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Chapter 14
Microbial degradation of plastics: Biofilms and degradation pathways

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Chapter contents

Abstract

Plastics are recalcitrant polymers released in the environment through unpredicted use leading to accumulation and increased water and soil pollution. Transportation of these recalcitrant polymers in agricultural soil, sediment, and water has been causing concerns for environmentalists. Biofilm community adhered on plastic polymers have a significant contribution in their degradation as they warrant bioavailability of substrates, sharing of metabolites and increased cell viability thereby accelerating biodegradation. Metabolic enzymes of the microbes can be exploited as a potent tool for polymer degradation. However very little or

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no reports are available about the influence of biofilm and plastic degradation and vice versa. The present chapter reports the impact of biofilm microbes in the degradation of commonly used plastics. Furthermore, potent microorganisms and their interactions with the plastic surface has been deciphered, which would serve as a better understanding of the utilization of biofilm-based methods in the development of plastic waste management.

Keywords: Plastics, Biofilm, Degradation, Pathways, Microbes

Introduction

Plastics are being contemplated as one of the most recalcitrant pollutants in the environment (Bonhomme *et al*., 2004). It comprises around 80% litter in agricultural lands, landfills and water bodies resulting in its accumulation (Rummel *et al*., 2017; Pathak and Navneet, 2017). About 110,000 and 730,000 tonnes of plastics are transported to agricultural landscapes accounting to a more considerable amount than ocean waters. Plastics produced through household activities get runoff and accumulated in the sludge of WTPs (waste treatment plants). It is then carried to agricultural soils leading to accumulation (Nizetto *et al*., 2016). Accumulation and adsorption of these recalcitrant polymers lead to the transportation of invasive and harmful species. Furthermore, the hazardous after-effects involve swallowing by animals due to mistaken as food resulting in entanglement (Rios *et al*., 2007; Yoshida *et al*., 2016). Therefore, many attempts have been made to reduce plastic wastes. Several physical and chemical degradation methods such as UV treatment, physical stress, oxidants, methanolysis, ammonolysis, hydrolysis, etc. have been developed (Kamini *et al*., 2001; Gewert *et al*., 2015). But, these processes usually require elevated temperatures and generally produces toxic substances (Hauenstein *et al*., 2016).

However, biocatalytic degradation is an eco-friendly process which eliminates the accumulation of harmful metabolic byproducts (Florez *et al*., 2015). However, the extent of plastic biodegradability confides on their physical and chemical properties (Das and Kumar, 2013). Microbes can degrade ester bonds in the plastics via enzymatic hydrolysis by attaching and colonizing onto the surface (Uchida *et al*., 2000: Arutchelvi *et al*., 2008). Consequently, the degradation mechanism must be understood and their products should be identified to ascertain probable environmental hazards.

Moreover, the effect of persistent organic pollutants and additives that adsorb to the plastic surface were also considered (Gewert *et al*., 2015). However, the chemicals produced by biodegradation of the plastic polymers themselves are not adequately investigated from an environmental aspect. Microorganisms adhered on the plastic surface forms a biofilm which degrades both natural and synthetic polymers (Gu, 2003). Biofilms are functionally, and phylogenetically diverse communities of bacteria, fungi, and algae, conjointly termed as a microbial conglomeration, attached to a surface (Ghosh *et al*., 2017b). They are mostly embedded in extracellular polymeric substance (EPS) (Ghosh *et al*., 2016; Ghosh *et al*., 2019). Biofilm provides

a plethora of benefits for survival and competition strategies, which includes bioavailability of nutrients, horizontal gene transfer, cell viability and prevents toxic shock (Qureshi *et al*., 2015; Ghosh *et al*., 2017a). They accelerate plastic surface utilization either as a substrate or support. However, microbes adhered depends on the organism source as well as film conditioning. Any kind of plastic in contact with water is being accessible to sunlight, physical stress, oxidants. They are colonized by microorganisms, may over time influence degradation and weathering (Mincer *et al*., 2016). However, floating plastic debris undergoes fouling, which diminishes the buoyancy and renders the polymer to sink (Eich *et al*., 2015). Hence, the biofilm community composition and its activity concerning plastic degradation required to be thoroughly investigated. Although little or no reports are available about the significance of microbial biofilm in plastic degradation. In the present chapter, the available information of the natural and engineered degradation pathways and metabolic products formed during degradation of microplastics which are usually found in agricultural soil and water are reviewed. Degradation products turn out to be low molecular weight oligomers and monomers where new end group formation takes place, i.e., carboxylic acids. We further summarize the influence of the adhered biofilm community in plastic degradation and their interactions, which serves a better understanding of the development of biofilm-based remediation methods in curbing plastic pollution.

Plastic degradation pathways in bacteria

Synthetic polymers serve as nutrient (energy and carbon) source for heterotrophs such as fungi and bacteria in many ways (Dey *et al*., 2012). Synthetic polymers such as homo or heteropolymer, which may contains same or different kinds of monomers. It includes PET (polyethylene terepthalate), PUR (polyurethane), PS(polystyrene), LDPE/HDPE(Low-density polyethylene, High density polyethylene) are commonly found in agricultural soils as microplastics (Nizetto *et al*., 2016). When microbes do not get into contact with the plastic, oxygen, and UV-radiation are the most crucial determinants that initiate chain scission in a carbon-carbon backbone. Shorter polymer fragments or oligomers generated during this process are susceptible to get attacked by microbes. Therefore abiotic degradation is preceded over biodegradation (Gewert *et al*., 2015). The degradation process is achieved by microbes having different bond cleavage and enzymatic activities. Two kinds of enzymes, namely extracellular and intracellular depolymerases are involved. Exo-enzymes produces monomers or short chains which are short enough to penetrate through the cells. It undergoes subsequent chain cleavage to be further metabolized. (Dey *et al*., 2012). The microbial attack on the polymer surface can be direct or indirect (Shalini and Sasikumar, 2015). In the direct mechanism microbe attacks and degrade the polymer for its nutrition and growth. On the contrary, in the indirect mechanism, the metabolic products produced by microbes degrade or deteriorate the polymer. It occurs in a consecutive manner, where physical and chemical traits of the polymer are altered (biodeterioration) followed by enzymatic cleavage (fragmentation), assimilation and mineralization (Singh and Sharma, 2008).

Both aerobic and anaerobic degradation could occur during an indirect mechanism. During aerobic degradation, CO_2 and H_2O are formed, whereas CO_2, CH_4, and H_2O are produced under anaerobic mode (Singh and Sharma, 2008).

Both bacteria and fungi synergistically play an important role in polymer degradation in the natural environment. TCA cycle is employed as the main central metabolic pathway for energy generation from most of the plastic polymers (Upreti and Srivastava, 2003; Ghosh *et al*., 2017a).

Natural metabolic pathways

Depolymerases are mainly employed in plastic degradation. (Gu, 2003). Extracellular enzymes are secreted by microorganisms which cleaves complex polymers to their corresponding monomers and dimers. They generally undergo hydrolytic cleavage in the periplasmic space or the cell membrane (Koutny *et al*., 2006). Consequently, short sized oligomers can be transported across the cytoplasmic membrane (Shah *et al*., 2008). These are further exploited as carbon and energy sources by the intracellular enzymes (Koutny *et al*., 2006). Oligomers can be directly internalized, presumably with the aid of biosurfactants produced by microbes. Thus, entering beta-oxidation (Kawai *et al*., 2004; Kawai *et al*., 2002) or can be further cleaved by abiotic processes before internalization (Albertsson and Banhidi, 1980). Biosurfactants are produced during biofilm formation. Alternatively, these monomers can also undergo sequential degradation into a common metabolite of the TCA cycle and enters into central carbon metabolism (Figure 14.1). Also, Mooney *et al*. (2006) reported the appearance of acetaldehyde, pyruvate, 2-vinylmuconate, and 2-phenyl ethanol, during biodegradation of styrene. These compounds are further metabolized to phenyl-acetyl-CoA and enter into the central carbon metabolism or tricarboxylic acid (TCA) cycle. However, the degree of degradability of PCL is dependent on its degree of crystallinity and molecular weight. The amorphous region was rapidly degraded than the crystalline region by two fungal strains. Also, the participation of several proteases towards plastics biodegradation cannot be ignored, where Williams, 1981 tested the degradation of Poly (L-lactide) PLA using three proteases such as bromelain, pronase and proteinase K. Among these, proteinase K from *Tritirachium album* was proved to be most efficient for cleavage of polymer chains. Proteinase K favored the hydrolysis of an amorphous section of L-PLA and thereby accelerated the degradation rate. But it was decreased in the crystalline region (Chaignon *et al*., 2007; Gilan and Sivan, 2013). However, some strains possess specific enzymes for a particular polymer. In the case of PET degradation, *Ideonella sakaiensis* secretes PETase. It has a Ser-His-Asp catalytic triad at its active site which could hydrolyze PET to monohydroxyethyl terephthalate (MHET), terephthalate and ethylene glycol which further metabolizes to protocatechuate and beta-oxidation pathway (Joo *et al*., 2018).

Engineered pathways

During the degradation of homopolymeric plastic materials, one kind of monomer is being produced, which either undergoes beta-oxidation or TCA cycle (Shah *et al*., 2008; Koutny *et al*.,

2006). When the polymer is comprised of two or more monomer, the degradation becomes difficult. In those cases, a single species could carry out some stages of degradation, but not all. Generally, the complete degradation pathways genes are complemented by engineering different bacterial species.

Additionally, a European website has reported during PET degradation, *E.coli* BL-21 synthesizes LC-cutinase which hydrolyzes the polymer to yield terephthalate and ethylene glycol as two principal monomers, this is the first step in the degradation pathway (iGEM, 2016). Polymers harboring hydrocarbon chains are degraded by polyurethenase alkane monoxygenase cutinase and amylase commonly termed as depolymerases (Seneviratne *et al*., 2006). A strain derived from *Commamonas testosteroni* degrades terephthalate and terminates in a toxic molecule, protocatechuate. Consequently, *P. putida* utilizes protocatechuate and undergoes central metabolism route by recruiting various dioxygenases to utilize it as a nutrient source (Jimenez *et al*., 2002).

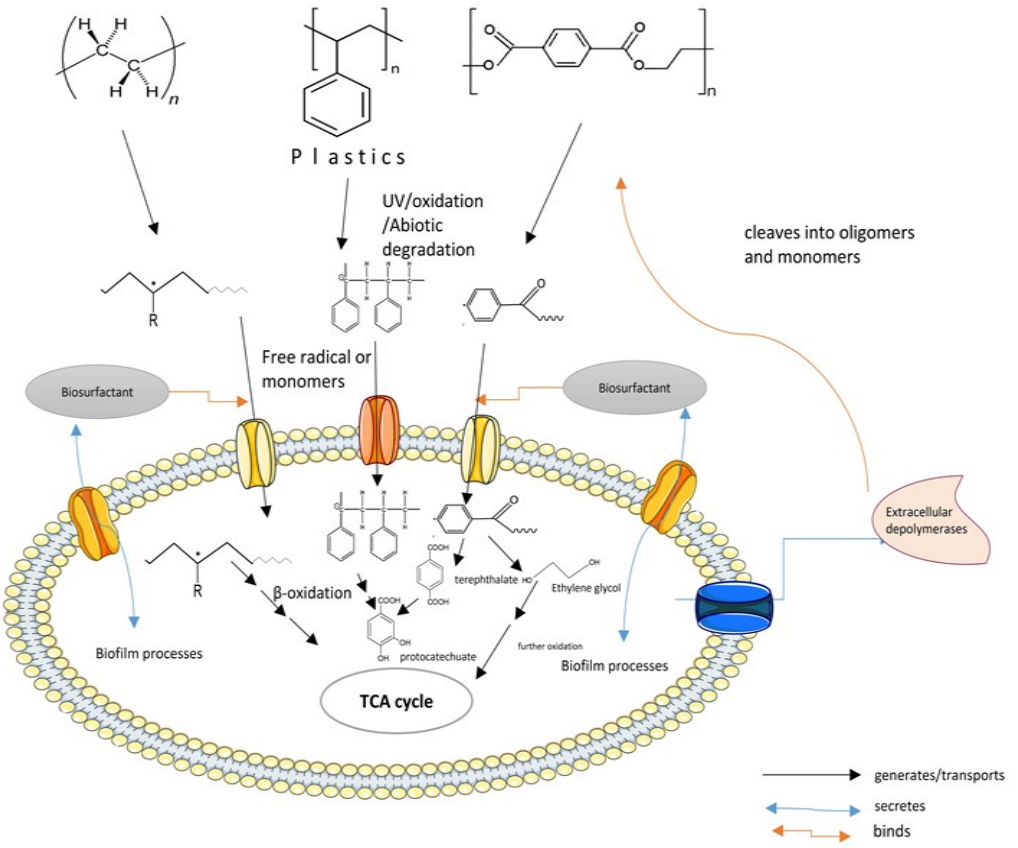

Figure 14.1. *Metabolic pathways for plastic degradation by biofilm forming microbes.*

The ethylene glycol is further degraded and mineralized by *E.coli* BL-21 to CO_2 and H_2O. However, in some cases, bacterial strains are genetically modified and complemented with other genes of the pathway to carry out degradation (iGEM, 2016). The polymer can be cleaved outside the cell into its corresponding monomers, or the engineered strain may possess transporters which are coupled with the degradation pathway genes to transport as well as degrade the molecule inside the cell (iGEM, 2016). As in the case of polyurethane (PUR) degradation, polyurethane esterase cleaves PUR polymer into ethylene glycol, which can diffuse across the membrane of the bacterium (Kang *et al*., 2011). However, osmY, encodes osmotic inducible protein Y, that fuses with PUR esterase and exports the fused enzyme outside the cell (Bokinsky *et al*., 2011; Kang *et al*., 2011). The engineered strain also contains an operon in a second plasmid composed of glycoaldehyde dehydrogenase (aldA) and glycolaldehyde reductase that allows the bacterium to use ethylene glycol as its central metabolite.

Hence complete degradation enzymes are present in a single species (Boronat *et al*., 1983). It allows the species to be self-sufficient in utilizing PUR as a nutrient source to convert the plastics into bacterial biomass which would, in turn, degrade more PUR. (iGEM, 2012). iGEM teams have designed a bioreactor where they have used *E. coli* engineered construct to degrade PUR. The construct is equipped with PUR esterase transport apparatus and secretion tags. With this apparatus, PUR esterase will be released from the cell. It then attacks the polymer and cleaves the ester bond to release ethylene glycol and sugars. Ethylene glycol will be utilized by a different organism and sugars are subsequently consumed to produce biomass.

Biofilm forming microbes involved in degradation

Microbial communities accumulate on the solid surface thereby forming a biofilm (Zettler *et al*., 2013). Abiotic degradation allows chain scission to generate oligomers and monomers and their radicals followed by their biodegradation. (Gewert *et al*., 2015). Although, chemical and physical properties of the polymer, determine the degree of degradation. At the same, the floating plastic gets fouled due to the settling of biomass (Van Sebille *et al*., 2015). Biofouling involves adsorption, biomass immobilization followed by micro and macrofouling. Bacteria serve as primary colonizers which entraps other organisms such as fungi, diatoms, etc (Selim *et al*., 2017). Metabolic activity of the attached biomass leads to desorption, adsorption, and fragmentation of polymer chain or degradation of the debris (Harrison *et al*., 2011). Other factors such as molecular weight, hydrophobicity, temperature chemical structure, elasticity, transition state, etc affect the degradation process (Balasubramanian *et al*., 2010).

Bacteria

Around 90 microbial genera were reported to degrade plastics (Chee *et al*., 2010). Microbial community composition varies in regions. Reports on PCL degradation suggests the involvement of *Pseudomonas* and *Rhodococcus* sp along with two fungal strains. Together they have degraded

PCL films up to 53% (w/w) in 30 days of incubation (Urbanek *et al*., 2017). Hence, *Pseudomonas* and *Rhodococcus* sp are salient bacterial candidate involved in biodegradation. Temperature plays a crucial role in degradation. During the degradation of biodegradable plastics such as PBS, PBSA, PLA and polyhydroxybutyrate (PHB), the microbial activity proceeded at 4°C but no degradation was observed at normal temperatures (Sekiguchi *et al*., 2011). Microbial genera responsible are *Pseudomonas, Tenacibaculum* and *Alcanivorax sp* where *Pseudomonas* spp. strains were active at low temperature (Sekiguchi *et al*., 2011).

Pseudomonas sp.

Biodegradation depends on the organism type in addition to the nature of pretreatment and polymer characteristics (Shah *et al*., 2008). In polyethylene degradation, *Pseudomonas sp* formed most viscous and flocculent biofilms on the surface among the other species in three week period. It was assumed that bacteria selectively utilized basal nutrients when they got depleted in the medium polyethylene then acted as the readily available nutrient source. As the medium was unperturbed without incorporation or elimination of nutrients. (Nanda and Sahu, 2010). *Pseudomonas sp* along with *Actinomycetes sp* degraded treated (UV and HNO_3) polypropylene and formed crystals (Sepperumal and Markandan, 2014). The presence of flocculent microcolonies of *Pseudomonas sp*. and *Actinomycetes sp* on the PP surface is also well supported by Arkatkar *et al*. (2010) during polypropylene (PP) degradation. *Pseudomonas sp* undoubtedly gets attached to biodegradable PE films. In this study, biofilm formed on various plastic films from flask experiments were subjected to CFU counts. Results point out that *Pseudomonas sp*. was significantly found and have maximum CFU among the two bacteria of 1.9×10^{10} /plastic strip on UK BD PE selected native microorganisms, in three months (Poonam *et al*., 2013). Some strains of *Pseudomonas sp,* i.e., *Pseudomonas azotoformans* and *P. stutzeri* secrete biosurfactant rendering PP films relatively more hydrophilic. It allows the subsequent degradation of the polymer (Sepperumal and Markandan, 2014). The degradation ability of *Pseudomonas* is dissimilar among the strains. *Pseudomonas* sp. strain accounted to 20% weight loss in the tested PE in 120 days (Yang *et al*., 2014), while another strain of *Pseudomonas sp.* AKS2 could degrade PE films up to 5 % in 45 days without initial oxidation. Available reports articulate that PE biodegradation by *Pseudomonas sp.* could be attuned by modulating the hydrophobic interaction between the PE film and the microbe. Certain agents such as mineral oil stimulated hydrophobic interactions, resulting in increased bacterial attachment and biofilm formation. This enhanced attachment accelerated polymer degradation. Unlikely, Tween 80 reduced biofilm formation by lowering hydrophobic interactions and thereby reduces bacterial attachment and PE degradation. (Tribedi *et al*., 2013).

Rhodococcus sp.

Apart from *Pseudomonas sp*, other bacterial species were also found to be potent in the plastic degradation process. Gilan *et al*. (2004) reported that *Rhodococcus ruber* (C208), used PE as a sole

carbon source and formed a dense biofilm on its surface in flask culture experiment. Weight loss analysis revealed polymer degradation up to 8% within 30 days of incubation. It is also reported to degrade polyolefins. Initially adhered cells in the biofilm transforms into cellular aggregate forming microcolonies. Further differentiation of the biofilm generates "mushroom-like" three-dimensional structures (Sivan *et al*., 2006). EPS of *Rhodococcus sp* is mainly comprised of proteins. As the addition of proteases hampers biofilm formation followed by plastic biodegradation (Gilan and Sivan, 2013).

Rhodococcus sp possess distinct polymer degrading characteristics. However, degradation also depends on isolation sites. *Rhodococcus* sp 36 isolated from soil sediments could degrade PP efficiently than *Bacillus sp*. Both strains could utilize PP (polypropylene) microplastic for growth. *Rhodococcus sp*. strain 36 degraded PP up to 6.4% while *Bacillus sp* up to 4.0% in 40 days incubation time (Auta *et al*., 2018). On the contrary, in a report, *Rhodococcus sp* showed the lowest degradation of PE compared to *Pseudomonas* and *Brevibacillus* sp respectively (Nanda and Sahu, 2010).

Other bacteria

It has been known that microbes of varying genera are responsible for polymer degradation in addition to *Pseudomonas* and *Rhodococcus* sp. It was observed in a report that the maximum amount of polyethylene degradation was observed in *Staphylococcus* sp (52%) and 11% by *Pseudomonas* sp (Vatsaldutt and Anbuselvi, 2014). However, Yang *et al*. (2014) provided strong evidence for the involvement of *Enterobacter asburiae* YT1 and *Bacillus sp*. YP1 isolated from the guts of plastic-ingesting waxworms, in PEA degradation. (Yang *et al*., 2014). However, they have mentioned earlier that microbial colonization and degradation depends on the material type. The characterization of PET degrading communities showed an abundance of *Tenacibaculum* and different members of *Flavobacteriaceae* and *Bacteriodetes*.

The genera *Owenweeksia* and *Crocinitomix* belonging to *Cryomorphaceae* and *Bacteriodetes* were also strongly represented on PET. Furthermore, available reports also suggested the abundance of *Saprospiraceae, Cryomorphaceae, Flavobacteriaceae*, in PET degradation (Oberbeckmann *et al*., 2016). Moreover, many strains of *Bacillus sp* were indicated in several reports as Wasserbauer *et al*. (1990) pointed out that PE foils, when exposed to *Bacillus brevis* showed carbonyl-like groups and signs of oxidation in FTIR spectra (Wasserbauer *et al*., 1990). Other bacterial species important to biodegradation process include *Ideonella, Actinomycetes, Klebsiella,, Streptomyces, Thermoactinomycetes, Nocardia, Mycobacterium, Micromonospora, Flavobacterium, Rhodococcus, Escherichia, Comamonas, Alcaligenes,* and *Azotobacter*. Some of them were reported to sequester the polymer up to 90% of the dry weight and reported to degrade plastic films (Leja *et al*., 2010; Joo *et al*., 2018).

Raghul *et al*., 2014 observed the involvement of consortium containing *Vibrio alginolyticus* and *V. parahaemolyticus* towards degradation of polyvinyl alcohol-low linear density polyethylene (PVA-LLDPE) blend film while LLDPE film did not have *Vibrio sp*. (Raghul *et al*., 2014).

Fungus

Both bacteria and fungi are reported to be involved in the biodegradation process. (Bonhomme *et al*., 2003; Yamada-Onodera *et al*., 2001; EI-Shafei *et al*., 1998). During the fouling process on the plastic surface, bacteria are the pioneer invaders. After colonizing onto the plastic surface. They allow entrapment of fungi and succession of other species (Mathur *et al*., 2011). It allows sharing of metabolic intermediates and accelerates degradation (Gilan *et al*., 2004). Fungus from agricultural soils was reported in degradation, the plastic pieces buried in agricultural soil mixed with sewage sludge.

Community analysis revealed bacterial and fungal attachment on the plastic surface, indicating probable usage of LDPE as a nutrient source. The isolated fungi are species of *Penicillium*, *Aspergillus,* and *Fusarium* (Shah, 2007). Consequently, in most of the degradation studies *Aspergillus*, *Penicillium* sp are indicated. Unlike bacteria, the capability of biofilm formation by fungal species on polyethylene was associated with a progressive decline in hydrophobicity of the surface (Gilan *et al*., 2004). Reports point out that *Fusarium sp* and other fungal species eroded the surface after their attachment (Shah, 2007; Bonhomme *et al*. 2003). However, some strains of *Mucor sp.* along with other fungal species such as *Aspergillus* are associated in fouling and degradation of polyethylene blended with 6% starch (Premraj and Mukesh, 2005).

Aspergillus sp.

Many species of *Aspergillus* have the potency to degrade polyethylene. A study conducted on the isolation of fungi from polyethylene polluted sites revealed mostly identified organisms as *Aspergillus niger* and *A. japonicus*. However, the degrading ability varies among the species where *A. niger* degraded LDPE up to 5.8%, and *A. japonicas* were more potent in degrading up to 11.11% in one month in vitro (Raaman *et al*., 2012). However, the degradation ability of *A. niger* was highest and degraded up to 38% in 60 days than 31% by *A. flavus* respectively. Mostly, fungi were utilized for the degradation of highly resistant plastics such as low-density polyethylene (LDPE) due to their capability to secrete hydrophobic proteins for attachment with the other organism for colonization (Mohan & Suresh, 2015). Some strains of *A. niger* (ITCC 6052) could also degrade modified polyethylene. Approximately 3.44% weight reduction and 61% decline in tensile strength was detected after 30 days of incubation in thermally oxidized polyethylene SEM analysis indicated to fissures and dense network of biofilm on the polymer surface.

Penicillium sp.

Aspergillus sp are mainly involved in the LDPE degradation, while species of *Penicillium* could degrade both LDPE and HDPE. As available reports suggest the involvement of *P. chrysogenum* and *P. oxalicum* towards LDPE and HDPE degradation. It degraded HDPE and LDPE to 55.598% and 34.35% in 90 days of incubation (Ojha *et al*., 2017). The degradation is followed by pH reduction which indicated that the culture is producing metabolic products by utilizing the

polymer LDPE or HDPE for its growth as compared its positive control (media with sucrose). Since isolates could carry out the degradation without initial treatment or oxidation, it is probable that these species possess enzyme(s) with alkene bonds oxidizing ability to generate carboxylic acids and carbonyl compounds. Thus, eliminating the need for initial oxidation (Yoon *et al*., 2012). Some species of *Penicillium* possess initial degrading enzymes which are responsible for the generation short chain oligomers which get degraded further. Strains of *P. simplicissimum* produces laccase and manganese peroxidase (Ojha *et al*., 2017). During PHB degradation, both fungi and bacteria secrete PHB depolymerase which hydrolyzes PHB into mono (3-hydroxybutyrate) and short chain oligomers. The enzyme of 35 kDa, binds to the polymer surface with its substrate binding domain and carry out the catalysis (Ojha *et al*., 2017), which are further degraded and assimilated to carbon dioxide and water. PHB-decarboxylase is produced by two *Penicillium sp*., namely, *P. pinophilum* and *Penicillium sp.* (Panagiotidou *et al*., 2014). *Penicillium sp*, when present in a consortium of *Bacillus megaterium*, *Pseudomonas mediterranea*, *Aspergillus sp*., *Pseudomonas putida,* and *Phanerochaete sp*., increased the degradation of PE films within 45 days of incubation compared to individual cultures under 90 days incubation period (Mahalakhshmi and Siddiq, 2015). Also, EDAX results indicated the use of PE film as a carbon source. FTIR and GC-MS analysis confirmed the presence of aromatic compounds such as 1-methyl-4-{1-methylethenyl}-acetate Cyclohexanol, Benzene,1,2-[methylene dioxy]-4-propenyl-,[E] and Cyclohexene,1-methyl1-3-{1-methylethenyl}-[n] suggesting that the degradation followed central catabolic pathway (Mahalakhshmi and Siddiq, 2015).

Mucor sp.

Mucor sp. are generally found associated with other microbes and carry out PE biodegradation synergistically. *Aspergillus flavus* and *Mucor circinilloides* isolated from municipal landfill area showed promising LDPE degradation with a maximum weight loss of 18.1 and 6% when mixed with cow dung and poultry dropping after nine months (Pramila and VijayaRamesh, 2011). The degradation potential varies depending upon the type of consortia used. In some cases, consortia may decrease the degrading ability of the fungi as in a study carried out by Singh and Gupta (2014)where fungal consortia comprised of *A. flavus* F1 (30%) *Fusarium sp* F6 (32%), *A. japonicas* F3 (36%), showed significant biodegradation results in four weeks as compared to 24,20,16% by *Penicillium* sp F5 , *A. niger* F2 , *Mucor sp.* F4 in terms of LDPE weight loss measurements. (Singh and Gupta, 2014).

Influence of biofilm on plastic degradation

Biofouling plays a crucial role in governing the buoyancy of plastic debris (Moore *et al*., 2001; Ye and Andrady, 1991). Biofilm formation on plastic surface is a preferred mode of growth by plastic degraders. They are in charge of significant physicochemical changes in the properties of plastic. As Morét-Ferguson *et al*. (2010) concluded that attached biomass degraded the polymer chains

and rapid defouling hindered degradation and density loss (Ye and Andrady, 1991; Yokota *et al*., 2017). Hydrophobicity is an important factor in bacterial attachment and degradation. As the plastic polymers are hydrophobic, bacteria have to initiate hydrophobic interactions with the plastic surface (Sivan, 2011). The current report stated that the hydrophobicity could be increased by starving the bacterial culture. It was shown that with carbon starved *R. corallinus* became more hydrophobic and adhered strongly than the non-starved cells thereby aggravated degradation (Sanin *et al*., 2003). These findings also support the increased affinity of *R. ruber* cells for attachment with the PE surface and raise the possibility that low carbon availability promotes hydrophobic interactions and biofilm establishment (Sivan *et al*., 2006). Biofilms from other bacterial species from soil and marine microflora also represent a similar scenario. Adhered microbes were reported to be hydrocarbon degraders, as the synthetic polymers are mainly comprised of hydrocarbons so their enzymes may be employed in the degradation (Harrison *et al*., 2014). Non-specific chemical bonds and several functional groups are introduced into the polymer by the adhered microbial flora which increase degradation and hydrophilicity (Fotopoulou *et al*., 2015). After attachment, the plethora of process occurs in different types of plastics facilitating abiotic biodegradation. Abiotic degradation by UV light allows the plastic surface to get weathered by introducing polar hydrophilic groups into the polymer resulting in a modification of its topography, increased roughness. (Fotopoulou *et al*., 2015; Cooper and Concoran, 2010). Increased roughness favors the microbial attachment which further modifies the polymer structure and composition and vice versa. Taken together, these processes allow polymer fragmentation with a high surface-to-volume ratio, which is also an essential aspect of the degradation process (Rummel *et al*., 2017).

Biofilm-plastic interactions

Attached microbes initiate hydrophobic interactions upon its contact with the polymer surface (Gilan *et al*., 2004; Sivan *et al*., 2006). It inevitably changes particle properties. Plastic was adsorbed by inorganic ions and molecules which promotes microbial attachment. Film conditioning customizes community colonization by governing material-specific surface attributes resulting in leaching of carbon compounds. Michels *et al*. (2018) and Rogers *et al*. (1990) observed elevated bacterial numbers on PVC and PE than stainless steel, which they speculated to leaching of additives, served as a possible nutrient source. Polysaccharides and nucleic acids of the EPS secreted by initiator organisms adhered to the film are known to be relatively sticky, which also conditions the film. It facilitates colonization for other organisms. (Flemming, 1998; Ghosh *et al*., 2016; Ghosh *et al*., 2017b; Michels *et al*., 2018). Furthermore, biofilm interacts with the synthetic polymer in several ways. Microbes get adhered to the surface, thereby masking surface properties and contaminates the surrounding fluid by organisms which failed to adhere. The enzymatic attack leads breakage of polymer chains and loss of mechanical stability. However, microbial filaments delve deep inside the polymer synthesizes biosurfactants and accumulate water for

further hydrolysis, which also leads to increased conductivity. Finally, lipophilic pigments are released assisting to discoloration of the plastic (Zettler *et al*., 2013).

Conclusion

Plastics are thermo-elastic, water-insoluble, polymers are posing a great environmental challenge. Microbial degradation is better than physical and chemical methods as the degradation pathway leads to complete degradation and mineralization of polymer. However, biodegradability depends upon the microbial biofilm community adhered in it. Biofilm community plays a significant role in modifying the physicochemical properties and degradation of plastics. As biofilm offers bioavailability of nutrients, sharing of metabolites without accumulation of metabolic products resulting in increased cell viability and degradation efficiency. However, fewer reports are available about the interconnection of biofilm with plastic degradation and vice versa. In the present chapter, we review the influence of biofilm microbes in the degradation of commonly used plastics. Both natural and engineered biodegradation pathways employed by the adhered microbes to execute degradation are deciphered. Furthermore, potent microbes and their interactions with the plastic surface has also been summarized. Hence, this would serve as a better understanding of the development of plastic remediation.

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