Chapter 6
Bioremediation of Polythenes and Plastics: A Microbial Approach

Shubha Rani Sharma

Contents

6.1 Introduction

Human beings have always endeavored to make life easier. They have devised ways and means to have a comfortable and luxurious life. They have tried to maintain everything around us by packaging like food packaging, water packaging, etc., and for this purpose, they have used plastics and polythenes. Plastics can be defined as polymer that has the capacity of being shaped into any form with the aid of high temperature and pressure. Polythene comprises of polymer of the ethylene monomers that is the major nondegradable solid waste produced from daily use. Plastics being typically inert and resistant to attack by microbes continue to exist for years. Exploiting the additional properties of plastics like low density and toughness, we can mold plastics into different types of products. Due to their durability and low cost, synthetic polymers are widely used, but discarding of these materials has cropped up as a major issue in the area of solid waste management causing main threat to environmental pollution.

S. R. Sharma (✉)
Department of Bio-engineering, Birla Institute of Technology, Mesra, Ranchi, India
e-mail: srsharma@bitmesra.ac.in

R. Prasad, E. Aranda (eds.), *Approaches in Bioremediation*, Nanotechnology in the Life Sciences, https://doi.org/10.1007/978-3-030-02369-0_6

Now, the use of polythenes and plastics has been so profuse that it has become a serious problem as to how to decompose them. Bakelite was invented by Leo Baekeland in 1907 as the first fully synthetic plastic. As the plastics are non-biodegradable, they have become a matter of serious concern for the proper disposal of these plastics and polythenes. The industrial and urban wastes produced by the human activities are alarmingly increasing the environmental contamination. The use of plants to convert the poisonous compounds into nonpoisonous forms is known as phytoremediation (Ghosh and Singh 2005). The use of plants in bioremediation for cleanup of contaminated environment dates back about 300 years. Polythene or polyethylene which is commonly used in our day-to-day life like grocery bags, carry bags, sachets, etc. is the product of polymerization of ethylene. The general formula for polythene is $(C_2H_4)_nH_2$.

The various classes of polyethylene used are low-density polyethylene, medium-density polyethylene, high-density polyethylene, and very low-density polyethylene, out of which the low-density polyethylene is widely used for common purposes like carry bags used in grocery, packaging materials, electrical casing, etc. Plastic containers in laboratory and kitchen are very versatile as they are acid resistant. Now the versatility of the plastic which makes it so useful in every scenario has become a headache for the environmentalists as the profuse use of the versatile material in every field is resulting into heaping and piling of non-biodegradable material in the environment causing pollution. Now the time has come when we should ponder over the concerns related to plastic and polythene pollution. The physical and chemical methods of degradation of plastics may be very cumbersome and may result into production of more toxic substances. So we should go for an alternative method of plastic degradation which is safe and easy. The answer to this problem is the microbial degradation of plastics that is the use of microbial enzymes and bioactive compounds for the degradation. There are different types of plastics found in our environment. Each type of plastic has unique type of properties as well as find use in different areas. Some of them are enumerated in the Table 6.1.

6.2 Environmental Effects of Plastic Pollution

Plastics being very less degradable can accumulate in the environment, making it polluted. If at all they are partially degradable, their chemical by-products are more toxic, producing adverse effects. Plastic pollution in present scenario is one of the biggest environmental problems. Nowadays we have become so much used to plastics and polythenes that it seems that life without plastics is not possible. Although plastics are very helpful to us in daily life, if we come down to review the after effects of plastic pollution, we would rather quit using plastics and polythenes. As the population grows, the quantity of plastic refuse accumulates in the world causing plastic pollution (Song et al. 2009). The toxic pollutants in the plastics have adverse effect on environment. The decomposition of plastics takes a very long time say thousands of years causing serious damage to the environment. The effect of

Table 6.1 Types of plastics, properties, and their uses

No	Types	Name	Properties	Uses
1	PET	Polyethylene terephthalate	Absorbs odors and flavors from food and drinks that are stored in them, high heat resistant, extremely effective moisture barrier, shatterproof	Beverage bottles, medicine jars, rope, clothing, and carpet fiber
2	HDPE	High-density polyethylene	Excellent moisture barrier properties, chemical resistance	Containers for milk, motor oil, shampoos and conditioners, soap bottles, detergents, and bleaches
3	PVC	Polyvinyl chloride	Excellent transparency, hard, rigid, long-term stability, brittle, waxy surface	Plumbing pipes, credit cards, carpet backings, window or door frames, synthetic leather products
4	LDPE	Low-density polyethylene	Tough, flexible, good transparency, low melting point	Cling film, sandwich bags, squeezable bottles, and plastic grocery bags
5	PP	Polypropylene	Excellent chemical resistance, high melting point, hard but flexible, strong, translucent	Lunch boxes, margarine containers, yogurt pots, syrup bottles, prescription bottles, plastic bottle caps
6	PS	Polystyrene	Clear to opaque, glassy surface, rigid or foamed, brittle, high clarity	Disposable coffee cups, plastic food boxes, plastic cutlery, and packing foam
7	Others	Polycarbonate and polylactide	Layered or multi-material mixed polymers	Baby bottles, compact discs, and medical storage containers

sunlight on plastic is immense which leads to photodecomposition resulting in trash and toxin-like chemicals, and these are the main cause of soil and water pollution by plastics. Plastic, synthetic polymer consisting of carbon, hydrogen, silicon, oxygen, chloride, and nitrogen, is manufactured from oil, coal, natural gas, etc. Since plastics are extremely stable and robust, they are extensively used. There are various types of plastics, e.g., polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), nylons, polyethylene terephthalate (PET), polypropylene (PP), and polyurethane (PUR). These polymers accumulate in the surroundings as there is no efficient method for degradation or method for safe disposal, thus posing constantly aggravating threat to the flora and fauna.

A new study suggests that as of the 1950s, over 9 billion tons of plastic has been produced, and most of them have been converted into scrap which permanently persists in our environment. Around 6300 Mt of plastic waste had been produced till 2018, out of which only 9% have been recycled, 12% were incinerated, and the residual 79% have their fate in landfills thrown into the environment (Geyer et al. 2017). According to the Global Industry Analysts, plastic consumption throughout the world has been approximated roughly equal to 260 million tons in 2008, and it was anticipated to be over 300 tons in 2015. These plastics end up in the environment as a permanent disaster producing threat to wildlife and humans. Worldwide utilization of plastic material in 2015 was expected to be 45 kg/person. The highest consumption of plastic material per person for the year 2015 was expected to be in NAFTA region which was 139 kg/person followed by Western Europe where 136 kg use of plastic per person was observed. The lowest consumption of 16 kg/person was found in the Middle East and African region. If existing production and the inadequate waste management trends persist, then by 2050, we will have a burden of approximately 12,000 Mt of plastic waste. By the data given by Smithers Rapra, worldwide plastic consumption hiked during the period 2007–2012, reaching to nearly 19.6 million tons. Global specialty plastic utilization has been predicted to upsurge in the period 2012–2017 to reach 25.3 million tons. Worldwide, about 380 million tons of plastic will be produced till 2018. Over 5 million tons of plastic is consumed each year in the UK only, out of which one-quarter is only recycled and the remaining are directed to landfills. There are studies which announce that the bodies of 90% of seabirds are found to contain plastic waste. Now that people have understood the havoc caused by the accumulation of the non-biodegradable plastics in the environment, major steps are being taken to trim down the aftereffects of plastic pollution, by dropping plastic consumption and enhancing plastic recycling.

There are many hazards of plastic pollution. Let's ponder on some major aspects of this plastic pollution. Garbage dumps and landfills containing plastic refuse are one of the major problems which let the pollutants to enter the earth affecting the flora and fauna as well as groundwater for long terms. The nets used for commercial fishing are usually made of plastic. When these nets are kept underwater for the purpose of fishing for long hours, the toxins are released affecting the water and fish of the area. When the water organisms are intoxicated with the poison in the plastic ingested by them, it results into greater problem for larger organisms, which make the higher part of the food chain, thus affecting the whole food chain and web. Plastics in the landfills interact with water forming hazardous toxins which in their turn seep into the earth affecting the water quality of groundwater.

Plastics can affect animals that may come in the surrounding area and might choke them to death. When plastic is burnt in open air, it releases toxic substances leading to environmental pollution. The humans and animals are forced to inhale the polluted air which results in health hazards and can cause respiratory troubles. Due to exposure to sunlight, rainfall, and other ecological conditions, the plastics disposed in the ocean decompose at a faster rate, releasing toxic chemicals such as bisphenol A (Teuten et al. 2009). Plastic bits are taken in by filter-feeding organisms and planktons in the ocean which in turn are taken up by the higher animals leading to bioaccumulation of these toxins in the food chain and food web. The most recognized undesirable environmental effects of polyethylene films are when they are swallowed by wild animals. The most general types of plastic pollution in oceans are the polystyrene, plastic pellets, plastic bags, and food containers. Chlorinated plastics release harmful chemicals which can then seep into groundwater causing serious injury to the species that consume this water. Different types of plastics continuously accumulate in the landfills.

In the manufacture of plastics, some of the chemicals that are used are easily absorbed by human beings through skin absorption and cause different types of diseases like dermatitis, etc. There are innumerable side effects of plastics and polythenes which we are not even aware of. Now the most important need of the hour is the alternative to plastics and the biodegradation of plastic heaps which have already accumulated and clogged our environment causing serious threats. Most of the countries restrict the use of incineration process for disposal of wastes as plastics are the major constituent. As an alternative, uncontrolled burning and landfilling are used to get rid of the plastics. Now the open burning can pose various health hazards. Some of the organic pollutants like furans and dioxins are produced as the by-product of polyvinyl chloride (PVC) plastic burning. Various immune diseases and lung disorders are produced in the burning of polyethylene, polyurethane, polyvinyl chloride, and polystyrene.

6.3 Microbial Role in Biodegradation of Plastics

Some of the living organisms are instrumental in the degradation of plastics, and, as a result, the degraded stuff is returned back to the environment. Both aerobic and anaerobic microbes processes are employed for degradation of plastics. Methane, which is considered as the greenhouse gas, is released in the landfills, resulting into global warming. Fungal degradation can proceed strictly under aerobic conditions, whereas bacterial degradation can occur both in aerobic and anaerobic conditions (Kumar et al. 2011). The polymer decomposes and produces chemicals which act as nutrients and support the microbial growth. Biological degradation is the process of biological conversion of organic compounds into some other specific product by the use of microbes (Restrepo-Florez et al. 2014).

Biodegradation is the practice in which organic substances are broken down by living organisms. It is anticipated that biodegradation is one of the chief methods for the release of most of the chemicals into the atmosphere. The ability of microorganisms to control degradation through physical, chemical, or enzymatic action can be aptly named as biodegradation. Here the microbes use the components in the plastics as carbon and energy source for their growth. Cracking, erosion, discoloration, and phase separation are the different methods by which plastics are degraded. Organic materials can be degraded aerobically with oxygen or anaerobically without oxygen. Microorganisms play a major role in the degradation of both synthetic and natural polymers which is depicted in Fig. 6.1. The microbes can biodegrade the polymers but not use it directly as a nutrient. The decomposition of plastics is considered to be an extremely slow process which is first started by the natural forces and effects like temperature, pH, and ultraviolet rays, and then the microbes adopt a special strategy to completely decompose the remains of the plastics.

In order to degrade the plastics, we can employ different types of methods like chemical, thermal, photooxidation, and biodegradation. Depending on the type of polymer, different time periods are witnessed to degrade them. Different types of

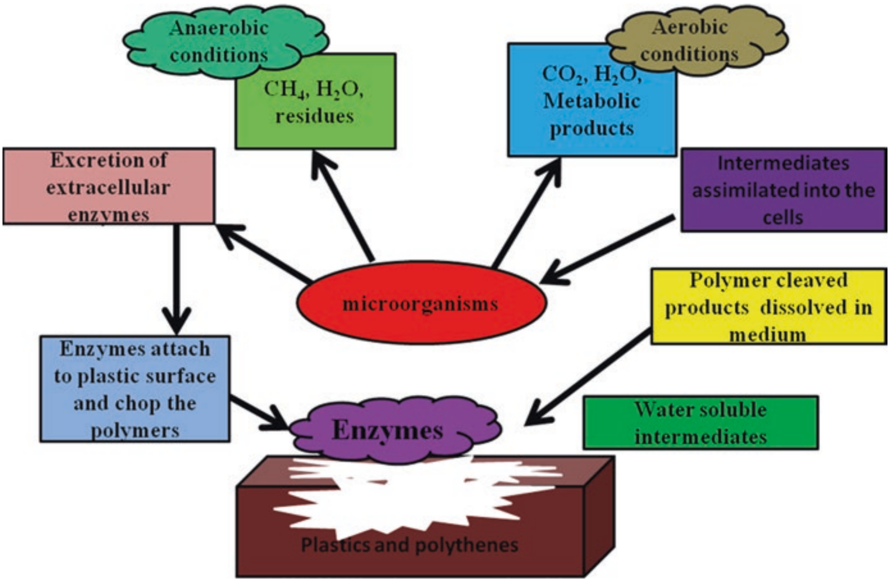

Fig. 6.1 Mechanism of plastic degradation

research reveal that microorganisms can be used for the biodegradation and enormous genera of bacteria (including Actinomycetes) and fungi have proved to degrade plastics (Mahdiyah and Mukti 2013). Usually, microorganisms biodegrade plastic very slowly, and also certain specific plastics cannot be degraded by them (Singh and Gupta 2014). Biodegradable plastics are especially fabricated so that they are ready to be degraded under environmental conditions; thus new avenues were targeted for waste management. Polyhydroxyalkanoates (PHAs) are a bioplastic produced by many of the microbes. It is considered very safe and nontoxic. These can be biodegraded. PHAs are completely biodegradable and biocompatible. They resemble to the petrochemical-based traditional plastics, and they are available in a variety of polymers.

6.4 Bacteria Involved in Biodegradation of Plastics and Polythenes

Microorganisms, chiefly bacteria, are responsible for the biodegradation of substances (Shah and Fariha 2008). As bacteria remineralize the organic carbon in the wastes, they can be considered as saprophytic scavengers. The hydrocarbons which are the major components of plastics are degraded by bacteria which are specialized for the purpose. *Ideonella sakaiensis*, a bacterium from the genus *Ideonella* and family *Comamonadaceae*, was found to be capable of breaking down PET (polyethylene terephthalate) plastic which was a by-product from a plastic recycling plant in Sakai, Japan (Yoshida et al. 2016). The bacterium gets nourished solely on PET and uses enzymes to break them. PETase is the most important enzyme secreted by *I. sakaiensis* 201-F6, a plastic-feeding bacterium. PETase degrades PET into mono(2-hydroxyethyl) terephthalic acid (MHET) which is then further broken down into terephthalic acid and ethylene glycol. The final products are used as food source by the bacterium (Austin et al. 2018).

Chemically different kinds of plastic bags HL (plastic bags containing nano-additives from the Netherlands) and VN1 (plastic bags with additives sold at supermarkets in Vietnam) were found to be degraded by a new thermophilic bacterial strain *Bacillus* sp. BCBT21 (Dang et al. 2018). This species that was isolated from agricultural waste undergoing composting in Vietnam was found to produce extracellular hydrolase enzymes including lipase, CMCase, xylanase, chitinase, and protease (Dang et al. 2018).

Microbes responsible for degradation of plastics were isolated from two different soil sample plastic strips. The isolates were identified as *Streptococcus* sp., *Pseudomonas* sp., and *Bacillus* sp. for bacteria and *Aspergillus* sp. and *Fusarium* sp. for fungi. The isolates were tested for their biodegradative ability, and after about 30 days, it was observed that 23% degradation was by bacterial sp. and 44% by fungal sp. (Vignesh et al. 2016).

The decomposed vulcanized natural rubber and synthetic poly(cis-1,4-isoprene) are used by the bacterial strains, i.e., *Streptomyces coelicolor* 1A and *Pseudomonas citronellolis*. Five Gram-positive and two Gram-negative bacteria and eight fungal species of *Aspergillus* were found associated with the degrading plastics. The chief species were *Streptococcus*, *Staphylococcus*, *Micrococcus* (Gram positive), *Moraxella*, and *Pseudomonas* (Gram negative) and two species of fungi (*Aspergillus glaucus* and *A. niger*). Shaker cultures were employed to check the efficiency of the microbial species in degradation of plastics and polythene. The 20.54% of polythene and 8.16% of plastics were found to be degraded by *Pseudomonas* species, degraded in 1-month period. *A. glaucus* among the fungal species was found to degrade 28.80% of polythene and 7.26% of plastics in 1-month period (Kathiresan 2003). The bacterium *Alcaligenes faecalis* produces polycaprolactone depolymerase (Oda et al. 1997) which degraded polycaprolactone (PCL) which is a biopolymer. Biodegradation of plastics was found to be efficiently done by fungal species like *Phanerochaete* and bacterial species *Streptomyces* (Lee et al. 1991). This was the first report indicating bacterial degradation by oxidization of polyethylenes in pure culture. As an electron acceptor, the aerobic bacteria use oxygen and decompose the organic chemicals finally into CO_2 and water.

It has been found in the recent studies that nitrate, sulfate, iron, manganese, and carbon dioxide are utilized as their electron acceptors by some anaerobic microbes and thus decompose the organic chemicals into smaller compounds (Datta et al. 1998). *Serratia marcescens marcescens* is found to degrade polyethylene plastic films like linear low-density polyethylene (LLDPE) which is used in the packaged water. A novel process of dissolving technique for plastics was used causing an increase in the surface area as well as creating an interface with the environment that may be the cause of microbial growth (Odusanya et al. 2013). Wax worms, or Indian meal moths, were found to be capable of degrading polyethylene films. *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1 were the two bacterial strains capable of degrading PE, isolated from this worm's gut (Yang et al. 2014).

6.5 Fungi Involved in Plastic Biodegradation

The fungus *Penicillium simplicissimum* YK was found to utilize the integral polyethylene as the carbon source and grew better on agar plates containing irradiated polythene (which contained carbonyl groups) than intact polyethylene having no carbonyl groups. It was also observed that hyphae of the fungus were more efficient in degrading intact polyethylene than the spores (Yamada-Onodera et al. 2001). PCL (polycaprolactone) is a biodegradable polymer, which is degraded by microorganisms aerobically as well as anaerobically. Fungi like *Penicillium* and *Aspergillus* are very active in degrading PCL. A report was furnished where they showed that the *Aspergillus* strain ST-01 degraded PCL when incubated at 50 °C for 6 days. It took 12 days for the *Penicillium* species strain 26-1 to degrade PCL (Bhardwaj et al. 2013). *Penicillium funiculosum* and *Aspergillus flavus* were shown to produce enzymes which cleave the PCL. Lipases and esterases are the enzymes which are involved in the degradation of PCL. Organic acid esters like dioctyl adipate (DOA) and dioctyl phthalate (DOP) existing in plasticized polyvinyl chloride (pPVC) make it susceptible to microbial attack (Webb et al. 1999).

Colonization of fungus and biodegradation of pPVC under in situ and ex situ conditions were investigated by Webb and his colleagues in 1999. They reported that 25 and 40 weeks were required for *Aureobasidium pullulans* attached itself on pPVC. It was demonstrated that *A. pullulans* can use pure pPVC as source of carbon. It produces extracellular esterase by degrading DOA. This suggested that fungus *A. pullulans* is trusted to be essential in degrading pPVC. Both fungus and bacteria are found to be instrumental in degrading polyester polyurethane (PUR). The endophytic fungi can degrade synthetic polymer polyester polyurethane (PUR) (Russell et al. 2011). In both aerobic and anaerobic conditions, *Pestalotiopsis microspora* was the fungus which was growing on PUR by using it as sole source of carbon. It was shown that serine hydrolase from the fungus was instrumental in degrading PUR. Here we must make a mention that the endophytes can be considered as the microorganisms which live in the plant tissues and actively participate in decomposition of the plant after the host plant dies.

In 2003, Kathiresan conducted experiments and concluded that the microbes obtained from mangrove soil were also slowly able to degrade plastics. Thus, the work revealed that many plastic-degrading microbes can be isolated from mangrove soil. Moreover, mangrove soil from the Niger Delta was also studied for plastic-degrading microbe population. Two *Aspergillus* species were isolated which were studied for degradation of low-density polyethylene (LDPE) (Pramila Vijaya Ramesh, 2012), and some of the fungal isolates have been found to degrade polyethylene sheets (high-density polyethylene (HDPE) and low-density polyethylene, LDPE). *Penicillium oxalicum* NS4 (KU559906) and *Penicillium chrysogenum* NS10 (KU559907) are the two potential strains which have been witnessed to have the plastic-degrading potential. The degradation of the polythenes like HDPE and LDPE films was found to be done by the unique enzymatic activities in the two fungal strains like *Penicillium oxalicum* NS4 (KU559906) and *Penicillium chrysogenum* NS10 (KU559907) (Ojha et al. 2017). Here prerequisites like prior oxidation or other chemical treatments are not required in the process of degradation. Endophytic fungi were researched for their capability to degrade the synthetic polymer polyester polyurethane (PUR) (Álvarez-Barragán et al. 2016). Under aerobic as well as anaerobic conditions, *Pestalotiopsis microspora* was found to grow on PUR as the sole carbon source. When the molecular characterization of the organism was done, it was found that a particular enzyme, known as serine hydrolase, is accountable for degradation of PUR (Russell et al. 2011). Fungi were found to be outstanding in degrading of polymers in soil as they produce a plethora of enzymes such as glucosidase, cutinase, amylase, lipase, esterase, cellulase, pectinase, and hemicellulase. Fungal strains like *Aspergillus niger* and *A. japonicus* were found to be effective in polythene carry bags of low-density polyethylene (LDPE) degradation (Raaman et al. 2012). As fungi are able to produce an array of enzymes such as glucosidase, cutinase, amylase, lipase, esterase, cellulase, pectinase, and hemicellulase, they would be very instrumental in degrading of polymers (Anastasi et al. 2013).

A fungus with the potential to destroy non-biodegradable plastics was found by the scientists at the Chinese Academy of Sciences' Kunming Institute of Botany. Waste plastics were degraded in a week's time by the fungus. *Aspergillus tubingensis* was a soil fungus which could happily live on the plastic surface by secreting enzymes (Khan et al. 2017). The fungi isolated from El-Sharqia soil, Egypt, were found to produce protease, esterase, and lipase followed by those isolated from Ismailia soil. The fungi with high esterase activity were recognized as *Monascus ruber*, *Monascus sanguineus*, and *Monascus* sp. Maximum esterase was found to be produced by *M. ruber* followed by *M. sanguineus*. *Monascus* sp. isolated from El-Sharqia was the most efficient isolate in degradation of polyurethane to Impranil DLN. Among the ten fungal strains that were isolated from Red Sea water, Jeddah, Saudi Arabia, *Aspergillus* and *Penicillium* species were found to biodegrade the low-density polyethylene, like polyethylene films and powder. The highest percentage in reduction of polyethylene weight was exhibited by *Penicillium* sp. (Alshehrei 2017). Two fungal strains isolated from municipal landfill area were tested for the biodegradation of low-density polyethylene. The degrading ability of the two fungal strains exhibited promising degradation pattern as analyzed by colonization studies, SEM and Sturm test analysis. The fungi were recognized as *Mucor circinelloides* and *Aspergillus flavus*.

6.6 Factors Involved in Biodegradation of Plastics

The biodegradation of plastics by bacteria and fungi takes place at different rates depending upon the soil conditions as well as the types and constitution of the plastics. The different factors that direct the biodegradation process of plastics depend mostly on the type of organism involved in the degradation, the properties of the polymer, and the type of pretreatment given to the polymer.

There are many factors responsible for plastic degradation by microbes which are enumerated in Fig. 6.2. The physicochemical parameters that decide the plastic degradation can be enumerated as the melting temperature, molecular weight, surface area, hydrophilic and hydrophobic nature of polymer, crystallinity, chemical structure, etc. Chemical and physical properties of plastics play important role in their biodegradation. The polymers with side chains are less degradable when compared to polymers with no side chains. Along with the high molecular weight, the morphology, the melting temperature, and the crystal property play a crucial role in the degree of biodegradation of plastics. The amorphous polymer is degraded easily in contrast to crystalline polymer.

Polymers with high melting temperatures are difficult to biodegrade. Polycaprolactone with higher molecular weight was degraded slowly by *Rhizopus delemar* lipase (endo-cleavage type) than that with low molecular weight (Tokiwa and Suzuki 1978). The various factors affecting the biodegradation of plastics and polythenes may be enumerated as composition that defines its chemical and physical properties; concentration; abiotic factors like temperature, salinity, presence of water, etc.; and biotic factors which include the composition of microbial community.

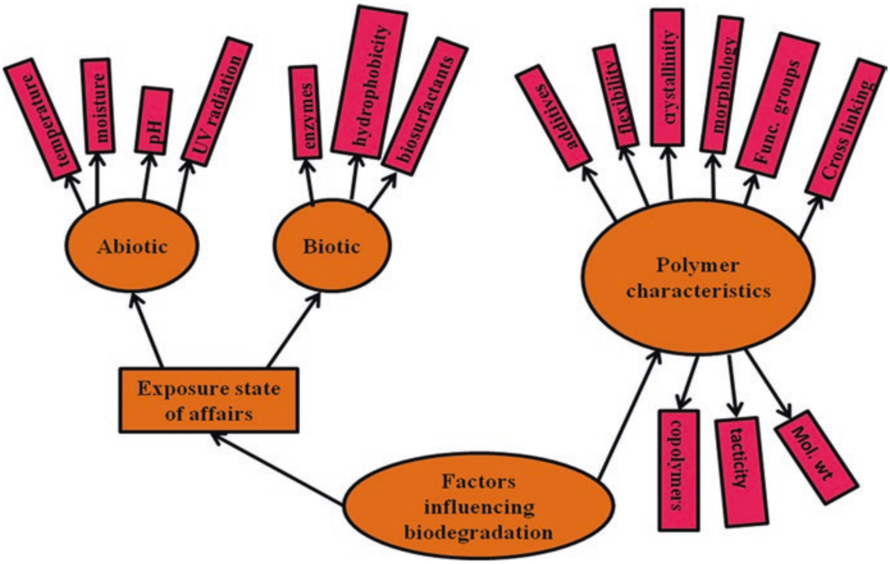

Fig. 6.2 Various factors affecting the biodegradation of plastics and polythenes

The knowledge composition of the hydrocarbons is very crucial for the rate of degradation of the plastics and polythenes (Gajendiran et al. 2016). The hydrocarbons present in the plastics and polythenes are of different types and have been placed in the decreasing order of vulnerability to biodegradation. The n-alkanes are the most susceptible ones for biodegradation by the microbes than the branched alkanes followed by low molecular weight aromatics and the cyclic alkanes, with high molecular weight aromatics and polar compounds being extremely resistant. Nevertheless the order of biodegradation can vary under some conditions depending upon the composition of microbial community or the abiotic factors. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus, and in some cases iron. The rate of biodegradation of the plastics follows the Michaelis-Menten kinetics (Chinaglia et al. 2018).

The microbial populations can degrade the substrates depending upon concentration of the hydrocarbons. The microbial degradation of high molecular weight hydrocarbons, such as long (>C12) alkanes with solubility less than 0.01 mg/l, occurs at rates that exceed the rates of their dissolution and are a function of the hydrocarbon surface area available for emulsification or physical attachment by cells and therefore do not display the dependence on concentration. At lower temperatures, the speed of plastic biodegradation generally decreases (Tokiwa et al. 2009). This may be accounted due to the decrease in enzymatic activity at lower temperatures. The rates of hydrocarbon metabolism are increased by higher temperatures, the optimum range being 30–40 °C. The oxidation of the substrate by oxygenases is the prerequisite in the biodegradation process of plastics by bacteria and fungi. Scarcity of nitrogen and phosphorus inhibits microbial plastic degradation in the environment, so for the process of biodegradation of plastics, the nitrogen and phosphorus concentration should be high in the environment (Ong et al. 2017).

The presence of salinity is inversely related with the biodegradation of plastics as the high salinity decreases the existence of microbes (Le Borgne et al. 2008). It was witnessed that rates of hydrocarbon consumption start to reduce when the salinity is between 3.3% and 28.4%. Crude oil-degrading *Streptomyces albiaxialis* and an n-alkane (C10–C30)-degrading member of the *Halobacterium* group however are capable of biodegrading plastics at high salinity. The effect of pressure on biodegradation of hydrocarbons is also observed (Schedler et al. 2014). It was seen that microorganisms degrade the plastic at the bottom of the sea or ocean extremely slowly, and so they continue to stay there for years and years altogether.

Most favorable rates of biodegradation of hydrocarbons are witnessed at 30–90% of water availability (Sudhakar et al. 2007). Thus the biodegradation of hydrocarbon in terrestrial ecosystems may be restricted by the amount of available water. The bacterial degradation is increased in the moist conditions, thus increasing the evolution of gas. Most of the bacteria and fungi involved in biodegradation prefer neutral pH (Siddique et al. 2002), and most of the times, the fungi are seen to be more tolerant to acidic conditions. Bacteria and fungi are the pioneers in biodegradation of hydrocarbons in the environment, and although the algae and protozoa both belong to the community of microorganisms of aquatic and terrestrial ecosystems, their role in hydrocarbon biodegradation is still a mystery.

6.7 Different Steps of Plastic Degradation by Microorganisms

The polymer compounds are broken down by the microorganisms into simpler forms by the help of biochemical reactions. Biodegradation of polymer is carried by digestion by microbial enzymes leading to decrease in molecular weight, loss of properties like mechanical strength, etc. Microorganisms produce catalytic enzymes for the purpose of biodegradation (Nigam 2013). This proves to be very instrumental in environmental waste management. Different enzymatic reactions of the microbial enzymes and breaking of bonds result into biodegradation. Sequential degradation steps are involved in the biodegradation process like bio-fragmentation by enzymatic cleavage and assimilation of the products by microorganisms and mineralization which involves formation of oxidized metabolites that is shown in Fig. 6.3. Both aerobic and anaerobic conditions are essential for mineralization of polymers. CO_2 and H_2O are the products of aerobic oxidation, while CH_4, CO_2, and H_2O are produced under anaerobic conditions (Singh and Sharma 2008).

Different steps involved in the plastic biodegradation process are:

1. *Biodeterioration*: Decomposing microbial communities are accountable for the physical and chemical degradation which results in modification of different properties of plastics like mechanical, physical, and chemical properties. Most of the times, abiotic parameters are useful either as a synergistic factor or to initiate

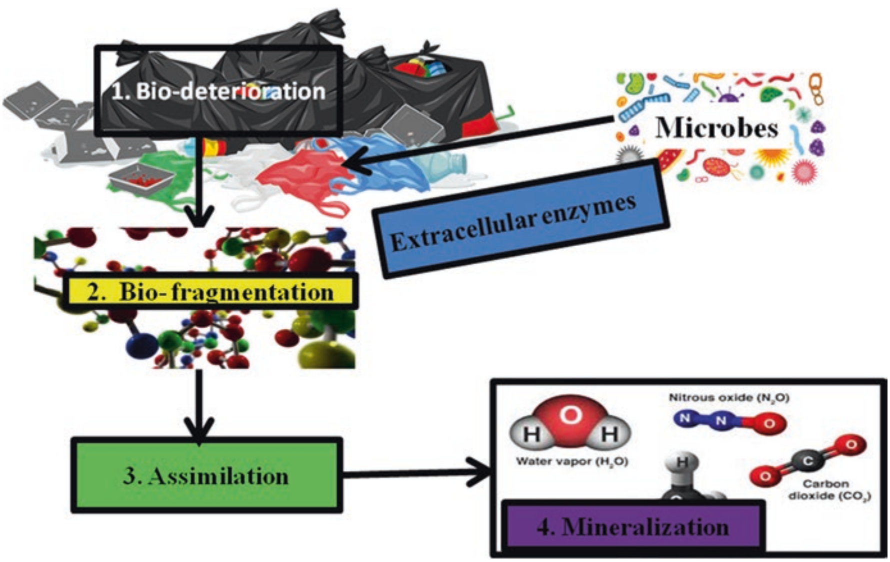

Fig. 6.3 Different steps involved in plastic biodegradation

the biodegradation process and deteriorate the polymeric structure (Helbling et al. 2006; Ipekoglu et al. 2007). The formation of a microbial biofilm on the surface aggravates biodeterioration.

2. *Bio-fragmentation*: It is the catalytic events where enzymes secreted by microorganisms cleave the plastics into monomers. Microorganisms secrete extracellular enzymes which catalyze reactions at the margins of the plastic polymer. The bacteria that are capable of degrading the plastics generally contain oxygenase enzyme which can add oxygen to a long carbon chain making it susceptible to cleavage. Post-formation of carboxylic groups, the lipases and esterases result in further degradation of the substrates.
3. *Assimilation*: Only those monomers which can be assimilated by the microbes, which are able to cross the cell wall. Those monomers which use particular carriers to cross the cell wall are able to get assimilated; others are left behind as they are. The assimilation produces a number of secondary metabolites which is carried out of cell to be utilized for further degradation. The absolute degradation of primary and secondary metabolites results in production of CO_2, N_2, CH_4, H_2O, etc. which is better known as mineralization of metabolites.
4. *Mineralization*: The final step in the process of biodegradation is the absolute degradation of molecules of the products of biodegradation that results in release of CO_2, N_2, CH_4, H_2O, etc.

6.8 Enzymes Involved in Biodegradation of Plastics and Polythenes

The biodegradation of polyethylene requires the set of microbial enzymes capable of degrading lignin in plant cells, which is a heterogeneous cross-linked phenolic polymer (Restrepo-Flórez et al. 2014). These microbial enzymes comprise of laccases, manganese peroxidase, and lignin peroxidases. It was found that, a copper-binding thermozyme, laccase obtained from *Rhodococcus ruber* C208, was very effective in degradation of the UV-treated polyethylene films in the presence of copper (Sivan et al. 2006). A laccase from *Trametes versicolor* in the presence of 1-hydroxybenzotriazole was instrumental in oxidation of non-phenolic substrates to degrade polyethylene membrane (Fujisawa et al. 2001).

The key enzyme involved in the degradation of a high molecular weight PE membrane is named as manganese peroxidase (MnP) which has been obtained from the white rot fungus *Phanerochaete chrysosporium* ME-446 and isolate IZU-154 (Iiyoshi et al. 1998). Extracellular laccases as well as MnP produced from *Bacillus cereus* were found to degrade UV-irradiated polyethylene (Sowmya et al. 2014). Seventy percent of a preoxidized high molecular weight PE was found to be degraded by lignin peroxidase and MnP that were produced by *Phanerochaete chrysosporium* MTCC-787 (Mukherjee and Kundu 2014).

Terminal or subterminal oxidation of hydrocarbon oligomers by alkane hydroxylases can catalyze the degradation (Rojo 2010). Alkane hydrolases, alkane monooxygenase, rubredoxin, and rubredoxin reductase from *Pseudomonas aeruginosa* E7 were very instrumental in degrading polyethylenes (Jeon and Kim 2015). It has been witnessed that whole cells rather than the isolated enzymes have more potential for biodegradation of plastic and polythenes. A number of lipases, esterases, and cutinases from fungal and actinomycetes species hydrolyze amorphous PET and modify the surface of PET films and fibers (Zimmermann and Billig 2011). Polyethylene terephthalate (PET) fibers were seen to be partially degraded by carboxylesterases obtained from *Bacillus licheniformis*, *Bacillus subtilis*, and *Thermobifida fusca* (Barth et al. 2016). The most active fungal polyester hydrolase is cutinase HiC, which is thermostable and is obtained from *Thermomyces* (formerly *Humicola*) *insolens* (Ronkvist et al. 2009).

There is a plethora of microbial enzymes for the degradation of the plastic which are reflected in Fig. 6.4. Some examples are alkane hydroxylase obtained from *Pseudomonas aeruginosa* for biodegradation of polyethylene (Jeon and Kim 2015), lipases (Bhardwaj et al. 2012), laccase (*Rhodococcus ruber*), lignin- and manganese-dependent peroxidase (LiP and MnP), serine hydrolases, esterases, polyurethanases, heme peroxidase (lignin peroxidase), protease, etc. The enzyme such as the oxygenase increases the solubility of plastic polymer, rendering them more readily degradable by bacteria. Microbial lipases and esterases specifically attack carboxylic groups and amine group's endopeptidases. The humification of various phenolic substances produced from the decomposition of lignin is carried by the

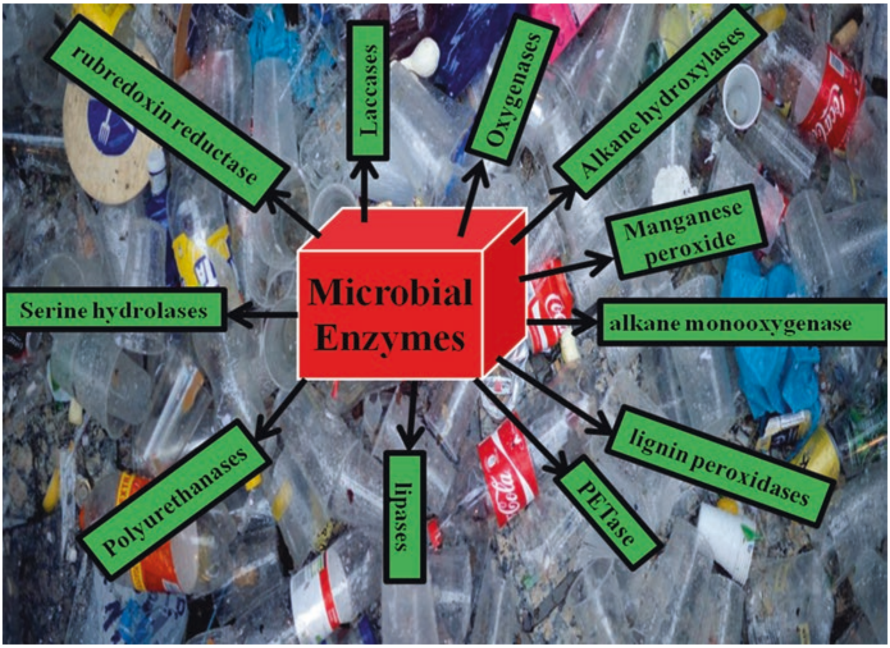

Fig. 6.4 Microbial enzymes used in biodegradation of plastics and polythenes

oxidoreductases. Different kinds of enzymes like laccase, manganese peroxidase, and lignin peroxidase are the main weapons of the fungal population that they use for biodegradation of the plastics and polythenes. The main extracellular oxidoreductase enzyme is the chief enzyme which helps the fungi in the process of biodegradation. The monooxygenases oxidize substrates ranging from alkanes to complex endogenous molecules such as steroids and fatty acids.

6.9 Conclusion

Synthetic or man-made plastics can be considered as xenobiotics. They are very extensively used materials in our day-to-day life and are omnipresent. They have become a matter of ponderance as they clog the various sites of the environment. They are so recalcitrant and nondegradable that it has become a burning issue as to how to get rid of them. They are causing a great environmental havoc, and there is an urgent need to address the problem of accumulation of the plastics and polythenes in the nature. The different methods of degradation of the synthetic polymers have been researched on, be it physical methods, chemical methods, as well as microbial methods. Out of these we found that the participation of the microbes has come up with wonderful results for biodegradation of plastics and polymers.

Pseudomonas which is the member of metabolically active species shows special performance in the biodegradation of synthetic polymers due to their capability to degrade as well as metabolize the synthetic substrates. Microbial species isolated from different ecological areas have been witnessed to degrade polyethylene, polyethylene terephthalate, polypropylene, polyethylene glycol, polystyrene, polyurethane, polyethylene succinate, polyvinyl alcohol, and polyvinyl chloride. Spotlight of research should be on the safe disposal of most common constituent of the plastics and polythenes like polyethylene, polypropylene, polyurethane, polystyrene, etc. These polymers are also long-lasting as well as the most durable plastics. Considering these problems, different types of strategies have been suggested to come to the rescue of the problem of safe waste disposal. The most efficient technique to overcome the problem is the biodegradation of plastic by enzymes obtained from the microbes. Lots and lots of research activities have been started on a large scale be it at international level or national level to overcome the problem of pollution that is being caused by the plastics.

The mechanism of biodegradation of the synthetic products by the microbial enzymes has been studied and employed, and more research in this field is being carried. The study of molecular mechanisms involved in the process of biodegradation like bio-fragmentation, bio-assimilation, and bio-mineralization is still in their infancy, and thus a lot of investigations are still on their way to explore these phenomenons.

There is a diverse population of microbes involved in the process of biodegradation which are very efficient in producing different types of enzymes for the biodegradation of recalcitrant substrates. *R. ruber*, *C. thermocellum*, *P. aeruginosa*, *P.*

stutzeri, *S. badius*, *S. setonii*, *C. acidovorans*, etc. some of them are the chief bacterial spp. related with the biodegradation of polymers. *P. aeruginosa* is one of the most studied microorganisms for polymer. Low-density polyethylenes are degraded very efficiently by *P. aeruginosa* CA9. Hydrolyzing enzymes produced by *Rhodococcus ruber* have been shown to degrade polyethylene by formation of a biofilm. Microbial peroxidases have also been found to enhance the polyethylene biodegradation by introducing in vitro thermal and photochemical mineralization.

Hence, further future studies on the screening of effective microbial strains which produce an array of hydrolytic enzymes are essential to eliminate the plastic pollution from the environment. The future prospects for a cleaner and healthy environment will lie on the product of only biodegradable products in the time to come, but our focus should be to get rid of the pile of tough plastics and polymers that have previously been designed and produced by exploring new enzymes and new microorganisms.

References

Alshehrei F (2017) Biodegradation of low density polyethylene by fungi isolated from Red Sea water. Int J Curr Microbiol App Sci 6(8):1703–1709

Álvarez-Barragán J, Domínguez-Malfavón L, Vargas-Suárez M, González-Hernández R et al (2016) Biodegradative activities of selected environmental fungi on a polyester polyurethane varnish and polyether polyurethane foams. Appl Environ Microbiol 82:5225–5235

Anastasi A, Tigini V, Varese GC (2013) The bioremediation potential of different ecophysiological groups of fungi, Fungi as Bioremediators, soil biology, vol 32. Springer, Berlin

Austin HP, Allen MD, Donohoe BS, Rorrer NA, Kearns FL, Silveira RL, Pollard BC, Dominick G, Duman R, El Omari K, Mykhaylyk V, Wagner A, Michener WE, Amore A, Skaf MS, Crowley MF, Thorne AW, Johnson CW, Woodcock HL, McGeehan JE, Beckham GT (2018) Characterization and engineering of a plastic-degrading aromatic polyesterase. Proc Natl Acad Sci U S A 115:E4350–E4357

Barth M, Honak A, Oeser T, Wei R, Belisario-Ferrari MR, Then J et al (2016) A dual enzyme system composed of a polyester hydrolase and a carboxylesterase enhances the biocatalytic degradation of polyethylene terephthalate films. Biotechnol J 11:1082–1087

Bhardwaj H, Gupta R, Tiwari A (2012) Microbial population associated with plastic degradation. Sci Rep 5:272–274

Bhardwaj H, Gupta R, Tiwari A (2013) Communities of microbial enzymes associated with biodegradation of plastics. J Polym Environ 21:575–579

Dang TCH et al (2018) Plastic degradation by thermophilic Bacillus sp. BCBT21 isolated from composting agricultural residual in Vietnam. Adv Nat Sci: Nanosci Nanotechnol 9(1):015014. https://doi.org/10.1088/2043-6254/aaabaf

Chinaglia S, Maurizio T, Degli-Innocenti F (2018) Biodegradation rate of biodegradable plastics at molecular level. Polym Degrad Stab 147:237–244

Datta PK, Mishra K, Kumar MNVR (1998) Popular plastics and packaging. Mahindra Publishers, New Delhi, p 73

Fujisawa M, Hirai H, Nishida T (2001) Degradation of polyethylene and nylon-66 by the laccase-mediator system. J Polym Environ 9:103–108

Gajendiran A, Krishnamoorthy S, Abraham J (2016) Microbial degradation of low-density polyethylene (LDPE) by *Aspergillus clavatus* strain JASK1 isolated from landfill soil. 3 Biotech 6(1):52

Geyer R, Jambeck JR, Law KL (2017) Production, use, and fate of all plastics ever made. Sci Adv 3:e1700782
Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3(1):1–18
Helbling C, Abanilla M, Lee L, Karbhari VM (2006) Issues of variability and durability under synergistic exposure conditions related to advanced polymer composites in civil infrastructure. Compos Part A Appl Sci Manuf 37(8):1102–1110
Iiyoshi Y, Tsutsumi Y, Nishida T (1998) Polyethylene degradation by lignin-degrading fungi and manganese peroxidase. J Wood Sci 44(3):222–229
Ipekoglu B, Böke H, Cizer O (2007) Assessment of material use in relation to climate in historical buildings. Build Environ 42:970–978
Jeon HJ, Kim MN (2015) Functional analysis of alkane hydroxylase system derived from Pseudomonas aeruginosa E7 for low molecular weight polyethylene biodegradation. Int Biodeterior Biodegrad 103:141–146
Kathiresan K (2003) Polythene and plastics-degrading microbes from the mangrove soil. Rev Biol Trop 51:3–4
Khan S, Nadir S, Shah ZU, Shah AA, Karunarathna SC, Xu J, Khan A, Munir S, Hasan F (2017) Biodegradation of polyester polyurethane by Aspergillus tubingensis. Environ Pollut 225:469–480
Kumar AA, Karthick K, Arumugam KP (2011) Biodegradable polymers and its applications. Int J Biosci Biochem Bioinforma 1(3):173–176
Le Borgne S, Paniagua D, Vazquez-Duhalt R (2008) Biodegradation of organic pollutants by halophilic bacteria and archaea. J Mol Microbiol Biotechnol 15:74–92
Lee B, Pometto AL, Fratzke A, Bailey TB (1991) Biodegradation of degradable plastic polyethylene by phanerochaete and streptomyces species. Appl Environ Microbiol 57:678–685
Mahdiyah D, Mukti BH (2013) Isolation of polyethylene plastic degrading-bacteria. Biosci Int 2:29–32
Mukherjee S, Kundu PP (2014) Alkaline fungal degradation of oxidized polyethylene in black liquor: studies on the effect of lignin peroxidases and manganese peroxidases. J Appl Polym Sci 131:40738
Nigam PS (2013) Microbial enzymes with special characteristics for biotechnological applications. Biomol Ther 3(3):597–611
Oda Y, Oida N, Urakami T, Tonomura K (1997) Polycaprolactone depolymerase produced by the bacterium *Alcaligenes faecalis*. FEMS Microbiol Lett 152:339–343
Odusanya SA, Nkwogu JV, Alu N, Udo GE, Ajao JA, Osinkolu GA, Uzomah AC (2013) Preliminary studies on microbial degradation of plastics used in packaging potable water in Nigeria. Niger Food J 31(2):63–72
Ojha N, Pradhan N, Singh S, Barla A, Shrivastava A, Khatua P, Rai V, Bose S (2017) Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization. Sci Rep 7:39515
Ong SY, Chee JY, Sudesh K (2017) Degradation of Polyhydroxyalkanoate (PHA): a review. J SibFU 10(2):211
Pramila R, Vijaya Ramesh K (2012) Biodegradation of low density polyethylene (LDPE) by fungi isolated from municipal landfill area. J Microbiol Biotechnol Res 1(4):131–136
Raaman N, Rajitha N, Jayshree A, Raman J (2012) Biodegradation of plastic by *Aspergillus* spp. isolated from polythene polluted sites around Chennai. J Acad Ind Res 1:313–316
Restrepo-Florez JM, Bassi A, Thompson MR (2014) Microbial degradation and deterioration of polyethylene – a review. Int Biodeterior Biodegrad 88:83–90
Rojo F (2010) Enzymes for aerobic degradation of alkanes. In: Handbook of hydrocarbon and lipid microbiology, 2, 781–797, https://doi.org/10.1007/978-3-540-77587-4_59
Ronkvist ASM, Xie W, Lu W, Gross RA (2009) Cutinase-catalyzed hydrolysis of poly(ethylene terephthalate). Macromolecules 42:5128–5138

Russell JR, Huang J, Anand P, Kucera K, Sandoval AG, Dantzler KW, Hickman D, Jee J, Kimovec FM, Koppstein D, Marks DH, Mittermiller PA, Nunez SJ, Santiago M, Townes MA, Vishnevetsky M, Williams NE, Vargas MP, Boulanger LA, Bascom-Slack C, Strobel SA (2011) Biodegradation of polyester polyurethane by endophytic fungi. Appl Environ Microbiol 77:6076–6084

Schedler M, Hiessl R, Valladares Juárez AG, Gust G, Müller R (2014) Effect of high pressure on hydrocarbon-degrading bacteria. AMB Express 4:77

Shah AA, Fariha H (2008) Biological degradation of plastics: a comprehensive review. Biotechnol Adv 26:246–265

Siddique T, Okeke BC, Arshad M, Frankenberger WT (2002) Temperature and pH effects on biodegradation of hexachlorocyclohexane isomers in water and a soil slurry. J Agric Food Chem 50:5070–5076

Singh J, Gupta K (2014) Screening and identification of low density polyethylene (LDPE) degrading soil fungi isolated from polythene polluted sites around Gwalior city (MP). Int Curr Microbiol Appl Sci 3:443–448

Singh B, Sharma N (2008) Mechanistic implications of plastic degradation. Polym Degrad Stab 93:561–584

Sivan A, Szanto M, Pavlov V (2006) Biofilm development of the polyethylene-degrading bacterium *Rhodococcus ruber*. Appl Microbiol Biotechnol 72:346–352

Song JH, Murphy RJ, Narayan R, Davies GBH (2009) Biodegradable and compostable alternatives to conventional plastics. Philos Trans R Soc Biol 364:2127–2139

Sowmya HV, Ramalingappa MK, Thippeswamy B (2014) Biodegradation of polyethylene by *Bacillus cereus*. Adv Polym Sci Technol-Int J 4:28–32

Sudhakar M, Trishul A, Doble M, Suresh Kumar K, Syed Jahan S et al (2007) Biofouling and biodegradation of polyolefins in ocean waters. Polym Degrad Stab 92:1743–1752

Teuten EL, Saquing JM, Knappe DRU, Barlaz MA, Jonsson S, Björn A et al (2009) Transport and release of chemicals from plastics to the environment and to wildlife. Philos Trans R Soc Lond Ser B Biol Sci 364(1526):2027–2045

Tokiwa Y, Suzuki T (1978) Hydrolysis of polyesters by *Rhizopus delemar* lipase. Agric Biol Chem 42:1071–1072

Tokiwa Y, Calabia BP, Ugwu CU, Aiba S (2009) Biodegradability of plastics. Int J Mol Sci 10(9):3722–3742

Vignesh R, Deepika RC, Manigandan P, Janani R (2016) Screening of plastic degrading microbes from various dumped soil samples. Int Res J Eng Tech 3(4):2493–2498

Webb JS, Van der Mei HC, Nixon M, Eastwood IM, Greenhalgh M, Read SJ, Robson GD, Handley PS (1999) Plasticizers increase adhesion of the deteriogenic fungus *Aureobasidium pullulans* to PVC. Appl Environ Microbiol 65:3575–3581

Yamada-Onodera K, Mukumoto H, Katsuyaya Y, Saiganji A, Tani Y (2001) Degradation of polyethylene by a fungus, *Penicillium simplicissimum* YK. Polym Degrad Stab 72:323–327

Yang J, Yang Y, Wu W-M, Zhao J, Jiang L (2014) Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. Environ Sci Technol 48:13776–13784

Yoshida S, Hiraga K, Talehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). Science 351(6278):1196–1199

Zimmermann W, Billig S (2011) Enzymes for the biofunctionalization of poly(ethylene terephthalate). Adv Biochem Eng Biotechnol 125:97–120