Microbial-Mediated Plastic Degradation: A Sustainable Pathway to Environmental Health

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Abstract

Plastic pollution has surged due to excessive use and poor disposal of non-biodegradable polymers. Physical and chemical breakdown methods are inefficient and release toxic by-products. An alternate and eco-friendly approach to overcome plastic contamination is the utilization of plastic degrading microbes. These microbial species degrade plastic by the action of variety of enzymes, synthesized in their mircofactories. The plastic degrading enzymes include esterases, cutinases, lipases, protease, peroxidases and laccases. The biochemical process of plastic biodegradation comprised of four phases i.e.,biodeterioration, biofragmentation, assimilation and mineralization. Plastic is utilized by microorganisms as essential carbon and energy source, which is followed by the production of non-toxic and neutral substances like CO_2, H_2O, CH_4 along with biomass. This comprehensive review reveals the role of different microbes in the biodegradation of plastic, various enzymes involved in polymer degradation, underlying metabolic pathways associated with plastic depolymerization, molecular strategies and protein engineering methodologies for the enhancement of microbial plastic degradation process.

Keywords: Biodegradation, Depolymerization, Enzymes, Genes, Plastic, Microorganism.

Cite this Article as: Ullah AA, Riaz A, Abdali U, Siddiqui A, Kazmi SK, Qadeer K, Naeem M, Khan DM and Ayub A, 2025. Microbial-Mediated plastic degradation: A sustainable pathway to environmental health. In: Abbas RZ, Akhtar T and Arshad J (eds), One Health in a Changing World: Climate, Disease, Policy, and Innovation. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 310-318. https://doi.org/10.47278/book.HH/2025.170

A Publication of Unique Scientific Publishers	**Chapter No:** 25-044	**Received:** 04-Jan-2025 **Revised:** 19-March-2025 **Accepted:** 22-May-2025

Introduction

Plastic pollution has indeed turned into a noteworthy environmental concern as plastics are resistant to decomposition in nature, leading to their piling up in landfills, soil leakage, and unfavorable effects on ecosystem. Non-biodegradable plastics, often regarded as solid waste, accumulate on the ground which are mistakenly consumed as food by terrestrial animals such as buffaloes and cows. Engulfing these plastics can be fatal for these animals (Singh, 2005). As a result of improper plastic waste disposal in environment, toxic chemicals such as PCBs (polychlorinated biphenyls), PBDE (polybrominated diphenyl esters), BPA (bisphenol A), NP (nonylphenol), PAH (polycyclic aromatic hydrocarbons) and DDT (dichlorodiphenyltrichloroethane) are buildup in oceans (Bryant et al., 2016). These hazardous substances can severely threaten marine animals as they can cause indigestion, blockages in GIT tract and reproductive disorders (McFall et al., 2024). Globally, more than 400 million tons plastic waste is produced annually; and almost 5-13 million tons of this waste is disposed in to ocean, thus endangering the ecosystem. Traditional methods of plastic waste management like incineration and land filling also pose negative impacts on environmental health. These methods can contribute ecological imbalances that ultimately influence human health. Landfilling can lead to the soil contamination. The presence of fragments of microplastics in deep layers of soil cause pollution in groundwater there by disrupt the natural properties of soil. Lozano & Rillig (2020) reported a study in which they incorporated the microfibers of plastic in soil, as a result the biodiversity and living communities of soil was extremely affected which eventually induce imbalances in natural environment. Similarly, burning (incineration) of plastic waste give off harmful pollutants in air, which is considered to be one of the major reasons of air pollution. Incineration can trigger greenhouse effect, cytotoxicity, contamination of soil, depletion of ozone layer and acidification of water and soil. Incineration releases carbon monoxide, nitrogen oxides, carbon dioxide, aldehyde, furan, PAHs, voltaic organic substances, methane gas and particulate matter (Dimassi et al., 2022). The adverse impacts stimulated by land filling, ocean dumping and incineration are illustrated in Figure 1.

Waste plastic significantly impacts human health directly or indirectly through engulfment, inhalation or skin exposure (Figure 2). Specifically, the persistent exposure to microplastics can result to several physiological responses for instance oxidative stress, genotoxicity, cytotoxicity, necrosis, apoptosis and inflammation (Prata et al., 2020). Many studies reported more severe conditions like tissue damage, fibrosis and cancer upon constant exposure to plastics (Wright and Kelly, 2017; Dzierżyński et al., 2024; Winiarska et al., 2024; Vasse et al., 2024). The cellular uptake of endogenous pollutants can be potentially facilitated by swallowing of microplastics and nanoplastics by humans and animals (Prata et al., 2020). Moreover, the inhalation of particulate matter can cause oxidative stress that ultimately lead to acute inflammation and intestinal fibrosis (Nelet al., 2006). Lithner et al. (2011) reported that different plastic types like polyurethane, polyvinyl chloride and polystyrene can liberate harmful monomeric subunits which may be carcinogenic and mutagenic.

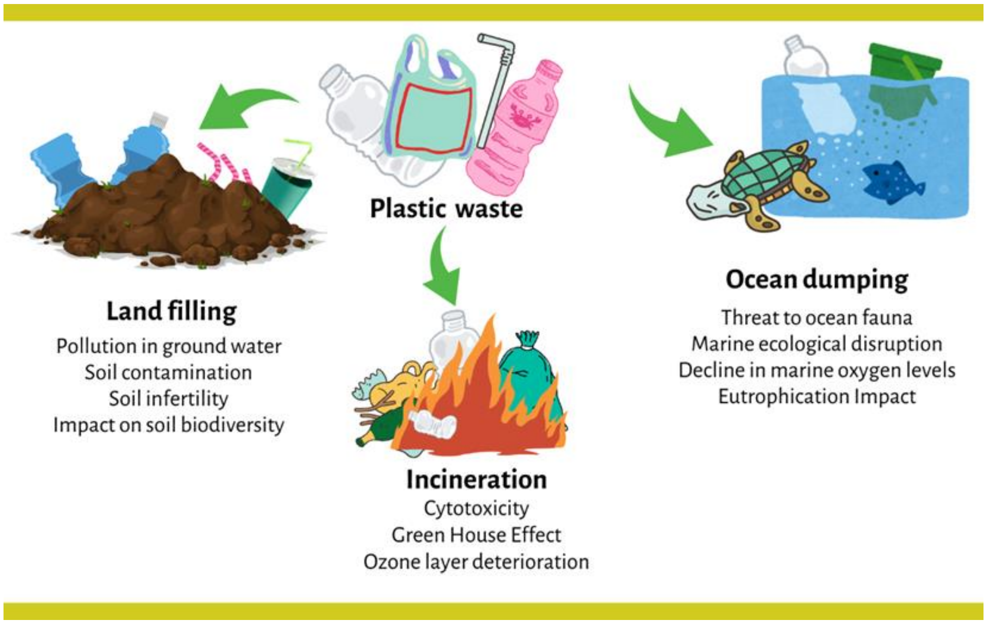

Fig. 1: Effects of traditional plastic disposal strategies on environment

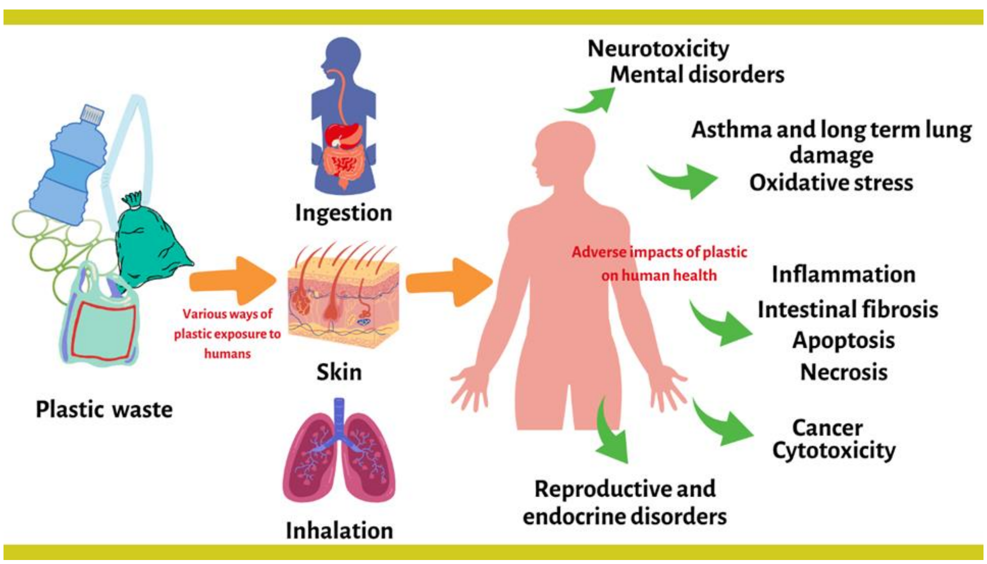

Fig. 2: Effects of plastic pollution on human health

Plastics, derived predominantly from hydrocarbon and petroleum derivatives, are extensively utilized due to their diverse applications. Different kinds of petrochemical plastic polymer include polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyethylene, (PE), nylon, polypropylene (PP), polycarbonate, polystyrene (PS), polyurethane (PUR) and polytetrafluoroethylene (PTFE). The packaging of products such as chemicals, detergents, drugs, food and beauty products frequently relies on polymers made from synthetic materials. Worldwide, packaging products use about 30% of all plastics (Sabir, 2004).

They have excellent thermal and electrical insulating qualities in addition to being durable, affordable, lightweight, and resistant to corrosion. However, the non-decomposable nature of most polymers poses a notable environmental threat. The disposal of synthetic plastics through methods such as burning, burying, and recycling has proven to be unsustainable and costly, necessitating a shift towards alternative, ecologically sound, and environmentally friendly approaches. However, the extensive use of plastics and inadequate waste management practices have led to a surge in plastic waste, surpassing efforts to mitigate plastic pollution (Borrelle et al., 2020). This highlights the urgent need to control plastic pollution with special emphasis on the execution of efficient plastic waste disposal technologies and manufacturing of bio-plastics (Samper et al., 2018).

Plastic degradation by microbes is one of the most promising approach to overcome plastic pollution. The microbial biodegradation is generally preferred over other traditional degradation strategies because of its ecologically safe nature and free-pollution mechanism. Environmental biotechnology primarily focuses on the consumption of microbial factories for the deterioration of plastic waste. Microorganisms like bacteria and fungi play a crucial role in biological breakdown of plastic waste thus there is need to understand their mechanisms of biodegradation which is critical for the development of effective plastic waste management strategies (Qin et al., 2021).

1. Microbial Breakdown of Plastic Polymers

For the biodegradation of different plastics, several strains of bacteria, algae, and fungi have been isolated and studied. The various microbial specie responsible for plastic biodegradation are presented in Table 1.

Table 1: Microbial species reported to degrade plastics (Khandare et al., 2022)

Microorganisms	Specie
Bacteria	*Mycobacterium*
	Bacillus
	Pseudomonas
	Flavobacterium
	Azotobacter
Fungi	*Aspergillus*
	Fusarium
	Alternaria
	Penicillium
Algae	*Anabaena*
	Chlorella
Actinomycetes	*Rhodococcus*

The general biochemical pathway of plastic degradation by microorganisms consists of three phases (a) adherence of microbes onto the polymer surface; (b) consumption of plastic polymer as a carbon source; and (c) the breakdown of plastic polymer in to CO_2, H_2O, CH_4 etc. Generally, microorganisms bind to the surface of polymer and catalyze the plastic molecules by releasing enzyme in order to acquire energy for their multiplication and growth(Danso et al., 2018) (Figure 3). Large plastic polymers catalyze into low molecular weight molecules called monomers and oligomers. Certain oligomers may diffuse inside microbial cells which are then absorbed in their microfactories.

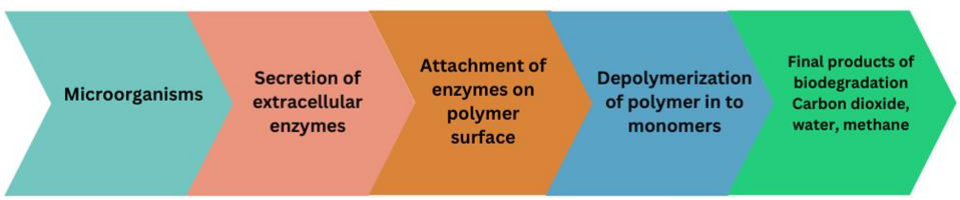

Fig. 3: General scheme of biochemical process of microbial-mediated plastic degradation

The primary breakdown of plastic polymer can be induced by a diverse biological and physical forces (Balangao, 2023). Physical processes like freezing/thawing, heating/cooling or wetting/drying can result in mechanical damage that ultimately leads to the cracks formation within polymer-(Shah et al., 2008).

The rate of breakdown of plastic polymer with high molecular weight by microorganisms is generally slow. Conversely, microbial cells can easily decompose and mineralize the monomers, dimers, and oligomers that make up the polymer's repeating units. High molecular-weight polymers bear low solubility thus making them less susceptible to microbial action because microorganisms require the substrate to be absorbed through the bio-membrane before being metabolized by enzymes. Extracellular and intracellular depolymerases are among the two types of enzymes that take an active role in the biochemical decomposition of polymers (Mohanan et al., 2023). During biodegradation, microbial extracellular enzymes catalyze complex polymers into smaller molecules like oligomers, dimers, and monomers that can easily diffuse across the semi-permeable membranes of microbes and later serve as a source of energy and carbon. This phenomenon is referred as depolymerization. On the other hand, mineralization is the deterioration process when the final products of plastic degradation are carbon dioxide, water and methane gas-(Mohanan et al., 2020). It is crucial to note that breakdown of polymeric compound typically do not reach to 100% because small part of the polymers is assimilated into humus, the biomass of microorganisms, and other organic compounds. External factors frequently affect prevalent microbial groups and their degradative mechanisms involved in the breakdown of polymers. Aerobic microbes primarily break down complex substances in to carbon dioxide and water along with microbial biomass (end products) in the presence of oxygen. In contrast, anaerobic microbes degrade polymers without the presence of oxygen, producing microbial biomass, carbon dioxide, methane, and water as the end products-(Zeenat et al., 2021).

1.1. Plastic degradation by bacteria

Bacteria are considered as one of the most manifesting microbes as they are responsible for biotransformation and recycling of nutrients in the ecosystem. The metabolic machinery of plastic degrading bacteria is equipped with specialized cellular enzymes that allow them to degrade polymers efficiently. The process of plastic biodegradation by bacteria involves the colonization of bacterial cells on plastic surface leading to the formation of biofilm. Along with biofilm formation, bacteria secrete extracellular enzymes that depolymerize the plastic in to monomers which further assimilated into carbon dioxide and water (Rüthi, 2023).

Marine environment is the best source for the isolation of novel bacteria for degrading complex organic compounds as well as plastic polymers. Zhao et al (2023) reported five bacterial specie namely *Marinobacter sediminum* BC31_3_A1, *Alcanivorax xenomutans* BC02_1_A5, *Marinobacter gudaonensis* BC06_2_A6, *Nocardioides marinus* BC14_2_R3 and *Thalassospira xiamenensis* BC02_2_A1 that were obtained from deep-sea sediments of Eastern Central Pacific Ocean. These strains were capable of degrading the PET plastic which was confirmed by the loss of weight about 1.3% to 1.8% of plastic after about 30 days of incubation time.

The plastic eating microbes have been found in various ecological habitats such as dumpsites (Muhonja et al., 2018), landfills (Gaytán et al., 2020), recycling locations (Yoshida et al., 2016), guts of insects (Ren et al., 2019) and low temperature marine zones (Urbanek et al., 2018).As reported in many studies, *Pseudomonas* is considered as one of the noticeable genera of bacteria characterized for plastic degradability that depolymerize the fatty acids long chains (Cai et al., 2023; Park et al., 2023; Zhang et al., 2023; Wilkes and Aristilde, 2017). Although some bacterial strains also showed resistance towards the biodegradation of thermoplastic such as polystyrene and polypropylene due to their homopolymeric nature but in recent years some potential findings have also been reported regarding the isolation of novel species like *Ideonella sakaiensis* which is able to degrade the PET (polyethylene terepthalate) plastic along with efficient consumption of polymer as exclusive source of energy and carbon (Swamy et al., 2024).

1.2. Plastic degradation by fungi

Another attractive microbial candidate that showed remarkable plastic degradation is fungi.Several studies have been done in this regard, indicating the isolation of fungal strains from both aquatic and terrestrial environment and their potential of depolymerizing toxic plastic pollutants (Ekanayaka et al., 2022; Srikanth, 2022; Bautista-Zamudio et al., 2023; Okal et al., 2023; Khatua et al., 2024). According to recently reported studies, among different categories of fungi Genus *Aspergillus* is the most potential and common type to be used for the degradation of synthetic plastic. *Aspergillus fumigatus* (Osman et al., 2018), *Aspergillus clavatus* (Gajendiran et al., 2016) and *Aspergillus niger* are some examples of *Aspergillus* species which showed degradation of plastic including PE, PU and PP. A synthetic polymer poly propylene (PP) was degraded by two fungal strains *Coniochaeta hoffmanni* and *Pleurostoma ruchardsiae*. These fungal candidates showed possible active colonization on pure propylene film(Porter, 2023).

Like other microorganisms, the ability of fungi to depolymerize plastic is also affected by various factors. In fungi, the penetration proficiency of fungal hyphae to colonize and dispense on the polymers prior to degradation and their property of hydrophobins production for the improvement of hyphal attachment to hydrophobic substrate have been considered as the important factor in plastic degradation (Sánchez, 2009).

1.3. Plastic degradation by algae

Several algal species have been identified and investigated for their role to eliminate toxic pollutants from the environment (Agate et al., 2024). However, the area of algae-mediated bioremediation of plastic remains underexplored, with research still ongoing. Falah (2020) proposed the effective depolymerization of PET plastic by *Chlorella vulgaris* after pre-treatment of plastic samples. The study demonstrates the more efficient plastic degradation (5.57%) with pretreated PET film than the one without pretreatment (5.45%). Other types of algal species such as *Anabaena*, *Spirulina, Spirogyra*, *Oscillatoria* and *Nostoc* have displayed the potential of colonization on various plastic surfaces but there is no evidence indicating their capability to metabolize plastic polymers (Sarmah and Rout, 2018).

2. Microbial Enzymes: Biocatalysts for plastic Decomposition

Microorganisms produce both extracellular and intracellular enzymes that play a critical role in plastic biodegradation. Extracellular enzymes are the most studied group of plastic degrading enzymes with high rate of reactivity (Glaser, 2019). These microbial plastic degrading enzymes include laccases, peroxidases, lipases, esterases and cutinases (Gan and Zhang, 2019).

2.1. Esterases

Petro-plastic polymers and other polysters have same ester bonds. Esterases are the hydrolase enzymes that cleave ester bonds. In the presence of water molecule, it splits ester into two compounds i.e. alcohol and acid (Mohanan et al., 2020).

Variety of fungal and bacterial species are reported for production of esterases. Bacterial specie *Comamonas acidovorans* has been observed for its ability to degrade low molecular weight PLA plastic using esterase enzyme. Poly (butylene succinate-co-adipate) and polyurethane have been reported to be attacked by the fungal esterases from *Purpureocillium lilacinum* and *Curvularia*(Yamamoto-Tamura et al., 2015).

2.2. *Cutinases*

Cutinases are enzymes with specificity towards high molecular weight polyesters (Chen et al., 2013). This specialized class of esterases is known to produce from variety of different fungal strains ranging from pathogenic plant fungi, e.g. *Fusarium solanipisi* (Heredia, 2003) to soil living fungal species including *Fusarium solani*(O'Neill et al., 2007) and *Penicillium citrinum*(Liebminger et al., 2007). Furukawa et al. (2019) worked on decomposition of mats made from electrospun polycaprolactone (PCL) using cutinase 2p from *Arxul aadeninivorans*.

Moreover, thermophilic actinomycetes have also been reported to be the producers of cutinases such as *Thermobifida cellulosilytica* (Herrero Acero et al., 2011), and *Thermomonospora curvata* (Islam et al., 2019).

2.3. *Laccases*

Laccases, belong to the class of oxidases having multiple copper ions at the catalytic site to oxidize phenolic compounds and reduce molecular oxygen to produce water (Nunes and Kunamneni, 2018). Laccases are known for degradation of lignin, polyethylene (PE) and variety of different types of polymers such as laccase producing *Cochliobolus* sp. has documented to degradepolyvinyl chloride(Tirupatiet al., 2016) and *Bjerkanderaadusta*TBB-03 has been reported as the efficient degrader of high-density polyethylene (Bo Ram Kang, 2019).

2.4. *Lipases*

Lipases belong to sub-class of esterase enzyme. They promote lipid hydrolysis. Various fungal species have gained importance due to plastic degradation through production of lipases enzyme.

Polybutylene succinate (PBS) and polybutylene succinate-*co*-adipate (PBSA), the two commonly used packaging materials have reported to be hydrolyzed by the lipase enzyme produced from *Cryptococcus* sp (Thirunavukarasua et al., 2008). Lipase FE-01 from *Thermomyces languinosus* has been reported to catalyze decomposition of electro spun polycaprolactone fiber (Furukawa et al.,2019).

2.5. Peroxidases

Peroxidases belong to the class of oxidoreductase enzymes. They catalyze reduction of peroxides particularly hydrogen peroxide and simultaneous oxidation of electron donating substrate. The different classes of peroxidases including lignin peroxidases (LiP), manganese peroxidases (MnP), and versatile peroxidases (VP) are known to be expressed in white-rot fungi (Hofrichter and Ullrich, 2006). Similarly, peroxidases from *Fusarium graminearum* were capable of doing the same job (Ganesh et al., 2017).

3. Mechanisms Underlying the Biodegradation of Synthetic Polymers

Microbial degradation of complex polymers can be broadly categorized into four different mechanisms i.e., biodeterioration, biofragmentation, assimilation, and mineralization (McFall et al., 2024).

3.1. Biodeterioration

Biodeterioration is the mechanism that affect plastic surface resulting in change of its chemical, physical and mechanical characteristics. In this process, microbes first bind and colonize on to the plastic surface with the purpose of reducing resistance and plastic durability. Due to the hydrophobic nature of plastic, an attachment of microorganisms is facilitated through hydrophilic functional groups (Nauendorf et al., 2016).The attachment and successive growth of microorganism on polymer surface cause swelling resulting in reduced mechanical properties. Once the microbes get attach to the plastic surface, their proliferation continues as they use polymers as carbon source. Exopolysaccharides play crucial role in attachment and biodeterioration of plastic polymers as they are strong adherent (Anjana et al., 2020).

3.2. Bio-fragmentation

After biodeterioration, bio-fragmentation occurs, involving enzymatic action on plastic polymers. Bacterial oxygenases introduce oxygen into carbon chains, producing less harmful alcohol and peroxyl products. Lipases, esterases, and endopeptidases further catalyze the transformation of carboxylic and amide groups. This process involves two key reactions: reducing polymer molecular weight and oxidizing lower-weight molecules, enabling microbial enzymes to degrade these smaller compounds. Hydrolytic cleavage, through exo- and endo-attacks on bonds like glycosidic, ester, and peptide, yields monomers or oligomers. Exo-attacks produce assimilable compounds, while endo-attacks generate intermediates requiring further breakdown (Pathak and Navneet, 2017).

The considerable impacts of the oxidation reactions were demonstrated in *Rhodococcus rhodocrous*, which was able to breakdown almost all of the previously oxidized oligomers from polyethylene (Gravouil et al., 2017). Furthermore, various inorganic and organic chemicals generated by bacteria may have a role in promoting the biofragmentation process. Several inorganic compounds (ammonia, hydrogen sulphide, nitrites, thiosulphates) and organic acids (citric, fumaric, gluconic, glutaric, glyoxalic, oxalic acids, etc.) have been shown to scavenge cations from the polymer surface, forming stable complexes that can induce surface erosion and fragmentation (Krause et al., 2020).

3.3. Assimilation

In assimilation, the lower molecular weight substances generated from biofragmentation are translocated into the cytoplasm of microbe. This process can include both passive and active transportation. In *Pseudomonas* sp. DG17 , the transportation of octadecane (metabolized product of plastic) across the membrane was achieved through facilitated passive transport systems at higher concentrations, while it is absorbed through active transportation at lower concentrations(Shahnawaz et al., 2019) . A specialized transporter protein has been reported to move terephthalic acid which is an intermediate product of polyethylene terephthalate (PET) (Hosaka et al., 2013).

3.4. Mineralization

Once these plastic hydrolyzed products are efficiently assimilated in to the cells, they are further processed through a series of various biochemical reactions involving enzymes which result in the complete degradation in to oxidized derivatives like CO_2, N_2, CH_4, and H_2O.

On the other hand, the intermediate substances can be utilized in to different metabolic pathways. For instance, it can be proposed that biodegradation of polyethylene leads to the production of acetic acid which can either enter the Kreb's cycle via acetyl-CoA formation or be directed towards lipid biosynthesis (Wilkes and Aristilde, 2017). The phases of plastic degradation are summarized in Figure 4.

4. Genetic and molecular Perspectives on Plastic Biodegradation

Various strategies of genetic engineering were successfully employed in order to enhance the production and activities of different plastic degrading enzymes by manipulating genes associated with these enzymes. Till now, very few studies have been recorded elaborating the genes encoding plastic biodegradation and its pathways.

The PET degrading activity of microbe was improved by transferring a gene of polyster hydrolase from *Pseudomonas aestusnigri* to *Escherichia coli* (Bollinger et al., 2020). Several studies highlighted the exceptional PET plastic degradation capabilities of *Ideonella sakaiensis*. In this context, considerable studies on genetic manipulation associated with PETase gene have been carried out. Various researches elucidated the cloning and expression of PETase gene from *Ideonella sakaiensis* in multiple host expression systems such as *E. coli* (Joo et al., 2018) and *P.tricornutum* (Moog et al., 2019). To stimulate the complete degradation of PET, the gene encoding MHETase, a tannase-like enzyme known to work synergistically with PETase for optimal degradation, was expressed in *E. coli* after being purified from *Ideonella sakaiensis* (Janatunaim and Fibriani, 2020).

The genes encoding alkane hydroxylase responsible for LDPE degradation have been identified and reported by Muhonja et al. (2018)in diverse fungal and bacterial isolates Recently, the plastic degrading genes have also been identified in algae specie. The gene encoded PETase enzyme was taken from green microalgae *Phaeodactylumtricornutum* and cloned in rapidly growing and ecofriendly algae specie *Chlamydomonas reinhardtii* (Kim et al., 2020).

Additionally, numerous protein engineering strategies have been applied to enhance the enzymatic efficacy of plastic-degrading enzymes. Ma et al.(2018) adopted site-directed mutagenesis to considerably lower the km value by enhancing the activities of *Ideonella sakaiensis* by over 100%. On the other hand, protein engineering techniques are employed to expand the enzyme's substrate specificity to allow the metabolism of a wide range of synthetic polymers, such as polyethylene-2,5-furandicarboxylate (PEF) beyond PET (Austin et al., 2018).

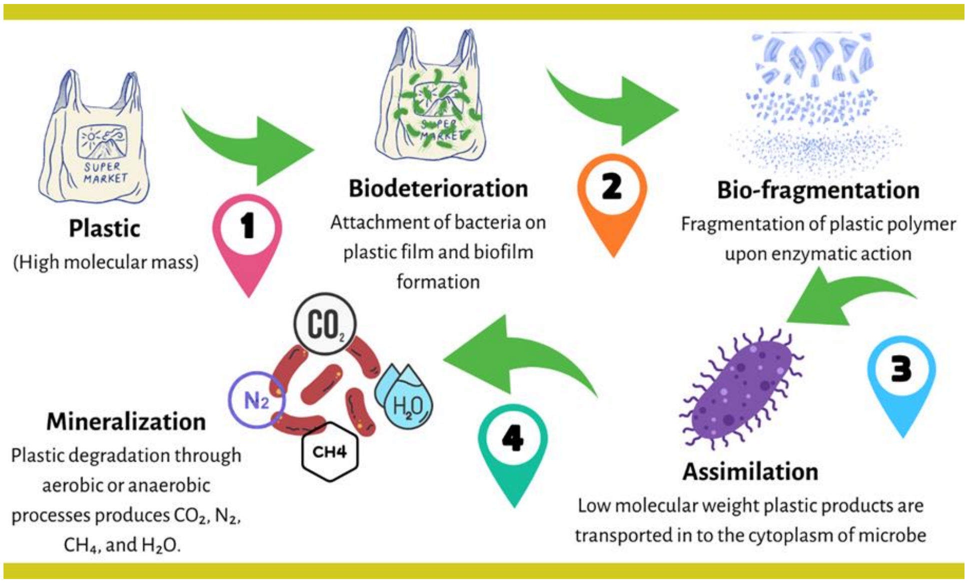

Fig. 4: Phases of microbial plastic degradation

Conclusion

Uncontrolled disposal of plastic waste in environment has led to significant global concern. Conventional plastic waste management approaches like land filling, ocean dumping and incineration pose adverse impacts on environment so there is need to find out eco-friendly strategies to manage plastic waste. Microbial-mediated plastic degradation is an efficient and sustainable approach to eliminate plastic waste from environment. Various bacterial, fungal and algal specie have shown great potential to depolymerize a wide range of plastics. These promising candidates include *Ideonella* sp., *Bacillus* sp., *Streptomyces* sp., *Pseudomonas* sp., etc. Though, research in this domain is not fully explored however, extensive investigation is still needed to understand the biochemical pathways associated with plastic biodegradation.

Furthermore, employing various approaches such as genomics, transcriptomics, and proteomics can aid in exploring the upregulation and downregulation of genes under specific cultural conditions during plastic depolymerization. Research focused on analyzing gene-protein interactions and uncovering the biological functions of target genes can provide deeper insights into the mechanisms underlying plastic biodegradation.

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