

Microbial degradation of plastic: a review

Swapnil K. Kale, Amit G. Deshmukh*, Mahendra S. Dudhare, Vikram B. Patil

Received: 28 May 2015 / Received in revised form: 29 September 2015, Accepted: 04 November 2015,
Published online: 25 December 2015 © Biochemical Technology Society 2014-2015

Abstract

Since last few decades the uncontrolled use of plastics for various purposes such as packaging, transportation, industry and agriculture in rural as well as urban areas, has elevated serious issue of plastic waste disposal and its pollution. The efficient decomposition of plastic bags takes about 1000 years. Plastic causes pollution and global warming not only because of increase in the problem of waste disposal and land filling but also release CO₂ and dioxins due to burning. Commonly used methods for plastic disposal were proved to be inadequate for effective plastic waste management, and hence there is growing concern for use of efficient microorganisms meant for biodegradation of non-degradable synthetic polymer. The biodegradable polymers are designed to degrade fast by microbes due their ability to degrade the most of the organic and inorganic materials, including lignin, starch, cellulose and hemicelluloses. The present review discusses the current status, mechanisms of biodegradation of plastics, techniques for characterizing degraded plastics and factors affecting their biodegradation.

Keywords: plastic degradation, microbial degradation, methods of degradation study, analysis of degradation

Introduction

The word plastic comes from the Greek word *plastikos*, which means 'able to be molded into varied shapes (Joel 1995). Plastic is defined as the polymer which become mobile on heating and thus

Swapnil K. Kale, Amit G. Deshmukh*,

Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh
Agricultural University, Akola- 444 104 India

*Tel: +91-9028493243; *E-mail: agd4in@yahoo.com

Mahendra S. Dudhare

Vasandrao Naik College of Agricultural Biotechnology, Yavatmal-
445 001, India

Vikram B. Patil

Yeshwant College, SRTMU, Nanded- 431 602, India

can be cast into moulds. The plastic is made up of carbon, hydrogen, silicon, oxygen, chloride and nitrogen. For extraction of the basic materials of plastics oil, coal and natural gas are used (Seymour 1989). Plastics are made up of linking of monomers together by chemical bonds. Polythene comprises of 64% of total plastic, which is a linear hydrocarbon polymers consisting of long chains of the ethylene monomers (C₂H₄). General formula of polyethylene is C_nH_{2n}, where 'n' is the number of carbon atoms (Sangale et al. 2012).

Recalcitrant nature of plastic is due to its high molecular weight, complex three-dimensional structure, and hydrophobic nature, all of them hampers its availability to microorganisms (Hadad et al. 2005). Plastics include polythene, propylene, polystyrene, polyurethane, nylon etc. Polyethylene either LDPE (low density polyethylene) or HDPE (high density polyethylene) is a thermoplastic polymer made by monomers of ethylene, used mostly as thin films and packaging sheets (Kumar et al. 2013). Among these LDPE materials are strong, light-weight and durable thus are having wide uses.

From last three decades uncontrolled use of the plastics for packaging (e.g. fast food), transportation, industry and agriculture in rural as well as urban areas, has elevated serious issue of plastic waste disposal and its pollution. Light-weight, inertness, durability, strongness and low cost are the main advantages of plastic while it has disadvantages such as, it is recalcitrant to biodegradation and difficult to degrade naturally. The global use of plastic is growing at a rate of 12% per year and around 0.15 billion tones of synthetic polymers are produced worldwide every year (Premraj and Doble 2005; Leja and Lewandowicz 2010; Das and Kumar 2014). Accumulation rate of plastic waste in the environment is 25 million tons/year (Orhan and Buyukgungor 2000; Nayak and Tiwari 2011; Baruah 2011; Kaseem et al. 2012) and is consequently considered a serious environmental danger (Sivan et al. 2006; Thompson 2004). Plastic are estimated to be 20% munciple solid waste (MSW) in United States and Germany (Leja and Lewandowicz 2010), 7.5% of MSW in Western Europe and 25% in Australia. In Turkey, 11 million tons of plastic were disposed per year (Orhan et al. 2004). In the year 1999-2000, India imported

more than 120,000 tons of plastic (Tiwari et al. 2009). Annually, India generates 5.6 million metric tonnes of plastic waste with Delhi accounting for a shocking 689.5 metric tonnes per day. According to Central Pollution Control Board (CPCB) of India, total plastic waste which is collected and recycled in the country is likely to be 9,205 tonnes per day (approximately 60% of total plastic waste) and 6,137 tonnes remain uncollected and littered. Major offender in generating such waste are four metros with Delhi contributing 689.5 tonnes a day, followed by Chennai (429.4 tonnes), Kolkata (425.7 tonnes) and Mumbai (408.3 tonnes). The figures only serve to confirm the common areas of masses of plastic in industrial, residential and slum areas of Indian cities and towns (CPCB Annual report 2011-12). The efficient decomposition of plastic bags takes about 1000 years (Pramila and Vijaya Ramesh 2011; Usha et al. 2011). Plastic causes pollution and global warming not only because of increase in the problem of waste disposal and land filling but also release CO₂ and dioxins due to burning (Ali et al. 2009). The burning of waste plastic material produces toxic gases posing health hazard by causing lung diseases and cancer after inhalation (Pramila and Vijaya Ramesh 2011). Undisposed, improperly disposed or used plastic packing material & bags do not let water and air to go into earth which causes depletion of underground water source and danger to animal life. Additionally, plastic degraded by sunlight into smaller toxic parts contaminating soil and water where they can be ingested by animals and hence enters the food chain especially in the aquatic animal. For aquatic biota like mammals, sea turtles and seabirds, polythene waste is considered as a main risk that causes intestinal blockage when ingested unintentionally (Spear et al. 1995; Secchi & Zarzur 1999; Denuncio et al. 2011). It also affects soil fertility, preventing degradation of other normal substances, which poses threat to whole world. The biodegradable polymers are designed to degrade quickly by the microbes due to their ability to degrade the majority of the organic and inorganic materials, including lignin, starch, cellulose and hemicelluloses (Kumar et al. 2013). The problem of waste can be solved to some extent by using biodegradable plastics; consequently there is growing attention in degradable plastics. Starch-based degradable plastics is most commonly suggested for uses in composting of lawn, garden and shrub litter, which could lessen the volume of material entering the landfills by up to 20% (Lee et al. 1991). Attention in using biodegradable plastics for packaging, medical, agricultural and fisheries applications has increased in last decades (Orhan et al. 2004; Leja and Lewandowicz 2010). However, none of biodegradable of plastics was efficiently biodegradable in landfills, therefore none of the products has gained extensive use (Anonymous 1999; Kathiresan 2003).

Table 1: Overview of Methods for study of biodegradation

	Field (In vivo)	Simulation	Laboratory (In vitro)
Process	a. Burying plastics samples in soil. b. Placing it in a lake or river	Burying plastics samples in compost, soil or sea-water placed in a controlled (temperature, pH, humidity) condition.	Defined media inoculated with mixed microbial population (e.g., from waste water) or individual microbial strains or enzymes which may have been especially screened for a particular polymer.
Advantages	Most easy & Widely used	<ul style="list-style-type: none"> • Practically most suitable; • Better analytical tools available than would be used for field tests. 	<ol style="list-style-type: none"> 1. Faster rate of degradation than under natural conditions. 2. Preferred for many systematic investigations.
Disadvantages	a. Environmental conditions cannot be well controlled. b. Analytical opportunities to monitor the degradation process are limited.	Lacks reproducibility due to variable microbial population	This method cannot be used to prove biodegradation in terms of metabolism by a microorganism

Available methods for study of plastic biodegradation

Any physical or chemical change in polymer are due to environmental factors such as light, heat, moisture, chemical conditions and biological activity is termed as degradation of plastic. Decomposition or destruction of contaminant molecules by the action of the enzyme secreted by microorganisms is known as biodegradation. General representation of microbial degradation of plastic is given in figure 1, and overviews of methods for plastic biodegradation are given in Table 1.

Factor affecting Plastic Degradation

Environmental parameters such as humidity, temperature, pH, salinity, the presence or absence of oxygen, sunlight, water, stress and culture conditions not only affect the polymer degradation, but also have a crucial influence on the microbial population and enzyme activity (Gu 2003). Maximum CO₂ evolution and optimal lignolytic activity occurred when fungi grow at lowest pH (Glass and Swift 1990). The chemical and physical properties of polyester have a strong influence on its biodegradability. Molecular weight (*M_n*) is one of the factors determining the biodegradation of plastics. Low molecular weight is favorable for biodegradation. The rate of enzymatic hydrolysis of poly (ε-caprolactone) PCL diol by *Rhizopus delemar* lipase was faster at the smaller molecular weight (Tokiwa & Suzuki 1977). The melting temperature (*T_m*) of a polymer has a great effect on enzymatic degradability. Generally, the higher the melting point of polyester, the lower the biodegradability tends to be. The enzymatic degradability decreases with increasing time. The higher order structure properties like crystallinity and modulus of elasticity, suppressed the polymer degradability (Tokiwa and Calabia 2004). Additives, antioxidants and stabilizers used in manufacturing of polymer can slow down the rate of degradation and may be toxic to microorganisms (Arutchelvi et al. 2008). Besides all above mentioned factor structural (linearity and branching in polymer, type of bond like c-c, amide & ester), molecular composition and physical form of polymer like powder, films, pellets and fibres may also influences the biodegradability polymer. Ultimately, the way and rate of polymer degradation depends on the mechanism of degradation and acceleration of process.

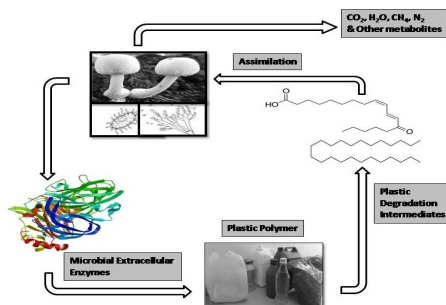


Figure 1: General representation of microbial degradation of plastic

Plastic Degrading Microbes

Commonly used methods for plastic disposal such as land-filling, incineration and recycling were proved to be inadequate for effective plastic waste management, and hence there is growing concern for use of efficient microorganisms meant for biodegradation of recalcitrant synthetic polymer biodegradation using efficient microorganisms (Seneviratne et al. 2006).

The potential of polyethylenes degrading microbes had been curiosity since 1961 when Fuhs (1961) reported that several microorganisms can consume paraffin as a carbon source. Plastic degrading microbes were isolated from a variety of resources such as rhizosphere soil of mangroves, polythene buried in the soil, marine water, plastic and soil at the dumping sites. Bacteria, fungi and algae are the biological factor that degrades plastic naturally (Rutkowska et al. 2002). Bacterial degradation of polyethylene and

paraffins compared and found that bacteria utilized polyethylene as carbon source (Jen-hou and Schwartz 1961). *Mucor circinilloides* and *Aspergillus flavus* isolated from municipal landfill area, showed promising degradation of low density polythene degradation by analyzing CO₂ evolution test, scanning electron microscopy (SEM) and colonization studies (Pramila and Vijaya Ramesh 2011). In vitro degradation of LDPE by using six strains of *Aspergillus* spp. and two *Fusarium* spp., revealed that plastic biodegradation were due to active enzymes produced by this fungal strain (Kumar et al. 2013). Polythene and plastic degraded to various extent by *Pseudomonas* spp. (37.09% and 28.42%) *Streptomyces* spp. (46.16% and 35.78%) and *Aspergillus* spp. (20.96% and 16.84%) in 6 month period in liquid (shaker) culture (Usha et al. 2011). Degradation of plastic cups and polythene bags studied using bacteria and fungi for one month period, among which bacteria, *Pseudomonas* spp. degraded 20.54% of polythene and 8.16% of plastics, while fungal species, *Aspergillus glaucus* degraded 28.80% of polythene and 7.26% of plastics (Kathiresan 2003). Summary of some studies on biodegradation of plastics are given in Table 2. The capacity of some fungal strains isolated from plastic contaminated site was evaluated for ability to grow on polyurethane and urease, protease, esterase and laccase activity (Loredo-Trevino et al. 2011).

Brevibaccillus borstelensis and *Rhodococcus ruber* were reported to have capacity to degrade the CH₂ backbone and use polyethylene as its sole carbon source (Hadad et al. 2005). Fungal strains like, *Mucor rouxii* NRRL 1835 and *Aspergillus flavus* (El-Shafei et al. 1998), *Penicillium simplicissimum* YK

Table 2: Summary of studies on microbial degradation of plastic (blended and non-blended)

Sr No	Plastic used	Microorganisms/ Culture method	Evaluation of biodegradation (Technique / Observation)	Results achieved/ Maximum Degradation Achieved	Reference
1	Cellulose blended PVC films	<i>Phanerochaete chrysosporium</i> PV1 Soil burial treatment and shake flask treatment	FTIR (Visual changes in the polymer). Plate assay and CO ₂ production	The surface of the PVC Cellulose blend film were discolored with, some cracks and film erosion was also visible. FTIR confirmed the shortening of peaks was due to degradation of the polymer. The CO ₂ (10.21g/L) produced after 30 days showed significant degradation of the polymer.	Ali et al. 2009
2	Copolyesters films	Composting using raw material for mushroom cultivation. Agar plate cultivation (<i>Pleurotus Ostreatus</i> strains, <i>Inonotus hispidus</i> , <i>Phanerochaete chrysosporium</i> , <i>Irpex lacteus</i> , <i>Agaricus bisporus</i> , <i>Stropharia rugosoannulata</i> , <i>Polyporus squamosus</i>) Composting under controlled conditions	SEM, Gel Permeation Chromatography (GPC). Reduction in viscosity	SEM study revealed <i>I. hispidus</i> , caused filament perforation and its partial destruction amongst various fungi studied. The significant decrease of reduced viscosity during degradation indicated active participation of hydrolysis in polymer backbone cleavage. Intensive degradation of aromatic-aliphatic copolyesters were observed.	Sasek et al. 2006
3	Emulsified poly-(butylenes succinate) (PBS) and emulsified poly-(butylenes succinate-co adipate) (PBSA)	<i>Aspergillus oryzae</i> RIB40 (ATCC-42149) Shake flask treatment	Clearing of the culture supernatant.	The PBA degrading enzyme was identified as cutinase. Butyric acid and n-hexanol were observed to be most preferred substrate for biodegradable plastic degrading enzyme.	Maeda et al. 2005
4	Poly-3-hydroxyalkanoates (PHA), Poly-b-hydroxybutyrate (PHB) i.e. BIOPOL™	134 pure strains of marine fungi or marine fungal isolates (Culture collection Botanical Institute, University of Regensburg). <i>Penicillium simplicissimum</i> (IMI 300465) as reference culture Agar Plate assay	Clear zone formed in the turbid medium in agar plates or the depth of clearing	Out of 32 strains of marine yeasts and 102 strains of marine fungi, only about 4% of the strains degraded BIOPOLTM and about 6% depolymerised pure PHB homopolymer.	Matavuly and Molitoris 2009

5	PHB (poly-3-hydroxybutyric acid)	Fungi isolated from lichen in natural habitats and soil compost. (<i>Aspergillus fumigates</i> , <i>Penicillium</i> spp., Protozoa, lichens) Agar plate cultivation	Clear zone in test medium	From 15 natural habitats and 8 lichens total 105 fungi were isolated out of which 41 strains showed PHB degradation. In PHB degrader <i>Penicillium</i> and <i>Aspergillus</i> were found to be common species which belongs to deuteromycetes (fungi imperfecti). Out of 67 isolates, 31 bacterial strains showed PHB degradation confirmed by clear zones on assay medium. Protozoa were also found in several samples such as pond, soil, hay, horse dung, and lichen having PHB degrading potential.	Lee et al. 2005
6	Polyester-polyurethane (PS-PUR).	Fungi isolated from soil, Wall paint (latex) coated with polyurethane, and plastic waste from different habitats in Jordan. Agar plate cultivation, Liquid shake flask	Clear zone in test medium, PS-PUR weight loss	4 isolates (<i>Fusarium solani</i> , <i>Spicaria</i> spp., <i>Alternaria solani</i> , and <i>Aspergillus flavus</i>) yielded positive results of biodegradation, indicated by clear zones created due to PS-PUR hydrolysis in 2-layered agar culture plate media. <i>Fusarium solani</i> showed 100% weight loss in the PS-PUR blocks in the shaken cultures.	Ibrahim et al. 2011
7	Polyethylene (PE)	Sewage sludge was collected from sludge treatment plant, Islamabad, Pakistan (<i>Fusarium</i> spp. AF4) Shake flask	SEM, CO ₂ evolution test	Erosions and extensive roughening of the surface with pits formation observed, which were not detected in untreated PE pieces in SEM. 1.85 g/l of CO ₂ was produced (0.45 g/l in case of control) after 2 months	Shah et al. 2009
8	Polyurethane	<i>Trichoderma</i> DIA-T spp. Shake flask	Protease, esterase and laccase Activity assay	95%, 86%, 50%, 36% of the strains showed urease, protease, esterase and laccase activities resp. during biodegradation.	Loredo-Trevino et al. 2011
9	HDPE, LDPE, and degradable polythene bag (9% starch, NP)	MSW 50% (w/w) of mature MSW & films were buried during 15 months at room temperature in 2 L desiccator jars.	FTIR Weight loss, tensile strength, carbon dioxide production	FTIR spectrum revealed various types of oxidation products formed during the biodegradation of polyethylene. Weight loss of 2.1% (LDPE) and 1.3% (HDPE) and 36% (NP) were observed. In NP, HDPE & LDPE 82.76%, 5.33% and 13.04% loss of tensile strength, were recorded. Highest CO ₂ evolution rate of 46.2 mg /L recorded within 28 days in NP.	Orhan et al. 2004
10	Polythene Bag	<i>Brevibacillus</i> , <i>Pseudomonas</i> , and <i>Rhodococcus</i> spp. from textile effluent drainage soil, sewage sludge and household garbage dump Shake flask	Weight loss	<i>Pseudomonas</i> spp. from sewage sludge was found most efficient in biodegradability @ 46.2% for natural and 29.1% for synthetic polythene respectively as compared to strains from other sources.	Nanda et al. 2010
11	Plastic and polythene	Fadama Soil (Bacteria- <i>S. aureus</i> , <i>Micrococcus</i> , <i>S. pyogenes</i> , <i>Pseudomonas aerogenosa</i> , <i>B. subtilis</i> , <i>B. Cereus</i> , <i>E. Coli</i> , <i>Klebsella Pneumonia</i> . Fungi- <i>A. niger</i> , <i>A. fumigates</i> , <i>A. flavus</i> , <i>Fusarium</i> , <i>Mucor</i> , <i>Penicillium</i> , <i>Candida</i>) amended with poultry droppings, cow dung and inorganic fertilizer (NPK) Soil Burial treatment	Weight loss and Physico-Chemical analysis of soil	Maximum % weight loss of polythene bags and plastics were 18.1% (cow dung) & 6% (poultry dropping) after nine months. The result of physicochemical properties of Fadama Soil mixed with organic and inorganic fertilizers after 9 months of the analysis showed an increase in pH. The total nitrogen decreased and available P increased.	Abdullhi and Saidu 2013
12	Polyester polyurethane (PUR)	Endophytic fungi isolated from woody plants of various families were collected in the Yasuni National Forest in the Ecuadorian Amazonian rainforest.	FTIR	Solid agar clearing organism was identified as a <i>Lasiodiplodia</i> spp. (strain E2611A). Four active organisms belonged to the <i>Pestalotiopsis</i> genus, with high relatedness at the species level to <i>Pestalotiopsis microspora</i> . Total Six organisms all cleared PUR more efficiently than the positive control fungus <i>Aspergillus niger</i> . A <i>Lasiodiplodia</i> spp. (strain E2611A) was the most active organism in the liquid medium clearance screen, along with a <i>Pleosporales</i> spp. (strain E2812A) and a <i>Bionectria</i> spp. (strain E2910B), followed by <i>Pestalotiopsis microspora</i> (E2712A) and <i>Pestalotiopsis microspora</i> (E3317B). As per FTIR study, a complete loss of the absorbance peak at 1,735 cm ⁻¹ which corresponds to the C(O)-O ester linkage in the polyurethane polymer. Serine hydrolase was found to be responsible for degradation of PUR. In both aerobic and anaerobic conditions, two <i>Pestalotiopsis microspora</i> isolates found to have unique ability to grow on PUR as the sole carbon	Russell et al. 2011

13	Low Density Polyethylene Film	Soil samples were collected from municipal solid waste [<i>Aspergillus</i> spp. (FSM-3, 5, 6, 8) and <i>Fusarium</i> spp. (FSM-10)] Plastic strip weight loss in Shake Flask	SEM, FTIR Weight loss, change in pH and CO ₂ Evolution test	Maximum weight loss of 9% recorded with <i>Fusarium</i> spp. FSM-10, while highest CO ₂ evolution rate (20.26 g/L) and mineralization level (10.3%) observed with <i>Aspergillus</i> spp. FSM-3 Surface chemical changes were observed and confirmed by FTIR. In all the cases media became acidic. In SEM study, control film was appeared with smooth surface without any pits, cracks or any particles attached on its surface while in treated, the attachment of fungi on LDPE surface and formation of various holes and irregularities were observed. In FTIR study, increase in 1079 cm ⁻¹ and 2418 cm ⁻¹ band due to formation of C=O and O-H stretch were observed. Treatment of fungal isolates resulted in to distortion of peak at 2920 cm ⁻¹ . Isolate FSM-10,3,6 found most efficient than the other fungi.	Das and Kumar 2014
14	Low Density Polyethylene Film	<i>Aspergillus Versicolor</i> and <i>Aspergillus</i> spp. (Isolated from Sea water) Plate assay to study colonization, Shake flask	CO ₂ Evolution test	LDPE totally degraded into carbon dioxide with maximum 4g/l CO ₂ evolution after 1 week during biodegradation of LDPE by <i>Aspergillus</i> spp.	Sindujaa et al. 2011
15	Low Density Polyethylene Film	Bacterial Consortia- <i>Microbacterium</i> spp. strain MK3 (DQ31 8884), <i>Pseudomonas putida</i> strain MK4 (DQ31 8885) & PW1 (EU741 798), <i>Bacterium</i> Te68R strain PN1 2 (DQ423487) <i>Pseudomonas aeruginosa</i> strain PS1 (EU741 797) & C1 (EU753182) In situ biodegradation study in laboratory and natural condition	SEM, FTIR, DSC (Diferential scanning calorimetry)	FTIR, SEM & DSC revealed that the consortia caused significant surfacial degradation of LDPE film and also change in bulk structural characteristics. Environmental factors like sun-light, temperature and rainfall may have role in enhancement of biodegradation were studied by comparative study <i>in situ</i> .	Negiet al. 2011
16	Polythene bag	<i>Bacillus subtilis</i> , <i>Bacillus amylolyticus</i> , <i>Arthobacter defluvii</i> liquid (shaker) culture method	Weight loss study	30% and 20% weight loss/month observed in liquid culture by <i>Bacillus amylolyticus</i> & <i>Bacillus subtilis</i> res.	Thakur 2012
17	Polythene	Waste Disposal Site dumped with polythene bags (<i>Brevibacillus</i> , <i>Pseudomonas</i> , and <i>Rhodococcus</i> spp.) Shake-flask study	Weight loss study	<i>Pseudomonas</i> , <i>Brevibacillus</i> and <i>Rhodococcus</i> degraded polythene to 40.5%, 37.5% , 33% respectively in terms of weight loss.	Nanda and Sahu 2010
18	Disposable plastic films	<i>Streptomyces</i> spp. , <i>Mucor rouxii</i> 1835, <i>Aspergillus flavus</i>	Average weight loss, change in tensile strength and percent elongation	Reduction in the % elongation with bacterial and fungal cultures were recorded as 28.5% and 46.5% respectively.	El-Shafei et al. 1998
19	Polyethylene (low density)	<i>Aspergillus fumigatus</i> and <i>Penicillium</i> spp. Shake flask study in liquid culture	Weight loss study	<i>Penicillium</i> spp. was found most effective in degrading LDPE with 6.58 % degradation	Singh et al. 2012
20	Low density polyethylene (LDPE) sheets	Soil Sample from plastic waste disposable site (<i>Fusarium</i> spp., <i>Penicillium</i> spp., <i>Mucor</i> spp., <i>Aspergillus niger</i> , <i>A. japonicas</i> and <i>A. flavus</i>) Agar plate cultivation	Weight loss study	Strain <i>Aspergillus japonicas</i> F3 (36%), <i>Fusarium</i> spp. F6(32%), <i>Aspergillus flavus</i> F1(30%) found efficient in biodegradation of plastic in 4 weeks as compared to rest of fungi in terms of weight loss.	Singh and Gupta 2014

(Yamada-Onodera et al. 2001) were already reported to be involved in degradation of polyethylene. Maximum 61.0% (*Microbacterium paraoxydans*) and 50.5% (*Pseudomonas aeruginosa*) polythene degradation recorded using Fourier Transform Infrared Spectroscopy (FTIR) within two months (Rajandas et al. 2012), while in another report 47.2% weight loss recorded after 3 months of incubation with the *A. oryzae* (Konduri et al. 2011). Polythene discs treated with *Phanerochaete chrysosporium* for 8 month under shaking, pH= 4.00 & room temperature condition gave 50% weight loss (Aswale 2010). Effect of UV irradiation on biodegradation of LDPE using *Aspergillus* spp. and *Lysinibacillus* spp. were monitored, that showed 29.5% of biodegradation of UV-irradiated and 15.8% non-UV-irradiated films (Esmaili 2013). The percentage decrease in the carbonyl index (CI) was higher for the UV-irradiated LDPE, when performed in soil inoculated with the selected microorganisms. In this study, the ability of microorganisms to utilize virgin polyethylene without pro-oxidant additives and oxidation pretreatment, as the carbon source were investigated. Thermophilic bacterium *Brevibacillus borstelensis* strain 707 isolated from soil and utilized for degradation of low-density polyethylene for 30 days at 50°C, which resulted in reduction of gravimetric and molecular weights by 11 and 30% respectively, it was also observed that FTIR analysis of photooxidized polyethylene revealed a reduction in carbonyl groups after bacterial treatment. Thermophilic bacterium *Brevibacillus borstelensis* strain 707 isolated from soil and utilized for degradation of low-density polyethylene for 30 days at 50°C, which resulted in reduction of gravimetric and molecular weights by 11 and 30% respectively, it was also observed that FTIR analysis of photooxidized polyethylene revealed a reduction in carbonyl groups after bacterial treatment (Hadad et al. 2005). *Microbacterium paraoxydans* and *Pseudomonas aeruginosa* were observed to play a role for polythene degradation as confirmed by Fourier Transform Infrared coupled Attenuated Total Reflectance (FTIR-ATR) showed maximum 61.0% and 50.5% degradation (Rajandas et al. 2012). In another study (Mahdiyah and Mukti 2013) 25 bacteria were isolated from landfill, out of which 8 isolates identified as plastic degrader. One of those showed maximum 17% degradation after 1 month incubation in liquid culture at 37°C with agitation 130 rpm.

Lignin degrading microbes and associated enzymes in plastic degradation

Lignin is a natural aromatic polymer of plant woody biomass and recalcitrant to degradation in nature. White rot fungi are most effective lignin degrading microorganisms were widely studied for plastic degradation. Degradation of Polyethylene membrane by lignin-degrading fungi, IZU-154, *Phanerochaete chrysosporium*, *Trametes versicolor* under various nutritional conditions, demonstrated that manganese peroxidase (MnP) is the main enzyme in polyethylene degradation. Partially purified MnP used for treatment of polyethylene membrane in the presence of Tween 80, Mn (II), and Mn (III) chelator resulted in significant degradation (Iiyoshi et al. 1998). Nuclear Magnetic Resonance (NMR) analysis of biodegraded nylon-66 using lignin-degrading fungus IZU-154 showed that four end groups, CHO, NHCHO, CH₃, and CONH₂, were formed in the biodegraded membranes, suggesting that nylon-66 was degraded oxidatively (Deguchi et al. 1997). Laccases are mainly secreted by lignin-degrading fungi, where they catalyzes the oxidation of various polyaromatic compounds. Laccase (La) is also well-known to act on non-aromatic substrates (Mayer and Staple 2002). Oxidation of the hydro-carbon backbone of polyethylene can be carried out by laccase. Cell-free laccase incubated with polyethylene causes reduction of average molecular weight and molecular number of polyethylene by 20% and 15 % respectively (Bhardwaj et al. 2012). Biodegradable plastic (containing pro-

oxidant and 6% starch) was treated with lignin degrading microbes *Phanerochaete chrysosporium*, *Streptomyces viridosporus* T7A, *S. badius* 252, and *S. setonii* 75Vi2 amongst them *Streptomyces viridosporus* T7A treated plastic showed 50% reduction in tensile strength (Lee et al. 1991). Capability of *Pleurotus ostreatus* to degrade oxo-biodegradable plastic without prior physical treatment, like UV or thermal heating, racks and small holes were developed in the plastic surface as a consequence of the formation of hydroxyl groups and carbon-oxygen bonds, after 45 days incubation. In addition, degradation of the dye in these bags were observed (Da Luz 2013). La & MnP enzymes secreted by fungus *Chaetomium globosum* were responsible for degradation of polyethylene (Sowmya et al. 2014).

Polyethylene degradation can take place by different molecular mechanisms; chemical, thermal, photo and bio-degradation (Shah et al. 2008). As a part of secondary metabolism, microorganisms have a natural ability to transform or accumulate a sundry of compounds including hydrocarbons (PAHs), pharmaceutical substances and metals. Plastic is the hydrolysed by enzymes to create functional groups for improvement of hydrophilicity, then the main chains of polymer are degraded resulting in polymer of low molecular weight and weak mechanical properties, thus, making it more accessible for further microbial assimilation (Shah et al. 2009). Plastics biodegradation by certain enzymatic system also leads to breaking of the polymer into oligomer and monomers or further converted to organic intermediates like acids, alcohols and ketones (Arutchevli et al. 2008). These water soluble cleaved products are absorbed by the microbial cells where they are metabolized. Carbon dioxide and water were formed after aerobic metabolism (Starnecker and Menner 1996), while anaerobic metabolism results in carbon dioxide, water and methane as the end products, respectively (Gu et al. 2000). Physical parameters like temperature, pressure and moisture mechanically breaks the polymers due to which the biological agents like enzymes and other metabolites stimulate the process (Yang et al. 2004). The biodegradation of plastics is typically a surface erosion process due to difficulty in penetration of extracellular enzymes into the polymer and so act only on the polymer surface. Plastic degradation carried out when the pro-oxidants catalyze the formation of free radicals in polyethylene, which react with molecular oxygen to attack the polyethylene matrix (Gnanavel et al. 2013). The enzymatic degradation of polymers by hydrolysis is a two-step process: first, the enzyme binds to the polymer substrate then subsequently catalyzes a hydrolytic cleavage. Intracellular degradation is the hydrolysis of an endogenous carbon reservoir by the accumulating microorganism themselves while extracellular degradation is the utilization of an exogenous carbon source not necessarily by the accumulating microorganisms (Tokiwai and Calabria 2004).

Techniques used for analysis of plastic biodegradation

Biodegradability of polymer can be characterized by monitoring CO₂ evolution rate, O₂ uptake, change in properties of polymer (chemical & physical), growth rate of organisms. Multiple tests should be followed in evaluating plastic degradation due to following reasons (Mohan and Shrivastava 2010)-

1. Weight loss may be due to leaching of additives, including plasticizers.

2. Carbon dioxide production might result from the degradation of low molecular weight fraction of the polymer, with no degradation of longer chains.
3. Loss of additives or very small change in chemical makeup plastic may affect strength of plastic

There are several techniques available for checking the degree and nature of degradation (Figure 2 and Table 3).

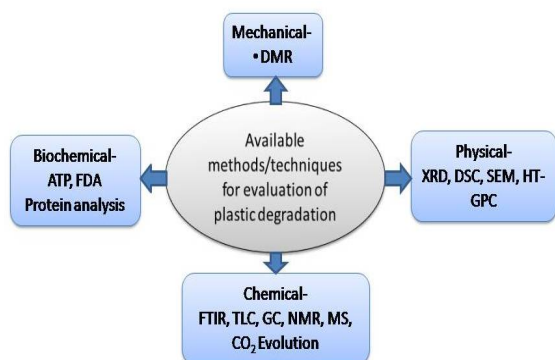


Figure 2 : Techniques for evaluation of plastic degradation

Products of microbially degraded/treated plastics

The literature shows that CO₂ gas is a major product emitted during biodegradation of polythene (Seneviratne et al. 2006; Pramila and Ramesh 2011; Abrusci et al. 2011). Generation of aldehydes, ketones and carboxylic acids was recorded in smoke of film extrusion of LDPE in an extrusion coating process (Andersson et al. 2002). *Rhodococcus ruber* (C208) produced polysaccharides and proteins by using polythene as carbon source (Sivan et al. 2006). In another report, *Rhodococcus rhodochrous* ATCC29672 (Bacterium) and *Cladosporium cladosporioide* ATCC 20251 (Fungus) used

in an extrusion coating process (Andersson et al. 2002). *Rhodococcus ruber* (C208) produced polysaccharides and proteins by using polythene as carbon source (Sivan et al. 2006). In another report, *Rhodococcus rhodochrous* ATCC29672 (Bacterium) and *Cladosporium cladosporioide* ATCC 20251(Fungus) used polythene with production of polysaccharides and proteins, while *Nocardia asteroides* GK911 (Bacterium) produced proteins only (Bonhomme 2003). Table 4 lists a number of products that are generated during biodegradation of plastics.

Toxicity of biodegraded plastic

Insolubility in water and relative chemical inertness of pure plastics make them low toxic. Several plastic products can be toxic due to the presence of some additives in them, e. g. plasticizers like adipates and phthalates are frequently added to brittle plastics like polyvinyl chloride to make them bendable enough. Traces of these compounds can percolate out of the product. The compounds leaching from polystyrene food containers have been predicted to interfere with hormone functions and are supposed to have carcinogenic effect. The finished plastic is non-toxic but the monomers that are used in the production of the parent polymers can be toxic. Toxicity study of biodegraded polythene on plants was monitored by observing their effect on seed germination in seeds like ground nut, sunflower, safflower, sesame and soybean (Aswale 2010). It was recorded that seed germination (%) decreases in treated seeds, while in case of larvae (*Chironomous* spp.) no toxicity was detected in terms of decreases in mortality rate. *S. aureus*, *P. aeruginosa*, *A. niger*, *Rhizopus* spp. and *Streptomyces* spp. were used for degradation of polythene bags and plastic cups and toxicity level of biodegraded polythene was studied using *Vigna radiata*. It was observed that addition of biotreated polythene granules reduced soil pores size, which may have negative effect on the nutrient uptake by the root of plant

Table 3: Existing techniques for assessment of plastic degradation

SN	Changes in properties of polymer	Type of technique	Reference
1	Mechanical: Tensile strength -Elongation at fail and modulus of the polymer	DMR (Dynamic Mechanical Analysis)	Huang et al. 2005; Kathiresan 2003
2	Physical: • Morphology- Microcracks • Density, Contact angle, Viscosity, Molecular Weight Distribution • Melting and Glass Transition temperature • Crystalline and amorphous region	SEM HT-GPC (High Temperature Gel Permeation Chromatography) Thermogravimetric analysis, DSC X-diffraction, Small and Wide angle X ray Scattering	Da Luz et al. 2013; Sasek et al. 2006 Kathiresan 2003 Ramis et al. 2004; Zuchoswka et al. 1999 Albertson et al. 1995
3	Chemical properties	Fourier Transformed Infra red Spectroscopy (FTIR)	Da Luz et al. 2013; Doble et al. 2008
4	Molecular Weight	Thin layer Chromatography (TLC), Gas Chromatography (GC), Nuclear Magnetic Resonance (NMR), Matrix Assisted Laser Desorption Ionization-Time Of Flight, Chemilluminence, Gas Chromatography-Mass Spectrometry (GC-MS),	Deguchi et al. 1997; Albertson et al. 1995; Cacciari et al. 1993
5	CO ₂ evolution test	GC	Seneviratne et al. 2006; Albertson et al. 1995
6	Metabolic activity of the cell	Adenosine triphosphate (ATP), Fluorescien Diacetate (FDA), protein analysis	Kouny et al. 2006; Gilan et al. 2004

polythene with production of polysaccharides and proteins, while *Nocardia asteroides* GK911 (Bacterium) produced proteins only (Bonhomme 2003). Table 4 lists a number of products that are generated during biodegradation of plastics. The literature shows that CO₂ gas is a major product emitted during biodegradation of polythene (Seneviratne et al. 2006; Pramila and Ramesh 2011; Abrusci et al. 2011). Generation of aldehydes, ketones and carboxylic acids was recorded in smoke of film extrusion of LDPE

(Kannahi and Sudha 2013).

Conclusions

It is obvious that without plastic we can't meet our day to daylife needs, but in view of its detrimental effect it is required to develop competent process for its safe disposal and explore alternative material like starch based and blended plastic.

Table 4: Products from microbial degradation of plastics

Sr No	Organisms used	Product	Reference
1	<i>Chaetomium globosum</i> (autoclaved, UV treated and surface sterilized polyethylene)	Carboxylic acids, aldehydes, aromatics, alcohols, phenols, esters, ethers, alkyl halides and alkenes were formed at different frequencies.	Sowmya et al. 2014
2	<i>Bacillus</i> , <i>Aspergillus</i> , <i>Penicillium</i> , <i>Pseudomonas</i>	Octadecadienoic acid, Octadecatrienoic acid, Benzene Dicarboxylic acid, Cyclopropanebutanoic	Mahalakshmi et al. 2012
3	<i>Pseudomonas aeruginosa</i> PAO1	Benzene, methyl; Docosane; Tetrachloroethylene; 3-Chloropropionic acid, heptadecyl ester; Benzene, 1,3-dimethyl; Tricosane; Octadecanoic acid, butyl ester; 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione; Alkanes (Octadecane, Tetracosane, Pentacosane, Hexacosane); Fatty acids (Hexadecanoic acid, Octanoic acid); Hexadecanoic acid, ethyl ester; 1-Nonadecene; 1,2 Benxenedicarboxylic acid, diisostyl ester; Eicosane	Kyaw et al. 2012
4	<i>Serratia marcescens</i> 724, <i>Bacillus cereus</i> , <i>Pseudomonas aeruginosa</i> , <i>Streptococcus aureus</i> B-324, <i>Micrococcus lylae</i> B-429, <i>Phanerochaete chrysosporiu</i> , <i>Pleurotus ostretus</i> , <i>Aspergillus niger</i> and <i>Aspergillus glaucus</i>	Ergosta-5,22-dien-3-ol, acetate (3, 22 E), 1-Monanalinoeoglycerol trimethylsilyl ether, Betamethasone acetate, Azafrin, 9, 12, 15-Octadecatrienoic acid, 2,3-bis [(trimethylsilyl) oxy] propyl ester, (Z,Z,Z)-C27H52O4Si2)	Aswale 2010

Though there are lots of reports demonstrating the potential of plastic degrading microbes, but none of them found to have practical application, thus there is a strong need to screen efficient organisms and developing technologies capable of degrading plastic efficiently without affecting environment.

Acknowledgement

The authors acknowledge the financial support (BT/PR15145/GBD/27/336/2011) provided by Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi, India. Authors also wish to thank the anonymous reviewers who gave valuable inputs into the review and their constructive comments.

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