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# Challenges and opportunities in bioremediation of micro-nano plastics: A review



Yuwen Zhou <sup>a</sup>, Manish Kumar <sup>b</sup>, Surendra Sarsaiya <sup>c</sup>, Ranjna Sirohi <sup>d</sup>, Sanjeev Kumar Awasthi <sup>a</sup>, Raveendran Sindhu <sup>e</sup>, Parameswaran Binod <sup>e</sup>, Ashok Pandey <sup>f</sup>, Nanthi S. Bolan <sup>g,h,i</sup>, Zengqiang Zhang <sup>a</sup>, Lal Singh <sup>b</sup>, Sunil Kumar <sup>b</sup>, Mukesh Kumar Awasthi <sup>a,\*</sup>

a *College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi Province 712100, PR China*

b *CSIR-National Environmental Engineering Research Institute (CSIR-NEERI), Nehru Marg, Nagpur 440020, Maharashtra, India*

c *Key Laboratory of Basic Pharmacology and Joint International Research Laboratory of Ethnomedicine of Ministry of Education, Zunyi Medical University, Zunyi, Guizhou, China*

<sup>d</sup> *Department of Chemical and Biological Engineering, Korea University, Seoul, South Korea*

<sup>e</sup> *Microbial Processes and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram, Kerala 695019, India*

f *Centre for Innovation and Translational Research, CSIR-Indian Institute of Toxicology Research, Lucknow 226 001, India*

g *School of Agriculture and Environment, The University of Western Australia, Perth, WA 6001, Australia*

h *The UWA Institute of Agriculture, The University of Western Australia, Perth, WA 6001, Australia*

i *School of Engineering, College of Engineering, Science and Environment, University of Newcastle, Callaghan, NSW 2308, Australia*

# HIGHLIGHTS

# GRAPHICAL ABSTRACT

- Microbial degradation of micro-nano plastics (MNPs) is considered as clean technologies.
- Biotic and abiotic factors of the environment control the biodegradation of MNPs.
- Microbial enzymatic machinery plays an important role in biodegradation of MNPs.
- Implication of advanced molecular for biodegradation of MNPs is desirable.
- Challenges and opportunities in bioremediation of MNPs are reviewed.

# article info abstract

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Rising level of micro-nano plastics (MNPs) in the natural ecosystem adversely impact the health of the environment and living organisms globally. MNPs enter in to the agro-ecosystem, flora and fauna, and human body via trophic transfer, ingestion and inhalation, resulting impediment in blood vessel, infertility, and abnormal behaviors. Therefore, it becomes indispensable to apply a novel approach to remediate MNPs from natural environment. Amongst the several prevailing technologies of MNPs remediation, microbial remediation is considered as greener technology. Microbial degradation of plastics is typically influenced by several biotic as well as abiotic factors, such as enzymatic mechanisms, substrates and co-substrates concentration, temperature, pH, oxidative stress, etc. Therefore, it is pivotal to recognize the key pathways adopted by microbes to utilize plastic fragments as a sole carbon source for the growth and development. In this context, this review critically discussed the role of various microbes and their enzymatic mechanisms involved in biodegradation of MNPs in wastewater (WW) stream, municipal sludge, municipal solid waste (MSW), and composting starting with biological and toxicological impacts of MNPs. Moreover, this review comprehensively discussed the deployment of various MNPs

Corresponding author.

*E-mail addresses:* mukesh\_awasthi45@yahoo.com, mukeshawasthi85@nwafu.edu.cn (M.K. Awasthi).



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remediation technologies, such as enzymatic, advanced molecular, and bio-membrane technologies in fostering the bioremediation of MNPs from various environmental compartments along with their pros and cons and prospects for future research.

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#### **Contents**



## 1. Introduction

Plastics as a readily available, cost-effective, and convenient material are widely used in industries and our day-to-day life worldwide, and bring a lot of convenience for mankind. Along with its convenient application, recently, it has aroused as a global environment menace (Ali et al., 2021a; Ali et al., 2021b; Cheng et al., 2021). It is certainty due to the increasing demand and consumption of plastic products leading to accumulation of a large number of used or spent plastics in the environment (Kumar et al., 2021c). Plastics waste become environmental contaminants and cannot be degraded in the natural surrounding even in 100 year (Ricardo et al., 2021; Tiwari et al., 2020). Brahney et al. (2020) reported and estimated that 11 billion tons of plastics will be expected to accumulate in the environment by 2025. However, currently, the recovery rate of plastics is no more than 5%. Moreover, the plastics that we call "white pollution" due to various physical, chemical and biological activities will be disintegrated into smaller fragments collectively termed as MNPs. Plastic fragments less than 5 mm in diameter are defined as micro-plastics (MPs) (Kumar et al., 2020a).

The size of nano-plastics (NPs) is smaller (<100 nm), therefore, it has more serious impacts on living tissue than MPs. NPs can readily pass through the cell membrane and adversely impact the cell and tissues (Wu et al., 2021). MNPs often tend to be difficult to handle and remove, but also, displayed greater impact on living organisms than macro particulate plastics (Kumar et al., 2021b). MNPs cannot only release organic toxicants but accumulate in living beings and also act as carriers of organic and inorganic toxicants (Bradney et al., 2019; Wang et al., 2021b). MNPs also interact and react with organic and inorganic environmental pollutants and even they can facilitate their transport to environmental compartments (Sridharan et al., 2021a; Sridharan et al., 2021b). These reactions enable MNPs to serve as substrates for accumulating and vector for transporting the organic and inorganic contaminants (Horton et al., 2017; Ricardo et al., 2021). Therefore, MNPs enhance the bioaccumulation of other pollutants in the environment. The sources of MNPs commonly recognized by researchers mainly come from the crushing of large pieces of plastics and the specialized production of micro-beads for various applications along with natural disintegration of larger plastic fragments (Camins et al., 2020; Cheng et al., 2021). The ultimate destination of MNPs is deposition in soil or sink into the ocean via rivers and atmospheric transportation (Hurley and Nizzetto, 2018; Sridharan et al., 2021b).

The presence of MNPs in the natural environment triggers the adaptation mechanism of microorganisms to cope up with adverse impacts of MNPs (Yang et al., 2020). Microbes respond to environmental stress in various ways, such as increasing or decreasing the growth, metabolic rate (Fuke et al., 2021; Kumar et al., 2021a; Mishra et al., 2021), and biosynthesis of new microbial bioproducts to avoid the environmental stress (Guan and Liu, 2020; Kumar et al., 2016; Thakur et al., 2018). These abiotic and biotic stress responses are meticulously associated with change in genes expression and enzymatic activities (Othman et al., 2021). These microbial enzymes are not only associated with cellular response, but simultaneously facilitate the microbial degradation of environmental pollutants, including MPs. For instance, the microbial assisted enzymatic degradation of plastics polymer breaks polymers into monomers which can be further used by microbes as carbon and energy source. Moreover, bacterial assisted composting reduced the abundance of polyhydroxyalkanoates (PHAs) and polyethylene (PE) MNPs by 13–29% (Sun et al., 2021). Similarly, MNPs in sewage sludge could be effectively degraded during the composting process under the action of bacterial bio-oxidation (Chen et al., 2020). Studies also claimed that green algae may be one of the natural degraders of MNPs, especially under aquatic marine ecosystem (Kiendrebeogo et al., 2021).

Currently, the more mature technology to remove MNPs from terrestrial environment includes physical and chemical technologies, such as micro/nano filtration in sewage treatment plants (STPs) (Dey et al., 2021; Sun et al., 2019). However, physical remediation technology is considered as ineffective in remediation of MNPs from polluted areas. Similarly, the application of synthetic chemical to remediate MNPs contaminated site is considered as a less attractive approach due to its complexity, non-greener nature, and heterogeneity of the polymers and environmental setting (Arpia et al., 2021). Therefore, considering the ecological threat of MNPs, there is an urgent need to develop a costeffective and environmentally sustainable remediation technology. It is known that there are few microorganisms that can degrade MNPs, and there are also few enzymes that play a specific role in the biodegradation of MNP polymers (Danso et al., 2019; Jacquin et al., 2019; Jenkins et al., 2019). Biotechnology is an emerging bioremediation technology,

which is gaining attention these days. As an environment-friendly technology, bioremediation warrants more researching to solve the problem of MNP pollution in terrestrial and aquatic ecosystem (Fig. 1). Only a few research and reviews have discussed the degradation of MNPs in detail by biological means (Othman et al., 2021; Priya et al., 2021; Sun et al., 2021; Yang et al., 2021; Yuan et al., 2020), but still, the mechanism and potential routes of MNPs degradation in terrestrial and aquatic environments are unexplored.

Therefore, the current review critically emphasized the biological and toxicological impacts of MNPs, role of diverse microbes, their enzymes, and diverse mechanisms involved in biodegradation of MNPs in wastewater stream, MSW and composting, starting with their ecotoxicological impacts on living well-being. Furthermore, this review firstly discussed the enzymatic, advanced molecular, and biomembrane technologies deployed in remediation of MNPs along with their advantages and disadvantages. Finally, this review discussed the



Fig. 1. An overview of microbial degradation of MNPs.

research gaps, which need further consideration in future research to make biodegradation of MNPs more effective and sustainable.

#### 2. Biological and toxicological effect of micro-nano plastics

The eco-toxicity of MNPs in organisms is a persistent threat to the ecosystem. MNPs can attract pollutants with positive charges because most of their surfaces are negatively charged (Bradney et al., 2019). Moreover, they can also be absorbed by microorganisms or animals. MNPs can trigger anomalous metabolic reactions in body of living organisms, as well as abnormal immune reactions that can damage the health (Allouzi et al., 2021). Zebrafish exposure to polystyrene (PS) results in oxidative stress and immune function limitation (Xu et al., 2021). Low density polyethylene (LDPE) increases the oxidative stress, neurotoxicity, and skin impairment in *Eisenia fetida* within 28 days (Chen et al., 2020) (Table 1). Similarly, Jiang et al. (2020) observed DNA damage, oxidative stress, hepatological disparities, reduced growth and increased mortality, when *Eisenia fetida* exposed to PS for 14 days. Chae and An (2020) also observed variations in the nursing and scavenging behaviors, inhibited growth and development in the *Achatina fulica* when exposed to PS polymers for 14 days. MPs will produce reproductive toxicity to fish and animals, and even have lethal effect if the dosage exceeds a certain limit. The hatching rate of fertilized eggs and the body length of fish larvae decreased significantly when exposed to MPs. Gut obstruction by MPs can cause abnormal animal behavior. Huang et al. (2021) studied different impacts of new and aged MPs on the composition of red tilapia. MPs are accumulated in fish body, and the content in the liver is always higher than that in other tissues.

MNPs pollution changes the physico-chemical characteristics of soil. Toxic additives in the manufacturing process of plastics and pollutants carried on the surface of MPs enter the soil environment with MPs and adversely affect the microbial habitat of the soil (Guo et al., 2020). MNPs along with the release of associated toxic chemicals can affect various soil properties including soil pH, conductivity, texture, nutrients, such as ammonium nitrogen, and organic carbon (OC) (de Souza Machado et al., 2018; Kim et al., 2020b). The mobility of plastic fragments increases with decreasing their size. MNPs are absorbed or adsorbed by the roots of plants in the soil and enter the plant system (Rillig, 2020; Rillig et al., 2019; Wu et al., 2021). The degree of influence of plants by MNPs is in the order of roots, leaves, buds and stems (Zhang et al., 2020b). The MNPs suspended in the air settles on the surface of

Table 1

Selected references for impacts of plastic polymers and their additives on living organisms and plants.

Polymers and/or additives	Species	Media	(days)	Duration Experimental dose	Observation	References
Polymers						
PS	Achatina fulica	Soil	14	10 mg $kg^{-1}$ w/w	Variations in the nursing and scavenging behaviors, inhibited development	(Chae and An, 2020)
PS	Caenorhabditis elegans	Synthetic media	3	100 µg $L^{-1}$	Intestinal damage	(Yu et al., 2020)
Low density polyethylene (LDPE)	Eisenia fetida	Soil	28	1.5 g $kg^{-1}$	Oxidative stress, neurotoxicity, skin impairment	(Chen et al., 2020)
PS	E. fetida	Soil	14	1000 $\mu$ g kg <sup>-1</sup>	DNA damage, oxidative stress and hepatological disparities, reduced growth and increased mortality	(Jiang et al., 2020)
PET	A. fulica	Soil	28	$0.71$ g kg <sup>-1</sup>	Variations in ingestion habits and deprived nutrient intake	(Song et al., 2019)
PS	Vicia faba	Soil	$\overline{2}$	10-100 m $L^{-1}$	Oxidative injury, reduced development	(Jiang et al., 2019a)
PS	C. elegans	Synthetic media	$\mathbf{1}$	17.3-86.8 mg $L^{-1}$	Retard movement, and metabolism, infertility	(Kim and An, 2019)
PS	Daphnia magna	Freshwater -		$\overline{a}$	Oxidative injury	(Zhang et al., 2019)
LDPE	E. fetida	Soil	14	16-200 particles $kg^{-1}$	Behavioral changes	(Rodríguez-Seijo et al., 2019)
Poly vinyl chloride (PVC)	Paracentrotus lividus	Marine water	$\mathbf{1}$	0.3-30 mg $L^{-1}$	Retard larval growth	(Oliviero et al., 2019)
PP, PVC	C. pyrenoidosa, Microcystis	Synthetic media	3	5-500 mg $L^{-1}$	Significantly decreased chlorophyll a, Impaired development	(Wu et al., 2019)
<b>PVC</b>	Metaphire californica	Soil	28	2000 mg $kg^{-1}$	Variations in the gut microbial diversity	(Zocchi and Sommaruga, 2019)
PE	F. candida	Synthetic media	28	$1\%$ w/w	Variation in intestinal microbial diversity, change in nourishing behavior, infertility	(Ju et al., 2019)
Mixture polymers	Tetraselmis chuii	Synthetic media	$\overline{4}$	4 mg $L^{-1}$	Inhibited development	(Davarpanah and Guilhermino, 2019)
Additives						
Phenanthrene	Gammarus roeseli	Freshwater -			Impaired locomotion, induced neurotoxicity	(Bartonitz et al., 2020)
Tetracycline	Enchytraeus crypticus	Soil		1000 mg $kg^{-1}$	Improved antibiotic resistant genes	(Ma et al., 2020)
Butylated hydroxy anisole	D. rerio	Freshwater -			Stunted development with abnormal larval growth	(Zhao et al., 2020)
Polyaromatic hydrocarbons (PAHs)	Danio rerio	Freshwater -		10 mg $L^{-1}$	Decreased vascular growth and energy generation mechanism	(Trevisan et al., 2019)
Fluoranthene	Mytilus edulis	Marine water			Change in enzymatic secretion	(Magara et al., 2019)
Polychlorinated biphenyls	D. magna	Freshwater -		1 mg $L^{-1}$	Induced mortality	(Latha and Lalithakumari, 2001; Lin et al., 2019)
PAHs	Perinereis aibuhitensis	Water sediment		0.4 mg $L^{-1}$	PS heightened the accumulation of PAHs	(Jiang et al., 2019b)
Glyphosate	D. magna	Freshwater -			Induced mortality rate	(Zocchi and Sommaruga, 2019)

the leaves of the aboveground part of the plants (Sridharan et al., 2021b). Small particles, such as NPs can enter the plants and pass through the cell wall and cell membrane easily (de Souza Machado et al., 2018). More mature, larger trees and arboreal plants are more enriched in MNPs, which may be attributed to their larger and stout structures of roots (Khalid et al., 2020). Boots et al. (2019) have confirmed that the accumulation of MNPs adversely impact the photosynthesis rate of the plants and each and every plant showed different responses against the MNPs pollution.

### 3. Microbial-degradation of micro-nano plastics

As a green approach, microbial assisted degradation of plastics fragments leads to degeneration of the MNPs. It is easier to control changes in plastic pollution because microbial degradation procedures are strongly dependent on both biotic and abiotic variables, such as pH, temperature, oxidative strain, etc. By using plastic fragments as a sole carbon source for the growth of microbes, the full degradation/elimination of MNPs can be predicted using cutting-edge technologies, such as omics (Knott et al., 2020; Tiwari et al., 2020). Although, the use of microorganisms in biodegradation of MNPs is a promising approach but due to its slower pace, incomplete mineralization, and unexplored degradation mechanism, this technology is in infancy stage and simultaneously gaining attention (Ru et al., 2020; Silva et al., 2018; Anastopoulos and Pashalidis, 2021) (Fig. 2 and Table 2).

# *3.1. Mechanism of micro-nano plastics biodegradation*

The chemical structure of MPs and their molecular weight as well as types of microorganisms and other environmental conditions influence degradation of microbe-driven MPs. Different properties of MNPs, such as, density, types of functional groups and their bioavailability as well as plasticizers or chemical additives incorporated to MNPs during processing influence their biodegradability (Yuan et al., 2020). Microbial degradation of MNPs involves a number of biochemical reactions. Biodeterioration (change in polymers size, shape and chemical properties), bio-fragmentation, biosynthesis, and mineralization are the steps involved in microbial MNP degradation (Tiwari et al., 2020). Hydrolase (extracellular) enzymes are released by bacteria and have the capacity to transform very complex molecules into polymers that can then be



Fig. 2. A schematic representation of recent development in bioremediation of MNPs.

#### Table 2

Microbial degradation of MNPs and key outcomes.



converted into hydroxyl acid monomers (Kamrannejad et al., 2014; Lear et al., 2021). Polyhydroxy butyrate (PHB) hydrolysis yields R-3 hydroxybutyric acid (HBA) whereas 3-hydroxybutyrate and 3 hydroxy valerate (PHBV) are extraterrestrial PHBV degradation yields. Under aerobic conditions, water soluble monomers are subtly spread by the metabolic mechanism of living organisms, like oxidation and the tricarboxylic acid cycle (TCA), leading to the generation of carbon dioxide  $(CO_2)$  and water  $(H_2O)$ . Under anaerobic environments, methane (CH4) is produced, and no harmful molecules are generated during the degradation of PHA.

Chitinases are enzymes biosynthesized by diverse groups of bacteria, such as *Achromobacter*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, and *Vibrio* sp. They hydrolyze the polymer and break it down. *Pseudomonas* sp. has been discovered to degrade MPs particles, but the precise mechanism remains unknown. Further research revealed the application of chitinase enzyme in enzymatic degradation of plastic polymer (Rogers et al., 2020). Larger fragments size of MPs restricts their entry into the microbial cells via cell membrane. Therefore, it must be broken down

into smaller fragments before being absorbed and biodegrade within the cell. MPs are broken down by microbial enzymes and microorganisms by a mechanism that typically entails hydrolysis, which is a common degradation process. The enzymatic hydrolysis and depolymerization of MP polymers trigger the biodegradation of MPs. MPs cannot be broken down without the aid of microbial enzymes (Yuan et al., 2020). Microorganisms degraded high molecular weight (HMW) plastics via a number of mechanisms, including the use of MNPs as a carbon source for growth and development (Othman et al., 2021).

## *3.2. Microbial degradation of micro-nano plastics in wastewater*

MNPs have been observed in vast quantities in contaminated and filtered water, surface water, such as oceanic water, streams, and rain water (Chandra et al., 2020; Kelly et al., 2021). The rate of biodegradation of plastics depends on their molecular weight, crystal structure, organic functional groups, and additives (Arpia et al., 2021). Their biodegradation either via anaerobic or aerobic processes leads to the

generation of  $CH<sub>4</sub>$  along with  $CO<sub>2</sub>$  and  $H<sub>2</sub>O$  (Mitrano and Wohlleben, 2020). Aerobic biodegradation of plastic fragments is profoundly impacted by abiotic factors, such as climate, salinity, UV-radiation, and co-contaminants that can either encourage or impede microbial colonization and bio-degradation (Arpia et al., 2021). Many bacterial taxa may form biofilms on the surface of plastic fragments via biosynthesis of extracellular polysaccharides (EPS). Biofilm formation on the surface of MNPs results fragmentation of MNPs into smaller size and finally its biodegradation. Several bacterial taxa, such as *Vibrio*, *Campylobacter*, and *Arcobacter* are well reported which can degrade MNPs during the treatment of WW (Kelly et al., 2021). Bacteria can degrade MNPs in contaminated water by producing enzymes like PETase and MHETase. As a result, it's a good idea to look at the very tiny molecules that can shape these kinds of enzymes in order to eliminate MNP from WW streams (Dey et al., 2021). MNPs have a higher surface area to volume ratio, providing a larger area for microbial colonization and their possible degradation.

The quantification of  $CO<sub>2</sub>$  and CH<sub>4</sub> emission during mineralization of MNPs can be used to quantify the percentage of biodegradation of MNPs (Hussain, 2019; Ariza-Tarazona et al., 2020). MNPs with organic additives contain oxygen (O), N, and sulphur (S) heteroatoms serve as hydrolytic and enzymatic action targets. The hydrolyzed and depolymerized MNPs fragments by the action of the extracellular microbial enzymes can be further used by microorganism as carbon and energy source (Mammo et al., 2020). Several bacterial groups have been involved in biodegradation of poly (3-hydroxybutyrate-*co*-3 hydroxyhexanoate) (PHBH), such as *Alteromonadaceae* and *Burkholderiales*. Furthermore, *Erythrobacter* sp. and *Alcanivorax borkum* growing on MP biofilms have been shown to be involved in eliminating low-density PE in aquatic environment (Yang et al., 2020). Studies related to MNPs removal from WW via primary (50–98%), secondary (0.2–14%), and tertiary (0.2–2%) treatments have been reported recently (Dey et al., 2021). Nevertheless, in most of the studies, limited evidence was provided about microbial degradation of MNPs in WW via biological/secondary treatment. Therefore, future studies must explore the roles of microbes in biodegradation of MNPs from WW stream.

# *3.3. Microbial degradation of micro-nano plastics in sewage sludge and municipal solid waste*

Generally, most of the MNPs are eliminated from WW after treatment but retained in the sludge (Liu et al., 2021). It was observed that the occurrence of MNPs in sludge is higher than WW (Sun et al., 2019; Aslam et al., 2020). Similarly, abundance of MNPs was reported higher in primary sludge than secondary sludge. Gies et al. (2018) assessed occurrence of 0.54–1.28 trillion MNPs in primary sludge and 0.22–0.36 trillion in activated/secondary sludge in wastewater treatment plant (WWTP) in Vancouver, Canada, which showed the importance of microbial assisted remediation of MNPs in secondary treatment. Similar to the sludge, MSW is also considered as a potential source of MNPs to environment. MNPs found in MSW can be linked with various micro inorganic and organic pollutants that can impose harmful impacts on the environment and human health as they enter the food chain (Golwala et al., 2021).

Indigenous microbial communities reside in MSW and sewage sludge are known to their plastic degradation potency. Ru et al. (2020) recently reported that, after 90 days, the mesophilic, *Stenotrophomonas panacihumi*, has been confirmed to break polypropylene (PP) into low (Mn: 2800, 3600 Da) and high (Mn: 44,000 Da) molecular weight form. Similarly, the bacterial strain *Rhodococcus* degraded 6.4% of the PP polymer mass in 40 days (Ariza-Tarazona et al., 2019). Moreover, the complete degradation of polyethylene terephthalate (PET) film by *Ideonella sakaiensis* within 6 weeks was reported by Yoshida et al. (2016) and 28% particle diameter reduction was found after 60 days using wild bacterial strains *Bacillus* sp., and *Paenibacillus* sp., isolated from landfill site by Park and Kim (2019). In comparison to pure microbial strains, microbial consortia are being effectively used to degrade the plastic polymers from the environment (Hussain, 2019; Li et al., 2021). The development of microbial consortia using two *Pseudomonas* sp. demonstrated better degrading abilities than established species which are the subject of this research. Consortia may also be formed with actinomycetes and fungi, which can have improved outcomes in terms of plastic and polymer degradation (Skariyachan et al., 2018; Tsering et al., 2021).

Plastic resistant bacterial strains, such as *Bacillus amyloliquefaciens* 1 and *B. Amyloliquefaciens* 2 have been isolated from solid waste sample and have been applied for plastic degradation (Pathak and Navneet, 2017; Wang et al., 2019). The study demonstrated the degradation of plastic by both the bacterial strains as monitored by  $CO<sub>2</sub>$  emission, weight loss, Fourier Transform Infrared Spectroscopy (FT-IR), and Scanning Electron Microscopy (SEM), analysis. They also isolated a fungal strain, which has been known as *A. clavatus*, from a local waste disposal site. This study revealed the degradation of LDPE by *A. clavatus* observed by SEM, and Atomic Force Microscopy (AFM) (Pathak and Navneet, 2017). Microorganisms use a variety of strategies to degrade high-density plastics, as carbon source or indirect action of microbial enzymes. *P.* fl*uorescence*, *P. aeruginosa*, and *Penicillium simplicissimum* are bacterial and fungal species, respectively that are well known for their plastic degradation ability. However, certain enzymes, such as lipases, esterases, and cutinases, have a remarkable ability to hydrolyze polymers like polyethylene adipate and polycaprolactone. Fungal species, such as *Rhizopus delemar*, *Achromobacter* sp., *R. arrhizus*, and *Candida cylindracea* are also sources of enzymes like lipases and esters which are involved in MNP degradation (Rana, 2019).

### *3.4. Microbial degradation of micro-nano plastics in compost*

Contamination due to smaller fragments of plastic waste is one of the most serious issues confronting the compost industry (Esan et al., 2019). In-situ biodegradation for field-based MNPs using hyperthermophilic composting (hTC) approach has been projected and thoroughly verified. 43.7% of MNPs are remediated from WW sludge after 45 days hTC action, the largest percentage ever recorded by biodegradation in MNPs. According to high-throughput sequences, *Thermus*, *Bacillus*, and *Geobacillus* are the most common biodegradation bacteria in the hTC age. These findings show that hyper-thermophilic bacteria play a crucial role in MNP bio-degradation during the hTC, pointing to a potential technique for removing MNPs (Chen et al., 2019). At 50 °C, LCcutinase, which is encapsulated in a specific metagenomic inventory of leaf-branch composting, can hydrolyzes low-crystallinity PET box film (lcPET-P, 8.4%) and yield up to 50% of weight in 7 days. Furthermore, at 63 °C, cutinase Cut190 from *Saccharomonospora viridis* can hydrolyze lcPET (7%) and lcPET-P (8.4%), ensuing in weight reduction of 13.5 and 27.0% of lcPET and lcPET-P, correspondingly, for more than 3 days. It was recently discovered that *Recmini*fi*da fusca* cutinase TfCut2 secreted by *B. subtilis* can minimize lcPET films (7%) by weight reduction to 97.0% and low PET observations with crystallinity from postconsumer bundles (AP-PET, 5%; CP-PET, 6%) by weight loss to 50.5 and 56.6%, correspondingly within 5 days at 70 °C (Ru et al., 2020). About 10% of independent plastic degradation tests have recorded polymer biodegradation below 50 °C, resulting 0.5% biodegraded plastics obtained in the extreme environmental settings like hot springs, compost, and anaerobic mounds (Ru et al., 2020). That extreme environmental setting could be a potential source of plastic degrading microbes and enzymes.

Scientific research is gradually focusing on implementing the regulations that allow biological approach of bio-plastics degradation in both aerobic and anaerobic natural waste treatment processes (Bhatia et al., 2013; Ruggero et al., 2019). Small plastics are naturally disintegrated using a number of processes, and thermophilic compost is a promising way to remove unwanted plastic fragments from the setting. *Streptomyces* sp., a thermophilic microbe that emerges from the soil alone and is documented to degrade poly (D-3-hydroxybutyrate) at 50 °C;

*Streptomyces* sp. demonstrated the rate of degradation of PE succinate. Similarly, *Bacillus* sp. TT96, a thermophilic bacterial strain showed the potency of PE succinate degradation during composting (Pathak and Navneet, 2017).

Polyurethane foam (PUR foam) is also a significant environmental contaminant and creates a most difficult waste disposal issue. In vitro, *Pseudomonas chlororaphis* was able to eliminate polyester PUR foam (Pathak and Navneet, 2017). Gram-negative *Pseudomonas*, a multi-species aerobic proteo-bacteria, is one of the few organisms that usually degrade polymers in the soil. Other Gram-positive bacteria, such as *Rhodococcus ruber* (aerobic, non-sporulating and, non-motile) and *Brevibacillus borstelensis*, have been found to eliminate polymers by using them as a carbon source. According to Skariyachan et al. (2018), fungal species like *Aspergillus niger* and bacteria species like *Pseudomonas* and *Vibrio* are able to make a consortium which improve the biodegradation of plastic fragments by syntrophic mechanism. Furthermore *Flavobacterium* sp., *Micrococcus* sp., *Staphylococcus* sp., *Bacillus* sp., *Chelatococcus* sp., and a few thermophilic bacteria are well known to biodegrade plastic in compost (Skariyachan et al., 2018; Skariyachan et al., 2015).

# 4. Recent advancement in micro-nano plastics degradation

The key properties of particulate plastic which make them inert toward biodegradation include their hydrophobic nature, HMW, and longer polymeric chain length (Sridharan et al., 2021a). The HMW of the organic compound bids a hindrance in facile transportation transversely to the microbial cell membrane and hence its depolymerization becomes critical (Oberbeckmann and Labrenz, 2020). To boost the biodegradation of plastic particulates, various advanced methods have been espoused recently like enzymatic/enzyme-assisted degradation (Priya et al., 2021), advance molecular tools and technologies (Sudhakar et al., 2007; Purohit et al., 2020), membrane bioreactor (MBR)-assisted remediation (Poerio et al., 2019), nano-technologiesbased remediation (Uheida et al., 2021) etc. The discussion relating to all these technologies is presented in this section.

# *4.1. Enzyme-assisted degradation*

It is implicit that once plastic wastes are dumped into the natural ecosystem, they may undergo disintegration/degradation as a result of the synergic impact of abiotic and biotic factors (Kumar et al., 2020a). Microbial-assisted biodegradation of plastic waste has emerged out as a greener and effective strategy as discussed in Section 3 comprehensively. A microbial diversity of genes, proteins, enzymes, and their involvement in diverse metabolic routes have been recognized significantly to alter the plastic polymers and enable the process of depolymerization (Priya et al., 2021; Yuan et al., 2020) (Fig. 3). Diverse groups of microbial enzymes, such as cutinases, lipases, esterases,



Fig. 3. Microorganisms and their respective enzymes in de-polymerization of synthetic plastics.

carboxylesterases, and etc. have been reported to alter and/or degrade range of plastic fragments (Zhang et al., 2020a) (Table 3). Also, various oxygenases, such as monooxygenases and dioxygenases have been reported to facilitate the enzymatic alteration of synthetic plastic via oxidation process (Jaiswal et al., 2020; Xu et al., 2020).

The oxidation improves the hydrophilic property of the plastic polymer, which promotes further colonization of microbes on it and release of various plastic degrading enzymes, such as esterases, hydrolases, and, lipases (Kim et al., 2020a; Onda et al., 2020; Puglisi et al., 2019). The Cubinding enzyme (laccase), extracted from *R. ruber* and *A.* fl*avus* is well known for its role in biodegradation of PE (Zhang and Chen, 2020; Priya et al., 2021). Similarly, bacterial species, *R. rhodochrous*, was tested to biodegrade and utilize PE oligomers with using specific carrier proteins, such as ATP binding cassettes (ABC) or major facilitator superfamily (MFS) (Eyheraguibel et al., 2017; Gravouil et al., 2017). Gravouil et al. (2017) studied, growth of the bacteria in PE-supplemented media along with their enzymatic expression. The consumption of PE by the microbes involved a sequence of actions, starting from the participation of acetyl coA and succinyl coA in the TCA cycle, then generation of energy currency as nicotinamide adenine dinucleotide hydrogen (NADH). The generated energy packet is further applied in the generation of adenosine triphosphate (ATP) through the electron transport chain (ETC),  $CO<sub>2</sub>$  and H<sub>2</sub>O as by product in the process of PE mineralization (Fig. 4). The basic findings related to PE biodegradation revealed the involvement of specific genes, enzymes, and various transporter proteins (Gravouil et al., 2017; Kumari and Chaudhary, 2020). Specific genes, like alkane hydroxylase (alkB) of *Pseudomonas* sp. E4 strain enabled to degrade the PE up to 28.6% in 80 days. Furthermore, to verify the potency of alkB gene, *Escherichia coli* BL21 strain was selected as host in which the expression of gene was performed and achieved 19.3% enzymatic degradation of the PE (Llorente-García et al., 2020; Yoon et al., 2012).

Diverse groups of marine and soil bacteria have been reported for production of hydrolases and their role in the enzymatic degradation of plastic wastes (Kawai et al., 2019; Tourova et al., 2020). The PET hydrolase produced by *Ideonella sakaiensis* has been investigated to degrade MHET, and showed similar activity like tannases (Palm et al., 2019). The bacterium strain *Ideonella sakaiensis* has enabled to produce PETase (hydrolase) and MHETase when subject to PET as a carbon source. These two biological catalysts enable to degraded PET into more facile compounds, like terephthalic acid (TPA), mono(2 hydroxyethyl) terephthalate (MHET), and bis(2-hydroxyethyl) terephthalate (BHET). Furthermore, MHET is hydrolyzed to TPA and ethylene glycol (EG) by the activity of MHETase (Kim et al., 2020a; Xu et al., 2020). Moreover, TPA is converted into protocatechuic acid (PCA), then PCA 3, 4 dioxygenase (PCA34) act on PCA and generate 4 carboxy-2-hydroxymuconic, which is further dehydrogenated and generate 2-pyrone-4-6-dicarboxylic acid. The generated 2-pyrone-4,6-dicarboxylic acid used in the TCA cycle and converted into pyruvate and oxaloacetate, and finally mineralized and released as  $CO<sub>2</sub>$  and  $H<sub>2</sub>O$ (Mahdi et al., 2016; Tourova et al., 2020).

Several fungal genera, such as *Fusarium*, *Humicola*, and *Penicillium* are well known as PET bio-degraders with the help of various enzymes, such as cutinase, polyesterase, and hydrolase (da Costa et al., 2020; Kawai et al., 2019; Palm et al., 2019). The most preferable enzyme was cutinases obtained from *Fusarium* and *Humicola*. Due to the accumulation of MHET as intermediate during the degradation of PET, *Humicola* cutinase action is inadequate, therefore, to overcome this, lipase obtained from *C. antarctica* is applied which entirely converts MHET into TPA (Carniel et al., 2017; Moharir and Kumar, 2019). PET esterases have also been observed to facilitate the hydrolysis of bis (benzoyloxyethyl)-terephthalate and polycaprolactone just like PET hydrolases (Hajighasemi et al., 2018; Nabi et al., 2020). A study performed by Luu et al. (2013) using styrene monooxygenase (extracted from *P. putida* F1 strain) assisted degradation of PS along styrene epoxide assisted oxidation. In the second step of oxidation, monooxygenase generate phenylacetaldehyde from styrene epoxide which further converts into phenylacetic acid (PAA). Furthermore, PAA is converted into phenylacetyl-CoA due to action of various enzymes and enters into the TCA cycle as acetyl-Co A and succinyl-CoA. Bacterial strain *P. putida* CA-3 was applied to degrade PS via specific rout known as Phenylacetyl-CoA catabolon. This route employs the action of catabolic operon, which facilitates the utilization of PS by *P. putida* CA-3, and production of PHAs (O'Leary et al., 2005).

Synthetic plastic variants, such as PUR, have also been well reported to undergo enzymatic degradation (Jenkins et al., 2019). *Comamonas*

#### Table 3







Fig. 4. A schematic representation of bio-mineralization of plastic waste.

*acidovorans* TB-35 bacterial strain has been deployed to biodegrade PUR via action of an esterase called as pudA (Yuan et al., 2020). Moreover, diverse groups of fungal species, such as *Candida ethanolica*, *Aspergillus fumigates*, *Penicillium chrysogenum*, *Fusarium solani*, and *Candida rugosa* have also been well characterized and reported to degrade PUR via various enzymes like lipase, esterases, and hydrolases (Jenkins et al., 2019; Kalita et al., 2020; Vanleeuw et al., 2019). *Arthrobacter* bacterial species have been reported for production of various hydrolases and aminotransferases which have capability to degrade nylon oligomer. The whole genome sequence of this bacterial strain confirms the presence of nylD1 and nylE1 genes, which encodes 6-aminohexanoate aminotransferase and adipate semialdehyde dehydrogenase, respectively that jointly facilitated the metabolization of 6-aminohexanoate and adipate semialdehyde to adipate semialdehyde and adipate, respectively (Gatz-Schrupp et al., 2020; Yuan et al., 2020).

#### *4.2. Advance molecular technologies*

The advancements in metabolic engineering and synthetic biology led to the development of robust microbial strains, which showed improved biotransformation potency and recycling of synthetic plastics in greener way. Genetic modification strategies act as influential technologies to modify the inherent characteristics of microbes and enhance their efficiency to biodegrade plastic wastes (Gu, 2021). Furthermore, the systems biology strategy applied various omics strategies, such as genomics, metabolomics, proteomics, transcriptomics, proteomics, etc. to improve the monitoring of degradation of diverse range of environmental contaminants (Basu et al., 2018; Kumar et al., 2019; Kumar et al., 2017; Kumar et al., 2018a; Kumar et al., 2018b) (Fig. 5).

Various metabolic engineering approaches have been developed recently and executed either individualistically or in association with genetically engineered construct well known to degrade recalcitrant pollutants (Kumar et al., 2020b; Taha et al., 2021). Systems metabolomic engineering has appeared as a significant strategy that assists the success of engineered microbes with improved cellular growth and achieves better plastic degradation efficiency (Yang et al., 2017). The progression in gene editing technologies and tools (TALENs, and the CRISPR/Cas9) also leads to improvement in the plastic degradation potency microbes (Gaj et al., 2013; Priya et al., 2021). These approaches can be practically applied for the introduction of genes in the genomes of microbes which encodes various plastic degrading enzymes, such as PETase, esterase, depolymerase, laccase and etc. (Gaj et al., 2013; Tofa et al., 2019).

Various investigations have emphasized that the biodegradation of plastic waste by the wild microbes is slow in comparison to the engineered constructs (Gu, 2021). For example, engineered enzyme construct cutinase was reported to reduce the degradation time of PUR 41.8 to 6.2 h in comparison to their wild counterpart (Islam et al., 2019). Syranidou et al. (2019) have confirmed the enhanced plastic biodegradation capability of manipulated marine microbes' consortia. The modified plastic degrader microbes can be applied in depolymerization of plastic polymers. In spite of several triumphs associated with genetic engineering of the microorganism at lab-scales, most of the engineered microorganisms have displayed unsatisfactory outcomes at field-scale.

The comprehensive knowledge about this information may improve the biodegradation of plastic polymers. Bioinformatics has arisen as an effective tool for enhancing the biodegradation of contaminants including MNPs (Purohit et al., 2020). Various databases related to



Fig. 5. Application of advanced molecular technologies/genetic engineering in biodegradation of plastic waste.

biodegradation pathways and toxicities have been established to evaluate the biodegradation. Few well-known databases, such as UM-BBD, MetaCyc, BioCyc provide valuable information associated with microbial metabolic routes, microbial genes and enzymes, and their complex enzymatic reactions which facilitate the biodegradation of recalibrates compounds like plastic polymers (Priya et al., 2021; Tourova et al., 2020). These computational methods are specifically advantageous, not only in investigating and recognizing the responsible enzymes, but simultaneously forecasting the biodegradation routes of toxic chemicals which were not previously recognized. This bioinformatics method certainly created a joint platform at which metabolic engineering and synthetic biology could be enabled to build a novel approach for biodegradation of plastics (Ali et al., 2021a; Ali et al., 2021b). Nevertheless, the major disadvantages associated with bioinformatics and experimental data are their unavailability, inaccessibility, and validation, which need consideration in future research.

Synthetic biology, most importantly 'omic' investigations along with computational biology and high throughput sequencing have consistently played a major role in enlightening the microbial-plastic-sphere interactions and subsequent degradation of polymers (Bouhajja et al., 2016; Wagner and Lambert, 2018). Moreover, designing a metabolic route for biodegradation of the synthetic polymers is also a key feature of synthetic biology (Purohit et al., 2020; Wang et al., 2021a). Nevertheless, there are knowledge gaps related to the diverse groups of synthetic polymer degrader microorganisms and their responsible enzymes. Hence, future investigations must emphasize the characterization and identification techniques of a robust polymer degrading microorganism and their enzymes. An extensive investigation is essential at environmental microbiology and biotechnology level, a gene manipulation and a protein engineering levels to overcome the stumbling block in the field of biodegradation of plastic polymers. In the near future, combinatorial approaches, such as unification of bioinformatic tools, metabolic engineering, genetics, molecular, and system biology may deliver ground-breaking insight in the field of biodegradation of plastic polymers.

# *4.3. Bio-membrane technology*

Bio-membrane technology or membrane bioreactor (MBR) technology is a set-up in which biological catalyst either microbes or enzymes, or both is linked with a partition method, ran by a film derived system, such as microfiltration and ultrafiltration (Dey et al., 2021; Xiao et al., 2019). These days MBR is considered as an emerging technology for effective treatment of industrial and municipal WW globally. Also, MBR has also been applied immensely in the field of food, pharmacology, biorefinery, and biodiesel production (Judd, 2016). In the treatment of MPs, the MBR is applied to reduce the complexity of the MPs contaminated media by biodegrading the organic matters (OMs), resulting into improved degradation of MPs. The biodegradation procedure commonly initiated, when a stream of pre-treated WW enters in the bioreactor, then biodegradation of OMs is performed over there. The remnant of treated liquid is impelled in a semi-cross flow filtration set-up for the removal procedure and concentrated in the retentate flow (Poerio et al., 2019).

Recently, Talvitie et al. (2017b) compared the efficiency of MBR with few others WW treatment methods like, disc-filter, rapid sand filtration, and dissolved air flotation for separation of MPs. In comparison to the above-mentioned technologies, an MBR technology displayed a substantial removal efficiency (99%) of MPs, improved quality of final effluent, and reduced treatment steps. Smith (2018) and Talvitie et al. (2017b) demonstrated a relative investigation related to separation of MPs via Rapid Sand Filters (RSF), Reverse Osmosis (RO), Dissolved Air Flotation (DAF), and MBR. Finding of these studies established that the MBR technology was the most effective in treatment of MPs polluted WW. Although MPs from WW could be separated out via MBR during WWT operation (Talvitie et al., 2017a), MPs treatment technologies which assist in the removal of the MPs from prevailing WWTP, are presently at the infancy phase of research.

MBRs are found to be effective in degradation of organic contaminants in MNPs. For example, the application of MBR in biodegradation of phthalate esters has been investigated at a laboratory-scale alone or liked with secondary sludge, starting with various synthetic or natural WW (Camacho-Munoz et al., 2012), paper mill WW (Yoshida et al., 2016), MSW leachate (Boonyaroj et al., 2012), etc. In WWTP, the application of MBR displayed around 70% more elimination of Di(2-ethylhexyl) phthalate (DEHP) in comparison to traditional treatment technology (3%) (Camacho-Munoz et al., 2012) and further enhanced (83%) if linked with primary adsorption. A comprehensive MPs biodegradation was achieved if MBR was linked with a primary anaerobic digestion along with RO filtration (Balabanic et al., 2012). Nevertheless, the degradation efficiency of the MBR is firmly governed by the physico-chemical characteristics of pollutants, and operation conditions, such as initial feeding rate and concentration, hydraulic retention time (HRT) etc. A significant finding, which could be in nearby future application linked with MBR, is the isolated bacterium species, *Ideonella sakaiensis* enable to utilize PET as a carbon source (Yoshida et al., 2016). Particularly, this bacterium species has specific enzyme mechanism which efficiently transformed PET into less toxic monomeric forms, such as TPA and EG. More recently, Dawson et al. (2018) observed a reduction in size of MP from 31.5 to <1 μm when Antarctic Krill (*Euphasia superba*) act on it. An in-depth investigation of size reduction of MPs by Antarctic Krill revealed the involvement of complex enzymatic mechanism. These enzymes will be effortlessly amalgamated with the MBR in near future and it will undoubtedly biodegrade the MP as previously confirmed by Barth et al. (2015) for PET biodegradation.

#### 5. Conclusion and future prospects

MNPs are recognized as emerging persistent pollutants in the environment, which seriously pollute the terrestrial and aquatic ecosystems. WW, sewage sludge, MSW, and composts are considered as potential sources of MNPs pollution in the environment. There are several technologies, such as physical, chemical, biological/microbial adopted so far to mitigate the MNPs menace. Biodegradation of plastics fragments is considered as one of the impending solutions to cartel the environmental menace generated by MNPs due to its greener nature. Therefore, this review spotted light on the various sources of MNPs in the environment, mainly focusing on the MNPs in WW, sewage sludge, MSW, and compost along with their toxicological impacts. Moreover, this review comprehensively covered various approaches adopted by the scientific community recently to mitigate MNPs pollution in WW, sewage sludge, MSW, and compost through bioremediation. Moreover, several MNPs remediation approaches, such as enzymatic, bio-membrane, advanced molecular and nanoparticle technologies have been described comprehensively in this review. Although, various research investigations have been performed so far to mitigate MNPs via bioremediation and subsequently minimizing their ecotoxicological impacts, still future investigations covering the aspects related to MNPs pollution, their impacts and mitigation strategies must be conducted.

- As for the sources and fate of MNPs in soil environment, and interactions with microorganisms, food crops and soil microbe, the scale of existing scientific research achievements is limited. The distribution, transportation and degradation of MNPs needs to be assessed in order to fully understand the long-term fate of MNPs in soil ecosystem.
- As carriers of various environmental pollutants, MNPs may promote or inhibit the mobility and bioavailability of the persistent and potentially hazardous pollutants in agricultural soils. But specific mechanisms of MNP and pollutant interactions are still unclear which require consideration in the future work. Additionally, future research should be more focused on NPs, as it has high penetration potential than MPs.
- The diversity of microorganisms and enzymes on the plastic-sphere is still limited. Therefore, future work must focus on identification of microorganisms on the plastic-sphere. In addition, it is a challenge for future microbiology researchers to select the effective microorganisms used in the degradation process of MNPs.
- It is a very beneficial research task to obtain MNP degrading microbes and its further application in production of biopolymer, which is worthy of further investigation in order to promote its application in biodegradation process. Furthermore, the recycling and synthesis of value-added products from the degraded plastics need extensive investigation to manage bio-valorization of synthetic plastic in a circular economy model.
- PVC is a thermoplastic. Only few fungal sp. has been reported which can degrade the PVC. Still bacterial and enzymatic-assisted degradation of PVC has not been reported that would be explored in future research investigations.
- PP is generally used in cosmetics and personal care products (PCPs). Abundant groups of microbes are reported, which effectively degrade the PP polymers. With various findings associated with microbial degradation of the PP, there are no reports available to enzymatic assisted degradation of PP and their mechanism, which need consideration in future research work.

#### 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.

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