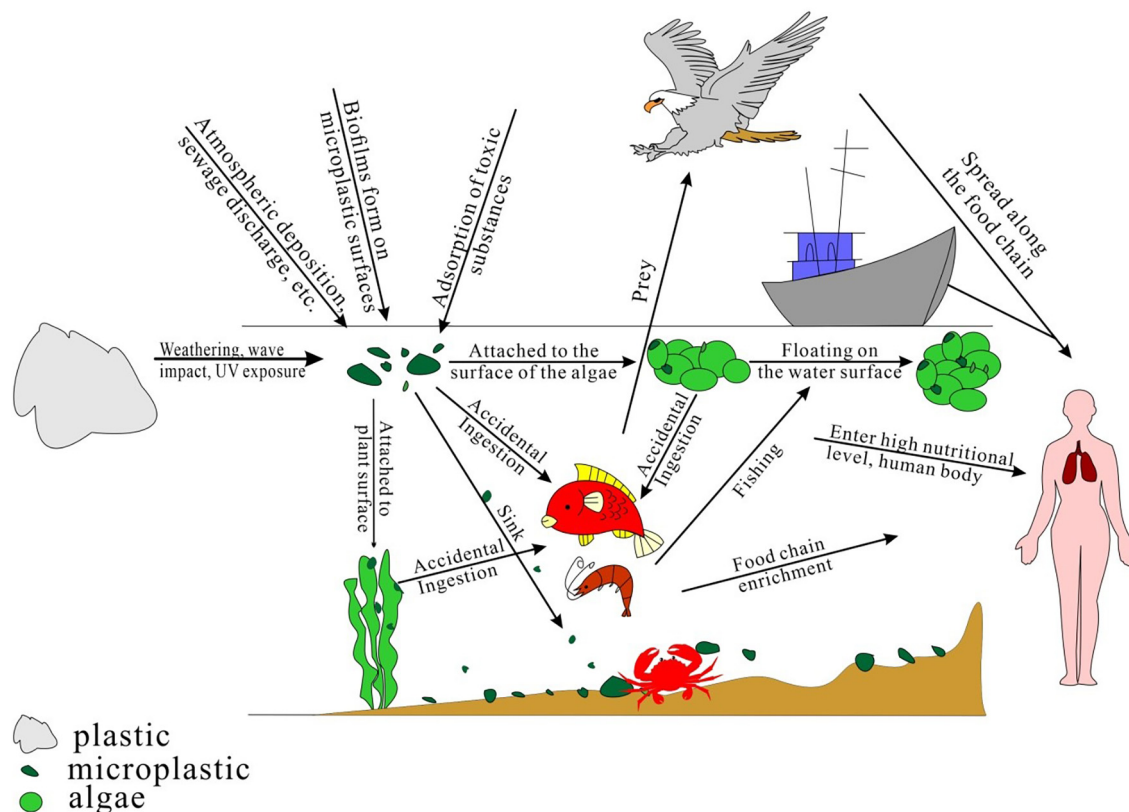


Fig. 1. Schematic model illustrating the cycling of microplastics in aquatic environments.

a lengthy irradiation period to produce measurable chemical or physical changes on the microplastic surface. Microbial decomposition takes a long time and has a low degradation rate (0–15 %) (Ebrahimbabaei et al., 2022). Although ultra-high-temperature composting (HTC) is an effective approach to microplastic degradation, the safety of hyperthermophilic microorganisms in humans, animals, and agricultural plants requires further research (Wang and Wu, 2021). In a study of the behaviour of microplastics in the natural environment, it was found that once microplastics enter the aquatic environment, microorganisms quickly colonise the surface of microplastics, thereby forming a stable biofilm (Harrison et al., 2014). In addition to enhancing the adsorption of pollutants by microplastics (Qiongjie et al., 2022), specific microorganisms in biofilms can also degrade organic pollutants (Rummel et al., 2017). More importantly, the interaction between biofilms and microplastics can lead to changes in the physicochemical properties of the polymer surface, resulting in biodegradation of microplastics.

Early research on biofilms and microplastics has focused on how biofilm colonization affects the deposition of microplastics (Elagami et al., 2022; Miao et al., 2021a; Semcesen and Wells, 2021), sorption of heavy metals on the surface of plastics (Qi et al., 2021; Richard et al., 2019), relationship between biofilms and toxic substances (Chen et al., 2021), microbial community structure on microplastics (Gong et al., 2019; Mughini-Gras et al., 2021; Qiang et al., 2021; Yang et al., 2021), and speed of biofilm colonization on microplastics of different sizes (Wu et al., 2022). Recently, researchers have begun to study the influence of biofilms on the degradation of microplastics as an eco-friendly biodegradation method. Hadad et al. (2005) isolated a strain of *Rhodococcus ruber* that colonised and formed biofilms on polyethylene (PE) surfaces and found that the weight and average molecular weight of the PE samples decreased by 14 % and 21 %, respectively. Biofilm formation may also cause physicochemical changes on the microplastics (Ganesan et al., 2022). Glucose, applied as an external carbon source, has been shown to enhance the degradation of microplastics compared with natural biofilms (Shabbir et al., 2020).

Significant degradation has also been observed on the surface of biofilm-treated microplastics in an environment containing high levels of methane gas (which can promote the growth of bacterial aggregates) (Faheem et al., 2020).

Biofilms are readily available and widely distributed in the natural environment (Faheem et al., 2020; Zhang et al., 2021a); the final products of microplastic decomposition are carbon dioxide and water (Lin et al., 2022), which, of course, pose no harm to the environment. However, some studies have also suggested that biofilms may increase the adsorption of microplastics to environmental pollutants, becoming carriers and aggravating the ecological risk of microplastics in the environment (Richard et al., 2019; Stabnikova et al., 2022; Wang et al., 2021b). It has been established that as soon as microplastics enter the aqueous environment, microorganisms rapidly colonise the surface of microplastics to form biofilms (Chen et al., 2020a), and this process increases the adsorption of microplastics to environmental pollutants. However, this concern can be largely alleviated if the biofilm is cultivated and matured in advance under artificial conditions before treatment with microplastics. Applying the technology of biofilm degradation of microplastics to the *in situ* treatment of microplastics in the freshwater environment or applying it to the source treatment of microplastics can not only degrade microplastics in the aqueous environment but also adsorb and even metabolise other environmental pollutants. More research is needed on how adsorbed pollutants should be effectively treated. Therefore, biofilm degradation of microplastics may be a relatively environmentally friendly emerging technology (Faheem et al., 2020; Shabbir et al., 2020).

Previous studies have also summarised the biofilm degradation of microplastics, but they only included biofilm degradation of microplastics as part of their articles, lacking a systematic summary of the technology (Luo et al., 2022; Yuan et al., 2020) or focused more on the nutrient transfer of microplastics, threats to ecosystems, and the interaction between microplastics and microorganisms in the marine environment. The introduction of biofilm degradation of microplastics was limited to the

description of the degradation process (Debroy et al., 2022). There is a lack of systematic research on analytical test methods for biofilm degradation of microplastics, biofilm culture, and feasible methods to improve the degradation efficiency of microplastics. This paper reviews past research on the formation of biofilms on the surface of microplastics and the degradation of microplastics by biofilms in an aqueous environment, summarises the factors affecting the degradation of microplastics by biofilms, the mechanisms of biofilm degradation of microplastics, and the analytical test methods to assess the degradation of microplastics, proposes feasible methods to improve the degradation of microplastics by biofilms, and discusses the types of future research and strategies that need to be conducted to better understand the issues surrounding the use of biofilms to degrade microplastics.

2. Microplastics in freshwater environments

In recent years, microplastics have been found in freshwater environments (Table 1). Di and Wang (2018) investigated the level of microplastic pollution in China's Three Gorges Reservoir and found that polystyrene (PS) was the most common type (38.5 %), followed by polypropylene (PP; 29.4 %), and PE (21 %). Su et al. (2018) conducted a large-scale survey of microplastic pollution in the middle and lower reaches of the Yangtze River and found that the primary polymer was polyester (PES; 33 %), followed by PP (19 %), and PE (9 %). Yan et al. (2019) investigated the distribution of microplastics in Pearl River water and found that the most common polymer type was polyamide (26.2 %), followed by cellophane (23.1 %), PP (13.1 %), and PE (10.0 %). Yuan et al. (2019) investigated microplastic pollution in Poyang Lake, the largest freshwater lake in

China, in which PP (37 %) and PE (30 %) were the main plastic types, followed by nylon (15 %) and polyvinyl chloride (PVC; 8 %). Mao et al. (2020) studied microplastic pollution in the Yulin River's surface water, which is a typical tributary upstream of the Three Gorges Reservoir. The main polymers identified were PE (39 %), PP (31 %), and PS (23 %).

Freshwater is indispensable for the survival of humans and most living organisms, but many freshwater rivers and lakes worldwide are now contaminated with microplastics (Table 1). Microplastics may cause incalculable damage to the ecosystem (Xu et al., 2021). Therefore, solving microplastic pollution is a problem that needs to be solved in the future.

3. Biofilm formation and culture

3.1. Biofilm formation

Biofilms can be subdivided into five types according to the substrate to which they are attached: epiphyton (plants), epilithon (rocks), epipelton (sediments), epixylon (wood), and epipsammon (sand) (Faheem et al., 2020). Biofilms are formed by extracellular polymers (EPS) secreted by microorganisms, including proteins, glycoproteins, and glycolipids (Wang et al., 2021a). They are phylogenetically and functionally diverse communities of bacteria, algae, protozoa, and fungi, collectively referred to as microbial assemblages, biofouling communities, or epiphytes (Cooksey and Wigglesworth-Cooksey, 1995). Biofilm formation is a dynamic process involving microbial adhesion, EPS secretion, and microbial proliferation (He et al., 2022). The formation of biofilms includes four primary stages (as shown in Fig. 2): (1) the attachment of microorganisms to the surface of the substrate in the water environment, (2) the secretion of EPS by the

Table 1
Microplastics in freshwater ecosystems around the world.

Location	Sample	Microplastic types	Microplastic shapes	Microplastic size rang	Microplastic colour	Reference
Three Gorges Reservoir Region, China	Surface waters, sediments	PS, PP, PE,	Fibres, fragments, and pellets	<1 mm, 1–2 mm	White, blue	(Di and Wang, 2018)
Saigon River, Vietnam	Surface water	PE, PP, PET	Fibres, fragments	50–250 µm	Blue	(Lahens et al., 2018)
Antuã River, Portugal	Waters, sediments	PE, PP, PS, PET, PVA	Foam, fibres, fragments, pellets	–	White, black, transparent, blue, green, brown, black, red	(Rodrigues et al., 2018)
Lake Guaíba, Porto Alegre, Brazil	Freshwater	PP, PE, PTFE, PA, PU, PS	Fragments, fibres, microbead	5–100 µm, 100–250 µm, 250–500 µm	White, transparent, yellow, red, green, blue, black	(Bertoldi et al., 2021)
Dahan River, Xindian River, Keelung River, Taiwan	Surface waters	–	Fragment, Film, foam	0.3–5 mm	White, transparent/translucent, blue, green	(Wong et al., 2020)
Red Hills Lake, Chennai, Tamil Nadu, India	Surface waters, sediments	HDPE, LDPE, PP, PS	Fibres, fragments, films, pellets	1 mm, 2 mm, 1–2 mm	White, green, blue, red	(Gopinath et al., 2020)
Rawal Lake, Pakistan	Surface waters, sediments	PE, PP, PEST, PET, PVC	Fibres, fragments	<1 mm	Blue, red, transparent, black	(Irfan et al., 2020)
Changsha, China	Drinking water	PET, PE, PP, PS	Fragments, fibres, spheres	1–10 µm, 10–50 µm	–	(Shen et al., 2021)
Antarctic Specially Protected Area	Stream	Acrylic, PES, PTFE	Fibres, films	869, 3546 µm, 400–1327 µm, 0–26 µm	Transparent, red, black	(González-Pleiter et al., 2020a)
Northwest Himalaya, India	Freshwater lake, sediments	PA, PET, PS, PVC, PP	Fibres, fragments/films, pellets	0.3–1 mm, 1–2 mm, 2 mm, >5 mm	Green, red, blue, white, yellow, black	(Neelavannan et al., 2022)
Florentino Ameghino Dam, Pico 1 Lake, Los Niños Lake, Vintter Lake, Pico 4 Lake, La Plata Lake, Fontana Lake, Toro Lake, Musters Lake, Argentine	Surface waters	PET, PU, PP, PS	Fibres	≤ 1 mm, 0.2–0.4 mm, 0.4–0.6 mm	Blue, black	(Alfonso et al., 2020)
Freshwater lake, Arctic	Sediments	PET, PES	Fibres, fragments, films, paint sheets, filaments	0.3–0.5 mm, 0.5–1 mm, 1–2 mm, 2–5 mm	Blue, red	(González-Pleiter et al., 2020b)
River Zala, fish ponds, Hungary	Freshwater, sediments	PE, PP, PS, PTFE, PAC, PES	Fragments, fibres	–	–	(Bordós et al., 2019)

Note polypropylene (PP), polyvinyl chloride (PVC), polyamide (PA), polyethylene (PE), polystyrene (PS), polyester (PES), polyethylene terephthalate (PET), polyvinyl acetate (PVA), polytetrafluoroethylene (PTFE), polyurethane (PU), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyesters (PEST), polyacrylate (PAC).

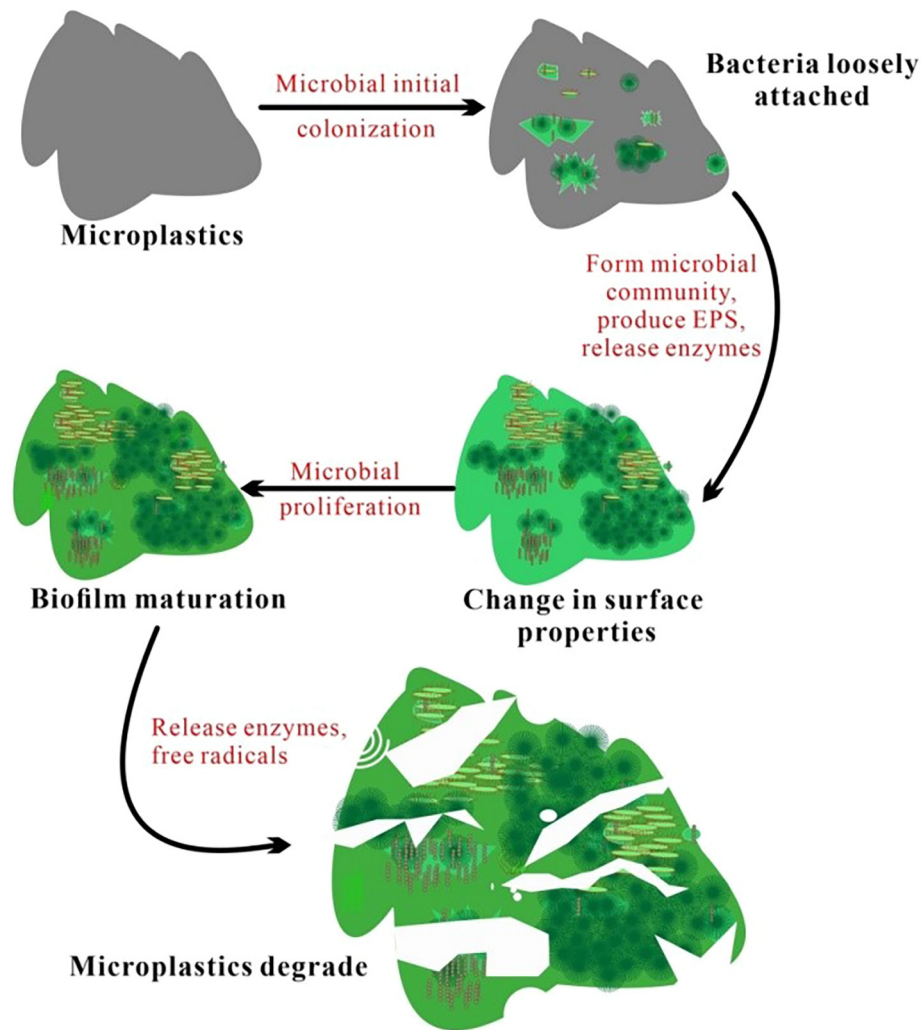


Fig. 2. Stages involved in the formation and degradation of biofilms on the surface of microplastic particles.

microorganisms attached to the surface of the substrate, followed by (3) the proliferation of these microorganisms on the surface of the substrate, and (4) the formation of biofilms on the surface of the substrate (Katakya and Knowles, 2018; Tu et al., 2020). As relatively large plastic items are converted into microplastics in the water environment, the specific surface area and adsorption capacity becomes greater (Anastopoulos et al., 2022; Sorasan et al., 2022).

3.2. Biofilm cultivation

Currently, the methods used to cultivate biofilms can be divided into two main types: *in situ* cultivation and laboratory cultivation. In general, *in situ* cultures are used to study the environmental behaviour of microplastics after their binding to biofilms. Laboratory cultivation is used to assess wastewater treatment technologies for biofilm degradation of microplastics; it has also been used in some studies on environmental behaviour (Table 2).

3.2.1. *In situ* cultivation

The *in situ* cultivation of biofilms on microplastics in natural water bodies, combined with regular sampling and analysis, mimics natural environmental conditions. The advantages of this approach include fast colonization and growth of diverse bacterial flora. Microplastics were placed in cylindrical stainless-steel cages, which were fixed in the Niushoushan River, Qinhuai River, and Donghu Lake in Nanjing, East China, and cultured *in situ* for 44 d to obtain mature biofilms for each

substrate (Miao et al., 2021b). However, the reproducibility of *in situ* experimental data is relatively low (Xie et al., 2021), and it is generally used to study the environmental behaviour and processes of microplastic degradation.

3.2.2. Laboratory cultivation

Laboratory cultivation refers to the collection of epiphytes from natural water bodies and their shipment to the laboratory for artificial cultivation of biofilms. After biofilms or cultures were formed, microplastics were added, and degradation of the microplastics was observed. For example, Faheem et al. (2020) obtained epiphytes from natural water bodies and brought them back to the laboratory, where they were placed in a low-temperature environment. Then, using modified Woods Hole culture (WC) media, biodegradability was determined after biofilm growth had stabilised.

Among the above culture methods, *in situ* culture can obtain flora similar to that in nature, but the culture time is relatively long, and the quality of the formed biofilm cannot be controlled. In contrast, laboratory culture can shorten the culture time to a great extent, and external conditions can be added to control the rate and quality of biofilm formation; however, the biofilm flora may be different from that of *in situ* culture. Environmental conditions strongly influence the growth of microorganisms, and microbes cultured in the laboratory will change in engineering applications. Therefore, strains that were not sensitive to the environment and played an efficient role in microplastic degradation were selected as the main strains for culturing biofilms. This is extremely helpful for the formation of biofilms

Table 2
Different biofilm cultivation methods.

Cultivation method	Training time	Carrier	Experiment method	Literature
<i>In situ</i> culture	20 d–30 d	Microplastics, glass beads, feathers	Carrier is placed in a natural water body until biofilm growth is complete, upon which it is taken out.	(Miao et al., 2019; Richard et al., 2019; Salomez et al., 2019)
	30 d, 75 d, 135 d	Microplastics	Microplastics in stainless-steel cages are suspended in seawater at various depths and retrieved after incubation.	(Tu et al., 2020)
	90 d	modified WC medium	Epiphytes are obtained from natural water bodies and brought back at low temperatures. Cultured using modified WC medium.	(Faheem et al., 2020)
Laboratory culture	21 d	WC medium	Natural water is collected and brought back to a water tank. Then it is exposed to natural light in the greenhouse, and the solar radiation is blocked with a black cloth. Dechlorinated tap water is added daily. The WC medium is pre-added to the experimental jar.	(Miao et al., 2019)
	7 d	Microplastics	Microplastics are packed into custom-made plexiglass columns blocked on both sides with nylon filters. Wastewater is mixed with glucose in glass bottles. The wastewater is slowly stirred and aerated. The glass bottle and the plexiglass column are connected using PTE tubing. Used a peristaltic pump to pump water through the packed bed column. The flow rate is adjusted to 1 cm/min. The column is periodically inverted until the incubation is complete.	(Sturm et al., 2022)

and the degradation of microplastics. Specific solutions for improving biofilm culture conditions to provide high-quality biofilms in a short period of time are presented in Section 4.2.

4. Factors affecting biofilm degradation of microplastics

The biofilm degradation process of microplastics is influenced by many factors (Fig. 3), mainly by two aspects: the properties of the microplastic itself and the rate and quality of biofilm formation on the surface of the microplastics. The characteristics of microplastics include chemical and physical properties. The factors affecting the rate of biofilm formation on the surface of microplastics include the physicochemical properties of microplastics and environmental factors (temperature, pH, UV radiation, and nutrients).

4.1. Characteristics of microplastics

Microplastics are essentially the same as plastics (Liu et al., 2022), and thus have a plastic-identical structure, chemical composition, and other essential characteristics. The composition of the microplastic polymer plays an important role in the biodegradation process. The presence of some functional groups is not conducive to the degradation of microplastics, for example, because the backbone of many polymers is completely composed of highly stable carbon-carbon (C—C) bonds (Chamas et al., 2020; Taniguchi et al., 2019), and there is a lack of enzymes in nature that can directly cleave C—C bonds (Taniguchi et al., 2019), so polymers are difficult to degrade in nature. However, the presence of some functional groups is beneficial for the degradation of microplastics. For example, biofilms preferentially hydrolyse plastics containing ester bonds and polyurethane components (Barlow et al., 2020), which are easily biodegraded by different microorganisms.

Likewise, the structure of microplastics themselves also affects degradation, and the degradation of plastics mainly depends on the amorphous regions of the polymer, which are more vulnerable to microbial attack than the crystalline regions (Restrepo-Flórez et al., 2014). In addition, owing to the hydrophobicity and high molecular weight of microplastics, which are hardly degraded in aquatic environments (Khoirani et al., 2020), one of the main limiting factors in the biodegradation of plastics is their high molecular weight, as they cannot be directly utilised by microorganisms, requiring extracellular enzymes to break down macromolecular polymers into small molecular products, which can be taken up and further metabolised by cells. Molecular weight is primarily affected by abiotic factors, such as ultraviolet radiation, rather than direct microbial attack (Restrepo-Flórez et al., 2014). Abiotic factors can produce low-molecular-weight degradation products and create cracks and pores on the polymer surface, thereby accelerating biodegradation (Zhang et al., 2021b). Plastics (such as PE, PP, PS, PET, and PVC) are highly hydrophobic, and the

hydrophobicity reflects the ability of the plastic surface to repel water (Zhang et al., 2021b), so the plastic itself is difficult to degrade without changing its hydrophobicity. In addition, polymer size affects the rate of polymer degradation, and it has been demonstrated that the smaller the size of the microplastics, the faster the degradation (Jiang et al., 2021).

Removing microplastics from the water environment has always been difficult and has a strong relationship with the characteristics of microplastics. The stability, hydrophobicity, high molecular weight, and other skeleton characteristics of the skeleton render the removal of microplastics difficult. The change in hydrophobicity and degradation of the crystalline region can be changed by screening and optimisation of specific strains, whereas the change in high molecular weight can only be changed by UV radiation. UV radiation can also make the microplastics age into smaller sizes; the change of these inherent characteristics can be beneficial for the degradation of microplastics. In addition, because of the small size of microplastics, it is difficult to collect them for centralised treatment. However, biofilms also have innate advantages. During the treatment process, the biofilm can enrich microplastics in the water, solving the problem that microplastics are difficult to collect.

4.2. Factors affecting biofilm formation on the surface of microplastics

The formation of biofilm on the surface of microplastics is a complex process influenced by several factors, mainly the physicochemical properties of microplastics (polymer type, roughness, hydrophobicity) and environmental factors (nutrients, temperature, salinity, pH) (Frère et al., 2018; Wang et al., 2020a; Yang et al., 2021).

4.2.1. Physicochemical properties of microplastics

The type (chemical composition) of the plastic polymer affects the biofilm growth. Bacterial communities tend to colonise a set of preferred substrates (Li et al., 2019). However, polymer type is not the primary driver of biofilm formation, nor does it control the later stages of biofilm formation and development (Rummel et al., 2017). Only a small fraction of the bacterial community exhibits polymer-type specificity during the initial stages of biofilm succession, and the colonization substrate is essential. Bhagwat et al. (2021), for example, studied the functional diversity of carbon metabolism in biofilms after *in situ* culture of PVC, PET, and glass carriers in three different locations for 44 d. Their results showed that the Shannon–Wiener diversity, Simpson diversity, and Shannon uniformity index varied in the order of glass > PVC > PET at all three sites. However, the biofilm and carbon metabolism rates were the lowest on the PET carrier.

The surface chemistry and structure of microplastics, such as surface roughness, hydrophobicity, crystallinity, and particle size, are essential factors affecting biofilm colonization. Rough surfaces provide more attachment sites for microorganisms than smooth surfaces, and microorganisms prefer to colonise rough and creviced areas (Feng et al., 2020; Parrish and

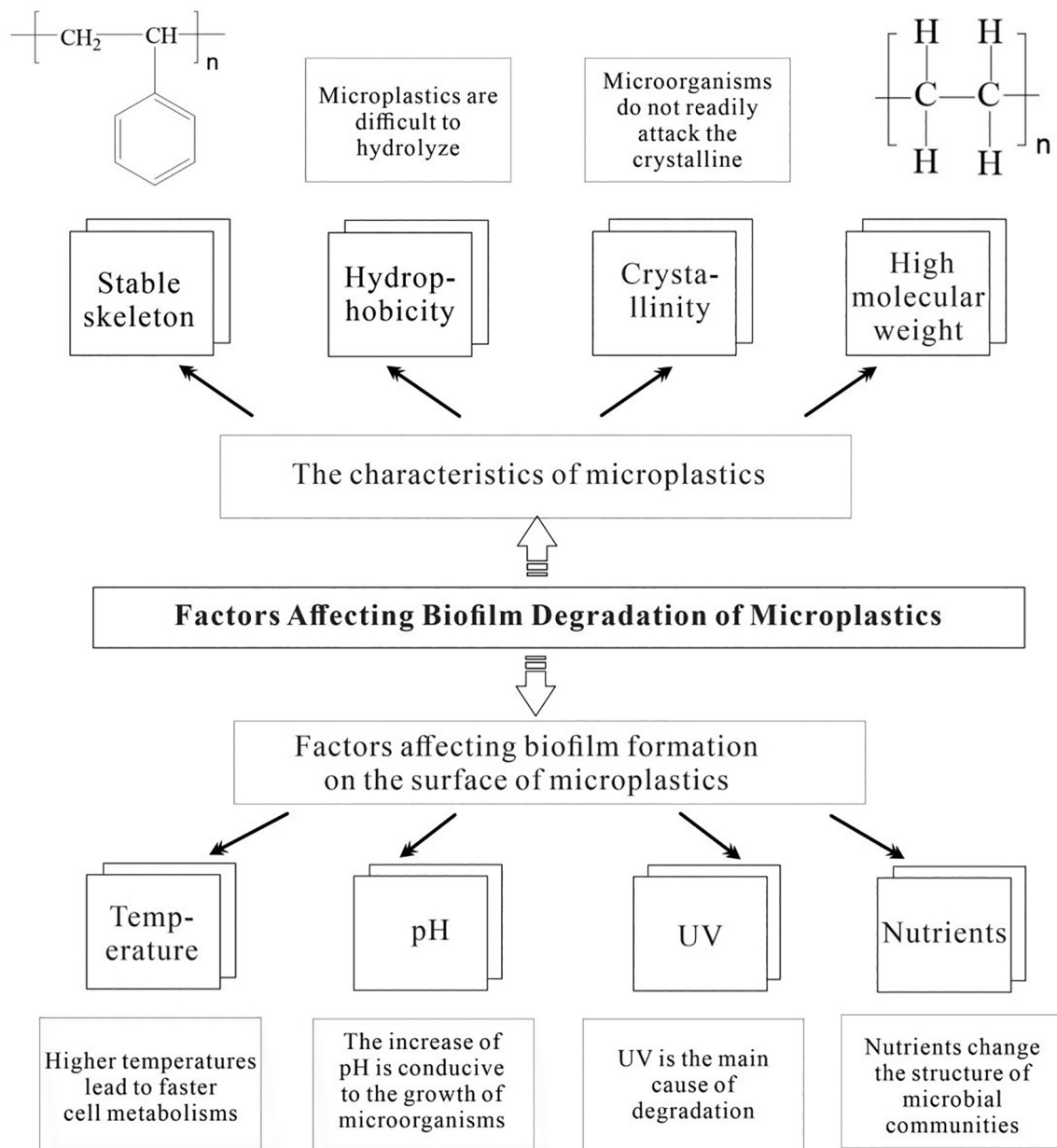


Fig. 3. Factors affecting biofilm degradation of microplastics.

Fahrenfeld, 2019). Inert surfaces can evenly distribute nutrients in the environment, providing more homogenised nutrient conditions for microorganisms (Zhao et al., 2021). After aging, the carbonyl index and surface area of the microplastics increased, and the cracks and roughness of the surface further increased. These changes are conducive to microbial colonization and biofilm growth (Luo et al., 2020). Many microplastics have hydrophobic surfaces that are not conducive to close contact between microorganisms and microplastics; as such, the colonization of microorganisms on the surface of microplastics and the formation of biofilms is slow (Gong et al., 2019). Higher bacterial diversity was also observed on substrates with low crystallinity but with higher hardness and surface roughness relative to smooth surfaces (McGivney et al., 2020). In terms of grain size, smaller microplastic surfaces are more prone to biofilm formation (Wu et al., 2022).

4.2.2. Environmental factors

Environmental factors have a greater and more long-term influence on microplastic surface biofilm formation than physicochemical properties (Wang et al., 2020a), particularly in terms of microbial colonization in both

freshwater and marine environments (Kesy et al., 2019). pH is the first step that directly affects biofilm formation, as it affects the production of bacterial biofilm mucilage, the activity of enzymes and microorganisms in the biofilm, and the degradation of microplastics (Auta et al., 2018). The biofilm growth rate is positively correlated with nutrient levels (TN and TP), and TN and TP are favourable for biofilm formation (Li et al., 2019). When the nutrient level was higher, the amount of biofilm on the surface of microplastics was significantly higher than that at a low nutrient level (Miao et al., 2020). Nutrient levels also change the microbial community structure, which influences the degradation of microplastics by microorganisms. Salinity is negatively correlated with biofilm growth and positively correlated with the bacterial community diversity. High salinity inhibits microbial activity and proliferation (Li et al., 2019; Miao et al., 2021b). Biofilm growth also varies with season. Summer biofilms are thicker and darker than winter biofilms because summer temperatures are higher and dissolved oxygen levels are lower (Chen et al., 2020b). Higher temperatures result in a higher diversity of microorganisms in the biofilm, leading to faster cell metabolism and degradation of microplastics (Chen et al., 2019; De Tender et al., 2017; Jin et al., 2020).

Finally, UV radiation indirectly affects biofilm growth through the effect of light on the organisms involved (Royer et al., 2018). In addition, UV radiation is considered the leading cause of the onset of microplastic degradation, where the molecular weight of microplastics changes under its action (Wang et al., 2021c; Zhang et al., 2021b).

In summary, to improve the efficiency of microplastic biofilm degradation, the following aspects should be considered: 1) Provide good environmental conditions. Environmental factors such as temperature, pH, and ultraviolet rays should be in the optimal range for the degradation process. 2) Change the composition of the microplastics. Biofilms preferentially degrade certain functional groups and amorphous regions in microplastics, making plastics containing such properties the focus of production. 3) Pre-treat microplastics at the beginning of degradation to change their hydrophobicity, molecular weight, roughness, size, and other conditions by physical and chemical means. Alternatively, strains of bacteria that affect the properties of the microplastic itself should be specifically cultivated to treat the microplastic before degradation begins. 4) Increase the rate of biofilm formation on the surface of microplastics. After screening for strains that are insensitive to environmental conditions and efficient for microplastic degradation, optimal environmental conditions should be provided to promote the growth of biofilms on the surface of microplastics. The efficiency of biofilm degradation of microplastics determines whether the technology can be engineered and applied; however, this is currently the biggest challenge. Therefore, further research in this area is required.

5. Mechanisms of microplastics degradation by biofilms

Biofilm degradation of microplastics is a function of the biofilm microorganisms, which cannot directly utilise macropolymers. After microplastics enter the biofilm, various extracellular oxidases and hydrolases break down the macromolecular polymers into oligomers and monomers before the microorganisms take up and metabolise these short-chain polymers further (Zhang et al., 2021b). Finally, in the presence of microorganisms, microplastics can be mineralised to produce carbon dioxide and water in the presence of microorganisms.

The process of biofilm degradation by microplastics is generally divided into four stages (as shown in Figs. 2 and 4). During the first stage, microorganisms (bacteria, fungi, prokaryotes) aggregate on the surface of microplastics and change their surface properties. The second stage of microbial degradation involves leaching of additives and monomers from microplastics. During the third stage, biologically derived enzymes or free radicals attack microplastics and their additives, resulting in microplastic embrittlement and loss of mechanical stability. The fourth stage is characterised by the penetration of water and microbial filaments into the polymer matrix, causing microplastics to be degraded by microorganisms (Flemming, 1998).

The second stage is considered to be the critical stage of degradation. Various additives are usually added to plastic products to improve or adjust their mechanical and chemical performance. When plastic waste is converted into microplastics, these additives remain; they are not easily leached by weak solvents, and their presence largely hinders the degradation of microplastics. Only after the additives are leached from the interior of the microplastics can they become embrittled or degraded during the subsequent degradation process. Other methods may be difficult to perform at this step, but the use of biofilms to degrade microplastics has excellent advantages. Microorganisms can metabolise polymer additives to promote the initial attachment of microbes to the particle surface and initiate the growth of biofilms (Wen et al., 2015). In this process, not only are the additives metabolised, but the growth of biofilms is also promoted, which assists in the process of biofilm degradation of microplastics. Screening and culturing of microorganisms that play a large role in the biofilm degradation of microplastics and applying them to microplastic degradation after obtaining optimal results will reduce the difficulty of microplastic degradation and increase the efficiency of biofilm degradation of microplastics.

6. Methods of analysing the biofilm degradation of microplastics

Biofilms can degrade microplastics by changing their physical, structural, and functional properties (Rummel et al., 2017). However, because of the solid form of microplastics, quantitative analysis and expression of the microplastic degradation rate have proven difficult and remain a hot topic in microplastic research. The analytical test methods for evaluating microplastic degradation are summarised in Table 3.

6.1. Weight loss method

The current method for assessing the degradation of microplastics involves measuring the loss in particle weight during a set period over which the degradational processes have been operating. Shabbir et al. (2020), for example, exposed three microplastics (PE, PP, and polyethylene terephthalate) for 60 d and determined that PP was degraded (in terms of weight loss) by 18 % in the presence of only glucose as an additional carbon source. In the absence of an additional carbon source, it degraded by only 13 %, demonstrating the importance of carbon source supplementation for the enhanced degradation of microplastics. Auta et al. (2022) mixed microbial degrading microbial strains isolated from mangrove soil with microplastics and found that after 90 d, the weight loss of PET was 18 %, and that of PS was 19 %.

6.2. Attenuated total reflection-Fourier transform infrared spectroscopy (FTIR-ATR)

The second approach to documenting the degradation of microplastics is to use FTIR-ATR to evaluate the structural changes in the polymer structure before and after a given degradation process. The appearance of new spectral peaks, stretching of C—C bonds, and formation of carbonyl compounds are all regarded as characteristics of microplastic degradation. Zhao et al. (2021) compared the surface of fresh PVC with that of PVC after 28 and 84 d of exposure to microbial cultures and found that the surface of the fresh PVC was relatively smooth. The treated microplastics exhibited bioadhesion, in which a layered biofilm structure appeared on the surface of the particle, along with some irregular cracks and pits, indicating that the surface texture of the microplastic particles was affected by the biofilms. Simultaneously, FTIR-ATR revealed a weakening of the functional group signal, suggesting that the microplastic surface was aging and biodegrading.

6.3. Scanning electron microscopy (SEM)

The third approach to assessing microplastic degradation is to compare the morphological characteristics of the surface of the microplastics before and after treatment using SEM. The development of cracks, grooves, pores, and other changes in surface roughness on the treated particle surfaces was considered an indicator of erosion and degradation by the biofilms. Faheem et al. (2020) injected microplastic particles, methane gas, and heavy metals into a serum bottle containing biofilms. After culturing for 20 d at a methane level of ≤ 100 ppm, SEM analysis showed that the particle surfaces developed visible concave concavities, such as grooves, cracks, and scratches. Similarly, Xie et al. (2021) exposed nine types of microplastics (PE, PS, expanded polystyrene, PP, polycarbonate, polyamide 6, PVC, polyethylene terephthalate, acrylonitrile butadiene styrene) from a mangrove nature reserve for a period of 1 month and 3 months. SEM revealed erosion marks on the surfaces of (polyamide 6, PE, and PVC samples after 3 months of *in situ* culture). Different *Bacillus* species were also found in the eroded pits, indicating biodegradation of the polymer.

6.4. Gel permeation chromatography (GPC)

The fourth analytical approach used GPC to measure the number-average molar mass (M_n), and weight-average molar mass (M_w) changes before and after degradation by the biofilms. For instance, Shabbir et al.

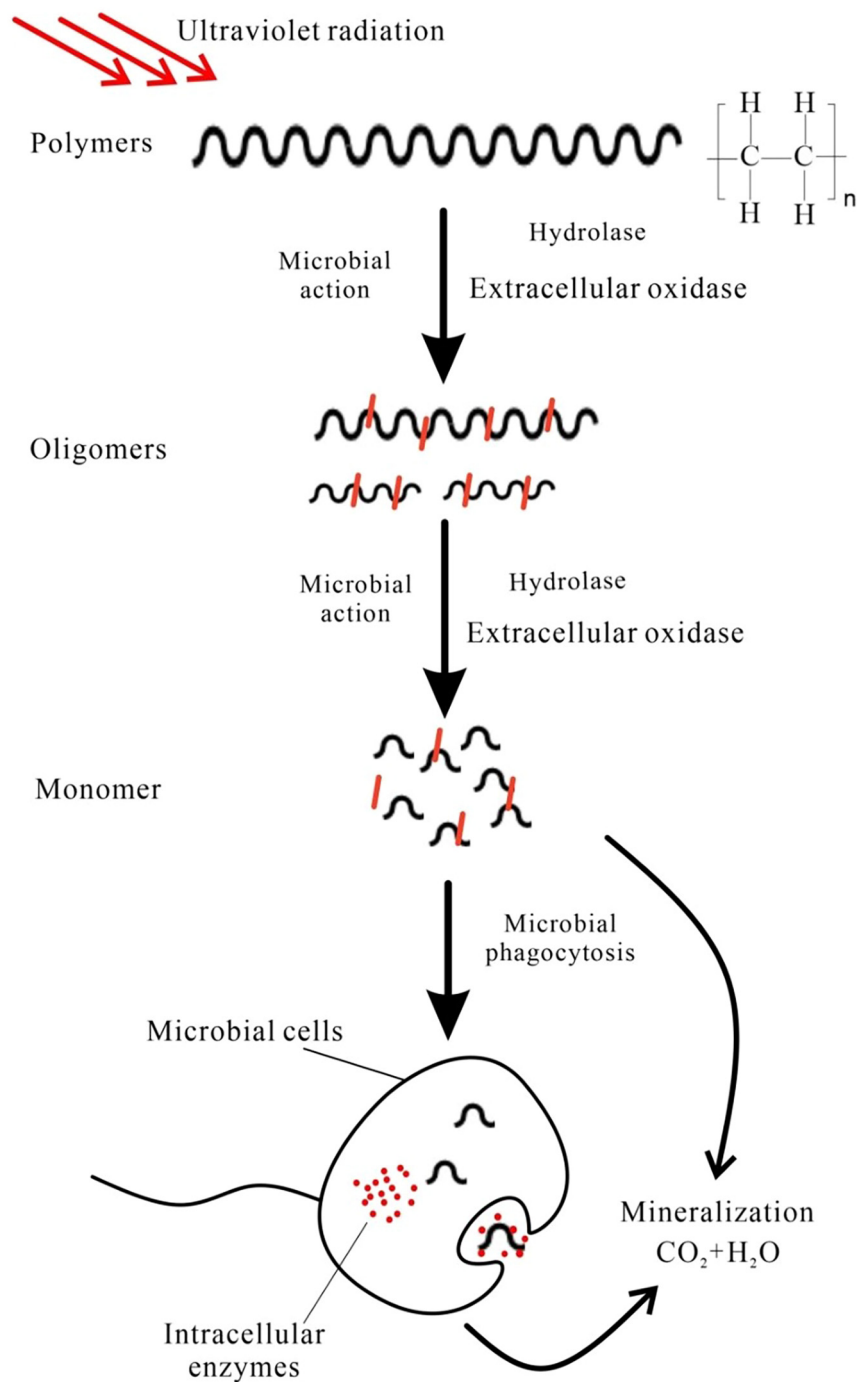


Fig. 4. Microplastic degradation mechanism.

(2020) used GPC to evaluate the biodegradation of three microplastics (PP, PE, and PET) by biofilms with and without different C sources. The M_n and M_w values decreased after 60 d, indicating that the microplastics were degraded by the biofilms. Among them, the changes in M_w and M_n were most apparent when glucose was used as an additional C source. The M_w and M_n values of PP decreased by 25 % and 14 %, respectively; of PE, by 35 % and 25 %, respectively; and of PET, by 28 % and 23 %, respectively. Other C-source processing cases M_w and M_n values also showed a reduction. Faheem et al. (2020) evaluated the effect of metal addition and methane oxidation on PE biodegradation after 20 d of GPC treatment. The M_n and M_w values were determined by GPC, indicating that the microplastics were biodegraded.

6.5. Assessment of microplastic degradation by physicochemical changes

The degradation of microplastics can also be assessed by changes in the physical (hydrophobicity, compressibility, crystallinity, and stiffness) and chemical properties of the microplastic surfaces. McGivney et al. (2020) incubated microplastics to create biofilms in experimental containers for 2 weeks and then tested the samples for compressibility (to detect changes in microplastic brittleness after biofilm formation), crystallinity (in which an increase in polymer crystallinity may promote the loss of compounds), and stiffness (a measure of how much force a material can withstand before it deforms). Compared to the control group, the crystallinity of PE increased, the stiffness of PP decreased, and the maximum compressibility

Table 3
Analytical test methods for evaluating microplastic degradation.

Analytical test methods	Test parameter	Degradation effect	Microplastic changes	Advantage	Limitation	Representational meaning	References
Gravimetric method	Microplastic weight	Weight loss	Microplastics are biodegraded	Degradation can be described intuitively	Number of samples required is high	Quantitatively describes the degree of degradation of microplastics	(Mughini-Gras et al., 2021; Shabbir et al., 2020)
Fourier transform infrared spectroscopy (FTIR-ATR)	Surface functional group changes	Appearance of new spectral peaks; double bond structure; formation of carbonyl, alcohol, and phenolic groups; weakening of functional group signals	Structural changes in polymers suggest the biodegradation of microplastics	Reflect changes in internal chemical bonds	Can only detect the nature of microplastic degradation, but cannot quantify the degree of degradation	Demonstrates the degradation of microplastics at the chemical structure level	(McGivney et al., 2020; Zhao et al., 2021)
Scanning Electron Microscope (SEM)	Surface morphological changes	Features, such as indentations, scratches, cracks, pits, and holes, etc.	Biofilm can degrade microplastics and can erode microplastics	Intuitive and clear image. Developed features can be used to study the structure of the biofilm	Physical analysis of microplastics only	Type of degradation indicated by characteristic changes in the surface structure of microplastics	(Xie et al., 2021; Zhao et al., 2021)
Energy Dispersive Spectroscopy Combined with Scanning Electron Microscopy (SEM-EDS)	Changes in microplastic surface elements	Incorporates changes in surface oxygen content by SEM surface morphology	Changes in surface morphology and elements	Reveals the correlation between changes in certain elements and biofilms	Only changes in surface elements of microplastics can be detected	Identification of microplastic samples, especially between carbon-based plastics and inorganic particles	(Wu et al., 2022)
Gel Permeation Chromatography (GPC)	Molecular weight change	Decrease in average molar mass (M_n) and weight-average molar mass (M_w)	Molecular weight reduction, polymer long-chain polymer depolymerisation	Characterisation of microplastic degradation from changes in molecular weight	Unable to indicate the degree of degradation of microplastics	Changes in the molecular dispersion index indicative of chain scission	(Faheem et al., 2020; Shabbir et al., 2020)
Differential scanning calorimetry (DSC)	Changes in crystallinity	Amorphous regions of microplastics degrade preferentially over crystalline parts	Amorphous region degrades first, followed by the degradation of the crystalline part	Reactive degradation changes inside microplastics	Degree of degradation of microplastics cannot be described.	Important criteria for assessing the biodegradation of microplastics.	(Tarafdar et al., 2021)
Contact Angle Tester	Changes in surface hydrophilicity	Change in contact angle	Changes in hydrophobicity of microplastics	Physically reflects the degradation of microplastics	Can only reflect changes in microplastics from a single perspective	Indicates biodegradable behaviour	(Sturm et al., 2022)
Confocal Laser Scanning Microscopy (CLSM)	Scope of microplastic biofilm coverage	Bacteria and EPS attached to microplastics	Biofilms are covered by bacteria, and EPS	Determines matrix composition in biofilms	Only a specific area can be observed	Observe different morphological characteristics of microorganisms; screening for biofilm coverage	(Salomez et al., 2019; Sturm et al., 2022)

of PS increased. These results show that microplastics biodegraded under the action of microorganisms within 90 d of exposure. In addition, the change in the contact angle (a parameter that measures the wettability of the liquid to the surface of the material) can also reflect the degradation of microplastics. The contact angle can indicate the hydrophobicity of different materials and is one of the key parameters driving microbial adhesion and controlling biofilm formation on material surfaces (Salomez et al., 2019). A larger contact angle indicates that the test material is more hydrophobic. Biofilms can reduce the hydrophobicity of microplastics and change their functional groups, thereby increasing the abundance of hydrophilic C—O and C=O groups (Shan et al., 2022; Tu et al., 2020). Sturm et al. (2022) cultured five types of microplastics in a packed bed column enriched in municipal sewage for 1 week. Biofilms formed on the surface of the microplastics. Contact angle measurements showed that PA and PES exhibited larger changes and became hydrophilic, whereas the contact angle of PVC increased, such that they became hydrophobic. The observed change may have been caused by biofilm biodegradation processes, including the potential degradation of additives contained within PVC.

Of the above approaches used to assess the degradation of microplastics by biofilms, the gravimetric method is the simplest and easiest. This is also the most widely used analytical method. However, this method requires a large number of samples. Infrared spectroscopy is used to detect changes in functional groups inside microplastics; it can only detect the degradation behaviour of microplastics but cannot quantify the degree of degradation. SEM

is used to observe changes on the surface of microplastics, and this method can only perform physical analysis of microplastics. However, some researchers believe that observing the number-average molar mass (M_n) may not be the best way to study the molecular weight reduction (Castro-Aguirre et al., 2018b). This is because M_n only reflects the change in molecular weight of the polymer, while the molecular weight distribution (MWD) can reflect more degradation behaviour. The shift of the MWD peak to the left represents a decrease in molecular weight and the broadening of the peak represents a higher polydispersity index (PI). The change from single to multiple peaks represents the rearrangement of newly formed short polymer chains into crystal structures. Higher and sharper peaks represent that the amorphous regions are being preferably degraded (Castro-Aguirre et al., 2018a). Therefore, measuring changes in MWD may provide a better understanding of biodegradation behaviour. However, this remains to be determined in future research. The use of biofilms to degrade microplastics is an extremely complex process. Future research should not only be limited to analysing conventional data such as weight loss, surface morphology changes, and changes in internal functional groups. It should also focus on the changes in the internal structure of microplastics and the study of their degradation behaviour.

7. The main flora of microplastic degradation

Biofilm degradation by microplastics relies on the combined action of many microorganisms (Table 4). Many strains can form biofilms that

Table 4
Bacteria responsible for microplastic degradation in biofilms.

Strain	Degradation time	Types of microplastics	Degradation effect	Literature
Proteobacteria, Deinococcus-Thermus, Cyanobacteria, Gammaproteobacteria, Alphaproteobacteria	20–60 d	PE	≥ 15 %	(Faheem et al., 2020; Shabbir et al., 2020)
Proteobacteria, Deinococcus-Thermus, Cyanobacteria	60 d	PP	≥ 10 %	(Shabbir et al., 2020)
Proteobacteria, Deinococcus-Thermus, Cyanobacteria	60 d	PET	≥ 15 %	(Shabbir et al., 2020)
<i>Sphingobium</i> , <i>Novosphingobium</i>	14 d	PS	–	(McGivney et al., 2020)
Alteromonadaceae, Cellvibrionaceae, Oceanospirillaceae, Proteobacteria, Bacteroidetes	28–84 d	PVC	–	(Zhao et al., 2021)

degrade microplastics (Table 4). For example, Shabbir et al. (2020) placed biofilms in flasks containing microplastics and an external carbon source for 60 d and detected 29 phyla in biofilms by MiSeq sequencing. The biofilms were dominated by four phyla: Proteobacteria, Cyanobacteria, Deinococcus-Thermus, and Bacteroidetes. The higher abundance of these phyla may be responsible for the higher biodegradation of microplastics. Zhao et al. (2021) identified 47 phyla in biofilms, of which 12 were dominant. Of these 12, the most abundant were Proteobacteria, followed by Bacteroidetes. Auta et al. (2022) isolated 22 bacteria from Matang, Cherating, Tanjung Piai, Sekam, Sedili Besar, and Pasir Puteh mangrove soils and found *Bacillus cereus*, *Alcaligenes faecalis*, *Bacillus sonorensis*, *Staphylococcus epidermidis*, *Bacillus vietnamensis*, *Rhodococcus ruber*, *Bacillus flexus*, *Sporosarcina globispora*, and *Bacillus gottheilii*, these nine bacteria can grow on Bushnell–Haas medium and can use PET and PS polymers as carbon sources, causing the degradation of microplastics.

Numerous studies have identified the flora in biofilms. However, most researchers have primarily used biofilms to study the degradation effect of microplastics; only a few have examined the actual bacterial species in biofilms and their importance in microplastic degradation. The lack of relevant studies may be because: (1) many species of bacteria occur in biofilms, and no single species of bacteria is responsible for the degradation of microplastics. This can lead to technical difficulties in isolating the flora; and (2) although there are many bacterial groups in the biofilm, the various microbial groups may play different roles. Some may change the surface properties of the particles (e.g., hydrophobicity and roughness), whereas others may release additives or promote microbial growth. Determining the role of each microbial group and cultivating it on demand is currently problematic.

Researchers have isolated specific flora and used them in degradation experiments. However, these bacteria originate from nature and animals. For example, Auta et al. (2022) successfully isolated nine bacteria that could degrade PET and PS from 22 bacterial groups isolated from mangrove soils. After 90 d of culture, the weight loss of PET in the experimental group was 18 %, whereas that of PS was 15 %. Lwanga et al. (2018) isolated bacteria from earthworm guts after 60 d of treatment with microplastics. After inoculating the isolated bacteria in a microplastic-containing mixture, bacterial consortia isolated from the earthworm gut were found to significantly reduced the size of microplastics within 4 weeks.

Researchers have yet to isolate microplastic-degrading strains in biofilms, cultivate them, and use them for microplastic degradation. The complex biological community formed by biofilms on microplastics is called a “plastisphere”. It is also the primary source of microorganisms that degrade microplastics (Rummel et al., 2017; Zettler et al., 2013). Biofilms formed on the surface of microplastics have a more stronger ability to degrade plastics than planktonic bacteria (Debroy et al., 2021). Therefore, in future research, microplastics can be considered a substrate for culturing biofilms. The biofilm strains that play a role in microplastic degradation can be extracted for targeted cultivation and applied to the degradation process of microplastics, improving the degradation efficiency of biofilm-degraded microplastics. However, obtaining strains suitable for commercial development remains challenging, and effective screening techniques are a prerequisite for isolating microplastic-degrading bacterial strains or consortia. The next question that should be addressed is how to isolate these bacterial groups efficiently and individually and cultivate

them in large quantities by artificial means. Using these specific bacterial groups for microplastic degradation may be an effective way to solve microplastic pollution.

8. Another possible use for biofilms

Although the advantages of biofilm-degrading microplastics are clear, they also have disadvantages. If the degradation efficiency cannot be improved, industrial applications are difficult. However, solving this problem in the short term may not be realistic. However, biofilms have another advantage: enrichment, which can be considered in freshwater environments, and in the tail water treatment of sewage treatment plants. Handling microplastics in freshwater environments is also challenging because of their small size. In wastewater treatment plants, treated wastewater still contains smaller microplastic particles (which may be more biologically toxic), and as the wastewater is discharged, they enter the circulation system of aquatic species, causing more serious ecological risks (Fang et al., 2022; Sun et al., 2019). Therefore, future research is expected to focus on the treatment of microplastics in freshwater environments and intercepting them from the source. Because adhesive EPS produced by biofilms can accumulate microplastics, researchers have designed a bacterial biofilm with a “capture-release mechanism”. The study grew strains of *Pseudomonas aeruginosa* under laboratory conditions, mixed their bacterial cultures with microplastics, and grew them together until they could colonise and capture the microplastic surfaces. It was found that all microplastics almost completely accumulated within 24 h, and the ability of the biofilm to accumulate microplastics did not differ from that in the laboratory (Liu et al., 2021). Although the method might have safety issues caused by genetically modified bacteria, it also proves the feasibility of using biofilms to enrich microplastics in an aqueous environment. Therefore, the most effective application of microplastic treatment with biofilms may be the enrichment of microplastics in freshwater environments and wastewater treatment plant tailwater with biofilms that have been cultured to maturity, in combination with other methods for microplastic treatment.

9. Conclusions and perspectives

In summary, the method of degrading microplastics using biofilms is feasible, but their degradation is currently not sufficiently significant. Previous studies have found that the highest degradation that can occur is approximately 20 %. The main reasons are as follows: 1. Structural stability: Microplastics are characterised by high molecular weight, hydrophobicity, structural stability, and large specific surface area, and microorganisms take a long time to change their intrinsic properties. 2. The biodegradation process of microplastics is carried out in multiple steps, which cannot occur simultaneously. The premise of microplastic degradation is to change its surface properties, such as hydrophobicity and roughness. This is the first step in the degradation of microplastic biofilms, followed by other processes. This results in slow progress in the degradation process. 3. Slow process of organism action: The biodegradation process is the physical degradation of plastics by organisms by biting, chewing, or digesting debris (Zhang et al., 2021b), which is also a slow process; thus, a long time is required for the entire degradation process. The rate and quality of biofilm formation on the surface of microplastics is secondary factor that also affects

microplastic degradation of microplastics to some extent; the onset of microplastic degradation depends on the time of biofilm formation. Based on these problems, this review summarises the advantages and disadvantages of existing technologies for biofilms and proposes feasible solutions.

This review divides the factors affecting the biofilm degradation of microplastics into two aspects: the inherent properties of microplastics and the rate of biofilm formation on the surface of microplastics. Regarding the intrinsic properties of microplastics, in addition to pre-treatment by physicochemical means, it may be more effective to prefer strains that have a specific effect on the intrinsic properties of microplastics. For example, strains that are efficient only in changing hydrophobicity and cultivating strains that are effective in degrading the crystalline zone of microplastics. In addition, good environmental conditions will positively affect the positive degradation of microplastics. The biofilm is the most important tool in the degradation process, and the rate and quality of its formation on the surface of microplastics is important. The faster the biofilm is formed, the earlier the process begins; the better it is formed, the better the degradation. Therefore, methods that can improve the efficiency of biofilm culture are summarised, such as providing the best environmental conditions and preferentially selecting strains for the culture that are not sensitive to the environment and play an efficient role in microplastic degradation. The degradation process is generally divided into four stages (microbial attachment changing surface properties, leaching of additives and monomers, embrittlement, and degradation). Additives in microplastics are very stable and are difficult to leach by normal techniques, whereas microorganisms in biofilms can use additives as nutrients to promote biofilm growth; therefore, we believe that the second stage may be the key stage for biofilm degradation. Optimising the culture of such microorganisms also improves the degradation efficiency of microplastics. After degradation, the analytical test methods used to evaluate the degree of degradation of microplastics were included. 1) describe the morphological changes on the surface - SEM, 2) evaluate the weight loss-weight method, 3) determine the internal chemical structure changes of microplastics (FTIR-ATR), 4) reflect the internal molecular weight changes of microplastics (GPC), and 5) indicate the physical and chemical changes of microplastics-contact angle (DSC). We believe that observing the internal structure of microplastics is more critical than observing visual factors, such as external morphology and weight changes. In addition, bacterial strains that play a role in the degradation of microplastic biofilms are summarised. A possible breakthrough problem for the realisation of microplastic degradation technology is the identification of the different roles of different microorganisms in biofilms and their targeted cultivation. Finally, because biofilms can enrich microplastics, possible applications of biofilms in freshwater environments and wastewater treatment plants are proposed.

Biofilm-degrading microplastics are ecologically environmentally friendly. However, there are inevitable shortcomings, such as long degradation times and unsatisfactory degradation degrees (amounts). Therefore, the development of this approach is in its infancy, and more research is required before its effective application.

Future research should consider the following:

- (1) Improving the speed and quality of biofilm formation: Biofilm formation requires a long time (1–3 months). The rates of formation rates differ under different experimental conditions, including changing environmental conditions, changes in the nature of the substrate, and differences in the cultivation methods. Methods that can be applied to improve the culture efficiency of biofilms will need to be devised in future research.
- (2) Efficiency improvement: The efficiency of using biofilms to degrade microplastics is currently not high enough to be applied as a remediation strategy. Obvious degradation characteristics have been observed on the surface of the microplastics after 10 d. Shortening the time for biofilms to degrade microplastics is another problem that researchers need to address.
- (3) Screening and effectively isolating different functional strains from biofilms and culturing them commercially: Current studies have

shown that specific microbial communities in biofilms can degrade microplastics, and strains with more efficient degradation effects may be obtained from biofilms cultured with microplastics as substrates, so microplastics can be considered as substrates for culturing biofilms, and then different functional strains from biofilms can be screened and cultured commercially, which may greatly improve microplastic degradation efficiency. However, obtaining strains suitable for commercial development remains a challenge. Effective isolation of microplastic-degrading bacterial strains is also a problem that must be addressed.

- (4) Development of new analytical test methods: The analytical test methods for microplastic degradation are still relatively fixed, and there is a need to develop analytical methods that can directly reflect the degree of microplastic degradation.
- (5) Using biofilms to enrich microplastics in an aqueous environment: Engineering technology for degrading microplastics using biofilms is still developing, and the current technology is not yet mature. In the short term, biofilms can be used to collect microplastics in freshwater environments and wastewater.

CRedit authorship contribution statement

writing—review and editing, X.-L.S.;
writing—original draft preparation, H.X.;
funding acquisition, H.-Q.X.;
conceptualization, Y.-C.F.;
review, Y.W.

All authors have read and agreed to the published version of the manuscript.

Funding

This study was sponsored by the General Project of the National Natural Science Foundation of China (31860126) and the Scientific Research Fund of the Yunnan Provincial Education Department, China (2022J0524).

Data availability

No data was used for the research described in the article.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Xiaolong SUN reports financial support was provided by the General Project of The National Natural Science Foundation of China (Grant number 31860126) and the Scientific Research Fund of Yunnan Provincial Education Department, China (Grant number 2022J0524). Xiaolong SUN reports a relationship with the General Project of The National Natural Science Foundation of China (Grant number 31860126) and the Scientific Research Fund of Yunnan Provincial Education Department, China (Grant number 2022J0524). that includes: funding grants.

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