Chapter 4
Microbial Depolymerization

Anvita Sheel and Deepak Pant

Abstract Depolymerization is a tertiary recycling technique which represents the transformation of polymer chain into monomer units along with oligomers. Depolymerization of polymers with heterofunctional groups can be targeted by microorganisms, especially by fungi. Polyethylene terephthalate (PET), polyurethane (PU), polyester are among such polymers. Hydrolysis is the main cause behind microbial degradation, and hydrophobic surface binding of involving enzyme is responsible for microscopic description. Microbial participation techniques can be modified by using a chemical-assisted hybrid technique, and even halogenated polymer is dechlorinated by this technique.

Keywords PET · PU · PVC, depolymerization · Hydrophobic surface binding
Chemical–biological

1 Introduction

Microbial depolymerization of polymers includes various techniques that involve microbes for transformation of polymer chain back into its monomer units along with oligomers. Polymer degradation alters the polymer properties because of the bond scissions and successive transformations via chemical, physical, or biological reactions.

Depolymerization of plastics can be categorized as:

(i) Photo-oxidative
(ii) Thermal
(iii) Mechanochemical
(iv) Ozone-induced

A. Sheel · D. Pant (✉)
Department of Environmental Sciences, Central University of Himachal Pradesh,
Dharamshala, Himachal Pradesh 176215, India
e-mail: dpant2003@gmail.com; dpant2003@yahoo.com

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(v) Catalytic
(vi) Biodegradation

Photo-oxidative degradation results in the decomposition of polymer by the action of light both UV and visible. The most damaging UV wavelength for a specific plastic depends on the bonds present, and therefore, the maximum degradation occurs at different wavelengths for different types of plastics, e.g., polyethylene (PE) degrades at around 300 nm, whereas polypropylene (PP) requires the wavelength of 370 nm for its degradation. The reaction initiates by the absorption of light energy by the suitable groups in the polymer chain; this results in chain scission leading to the formation of monomers and oligomers. Photodegradable polymers need to have a photo-responsive group (either already present in chain or by addition) (Kyrikou and Briassoulis 2007). Polymer degradation happens mainly in the ether parts of the soft segments, where photo-irradiation generates aldehyde, ester, propyl, formate, and end groups (Nagai et al. 2005). Both photochemical and thermal degradations are classified as **oxidative** degradation. The points of differences between these are (a) the sequence of initiation steps leading to auto-oxidation cycle and (b) thermal reactions occur throughout the bulk of the polymer sample, whereas photochemical reactions occur only on the surface (Singh and Sharma 2008). Many additional polymers depolymerize at high temperature, e.g., polymethylmethacrylate (PMMA) and PE have been converted almost quantitatively back to the monomer Thermal degradation (above 200 °C) leads to chain scission and mainly depends on impurities like head-to-head units, unsaturation sites (Ramis et al. 2004).

In **mechanochemical** degradation, breakdown of molecular chains under shear or mechanical force is often followed by a suitable chemical reaction. The mechanodegradation of polymers in melts occurs via free radical pathways. When polymers are subjected to shear, macroalkyl radicals are formed which accelerate oxidation. This may occur either in polymer melt during processing phase or under mechanical stress conditions (e.g., cross-linking rubber at lower temperatures). Under such conditions, chain-breaking electron acceptor (CB-A) antioxidants have noted to be relatively more effective than thermal oxidation (Scott 1984). When polymethylmethacrylate (PMMA) is degraded mechanochemically using nitroxides as chain termination agents, macroradicals are produced which are used in polymerization reactions (Schmidt-Naake et al. 2002). PVC when dechlorinated mechanochemically, using oxide powders such as Fe_2O_3, CaO, SiO_2 and Al_2O_3, in air, has shown to decrease the molecular weight of PVC (Inoue et al. 2004). Atmospheric ozone, even in every modest concentration, speeds up the aging of polymeric materials (Kefeli et al. 1971). When polymers are exposed to ozone, many carbonyl and unsaturated carbonyl products, based on ketones, aliphatic esters, and lactones as well as aromatic carbonyl associated with the styrene phase, are formed rapidly, followed by gradual formation of ether, hydroxyl, and terminal vinyl groups (Allen et al. 2003). Ozone mainly interacts with polymer chain aromatic rings, C=C bonds, or saturated hydrocarbon links. The degradation reaction proceeds via formation of unstable intermediates like peroxy radicals that can isomerize or degrade, thereby causing decomposition of macromolecules (Ghosh and Ray 2004). Ozone attacks polystyrene rather slowly compared to unsaturated polymers. Reaction products (i.e., 18% ketone, 35% peroxide, and 47% acid) are similar to those expected after free radical chain oxidation processes (Singh and Sharma 2008). The addition of a catalyst lowers the temperature of decomposition, improves the quality of products obtained from pyrolysis of plastic wastes, and also enables a given selectivity to a certain product to be achieved. Solid acid catalysts like **zeolites** favor hydrogen transfer reactions due to the presence of multiple active sites. The access of molecules to catalyst's reactive sites is limited to the pore size as well as the growth of end products inside the pores. Zeolite catalysts, therefore, may produce molecular sieving and shape selectivity (Marcilla et al. 2003). Polyolefins are thermally or catalytically degraded into gases and oils. Zeolite, non-zeolite catalyst (Lin and Yen 2005), transition metal catalysts, i.e., Cr, Mo, Ni, Fe, Co supported over Al_2O_3 and SiO_2 (Williams and Bargi 2004) and Pt–Co, Pt–Mo supported over SiO_2 (Gimouhopoulos et al. 2000), are major catalytic combinations employed for degradation. Biodegradation involves biochemical transformation and mineralization of polymers using microorganisms. It is nowadays described as the sustainable technique due to its easy and environmental-friendly nature.

2 Microbial Participation

Biodegradation of a polymeric material is a degradation brought about by the enzymatic action of naturally occurring microorganisms like bacteria; fungi yeast etc. into metabolites such as H_2O, CH_4, CO_2, biomass etc. (Lenz 1993; David et al. 1994; Chandra et al. 1998; Mohanty et al. 2000). In biodegradation of polymers, almost complete transformation of material happens, whereas in ***biodeterioration*** or ***biocorrosion,*** the change is observed only on polymer structure or in its composition (Gu et al. 2003). In both cases, structural integrity is lost due to reduction in molecular weight.

Microorganisms gain energy by catalyzing energy-producing chemical reactions that transport electrons away from the pollutant polymer. The organic part is oxidized, and the chemical that gains the electrons is reduced. The energy achieved from these electron transfers is then utilized along with some electrons and carbon from the contaminant, for the production of more cells. Several factors including accessibility to water, oxygen usage, minerals, temperature, pH, redox potential, carbon, and energy source influence the growth of microorganisms (Sand 2003) (Table 1).

Table 1 Conditions for microorganism survival (Mohan 2011)

S. no.	Factor	Condition range
1.	Temperature	−5 to +116 °C
2.	pH	0–13
3.	Redox potential	(−)55 to (+)850 mV
4.	Saltness	Ultrapure H_2O to saturated H_2O
5.	Radiation	Biofilms on UV lamps, irradiation units, and nuclear power plants
6.	Nutrient conc.	From 10 μg/L in drinking/purified water up to any carbon sources

2.1 Role of Enzymes

Enzymes are present in every living cell and therefore in all the microorganisms. Microorganisms are highly adaptive to environment and secrete both ***endo-enzymes*** and ***exo-enzymes*** that attack the substrate and cleave the molecular chains into segments (Lugauskas et al. 2003; Huang et al. 1990). Amounts of various enzymes produced by different microorganisms vary with species and even within the strains of the same species. The enzymes have proteins with complicated chemical structure, high molecular weights, and hydrophilic groups such as –OH, –COOH, and $–NH_2$ (Potts 1978) which can attack and eventually destroy almost anything.

In presence of enzymes, accelerated reaction rates (of 10^6–10^{20}) without creating production of undesirable products can be observed (Lenz 1993). Generally, over 2000 types of enzymes are present in a biological system, each one performing one specific chemical function. The following enzymatic factors greatly influence the susceptibility of polymers toward microbial attack:

(i) enzyme availability;
(ii) sites in the contaminant/polymer for enzyme attack;
(iii) enzyme specificity for that contaminant;
(iv) presence of co-enzyme if needed.

Microorganisms are incapable of directly transporting the lengthy and hydrophobic polymer chains into their cells via outer cell membranes. To utilize polymer molecules as carbon source, microbes excrete extracellular enzymes, which depolymerize the polymers outside the cells. Both extracellular and intracellular *depolymerases* enzymes are actively involved in biological degradation of polymers. A bacterium could constantly produce all of the enzymes required for degradation or else could start enzyme synthesis as required to metabolize when needed (Albertsson et al. 1987).

2.2 Degradation Mechanisms

During degradation, exo-enzymes from microorganisms break down complex polymers into short chains or smaller hydrophilic molecules such as dimers, oligomers, and monomers, able to pass the outer semi-permeable bacterial membranes and to be utilized as carbon and energy sources (Gu 2003). The following are the steps toward any polymeric degradation and can work individually or jointly (Fig. 1).

2.2.1 Solubilization

Solubilization is dependent on the hydrophilicity of the polymer. Hydration of polymer chains takes place when structure stabilized by van der Waals forces and hydrogen bonds disrupt. After hydration, the polymer chains may become water soluble and/or the polymer backbone may be cleaved by chemical- or enzyme-catalyzed hydrolysis to result in the loss of polymer strength (Ishigaki et al. 1999). In case of cross-linked polymers, the polymer strength may be reduced by cleavage of either of the polymer backbone, cross-linker, or pendent chains. In non-swellable (non-water absorbing) polymer systems, the decrease in molecular weight may lead to the loss of coherence between polymer chains (Park et al. 2011). In a study exploring biodegradation of poly aromatic hydrocarbons (PAHs), enhancement of solubility and biodegradability of hydrophobic hydrocarbons was achieved using biosurfactants (Barkay et al. 1999)

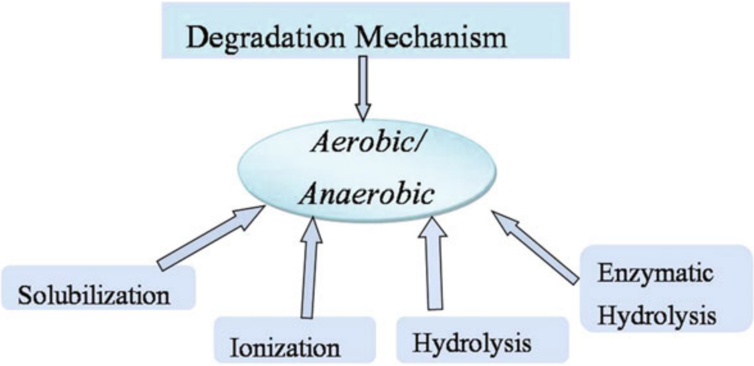


Fig. 1 Degradation mechanism

2.2.2 Ionization

Some water-insoluble polymers can be solubilized by ionization or by protonation of a pendent group. Poly acids become soluble at high pH (Chambliss 1983), and poly (vinyl acetate phthalate) (PVAP) and hydroxyl propyl methyl cellulose phthalate are ionized at a lower pH (cellulose acetate phthalate becomes water soluble at a pH > 6).

2.2.3 Hydrolysis

Water-insoluble polymers with pendent anhydride and/or ester groups may be solubilized if anhydride or esters hydrolyze to form ionized acids on the polymer chain. For example, poly (methacrylate) and poly (methyl methacrylate) are water insoluble but become water soluble upon hydrolysis of the pendent esters and subsequent ionization of the carboxylic group (Murthy et al. 1986). For hydrolysis to occur, the polymer has to contain hydrolytically unstable bonds, reasonably hydrophilic for contact with water. Polyesters are degraded mainly by simple hydrolysis via non-enzymatic, random hydrolytic ester cleavage, and its duration is determined by the initial molecular weight and chemical structure of the polymer.

2.2.4 Enzyme-Catalyzed Hydrolysis

Enzymes act as catalysts for particular reactions or a series of reactions, such as reduction, oxidation, esterification, hydrolysis, synthesis, and molecular interconversions. The enzymatic degradation of some natural polymers occurs by the unzipping or chain-end degradation mechanism. For example, β-amylase degrades starch to maltose, beginning with the chain ends. In the lipase-catalyzed degradation of poly (vinyl acetate) (PVAc), the ester bonds in the side chains are broken particularly to yield oligomers with acid and alcohol groups (Singh and Sharma 2008). Poly (3-caprolactone) has been enzymatically degraded in supercritical carbon dioxide ($scCO_2$) using *Candida antarctica* lipase to give oligo (3-caprolactone) (Takamoto et al. 2001). When the end products are inorganic species, e.g., CO_2, H_2O, or CH_4, the degradation is also called ***mineralization.*** Mineralization can come about either aerobically or anaerobically. Anaerobic and aerobic biodegradation pathways are given (Fig. 2), with their corresponding final products (Gu 2003).

2.3 *Aerobic Respiration*

In aerobic respiration, microbes use O_2 to oxidize part of the carbon in the polymer to CO_2, and rest of the carbon is used to produce new cell mass. In the process, O_2

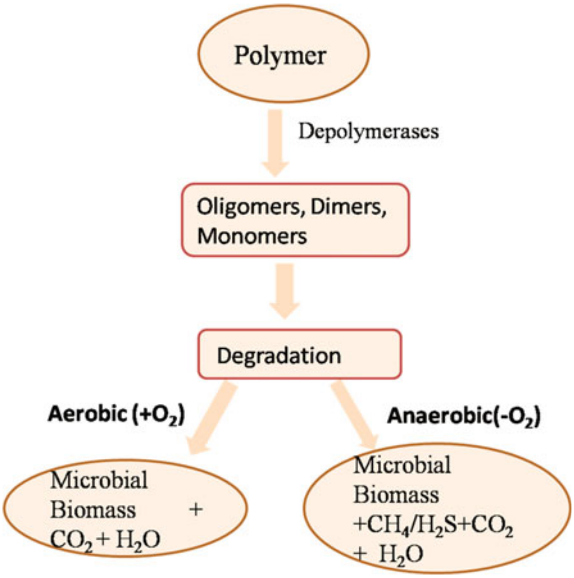


Fig. 2 Aerobic and anaerobic degradation pathways

gets reduced, producing H_2O. Thus, the major by-products of aerobic respiration are carbon dioxide, water, and an increased population of microorganisms. The by-products produced during conversion which enter into the microbial cell can be utilized as the energy source (Shimao 2001).

2.4 Anaerobic Respiration

In anaerobic respiration, nitrate ($NO3^-$), sulfate (SO_4^{2-}) metals such as iron (Fe^{3+}) and manganese (Mn^{4+}), or even CO_2 can accept electrons from the degraded contaminant (National Research Council 1993). Hence, anaerobic respiration uses inorganic chemicals as electron acceptors. By-products of anaerobic respiration are new cell matter, H_2S, N_2 gas, CH_4, and reduced forms of metals.

Given that thermodynamically, O_2 is a more efficient electron acceptor than both $SO_2^{4-}$and CO_2; **aerobic processes yield much more energy** and are capable of supporting a greater population of microorganisms than anaerobic processes. It is important to note that biodeterioration and degradation of polymer substrate can rarely arrive at 100% because a small fraction of the polymer will always be incorporated into microbial biomass, humus, and other natural products (Alexander 1977; Narayan 1993).

2.5 Formation of Biofilms

Microbes deteriorate and degrade both natural and synthetic polymers by adhering to and colonizing polymer surfaces forming biofilms (Gu et al. 2000; Mitchell 1996). The formation of a biofilm is a precondition for substantial corrosion and deterioration of these materials to take place. Polymers susceptible to biofilm formation include but not limited to paints, plastics, adhesives, sealants, lubricating materials, composites, fuels (Gross et al. 1995; Gross 1993; Lugauskas et al. 2003).

Biofilm is a slimy layer where bacterial cells can encase themselves in a hydrated matrix of polysaccharides and protein which is composed of water (80–95%), extracellular polymer substances that constitute 85–98% of the organic matter, the microorganisms, entrapped organic and inorganic particles (e.g., humic substances, debris, clay, silica, gypsum), substances sorbed to extracellular polymeric substances, cells or particles and substances dissolved in the interstitial water (Flemming 1998). *The process of establishment of complex community of microorganisms on surface attachment as biofilm is known as* ***biofouling*** *or* ***microfouling***

Main mechanism (Flemming 1998) (both direct and indirect) through which the structure and function of synthetic polymeric materials can be damaged by biofilms includes as follows:

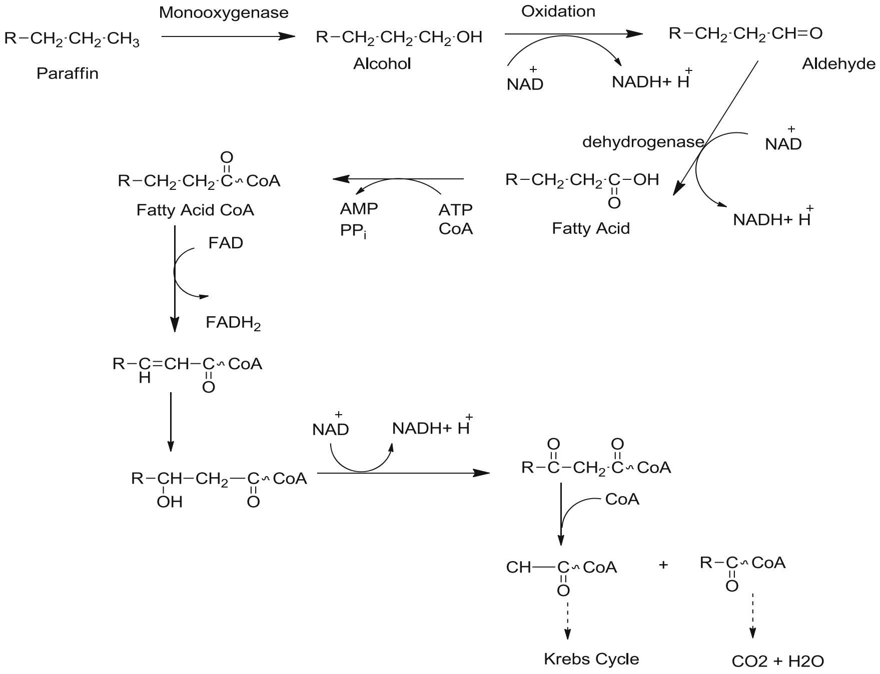
(i) coating the surface, masking surface properties, and contaminating adjacent media such as H_2O;
(ii) increasing the leaching additives and monomers out of the polymer matrix;
(iii) attack by enzymes or radicals of biological origin to polymer and additives, leading to both embrittlement and loss of mechanical stability;
(iv) accumulating water and penetrating the polymer matrix with microbial filaments, causing swelling and increased conductivity;
(v) excretion of lipophilic microbial pigments that lead to unwanted colors in the polymer.

2.6 Properties Deterioration

Mechanical properties of polymers are principally dictated by length of the polymer chains and are weakened by chain scissions. A single endo-cleavage in a polymer chain can reduce the molar mass up to 50% and hence cause significant alterations in the mechanical properties such as embrittlement, increased modulus, change in weight, dimensions. When plasticizers are removed from the plastic materials by microorganisms, embrittlement can also occur, whereas deterioration in electric properties such as dielectric constant, insulation resistance power factor, and dielectric strength are observed because of the surface growth and pH variations resulted by excreted metabolic products.

3 Biodegradation of Plastics Using Microorganisms

The potential of microbes for plastic degradation first came to light in 1961 when Fuhs reported that several microorganisms were capable of consuming paraffin as a carbon source. Plastic-degrading microbes were isolated from a multiple sources such as rhizosphere soil of mangroves, marine water, polythene buried in the soil, and soil at the dumping sites. Comparative study of polyethylene and paraffin degradation revealed that bacteria had utilized polyethylene as carbon source (Jen-hou and Schwartz 1961). The mechanism put forward by Gautam et al. (2007) reported that paraffin molecules first get converted to an alcohol by a *monooxygenase* enzyme; then alcohol is oxidized into an aldehyde by alcohol *dehydrogenase* enzyme. Further an aldehyde *dehydrogenase* coverts aldehyde to fatty acid, which then undergoes β-oxidation inside cells (Scheme 1).



Scheme 1 Paraffin biodegradation pathway (Gautam et al. 2007)

3.1 Polyethylene (PE)

Hydrophobic nature of polyethylene (PE) restricts its bioavailability (Mahalakshmi et al. 2012). It was found that lower molecular hydrocarbon (less than 620) oligomers favor the growth of microorganisms while this is not possible in high molecular weight PE. To increase the effectiveness of the process, it is often coupled with some form of physical and chemical treatments like photo-oxidation, nitric acid oxidation, UV irradiation, and thermal treatment which favor its biodegradation (Hadad et al. 2005). PE molecules when treated with UV radiations form radical. Oxygen absorbed from atmosphere results in formation of hyperoxides which ultimately bring in C=O group in polymer (Scheme 2).

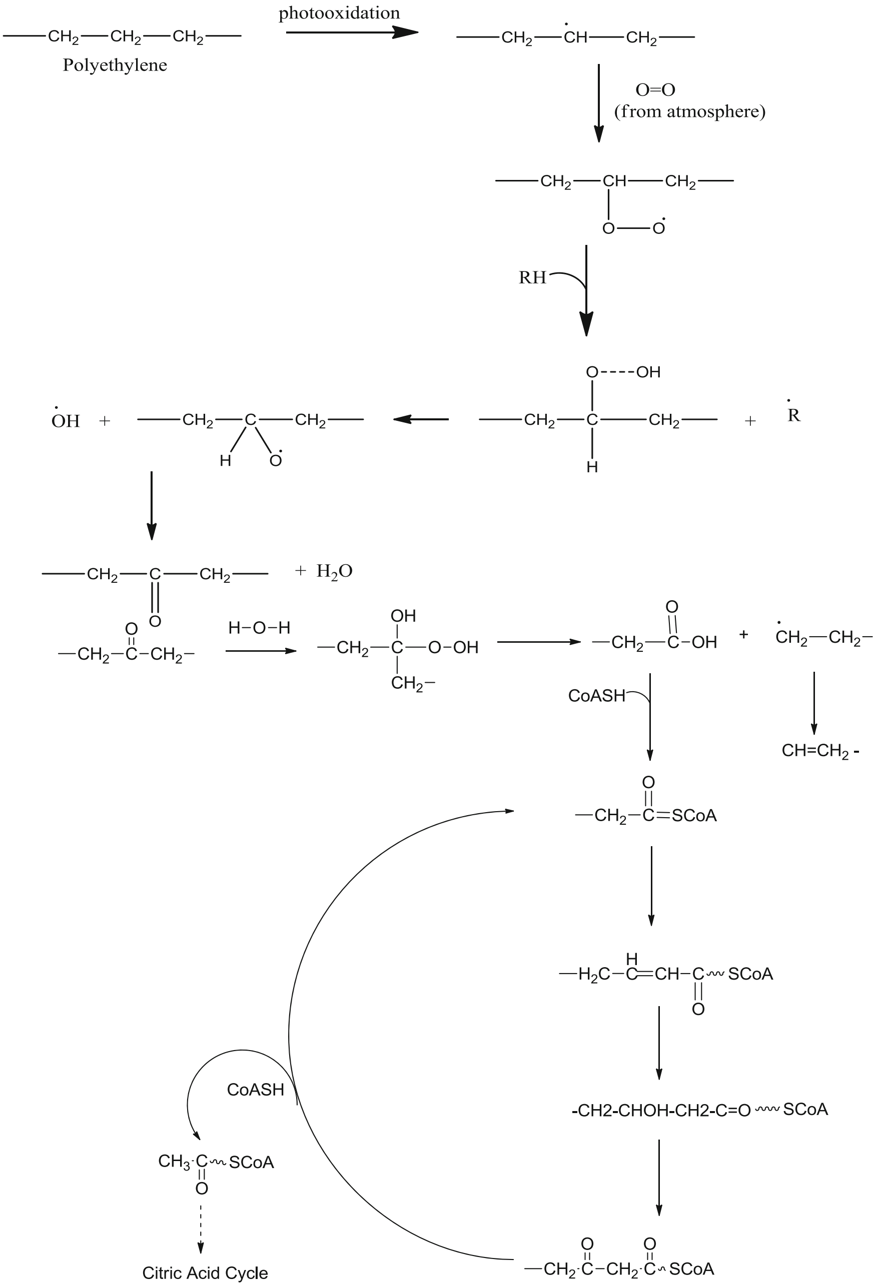
Various modifications affect PE biodegradation (Table 2). Chemical pretreatment of polyethylene with 0.5 molar nitric acid and sodium hydroxide speeds up the subsequent biodegradation using *Pseudomonas* sp. (Nwachkwu et al. 2010). Genera associated with PE biodegradation are *Gordonia* and *Nocardia, Bacillus, Lysinibacillus, pseudomonas, staphylococcus, streptococcus, micrococcus, streptomyces, rhodococcus, proteus, listeria, vibrio, bravibacillus, serratia, nocardia, diplococcus, moraxella, penicillium, arthrobacter, aspergillus, phanerochaete, chaetomium,* and *Gliocladium* (Bonhommea et al. 2003; Koutny et al. 2006a, b; Arutchelvi et al. 2008; Restrepo- Flórez et al. 2014; Grover et al. 2015). Non-ionic surfactants increase polymer hydrophilicity by providing better chances of surface adhesion to microbes (Hadad et al. 2005).

3.2 Nylon

Some types of nylon can be degraded by both fungi (Deguchi et al. 1997) and bacteria (Tomita et al. 2003). A number of studies for isolation, expression, and transformation of genes encoding nylon-degrading enzymes are available in the literature (Kakudo et al. 1993; Kanagawa et al. 1989). In a study, bacterium *Geobacillus thermocatenulatus* was effectively isolated and applied to degrade nylon 12 and nylon 66 (Tomita et al. 2003). This bacterium enzymatically hydrolyzed the amide bonds, which resulted in the production of 12-aminododecanoic acid (Scheme 3).

Lignin-degrading white rot fungus strain IZU-154 and extracellular enzyme fungus peroxidase from the same fungal strain degrade nylon 66 (Deguchi et al. 1997, 1998). Degradation products revealed the presence of CHO, NHCOH, CH_3, and $CONH_2$ end groups indicating the oxidative process (Scheme 4).

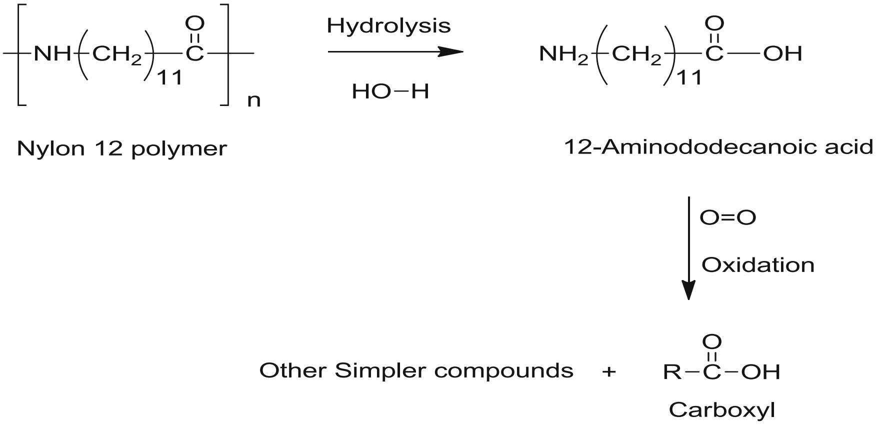
Bacterial biodegradation of nylon 66 has been noted to be a hydrolytic process. Proposed mechanism implied that initially, enzyme peroxidase oxidizes the methylene group next to N atom in polymer backbone forming –CH radical, which then undergoes oxidation (Nomura et al. 2001). The enzymes associated with nylon



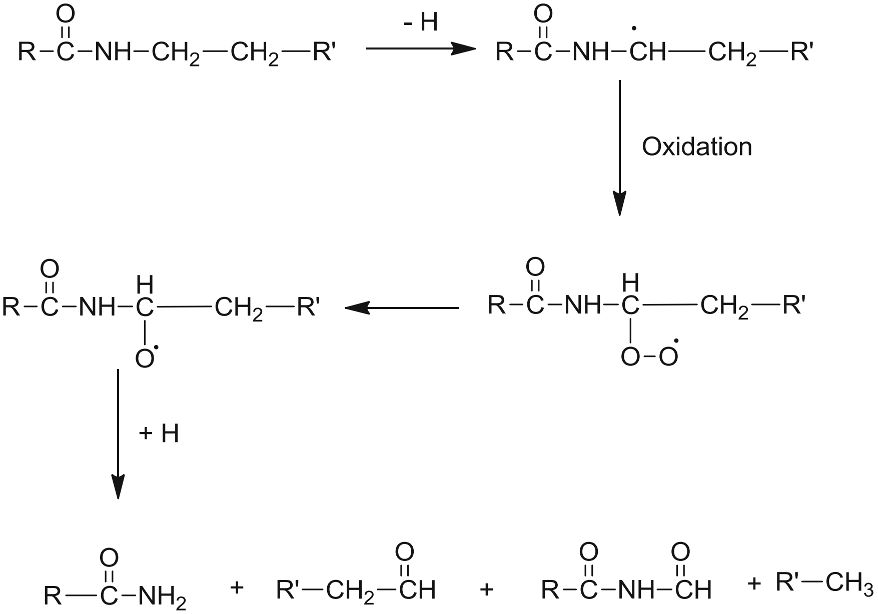
Scheme 2 Biodegradation of PE (Gautam et al. 2007)

Table 2 PE-degrading microbes

Polyethylene type/ modification	Microbes	References
Unmodified polyethylene	*Lysinibacillus fusiformis* VASB14/ WL *Bacillus cereus* strain VASB1/TS	Shahnawaz et al. (2016)
	P. fluorescens, S. aureus, and *A. niger*	Thomas et al. (2015)
	Bacillus amyloliquefaciens BSM-1, *B. amyloliquefaciens* BSM-2	Das and Kumar (2015)
	Copper-binding laccase from *R. Ruber*	Santo et al. (2013)
	S. marcescens	Odusanya et al. (2013)
Starch-based polyethylene	*Mucor rouxii* NRRL 1835, *A. flavus* and strains of *Streptomyces*	Mahalakshmi et al. (2012)
Lignocelluloses Heat treated degradable	*Streptomyces badius* 252 and *Streptomyces setonii* 75Vi2 *Streptomyces viridosporus* T7A	Pometto et al. (1992)
Starch blended	*P. chrysosporium*, *Streptomyces* sp.	Flavel et al. (2006), Arvanitoyannis et al. (1998)
Low molecular weight polyethylene	*Pseudomonas* sp. E4	Yoon et al. (2012)
Low-density polyethylene	*Pseudomonas* sp. AKS2 *clavatus* (strain JASK1) (landfill isolate)	Tribedi et al. (2015, 2014)
	Brevibacillus parabrevis PL-1, *Acinetobacterbaumannii* PL-2, *A. baumannii* PL-3, and *P. citronellolis* PL-4	Gajendiran et al. (2016), Pramila et al. (2012)
	Streptomyces coelicoflavus 15399T (isolated from oil-contaminated site)	Duddu et al. (2015)
Irradiated low-density polyethylene	*Aspergillus* sp., *P. Lilacinus* and *L. theobromae*	Sheik et al. (2015)
High-density polyethylene	*Methylobacter* sp.	Muenmee et al. (2015)
	Nitrosomonas sp. AL212, *Burkholderia* sp., *Methylobactor* sp., *Methylococcus capsulatus, Nitrobacter winogradkyi, Methylocystic* sp., *Methylocella* sp.	Muenmee et al. (2016)
	Aspergillus tubingensis VRKPT1 and *A. flavus* VRKPT2	Devi et al. (2015)
Photodegraded PE	*Bacillus cereus*, *B. megaterium, B. subtilis Brevibacillus borstelensis*	Abrusci et al. (2011)
	*B. borstelensis*707	Hadad et al. (2005)
Modified Low-density PE [blend of titania (TiO_2) and starch]	*P. aeruginosa*CA9, *Burkholderia seminalis* CB12, and *Stenotrophomonas pavanii* CC18	Mehmood et al. (2016)



Scheme 3 Nylon 12 degradation



Scheme 4 Oxidative process for Nylon degradation

degradation are 6-aminohexanoate cyclic dimer hydrolase (F-EI), 6-aminohexanoate-dimer hydrolase (F-EII), and 6-aminohexanoate oligomer hydrolase (F-EIII) (extracted from Flavobacterium sp. KI72; Kakudo et al. 1993; Kinoshita et al. 1977, 1981; Negoro 1992).

3.3 Polyethylene Adipate (PEA) ($[-OCH_2CH_2OOC(CH_2)_4CO-]_n$)

PEA with molecular weight 3000 can be degraded by using *penicillium sp*. as sole source of carbon. Out of the various other strains, 14-3 found to be showed greatest activities. This strain can also degrade aliphatic polyesters like polyethersulfone (PES), polybutylene succinate (PBS), and polybutylene adipate (PBA) (Tokiwa and Suzuki 1974). Furthermore, lipases from *Rhizopus delemar*, *Rhizopus arrhizus*, *Achromobacter* sp., and *Candida cylindracea* and esterase from hog liver are noted for showing PEA degradation (Tokiwa and Suzuki 1977a, b).

3.4 Polycaprolactone (PCL) ($[-OCH_2CH_2CH_2CH_2CH_2CO-]_n$)

Aliphatic ester linkages in PCL make it susceptible to enzymatic degradation, but hydrolysis of this polymer is time-consuming (Woodruff and Hutmacher 2010; Gajanand et al. 2014). Enzymatic cleavage of polymers is substrate specific (Shimao 2001; Murphy et al. 1996; Schink et al. 1992). *Penicillium funiculosum* and *A. Flavus* enzymatically hydrolyzed PCL in its amorphous region (Tokiwa et al. 2009b). PCL degradation was also achieved using enzyme esterases and lipases (Shimao 2001). Lipase-producing species such as *R. delemar, R. Arrizus, Achromobacter* sp., *Candida cylindracea*, *Penicillium* sp., have been reported for PCL degradation (Tokiwa et al. 2009). PCl when blended with % sebacic acid showed greater degradation rate (Salgado et al. 2012; Tokiwa et al. 2009). Aspergillus sp. strain ST-01 was used on multiple instances for PCL degradation (Tokiwa et al. 2009; Sanchez et al. 2000). Only 10% degradation of the polymer was noted by *P. lilacinus* (Oda et al. 1995). *P. lilacinus* D218 releases PCL depolymerase, whose optimum activity was observed at 30 °C in pH range of 3.5–4.5. Degradation under anaerobic conditions have also shown (Abou-Zeid et al. 2001; Yagi et al. 2009) good degradation results. Some other reported species for PCL degradation are *Bacillus pumilus (*Motiwalla et al. 2013); genus *Alcanivorax, Tenacibaculum* and *Pseudomonas* (isolated from marine environments) (Sekiguchi et al. 2009); yeast *Cryptococcus laurentii* (Benedict et al. 1983); *Clostridium botulinum, Alcaligenes faecalis* (Caruso 2015; Tokiwa et al. 2009). Activity of phytopathogenic fungi against PLC is suspected to be attributed to the presence of cutinases.

3.5 Polybutylene Succinate (PBS) ([–O$(CH_2)_4$OOC (CH2)$_2$CO–]$_n$) and Polyethylene Succinate (PES) ([–O$(CH_2)_2$OOC$(CH_2)_2$CO–]$_n$)

Strain *Amycolatopsis* sp. HT-6 has found to degrade PBS, PHB, PCL (Pranamuda et al. 1995). Actinomycetes (thermophilic) that degrade PBS and PES are *Microbispora rosea, Excellospora japonica*, *Excellospora viridilutea* (Jarerat and Tokiwa 2001; Duddu et al. 2015; Seretoudi et al. 2002; Hoang et al. 2007). Other microbes and genus reported are *Pseudomonas* sp. AKS2 strain (Qiu et al. 2003; Liu et al. 2012; Tribedi and Sil 2013), *Saccharomonospora*, *Streptomyces*, *Microbispora*, *Thermoactinomyces,* and *Actinomadura* (Tseng et al. 2007), *Streptomyces* sp. (Calabia and Tokiwa 2004) *Bacillus* sp. TT96 (Tokiwaet al. 2009), Mesophilic *Bacillus, Paenibacillus, Bacillus pumilus*, *B. subtilis,* and *Paenibacillus amylolyticus* (Tezuka et al. 2004), *Rhizopus delemar* (Seretoudi et al. 2002). Biostimulation helps in degradation by forming a new microbial community (Tribedi and Sil 2013). Hydrocarbon-degrading microbes from oil-contaminated marine environment have also been identified (Hazen et al. 2010). Microbial serine proteases have also been reported for PES biodegradation (Lim et al. 2005).

3.6 Poly (β-Propiolactone) (PPL) ([–OCH_2CH_2CO–]$_n$)

Microbes belonging to Bacillus sp. have been employed for degrading this polymer (Nishida et al. 1998). Some other strains from thermophilic *Streptomyces* identified for PPL degradation are *Variovorax paradoxus, Acidovorax* sp, *Sphingomonas paucimobilis, Rhizopus delemar* (Tokiwa and Suzuki 1977a, b), poly (3-hydroxybutyrate) depolymerase (Tokiwa and Calabia 2006).

3.7 Aliphatic–Aromatic Copolyesters (AACs)

Aliphatic–aromatic copolyesters generally consisting of **polyethylene terephthalate (PET), PCL, polyethylene isophthalate (PEIP), and polybutylene terephthalate** were hydrolyzed by *Rhizopus delemar* lipase (Tokiwa and Suzuki 1981), although the susceptibility decreased with increased aromatic content. Kleeberg et al. (1998) reported *Thermobifida fusca* and its enzyme hydrolase for AAC degradation. This enzyme is highly similar to triacylglycerol lipase from *Streptomyces albus* G and triacylglycerol acylhydrolase from *Streptomyces sp.* M11 (Kleeberg et al. 2005). PET films were degraded when treated with (a) cutinase from *Humilica insolens* and *Fusarium solani pisi* (Donelli et al. 2010) observed 97% weight loss; (b) Pseudomonas mendocina with 5% weight loss (Heumann et al. 2006) and cracks on fiber surface (Zhang et al. 2004). Enzymes from

Aspergillus sp., *Beauveria sp.*, *Thermobifida fusca*, and commercial enzymes (TEXAZYME PES sp5 and Lipase PS) increased the hydrophilicity of PET fibers (Heumann et al. 2006; Ronkvist et al. 2009; Naharjan et al. 2006). Besides, the microbial capability of forming biofilm over PET film pointed out the possibility of degradation under natural conditions (Webb et al. 2009). Furthermore, to increase biodegradability of PET, studies have been done to modify the polymer in order to decrease the intermolecular cohesion. Nowak et al. (2011) modified PET films with Bionolle polyester in the presence of filamentous fungi *Penicillium funiculosum.* After 84-day incubation period, considerable reduction in quantity of aromatic rings was observed. In another study, series of copolyesters were prepared using waste PET beverage bottles and L-lactic acid. Samples were incubated in thermophilic sludge at 55 °C in alkaline environment for a period of 394 days. This modified copolyester was more inclined to hydrolytic biodegradation than PET waste. Copolyester mineralization gave methane-rich biogas in 69% and 34% of theoretical amount for two samples, respectively. The rate of anaerobic biodegradation decreased with higher starting content of aromatic sequences (Hermanová et al. 2015). The degradation of transparent PET sheets by microbes of *Nocardia* species and esterase was studied. Esterase was found to be involved in the biodegradation. Although the biodegradation was slow and weak, microbes and esterase could act on the polyethylene terephthalate (Sharon and Sharon 2017).

3.8 Poly (3-Hydroxybutyrate) (PHB) ($[-O(CH_3)CHCH_2CO-]_n$)

First reports of PHB-degrading microbes were from Bacillus, Streptomyces, and Pseudomonas species (Chowdhury 1963). Mostly, microbes that utilize PHB do so at ambient temperatures and they have been isolated from soil *Pseudomonas lemoigne*, *Comamonas sp.*, *Acidovorax faecalis*, *Aspergillus fumigatus*, *Variovorax paradoxus,* sludge *Pseudomonas, Ilyobacter delafieldi*, *Alcaligenes faecalis,* and sea and lake water *Pseudomonas stutzeri*, *Comamonas testosterone* (Lee 1996). A thermophilic *Streptomyces* sp. shows higher PHB-degrading activity than thermotolerant and thermophilic strains from same species (Calabia and Tokiwa 2004). *Aspergillus* sp. degraded 90% of PHB film in five (Sanchez et al. 2000). Actinomycetes associated with PHB, PCL, and PES depolymerization are members of the genera *Streptomyces, Microbispora, Actinomadura, Saccharomonospora, and Thermoactinomyces* (Tseng et al. 2007). Another study done to assess the biodegradation pattern of blends of polylactic acid (PLA) and polyhydroxybutyrate (PHB) at different weight ratios showed biodegradability of blends increased as ratio of PHB increases (Zhang and Thomas 2011). Biocomposites prepared form PHB and potato peel waste fermentation residue also showed complete degradation in eight months (Wei et al. 2015).

3.9 Polylactic Acid (PLA) ($[-O(CH_3)CHCO-]_n$)

Polylactic acid, a biodegradable plastic is used freely in medicine industry. T16-1 strain of *Actinomadura keratinilytica* NBRC 104111 has been illustrated for producing enzyme that degrades PLA. 60% degradation of PLA films have been observed by *Amycolatopsis* sp. over a period of fourteen days (Babu et al. 2013; Sukhumaporn et al. 2012; Garlotta 2002; Pranamudaet al. 1997). *Bacillus amyloliquefaciens, a mesophilic bacteria,* are also known to degrade PLA (Prema and Uma 2013). Masaki et al. (2005) used lipase enzyme purified from strain *Cryptococcus* sp. S-2 for PLA degradation. When dye having PLA films as a constituent was inspected, enzymes from *Aneurinibacillus migulanus* showed the same activity as proteinaseK for PLA degradation (Chaisu et al. 2015). PLA depolymerase enzyme is also reportedly produced by *Pseudomonas tamsuii* TKU015 (Liang et al. 2016).

3.10 Polyurethanes (PUR)

Polyurethanes are both polyether PUR and polyester PUR (Shimao 2001). Enzyme proteases, esterases, ureases, and lipases have been noted to bring about PUR hydrolysis. *Aspergillus terreus, Trichoderma* sp., and *Chaetomium globosum* have also been reported for the producing ureases and esterase that hydrolyze PUR (Bhardwaj et al. 2013; Howard 2012). A total of 22 fungal strains were screened, and up to 95% urease activity was found in most of them (Loredo-Trevino et al. 2012). Microbial species identified as polyurethanes degraders are *Candida ethanolica, Fusarium solani* (Schink et al. 1992; Zafar et al. 2013), *Aspergillus fumigatus*, *Emericella*, *Arthrographis kalrae*, *Lichthemia*, *Fusarium solanii*, *Plectosphaerella, Phoma, Nectria, A. niger*, *Corynebacterium* sp., *Neonectria, Thermomyces*, *P. aeruginosa*, *Alternaria*, *Bacillus* and *Comamonas; C. acidovorans* TB-35, *Pestalotiopsis microspora* (Bhardwaj et al. 2013; Howard 2012; Akutsu et al. 1998; Zafar 2013; Flavel et al. 2006; Shimao 2001). Proteolytic enzymes—urease and papain—also degrade polyurethane used in medical devices. Ma and Wong (2013) investigated the esterase activity of A. Flavus. Different experiments have illustrated the important role of Genes*pue*A and *pue*B in PUR degradation (Howard 2012). *pue*A helps in enhancing PUR degradation by increasing the cellular density as shown by gene silencing experiments (Howard et al. 2007).

The various plastic-degrading microbes are summarized in Table 3.

Table 3 Plastic-degrading microbes

S. no.	Plastic	Microorganisms	References
1	Polyethylene adipate (PEA)	*Penicillium* sp., *Rhizopus delemar, Rhizopus arrhizus, Achromobacter* sp., and *Candida cylindracea*	Tokiwa and Suzuki (1977a, b)
2	Polycaprolactone (PCL)	*Penicillium funiculosum, A. flavus, R. delemar, R. arrizus, Achromobacter* sp., *Candida cylindracea*, *Penicillium* sp., *Aspergillus* sp., *Cryptococcus laurentii, Bacillus pumilus, Alcanivorax, Tenacibaculum, Pseudomonas, Clostridium botulinum, Alcaligenes faecalis, P. lilacinus*	Tokiwa et al. (2009), Benedict et al. (1983), Motiwalla et al. (2013), Caruso (2015), Tokiwa et al. (2009), Oda et al. (1995)
3	Polybutylene succinate (PBS) Polyethylene succinate (PES)	*Amycolatopsis* sp., *Microbispora rosea, Excellospora japonica, Excellospora viridilutea, Pseudomonas* sp., *Saccharomonospora*, *Streptomyces*, *Microbispora*, *Thermoactinomyces Actinomadura, Bacillus* sp., *Bacillus pumilus*, *B. subtilis*, *Paenibacillus amylolyticus, Rhizopus delemar*	Pranamuda et al. (1995), Qiu et al. (2003), Liu et al. (2012), Tribedi and Sil (2013), Tseng et al. (2007), Tokiwa et al. (2009), Tezuka et al. (2004), Hazen et al. (2010), Seretoudi et al. (2002)
4	Poly (βpropiolactone) (PPL)	*Bacillus* sp., *Acidovorax* sp., *Variovorax paradoxus, Sphingomonas paucimobilis, Rhizopus delemar, Streptomyces* sp., *Thermobifida fusca, Streptomyces sp., Humicola Insolens, Aspergillus sp., Beauveria sp. Fusarium solani, Pseudomonas mendocina, Penicillium funiculosum, Nocardia sp.*	Nishida et al. (1998), Tokiwa and Suzuki (1977a, b), Tokiwa and Calabia (2006), Tokiwa and Suzuki (1981), Kleeberg et al. (2005), Donelli et al. (2010), Heumann et al. (2006), Ronkvist et al. (2009), Zhang et al. (2004), Nowak et al. (2011), Sharon and Sharon (2017)
5	Poly (3-hydroxybutyrate) (PHB)	*Bacillus, Pseudomonas, Streptomyces* species, *Comamonas sp.*, *Pseudomonas lemoigne*, *Aspergillus fumigatus*, *Acidovorax faecalis*, *Variovorax paradoxus*, *Alcaligenes faecalis*, *Pseudomonas*, *Ilyobacter delafieldi*, *Comamonas testosterone*, *Pseudomonas*	Chowdhury (1963), Lee (1996), Calabia and Tokiwa (2004), Sanchez et al. (2000), Tseng et al. (2007)

(continued)

Table 3 (continued)

S. no.	Plastic	Microorganisms	References
		stutzeri, *Actinomadura*, *Microbispora*, *Streptomyces*, *Thermoactinomyces*, *Saccharomonospora*	
7	Polylactic Acid (PLA)	*Actinomadura keratinilytica*, *Bacillus amyloliliquefaciens*, *Amycolatopsis*, *Cryptococcus* sp, *Aneurinibacillus migulanus, Pseudomonas tamsuii*	Pranamudaet al. (1997), Garlotta (2001), Sukhumaporn et al. (2012), Babu et al. 2013), Prema and Uma (2013), Masaki et al. (2005), Chaisu et al. (2015), Liang et al. (2016)
8	Polyurethanes (PUR)	*Trichoderma* sp., *Aspergillus terreus*, *Chaetomium globosu, Fusarium solani*, *Candida ethanolic, C. acidovorans, A. niger*, *P. aeruginosa*, *Emericella*, *Nectria*, *Lichthemia*, *Fusarium solanii*, *Corynebacterium* sp., *Neonectria, Arthrographis kalrae*, *Plectosphaerella, Phoma, A. flavus, Pestalotiopsis microspore*, *Comamonas acidovorans* TB-35, *Aspergillus fumigatus*, *E. coli, P. chlororaphis, Seudomonas chlororaphis, Thermomyces, Alternaria*	Bhardwaj et al. (2013), Howard (2012), Schink et al. (1992,b), Zafar et al. (2013), Howard (2012), Shimao (2001), Flavel et al. (2006), Zafar (2013), Akutsu et al. (1998), Ma and Wong (2013), Howard (2012), Shigeno-Akutsu et al. (1999)
9	Polyethylene (PE)	*Pseudomonas* sp., *Gordonia Nocardia, Staphylococcus, Serratia, Phanerochaete, Penicillium, Arthrobacter, Aspergillus, Streptomyces, Lysinibacillus, Nocardia, Gliocladium, Listeria, Vibrio, Streptococcus, Bacillus, Rhodococcus, Micrococcus, Proteus, Diplococcus, Chaetomium, Bravibacillus, Moraxella, Mucorrouxii, A. flavus, Streptomyces badius* 252 and *Streptomyces setonii*75Vi2, *Streptomyces viridosporus* T7A, *R.ruber*, *P. chrysosporium*, *Streptomyces* sp., *S. marcescens, Bacillus*	Nwachkwu et al. (2010), Restrepo- Flórez et al. (2014), Bonhommea et al. (2003), Grover et al. (2015), Koutny et al. (2006a, b), Arutchelvi et al. (2008) Mahalakshmi et al. (2012), Pometto et al. (1992), Basnett et al. (2012), Yoon et al. (2012), Odusanya et al. (2013), Das and Kumar (2015), Gajendiran et al. (2016), Thomas et al. (2015) Pramila et al. (2012), Flavel et al. (2006), Muenmee et al. (2015, 2016), Duddu et al. (2015); Abrusci et al. (2011) Devi et al. (2015), Hadad et al. (2005)

(continued)

Table 3 (continued)

S. no.	Plastic	Microorganisms	References
		amyloliquefaciens BSM-1, *B. amyloliquefaciens* BSM-2, *A. clavatus*., *P. fluorescens, S. aureusA. niger, Brevibacillus parabrevis* PL-1, *Acinetobacter baumannii* PL-2, *A. baumannii* PL-3 and *P. citronellolis* PL-4, *Methylobacter*sp. or *Methylocella*sp., *Nitrosomonas*sp AL212, *Nitrobacter winogradkyi, Burkholderia*sp., *Methylobactor*sp., *Methylococcus capsulatus, Methylocystic*sp. *Streptomyces coelicoflavas*, *Bacilluscereus*, *B.megaterium, B. subtilis*, *Brevibacillus borstelensis*, *B. megaterium*, *Aspergillus tubingensis*VRKPT1, *B. borstelensis*707, *P. aeruginosa* CA9, *Burkholderia seminalis* CB12, *Stenotrophomonas pavanii* CC18; *P. lilacinus, L. theobromae*, *Lysinibacillus fusiformis* VASB14/WL and *Bacillus cereus* VASB1/TS.	Mehmood et al. (2016), Shahnawaz et al. (2016)
10	Polypropylene	*Pseudomonas chlororaphis, Pseudomonas stuzeri, Vibrio species*	Alariqi et al. (2006)
11	Polyvinyl Chloride	*As. Fumigatus, Phanerochaete chrysosporium, Lentinus tigrinus, As. Niger, Aspergillus sydowii*	Kirbas et al. (1999), Ali et al. (2014)
12	Polystyrene	*Azotobacter beijerinckii* HM121	Nakamiya et al. (1997)
13	Nylon	*Geobacillus thermocatenulatus, Flavo bacterium* sp. KI72, *Pseudomonas* sp. strain NK87	Tomita et al. (2003), Kakudo et al. (1993), Kanagawa et al. (1993), Kinoshita et al. (1981), (1977), Negoro et al. (1992)
14	PUR Foam & PS Foam	*Pseudomonas aeruginosa*, sp. *Cladosporium resina, Aspergillus niger, Aspergillus versicolor, Penicillium funiculosum, Chaetomium globosum, Aspergillus flavus*	Edmonds and Cooney (1968), Expandable Polystyrene Molders Association (2004)

(continued)

Table 3 (continued)

S. no.	Plastic	Microorganisms	References
15	PET and other Aliphatic–aromatic copolyesters	*Rhizopus delemar* lipase, *Thermobifida fusca*, *Humilica insolens* *Aspergillus sp*., *Beauveria sp*., *Thermobifida fusca*, commercial enzymes (TEXAZYME PES sp5 and Lipase PS), *Fusarium solani pisi Pseudomonas mendocina* and *Fusarium solani*, *Penicillium funiculosum* *Nocardia* sp., esterase	Tokiwa and Suzuki (1981), Kleeberg et al. (1998), Donelli et al. (2010), Heumann et al. (2006), Ronkvist et al. (2009), Heumann et al. (2006), Nowak et al. (2011), Sharon and Sharon (2017)

4 Physiochemical Characteristics Influencing Depolymerization

Polymer biodegradation is a heterogeneous process since polymers are multicomponent systems. These components can provide nutrients for some microbes (Kyrikou and Briassoulis 2007). All polymers are biodegradable to some degree because of the organic fraction of their composition like resins, hardener. Polymeric material structures and compositions govern their biodegradation. Single polymer can contain different structural elements, e.g., random or alternating copolymers. Also, varying structures like branched network can directly impact the polymer chain cleavage. Substantial amount of qualitative and semi-quantitative information has been gathered documenting the factors that impact the rate of degradation of synthetic polymers in biological environments (Fig. 3).

4.1 Abiotic Factors

Environmental conditions such as temperature, humidity, pH, salinity, sunlight, water, presence/absence of oxygen, culture conditions, and stress significantly impact the degradation process and also control microbial population and enzyme activity (Gu 200). Increased temperature and moisture content increase the rate of hydrolysis and microbial activity, which in turn amplify the chain scissions subsequently increasing the available polymer sites for attack. This results in faster degradation (Ho et al. 1999). Chain scissions from photodegradation reduce the molecular weight of polymer and improve the approachability of microbes to the polymer (Stevens 2003).

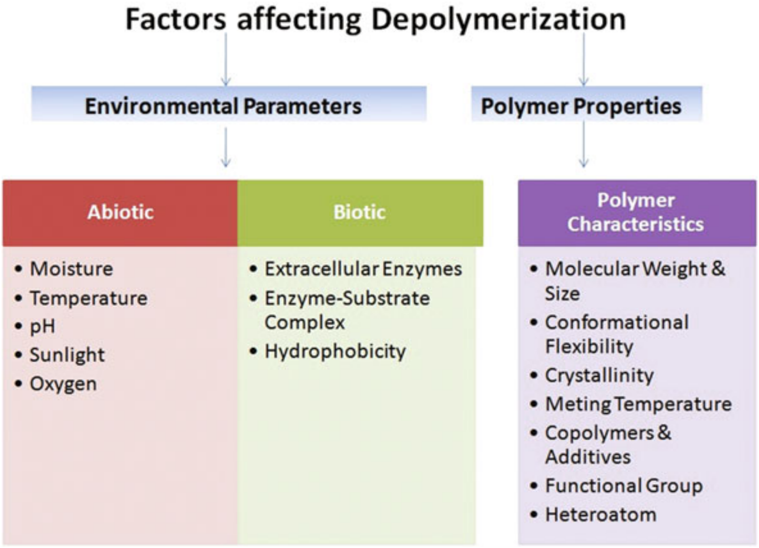


Fig. 3 Factors affecting depolymerization

4.2 *Biotic Factors*

4.2.1 Production of Extracellular Enzymes

Extracellular enzymes excreted by different microbes may have active sites with different structure/configuration and biodegrade certain polymers better than others. For example, *Aspergillus niger* and *Aspergillus flavus* fungi produce enzymes which are known to better digest 6-12 carbon di-acid-derived aliphatic polyesters as opposed to those from other monomers (Chandra and Rustgi 1997).

4.2.2 Enzyme–Substrate Complex Formation

Enzyme–substrate complex formation is generally explained by either ***lock and key fit*** or by ***induced-fit*** mechanisms (Donald et al. 2006; Koshland 1995). For a reaction to take place and enzyme–substrate complex to be formed, some geometrically similar pattern between enzyme and substrate is required. The lack of these similar geometric patterns between plastic substrate and available enzymes makes the succeeding biodegradation difficult.

4.2.3 Hydrophobicity

Although water solubility does not guarantee biodegradability, the particularly hydrophobic nature of plastics makes them harder to biodegrade. Enzymes and microbes often have complex metabolic pathways for mineralizing such extremely hydrophobic materials (Nakajima-Kambe et al. 1999).

4.3 Polymer Characteristics

4.3.1 Conformational Flexibility

Flexibility in a polymer makes it easily reachable for the microbes. Most polymers have highly cross-linked branched structures, which make formation of an enzyme–substrate complex harder. For example, flexible aliphatic polyesters are comparatively easier to biodegrade than rigid aromatics like polyethylene terephthalate (PET) (Omichi 1992).

4.3.2 Molecular Weight (M_W)

Microorganisms can only digest lower molecular weight segments of polymer and convert them into metabolites. Synthetic polymers with $M_W > 1000$ are found to be less susceptible to biodegradation than those with $M_W < 1000$ (Suzuki et al. 1978).

4.3.3 Degree of Crystallinity

The amorphous part of polymeric chain is prone to enzymatic attack than the crystalline part because

(a) molecules in amorphous part are freely packed, making the enzymatic attack easier and more susceptible. For example, the rate of polylactide acid degradation decreases with increasing crystallinity (Tsuji and Miyauchi 2001).

(b) Amorphous regions show high permeability to O_2. Oxidation rates depend on the reactivity of formed peroxy radicals and dissociation energies of available C–H bonds of polymer substrate. A polymer with unreactive methyl and phenyl groups or with no *H* at all shows resistance to oxidation (Bovey and Winslow 1979).

4.3.4 Melting Temperature (T_m)

Melting temperatures of polyesters is an important parameter for the enzymatic depolymerization. Higher melting temperatures imply lower polymer biodegradation (Tokiwa and Suzuki 1978, 1981; Tokiwa et al. 1979).

4.3.5 Size of the Polymer

In order to avoid mass transfer restrictions and to increase surface area available to microorganism for interaction, the polymer must be reduced in size before subjecting it to biodegradation.

4.3.6 Copolymers and Additives

Naturally occurring polymers are biodegradable but biodegradation of chemically tailored natural polymers is conditioned on type and degree of modification. Adding comonomers in a polymer increases chain irregularity, thereby reducing its crystallinity and providing easy access to microbes and H_2O. Impurities such as catalytic residues, fillers, pigments, and altered products of additives also influence the resistance to degradation. Metals like Mn act as good pro-oxidants in polyolefins making them susceptible for thermo-oxidative degradation. After heat activation in the presence of oxygen, pro-oxidants produce free radicals on hydrocarbon chain which undergo oxidation (Orhan et al. 2004). Furthermore, the pro-oxidant catalyzes chain scissions giving low molecular mass oxidation products containing –OH, –COOH, C=O groups (Jakubowicz 2003). Traces of transition metals present also speed up thermal oxidative processes inducing hydroperoxide decomposition (Zheng et al. 2005, Zhang and Thomas 2011). For example, TiO_2 delustrant makes polyamides more prone for light- and heat-induced oxidation. Making polyolefins blend with other polyester or starch also increases its susceptibility for degradation (Erlandsson et al. 1997).

4.3.7 Role of Heteroatom

It has been observed that polymers that include heteroatoms in the backbones such as polyamines and polyesters show higher susceptibility to degradation than polymers with pure carbon backbones (Zheng et al. 2005). Incorporating heterogroups like oxygen in polymer chains influences bond strength (of neighboring C–H bonds) promoting carbanion formation (e.g., in presence of bases) subsequently making them labile for thermal and biodegradation (Gowariker et al. 2000). Similarly, unsaturation in polymer chains makes polymer prone to oxidation, i.e., natural rubber is more vulnerable to degradation than polyethylene (Seymour 1971). Aromatic polyesters despite of having easily hydrolysable ester bonds have seen to be resistant to degradation. For example, PET despite having ester bonds in its polymer chains still remains non-degradable (in normal conditions) due to its aromatic content (Webb et al. 2012).

4.3.8 Introduction of Functionality

Introducing carbonyl groups like C=O in polyolefins makes them more vulnerable for photodegradation. Increase in the number of chromophores results in increased sites for photon absorption and hence increases the rate of photodegradation. Incorporation of additional chromophores like metal–metal bonds in polymer backbone too induces photodegradability. In such case, metal–metal bond can cleave homolytically when irradiated (Meyer and Caspar 1985).

4.3.9 Chemical Bonding

In thermoplastics, during addition polymerization head-to-head or tail-to-tail addition of monomers results in weak spots making plastic susceptible for degradation. For example, head-to-head linkage in poly (methyl methacrylate) improves its thermal degradation (Holland and Rae 1987). Similarly, branching in polymeric chains too increases thermal degradation (Gowariker et al. 2000).

4.3.10 Mode of Synthesis

Methods of synthesis exhibit an effect on the stability of the polymers (Hendrickson and Connole 1995), e.g., polystyrene (anionic polymerized) shows more photo-stability than its free radically formed equivalent because of the presence of peroxide residue in the latter, which is labile for photodegradation (Pospisil et al. 2006). Similarly, copolymerized polypropylene (PP) is less inclined to photodegradation than PP synthesized via bulk polymerization and by using Ziegler–Natta catalysts (Tang et al. 2005).

5 ASTM Standards for Plastic Biodegradation

The American Society for Testing and Materials (ASTM) has recognized an assortment of scientific and technological tests to assess true biodegradation in numerous plastic articles under varying environments using different analytical methods (Table 4). ASTM (standard D-5488-94d) recognizes biodegradation as "*process which is capable of decomposition of materials into carbon dioxide, methane, inorganic compounds or biomass and water in which the predominant mechanism is the enzymatic action of microorganism that can be measured as*

Table 4 ASTM standards for biodegradation of plastics

Test number	Methods
ASTM G21	Standard practice determining resistance of synthetic polymer materials to fungi
ASTM G29	Standard practice determining algal resistance of plastic films
ASTM D5511 Equivalent to ISO DIS 15985	To determine anaerobic biodegradation, under high solid conditions (total solids > 30%)
ASTM D5247	For determining the aerobic biodegradability of degradable plastics by specific microorganisms
ASTM D5526	To determine anaerobic biodegradation under accelerated landfill conditions
ASTM D5338	To determine aerobic biodegradation under controlled composting conditions
ASTM D6400	List specifications for compostable plastics
ASTM D6002	Standard guide to assess compost ability of environmentally degradable plastics
EN ISO 846	Evaluation of action of microorganism on plastics
ISO 846	Plastics: Determination of behavior under the action of fungi and bacteria. Evaluation by visual examination or measurement of changes in mass or physical properties

standard tests, in a specified period of time, reflecting available disposal conditions." Taking into account the fact that plastics more often than not have multi-faceted compositions and are generally degraded via heterogeneous surface mechanism, these tests are chiefly based on concepts used for assessing low molecular weight substances. Some modifications have been done over the years for different environments in which plastics might degrade.

5.1 Test Methods for Study of Biodegradation

These tests assess the changes in the material properties of polymers. Multiple test measures are required to evaluate the biodegradability of polymer for the reasons that

- Leaching of additives, plasticizers, etc., may also result in the observed weight loss and not only polymer degradation;
- Very small change in chemical makeup of polymer could result in high reduction in material strength, e.g., 5% of mineralization can cause 90% decrease of polymer strength;
- CO_2 production may be a result of degradation of low molecular weight part of the polymer, with no degradation of longer chains (Mohan 2011).

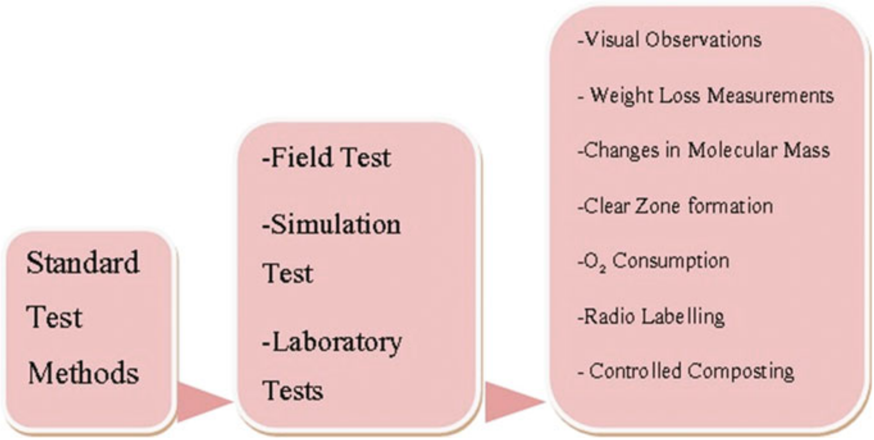


Fig. 4 Test methods used for evaluating polymer degradation

The current test methods are not sufficient and offer a very little flexibility to take into account the wide assortment of advanced polymers (like the ones used in electronics or aviation applications) consumed in different environmental conditions (Gu and Gu 2005). In principle, these tests can be divided into three types (Fig. 4).

5.1.1 Field Test(s)

These tests are carried out by burying the plastic sample in the soil, placing them in water sources like rivers, lakes, or by performing complete composting. Although these tests are easy and widely used, there are some associated disadvantages as well such as (i) the environmental factors like temperature, humidity, pH cannot be well controlled; (ii) analytical prospects to monitor the degradation are limited. In such situations, only possibility is to evaluate disintegration via measuring weight loss or to estimate visible changes in the polymer sample. Complex and open-ended environmental conditions make study of residues, intermediates, degradation pathways, CO_2 evolution, or O_2 consumption complicated.

5.1.2 Simulation Test(s)

As a substitute to field tests, a range of simulation test has been developed in which degradation process is done in a real environment like soil, in H_2O, or in compost, but the contact to the environment is provided in a laboratory reactor. The important abiotic factors such as temperature, pH, humidity that affect the degradation process can be controlled and adjusted. To speed up the degradation and thereby reducing the test durations, sometimes additional nutrients are added to the sample. Examples for such tests include test simulating landfills (McCartin et al. 1990; McCarthy et al. 1992; Smith et al. 1990), the controlled composting test (Pagga et al. 1995; Tosin et al. 1996; Degli-Innocenti et al. 1998; Ohtaki et al. 1998; Tuominen et al. 2002), the soil burial test (Pantke 1990), and aquarium tests (Püchner et al. 1995). The only disadvantage is that it lacks reproducibility because of varying microbial population.

5.1.3 Laboratory Tests

Most reproducible tests, here a pre decided growth media is used which is then inoculated either with individual microbial strains or with mixed microbial inoculums. Reaction conditions are tailored for the activity of a particular microbe. Polymers frequently show higher degradation rates in laboratory tests compared to natural conditions. Examples of such tests are petri dish screen, closed bottle test, rapid detection method, gravimetry, surface hydrolysis, respirometry, and measurement of biogas. These tests offer the advantage of systematic investigations into reaction mechanism but it is still not possible to prove biodegradation in terms of metabolization by microorganisms (Mohan 2011).

5.2 Standard Test Methods

5.2.1 Visual Observations

Visual observations can be made in almost all the tests. Effects used to express degradation comprise of changes in color, roughening of the surface, defragmentation, formation of holes or cracks, or formation of biofilms on the plastic surface. These parameters can be used as indications of microbial attack but they do not essentially prove the biodegradation in terms of metabolism. To get information about the reaction mechanism, more sophisticated observations can be made using various characterizations techniques including but not limited to scanning electron microscopy or atomic force microscopy (Ikada 1999).

5.2.2 Residual Polymer Determination by Weight Loss

The mass loss of test samples is commonly noted in degradation tests (mainly in field and simulation tests), although this also does not give any direct proof for biodegradation. By combining structural analysis of residual material and low molecular weight intermediates, detailed information concerning degradation process can be gathered, especially when using a defined synthetic test medium (Witt et al. 2001).

5.2.3 Change in Molecular Mass and Subsequently in Mechanical Properties

If only small changes in the mass of samples are noted, then the changes in mechanical properties are normally used. Some properties like tensile strength are very receptive to changes in the molecular mass of polymers, which is also an indicator of degradation (Erlandsson et al. 1997).

5.2.4 Clear Zone Formation

It is a simple semi-quantitative method. The polymer size is reduced to fine particles and spread onto an agar medium. Microbes are introduced to the medium. The formation of clear halo around microbial colony is taken as an indication of capability of microbe to depolymerize the polymer. Although this method is typically employed to screen microbes for degradation potential for certain polymers (Nishida and Tokiwa 1993; Abou-Zeid 2001), it can also be utilized to gather semi-quantitative results by analyzing the growth of clear zones (Augusta et al. 1993).

5.2.5 O_2 Consumption or CO_2 Evolution

In aerobic respiration, microbes utilize oxygen and form CO_2. Consequently, the respirometric test, used for estimating the oxygen consumption (Hoffmann et al. 1997), and Sturm test, for estimating the formation of $CO_{2,}$ are often used as good evaluators to estimate polymer degradation in laboratory test with good accuracy.

5.2.6 Radio Labeling

Net CO_2 and $14CO_2$ evolution measurements are simple, non-destructive and measure ultimate biodegradation. Using this system for biodegradation studies in varying microbial environments exhibits precision and consistency (Sharabi and Bartha 1993). However, labeled materials are quite expensive and not easily available. The licensing and related radioactive waste disposal problems present some major disadvantages.

5.2.7 Controlled Composting Test

In this process, a definite weight of dry plastic is mixed with fixed amount of mature compost and incubated at 58 °C with 65% moisture content. Biodegradation is examined by measuring the amount of material carbon changed into gaseous carbon oxide, i.e., the CO_2 evolved from the polymer–compost mixture minus the CO_2

evolved from the unamended compost (as blank) which is tested in a different reactor (Bellina et al. 1999).

6 Characterization Techniques Used to Study Microbial Depolymerization

The biodegradability of the plastic can be characterized using several parameters such as oxygen uptake rate, carbon dioxide evolution rate, accumulation of biomass, surface changes, and changes in physiochemical properties of plastics (Albertsson and Huang 1995). Numerous analytical techniques have been used to investigate the degree and character of degradation. The examination of changes in physical and chemical properties of plastic both before and after degradation helps to understand reaction mechanisms and/or degradation pathway.

6.1 Dynamic Mechanical Analysis (DMR) Using Universal Testing Systems

Study the mechanical properties of plastics such as elongation at fail, tensile strength, and modulus of the polymer. Physical properties observed for changes are morphology (microcracks), contact angle, density, viscosity, glass transition temperatures, melting temperatures, amorphous part and crystalline part of the polymer, etc.

6.2 Scanning Electron Microscopy (SEM)

SEM helps in studying the surface morphology of the polymer films. The samples are generally have to be sputter-coated with gold or some metal ion before examination. Variable-pressure SEM is helpful for direct observation without using any surface metallization at suitable magnification (Pinzari et al. 2006).

6.3 Thermogravimetric Analysis (TGA)

It observes the changes in mass of sample due to heating. Dynamic thermogravimetry can be used to study the activation energy, rate of decomposition reactions, and temperature at maximum decomposition rate (Carrasco and Pages 1996).

6.4 Fourier Transform Infrared Spectroscopy (FTIR)

Chemical changes produced in polymers during or after microbial degradation processes are analyzed by FTIR (Mohamed et al. 2007; Singh and Sharma 2008; Elashmawi et al. 2008; Milstein et al. 1994). It helps to explain physical and chemical structures, degradation reactions, end group detection, hydrogen bonding, cross-linking behavior of molecules, and copolymer composition in solid- and liquid-form polymers.

6.5 Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectroscopy (both 1H and 13C) is the most versatile analytical tool used to understand degradation reaction pathways and formed intermediate compounds (Massardier-Nageotte et al. 2006; Schlemmer et al. 2009; Shah et al. 2008).

6.6 Gel Permeation Chromatography (GPC)

GPC also known as size-exclusion chromatography (SEC) is used to find out molecular mass distribution of polymers and changes in molecular weight after biodegradation (Peng and Shen 1999; Kale et al. 2006; Walter et al. 1995).

6.7 High-Pressure Liquid Chromatography (HPLC)

HPLC is extensively used to separate molecules on the basis of their size. It helps to analyze the metabolic degradation products of xenobiotics, e.g., biotransformation products of styrene, epoxy styrene, phenylacetaldehyde, and 2-phenylethanol were detected by using reverse-phase high-pressure liquid chromatography (Beltrametti et al. 1997; Marconi et al. 1996).

6.8 Gas Chromatography–Mass Spectrometry (GC/MS)

The GC/MS technique comprises a gas chromatograph coupled to a mass spectrometer and is used to identify and quantify residual monomers, trace contaminants, analyzing volatile components, gas mixtures, solvent in resins and coatings, verifying additive levels in polymers and for on-line or at-line monitoring of reactions.

6.9 Biochemical Analysis

The metabolic activity of the cells in the culture and in the biofilm can be determined by various biochemical assays, e.g., by adenosine triphosphate assays, by fluorescein diacetate analysis, and by protein analysis (Orr et al. 2004; Koutny et al. 2006a, b).

6.10 Molecular Techniques

Microbial depolymerization and degradation usually take longer time periods. Once the degradation pathway is known, genetic engineering allows us to (i) reduce the reaction time, (ii) make the plastic more susceptible for microbial attack, (iii) modify regulatory mechanisms, (iv) alter the properties of degradative enzymes, and (v) assemble degradative enzymes from different organisms within a single organism. This can be done by exploiting genetic engineering techniques such as DNA and protein sequencing and production of recombinant organisms, etc.

Some biosynthetic genes, e.g., *phbA* (for 3-ketothiolase), *phbB* (NADPH-dependent acetoacetyl-CoAreductase), and *phbC* (PHB synthase) from acetyl-CoA have been cloned to produce PHA and PHB acids (Slater et al. 1988). These genes are clustered, presumably organized in one operon, and expressed in *Escherichia coli* and in also in different species of *Pseudomonas*. The enzymes implicated in plastic degradation should be characterized, and further genes liable for those enzymes should be worked out. Once these genes are identified, they can be modified to boost their degrading capacity (Aswale and Ade 2008). Efficient degrading microbes should be multiplied at larger scale to decompose the plastic at commercial level.

7 Significance

Efficient technology, low cost, and relatively eco-friendly treatment capable of minimizing and even eliminating synthetic polymers hold a great environmental interest. Microbial population is now being extensively studied for biodegradation of synthetic polymers. Biodegradability tests are essential to assess and minimize the environment impacts and accumulation of plastics. If plastics can be made to degrade more effectively by microbes, it will reduce solid waste problems which raise a plethora of environmental concerns. Microbial enzymes are crucial to biodegradation of plastics. There is a great potential and scope in exploring these microbes and tailoring according to specific needs like to grow in varied environmental conditions, for better utilization of plastics carbon for their energy needs.

8 Future Perspective

The microbial depolymerization of synthetic polymers is a multifaceted phenomenon. Strict nature-like experiments are difficult to carry out in laboratory settings because of number of parameters occurring during the biogeochemical recycling which cannot be entirely replicated and directed in vitro. Information available so far regarding microbial ability for degrading synthetic plastics is build on a few bacteria (representing < 0.1% of total bacteria) that could grow on culture media. Thus, high diversity of microorganisms present in nature has not been even partially explored yet. Making use of molecular technologies such as genomics, proteomics, transcriptomics, metabolomics makes it feasible to discover novel microorganisms (even non-culturable ones) involved in plastic degradation and investigate properties of microorganisms arising from the interplay of proteins, genes, other macro–micro molecules and the environment.

9 Conclusion

Polymers are generally resistant toward biodegradation. Physicochemical methods of degradation have been more successful than biological ones. However, exploration of microbial depolymerization is a step toward plastics management by sustainable method.

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Author Biographies

Ms. Anvita Sheel is currently perusing Ph.D under the guidance of Prof. Deepak Pant in the subject of Environmental Science from Central University of Himachal Pradesh, Dharamshala, Himachal Pradesh, India. She received post graduation degree in Environment Science (Gold Medalist) from Panjab University, Chandigarh in 2014. She received first position (poster presentation) in National Seminar on Emerging Trends in Environmental Science and Technology-2017 and 2nd position in 8th Chandigarh Science Congress (oral presentation). She was also awarded with Certificate of Excellence in Rio+21 IYWC India Certification Program.

Dr. Deepak Pant is currently the Dean and Head of Environmental Science, Central University of Himachal Pradesh, Dharamshala. He received the Silver Jubilee Research Fellowship Award in 2003 from Kumaun University, Nainital, India; the UCOST Young Scientist Award 2009, INSA Fellow (2010), DST-SERC, Visiting Fellow (2010) and DST-SERC Young Scientist Award (2011). He has 5 patents in the area of waste management using green techniques and published 10 books, 45 research papers in various national and international journals. He recently received Visitor's Award 2017 from the President of India for his innovations in the area of waste management.