

## **Bioremediation of Microplastics by Bacteria: Trends, Challenges and Perspectives**

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### **Abstract:**

*Microplastics, defined as particles <5 mm in diameter, are emerging environmental pollutants that pose a threat to ecosystems and human health. Ecosystems are under serious threat from microplastics contamination, which affects both biotic and abiotic elements. Diverse and extremely complex pollutants known as microplastics spread other toxins and bacteria. There are now several ways to get rid of microplastics, including recycling, landfilling, incineration, and biodegradation. This review systematically summarizes the factors affecting degradation of microplastics and proposes feasible methods to improve the efficiency of microplastics 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 metabolize additives in microplastics. The biodegradation mechanism is also described; microorganisms secreting extracellular oxidases and hydrolases are key factors for degradation. In this article, the origins, toxicity, and biodegradation of microplastics are reviewed. This article also highlights how bacteria contribute to biodegradation and propose biotechnological techniques to speed up the process, such as gene editing tools and bioinformatics.*

**Keywords:** *Microplastics; degradation; microbeads, bacteria; human health.*

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### **I. INTRODUCTION**

As the time goes by, we encounter the harmful effect of compounds that have recently been identified as a major risk to both human health and the natural environment leading to a very toxic and non-biodegradable condition for the planet's well-being. To retain an ethical surroundings for the advantage of polluted environment, the term "Bioremediation" is considered to be counted as an only support that is defined as the process of using microorganisms such as bacteria, algae, fungi, and plants to break down, change, remove, immobilize, or detoxify various physical and chemical pollutants in the environment. In simple words, Bioremediation is an effective cleaning technique and known to be cost-effective and practical solution for removing the environmental contaminants who is eventually gaining a lot of popularity. Using this process, harmful substances can be detoxified or degraded by providing the organisms with the nutrients and other chemicals they need to function optimally. Enzymes play a critical role in every stage of the metabolic process. It is part of the family of oxidoreductases, hydrolases, lyases and transferases. There must be enzymatic action on the pollutants in order for bioremediation to be successful. In order to speed up microbial growth and degradation, environmental parameters must often be manipulated during bioremediation. This is because bioremediation only works when the environment is right for microbes to grow and move around (Sarojbala et al., 2022). This proves that a bioremediation approach requires the use of microbial enzymes to break down hydrocarbons into less harmful compounds. In order for microorganisms to combat pollutants, they must come into contact with compounds that provide them with the energy and nutrients they need to multiply.

There are several factors such as physical, chemical and biological where thermal desorption, verification, encapsulation, vapour stripping, soil washing, sorting, and Electrokinetic remediation are examples of physical methods. These methods are usually low-efficient. Whereas, the chemical methods include different techniques like distillation, electro dialysis, chemical precipitation, and ion exchange. Chemical precipitation includes sulphide precipitation, hydroxide precipitation, chelating precipitation, Xanthate precipitation, and carbonate precipitation. Additionally, biological methods, as the alternative strategies for the remediation of heavy metals, are commonly used for their high efficiency, fast-growing, and easy control. Their primary procedure is Biosorption, which is a physiochemical technique in which pollutants like metal ions from an aqueous environment bind onto the presented functional groups on the organism's surfaces. This method is efficient and selective. Some groups of bacteria have the ability to transform pollutants like metallic ions into less harmful structures (MotahareHaghighatjoo et al., 2013) soil-type, carbon and nitrogen source, type of microorganisms and others that affect the process of bioremediation. One of the most economical and environmentally favourable biotechnological innovations is bioremediation. Waste management mainly relies on bioremediation. It is capable of eliminating the persistent organic pollutants, which are hard to break down and are thought to be heterologous biological substances (Sarjobala et al., 2022). The most common example of waste management that is the main reason behind a polluted environment and existence of bioremediation techniques is the accumulation of microplastics.

The term "Microplastics" (MP) was formally introduced in 2004 by Thompson et al., who alerted us to the growing problem of the plastic surrounding us. Since then, its presence in the environment has gained an increasing attention among the scientists, authorities, general population and in the media. Although neither official definition within the author exists, MP's are generally defined as plastic fragments smaller than 5 mm in any dimension with an indeterminate lower limit. However, studies showed certain discrepancies on the range of MP sizes as well as an evolution of the terminology according to their impact. MPs are often classified into two categories: Primary and secondary. Primary MPs are those already manufactured with a micro size, including the microspheres (<500 micrometer) contained in some cosmetic products, mixtures used for sandblasting/shot blasting and MPs employed as pharmaceutical vectors and to form 3D printing. Secondary MPs are the products of degradation of larger plastic materials, from mechanical or photo-oxidative pathways. MPs can also be classified by their form, commonly in fibers, fragments, and spherical bead, as well as by their chemical composition, for example, polyethylene (PE), low-density polyethylene (LDPE), Polyethylene terephthalate (PET), Polyacrylates (PA) and so on. MPs can be divided into many groups depending on the characteristics considered, describing a diversified class of materials that includes a wide range of polymer types, particle sizes (ranging over 6 orders of magnitude), shapes (from sphere to fibers), and chemical formulations (thousands of different types), which are likely to be found in water (Yolanda pico et al., 2019). Bioremediation has come a long way in terms of efficiency, cost, and social acceptability. Bioremediation research has largely focused on bacterial processes, which have numerous applications including a main role in degradation of microplastics (Sarjobala et al., 2022). The primary sources of MPs are plastic powder used in cosmetics, paint and coating, and detergents, however, waste plastics, abrasion of tires, urban dust, and synthetic clothes are known to be the secondary sources. Other sources of MPs are the plastics used in households, industry, fishing, and agriculture (PoritoshRoy et al., 2022).

## **II. Historical perspective**

Bakelite, the first synthetic plastic, was discovered in 1907. It transformed polymer research and modern life by introducing a slew of new polymers and plastic formulations into our daily lives, many of which are still available today. However, what was once considered revolutionary has steadily evolved into a global environmental hazard due to its widespread distribution in marine and freshwater habitats. Plastic objects deteriorate and break into smaller particles known as microplastics due to naturally occurring environmental conditions in these habitats, such as ocean current dynamics, sun radiation, abrasion and interactions with vessels and aquatic organisms (Chaudhry et al., 2021). So far only little information is known about how microplastics accumulated in marine sediments in the past, creating a hiatus in the evaluation, prevention, and control of plastic pollution. As during the 2000s, the microplastics were estimated to eventually sink into the seafloor and simultaneously accumulate in the sedimentary sequence. Yet, the pollution history of microplastics documented in these sediments is still poorly understood (M. Chen et al., 2020).

## **III. Origin of microplastics**

As affirmed above in the introduction, microplastics have been popular from the mouth of ours because it has been found in almost all objects. These MPs are typically used for various scrubbing, polishing, and cleaning, which can be found in consumer skin care products such as body cleansing, liquid soap, facial cleanser and toothpaste. These resins have been patented since 1980, with the trade name "Microbeads", micro-exfoliator or scrub. These microbeads are manufactured from many different types of plastics such as PE, PP, PET,

Polymethyl methacrylate (PMMA) and nylon in different shapes and sizes depending on the usage characteristics. In addition, the industry also uses small plastic resins made from acrylic, melanin and polyester for scrubbing, cleaning surfaces, rusting or polishing off metal surfaces by using a tool called air blasting machine (Sukritapunyauppa-path et al., 2019). Microplastics are mostly found in:

**Synthetic textiles:**

Polyester, nylon, acrylic and other synthetic fibers - each a form of plastics - make up 60 percent of the fabric content of our clothes. These synthetic microplastics fibers are cheap and versatile. The fibers create stretch and breathability in active wear, and warmth and sturdiness in winter clothes. Natural fibers like cotton shed too but while many natural fibers biodegrade, synthetics don't.

**Urban dust:**

Weathering, abrasion and detergents create urban dust from manmade products. The urban dust includes losses from the abrasion of objects like synthetic soles of footwear and synthetic cooking utensils, the abrasion of infrastructure like household dust, artificial turfs, harbors and marina building coatings. It also includes particles from the blasting of abrasives, weathering of plastic materials and use of detergents.

**Road markings:**

Crews apply road markings while building and maintaining roadways. The substances used include polymer tape and paint. The loss of microplastics may result from weathering or abrasion by vehicles. The materials are either spread by wind or washed off the roads by rain before reaching the surface waters and potentially the oceans.

**Marine coatings:**

Operators apply marine coatings to all parts of seagoing vessels for protection. That includes the hull, the superstructure and on-deck equipment. The materials involve solid coating, anticorrosive paint or antifouling paint. Developers use several types of plastics for marine coatings, including mostly polyurethane and epoxy coatings, vinyl and lacquers. Weathering and spills during application, maintenance and disposal of these coatings cause the release of primary microplastics.

**Personal care products:**

Microbeads are manufactured polyethylene plastic. It acts as an exfoliate, delivers active ingredients, and controls viscosity in health and beauty products. Upto 10 percent of some personal care product's weight is plastics. That's more than the packaging material. Some items have several thousand microbeads per gram of product. Once the personal care item is used, it ends up in wastewater. These tiny particles easily pass through the water filtration system and end up in our waterways (Horiba scientific).

#### **IV. Impact of microplastics on water bodies**

Water is an essential resource on the surface of the earth crucial for all industrial, agricultural, and human activities, as well as the biological process of all non-human beings, to sustain life. One of the emerging contaminants that seriously affects the quality of water are microplastics because their small size allows them to easily penetrate living cells and reach remote locations, exacerbating their potential harm (Osman et al., 2023). The ubiquitous presence of microplastics has emerged as a significant aquatic hazard as worldwide plastic production continues to accelerate. They contaminate water columns, sediments and biota of coastal waters, Open Ocean, freshwater environments and wastewater treatment plants worldwide. Microplastics' propensity to adsorb inorganic and organic contaminants and then release them into marine and freshwater systems is another source of concern. Thus, aquatic ecosystems are the foundation of material movement and energy flow on the planet, and they have also become a significant sink for plastic garbage. Microplastics enter the marine environment, mainly through anthropogenic activities (eg. aquaculture, fishing, tourism, industrial and domestic wastewater systems) and their distribution is quite diverse. Microplastics' physico-chemical properties (eg., size, specific density, charge and chemical composition), hydrodynamic factors, and environmental factors (eg., velocity, water currents, turbidity, density of water mass, temperature, and wind) all affect their transport dynamics and as a result, their distribution and accumulation in various marine areas (Chaudhary et al., 2021). It also has been stated that plastics pollution would have long-term impacts on their ecosystems as toxicity in water would increase due to microplastics in saline water, leading to eutrophication which can have potential impacts on aquatic organisms and other fauna. In addition, ever-increasing accumulation of microplastics in water leads to natural resource depletion with resulting effects of poverty and unsustainable living standards (Rakesh Kumar et al., 2022). So where these microplastics are originated from that they end up in the large water bodies? The answer can be land-based sources which are responsible for 80-90% of microplastics in

aquatic life. These sources include plastic bags, bottles, personal care products, construction materials, and clothing. Household products, waste generated items, food and drink packaging waste, waste generated from shipbuilding, sewage sludge and industrial activities, particularly those using granules and small resin pallets, are other probable sources of microplastics sources of microplastics discharge into the water bodies. Furthermore, face washes, hand soaps, hand gels, laundry detergents, washing powder, toothpaste, facial creams, cosmetics and shower gels are some of the common examples of microplastics carriers. Even the seaside tourism, commercial fishing, marine vessels and offshore industries plays a very important role in discarding the microplastics in the environment. Along with many sources like discarded or lost fishing gear, such as plastic monofilament lines and nylon nets, tire wear and tear of cars are a significant source of microplastics that can float at different depths in the ocean. Additionally, the presence of microplastics in freshwater is influenced by varying rainfall patterns (Osman et al., 2023).

It is evident that humans are exposed to microplastics through the consumption of marine food stuff, including shellfish, fish and sea salt. In addition to seafood, humans may be exposed to microplastics via other routes, including drinking water, bathing waters, inhalation from air and/or via active contact with cosmetics. Microplastics have also been detected in a variety of terrestrial foodstuffs such as honey, drinking water, beer, sugar and table salt (Andrady et al., 2015). The effect of these microplastics on human beings is very severe as it causes unexpected conditions such as metabolism disturbances, neurotoxicity, and increases in the chances of cancer. These small size plastics can absorb mainly persistent organic pollutants (POP's) and living organisms come into contact when they get introduced into the food web. This microplastics has the ability to absorb pathogens too. These microplastics are small in size, and due to that, they are present in the air and through them, they enter the respiratory system (AnujSharma et al., 2023). The size and density of particles will affect their deposition in the respiratory system, with smaller and less dense particles penetrating deeper into the lungs. After deposition, particle translocation might occur due to macrophage clearance or migration into the circulation of the lymphatic system (Chaudharyand Sachdeva, 2021).

**Table 1.** Characteristics of the common chemicals found in the microplastics.

Name	Acronym	Products	Density (g/ml)	Life span (years)
Polyethylene terephthalate	PET	Water bottles, liquid hand soap, mouthwash	1.35 - 1.45	20
Polyester	PES	Polyester clothes, Bottles	1.40	>20
Low density polyethylene	LDPE	Plastic bags, Squeeze bottles	0.92 - 0.93	-
High density polyethylene	HDPE	Detergent bottles, Bleaches	0.93 - 0.97	>28
Polyvinyl Chloride	PVC	Pipes, Electric cables, Clothing	1.21 - 1.45	140
Polypropylene	PP	Clothing, Jars, Stoppers	0.88 - 0.95	>100
Polyamide	PA	Packaging, Textile (Nylon), Toothbrush	1.14 - 1.35/1.41	>20
Polystyrene	PS	Ready to - eat food, Disposable cutlery, CD's and DVD cases	1.04 - 1.10	50
Acrylonitrile butadiene-styrene	ABS	Pipe systems, Musical instruments	1.04 - 1.06	-
Polytetrafluoroethylene	PTFE	Plain bearings, Gears, Slide plates, Seals, Gaskets, Bushings	2.11 - 2.31	>140

## V. PREVENTION MEASUREMENTS FOR ERADICATION OF MICROPLASTICS

Water quality, human activities, urbanization, and wastewater treatment technologies are key factors that regulate microplastics pollution levels in freshwater systems. Wetlands are among the largest ecosystems that receive microplastics from municipal, agricultural, and industrial wastewater, making them a significant sink for microplastics. The main focus of treatment strategies for microplastics is their removal from aquatic

ecosystems, where they often end up. There are two broad categories of techniques for microplastics removal: conventional and innovative strategies.

### **Conventional treatment techniques**

#### **Coagulation:**

It is one of the most frequently utilized techniques for wastewater treatment. It uses various chemical agents (Coagulants) to destabilize the dissolved and suspended particles and enables their removal by sedimentation. Different coagulants, such as iron-based and aluminum-based coagulants, have varied removal pathways for microplastics. However, traditional methods of microplastics removal, such as charge neutralization, adsorption, and sweep flocculation, remain relevant in describing their removal mechanisms.

#### **Membrane bioreactor technology:**

It is a reliable method for treating municipal and industrial wastewater that usually contains various concentrations of different contaminants based on nitrifying bacteria and other microorganisms. The notable positive aspects of using membrane technology are high effluent quality and good removal efficiency with a high rejection potency towards target pollutants.

#### **Rapid sand filtration:**

This technique removes the different contaminants, such as viruses and suspended solids of clay particles along with microplastics from wastewater. Rapid sand filtration being low sensitive to water quality parameters and high flow rate is effective for microplastics removal without the use of costly flocculating agents which adds to the overall costs of the filtration process.

#### **Adsorption:**

The adsorption technique's superior efficacy in removing microplastics from wastewater has been proved by using various adsorbents, including chitin and graphene oxide. In addition, other materials exhibited significant adsorption efficiency, achieving up to 100 % for microplastics such as layered double hydroxides.

### **Innovative treatment techniques**

#### **Photocatalytic degradation:**

The utilization of photodegradation has been recognized as a highly effective and promising method for treating toxic organic pollutants, including microplastics, in wastewater. A semiconductor material absorbs visible or ultraviolet light in this process, generating free radicals, including reactive oxygen species such as singlet oxygen and superoxide radicals, which further degrade the microplastics. The Photocatalytic semiconductor material absorbs light energy that exceeds its bandgap energy. It triggers an electron transfer from the valence band to the conduction band, creating holes in the valence band. This process ultimately generates superoxide and hydroxyl radicals which break down the microplastics.

#### **Electrochemical Oxidation:**

It is a sustainable and cost-effective technique for wastewater treatment that includes two methods, anodic oxidation and indirect cathode oxidation. This technique has been shown to effectively degrade various organic pollutants, including microplastics, antibiotics, antipyretics, and dyes, into simple and non-toxic products such as carbon dioxide and water vapour without adding chemical agents.

#### **Electrocoagulation:**

This process is a prosperous, sustainable and highly efficient technique for removing microplastics from wastewater, integrating the positive aspects of coagulation and electrochemistry. Electrocoagulation produces flocs from the cations formed by metallic electrodes under an electric current. Subsequently, this process leads to the formation of "micro-coagulants" and the loss of suspended particle stability due to coagulation (Osman et al., 2023).

#### **Bioaccumulation**

Microplastics are also known for their stability and inability to degrade, meaning they can persist in the environment for decades. The life cycle of microplastics which involves, "Bioaccumulation" as one this cycle usually begins with the release of primary or secondary microplastics into the terrestrial and aquatic ecosystems, followed by their transport into water systems. Consequently microplastics enter the food chain of aquatic microorganisms and undergo bioaccumulation in their tissues, gradually working their way up the trophic levels as zooplankton, small fish, larger fish, and other organisms consume them. Swallowing these pollutants has been shown to have toxic effects on aquatic life, including fish, oysters, mussels, and sea turtles, such as

compromising their immune and digestive systems and potentially leading to their demise. Furthermore, the cycle of microplastics in the environment continues as they may be excreted by humans or discharged as plastic waste materials (Osman et al., 2023). Bioaccumulation is of a major environmental concern. Thus, monitoring chemical concentrations in biota are widely and increasingly used for assessing the chemical status of aquatic ecosystems. To create awareness for critical issues and to mutually benefit from technical expertise and scientific findings, communication between risk assessment and monitoring communities' needs to be improved (Schafer et al., 2015).

**Table 2.** Bacteria involved in bioremediation process.

Bacteria	Enzymes	Name of the Scientist
<i>Cyanobacteria</i>	Acyl-ACP Reductase(AAR) and Aldehyde-DeformylatingOxygenase(ADO)	Stanier and colleagues
<i>Rhodococcusrhodochrous</i>	Aldoximedehydratase, nitrilase, nitrile hydratase and amidases	Zelman Waksman and H. Boyd woodruffopf
<i>Streptomycetales</i>	L-asparaginase, uricase and cholesterol oxidase	Waksman and Henrici
<i>Bacteroidetes</i>	CAZymes(Carbohydrate-degrading enzymes)	Veillon and Zuber
<i>Vibrio sp</i>	Extracellular proteolytic	FilippoPacini
<i>Bacillus cereus</i>	Lecithinase, gelatinase, lipase and protease	Frankland
<i>Salmonella</i>	Lipase, cutinase and hydrolases	Theobald Smith
<i>Ideonellasakaiensis</i>	PETase	KoheiOda
<i>Escherichia coli</i>	MHETase(Monohydroxyethyl terephthalate hydrolases)	Theodor Escherich
<i>Pseudomonas aeruginosa</i>	Homeostasis enzyme algI	Carle Gessard

Genetically engineered bacteria species	Targeted heavy metals
<i>Deinococcusradiodurans</i> strains; <i>E. coli</i> strain; <i>E. coli</i> JM109; <i>Acidithiobacillusferrooxidans</i> strain; <i>Pseudomonas</i> K-62; <i>Achromobactersp</i> AO22	Hg
<i>Escherichia coli</i> and <i>moraxella sp.</i>	Cd and Hg
<i>P. fluorescens</i> 4F39; <i>E. coli</i> SE5000	Ni
<i>P. putida</i> strain; <i>Methylococcuscapsulatus</i> (Bath)	Cr
<i>Pseudomonas fluroscenes</i> OS8; <i>Escherichia coli</i> MC1061; <i>Bacillus Subtilis</i> BR151; <i>Staphylococcus aureus</i> RN4220	Cd, Zn, Hg and Pb
<i>P. putida</i> 06909; <i>Caulobactercrescentus</i> JS4022/p723-6H; <i>B. Subtilis</i> BR151 (pTOO24)	Cd

**Table 3.** Genetically engineered bacteria involved in bioremediation of heavy metals.

### Bioremediation of microplastics

Most of the conventional methods discussed for the reuse of microplastics degradation include a primary method where the plastic scrap is re-introduced in the heating cycle of the processing unit, followed by the conversion of waste to new plastic products by blending it with a virgin polymer which can considerably reduce the cost of production. Sometimes, plastic wastes are chemically or thermo chemically altered to be recycled in the industrial loop. Currently, several physical, as well as chemical methods are popularly used for disposing of microplastics particles including incineration, landfilling, and recycling. Chemical recycling processes such as pyrolysis are extremely popular at the commercial level (Thiounn et al. 2020). For efficient biodegradation, several factors are required which include the availability of potential microbial degrading organisms which possess suitable enzymes and metabolic pathways and other environmental factors such as temperature, pH, salinity, and moisture content (Syranidou et al., 2019).

### Techniques to monitor microplastics biodegradation

Different techniques have been applied to study microbial degradation of microplastics, which includes weight loss measurement due to leaching, CO<sub>2</sub> production due to degradation of low molecular weight polymers and loss of additives which affect the strength of microplastics (Moreno et al.,2022). To get direct proof of the degradation process, morphological, chemical, thermal, and structural properties are investigated using various techniques/methods such as scanning electron microscopy, laser diffraction particle, differential scanning calorimetric, dynamic light scattering, X-ray diffraction, etc. (Huang et al.,2022). Chemical changes are usually tracked by vibrational spectroscopy techniques, such as Fourier transform infrared spectroscopy, nuclear magnetic resonance, mass spectrometry, and gas chromatography (Donelli et al.,2009).

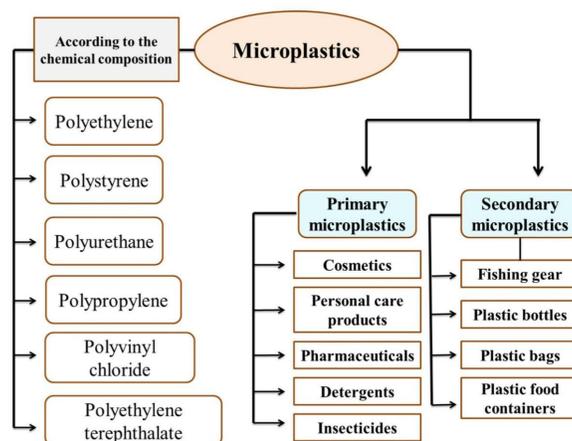


Fig 1. Classification of microplastics based on its chemical composition.  
(Ref - <https://doi.org/10.1007/s10311-023-01593-3>)

### Mechanisms of microplastics degradation by biofilms:

#### Biofilm Formation and Culture:

Biofilms can be subdivided into five types according to the substrate to which they are attached: Epiphyton (plants), epilithon (rocks), Epipelon(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).

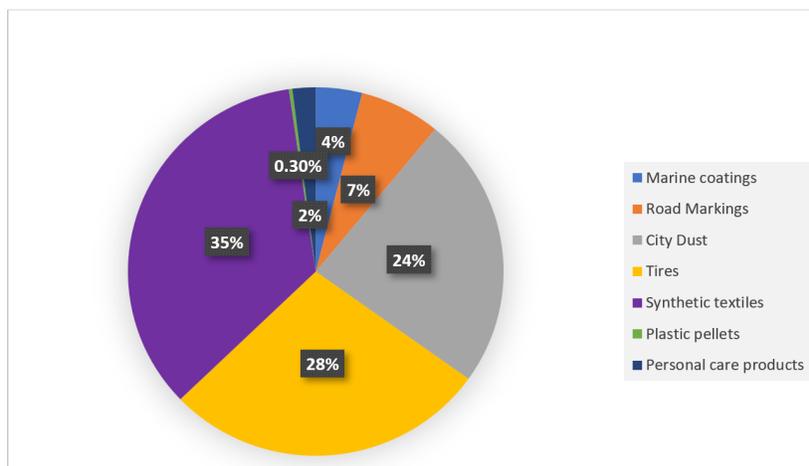


Fig 2. Distribution of microplastics sources.

### Biofilm cultivation:

Currently, the methods used to cultivate biofilms can be divided into two main types: In-situcultivation and Laboratory cultivation. In general, in situ cultures are used to study the environmental behavior of microplastics after their binding to biofilms. Laboratory cultivation is used to assesswastewater treatment technologies for biofilm degradation of microplastics.

### In-situ cultivation:

The in situ cultivation of biofilms on microplastics in natural water bodies, combined with regularsampling 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. 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.

### Laboratory cultivation:

Laboratory cultivation refers to the collection of epiphytes from natural water bodies and theirshipments to the laboratory for artificial cultivation of biofilms. After biofilms or cultures wereformed, 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 stabilized 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.

The process of biofilm degradation by microplastics is generally divided into four stages. 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 microplastics embrittlement and loss of mechanical stability. The fourth stage is characterized 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. Microorganisms can metabolize 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 metabolized, but the growth of biofilms is also promoted, which assists in the process of biofilm degradation of

microplastics. Screening and culturing of microorganisms that plays a large role in the biofilm degradation of microplastics and applying them to microplastics degradation after obtaining optimal results will reduce the difficulty of microplastics degradation and increase the efficiency of biofilm degradation of microplastics.

## VI. Bacterial Degradation of Microplastics

Bacteria capable of degrading microplastics have been isolated from a wide range of habitats including contaminated sediments, wastewater, sludge, compost, municipal landfills (Awasthi et al., 2020), and also from extreme climatic conditions like the Antarctic soils, mangrove, and marine sediments. Moreover, microplastics degrading microbes have also been isolated from the gut microflora of earthworms. It is generally reported that microbes living in polluted sites often develop an ability to activate the enzymatic system responsible for microplastics degradation. Both pure cultures and bacterial consortiums can be used for microplastics degradation. The pure cultures of bacteria isolated from most environments show that bacteria have diversity and functionality. Through the identification of a large amount of data, species belonging to various bacterial phyla, including Proteobacteria, Firmicutes and Actinobacteria, are able to degrade plastics. Most Potential bacteria that can biodegrade plastics were isolated from contaminated sites, such as landfill has been demonstrated that bacterial strains, such as *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Rhodococcus ruber*, and others may break down the thermoplastics PE and PET (Amaral-Zettler et al.). However, pure cultures present several advantages in the degradation process, offering a convenient way to study metabolic pathways involved in the process (Janssen et al. 2002). The main process of degradation is represented by physicochemical degradation which reduces the polymer length and alters the functional groups of microplastics, making them more susceptible to microbial enzyme activity. Biodegradation using enzymes involves the action of lipases, esterases, laccases, amidases, cutinases, hydrolases, and carboxylesterases (Barth et al., 2016). Thus, in-depth knowledge of the metabolic pathways and associated enzymes is necessary to perform an efficient biodegradation process. The earliest study of microplastics biodegrading microorganisms was conducted by Cacciari, using a consortium of *Pseudomonas chlororaphis*, *Pseudomonas stutzeri*, and *Vibrio sp.* to degrade polypropylene. A consortium of bacteria consisting of *Bacillus sp.* and *Paenibacillus sp.* was able to reduce the dry weight of microplastics by 14.7% in 60 days (Park and Kim et al. 2019). Moreover, Huerta Lwanga investigated the earthworm (*Lumbricus terrestris*)-mediated degradation of low-density polyethylene. The isolates from the gut included genera

Actinobacteria and Firmicutes which were also studied separately and observed to be able to degrade low-density polyethylene microplastics and release volatile compounds like eicosane, docosane, and tricosane. A consortium of *Enterobacter* and *Pseudomonas* from cow dung enhanced weight loss up to 15% within 120 days (Skariyachan et al., 2021). Several marine hydrocarbonoclastic bacteria such as *Alcanivorax borkumensis* showed the ability to degrade alkyl cycloalkanes, isoprenoid hydrocarbons, alkanes, and branched aliphatic compounds (Davoodi et al. 2020). It was also stated that the presence of alkanes modifies the cell membrane hydrophilicity and produces biosurfactants to interact with the plastic surface and the formation of COOH/OH and C=O functional groups. Several actinomycetes including *Rhodococcus ruber* and *Streptomyces* were also involved in polyethylene biodegradation (Sivan et al. 2011). Among the different genera of bacteria associated with microplastic degradation, 21% belonged to *Pseudomonas*, about 15% to *Bacillus* and 17% derived from mixtures of these two genera (Matjašič et al. 2021). Other bacteria associated with g biodegradation included *Enterobacter asburiae*, *Bacillus sp.*, *Nocardia asteroides*, *Rhodococcus rhodochrous* (Bonhomme et al. 2003), *Streptomyces badius*, *Rhodococcus ruber*, *Comamonas acidovorans* and *Clostridium thermocellum* (Paço et al. 2019), *Exiguobacterium sp.*, *Ideonella sakaiensis* (Tanasupawat et al. 2016), *Pseudomonas chlororaphis*, *Pseudomonas putida* AJ, and *Thermomonospora fusca* (Ghosh et al. 2013).

### Modern biotechnological method of degradation of microplastics

These techniques are used for the construction of novel pathways and can alter enzyme specificity and their affinity toward different microplastics. For successful gene editing, it is necessary to find suitable genes required for metabolizing and degrading microplastics and suitable host organisms like *E. Coli* in which these genes are expressed. The main processes involved are polymerase chain reaction, antisense ribonucleic acid (RNA) technology, and site-directed mutagenesis (Elsamahy et al).

### Gene editing tool:

Gene editing tools have been applied for genome engineering of plants, animals, and microorganisms for the expression of specific genes (Paço et al., 2019). Gene editing uses engineered nucleases, known as molecular scissors, to modify DNA sequences. CRISPR-Cas, ZFN, and TALENs are the main gene editing tools, and they work by introducing double strand breaks in the target gene sequence, which is then repaired by either a homology-directed repair (HRD) or a nonhomologous end joining (NHEJ) pathway (Arazoe, et. Al). Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated proteins (Cas) act as an

adaptive immune system of microbes by incorporating short sequences of invading genomes (spacers) into the CRISPR locus. There are three types of CRISPR systems and several subtypes which have been identified, of which the type II system is best characterized. It comprises Cas9 nuclease, the guide crRNA, and transactivatingcrRNA, which forms a CRISPR complex and associates with the target DNA using the mature guide crRNA. Cas9 endonuclease introduces double strand breaks which are then repaired by the host cell machinery, resulting in either insertion or deletion of genes, thus resulting in disruption of openreading frames of the genes. Thus, by using these gene-editing tools, knock-in or knock-out mutations can be introduced, wherein the expression of enzymes which play a crucial role in plastic degradation can be increased, or such genes coding for the enzymes could be introduced in the host microorganism (Agarwala, V et. al). CRISPR-Cas systems have been used by researchers in carrying out gene editing in *Pseudomonassp*(Vishwakarma et al., 2020) and *Escherichia coli* (Marshall et. al 2021).However, carrying out gene editing of indigenous microorganisms which are already present at the contaminated site would be more beneficial, as they have the ability to survive and harbor themselves in stress conditions.

#### **Bioinformatics:**

Bioinformatics has also become an effective tool for enhancing the biodegradation of plastic debris including microplastic particles (Purohit et al., 2020). Various databases, such as MetaCyc databases and BioCyc databases related to biodegradation pathways have been established to evaluate the process of biodegradation by providing information on the metabolic pathways, the microbial enzymes and genes associated with the process (Wicker et al. 2016). These databases and computational methods help to recognize enzymes involved in a metabolic pathway of interest and help in forecasting the biodegradation routes of toxic chemicals, providing a platform in which a novel approach for the biodegradation of plastic can be designed. The major disadvantage associated with bioinformatics is the lack of experimental data and its validation which is required for future research. Moreover, there is a wide knowledge gap between diverse groups of synthetic polymer degrader microorganisms and their responsible enzymes.(Abboud, 2022).

#### **Genetically modified organisms for bioremediation**

Bioremediation is a term that refers to a group of procedures that employ biological systems, such as indigenous or genetically modified organisms (GMOs) to restore or clean-up contaminated environments. The majority of indigenous bacteria are capable of successfully restoring the environment by oxidizing, immobilizing, and/or converting pollutants into carbon dioxide and water. As pollution level rise, researchers are looking at bioremediation using GMOs as for safer and more cost-effective than conventional treatment approaches. GMOs, such as bacteria, fungus, and algae can degrade toluene, naphthalene, camphor, halobenzoates, trichloroethylenes, etc. These designed GMOs appeared high effective than normal strains and have superior debasement adaptability, as well as the ability to quickly adapt to different contaminants as substrates or co-metabolize (Arunrajaet al., 2023).

Biodegradation is the main process for the removal of organic compounds from the environment, but proceeds slowly for many synthetic chemicals of environmental concern. Research on microbial biodegradation pathways revealed that recalcitrance is – among other factors – caused by biochemical blockages resulting in dysfunctional catabolic routes. This has raised interest in the possibility to construct microorganisms with improved catabolic activities by genetic engineering. Although this goal has been pursued for decades, no fill-scale applications have emerged, this perspective explores the lagging implementation of genetically engineered microorganisms in practical bioremediation (Jannsen et al., 2020).

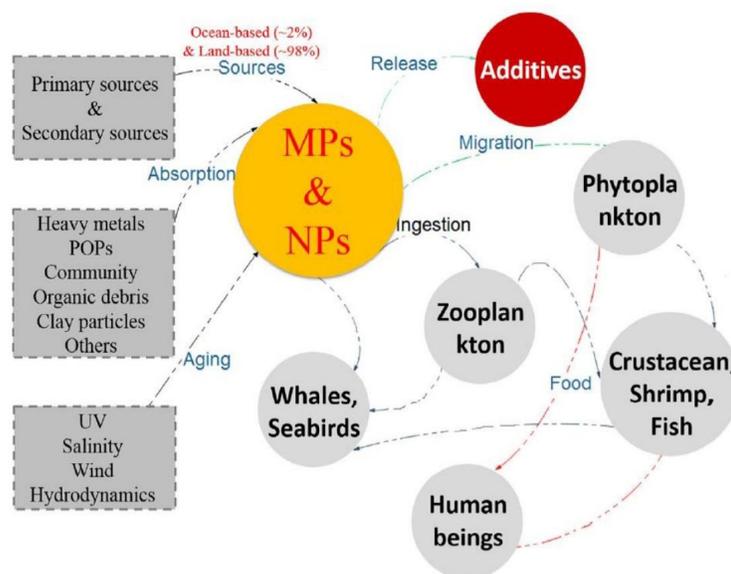


Fig 3. Bioaccumulation of microplastics from different sources (Sanjurjo, 2022).

### Advantages of Bioremediation

#### Eco-friendly:

The most significant advantage of adopting bioremediation technologies is the positive impact on the environment. Nature itself is used to fix nature in this process. Microbes that can degrade the pollutant multiply and produce harmless byproducts, unlike other waste removal techniques. The treatment's leftovers are often innocuous compounds such as carbon dioxide, water and cell biomass (Amrutha, 2023). By relying solely on natural processes, it minimizes damage to ecosystems (Sudrisha, 2022). Current way of remediating from large contaminates and acts as eco-friendly sustainable opportunities (Sharma, 2020).

#### Cost-effective:

Bioremediation is functional in a cost effective process as comparison to other conventional methods that are used for clean-up of toxic hazardous waste regularly for the treatment of oil contaminated sites. It also supports in complete degradation of the pollutants; many of the toxic hazardous compounds can be transformed to less harmful products and disposal of contaminated material (Sharma, 2020). Bioremediation is also a cost-effective alternative because it reduces the need for costly machinery and labor. The difference can be significant when compared to traditional environmental cleanup methods. By harnessing natural organisms, bioremediation achieve similar results with significantly lower costs. This is why bioremediation can be a great option when it comes to large-scale environmental remediation projects (Micheletty, 2023).

#### Scalable:

Bioremediation technology is easily scalable, allowing it to address a wide range of situations, from small landfills to large water treatment plants (Amrutha, 2023). Bioremediation is highly scalable and make sense regarding costs. It's the ideal solution for a wide range of environmental remediation needs. Regardless of project size or contamination present, bioremediation can accommodate the needs (Micheletty, 2023).

#### Highly Treatable:

As the microorganisms are employed in the process of bioremediation, they have the enzymes and the ability to degrade heavy contaminants. Organic pathogens, arsenic, fluoride, nitrate, volatile organic compounds, metals, and a variety of other pollutants such as ammonia and phosphates can all be treated by bioremediation. (Amrutha, 2023).

#### No risk of transportation:

In most cases, the process of cleaning requires huge equipment which also involves transportation and related risks. But this is not the case in bioremediation. For the most part, the work is done on-site by employing microorganisms. Thus, this method avoids the risks of transportation (Amrutha, 2023).

### **Disadvantages of Bioremediation**

#### **Biodegradable substances are only treated:**

The major shortcoming of bioremediation technology is that it can only deal with biodegradable substances (Amrutha, 2023). This method is susceptible to rapid and complete degradation. Not all compounds are disposed to quick and complete degradation process. Products of biodegradation may be more persistent or toxic than the parent compound in the environment (Sharma, 2020). It is limited to the compounds which are degradable because every compound in this biosphere is not degradable. It is not able to remove all kinds of impurities from the contaminated site. Like, some kind of inorganic contaminants cannot be treated with this bioremediation method (Srivastava, 2023).

#### **Harmful New Product:**

Researchers have also discovered that the new product created following biodegradation is sometimes more harmful to the environment than the original component (Amrutha, 2023). There are particular new products of biodegradation may be more toxic than the initial compounds and persist in the environment (Sharma, 2020). Some heavy metals cannot be completely broken down, resulting in toxic by-products (Srivastava, 2023).

#### **Time-Consuming:**

Bioremediation takes a large area and time from months to years (Srivastava, 2023). Finally, the procedure takes time, particularly ex-situ bioremediation, which necessitates excavation and pumping (Amrutha, 2023). It is demanding to encourage the process from bench and pilot-scale to large-scale field operations. Contaminants may be present as solids, liquids and gases. It often takes longer than other treatment preferences, such as excavation and removal of soil or incineration (Sharma, 2020).

## **VII. FUTURE PERSPECTIVES IN BIOREMEDIATION**

Bioremediation has the potential to restore contaminated environments inexpensively yet effectively. Lack of sufficient knowledge about the effect of various environmental factors on the rate and extent of biodegradation create a source of uncertainty. It is important to point out that many field tests have not been correctly designed, well controlled, or properly analyzed, leading to uncertainty when selecting response options. Hence, future fields' studies should invest serious efforts adopting scientifically legitimate approaches and acquiring the highest quality data possible. Moreover, a wide diversity of microbes with detoxification abilities is waiting to be explored. The inadequate knowledge about microbes and their natural role in the environment could affect the acceptability of their uses. The understanding of the diversity of microbial community's in petroleum contaminated environment is essential to get a better insight into potential oil degraders and to understand their genetics and biochemistry that will result in developing appropriate bioremediation strategies, thus, preserving the long-term sustainability of natural terrestrial and marine ecosystems.

Bioremediation is a collective phenomenon involving processes that use biological systems to either restore or clean-up contaminated sites. The microbial community is consistently reported for bioremediation. Most of the indigenous microbes have the ability to successfully bring up the environmental restoration via oxidizing, immobilizing, or transforming the contaminants. It aims to reduce or bring down pollutant levels up to undetectable, nontoxic or acceptable levels. The concept of bioremediation was first used on a large scale in 1972 for the cleaning of Sun Oil pipeline spill at Ambler, Pennsylvania. However, in laboratory-scale, George M. Robinson was the first to recognize this process during experiments on sewage and oil treatment. Subsequently, in 1992, Environmental Protection Agency (EPA) developed protocols for bioremediation on the basis of different case studies on bioremediation. The modern approaches of bioremediation are to search for a novel microorganism from contaminated sites. The isolated microbes are thought to have a strong potential to remediate pollutants. The use of genetically modified strains and also microbial consortium has been used directly or indirectly to increase the bioactivity of a bioremediant. Various mechanisms including bioaccumulation, biodegradation pathways and different modes for biosorption have also been investigated for the removal of pollutants.

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