

**BIODEGRADATION OF POLYTHENE AND PLASTIC BY THE HELP OF MICROBIAL TOOLS: A RECENT APPROACH**

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**Abstract**

Microorganism play a significant role in biological decomposition of materials including synthetic polymers in natural environments, this is called biodegradation. In this review microbial biodegradation of polythene and plastics are discussed. Here we aim to isolate and identify microorganism associated with various polyethylene and plastic degradation in the soil. Polythene and plastic are the two polymers with wide ranging applications. They are recalcitrant and hence remain inert to degradation and deterioration leading to their accumulation in the environment, and therefore creating serious environmental problems. In this context, an attempt was made to study the biodegradation of polythene and plastic strips inside the laboratory (under controlled condition) and outside the laboratory (under natural condition) with the help of microbial tools. In this review, biodegradation of these two polymers under *in vitro* conditions is reported. An attempt has been made to cover the mechanism of biodegradation, the various bacterial and fungal organisms that have been reported for the same, method adopted for the studies and different characterization techniques followed to measure the extent of degradation.

**Keywords:** Biodegradation, Polythene, Plastics, Environment, Microbial tools

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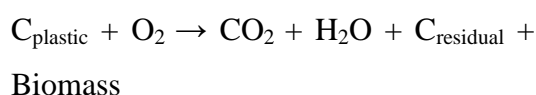
**1. Introduction**

Biological degradation is generally considered as a phenomenon of biological transformation of organic compounds by living organisms particularly microbes. It has been considered as a natural process in the microbial world as carbon and energy source for their growth and takes a key

role in the recycling of materials in the natural ecosystem<sup>1</sup>. Organic materials can be degraded aerobically with oxygen or an-aerobically without oxygen.

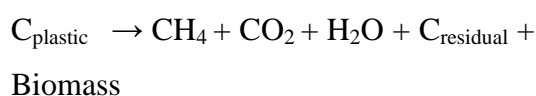
**1.1 Aerobic biodegradation:** Aerobic biodegradation is also known as aerobic respiration and it is an important

component of the natural attenuation of contaminants at many hazardous waste sites. Aerobic bacteria use oxygen as an electron acceptor, and break down organic chemicals into smaller organic compounds, often producing CO<sub>2</sub> and water as the final product.<sup>2</sup>



### 1.2 Anaerobic biodegradation:

Anaerobic biodegradation is an important component of the natural attenuation of contaminants at many hazardous waste sites. It is the breakdown of organic contaminants by microorganisms when oxygen is not present. Some anaerobic bacteria use nitrate, sulfate, iron, manganese, and carbon dioxide as their electron acceptors, and break down organic chemicals into smaller compounds.<sup>3</sup>



## 2. Polythene/Plastics

Plastic is one of the few new chemical material which pose environmental problems. Polyethylene, polypropinyl chloride, polystyrene is largely used in the manufacture of plastics. These chemicals are resistant to biodegradation

and persist in soil environment for a long time. And the degradable plastic is substance created from a polymer called Polyolefin combined with certain additives, in order for it decompose or degrade. High density and low density polyethylene's are the most commonly used synthetic plastics.<sup>7</sup> They are extremely hazardous particularly the thin color ones. They release toxic chemicals which contaminate food items.<sup>8</sup> A very visible portion of municipal and industrial waste consists of polyethylene (PE) films utilized on a massive scale as wrapping material, a typical example for the end consumer being shopping bags. Polyethylene is also used in large quantities in agriculture for green-house construction or directly applied on the soil surface as mulching films, and there is, therefore a growing concern as to whether the plastic litter does not compromises soil quality or not.<sup>4</sup> The drastic rise in the use of non biodegradable plastic materials during the past three decades has not been accompanied by corresponding development procedures for the safe disposal or degradation of these polymers. Polyolefin derived plastics, such as polyethylene, is currently used in the manufacture of plastic films for carry bags, cups,

packaging films, garbage bags, etc.<sup>5</sup> Plastic litter has become an omnipresent part of our environment. Although there are almost no data about the environmental fate of the fragments, it seems that their biodegradation is extremely slow and currently it is hardly possible to make even a rough estimation regarding the time necessary for their biodegradation to some substantial extent.<sup>3</sup> The only known adverse environmental effects of PE films are when they are swallowed by wild animals and encapsulation of material on landfills and in the soil, thus altering microbial processes towards anaerobiosis. For this type of contamination the term ‘‘macropollutants’’ is sometimes used. The polythene and plastic could sometimes caused blockage in intestine of fish, birds, cow, deer and various.<sup>6</sup>

### 3. Biodegradation of Polyethylene

As already noted, a significant amount of low MW compounds is released to aqueous media from oxidized PE film. It was shown that the compounds could be consumed by microorganisms<sup>9</sup>, followed release of low molecular compounds to water media from thermo and photo-oxidized HDPE and LDPE samples by

NMR. These substances were subsequently completely consumed by *Rhodococcus rhodochrous* strain during 4 days of cultivation. The same samples without oxidation pretreatment did not release any substances. In another study<sup>10</sup> extractable compounds up to 12 carbon lengths were completely removed by a culture of *Arthrobacter paraffineus* as demonstrated with the GC–MS technique. After cultivation a new series of signals produced by alkanes with twenty to twenty six carbon atoms appeared on the chromatogram, indicating that by the bacterial action, some compounds with higher MW and lower solubility could also be extracted. In this context the existence of microbial surface-active compounds enabling utilization of insoluble substrates could be of interest.<sup>11</sup> Such compounds were investigated for example with *Rhodococcus erythropolis* DSM 43215 growing on higher alkanes.<sup>12</sup> They are relatively firmly associated with the bacteria surface, increase its hydrophobicity, and mediate adhesion of the bacteria on the substrate surface and passive transport of the substrate molecules. This could be related with the very low critical micelle concentration of the biosurfactants compared to the common synthetic

surface-active compounds. For another poorly soluble substrate, phenantrene, it was shown that the phase transfer between the solid substrate and aqueous medium was the rate-controlling process of biodegradation.<sup>13</sup> In the case of oxidized PE the microbial surface-active compounds can play a very important part also. It seems that an addition of a synthetic detergent with physico-chemical properties different from the biosurfactants can affect biodegradation, more likely in a negative way, because it can probably increase mobility of poorly soluble compounds, but at the same time it can also compromise microbial adhesion on the material surface.<sup>14</sup> Two approaches exist in principle for biodegradation experiments. The first utilizing natural complex media, with established mixed microbial communities with a broad range of microbial strains and activities, enable to mimic biodegradation in situ, like in soil or compost. The second working with defined microbial strains in a synthetic medium where the experiments can be controlled and reproduced precisely, giving the possibility to compare experiments from different laboratories and to deduce information concerning the mechanism of biodegradation.

#### **4. Factors Affecting the Biodegradability**

The selection of suitable strains, which were tested for PE degradation, were based in principle on three ideas: (i) Collection strains of bacteria belonging to the *Streptomyces* genera and strains of fungi both producing lignolytic enzymes were used. The authors followed the idea that lignin as well as PE is an insoluble macromolecular substrate, during its biodegradation a broad range of oxidizing enzymes with unfocused substrate specificity is excreted which eventually could attack PE also. (ii) Collection strains of especially Gram-positive bacteria growing on higher n-alkanes were tested. In such strains we can expect the ability to utilize oxidized PE as a substrate of similar chemical structure; these strains can also produce biosurfactants necessary for mobilization of insoluble hydrophobic substrate molecules. (iii) Strains isolated from soil environment contaminated regularly over many years with PE, a classical approach in biodegradation studies. Previously discussed soil and especially composting experiments<sup>15</sup> showed that pre-thermooxidised prooxPE could be biodegraded to a great

extent with a time horizon of about one year. This could suggest that microorganisms present do not wait passively for the lower MW products of abiotic oxidation and contribute in some way to PE oxidation and chain cleavage or at least that the biotic environment accelerates abiotic oxidation processes. Some authors anticipate that the microorganism producing extracellular lignolytic enzymes may play an important role in the process.<sup>16</sup> Fungi and some bacteria produce various peroxidases and other enzymes which are able, as a consequence of their common action, to oxidise and break the structure of normally very recalcitrant insoluble high molecular lignin.<sup>17</sup> Lignolytic enzymes are produced in conditions of nutrient limitation<sup>18</sup> and thus may be present in a PE degrading culture. However lignin as a polymer, consisting of aromatic benzene rings connected by oxygen and carbon containing bridges, is very distant from PE both structurally and in its reactivity. The confrontation of the results from different studies reveals that probably the higher abiotic oxidation level and consequent decrease of the average MW to under about 5000 Da is the most important factor if some significant extent of biodegradation in a reasonable

time period is desired. The degradation of polythene can occur by different mechanisms, such as chemical, thermal, photo and biodegradation. Some studies have assessed the biodegradability of plastic films by measuring changes in physical properties or by observation of microbial growth after exposure to biological or enzymatic treatments. The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the microorganism responsible for the degradation differs from each other and they have their own optimal growth conditions in the soil.

## 5. Microbial Degradation

The microorganism associated with the plastic films and cups recovered from the soil were quantified and identified, they revealed the presence of both bacteria and fungi in large number. The microbial species identified from the sample polythene bags tested were *Bacillus sp.*, *Staphylococcus sp.*, *Streptococcus sp.*, *Diplococcus sp.*, *Micrococcus sp.*, *Pseudomonas sp.* and *Moraxella sp.* Among the fungal species identified, *Aspergillus niger*, *A. ornatus*, *A. nidulans*, *A. cremeus*, *A. flavus*, *A. candidus* and *A. glaucus* were the

predominant species.<sup>19</sup> With the advances in technology and the increase in the global population, plastic materials have found wide applications in every aspect of life and industries. However, most conventional plastics such as polyethylene, polypropylene, polystyrene, poly (vinyl chloride) and poly (ethylene terephthalate), are degrade very slowly (non biodegradable), and their increasing accumulation in the environment has been a threat to the planet. It involved the strategy as the production of plastics with high degree of degradability. Biodegradable plastics offer a lot of advantages such as increased soil fertility, low accumulation of bulky plastic materials in the environment and reduction in the cost of waste management. Furthermore, biodegradable plastics can be recycled to useful metabolites (monomers and oligomers) by microorganisms and enzymes. As the occurrence of polymer-degrading microorganisms vary depending on the environment, such as soil, sea, compost, activated sludge, etc. It is necessary to investigate the distribution and population of polymer-degrading microorganisms in various ecosystems. Generally, the adherence of microorganisms on the surface of

plastics followed by the colonization of the exposed surface is the major mechanisms involved in the microbial degradation of plastics. The enzymatic degradation of plastics by hydrolysis is a two-step process: first, the enzyme binds to the polymer substrate then subsequently catalyzes a hydrolytic cleavage. Polymers are degraded into low molecular weight oligomers, dimers and monomers and finally mineralized to CO<sub>2</sub> and H<sub>2</sub>O.<sup>20-23</sup> The properties of plastics are associated with their biodegradability. Both the chemical and physical properties of plastics influence the mechanism of biodegradation. The surface conditions (surface area, hydrophilic, and hydrophobic properties), the first order structures (chemical structure, molecular weight and molecular weight distribution) and the high order structures (glass transition temperature, melting temperature, modulus of elasticity, crystallinity and crystal structure) of polymers play important roles in the biodegradation processes. In general, polyesters with side chains are less assimilated than those without side chains.<sup>4</sup> The molecular weight is also important for the biodegradability because it determines many physical properties of the polymer. Increasing the molecular

weight of the polymer decreased its degradability.<sup>24</sup>

## 6. Enzymatic Degradation

### 6.1 Poly(Ethylene Adipate) (PEA)

PEA ( $[-OCH_2CH_2OOC(CH_2)_4CO-]_n$ ) is a pre-polymer of polyurethane. It is often blended with other polyesters to get specific desirable properties such as soft segments. PEA-degrading microorganisms were screened and isolated using PEA (Mn 3,000) as a sole source of carbon. Among the isolated PEA-degrading microorganisms, *Penicillium* species exhibited the strongest activity. The enzyme responsible for the degradation of PEA has been purified and is considered to be a kind of lipase with broad substrate specificity.<sup>25</sup>

### 6.2 Poly( $\epsilon$ -Caprolactone) (PCL)

PCL ( $[-OCH_2CH_2CH_2CH_2CH_2CO-]_n$ ) is a biodegradable synthetic partially-crystalline polyester with low melting point (60 °C) and a glass transition temperature of -60 °C. It is prepared by ring-opening polymerization of  $\epsilon$ -caprolactone. PCL has been shown to be degraded by the action of aerobic and anaerobic microorganisms that are widely distributed in various

ecosystems.<sup>26</sup> The degradation of high molecular weight PCL was investigated using *Penicillium* sp. strain 26-1 (ATCC 36507) isolated from soil. PCL was almost completely degraded in 12 days. PCL can be degraded by lipases and esterases.<sup>27</sup>

### 6.3 Poly ( $\beta$ -Propiolactone) PPL

PPL ( $[-OCH_2CH_2CO-]_n$ ) is a chemosynthetic biodegradable aliphatic polyester with good mechanical properties. The structural units of this polyester are similar to PHB and PCL, thus, it can be degraded both by PHB depolymerase and lipase.<sup>28,29</sup>

### 6.4 Poly(3-Hydroxybutyrate) (PHB)

PHB ( $[-O(CH_3)CHCH_2CO-]_n$ ) is a natural polymer produced by many bacteria as a means to store carbon and energy. It can be biodegraded in both aerobic and anaerobic environments, without forming any toxic products. PHB-degrading microorganisms from *Bacillus*, *Pseudomonas* and *Streptomyces* species.<sup>30</sup> From then on, several aerobic and anaerobic PHB-degrading microorganisms have been isolated from soil (*Pseudomonas lemoigne*, *Comamonas* sp. *Acidovorax faecalis*, *Aspergillus fumigates* and *Variovorax paradoxus*), activated and

anaerobic sludge (*Alcaligenes faecalis*, *Pseudomonas*, *Illyobacter delafieldi*), seawater and lakewater (*Comamonas testosteroni*, *Pseudomonas stutzeri*).<sup>31</sup> The percentage of PHB-degrading microorganisms in the environment was estimated to be 0.5-9.6% of the total colonies.<sup>32</sup>

### 6.5 Polyurethanes (PU)

PU have various applications such as in the manufacture of plastic foams, cushions, rubber goods, synthetic leathers, adhesives, paints and fibers. Darby and Kaplan reported that polyester-type polyurethanes (ES-PU) were more susceptible to fungal attack than polyether-type polyurethanes (ET-PU).<sup>33</sup> The amount of degradation products obtained from the ES-PU film with hog pancreatic lipase was approximately half of that produced by *R. delemar* lipase (53% degradation of the original ES-PU film) after 24 h reaction.

### 6.6 Polyethylene (PE)

PE is a stable polymer, and consists of long chains of ethylene monomers. PE cannot be easily degraded with microorganisms. However, it was reported that lower molecular weight PE oligomers (MW = 600-800) was

partially degraded by *Acinetobacter* sp. 351 upon dispersion, while high molecular weight PE could not be degraded.<sup>34</sup> Furthermore, the biodegradability of low density PE/starch blends was enhanced with compatibilizer.<sup>35</sup> Biodegradability of PE can also be improved by blending it with biodegradable additives, photo-initiators or copolymerization.<sup>36,37</sup> Environmental degradation of PE proceeds by synergistic action of photo-and thermo-oxidative degradation and biological activity (*i.e.*, microorganisms).

## 7. Conclusion

Biodegradable plastic is an innovative means of solving the plastic disposal problem from the standpoint of development of new materials. In general, plastics are water-insoluble, thermo-elastic polymeric materials. Biodegradability of plastics is affected by both their chemical and physical properties. Plastic wastes accumulating in the environment are posing an ever increasing ecological threat. The most problematic plastic in this regard is probably polythene. This review discusses the literature on biodegradation of polythene and plastics by enzymatic degradation. Most of the



examples deal with bacterial and fungal based degradation. Within the time scale of our experiment, the microorganism associated with the polythene & plastic films were identified which revealed the presence of both bacteria and fungi in large number. These microorganisms utilize polythene films as a sole source of carbon resulting in degradation of polythene & plastic. This research in biodegradation of polythene in the last few decades has increased our knowledge of degrading microorganism under natural condition, this enhance our understanding in developing new technologies or modifying the existing ones to degrade the plastics in an environmental friendly way to no toxic byproducts.

Hence, the future attention is on the commercial development and application of natural and eco- friendly polythene and plastic. Based on the literature one could conclude that in order to enhance biodegradation of polythene and plastics the following approaches could be adopted as the biodegradation studies of polythene and plastics in soil, inside the laboratory (under controlled condition) and outside the laboratory (under natural condition) by the help of microbial tools.

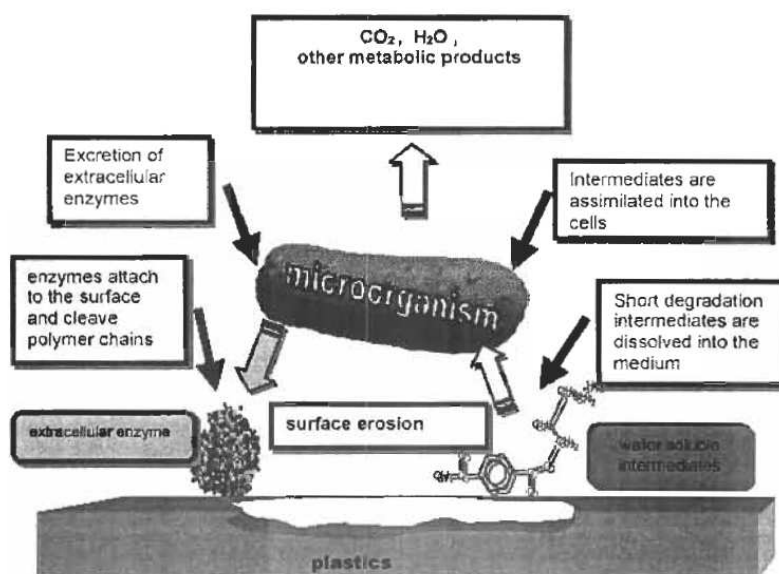
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**Fig 1:** (General mechanism of plastic biodegradation under aerobic condition)