UNVEILING THE POTENTIAL OF METAGENOMICS FOR ERADICATING MICROPLASTICS FROM DRINKING WATER

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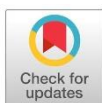*Corresponding Author: deepthisha2011@gmail.com

Abstract: *Plastic pollution is a critical environmental issue today, with microplastics emerging as persistent toxicants found in various samples. Recent studies have detected Microplastics in drinking water, raising concerns due to their association with toxicity in animals, including humans. Metagenomics has become instrumental in understanding the microbial communities responsible for plastic biodegradation. The text discusses the challenges associated with detecting and evaluating microplastics in drinking water, highlighting metagenomics as a viable instrument for devising enduring remedies for plastic contamination. There is a great deal of environmental concern due to the fact that microplastics pollution is pervasive and that it harms ecosystems and human health. Metagenomics, a powerful tool for studying microbial communities, has emerged as a promising approach for understanding the biodegradation of microplastics. This review explores how metagenomics helps identify the microbial communities that break down microplastics, showing promise in creating strategies to reduce microplastics pollution. It summarizes recent metagenomic research on microplastics, focusing on identifying important microbial groups and enzymes involved in degradation. Additionally, we discuss the challenges and future directions of using metagenomics to address microplastics pollution, emphasizing the need for interdisciplinary research efforts to develop sustainable solutions.*

Keywords: *Microplastics, Metagenomics, Microbial community, Plastic degrading microbes (E.coli,Pseudomonas aeruginosa), Biodegradation, Enzymes, Pollution.*

INTRODUCTION

Plastic contamination poses a critical danger to worldwide biological systems, affecting both abiotic and biotic components. The rise in urbanization and way of life changes has driven to expanded plastic utilization in later decades. Roughly 60% of marine squander is ascribed to plastics, with coasting squander bookkeeping for around 90% (Karim et al., 2020). The yearly stream of plastic from waterways to oceans ranges from 1.15 to 2.41 million tons. A few contaminated streams in Asia, covering 2.2% of the mainland surface region, contribute two-thirds of the worldwide yearly input of plastic squander (Anik et al.,2021). Because plastic is safe, it persists in the environment indefinitely (Rodrigues et al., 2018). The biogeochemical cycle of plastic includes its development among the climate, lithosphere (earthly frameworks), and hydrosphere (oceanic frameworks). Microplastics, characterized as plastic particles smaller than 5 mm, transcendently cycle through these frameworks (Lwanga et al., 2022). This little measure empowers them to travel to different situations, a result of over the top and unregulated plastic utilizationin later decades. MPs have been found in different situations (Kumar et al., 2024) and can a mass in nourishment, drinking water, and tissues, posturing a well-being chance to people (Prata et al., 2020).

Microplastics are categorized as essential or auxiliary. Essential Microplastics, like dots, pellets, and strands, are commonly found in restorative items, paints, etc., entering the environment a mid item transport or dealing with, dress washing, or family squander transfer (Bhardwaj et al., 2021). Auxiliary microplastics are formed Wastewater treatment plants are a major source of microplastics, releasing about 65 million particles into the environment each year (Oladoja et al., 2021). Microplastics are created when plastic objects break down into tiny bits through physical, chemical, or natural processes (Sharma et al., 2021).Other significant sources of MP contamination include stormwater, landfills, sewers, sewage, and sludge (Murphy et al., 2016). Family items like beauty care products contribute to the Microplastics generation (Zhou et al., 2023). Precipitation or water systems can cause Microplastics to total in soil and inevitably mobilize to other oceanic bodies. Microplastics pose different well-being dangers (Vasseghian et al., 2023), with their nearness in drinking water considered more unsafe than in fish. Microplastics can filter from landfills and reach groundwater, with the estimate, more thickness, and generation exercises being key components in their section into drinking water. Techniques for cleaning up Microplastics can be created based on a few basic parameters (Kumar et al., 2023).

Microplastics, due to their larger surface area, can harbor various toxins such as antibiotics, heavy metals, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polyfluoroalkyl substances, and more (Zhang et al., 2020). A high concentration of microplastics can have severe detrimental effects on human health, posing a global health risk due to microplastics pollution. Microplastics have ended up micropollutants that sully major water bodies, leading to poisonous impacts on living beings, including people, through drinking water defilement. Whereas a few surveys examine the wealth of Microplastics in water bodies, less reports are accessible on the debasement of Microplastics in drinking water. Plastics can contain added substances on their surfaces that increment their harmfulness, posturing dangers to earthbound and sea-going situations and straightforwardly influencing human well-being through ingestion and in a roundabout way through environment disintegration (Koelmans et

al., 2020). With an estimated half-life ranging from tens to hundreds of years, depending on the kind of polymer and environmental factors, plastic is known to be a growing toxin (Chamas et al., 2020). The nearness of microplastics in earthly and oceanic situations is concerning since they can discharge collected added substances and poisons, possibly influencing microbial composition and serving as a surface for the advancement of microbial pathogens in biofilms.

This review focuses on the origins, distribution, and methods for removing microplastics from drinking water. It also categorises the many kinds of microbes and enzymes that break down microplastics. Metagenomics investigates various biotechnological approaches to enhance the bacterial degradation efficiency of microplastics and their potential use in field research.

SOURCES OF MICROPLASTICS

There are two main sources of microplastics. The first is primary microplastics, which are produced from cosmetic products, household items, and drug delivery systems. The second source is polymer raw materials, such as pellets, flakes and powders, which are typically made from polyethylene, polystyrene, nylon 6, polypropylene and other polymers. Items like toothpaste and scrubs, as well as cleaning materials and cosmetics, contain microplastics that are irregularly shaped and range in diameter from <0.5 mm to <.1 mm (Fendall and Sewell 2009). These are a significant contributor to primary microplastics contamination and are frequently advertised as "micro-beams" or "micro-exfoliates." Because they efficiently remove these contaminants through skimming or settling procedures, existing wastewater treatment plants do not contribute to the development of microplastics (Carr et al., 2016).

When big plastic objects or particles are extensively broken down by environmental elements including high temperatures, UV rays, stress, reactivated ozone, oxidation, and atmospheric pressure, secondary microplastics are formed (Tiwari et al., 2020; John et al., 2021).Only when stabilisers and antioxidants are present can polymer materials withstand degradation. By generating enzymes that aid in their breakdown, bacteria, fungus, and algae contribute to the creation of these microplastics (Chia et al., 2020; Othman et al., 2021; Chen et al., 2022; Manzi et al., 2022; Miri et al., 2022; Zhu et al., 2022).Made of similar polymers, huge plastic objects that have been disposed of inappropriately are the principal source of microplastics. They are formed when synthetic fiber is broken down during clothes washing and by commercial activities such as thermal cutting polystyrene.Microplastics pollution is also caused by single-use plastic and single-use products from industries such as electronics, automobiles and textiles (Gerritse et al., 2020).

Table:1-Sources of Microplastics	
Primary Sources	**Secondary Sources**
Clothing industry	Plastic formation by mechanical friction
Shipping line	Plastic breakdown by wave
Fishing industry	Fragmentation by UV and Sunlight
Wastewater treatment plant	Degradation by Microorganisms
Cosmetic industry	Synthetic fibers
Air blasting	Foams

(Table 1 :Sources of Microplastics)

DETECTION OF MICROPLASTICS IN DRINKING WATER: ASSESSING MPs CONTAMINATION

Figuring out microplastics in consuming water is crucial because of the related health risks, such as the ability of Microplastics to carry dangerous chemicals and pathogens (Acarer et al., 2023). Accurate evaluation is important for assessing and mitigating these dangers (Koelmans et al.,2019). The vast presence of Microplastics in various water assets highlights the importance of information on their incidence and awareness to ensure water safety (Nirmala et al.,2023). Reading Microplastics in drinking water is imperative to address health and environmental concerns and to implement stringent rules for water useful resource safety (Sol et al.,2023).

METHODS OF MICROPLASTICS DEGRADATION

Reintroducing plastic scrap into a processing unit's heating cycle and then blending it with a virgin polymer to make new plastic products reduces production costs in many conventional methods for reusing degraded microplastics. Alternately, in industrial processes, plastic waste can be modified chemically or thermochemically for recycling.

Nonetheless, because of deficient administration, microplastics particles are frequently not appropriately discarded or isolated, prompting their blending in with natural parts in landfills utilized for fertilizing the soil or anaerobic processing (Verma et al., 2016). Microplastics are currently disposed of in a variety of physical and chemical ways, including recycling, landfilling, and incineration (Thiounn et al.,2020).

Pyrolysis and other chemical recycling procedures are particularly well-liked in the business world. In sluggish pyrolysis, plastic waste is changed over into a combination of burn and falter items at various temperatures(Dussud et al., 2018).

A few investigations center around pyrolyzing polypropylene, polystyrene, and polyethylene to recuperate heat energy. Microplastics corruption can happen through physical, compound, and natural strategies, with the organic interaction including different chemicals (Bacha andco2021).

The interaction normally includes corrupting polymers into more modest particles, then into oligomers, dimers, and monomers, trailed by mineralization helped by organisms. This mineralization brings about the advancement of carbon dioxide and the development of middle mixtures that act as an energy hotspot for microorganism development.

Hydrolase chemicals like lipases, esterases, and cutinases follow up on plastic surfaces, improving chain cleavage responses and making breaks. Following their incorporation into the cytoplasm of the microbe, the generated monomers enter a variety of metabolic pathways.
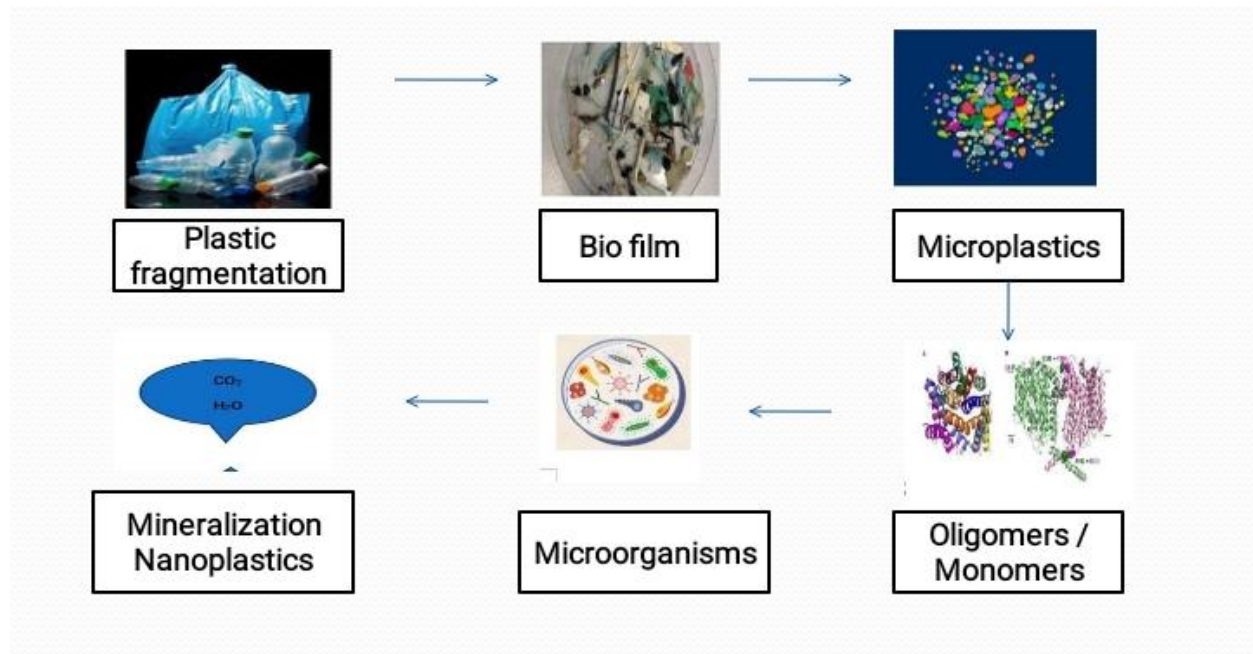
(Fig:1)   Process of Microplasticsdegradation

Microorganisms adhere to microplastics using processes similar to biofouling and plasticizer depletion, which allows them to penetrate the polymer structure by attacking its backbone. Environmental parameters like temperature, pH, salinity, and moisture content, as well as the existence of suitable microbial degrading organisms with the required enzymes and metabolic pathways, are all important for effective biodegradation (Raddadi and Fava 2019). The polymer's surface and structure, including its crystalline and amorphous areas, crystal size, and lamellar structure, all have an impact on the degrading process. Enzymes such as polyhydroxyalkanoates depolymerase have been observed to break down chains in the amorphous state on plastic film surfaces, followed by the degradation of crystalline state chains. Microplastics, arising from plastic debris fragmentation, can deteriorate further into smaller particles at the nanoscale (nano plastics) due to a mix of abiotic factors and extracellular enzymes, leading to bio-deterioration and bio-fragmentation processes (Vieira et al.,2023). Microorganisms use particular cell transport systems to absorb the oligomers and monomers released by this destruction, which they then use as a carbon source in catabolic pathways. The plastic is eventually mineralized by the aerobic metabolism of these monomers by the cells, resulting in the end products of carbon dioxide and water (Zhu et al., 2023).

CONTEMPORARY BIOTECHNOLOGICAL APPROACHES FOR IMPROVING MICROPLASTICS DEGRADATION

Genetic engineering Techniques have been employed to raise the microbiological biodegradation of various pollutants, including microplastics. Although these methods could improve the bioremediation capacity of hydrocarbons and heavy metals, limited research focused specifically on developing strains for the degradation of plastics.These approaches employ genetic engineering, gene cloning, and recombinant DNA technology to create new pathways and modify enzyme properties, improving their ability to break down microplastics (Naqash et al., 2020; Kumar et al., 2020). Methods include constructing fresh metabolic pathways and adjusting enzymes to enhance their efficacy against microplastics. Site-directed

mutagenesis, antisense RNA technology, and polymerase chain reaction are some of the methods used. Site-directed mutagenesis modifies the function of genes, whereas antisense RNA technology is helpful in controlling gene expression. Polyethylene terephthalate can also be targeted by enzymes such as cutinase, which was first employed by microbes to break down polyester (Shirke et al., 2018). It is possible to increase these enzymes' efficiency by genetic engineering, which would significantly shorten their degradation time. In contrast to the wild-type strain, modified cutinase shown a reduction in degradation time from 41.8 hours to 6.2 hours in experiments (Islam et al., 2019).
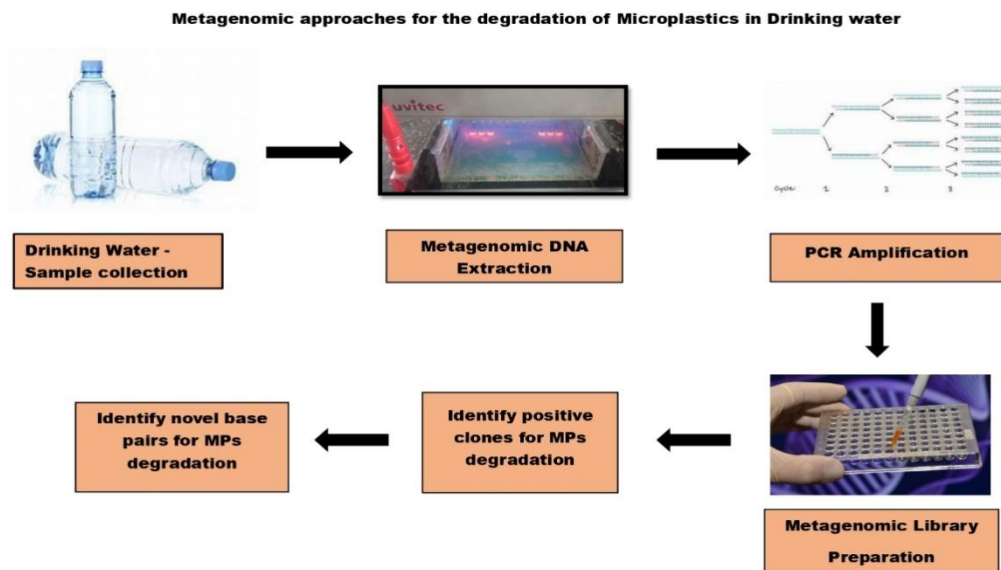
ENZYMATIC DEGRADATION OF MICROPLASTICS

The challenge of changing the breakdown of microplastics atoms and molecules is due to the coarsely ordered homoand heteroatomic composition of credit cards. Although microorganisms cause a significant pressure drop in a flexible polymer, it is notably more colorless than chemically bent biodegradation because it is related to the process of changing atoms and molecules. Proteins reported by organisms, as highlighted in studies (Lvet al., 2022), break the polymer chains of military forces according to the application rules. ATP-binding cassette transporters play a role in hydrolysis to control reactions and the efflux of specific molecules across membrane barriers in both prokaryotic and cells with eukaryotes. Toxin release is another function of these transporters. owing to atomic and molecular modifications including hydroxylation, hydrolysis, and degradation (Rana et al., 2022). Extreme protons and neutrons at first Extracellular proteins are considered disgraceful by the military and have so been converted to microbiological containers. Because starch hydrolyzes quickly when combined with prepared MPs, it enhances the rate of MP biodegradation and renders MPs incapable of being reduced by microbes.described in detail how starch and amylase might contaminate LDPE (Zheng et al., 2022). Chemicals that impact high molecular weight MP materials including PVC, PP, PS, and polyamide are the subject of little information (Luo et al., 2022).Despite the fact that mixed microbial communities cause difficulties for these MPs, the effectiveness of corrupt objects has not been fully captured (Hermida et al., 2022).The study of MP-degrading microorganisms and proteins is developed through metagenomic strategies.

METAGENOMICS IMPLICATIONS IN DEGRADATION OF MPs

The rise in microplastics pollution has emerged as a significant environmental and health concern, particularly in regions with substantial human and industrial presence(Geyer et al., 2017). Landfills and areas with a lot of waste greatly increase this pollution and create an environment where microbes can thrive to break down plastics and microplastics(Jambecket al., 2015). Although some microbial populations can be identified by conventional culture-based methods, the majority, approximately 98 percent, remain uncultivable, making it difficult to fully understand their biodegradation potential. However, advancements in next-generation sequencing and omics technologies have fundamentally changed how we can study these microorganisms (Nayfach et al., 2020). Metagenomics, a culture-independent method, allows us to reveal hidden microbial diversity and their functions in different environments(Sunagawa et al., 2020).Currently, two main approaches are used to analyze microbial communities, structural and functional metagenomics.The study also found several other enzymes, such as lipases, alkane hydroxylases and carboxylases, for the degradation of plastics in different habitats (Danso et al., 2018). Additionally, the research was concentrated on the degradation of

phthalate compounds in plastics using the metagenomically derived enzyme EstJ6 through a functional metagenomic approach (Qiu et al., 2020).

(Fig:2) Metagenomic approaches for the degradation of Microplasticsin Drinking water sample

ROLE OF METAGENOMICS IN MICROPLASTICS REMEDIATION

Global marine research has revealed around 40 million unique features from more than 30,000 species, with over 97% of the recorded 150 million surface soil features not yet incorporated into the existing inventory. This highlights the significant practical potential of microbiomes, especially those housing unculturable microorganisms that serve as protein sources (Bahram et al., 2018). Around 30,000 specific chemicals have been identified, showing similarities to known chemicals that have the ability to degrade plastics (Zrimec et al., 2021). (Chow et al., 2023) A new in-silico approach was developed to screen and characterize PETases from metagenomic datasets. Using next-generation sequencing in an in vitro expression system, researchers identified a novel PET esterase through metagenomic screening (Han et al., 2023). Metagenome-based research in the future revealed particular microbial communities that may break down hydrocarbon chains, which are the building blocks of plastic polymers (Hauptfeld et al., 2022). In a different study, scientists used 16S rRNA information from metagenomes to examine the taxonomic and functional characteristics of bacteria degrading polyethylene at a waste recycling facility in Tehran, Iran.

METAGENOME ANALYIS THROUGH COMPUTATIONAL TOOLS

The advancement of metagenome analysis has been greatly aided by the development of computer power and tools.Sequencing metagenome samples can help us find new microorganisms and genes that can break down contaminants (Turnbaugh and Ley 2007).

Shotgun MGs help us understand the microbes in a community and how they might be helping with different chemical processes. Because we can't control the collection of metagenomes from environments, the data we get tends to have a lot of the same organisms represented in it (Gilbert and Meyer2010). To make sure that all the different kinds of microbes are treated fairly, random shotgun sequencing is preferred by used. This method helps to evenly study .

all the organisms and find the ones that are not as common in the community. Metagenome data is usually very big and includes parts of DNA that are not organized and in their original form, the year is 2010. The tool helps sort and categorize organisms based on their functions and characteristics using a specific algorithm (Huson and Auch2007).MG-RAST is a big collection of metagenomes that is used to study and analyse different kinds of living things found in the environment. It helps scientists automatically analyse the functions of these living things (Meyer et al.,2008). In 2022, we thoroughly looked at the most computational tools used to analysemetagenome data sets.

SEQUENCE-BASEDSCREENING

Advances in DNA sequencing and bioinformatics now allow for consideration of unique genetic traits in the uncultured portion of host-associated microbial communities. Amplicon sequencing, commonly used for microbiome variation analysis, involves extracting DNA from tested cells.By analyzing clone sequences alongside phylogenetic remnants, researchers can identify the probable source of the DNA element for incorporation into sequence-based analyses. Alternatively, they can suggest random sequencing to determine the degree of interest, examining the phylogenetic relationships in adjacent DNA to establish a developmental network linking genes and advantageous trait(Geethanjali et al., 2024).

FUNCTIONAL BASED SCREENING

Metagenomic functional screening involves analysing the collective genetic material from a mixed community of microorganisms (metagenome) to identify specific functions or activities. (Sathya et al., 2014) This method is particularly useful for exploring the functional diversity present in complex microbial ecosystems, such as soil, water, or the human gut. This could involve screening for specific enzymatic activities, antibiotic resistance, or other desired functions. Sequence the DNA from the clones exhibiting the desired functions can be identified. This helps identify the specific genes responsible for the observed activities. Metagenomic functional screening allows researchers to discover novel genes and enzymes with potential industrial, agricultural, or medical applications. It provides a broader view of microbial diversity and functional capabilities in diverse environments. (Gokilavani et al.,2023)

BIOINFORMATICS

Microplastics and plastic breakdown are greatly aided by bioinformatics (Purohit et al., 2020). MetaCyc, BioCyc, Environmental Contaminant Biotransformation Pathway resource, and the University of Minnesota Biocatalysis/Biodegradation Database are a few databases that provide information on genes, microbial enzymes, and metabolic pathways related to degradation (Gao et al., 2010; Wicker et al., 2016; Caspi et al., 2020).These resources aid in enzyme identification within metabolic pathways and forecasting degradation pathways, laying the groundwork for innovative degradation strategies (Ali et al., 2021). Nevertheless, a major

limitation of bioinformatics is the lack of experimental data and validation, which is important for further studies. Furthermore, there is still a great deal to learn about the microbes and enzymes that can break down synthetic polymers, which emphasises the need for more research to identify the right metabolic pathways and enzymes. In the future, it is anticipated that the development of sustainable solutions for microplastics degradation will be streamlined by combining bioinformatics techniques with genetics, metabolic engineering, molecular biology, and systems biology (Wei et al., 2021).

## METAGENOMIC WAY AND MICROPLASTICSBIOREMEDIATION

Metagenomics can be employed in microplastics bioremediation by analysing microbial communities in diverse environments to identify potential plastic-degrading organisms. Understanding these communities helps design targeted strategies for bioremediation, harnessing the metabolic capabilities of microorganisms to break down plastics (Wright et al., 2018). Researchers explore metagenomic data to uncover key enzymatic pathways involved in plastic degradation (Zettler et al., 2019). By identifying microbial genes responsible for plastic breakdown, scientists can engineer or optimize microbes for more effective bioremediation (Oberbeckmann et al., 2021). Ultimately, metagenomic approaches offer valuable insights into the complex microbial ecosystems involved in tackling microplastics pollution (Kesy et al., 2020). In the context of microplastics bioremediation, a metagenomic approach involves studying the collective genetic material of microbial communities in various environments.

Collect samples from environments with microplastics pollution, such as oceans, soil, or wastewater. Extract genetic material (DNA) from the collected samples, capturing the diversity of microorganisms present. Use high-throughput sequencing technologies to analyze and sequence the genetic material, generating vast amounts of data. Employ bioinformatics tools to analyze the sequenced data, identifying microbial species and their functional genes (Dussud et al., 2018). Focus on genes related to plastic degradation, such as those encoding enzymes capable of breaking down plastic polymers (Zettler et al., 2019). Design targeted bioremediation strategies based on the identified genes and microorganisms. This could involve optimizing conditions for microbial growth, enhancing plastic-degrading enzymes, or even genetic engineering of microorganisms for improved plastic degradation.

CHALLENGES AND FUTURE PROSPECTS

In the quest to eradicate microplastics from drinking water, metagenomics emerges as a promising frontier offering innovative solutions. The future holds exciting prospects, including the engineering of microbial consortia with enhanced plastic-degrading capabilities through synthetic biology. Bioremediation strategies could be advanced, utilizing naturally occurring or engineered microbes for in situ bioremediation, while optimized bioreactor technology could be developed for water treatment plants. Metagenomic screening continues to be a key focus, seeking novel enzymes for specific microplastic types. Environmental monitoring, integrating metagenomics, could assess remediation efficacy and identify new plastic-degrading microbes. Furthermore, integrating metagenomics with nanotechnology or advanced materials may yield novel approaches for microplastic removal. However, these advancements must be accompanied by considerations of regulatory and policy frameworks to ensure safety, efficacy,

and minimal environmental impact. Through these avenues, metagenomics stands poised to revolutionize the eradication of microplastics, safeguarding the purity of our drinking water.
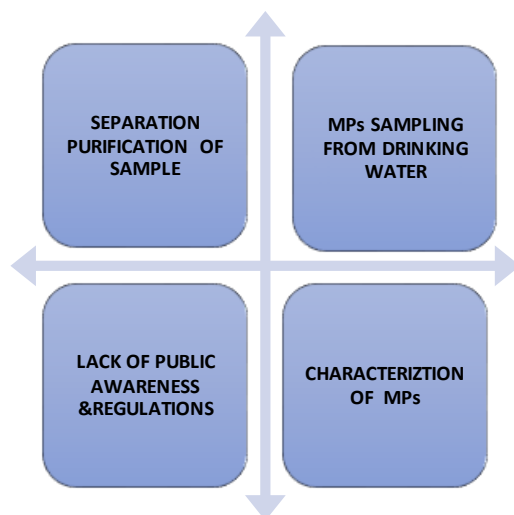
 (Fig:3) Challenges to identify Microplasticsin drinking water

CONCLUSION

Metagenomics offers a powerful and promising approach for understanding the microbial communities involved in plastic degradation in drinking water. Through the identification of key plastic-degrading genes and pathways, metagenomics enables the development of targeted strategies for mitigating microplastics pollution. By harnessing the diverse metabolic capabilities of these microbes, we can potentially engineer microbial consortia or enzymes for more efficient and eco-friendly degradation of microplastics in water sources. However, further research is needed to fully elucidate the complex interactions within these microbial communities and optimize their use in environmental remediation efforts. The application of metagenomics holds great potential in our quest to combat microplastic contamination and safeguard the quality of our drinking water sources.

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