Microbial Degradation of Plastics

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ABSTRACT

The main contributor to environmental pollution and a growing ecological danger is plastic accumulation. One of the key concerns of the twenty-first century is the sustainable usage of synthetic polymers. The traditional methods used to decompose plastic in the environment are insufficient because they produce toxic byproducts. Utilizing microorganisms to biodegrade plastic is one of the best method for minimizing plastic pollution. This paper deals with, potential of plastic degrading microbes reported by different investigators.

INTRODUCTION

Around the world, plastics are crucial to every economic sector.Plastics use ensures that they are in high demand in fields that are seeing rapid growth, such as agriculture, building and construction, health, and consumer products. Without plastics, no one could perform their jobs. Plastics, the foundation of many businesses, are utilized in the production of many objects we use every day, including defense supplies, sanitary items, tiles, plastic bottles, leather, and other domestic items.The manufacture of synthetic plastics is one of the sectors of the global economy that is expanding.Plastics are more superior than other materials due to their unique properties. These properties also led to increase the plastic production scale to 20 folds since 1964 (Ellen MacArthur Foundation 2016), and production scale exceeds 300 million tons/year (Plastics Europe 2015) in 2015 it reached to 335 million tons (Plastics Europe 2017) Plastics are strong, durable, and light weight. On the other hand,they are harmful to the natural environment, resistant to degradation and cause the environmental pollution.The accumulation of misadministered plastic waste in natural environments create a serious threat to our oceans, wildlife, and human health. As the global demand on plastics is growing continuously,the trend of plastic emission into open environments is unlikely to reduce until 2030 unless key policies, consumption behaviors and waste management measures regarding plastic products will be radically transformed immediately (Borrelle et al., 2020).A growing accumulation of plastic wastes has become a severe environmental and social issue. It is urgent need to develop innovative approaches for the disposal of plastic wastes.

The polythene is the most commonly found non-degradable solid waste that has been recently recognized as a major threat to marine life. The polythene could sometimes cause blockage in intestine of fish, birds and marine mammals (Spear *et a*l. 1995, Secchi and Zarzur 1999).In recent years, reports on biodegradation of synthetic plastics by microorganisms or enzymes have been arise suddenly ,and these offer a possibility to develop biological treatment technology for plastic wastes. the microorganisms and enzymes that are able to degrade a variety of generally used synthetic plastics, such as polyethylene (PE),polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR),and polyethylene terephthalate (PET). Plastics can be differentiated into degradable and nondegradable polymers on the basis of their chemical properties (Ghosh et al., 2013). Plastics that are obtained from renewable resources are biodegradable plastics. These are naturally degradable,as a source of cellulose, starch and algal material, an important component in plants, animals and algae. These polymers are also produced by microorganisms. Non-degradable plastics, typically known as synthetic plastics, are derived from petrochemicals and are higher in molecular weight due to the repetitions of small monomer units (Imre and Pukánszky, 2013).During plastic degradation the generation of plastic particles with a size of < 5 mm are known as microplastics (MPs) which lead to potential ecotoxicological effects (Chen et al.,2020; Wong et al., 2020). Fibrous MPs may be inhaled,may persist in the lung, and along with associated contaminants including dyes and plasticizers could lead to health effects like carcinogenicity and mutagenicity (Wong et al.,2020). Generally, it is accepted that plastic waste can always be eliminated through incineration. However, unburned material still exists in the bottom ash in the form of a solid residue from incinerators that can produce 360 to 102,000 microplastic particles per metric ton after incineration. This bottom ash is a probable source of MPs released into the environment (Yang et al., 2021).It is reported that plastic fragments in the <100 nm size range,referred to as nanoplastics (NPs), may also be formed in the aquatic environment and may cause serious health effects (Nolte et al.,2017; Revel et al., 2018).Suman et al. (2020) reported that the histopathology analysis indicated the deformation of epithelial cells in the midgut region after both chronic and acute exposures at 1 and 100 mg/L, respectively to polystyrene microplastics. In another study, Chen et al.(2020) reported that redclaw crayfish (Cherax quadricarinatus)were exposed to different concentrations

(0, 0.5, and 5 mg/L) of 200 nm-sized polystyrene microspheres for 21 days and the microplastics were distributed in the intestines and hepatopancreas after ingestion and inhibited the growth of Cherax quadricarinatus.

Xiao et al. (2020) reported that freshwater microalgae,Euglena gracilis, exposed to 1 mg/L of polystyrene microplastics(PS-MPs) for 24 h. The vacuoles of microalgae were induced and pigment contents were reduced significantly ($p < 0.05$). Plastics can be degraded in the environment by 4 mechanisms i.e. hydrolytic degradation, photodegradation, thermo-oxidative degradation and biodegradation (Webb et al., 2013). Plastics create serious threats to our environment and their removal from the environment is imperative. The plastics that are degraded by microorganisms are known as biodegradable plastics and microorganisms can degrade them into H_2O and CO_2 (Nakajima-Kambeet al., 2009). The rate of biodegradation of polymers can be increased by using thermo-oxidant and photo-degrading agents.Free radicals cause rupturing of the chains by oxidizing the polymeric molecules. Many physical and chemical changes occur due to photo oxidation including reduction in polymers molecular weight and production of carbonyl groups. In thermal oxidation high temperature more than melting point is given which decreases the fusion heat and increases level of carbonyl group production. As a result, polymers are more liable to be degraded by microorganisms. Phase separation, erosion,discoloration treatment types, cracking and types of polymers are the various factors that are responsible for the biodegradation and source of pollution in the environment.

While abiotic environmental degradation contributes considerably to the fragmentation of large plastic debris resulting in micro- and nanoplastic pollution (Min et al., 2020), the role of microorganisms in the plastic degradation under natural conditions is still poorly understood. In recent years, various microbes seemed capable of depolymerizing synthetic polymers at laboratory conditions (Wierckx et al., 2018). Neverthless, the microbial degradation extents and rates of conventional petroleum-based plastics such as polyethylene (PE) and polystyrene (PS) can differ remarkably to those of biodegradable polyesters such polylactic acids (PLA). Microbial biotechnology has been repeatedly proposed as an option of sustainable disposal approach of plastic waste although the reality and promise of biotechnological recycling methods is not yet clear among scientific communities, plastic end-users and policy makers (Wei et al., 2020).Ru et al.(2020) provided a complete review on the microorganisms and enzymes able to degrade mass-produced recalcitrant petrochemical plastics reported since the 1970s.While PE and PS exhibited to be degraded by several microorganisms, evenso very slowly, the key depolymerases involved in the breakdown of carbon-carbon backbones remain still unknown. For a better understanding of the enzymatic degradation of vinyl polymers, Xu et al. carried out quantum mechanism calculations to model the cleavage of the carbon-carbon bond at the Cß position under both acidic and alkaline conditions.

Microbial communities are a valuable source of enzymes with degrading activities on synthetic polymers. Pinnell and Turner reported the shotgun metagenomic sequencing of biofilms fouling polyethylene terephthalate (PET), polyhydroxyalkanoate (PHA) and ceramic placed at the sediment-water interface of a coastal lagoon. While PET plastic biofilms were indistinguishablecompared to the ceramic biofilm control, PHA bioplastic biofilms were distinct as indicated by the dominant presence of sulfate-reducing microorganisms (SRM) and a significant enrichment of phylogenetically diverse polyhydroxybutyrate (PHB) depolymerases. Their findings indicatethat the plastisphere SRM play an important role in PHA biodegradation.

Weinberger et al. developed a fungal library with aliphatic and aromatic polyesters, leading to the identification of new strains that produce polyester hydrolyzing enzymes. This method will enable exploring the available fungal diversity and thus broadening the spectrum of candidate enzymes for plastic recycling. The increasing commercial demand on bio-based and biodegradable plastics such as PLA requires also environment friendly disposal methods using microorganisms .The high-resolution structural elucidation and functional characterization of a novel PET hydrolyzing enzyme (PE-H) identified in the genome of the marine bacterium Pseudomonas aestusnigri. PE-H was shown to degrade amorphous PET at 30◦C. By structural modeling and mutagenesis study, a Y250S variant was constructed to exhibit increased PET hydrolytic activity as a result of the rearrangement of the active site conformation, thereby providing new knowledge on the structural features required for efficient polyester degradation. Based on previous reports regarding the PET polymer chain mobility associated with enzymatic degradation (Wei et al., 2019a,b), Falkenstein et al. could evaluate the feasibility of UV radiation as a potential pretreatment method to stimulate the subsequent biocatalytic depolymerization of PET. Although UV treatment has caused significant chain scissions at the surface layer of amorphous PET films, the resulting increased surface crystallinity drastically impaired the efficiency of enzymatic degradation.As engineered whole-cell catalysts have been recently considered with great prospects for plastic degradation (Yan et al., 2020), the microbial metabolism of plastic monomers and additives will become a research focus both in the contexts of environmental degradation of plastic pollution and of biotechnological plastic upcycling, i.e., utilization of plastic hydrolysates as raw material for microbial production of chemicals with high value (Salvador et al., 2019).

Environmental pollution by plastic waste was first reported in the 1970s (Carpenter and Smith, 1972). The expanding amount of plastic waste has become a global concern. Despite increasing efforts to reduce the plastic waste by disposing off through segregated collection and recycling, a sizeable amount of plastic solid waste is still landfilled. From whole plastic production by 2017 (8,300 million tons), after recycling, incineration (energy recovery) of wastes and calculating in-use plastics in domestic; around 60% have been left in the environment including 95% in landfills and 5% in the oceans and other terrestrial areas (Ragaert et al., 2017). Plastic debris in the environment is degraded in nature by photo-, bio-, and thermo-oxidative depolymerization as well as friction (Barnes et al., 2009; Browne et al., 2011). Although biodegradation of these plastics is feasible in the natural environment, it can take long periods of time: from 50 to more than 100 years (Table 1).

TABLE 1

Selected properties of major synthetic thermoplastic polymers (Ojeda, 2013).

Polymer	Density (g/L)	Crystallinity (%)	Life span (years)
PET	1.35	0–50	450
LDPE	0.91–0.93	50	10–600
HDPE	0.94–0.97	70	>600
PS	1.03–1.09	0	50–80

PP	0.90–0.91	50	10–600
PVC	1.35–1.45	0	50–150

PET, Polyethylene terephthalate; LDPE, Low density polyethylene; HDPE, High density polyethylene; PS, Polystyrene; PP, Polypropylene; PVC, Polyvinyl chloride.

Biodegradation has been measured by a wide-range of variables, including substrate weight loss, changes in the mechanical properties and/or the chemical structure of the polymer and the percentage of carbon dioxide emission. Early microbial biodegradation experiments attempted to demonstrate that microbial activity could result in changes in the physical characteristics of plastics, such as tensile strength, crystallinity, and water uptake (Pirt, 1980; Albertsson and Karlsson, 1990). The identification and genetic engineering of these plastic-degrading microorganisms and/or enzymes will provide an opportunity to improve plastic recycling and thereby reduce environmental plastic pollution by means of assimilation of plastic waste into carbon source or degradation of plastics waste into valuable alkane products via microbial biotechnology. The biodegradation mechanisms of petro-plastics are likely related to the types of bonds in the polymeric chains (since the active sites of related enzymes are individual for any specific bond). Thus, mechanisms of petro-plastic degradation can be classified into three groups: (i) Polymers with carbon back-bones; (ii) Polymers with ester-bond back-bones and side-chains; and (iii) Polymers with hetero/carbamate(urethane) bonds.

Investigating the techniques involved in the bioremediation of plastic.

Natural decomposition of the polymer can be characterized by uptake of O_2, the rate of CO_2 released alterations in the polymer's physical and chemical properties, and microbial growth rate (Mohan and Srivastava, 2010) used different assessments method for evaluating the polymer degradation based on the following reasons(Table 2)

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Table :2

S.NO	**Variations in characteristics of Polymer**	**Various Types of Technique**	**References**
1	Mechanical: Modulus of the polymer & Tensile strength - Elongation at fail	Dynamic Mechanical Analysis (DMR)	Huang et al., 2005 Kathiresan, 2003
2	Physical: Morphology- Microcracks Density, Molecular Weight Distribution, Contact angle, Viscosity Glass Transition temperature and Melting Amorphous region and Crystalline	Scanning Electron Microscope (SEM)	Zuchoswka et al., 1999 Albertson et al., 1995 Doble et al., 2008
3	Chemical properties	DSC, Thermogravimetric analysis	Albertson et al., 1995; Deguchi et al., 1997.
4	Molecular Weight	X-diffraction, Small and Wide-angle X ray Scattering Fourier Transformed Infrared	Zuchoswka et al., 1999 Albertson et al., 1995 Doble et al., 2008

		Spectroscopy (FTIR) Gas Chromatography-Mass Spectrometry (GC-MS), Gas Chromatography (GC)	
5	Evolution test of CO2	Gas Chromatography	Albertson et al., 1995 Seneviratne et al., 2006
6	Metabolic force of the cell	Protein analysis, Fluorescein Diacetate (FDA), Adenosine triphosphate (ATP)	Gilan et al., 2004 Kounty et.al.2006

Factors affecting Biodegradation of Plastics

The biodegradation of plastics by bacteria and fungi proceeds differently under different soil conditions according to their properties. The different factors that govern biodegradation are type of organism, polymer characteristics and nature of pre-treatment. Polymer characteristics refer to its tacticity, mobility, crystallinity,molecular weight, the type of functional groups and other substituents present in its structure and additives or plasticizers added to the polymer (Singh and Sharma,2007). It was noticed that smaller fragments of plastics were biodegraded faster than bigger ones(Sivan,2011). Biological degradability of polymers by microorganisms decreases with increase in the molecular weight of the polymer. With increase in molecular weight, there is decrease in polymer solubility which makes it unfavourable for microbial attack as the polymer needs to be assimilated into the bacterial cell membrane and broken down by cellular enzymes. Repeating units of polymers like monomers, dimers and oligomers are easily degraded and mineralized (Shah et al.,2007) Biodegradation is enhanced by abiotic hydrolysis, photo-oxidation and physical disintegration.These processes enhance the surface area of the polymer and reduce its molecular weight; facilitating microbial degradation (Singh and Sharma,2008).Amorphous regions are more susceptible to microbial degradation than crystalline regains. The presence of a glucose source reduces the rate of biodegradation as glucose is a more preferred carbon source than plastic(Jang et al.,2002).

Microorganisms involved in Biodegradation of Plastics

The microbial species associated with the degrading materials were identified as bacteria, fungi, actinomycetes sp. and *saccharomonospora* genus (Chee,et.al.,2010). The microorganism's growth is influenced by several factors including the availability of water, redox potential, temperature carbon and energy source (Sand,2003). Microorganisms secreted by both exoenzymes and endoenzymes that are attached to the surface of large molecular substrate and cleave in to smaller segments (Albinas and Dahlia Microorganisms recognize polymers as a source of the organic compounds (Premraj and Mukesh,2005)

Bacteria

Microorganisms such as *Bacillus megatserium*, *Pseudomonas* sp., *Azotobacter sp.*, *Ralstonia eutropha*, *Halomonas* sp., etc are used to degrade plastics ((Chee,et.al.,2010).In addition, *Bacillus brevis(Tomita,1999***)** *Acidovorax delafieldii* BS-3(Uchida,2000), *Paenibacillus amyloticus* TB-13 (Teeraphatpornchai,2003) *Bacillus pumilus* 1-A (Hayase,2004)**,** *Bordetella petrii* PLA-3 (Kim and Park,2010)**,** *Pseudomonas aeruginosa* PBSA-2 (Lee and kim,2010)**,** *Shewanella* sp. CT01 (Sekiguchi,2010) are examples of bioplastic degrading bacteria.In addition to these strains, a thermophilic bacterium, *Bacillus brevis*, with PLA-degrading properties has been isolated from soil.

In past years polyethylene degrading bacteria has been reported such as, *Acinetobacter baumannii, Arthrobacter* spp*, Viscosus spp*, *Pseudomonas* spp, *Arthrobacter viscosus, Bacillus amyloliquefaciens, thuringiensis, Mycoides, Cereus, pumilus, Staphylococcus cohnii, Xylosus spp, Pseudomonas fluorescens, Rahnella aquatills, Micrococcus luteus, Lylae, Paenibacillus macerans*, *Flavobacterium* spp*, Delftia acidovorans, Ralstonia* spp *Rhodococcus erythropolis, Pseudomonas aeruginosa* (Koutney et al.,2009)and *Bacillus brevies* (Watanabe ,2009)The microbial species that identified from the sample polythene bags tested were *Bacillus sp., Staphylococcus sp., Streptococcus sp., Diplococcus sp.,Micrococcus sp., Pseudomonas sp. and*

Moraxella sp. The microbial species are associated with the degrading materials were identified as bacterial genus like (*Pseudomonas, Streptococcus, Staphylococcus, Micrococcus, Moraxella*) (Swift ,1998). Kathiresan (2003) identified 9 microbial species identified from degrading polythene bags were *Bacillus* sp., *Staphylococcus* ,*Streptococcus* sp., *Diplococcus* sp., and *Micrococcus* sp. (belong to Gram- positive bacteria); *Moraxella* sp. and *Pseudomonas* sp. (belong to Gram-negative bacteria); and, *Aspergillus niger*, *A. ornatus, A. cremeus, A. flavus, A. candidus, A. ochraceus, A. nidulans,* and *A. glaucus* (belonging to fungi). Thus seven bacterial species and eight fungal species were obtained. These microbial species were also recorded from degrading plastic bags, except *Bacillus* sp., *Diplococcus* sp., *Aspergillus ornatu*s, *A. cremeus, A. flavus, A. candidus, A. ochraceus, A. nidulans.* There were five bacterial and two fungal species, commonly and predominantly found detected in both polythene and plastics,

Fungi

The growth of many fungi can also cause small-scale swelling and bursting, as the fungi penetrate the polymer solids. In recent years fungal strains have been reported for plastic degradation such as *Aspergillus versicolar* (Pramila and Ramesh,2011)*Aspergillus flavus ,Chaetomium* spp (Soumya et.al.,2012) *Mucor circinellodies* species etc. The polythene bags were degraded by some fungal species identified such as, *Aspergillus niger, A. ornatus,nidulans*, *A. cremeus, A. flavus, A. candidus and A. glaucus* were the predominant species. The microbial species are associated with the degrading materials were identified fungi (*Aspergillus niger*, *Aspergillus glaucus*), (Swift,1998) Sanchez *et al*., (2000) has reported that the PCL-degrading fungi, *Aspergillus* sp is effective in biodegradation as plastics studies.

Many studies on fungal degradation of the bioplastic have also been performed including *Paecilomyces lilacinus* D218 (Oda ,1995)*Fusarium moniliforme Fmm* (Torres, 1996) *Aspergillus flavus* ATCC9643 (Benedict,et.al,1983) *Thermoascus aurantiacus* IFO31910 (Sanchez,2000), *Tritirachium album* ATCC22563 ,*Paecilomyces verrucosum(JareratTokiwa,2001)*and *Aspergillus sp*.XH0501-a (Lif et al.,2011).On the other hand, polylactic acid (PLA) is subjected to degradation by only two genera of fungi (*Penicillium roqueforti* and *Tritirachium album*) (Mosilnitskii,1987)reported that *Aspergillus niger* van Tieghem F-1119 had the ability to degrade PVC. PHB and polyesters are degraded by many fungal genera such as *Acremonium, Cladosporium, Debaryomyces, Emericellopsis, Eupenicillium, Fusarium, Mucor*, *Paecilomyces, Penicillium, Pullularia, Rhodosporidium,* and *Verticillium*. Similarly, PCL is degraded by

Aspergillus, Aureobasidium, Chaetomium, Cryptococcus, Fusarium, Rhizopus, Penicillium, and Thermoascus. PEA is degraded by *Aspergillus, Aureobasidium, Penicillium, Pullularia*. Fungus like *Alternaria solani, Spicaria sp., Aspergillus terreus, Aspergillus fumigates, Aspergillus flavus* were isolated from soil where plastic have been dumped. These caused significant weight loss in the PS PUR blocks in the shaken cultures, reaching up to 100% in case of the isolate *Fusarium solani* (Ibrahimet.al.2013)

Actinomycetes

PLA-degrading actinomycete, and *Amycolatopsis* sp. Strain isolated from the sample, reduced 100 mg of PLA film by ~60% after 14 days in liquid culture at 30°C. There are many species of microorganisms which can degrade PLA, PCL and PBS such as actinomycetes. Several actinomycetes including *Amycolatopsis sp*. 3118 [Ikwra kudo,1999], *Amycolatopsis sp*. HT-6 , *Saccharothrix* JMC9114 (Pranamuda Tokiwa,2003), *Kibdelosporangium aridum* JMC7912 (Sukkhum,2011), *Actinomadura keratinilytica* (Sukkhum,2011), *Amycolatopsis thailandensis* PLA07 (Chomchoei et.al.,2011)], *Streptomyces bangladeshensis* 77T-4(Hsu.et.al.,2012), *Streptomyces thermoviolaceus* subsp. *thermoviolaceus*76T-2 (Chua .et.al.,2013) were reported as bioplastic degraders. *Cryptococcus sp.* S-2 (Masaki.Kamini.et.al.,2005) and *Pseudozyma antarctica* JCM10317 (Shinozaki.Y.Kikkana .et.al.,2013) were reported to be bioplastic-degradings yeasts.

CONCLUSION

For plastics degradation,microbial use is now considered as an eco-friendly method as compared to the conventional methods. Various physicochemical approaches i.e. photooxidative, thermal, ozone, mechanochemical and catalytic are used, although such methods are costly and are not suitable to use at low plastic concentration, to exterminate plastics from the environment to save living organisms. For plastics degradation,microbial use is now considered as an eco-friendly method and also cost effective approach as compared to the conventional methods. Low cost, efficient technology, eco-friendly treatments capable of reducing and even eliminating plastics, are of great environmental interest. Among biological agents, microbial enzymes are one of the most powerful tools for the biodegradation of plastics. The polluted environment containing plastic wastes can be cleaned easily with the inclusion of microbes without causing any detrimental effect to the environment.. Hence, more studies may be taken up for bringing out a package of practise for efficient management of plastic pollution. In this direction, Environmental Management and Policy Research Institute (EMPRI) has initiated research studies to screen the microbes from various sources to formulate a microbial product for plastic biodegradation.

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