

Review of microplastic degradation: Understanding metagenomic approaches for microplastic degrading organisms

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ABSTRACT

Environmental problems caused by plastic pollution are among the most pressing issues of our time. In recent years, metagenomics has become a powerful tool for understanding the microbial communities responsible for plastic biodegradation. In this review, recent developments and trends in metagenomics are discussed, and a comprehensive overview of the metagenomic methodology, analysis, and comparison of plastic-degrading bacteria is provided. In addition, the environmental consequences of plastic degradation are discussed, such as the impact on soil, water, and air quality, as well as the potential health risks posed by ingesting and inhaling microplastics. Possible solutions to the plastic degradation problem, such as using biodegradable materials and implementing recycling programs, are also explained. This review highlights the potential impact of metagenomics on the development of sustainable solutions to plastic pollution.

1. Introduction

Plastic waste, especially microplastics, has become one of the biggest contributors to environmental and health hazards in the last decade. They are found dispersed throughout the planet, contaminating all natural environments, including marine, terrestrial, and water bodies, from the deepest part of the sea, the Mariana trench, to the highest Himalayan mountains [1,109]. These contaminations have led to significant microplastic accumulation and distribution of plastic to a higher level in the food chain, eventually making its way into the human body [2]. The United Nations has classified plastic pollution as one of the ten

emerging environmental problems [3]. In 2015, more than six thousand metric tons of plastics were manufactured globally, of which 79% were accumulated in our environment, most notably in landfills. The growing worry surrounding the rise of plastic waste in ecosystems and its effects on organisms has prompted the development of biodegradable alternatives. Nonetheless, the extended degradation periods of these biodegradable plastics in natural environments indicate that they may still pose risks of ecological consequences. The precise impact of microplastics on organisms remains uncertain, particularly due to the utilization of particle concentrations in experimental exposures that greatly exceed those typically observed in natural ecosystems [116]. When this

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increasing rate of plastic production is paired with the current waste management system, by 2050, there will be about twice the amount of plastic waste in our environment [4]. To address this problem, an emphasis has been placed on using microorganisms and microbial enzymes to manage plastic waste sustainably. However, approximately 98% of microorganisms in microbial communities cannot be cultured under laboratory conditions, making the selection and characterization of countless plastic-degrading enzymes of microbial species arduous. With tremendous technological development and the collaboration of great minds in the field of bioinformatics and genome sequencing technologies, a new field has emerged, an amalgamation of the best of both worlds, metagenomics.

Metagenomics offers the solution to this microplastic crisis through next-generation sequencing (NGS), high throughput sequencing methods, shotgun metagenomics, and an array of modern omics, such as genomics, proteomics, and bioinformatics tools and software [110]. Analyzing metabolic pathways and microbial, phylogenetic, and functional diversity of uncultivable microbes using metagenomics provides crucial insights. The application of molecular biology and metagenomics has expanded our understanding and knowledge of the microbiome and its biological systems in polluted environments, allowing us to study microbial communities from highly contaminated sites [5,6]. This review article discusses the latest discoveries and trends in metagenomics, a comprehensive study on metagenomic methodology, analysis, and comparison of plastic-degrading microbes and their enzymes.

2. Elucidation of microplastics and their characteristics

2.1. Sources of microplastics

Plastics come in two varieties: large and small plastic waste less than 5 mm in size, known as microplastics. According to recent research, 8.3 billion tons of plastic have been generated worldwide since its created. An estimated 9% of this has been recycled, but the annual amount of plastic waste entering the ocean is between 4.8 and 12.7 million megatons. Considering these current estimations and efforts to quantify the issue, it is crucial to comprehend the connection between macro and microplastics [7]. Based on the annual garbage production per person, the proportion of plastic waste in that pollution and the proportion of poorly managed plastic waste that could end up in the ocean as plastic pollution indicate that there are more microplastics and larger plastics in the sea than the frequently stated average of 8 million metric tonnes [8]. The sources of microplastics considered here come from the roughly 300 million tons of plastics consumed worldwide. The primary uses are for plastic goods that start as pellets (85%), synthetic fabrics (11%), and synthetic rubber in tires (2%). The only losses that can be considered purposeful losses are losses from personal care products. Primary and secondary microplastics are the two types of microplastics that pollute the world's oceans. Primary microplastics are defined as plastics released directly into the environment as minute particles (Fig. 1). The first category of microplastics is purposefully added to water bodies. Whereas some secondary microplastics are the result of accidents during the synthesis, transportation, use, maintenance, or recycling of objects containing plastic through abrasion, weathering, or unintentional spills [9–11]. These fibers obtained through abrasion and fiber shedding, washing, etc., end up in the oceans. Significant numbers of these textile fibers have been discovered in both open-water and marine sediments through numerous in situ sampling studies [12]. The microplastics resulting from tires degradation are then dispersed by the wind or removed by rain into water bodies [108]. Marine coatings viz., Solid coatings, anticorrosive paint, and antifouling paint made from various plastics release microplastics during construction, maintenance, repair, and use (wear and tear) [13].

In personal care and cosmetic products, plastic microbeads cause plastic particles to be directly introduced into wastewater streams from residences, hotels, hospitals, and sporting venues such as beaches [14].

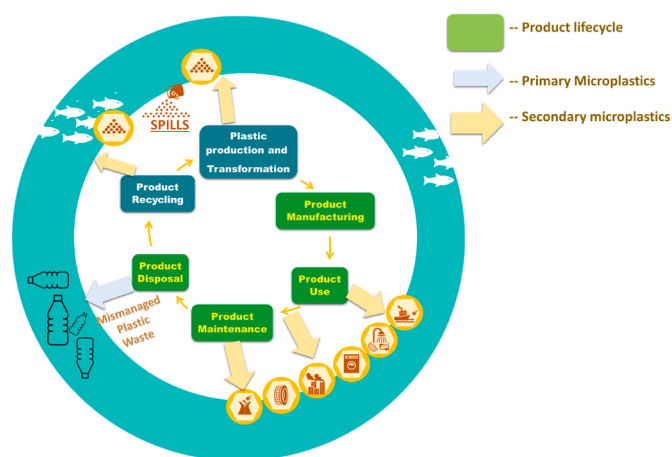


Fig. 1. Sources and distribution of various microplastics in the world's oceans.

(Fig. 2). City Dust is a catch-all name for a collection of nine causes recently identified in national assessments and most frequently seen in urban settings. City Dust includes losses from abrasion of infrastructure (home dust, city dust, artificial turfs, ports, and marinas, building coating), abrasion of objects (synthetic cooking utensils, synthetic shoe bottoms), abrasion of things, abrasive blasting, and deliberate pouring (detergents). These sources are combined because their separate contributions are negligible [15]. They eventually gather in gyres created by ocean currents. Estimates suggest that 93–268 kilotons of these microplastics floats in the waters [16]. Many microplastics will eventually accumulate in the deep sea and ultimately in food chains, as other types of microplastics, such as acrylic, are denser than saltwater and will most likely get deposited on the ocean bottom [17].

2.2. Types of microplastics

Microplastics are distinguished from larger plastic wastes, such as plastic bottles, containers, sheets, and waste plastic, by their size. Microplastics are currently classified into two types (Fig. 3). Any plastic pieces or particles that are 5.0 mm or smaller before entering the environment are considered the primary category of microplastics. Microfibers, beads, and plastic pellets used in clothing are a few examples (also known as nurdles). Secondary microplastics are created when larger plastic goods deteriorate in the environment due to normal weathering [18]. Secondary microplastics can come from various sources, including tea bags, fishing nets, water and soda bottles, plastic bags, microwave containers, and tire wear. Both types of contaminants are known to remain in the environment at high concentrations, especially in aquatic and marine ecosystems where they pollute the water. 35% of all ocean microplastics are made of textiles and apparel, mainly due to the normal evaporation of polyester, acrylic, or nylon garments. However, microplastics accumulate in the atmosphere and terrestrial ecosystems [19].

2.2.1. Primary microplastics

Small bits of plastic produced on purpose are known as primary microplastics. They are frequently discovered in cosmetics, facial cleaning products, and air-blasting technology. Their use as drug vectors in medicine has been described in some cases [20]. In exfoliating hand cleaners and facial scrubs, microplastic “scrubbers” have taken the role of naturally occurring substances such as powdered nut shells, oats, and pumice. For use in air blasting technology, primary microplastics have also been produced. Acrylic, melamine, or polyester microplastic scrubbers are fired at machinery, motors, and boat hulls to remove paint and rust (Gilbert, 2022). They are used repeatedly until their size decreases, and their cutting effectiveness is lost. These scrubbers usually

GLOBAL RELEASES TO THE WORLD OCEANS

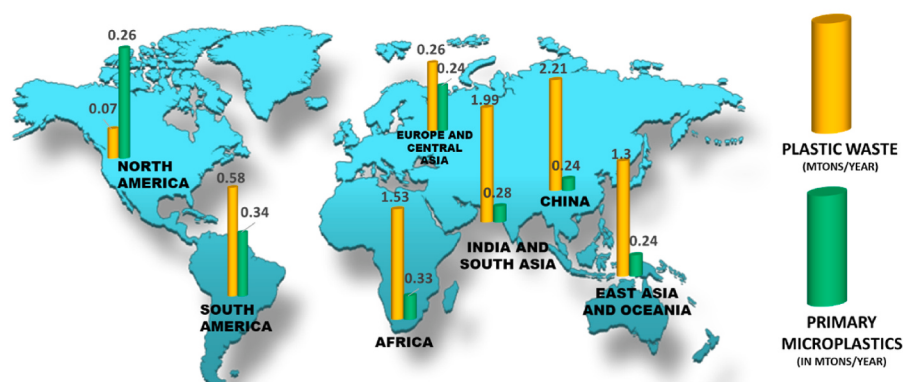


Fig. 2. Release of plastics, specifically primary microplastics, into the oceans by various parts of the world.

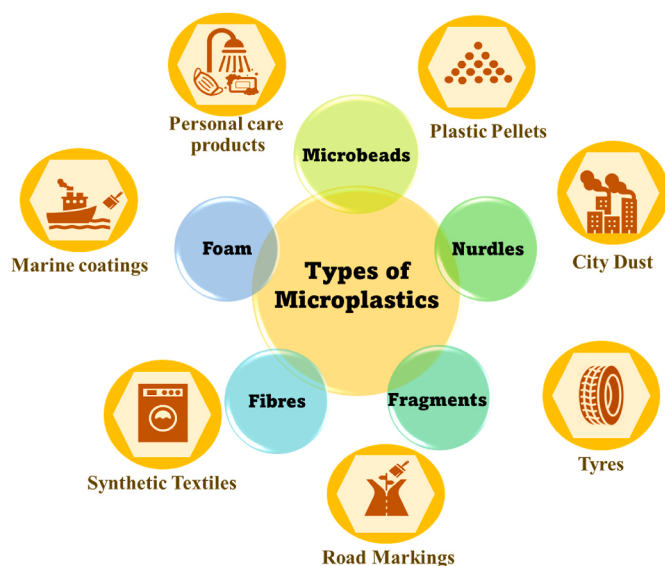


Fig. 3. Types of microplastics and where they are usually sourced from.

contain heavy metals, such as cadmium, chromium, and lead [21]. Although many businesses have pledged to cut back on microbead production, there are still plenty of bioplastic microbeads that degrade slowly, much like regular plastic [22].

2.2.2. Secondary microplastics

Secondary plastics are tiny fragments created as bigger pieces of plastic debris, both on land and at sea, break down [23]. A mixture of physical and biological means can gradually erode the structural integrity of plastic debris, and chemo-photodegradation processes, including photooxidation, are caused by sunlight exposure. Eventually, this can make the debris too small to be seen by the human eye. Fragmentation technique involves reducing large amounts of plastic material into significantly tiny pieces [24]. Although microplastics are believed to continue to break down and shrink in size, the smallest microplastic currently considered to have been found in the oceans has a diameter of 1.6 μm (6.31 in). The prevalence of microplastics in irregular forms indicates fragmentation is a significant source [25]. Some of them include Fibres [26,27], microbeads [28–30], polymer blends [113], Fragments [31], Nurdles [32–36], styrofoam [37–39]

2.3. Toxicity caused by microplastics

Microplastics, mainly in the range of 5 μm , are worldwide contaminants that are widely disseminated in the environment. They are constantly prevalent within human ecosystems. The scale of pollution, it is widespread and its long-term durability raises significant concerns about its impact on ecosystems, animals, and human health [40]. Microplastics have fatal impacts on the environment as they are abundantly distributed in the soil and aquatic ecosystems due to their minuscule particle size. They indirectly or directly affect plant life by clogging in plant parts such as roots, stems, or leaves, thus affecting their nutrient uptake. Toxic and hazardous chemicals are used during the process as additives to improve the properties of polymers and increase their useful life, thus transporting toxic chemicals across ecosystems [41,107]. The ecotoxicity of microplastics is seen not only in plants but also in animals. Microplastics are quickly taken up by the aquatic creatures and are transferred in the food chain to higher organisms, such as humans, thus becoming a source of concern for human health. Fish serve as one of the most integral biological models for assessing the toxicity of microplastics. Microplastic contamination of the aquatic ecosystem is one of the major concerns since they are easily ingested by the fauna in the waters and usually accumulate in their intestines. Several studies have proven the potential for microplastics to hinder reproduction capacity and cause fish organ failure [42,43].

At the apex of the food chain, humans are more vulnerable to microplastic contamination. Microplastics derived from the exhaust of gas and oil products tend to settle in household dust. They can be breathed due to their tiny size, and depending on individual sensitivity and particle qualities, they cause respiratory system lesions. Synthetic fibers of microplastics have been detected in human lungs by biopsies. Such airborne microplastics can cause injury or even death from chronic exposure due to their carcinogenic or mutagenic properties, potentially leading to cancer [44]. Microplastics have also been found to have a considerable influence on several regulatory enzymes, such as catalase, glutathione-S-transferase, and acetylcholinesterase [45]. Due to their ability to inhibit acetylcholinesterase, which results in an inflammatory reaction that may aid in the development of cancer, microplastics have also been shown to have neurotoxic effects. The interaction between humans and microplastics has been shown to affect cell function at the molecular level [46,47]. The inappropriate disposal of face masks worn during the COVID-19 pandemic contributes to the considerable amount of fibrous polypropylene microplastics found in non-woven fabrics. Again, this is another major factor responsible for the build-up of microplastics in air and water. Proper treatment of industrial effluents such as those of cosmetics, textiles, manufacturing, or other industries is

also crucial, as they tend to contain a high concentration of microplastics [48]. The impact of microplastics on living things may be broadly categorized into physical and chemical impacts. Physical effects include the size, structure, and concentration of microplastics, while chemical effects include their toxic traits [49].

3. Characterization of microplastic degrading microbes

3.1. Microbes and their enzyme action

The significance of microorganisms in plastic degradation in the natural environment is unclear. However, abiotic environmental degradation plays a significant role in the fragmentation of large plastic waste, leading to micro- and nano-plastic contamination. Recent reports suggest that several microorganisms can depolymerize artificial polymers in a laboratory environment [50,51]. Microbial biotechnology has frequently been suggested as a viable solution for sustainable plastic waste disposal, even if the actuality and potential of biotechnological recycling technologies are not yet clearly understood by scientific communities, plastic end users, and policymakers [52,111]. Microbial communities engaged in synthetic polymer degrading activities are a valuable source of enzymes. Biofilms that foul polyethylene terephthalate were reported to have undergone shotgun metagenomic sequencing by using ceramic, polyhydroxyalkanoate (PHA) and polyethylene terephthalate (PET) as the substrates at the sediment-water interface of a coastal lagoon [53]. PET plastic biofilms could not be distinguished from ceramic biofilm control. However, bioplastic biofilms of PHA could be identified because they were significantly enriched in phylogenetically diverse polyhydroxy butyrate (PHB) depolymerase and sulfate-reducing microorganisms (SRM). Here, it is seen how crucial the SRM of the plastisphere is to PHA biodegradation [53].

Numerous research studies have focused on the enzymatic breakdown of PET in the past ten years. The genome of the marine bacteria *Pseudomonas aestusnigri* included a unique PET hydrolyzing enzyme type IIa (PE-H), which was physically and functionally characterized. Amorphous PET was discovered to decompose at 30 °C via PE-H [54]. By rearranging the active site conformation in a Y250S variation, structural modeling and mutagenesis were used to gain new knowledge about the structural elements necessary for the effective degradation of polyester. This variant exhibit enhanced PET hydrolytic activity [105]. Although UV treatment significantly enhanced chain scissions at the surface layer of amorphous PET films, the resulting increase in surface crystallinity significantly decreased the effectiveness of enzymatic degradation [55]. The microbial metabolism of plastic monomers and additives will be a research focus on environmental degradation of plastic pollution and biotechnological plastic upcycling, i.e., the utilization of plastic hydrolysis as feedstocks for the microbial production of high-value chemicals. Engineered whole-cell catalysts have recently been identified to have a high potential for plastic degradation [56]. Depolymerases, which convert long-chain polymers into low molecular weight oligomers or monomers that can be taken up by microbial cells or broken down into CO₂, are secreted as the initial stage of the microbial degradation process. These depolymerization products could be utilized to manufacture high-value compounds via specific metabolic pathways, which align with the circular economy principle and could valorize plastic trash [57].

However, little is known about the depolymerase that aids in the decomposition of plastics. Therefore, future studies should focus on discovering additional depolymerase from microbes that degrade plastic. The efficiency of enzymatic breakdown must also be increased, which is a difficult task. On the one hand, the crystalline structures and cross-linking networks seen in the macromolecular aggregate structures of plastics prevent enzymatic breakdown [58]. These macromolecular aggregation formations may be disorganized and more amenable to enzymatic breakdown using physical pre-treatments like mechanical

grinding and irradiation. However, directed evolution and rational protein engineering are needed to increase depolymerization activity and stability, increasing the efficiency of enzymatic degradation [59]. Although depolymerases could break down long-chain polymer molecules into smaller pieces (monomers or oligomers), cells would have utilized these tiny depolymerization by-products as metabolic feedstocks [60]. The comparative list of plastic-degrading enzymes and microbes is given in Table 1.

3.2. Mechanism of plastic degradation

3.2.1. *Ideonella sakaiensis*

A novel strain of bacteria called *Ideonella sakaiensis* 201-F6 can use PET as its main source of carbon. Two hydrolytic enzymes, namely mono (2-hydroxyethyl) terephthalate hydrolase (MHETase) and PET hydrolase (PETase) produced by *I. sakaiensis*, can break down PET into its monomeric components [67]. PET is transformed into mono-(2-hydroxyethyl) terephthalate (MHET) by the enzyme PETase, a consensus α/β hydrolase enzyme with a well-characterized structural fold. The second most necessary enzyme, MHETase, hydrolyzes MHET to produce PET byproducts of terephthalate (TPA) and ethylene glycol. Together, PETase and MHETase break down PET through MHET in two stages, producing simpler components required for a new cycle of PET synthesis (Fig. 4). While MHETase is necessary to destroy PET, its exact function is unclear. PETase effectively acts on PET in its crystalline state and at the optimal temperature [68].

3.2.2. *Pseudomonas* sp.

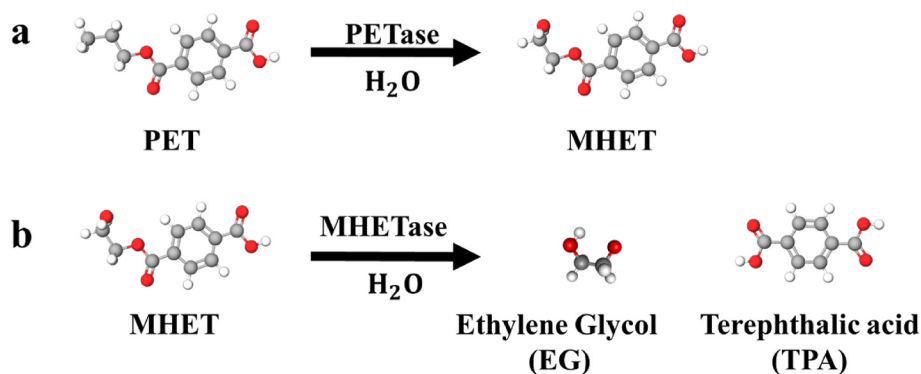
Pseudomonas sp. is a widespread and diverse microorganism genus with a total of 191 distinct species. As a result, they have a wide variety of properties, including plant growth factors, electroactivity, pathogenic activity, etc. Apart from these, the significant uses of *pseudomonas* sps. are bioremediation and plastic biodegradation. They produce simple enzymes to break down several types of polymers and aid in the degradation of PE, polystyrene (PS), polyurethane (PUR), and PET by employing primary enzymes such as hydrolase, alkene monooxygenase, esterase, lipase, and protease. The breakdown mechanism consists of two significant steps: the breakdown of macro-plastic into micro-plastic by extracellular enzymes such as depolymerase. This initial stage employs two distinct approaches, depending on the type of plastic: exo-attack (chain-terminal) for breaking smaller subunits and endo-attack (along the polymer chain) for molecular weight reduction. The tiny fragments of plastic traverse intracellular metabolic cycles such as the tricarboxylic acid (TCA) cycle and metabolic pathways, such as the catabolism pathway, in the second stage. Therefore, macroplastics would be completely decomposed into simpler compounds without toxicity [5,6].

PET is broken down into ethylene glycol (EG) and terephthalic acid (TPA) during the enzymatic degradation of polyethylene by *Pseudomonas*. Both products also gain new functional groups at the site of lysis. During hydrolysis, bis-(2-hydroxyethyl) terephthalate (BHET) and MHET are formed as intermediates that are converted into TPA and EG. The TPA is transported to the microbial cell by a TPA transporter, where it undergoes metabolic processes and is transformed into protocatechuic acid (PCA). PCA is metabolized in the TCA cycle. Likewise, EG is merged into the TCA cycle and biosynthesis process and metabolized. Enzymes such as hydrolase and polyethylene terephthalate are degraded by alkane hydroxylase (PET). The metabolic pathway consists of various phases, including oxidation, dehydrogenation, and breakage of carbon-carbon bonds, in the enzymatic degradation of PE. The PE is broken down into acetic acid, a new functional group, and then it is integrated into the TCA cycle. Metabolic hydrocarbon products of about 20 carbon atoms are transported directly into the microbial cell for the final breakdown. Hydrocarbons larger than 20 carbon atoms are metabolized outside the cell until through they can pass the microbial cell wall. And enzymes such as esterase and alkene monooxygenase will help in the

Table 1

List of plastic degrading microbes and enzymes.

Enzyme	Types of plastic	E.C number	Microorganism/s	Mechanism	Sample collection location	Reference
poly(3-hydroxyoctanoate) depolymerase	Polythene (PE)	EC 3.1.1.76	<i>Pseudomonas</i> sp., <i>Comamonas</i> sp	Hydrolysis	Gujrat, India	[5,6]
poly (ethylene terephthalate) hydrolase	Polyethylene Terephthalate (PET)	EC 3.1.1.101	<i>Fusarium oxysporum</i>	Hydrolysis	Gujrat, India	[5,6]
The cutinase-like enzyme (CLE)	PET	EC 3.1.1.74 (cutinase)	<i>Pseudozyma</i> and <i>Cryptococcus</i>	acid/base mechanism - acylation and deacylation processes	-	[61]
MHETase	PET	EC 3.1.1.102	<i>Ideonella sakaiensis</i>	Hydrolase	Sakai City, Japan	[62]
PET hydrolases	PET	EC 3.1.1.101	<i>Ideonella sakaiensis</i>	cleave internal ester bonds	Sakai City, Japan	[62]
Cut 190	PET	-	<i>Thermophilic actinomycetes</i>	Hydrolyze	-	[63]
T manganese peroxidase (MnP)	PE	EC 1.11.1.13	<i>Irpex lacteus</i>	Degradation of Polyethylene membrane	-	[64]
-	Polythene and other plastic	-	Bacteria: <i>Pseudomonas</i> sp., <i>Staphylococcus</i> sp., <i>Moraxella</i> sp., <i>Micrococcus</i> sp., <i>Streptococcus</i> sp. Fungi: <i>Aspergillus glaucus</i> , <i>Aspergillus niger</i>	-	Mangrove soil	[65]
-	Low-Density Polyethylene (LDPE)	-	Bacteria: <i>Bacillus</i> sp., <i>Pseudomonas</i> sp., <i>Streptococcus</i> sp. Fungi: <i>Aspergillus</i> sp., <i>Fusarium</i> sp	-	dumped soil, Chennai	[66]

**Fig. 4.** PET degradation involving 2 different enzymes.

degradation of polystyrene [69].

3.3. Recent discoveries

Due to the possible risk, they pose to aquatic life and human health, as previously indicated, and their link with invasive microorganisms, microplastics in the ecosystem are currently a significant source of environmental concern [70]. Microplastics are ubiquitous in many environments, particularly aquatic and soil biomes [70–72]. Recent studies have revealed that microplastics are essential vectors for microorganisms that could form fully developed biofilms on this artificial substrate [71,72]. Microorganisms play a vital role in the breakdown of microplastics because they control nutrient cycling in the immediate environment, which links biotic and abiotic processes [73]. They have evolved enzymes to digest plastic particles into assimilable carbon sources, as they have unfortunately become prevalent in the environment [74]. The breakdown of microplastic particles is significantly influenced by abiotic environmental deterioration. However, research on the effects of bacteria on biotic circumstances is ongoing [6,75,76]. Environmental conditions like pH, temperature, etc., should also go hand in hand with the available microplastic-degrading microbes in the

immediate circle [77].

Microplastic-associated biofilms are a prominent microplastic aggregation observed in the aquatic environment. Specific bacterial communities play an important role in the production of these biofilms. Microplastic biofilms selectively enhance certain pathogenic bacteria [70]. As we dig deeper into the processes, one fact is that biofilms have pros and cons. Both merge at the point where biofilms play an influential role in the development of microbial biocommunities [73]. In the absence of microbial activity, plastics slowly degrade, with half-lives ranging from hundreds to eons, depending on the polymeric material and the characteristics of the environment [74]. As mentioned above, microbial populations provide a rich source of enzymes that break down plastics. One such organism is *pseudomonas*, whose enzymes are extensively studied because of their ability, especially in the upcycling process [6,75]. The researchers found that a bacterial consortium significantly altered the surface topography and rheological properties of the degraded polyethylene surface by forming a biofilm. These findings showed that, like pure fungal cultures, bacterial consortia could accumulate as harmful biofilms on the surface of microplastics [77].

The degradation of plastic polymers is one of the most studied effects of microbial communities on microplastics. Recent research has

identified primary bacterial genera capable of dissolving poly (3-hydroxybutyrate-co-3-hydroxyhexanoate (PHBH) biofilms such as *Alteromonadaceae* and *Burkholderiales*. *Alcanivorax borkumensis* that grows in microplastic biofilms appeared significant in the degradation process of low-density polyethylene. Furthermore, it has been shown that *Erythrobacter* species in microplastic biofilms break down hydrocarbons [70]. According to a recent study, aquatic bacteria have adapted to plastics as a surface for colonization and may even break them down. For instance, numerous types of plastic, including macro- and microplastics, have been found to contain *Erythrobacteraceae*, a common aquatic bacterium that colonizes plastic [73]. PET plastics are one of the most common and widely used materials. In its macro or microform, PET degradation or hydrolysis is prompted by one of the most efficient enzymes, named IsPETase, isolated from *Ideonella sakaiensis*. In recent years, much research has been aimed at improving the stability and efficacy of IsPETase. Numerous recent reports have detailed point mutations that have been systematically engineered to make IsPETase a better fit for industrial use for the degradation of microplastics [74]. Another organism, *Pseudomonas aestusnigri*, with its enzyme PE-H at 30 °C was found to break down amorphous PET. A Y250S variant was created using structural modeling and mutagenesis due to the rearrangement of the active site conformation. This variant showed improved PET hydrolytic activity and novel structural qualities required for efficient polyester degradation [6,75].

An enzyme or a couple of enzymes with the capability of degradation are not enough for the enormous amount of microplastic that must be washed off from the biosystem. To at least achieve the primary goal of degrading the microplastics, the combination of different enzymes from a particular type of organism is highly preferred, i.e., a bacterial, fungal, etc. This application could eliminate toxicity or toxic metabolites formed during degradation [77]. In general, the composition of the microbial community and essential microbial respiratory processes of the bacteria are influenced by the redox environment. As a result, microbes can interact actively with microplastics, coupling accessible redox mediators to polymer breakdown [73]. As always said, no matter the numerous mechanisms and biodegradation processes, curbing the actual problem is to lessening the use of any type of plastic or at least decrease the habit of inconsiderable disposal of plastic waste [71,72]. Recent studies indicate that fish, crabs, and other aquatic creatures readily consume microplastics. This, in turn, leads to bio amplification if plastics enter the food chain [70]. Another critical research to improve sustainability in food and agriculture is innovation in the performance and economics of bioderived and biodegradable plastics that avoid microplastic accumulation. It is strongly recommended that the food and agriculture sectors invest heavily in biodegradation technology to reduce microplastics in food and agricultural goods [74].

4. Metagenomic analysis

Various microbial communities can be drawn out, which, possibly, with the help of the enzymes they manufacture, contribute to the breakdown of microplastics. Not all enzymes produced by the microbial population are efficient enough to participate in the degradation process. To understand the nature of the microbial community and anticipate its ability to participate in in-situ biodegradation, a metagenomic study of the microbial population participating in plastic biodegradation is recommended as a solution to this problem [78]. Detailed metagenomic analysis of microbiomes and mining of associated genes and enzymes involved in biodegradation is now possible because of advances in bioinformatics and sequencing methods. Therefore, metagenomics could be a valuable method to discover effective plastic-degrading genes and enzymes in uncultivable microbial populations [104,112]. The metagenomic analysis evaluated the genomic capabilities of aquatic plastic biofilms, as well as the levels of protein expression [79]. As the previous topics suggest, biofilms are one of the peculiar properties encountered in the process of degradation of

microplastics. The composition or characterization of these biofilms depends on the strata or atmospheric level at which they are formed. This can be proved by shotgun metagenomic studies. At the sediment-water interface of a coastal lagoon, biofilms that break down plastic and microcosms made of bioplastic were shotgun metagenomic sequenced. According to a study conducted by Pinnell and Turner in 2019, plastic biofilms showed the same community composition as the ceramic biofilm control. This finding suggests that plastic-degrading microorganisms can be investigated through metagenetic studies. By examining the microbial community within the “plastisphere” and identifying novel genes or enzymes involved in the degradation of various polymers, we can uncover a vast and unexplored microbial gene pool. This approach holds great potential for biotechnological applications and further advancement in the field of valorization, as highlighted by Kirstein et al. [84].

According to environmental microbiologists, only 2% of entire microbiomes can be grown in the research lab, leaving a large fraction of uncultured fungus, bacteria, and other microorganisms undiscovered [80]. New developments in computational tools and next-generation sequencing techniques have allowed the parallel investigation of many biological samples by processing millions of DNA/RNA fragments [81]. The metagenomic analysis of any microbial biosystem can be broadly divided into the following ways, irrespective of the state., (a) structural metagenomic approach, (b) functional metagenomic approach, and comparative metagenomic approach [82].

4.1. Structural metagenomic approach

The primary goal of a structural metagenomic approach is to reveal the microbial community structure of any specific ecosystem by sequencing environmental samples. This will primarily provide the taxonomic identity of the microbial community in a culture-independent manner. However, it may also be used to investigate other aspects, such as identifying novel genes, predicting gene functions, and involving genes in various metabolic processes. Additionally, it will help establish connections between community members' preferences for the environment. Assigning minor or significant geo-ecological functions to microbiomes in the evolution of the community structure also provides information about the microbial population dynamics of a particular ecosystem at various spatial and temporal scales [78].

4.2. Functional metagenomic approach

Functional metagenomics helps determine the expression of a gene based on its sequencing or information about the structure. Beginning with the extraction of DNA from ambient sources, it predicts the likely required genes from a metagenome library and then moves on to heterologous expression for activity-based screening and functional validation. As a result, functional metagenomics is utilized in conjunction with sequence-based structural metagenomics to aid gene identification from the massive metagenomic database [78,83].

4.3. Comparative metagenomic approach

Additionally, a comparative metagenomic analysis of the various “plastispheres” of broad ecosystems could help identify the core microbial community, or the microorganisms that consistently persist over time to carry out a significant portion of plastic degradation and appear to be common in “plastispheres” across various geographical locations. Methods of adaptability, viability, and survival in their varied biological contexts (from marine to terrestrial) with the ability to degrade plastic could also be studied. Therefore, it is feasible to accelerate the degradation process by altering the microbial community and its metabolic processes in the plastisphere [78,84].

4.4. Analysis of microbial community structures through metagenomics

Using the three techniques above, the community structure of a microbiome can be deciphered. Community structure, physiochemical structure, and habitats, among others, are known to vary among microbial community belonging to similar ecological communities. The microbial population associated with plastics has a different makeup and evolves as the plastic deteriorates. According to reports, the varied species in the “specific” assemblage are congruent with types of plastic and can be recognized from other communities [78]. There are mainly two types of colonizers attributed to biofilm formation, i.e. primary colonizers and secondary colonizers. After 24–36 h, depending on the type of substrate and habitats, the buildup of primary colonizers modifies the substratum, trying to make it favorable for further colonization by various secondary colonizers. Microorganisms arrive later in the biofilm formation process and may have unique features, indicating they are secondary colonizers. The structure of the microbial community evolves with time, and the relative proportions of secondary colonizers increase. The evolution of biofilm formation is represented by this progressive transition in community structure from primary to secondary colonizers over the duration [85].

Due to this variation in plastisphere characterization, metagenomic studies are limited to the structural analysis of the microbiome [84]. Although it helps the discovery of new microbial species, quantification of their abundance in the local microbial niche, and quantification of their rarity, the classification of these species into “core” to “specific” and “rare” species depends on their richness and specificity. Their functional involvement in the breakdown of polymers is still unknown. Purohit et al. [78] illustrated that some enzymes that can be used for the degradation of microplastics on the laboratory scale are not always suitable for performing the exact mechanism in a natural environment. For example, PETase hydrolysis is one of the most frequently used on disposed PET plastics in marine environments. A different form of the same enzyme can be obtained from *Ideonella sakaiensis* in iPETase. This enzyme is not suitable for the degradation of plastics in an aquatic environment. Researchers are now using a functional metagenomic method to address this problem by harvesting PET hydrolase homologs from various microbial sources. PET hydrolases are expected to be widely distributed in marine and terrestrial metagenomes based on conserved amino acids and can be exploited using various genetic engineering techniques for suitable modifications to the desired organism [78,83].

4.5. Deciphering a possible explication from metagenomic studies

The key benefit of metagenomic analysis is its rapid and effective means of highlighting the structural and functional importance of the relevant microbial niche [82]. A viable solution is developed based on all the data offered by metagenomics to meet the requirements of the microplastic breakdown process (Fig. 5). This answer can be divided into two categories, namely (a) modification of the external environment-microbial community and (b) modification of the internal cell primordia – genetic manipulation.

The first category describes the modification of the environment in which the microbial bio community is present. These modifications can be applied using various chemicals or molecular methods or by introducing different sources of nutrition to the microorganisms in the community. This modification will eventually enhance the structure of the microbial component, resulting in better degradation of plastics. The second describes the modification of genes or enzymes using microorganisms that are tailored to fit the need. In this way, the functional characterization of a particular microbial community can be altered by delivering the genes of interest into the organism's genome [78]. As a result, we can conclude that the development of metagenomic methods aids in the fabrication of proteins with enhanced characteristics, as well as the discovery of novel genes and the functional investigation of those

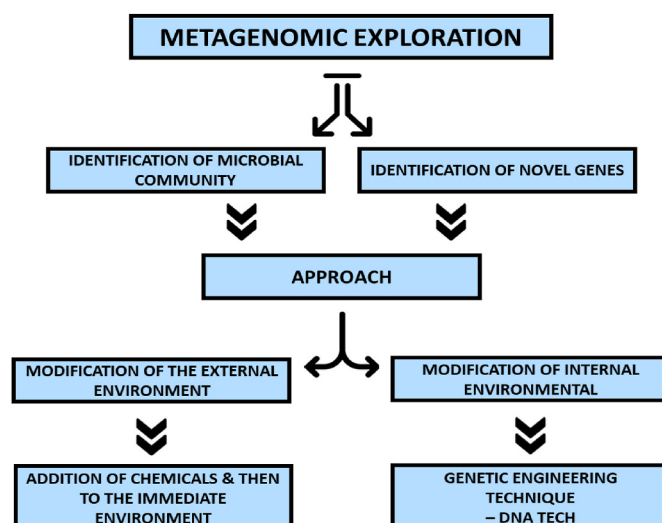


Fig. 5. Deciphering a possible explication of metagenomic study's.

genes through heterologous expression. To provide specialized solutions to problems, the biotechnological potential of metagenomics is emphasized, making it possible to obtain increasingly important information about the microbial world in order to address a range of societal and environmental problems.

4.6. Metagenomic analysis of plastic degrading enzymes in landfills

Around 21–42% of the plastic generated worldwide is reportedly disposed of in landfills [86]. Gaytán et al. [106], in their study on the polyurethane and xenobiotic biodegradation procedure and the evaluation of the product, revealed that the BP8 community breaks down various groups and bonds, including ester bonds, C-C bonds, aromatic recalcitrant urethanes, and ether groups. This was accomplished using oxidation and hydrolysis processes in all types of copolymer segments. The five genomes reconstructed using metagenomic analysis based on proximity ligation contained three genomes from the novel species. The identification of genes associated with different enzymes, such as putative enzymes and metabolic processes responsible for the biodegradative function of the BP8 population for additives and copolymers, was found in the metagenome. This work is the first to identify possible genes for microbial populations collected from landfills and water polyurethane dispersions systems that perform biodegradative processes. They also present viable solutions for the contaminations caused by xenobiotics and PU. Phylogenetic analysis identified well-supported clades for *Paracoccus*, *Chryseobacterium*, *Parapedobacter*, and *Ochrobactrum intermedium*, all of which are members of the Microbacteriaceae family.

Similarly, Kumar et al. [5,6] conducted a metagenomic analysis of the solid waste disposal site in Gujarat, India. Their study predicted thirty phyla, fifty-eight classes, 125 orders, 278 families, and 2468 distinct species. The most prevalent species in the soil and compost samples were Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria. However, the leachate samples exhibited a predominance of phyla Firmicutes (54.24%), Actinobacteria (43.67%), and Proteobacteria (1.02%). The study also identified probable genes related to the decomposition of other plastics, such as PE, PS, and PET. Their research found a link between numerous gene sources actively participating in plastic waste biodegradation at landfill sites and the structured microbial community. Through the analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG), they found a total of 11 significant metabolic pathways, out of which the top five metabolic pathways were carbohydrate metabolism (21.92%), lipid metabolism (6.95%), energy metabolism (14.53%), amino acid metabolism (18.12%), and

metabolism of cofactors and vitamins (10.34%). According to the KEGG annotations, in parallel to the above, only about 10.82% of the metabolic pathways predicted the general function, while 9.62% predicted amino acid transport and metabolism, 8.45% predicted ribosomal translation structure, and 7.67% indicated energy generation and conversion.

Sonnendecker et al. [91] studied PET plastic recycling by polyester hydrolase enzyme using metagenomic analysis at compost sites in Leipzig, Germany. Their study found that amorphous PET films are entirely hydrolyzed by polyester hydrolase PHL7, which was isolated from a compost metagenome. This enzyme releases 91 mg of terephthalic acid every hour. The PET film deterioration rates, measured by vertical scanning interferometry, were $6.8 \mu\text{m h}^{-1}$. According to structural studies, leucine at position 210 is crucial for PHL7's robust PET-hydrolyzing activity in any energy-intensive pre-treatments; $0.6 \text{ mg}_{\text{enzyme}} \text{ gPET}^{-1}$ destroys the post-consumer thermo-form PET packaging completely in 24 h at 70°C in an aqueous buffer. Polyester hydrolases can function as catalysts in environmentally friendly, sustainable PET recycling processes by recovering terephthalic acid from the enzymatic hydrolysate and using it to create virgin PET. In a study conducted by Zrimec et al. [115], the microbial potential of plastic degradation concerning the trends in plastic pollution, they developed a list of approximately thirty thousand non-redundant homologs enzymes that could break down ten distinct kinds of plastic. They also discovered that the quantity of enzymes in the ocean rises with distance downwards. After collecting further pollution measurements, they also discovered that the abundance of enzymes in soil and ocean ecosystems was strongly correlated with changes in marine and nation-specific plastic pollution. Thakur et al. [87] conducted a metagenomic study to search for novel enzymes in Delhi, India, solid waste landfills. They found that, based on operational taxonomic unit (OTUs), Proteobacteria were the most prevalent species in all samples, followed by Actinobacteria, Firmicutes, Bacteroidetes, and Chloroflexi. Verrucomicrobia and Acidobacteria were among the other relatively dominating species that were highly prevalent in other samples, while the Parcubacteria and Tenericutes species were enriched in other samples. It was discovered that the average percentage of the phylum proteobacteria in all samples was 40.54%, followed by Actinobacteria, the second dominating phylum, and firmicutes, the third most prevalent. They speculated that members of the phylum Firmicutes play a significant role in the breakdown of cellulose in landfills and are one of the most critical bio-degraders of biomass there. Other phyla included Chloroflexi and Bacteroidetes. Iodidimonas, a rare genus, was detected only in sample V. In contrast, Flavifex was present only in sample T. A different uncommon species from the phylum Bacteroidetes, Patricia, was discovered in samples V and T.

4.7. Metagenomic analysis of plastic degrading enzymes in marine

Anthropogenic activities and other causes have strained coastal and marine ecosystems for many years. The environment is being physically destroyed and polluted. Due to unsustainable development and building operations, one of the severe risks humans have posed to marine and coastal systems is the build-up of debris or litter. As a result of poor garbage disposal, the ocean's surface is covered with five trillion bits of floating plastic waste [88].

Pinnell et al. [53] conducted a study on the Benthic microbial community in the marine environment in Texas, USA, and found that Proteobacteria Operational taxonomic units of Proteobacteria (OTU) were the most prevalent across the four types of communities at the phylum level of all operational taxonomic units in saltwater, pottery, PET, and PHA samples, respectively. Cyanobacteria were the next most prevalent phylum in the saltwater community (25%). The PHA biofilm communities were the only ones where Chloroflexi (4%), Spirochaetes (4%), and Firmicutes (2%) were present among the most numerous phyla. Synechococcus and Prochlorococcus, the two genera of

Cyanobacteria, made up almost 20% of all operational taxonomic units in the seawater community but only accounted for 1% of the three biofilm communities. In the PET and ceramic biofilm communities, members of the uncultured genera Desulfobacteraceae, Rhodobacteraceae, and Flammeovirgaceae were among the five most prevalent genera, accounting for about 20% of all OTUs. 25% of all OTUs in the PHA biofilm were represented by three genera of Desulfobacteraceae and one genera of Desulfobulbaceae that were not cultivated, highlighting the dominance of SRM in that community. Six high-quality metagenome-assembled genomes were recovered from the co-assembled PHA biofilm metagenomes. The first genomes assembled with identified metagenomes were Desulfovibrio, the Desulfobacteraceae family, the Desulfobulbaceae family, the Spirochaetaceae family, and the Gammaproteobacteria order.

Bryant et al. [89] conducted a study on microbial diversity and activity in the North Pacific Subtropical Gyre. To ascertain the metabolic activities of the microplastic, the authors employed the Chlorophyll analysis approach in conjunction with a few additional methods and discovered that heterotrophic and photosynthetic microorganisms were attracted to plastic garbage. Additionally, they discovered that multi-species microbial biofilms, including pennate diatoms and coccus, rod and spiral-shaped cells, were connected to the frontal membranes of bryozoans. On bryozoan surfaces, bacteria with long filaments and prostheses were also observed. Similar cell morphologies could be visible on plastic particle surfaces, with some cells nesting inside the pores of the material. The Rhodobacteraceae and Cyanobacteria subsection III family I group, which includes Phormidium and Lepetolyngbya, were the most common microbial families in both tests, according to their research. Additionally, Hyphomonadaceae, Flavobacteraceae, Saprospiraceae, and Flammeovirgaceae regularly contributed to microbial plastic communities. Vibrionaceae, on the other hand, was rare in the samples from their analysis but quite frequent in one sample from the Atlantic.

Similarly, Meyer-Cifuentes et al. [90] researched the biodegradation of plastic by marine microbial populations. They conducted three separate experiments: detection of CO_2 synthesis and breakdown products, meta-omic analysis of various microorganisms, and identification of potential genes and proteins. A varied community was discovered by assembling and profiling metagenome, mainly made up of Alphaproteobacteria, Gammaproteobacteria, Flavobacteria, and Actinobacteria, albeit in smaller quantities. Throughout the duration of the time series experiment, the abundance profiles remained constant. In this experiment, the six most prevalent bins were three from the Rhodobacteraceae family, including two Pseudooceanicola spp., one unidentified Rhodobacteraceae bacteria, and one each from Marinobacter, Aequorivita, and Micavibrionaceae. Six orthologues of the three PETases were discovered, sakaiensis PETase (A0A0K8P6T7, GAP38373) (IsPETase), leaf compost cutinase (AEV21261.1) and Thermobifida fusca cutinase (ADV92528.1). Furthermore, four possible enzymes resemble MHETases and can break down polymers and waste products left behind by those breakdowns. Our findings demonstrate that only a few genes and organisms are active during biodegradation, although many can carry out each stage of degradation (Table 2)

5. Case studies

In a study carried out to establish and validate a suitable technique to extract and quantify microplastics of varying sizes and forms from sewage sludge samples, it was found that over 190 days, soils containing sludge with the highest microplastic concentration produced the least biomass and no mature fruits were borne. Several factors, such as soil humidity and temperature, precipitation, and air temperature, influence biomass production, the availability of soil nutrients being the most critical among them. Both surplus and deficit nutrients adversely affect biomass and tomato production [92,93]. Overall, alterations in the soil's C: N ratio, which changed the availability of nutrients, significantly

Table 2
Studies done in metagenomic analysis of plastic-degrading microbes and their enzymes.

Aim of the study	Type of plastic	Ecosystem	Phylum/Family	Discovery	Location of sampling	Reference
Degradation of Recalcitrant Polyurethane and Xenobiotic.	PU, PE-PU-A, N-methyl pyrrolidone, isopropanol and glycol ethers	Landfills	Microbacteriaceae, Paracoccus, Chryseobacterium, Parapedobacter, and Ochrobactrum intermedium.	BP8 community functions & three novel species metagenome.	Nezahualcóyotl Estado de México, México.	Gaytán et al. [106]
Metagenomic analysis of the solid waste disposal site.	Soil waste compost	Landfills	Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria. Firmicutes, Actinobacteria, and Proteobacteria.	2468 distinct species & eleven significant metabolic pathways.	Gujarat, India.	Kumar et al. [5, 6])
Use of metagenomic polyester hydrolase to recycle plastic.	PET	Landfills	-	PHL7's role in plastic degradation.	Leipzig, Germany.	Sonnendecker et al. [91]
Relation between global plastic degrading microbial populations and trends in pollution.	Plastic (10) PVA, PLA, PU, PHB, PBS, PET, PBAT, PE, PEG, PHO Additive (4) Phthalate PA, DBP, TP,	Landfills and marine	Acidobacteriota, Actinobacteriota, Alphaproteobacteria, Bacteroidota, Bdellovibrionota, Chloroflexota, Desulfobacterota, Gammaproteobacteria, Gemmatimonadota, Latescibacterota, Marinisomatota, Myxococcota, Planctomycetota, Poribacteria, Spirochaetota, Thermoplasmatota, and Verrucomicrobiota.	30,000 non-redundant enzyme homologs.	169 samples from 38 countries.	Zrimec et al. [115]
Using metagenomics for finding new microbial enzymes in the solid-waste dump.	compost	Landfills	Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, and Chloroflexi. Verrucomicrobia and Acidobacteri. Parcubacteria and Tenericutes.	Novel enzymes, and bacterial Communities.	Delhi, India.	Thakur et al. [87]
Shotgun metagenomic analysis of microbes acting on Coastal plastic and bioplastic.	biofilms of plastic and bioplastic	Marine	Proteobacteria, cyanobacteria, Chloroflexi, Spirochaetes, and Firmicutes.	novel species of Desulfovibrio, Desulfobacteraceae, and Desulfobulbaceae	Texas, USA.	Pinnell et al [53]
Study the diversity of the microbial population and their activities in North Pacific Gyre.	Microplastic	Marine	Rhodobacteraceae, Cyanobacteria, Phormidium, and Leptolyngbya. Hyphomonadaceae, Flavobacteraceae, Saprospiraceae, Flammeovirgaceae, and Vibrionaceae.	che genes, secretion system genes, and nifH genes.	North Atlantic Subtropical Gyre.	Bryant et al. [89]
Microbial symbiotic biodegradation of aromatic copolyester.	aromatic-aliphatic copolyester	Marine	Alphaproteobacteria, Gammaproteobacteria, Flavobacteria, and Actinobacteria. Rhodobacteraceae, Pseudoceanicola spp., Marinobacter, Aequorivita, and Micavibrionaceae.	6 PETase-like enzymes and 4 MHETase-like enzymes.	Helgoland, Germany. Athens, Greece. Elba, Italy	Meyer-Cifuentes et al. [90]

influenced the growth rate [94].

Microplastic contamination occurs not only in the soil but also in water bodies and in more significant proportions. Microplastics were found in every sample of water tested, including drinking water, according to research to determine the number of microplastic particles in freshwater and drinking water. Microplastics of 10 µm size were the most prevalent in both treated and untreated water. Even particles down to the size of 1 µm were also identified as polyethylene. PET, PP and PE formed the majority of 12 other microplastic materials that were obtained. However, raw water was shown to have a more significant proportion of microplastics than treated water [95]. The quantification of microplastics was also carried out for 16 months near the Ofanto River in the Apulia region of southeast Italy. Black flakes and transparent fragments of microplastics were found at various concentrations. Their origin was mostly found to be land-based [96]. The effects of microplastics and other pollutants of various types in the aquatic ecosystem

caused more significant damage to zebrafish than the contaminants were present alone, according to an investigation in which environmental circumstances were recreated in the laboratory. This combination resulted in adverse failure of the fish's internal organs [42,43]. In another similar study, the impacts of microplastics were studied at actual ambient levels where the microplastics were detected in the gills, intestines, and life of *Oryzias melastigma*, also called the marine medaka. These microplastics caused structural damage and increased oxidative stress in the tissues that made up the vital organs. The fecundity of the fish was also markedly reduced [9,10]. Similar toxicity effects were also observed in other aquatic beings, such as amphipods, crustaceans, and aquatic gastropods. Fish and other underwater organisms in areas that support mangrove growth serve as a food source for a large population. Recently, further research has been conducted to detect microplastic traces in these organisms. Among the other polymers, polycarbonates, and polystyrenes were detected in the highest

concentrations [97]. The mudskipper fish from India's Ulhas River estuary provided another set of findings. These fish are selective feeders, so micro-sized plastic particles were easily ingested. In contrast, the larger ones were inhibited, leading to a more significant accumulation of these microplastics in the gills. Microplastics were also found to accumulate in other fishes, such as long-tailed tuna (*Thunnus tonnggol*) and Sawtooth barracuda (*Sphyrna putnamiae*) [97].

Microplastics were also accumulated in several regions of the aquatic plant *Utricularia Vulgaris*, such as the leaves, shoots, and bladders of the plant. This accumulation elevates the antioxidative enzyme activity of the plant, thus increasing oxidative stress that leads to damage to plant parts [98]. Microplastics were detected in the roots of *Vicia faba* using laser confocal scanning electron microscopy, and a comparable impact of microplastics was also identified there [99]. A study conducted in the 11 most secluded and protected regions of the United States reported the accumulation of primary and secondary microplastics in dry and wet atmospheric depositions. A total of 339 samples (wet - 263, dry - 103) were taken and most of these particles were synthetic microfibers of size 20 to approximately 3 mm and other particles of size 4–188 mm [100]. Microplastics have also been found in air debris collected from many places [101,102]. The build-up of minute plastic particles in arable soils, which might have unanticipated effects on soil quality and output, is now causing significant concern. Four agricultural areas and a buffer zone of the riparian forest at Dian Lake in southern China were investigated for the prevalence and distribution of plastic particles in mixed soil fractions. In 50 soil samples, plastic particles were discovered in sizes ranging from 0.05 to 1 mm. Compared to buffer soil, the concentration of microplastics in vegetable soil was greater, indicating the use of soil additives [103]. Microplastics have also been found consistently in several of China's inland water systems. It was discovered that most of these had secondary origins [114]. A significant accumulation of microplastics was also detected in one of the most critical drinking water sources of China's Nanning city, both in the surface waters and in the sediments. Polypropylene and polyethylene were detected at the highest concentrations [71,72].

6. Conclusions

Microplastic pollution continues to pose a significant challenge in the fields of environmental engineering, ecology, and materials science. Exploring the biodegradation of microplastics through research can enhance our understanding of how to mitigate their presence in the environment and develop innovative technologies to combat pollution. The efficient degradation of microplastics by microbes using various enzymes highlights the importance of studying enzyme activities, particularly in the context of micropollutants, which have emerged as a major environmental concern. Applying such knowledge on a broader scale could lead to the development of more effective degradation mechanisms for heavily polluted areas facing multiple pollution problems. In-depth comparative studies focusing on identifying the most suitable enzymes for microplastic degradation are crucial, particularly when dealing with high volumes of micropollutants. While traditional methods are valuable for unlocking the potential of different microorganisms, interdisciplinary approaches like structural or functional metagenomics hold significant importance, especially in the face of escalating environmental pollution. By incorporating these approaches into projects, concrete conclusions can be drawn within a short time-frame. In the future, it is essential to extend these techniques to encompass the larger ecosystems that serve as primary carriers of microplastics.

Thoroughly investigating microplastics, including their distribution, associated risks, spatial dispersion, temporal trends, and the interplay of external factors with microbe behavior using chemical and visual cues, is crucial. Understanding the health risks posed by microplastics and other chemical pollutants in the environment is also of paramount importance. Furthermore, the development of beneficial microbial

agents is necessary to effectively reduce microplastic pollution. This comprehensive review provides valuable insights into the metagenomics of microplastic-degrading organisms, shedding light on their environmental roles. However, further investigations are still needed to explore the use of metagenomic approaches for identifying and characterizing microplastic-degrading organisms, as well as understanding the environmental factors influencing their growth and activity. Continued research in this area will yield useful insights and aid in the development of effective strategies to combat plastic pollution.

Authors contributions

All authors had full access to all the contents of the review paper and take responsibility for the integrity and accuracy of the text and analysis. Study concept and design: CNR. Acquisition of data: KVSSNM, AM, JCJR and ACVS. Analysis and interpretation of data: KVSSNM, AM, JCJR, ACVS, VP and CNR. Drafting of the manuscript: KVSSNM, AM, JCJR, ACVS, BM, YR, SKM, PK, VP, MH and CNR. Overall supervision: CNR, FB, MH.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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