



Biodegradability of Synthetic Plastics – A Review

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Abstract : Plastics are chemically synthesized polymers made up of two sets of plastics - thermosetting and thermoplastics. Their different properties have made it to enter in different sectors and replaced the conventional materials. Their durability has made it non biodegradable and has also affected the environment due to its large production according to the need of the growing population. The use of biological means to degrade these plastics has been extensively studied by using different microorganisms collected from mainly contaminated sites. This paper discusses about the different screening methods for the detection of plastic degrading microorganisms. The different enzymes synthesized by microorganisms degrade different types of plastics.

Keywords : Plastic degradation, Biodegradable, Enzymes, Environment.

Introduction

Plastics are synthetic carbon polymers from petrochemical industries. These are synthesized by mixing two different polymers, resulting in improved properties of both the parent polymers(1). They are made up of eight fundamental elements i.e. hydrogen, silicon, oxygen, carbon, fluorine, silicon, sulphur and nitrogen. The different properties of plastics such as being light, resistant, different shapes and sizes, corrosion free, insulators, colourful and inexpensive has increased its industrial application. Plastic serves as the mother to thousands of industrial products manufactured for our daily use. It is mixed in our life in such a way that it cannot be separated in any manner. More than 30% of plastic are used for packaging due to their physical resistance and water impermeability. Each plastic gives a specific property that makes it to serve in different range of sectors (2). With the increase in population, the necessity of durable things have also increased, which have paved way to the increase in the synthesis of materials from plastics.

The application of plastics ranges from tooth brush to drinking water bottles, car batteries to irrigation and drainage pipes and straws to helmets (3). Plastics being unavoidable, utilization of synthetic plastics made from petroleum has adversely affected the environment since the majority of these synthetic plastics do not degrade and their physical way of degradation generates polycyclic aromatic hydrocarbons, polychlorinated

biphenyls, heavy metals, toxic carbon- and oxygen-based free radicals, and greenhouse gases, (4) carbon dioxide and dioxin in the process of incineration (5, 6). The main non-biodegradable types of plastics are polyethylene, polyamide, polycarbonate, polypropylene, polystyrene, polyvinyl chloride, polyethylene terephthalate, polymethylpentene, polyoxymethylene and polysulfone.

Types of plastics

According to their technical and chemical behaviour, plastics have been differentiated into two types: **thermosetting plastics** and **thermoplastics**. Thermosetting plastics solidifies after melting by heating. This solidification process is also called curing, during this process the monomers are chemically linked, and obstruct the chains to slip and flow once melted. These thermosetting plastics doesn't melt after they have been solidified, they only undergo chemical breakdown (2). On the other hand, thermoplastics can be melted and solidified infinite times, but with increase in the number of cycles they impact the plastic properties. These thermoplastics have glassy behaviour in solid state (2).

Global Environmental issues

The huge amount of plastic production have brought about global warming, pollution, lowering soil fertility, decreasing water penetrating capacity of plants (7). Over 90% of the produced plastics uses fossil fuels for their synthesis. The decrease in the fossil fuels didnot affect the plastic production, while they are depending upon the renewable resources such as bioplastics. Therefore, the treatment of plastics has become genuine need of the hour (8). The concerning issue from marine environment are scarce with over 8 million tons of plastic per year is dumped into the ocean as estimated in 2016 and will likely increase to 150 million by 2050 (8). Plastics have been found to be the major non-degradable solid waste to marine life. Many highlighting documentaries have shown the dangerous impacts of plastic on marine ecosystem like blockage of intestines and ulceration of the stomach due to starvation etc. (9, 10, 11). Degradation of plastics have become a challenge due to the increasing use of materials. Also, the harmful chemicals leaching from plastic without constraint are contaminating the ground water (12).

The biological way of degradation of plastics happens both microbially and enzymatically. For microbial degradation, requires the growth of microorganisms according to the environmental conditions i.e. temperature, pH, oxygen, nutrients etc. and enzymatic degradation occurs by different sites of cleavage i.e. exo and endo cleavage types implies the intracellular and extracellular acting enzymes. The biodegradability of these plastics is governed by their chemical properties such as surface area, hydrophobicity, hydrophilic nature etc. (13). Microbial degradation occurs by the attachment of microorganism on the surface of the polymer and growth of the microbe by utilizing the polymer as carbon source leading to degradation of the polymer (14).

Diversity of microorganisms

Distribution of plastic degrading microorganism vary according to the geographical location and type of environment. In general, it is very important to examine the distribution of these organisms in different ecosystem. Foremost, the quality of degrading microorganisms attaching to the surface of the plastics and their colonization. The enzymatic hydrolysis process involves the binding of the enzyme to these plastics and their conversion into low molecular weight oligomers, dimers and monomers and further mineralization to carbondioxide and water. Table 1 shows different samples taken for isolation of plastic degrading microorganisms from different environmental samples.

Table 1: Samples collected for isolation of plastic degrading microorganisms from different environmental locations

S No	Sample Type	Name of Microorganism	Area collected	Plastic	Reference
1	Municipal Solid soil area	<i>Bacillus amyloliquefaciens</i>	Pallikaranai, Chennai	LDPE	(15)
2	Paddy field, weed field, roadside and dumping ground	<i>Amycolatopsis</i>	Tsukuba City, Ibaraki, Japan	PLA	(16)
3	Plastic dumped site	<i>Arthrobacter sp. and Pseudomonas sp.</i>	Gulf of Mannar, India	HDPE	(17)
4	Garden soil	<i>Geomyces pannoram, Nectria, Cyllindro cladiellaparva, Penicillium inflatum, and Plectosphaerella cucumerin</i>	Manchester, United Kingdom	PU	(18)
5	Garden Termite soil	<i>Xylaria</i>	Aanaikatti, Tamil Nadu	LDPE	(19)
6	Polythene soil landfills	<i>Aspergillusniger, Lysinibacillus xylanilyticus</i>	Tehran	LDPE	(20)
7	Soil garbage dump yard	<i>Staphylococcus arlettae</i>	Pitchavaram, Pallikarnai	Polythene bags	(21)
8	Polyethylene soil dumped sample	<i>Bacillus sp., Pseudomonas sp.</i>	Tamil Nadu	HDPE	(22)
9	Sludge from sewage plant	<i>Bacillus sp., Pseudomonas sp.</i>	Suyoung, Korea	PLA	(23)
10	Kitchen refuse	<i>Bacillus smithii</i>	Mag Suehiro, Oita	PLA	(24)
11.	Oil contaminated soil sample	<i>Streptomyces coelicoflavus</i>	Naval Dockyard, Vishakhapatnam	LDPE	(2,5)

Screening of plastic degraders

1. Clear zone method

A zone clearing method using agar with the emulsified plastic as substrate for screening plastic degrading microorganisms and also as semi-quantitative method. It confirms the potential of plastic degrading microorganisms by forming clear halo around the growing microorganism and also confers the amount of microorganism by the increase in growth of the halo. The microorganism having the capability of excreting extracellular enzymes can degrade these plastic polymers into water soluble materials (25, 26, 27, 47). The analysis of this method was done by taking 1 g of synthetic plastic and solubilising in aromatic solvents and added to the mineral medium having agar as solidifying agent as carried out by (28). The solubility of different synthetic plastics were known from chemical resistance of chemicals www.kuhnke.kendrion.com/industrial/ics/en/kuhnke-pneumatic-fluidtechnology/valves/fluid-isolation-valves-html?src=pdf.

2. Visual Experiments

To visualize the process happening during the degradation, different instruments can be used like atomic force microscopy (AFM) (29). During the initial degradation, spherulites appeared that was correlated to degradation of plastics. AFM micrographs were taken to investigate the surface erosion after they had been enzymatically degraded (30). Many other techniques have been used such as differential scanning calorimetry(DSC), Nuclear magnetic resonance spectroscopy (NMR),X-ray photoelectron spectroscopy (XPS),X-ray Diffraction (XRD), contact angle measurements and water uptake (3).

3. Scanning Electron Microscope

For topographical study of the changes brought by the microbes, scanning electron microscopy (SEM) is being used. The biofilm formation by the attachment of the microbes on the surface of the polythene is being visualized under this microscope. The changes brought before and after microbial attack during the certain period are being looked (31, 32).

4. Fourier Transform Infrared spectroscopy (FTIR)

The FT-IR spectra of the polythene sample show the destruction caused by the microbial activity. This instrument used can analyze the surface compounds present in the plastic initially and after treatment alterations brought by the microbes by appearance or disappearance of functional groups (33, 34).

5. In-situ degradation method

In this method, polythene needed to be degraded are cut into small strips, rinsed with tap water and with distilled water. Dry them at room temperature. Take the initial weight of the strips and incubated in the selected soil for a certain period. After the specific period, take readings of the strips after rinsing with tap water and distilled water and drying in room temperature (35). For accurate readings, the surface of the polythene is subjected to 2% sodium dodecyl sulphate to remove the colonizing bacteria (36).

Percentage of biodegradation can be calculated from the given formula:

$$\text{Insitu degradation (in \%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

6. Cell surface hydrophobicity assay

This assay determines the hydrophobicity of bacterial cell using bacterial adhesion to hydrocarbon (BATH) test (37). The bacteria in the logarithmic phase were washed with phosphate urea magnesium buffer and optically measured at 400nm. This assay shows the ability of a microorganism to utilize any substrate by its growth and adherence to the substrate. This assay determines the turbidity of microorganism with respect to hexadecane (38, 39). Gilan et al explained the more the hydrophobicity of the bacteria the more is the attachment of the microorganism to the polyethylene (38).

7. Test for Viability and metabolic activity

Degradation of plastic is a slow process and viability and metabolic activity of microorganism to be determined is very significant. Dehydrogenases are intracellular enzymes, involved in oxidoreductase activity taking place in respiration of bacteria. 2,3,5-triphenyltetrazolium chloride (TTC) is a probe that facilitates in direct quantification of viable microorganism. Colorless TTC is reduced to red colored insoluble triphenylformazan (TPF). TTC is reduced by aerobic cytochrome by replacing oxygen as the final H^+/e^- acceptor by the Electron Transport system (36, 40).

8. Biofilm formation

Biofilm is an aggregation of microorganisms (41) that are very resistant compared to free living organisms (42). The accumulation of microorganisms also result in the secretion of enzymes that help in the degradation of biodegradable substances and use it as nutrient source for its survival (43). Biofilm forming adherent cells are collected from plastic by washing it with phosphate buffer saline (PBS). Vortex the plastic with a speed of 2500 min^{-1} in a falcon tube until the dissolution of the biofilm into the PBS. Determine the OD at 600 nm with spectrophotometer (44).

9. Enzyme production

Different enzymes produced by plastic degrading microorganisms are Alkane hydroxylase, Lipases, Laccase, Lignin peroxidase, Cutinase, Serine hydroxylase, Esterases, polyurethanases, Protease,

Dehydrogenase, Depolymerase etc. Detection of enzymes be related to the plastic degraded by the particular microorganism. Tab. 2 shows the enzyme produced by microorganism and different methods of detection.

Table 2: Enzyme produced in the presence of plastic with the association of microorganism and their detection process

Sr. No.	Enzymes produced	Micro organisms	Plastic degraded	Detection	References
1.	Alkane hydroxylase (Alk B)	<i>Pseudomonas sp</i> , <i>Streptomyces</i> , <i>Geobacillus thermodenitrificans</i>	LMWPE	PCR Method, Scanning electron microscope (SEM)	45, 46
2.	Lipases	<i>Acidovorax delafieldii</i> ,	PBSA, PCL	Clear zone method	47
3.	Polyurethane esterases	<i>Comamonas acidovorans</i>	PUR	FTIR, Native PAGE GEL Electrophoresis	48, 49
4.	Cutinase	<i>Fusarium roseumsambucinum</i> , <i>Fusarium solanipisi</i> , <i>Streptomyces scabies</i>	PET, PCL, Polylactic acid, Poly butylenes succinate and PUR	Spectrophotometric method and Fluorescent method	50, 51
5.	Laccase	<i>Cochliobolus</i> sp	PVC	Guaiaucol method, Enzyme assay	52
6.	Lignin peroxidase	<i>Pseudomonas aeruginosa</i> , <i>Bacillus sp.</i> , <i>Aspergillus flavus</i>	PE	Lignin peroxidase assay	53
7.	Protease	<i>Trichoderma spp.</i> , <i>Brevibacillus sp.</i> , <i>Bacillus sp.</i>	PU, PE	Casein agar clearance method	32, 54 55
8.	Dehydrogenases	<i>Sphingopyxis</i> sp, <i>Alcaligenes</i> sp, <i>Pseudomonas sp</i> , and <i>P. strutzeri</i> , <i>Streptomyces coelicoflavus</i>	PVA, LDPE, PE, PCL, PEG, PEO	2,3,5–Triphenyltetrazolium chloride assay	36, 56, 57, 58
9.	Esterases	<i>Aspergillus flavus</i>	PCL, PBSA, PU	P- nitrophenyl acetate	45, 59, 60

Note:- PBSA - Poly(tetramethylene succinate)-co-(tetramethylene adipate), PVC - Polyvinyl chloride, PET - Polyethylene Terephthalate, PCL – Polycaprolactone, PUR – Polyester Polyurethane, LMWPE - Low Molecular weight polyethylene, PU – Polyurethane, PE – Polyethylene, PVA – Polyvinylalcohol, LDPE – Low density polyethylene, PEO – Polyethylene oxide.

10. Emulsification Index (E_{24})

Emulsification assay was done by using petroleum as described by Das et al and Cooper et al (61, 62). This assay is used for the detection of Biosurfactant by microorganism. The application of Biosurfactant by the microorganism has been proved in the degradation of plastics (63). This assay helps to confirm the presence of potent degraders that helps in the dissolution of hydrophobic hydrocarbon and also conversion of the toxic component to environment friendly substance. This confirms the presence of the biosurfactant in percentage.

$$(E_{24}\%) = \text{Height of emulsion (cm)} \times 100 / \text{Total height of liquid (cm)}$$

11. Carbon dioxide evolution

This is a method used to analyse the process of degradation of plastics by the evolution of carbon dioxide. The assay has been done based on procedure ISO9408 (1999), ISO9439 (1999), ISO14851 (1999), ISO 14852 (1999) (64-67). This assay even though seems to be a direct method for detection with respect to mineral media, other than plastic there is no other carbon source provided in the medium. This assay cannot be related only by the evolution of carbon dioxide evolved but in addition to the production of biomass and proteins needed for the survival of the microbe and also enzymes needed for degradation. This brings about a decrease in the evolution of carbon dioxide percentage in terms of biodegradation by microorganisms. Therefore, the CO₂ evolution assay should have freedom in its own way to be considered for biodegradation (65).

$$\text{Percentage of Biodegradation (\%)} = [\text{CO}_2]_T \times 100 / [\text{CO}_2]_{\text{Theor}}$$

Conclusion

Biodegradation of plastics is of major concern to the world. Plastics are insoluble synthetic products which makes it more complicated in its degradation and remediation process. In the present review, most of the microorganisms having plastic degrading ability were isolated from plastic dump yard soil. 20% of *Bacillus sp.* isolated had the capacity to degrade LDPE and PLA, whereas 15% of *Pseudomonas sp.* had the ability to degrade HDPE and PLA. The involvement of different enzymes in biodegradation is inevitable for microorganisms to remediate different types of plastics.

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