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Biodegradation of Polythene waste through soil Bacteria: A Review

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Abstract: ***Polyethylene waste accumulating in the environment poses an ever increasing ecological threat. They are usually a polymer of ethylene which forms an essential part in our everyday life. They are used in various sectors for wide applications such as packaging materials as films or sheets, as insulators or as consumer products and others. There is an increasing demand for these polymers; with almost 500 billion to 1 trillion tonnes have been used routinely worldwide. They consist of high molecular hydrocarbons with complex structures which can't be degraded easily and their disposals have aroused a great environmental threat. It takes nearly about thousand years for efficient decomposition of these polymers in nature. Widespread studies on biodegradation of plastics have been carried out to overcome the environmental problem associated with these synthetic plastic wastes. This review focus on recent findings on the isolation of synthetic polymer degrading bacterium from soil, the discovery of new degrading enzymes and the cloning of genes for biodegradation. The soil bacteria release the extracellular enzymes such as lignin peroxidase, manganese peroxides to degrade the polythene but the thorough characterization of these enzymes in relation to polythene degradation is still needed to be carried out. In addition, efficient polythene degrading soil bacteria need to be screened from various sources.***

Keywords: Bioremediation; Polythene waste; Soil Bacteria

1. Introduction

Plastics are non-biodegradable, strong, durable, moisture resistant, light weight polymers. They are composed of elements such as carbon, hydrogen, nitrogen, sulphur, and other elements. It is a man-made hazardous long-chain synthetic polymer produced from non-renewable sources such as fossil fuel and nuclear fuels. The annual global demand for plastics has increased over the recent years and presently stands at about 245 million tonnes.They are classified as thermoplastics and thermosetting plastics based on type of chemical reaction. According to their chemical structure they are classified as polycarbonate, polystyrene, polypropylene, low density polyethylene (LDPE), high density polyethylene (HDPE) (Kumari*et al*., 2013). Among them, LDPE is a widely used non-biodegradable thermoplastic. The review summarizes the recent studies on characterization of bacterial biodegradation of LDPE from all types of solid waste using soil bacteria.Environmental degradation can be evaded by using bioremediation methods. Bioremediation is a treatment processes that uses naturally occurring microorganisms and/or plants to breakdown, or degrade hazardous substances into less toxic or non-toxic substances. They can be applied by various methodologies such as on site (*in situ*), off site (*ex situ*) or mediated by either mixed microbial consortia or pure microbial strains.

2. Bioremediation of non-biodegradable waste – Low density polyethylene (LDPE)

Being a versatile, lightweight, strong and potentially transparent material, Plastics are best suited for a variety of applications. Their low cost, excellent oxygen/moisture bearing properties, bio-inertness and light weight make them exceptional packaging materials (Andrady, 2011). However, large amount of accumulation of plastic waste leadsto harmful effect on nature, causing environmental pollution as it is resistant to biodegradation. Most of the cases several chemicals have been added to plastics to give certain properties resulting in compounds such as bisphenol A, phthalates and flame retardants. These substances have known negative effects on human and animal health, mainly affecting the endocrine system. There are also toxic monomers, which have been linked to cancer and reproductive problems. Due to consciousness of wastesproblem among public and its impact on the environment has awakened new interest in the area of degradable polymers. The concern in environmental issues is growing and demands to develop materials which do not burden the environment significantly (Shah *et al*., 2008). Furthermore, these synthetic polymers are normally non-biodegradable until they are degraded into low molecular mass fragments that can be assimilated by microorganisms (Francis *et al*., 2010).

The biodegradable polymers are designed to degrade quickly by microbes, which have ability to degrade most of the organic and inorganic materials including lignin, starch, cellulose and hemicelluloses (Sadocco*et al*., 1997). In addition, biodegradable plastics offers lot of advantages such as increased soil fertility, low accumulation of bulky plastic materials in the environment (which invariably minimize injuries to wild animals) and reduction in the cost of waste management (Tokiwa*et al*., 2009). The microbial species associated with the degrading polymers includes bacteria such as *Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Moraxella*, *Actinomycetes* sp. - *Saccharomonospora* sp. and fungi such as *Aspergillus niger*, *Aspergillus glaucus* (Swift, 1997). Biodegradation has been considered as a natural process in the microbial world where polymers can be used as carbon and energy sources for their growth and plays a key role in the recycling of these materials in the natural ecosystem (Albertsson*et al*., 1987). The microbial degradation of plastics is carried out by certain enzymatic activities that lead to a chain cleavage of the polymer into oligomers and monomers. These water soluble enzymatically cleaved products are further absorbed by the microbial cells where they are metabolized aerobically resulting in carbon dioxide and water (Starnecker and Menner, 1996), whereas anaerobic metabolism results in methane as the end products (Gu*et al*., 2000). The aim of this research was to study the biodegradation of LDPE by selected and potent microorganism isolated from municipal solid waste using various *in vitro* techniques.

Recent years, environmental problem related to non-biodegradable thermoplastics and research to modify them to biodegradable materials is of great interest (Zheng *et al*., 2005). As per a case study, plastic contribute about 8% of the total solid waste generated (Sreedevi*et al*., 2015).

3. Polymer degrading microorganisms

Existence of polymer-degrading microorganisms varies depending on the environmental conditions such as soil, sea, compost, activated sludge, etc. It is necessary to explore 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 is a two-step process: first, the enzyme binds and subsequently catalyzes a hydrolytic cleavageof polymer substrate. Polymers are degraded into low molecular weight oligomers, dimers and monomers and finally metabolized to CO_2 and H_2O.

4. Isolation of bacteria from different solid wastes

Biodegradation of a polymeric material is brought by the action of naturally occurring bacteria and fungi via enzymatic action(Tokiwa*et al*., 2009). Further, Kathiresan (2003) ensured the degradation of plastic materials in mangrove soil having rich total heterotrophic bacterial and fungal counts and found that these materials were colonized commonly by five species of bacteria such as *Pseudomanas*spp., *Staphylococcus* spp., *Moraxella* spp., *Micrococcus* spp.and *Streptococcus* spp. and two species of fungi such as *A. glaucus*and *A. niger*. Yuksel*et al*. (2004) worked on biodegradation of plastic compost bags under controlled soil conditions and determined degradation efficiency by the weight loss of sample, tensile strength, carbon dioxide production, etc. and found the results of the study are in conformity with these previous findings and similar microbes were reported.

5. Studies on factors affecting the biodegradability of plastics

The properties of plastics are linked with their biodegradability. Both the chemical and physical properties of plastics influence the mechanism of biodegradation. Thesurface area, hydrophilic, hydrophobic properties, the first order structures such as chemical structure, molecular weight and molecular weight distribution and the high order structures such as melting temperature, glass transition temperature, modulus of elasticity, crystalline 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 (Tokiwa*et al*., 1976). The molecular weight is also important for the biodegradability because it determines the physical properties of the polymer, increasing the molecular weight of the polymer decreased its degradability. The aliphatic polyesters [ester bond (-CO-O-)] and polycarbonates [carbonate bond (-O-CO-O-)] are two important plastic polymers that show high potential for use as biodegradable plastics due to their susceptibility to lipolytic enzymes and microbial degradation. In comparisonof aliphatic polyesters and polycarbonates as well asaliphatic polyurethane and polyamides (nylon), latter showed higher melting temperature (T_m) due to large change in enthalpy (ΔH) value and presence of hydrogen bonds among polymer chains based on the urethane bond (-NH-CO-O-) and the amide bond (-NH-CO-) respectively (Tokiwa*et al*., 2009).

Hee-Soo*et al.,* (2006) carried out experiments on the biodegradability of bio-flour filled biodegradable poly butylene succinate (PBS) bio-composites in natural and compost soil. It was deduced that percentage weight loss, reduction in mechanical properties of PBS bio-composites in the compost soil burial test was significantly greater than those in the natural soil burial test. It was inferred that biodegradability was enhanced with increasing bio-flour content. Chlopek*et al.,* (2010) examine the influence of the environment on the degradation of polylactic acid (PLA) and their composites and found that the degradation speed is dependent on the viscosity of the applied fluids and ability of penetration in the polymer's structure and the interfacial boundaries. Kumar *et al*., (2010) carried out experiment on biodegradation of flax fiber reinforced PLA and found addition of different amphiphilic additives either delayed or accelerated biodegradability.

6. Methods used for Bacterial Biodegradation of Low-Density Polyethylene (LDPE)

The assessment of the degradation potential of different microorganisms towards a polymer can be analyzed using clear zone method. Agar plates containing emulsified polymers are inoculated with microorganisms and formation of clear halo zones around the coloniesindicated the presence of polymer degrading microorganisms. The polymer-degrading microorganisms excrete extracellular enzymes which diffuse through the agar and degrade the polymer into water soluble materials. Using this technique, distribution of Polyhydroxybutyrate (PHB), polypropiolactone (PPL) and Polycaprolactone (PCL) degraders in different environments was confirmed by several workers (Nishida and Tokiwa 1993; Mergaert and Swings 1996; Suyama *et al*., 1998). Majority of the strains that are able to degrade varied polymers belong to taxa such as *Streptomyces*, Gram-positive and Gram-negative bacteriaand fungi. It has been reported that 39 bacterial strains of the classes *Firmicutes* and *Proteobacteria* can degrade PHB, PCL, and PBSexcept PLA, whereas only few PLA degrading microorganisms have been isolated and identified. The population of aliphatic polymer-degrading microorganisms in diverse ecosystems was found to be in the following order: PHB >PCL > PBS > PLA (Nishida and Tokiwa, 1993).

Low-density polyethylene (LDPE) is a major cause of persistent and long-term environmental pollution. Das and Kumar (2015) examined two bacterial isolates *Bacillus amyloliquefaciens* (BSM-1) and *Bacillus amyloliquefaciens* (BSM-2) from municipal waste contaminated soil and used for polymer degradation studies. After 60 days of incubation, various parameters such as dry weight reduction of LDPE film, change in pH of culture media, CO_2emission was estimated. Film surface was analyzed using SEM which revealed that both the strains were exhibiting adherence and growth with LDPE, while FTIR images showed various surface chemical changes. Isolatedbacteriain the extracellular media showed the depolymerization of biodegraded products which indicateduring the degradation processLDPE as a sole carbon source. BSM-2 showed better degradation than BSM-1 which proves the potentiality of these strains to degrade LDPE films in a short span of time.

Ishigaka*et al*. (2000) reported that the abundance of polymer degrading microorganisms in sea-bed solid waste disposal site. Similarly, Imam *et al*. (1999) observed that significant biodegradation of plastic can be occurred only after colonization by resident microbial populations and concludes that an increase in the bacterial load has correlation with degradation of the polymer. The mechanism of biodegradation of polymer granules happened in three steps, initially microorganism attached to the polymer granule then they grow around the granule and finally degradation the polymer and utilized them as carbon source. Augusta *et al*. (1993) reported that the zone of clearance around the colony is due to presence of extracellular hydrolysing enzymes secreted by the target organism in the suspended polyesters agar medium. In another study by Arutchelvi*et al*., (2008), the microbes involved in forming a clear zone were selected to analyse weight gain/loss method in polymers. The minerals were supplemented along with polyethylene as carbon source for the growth of the organism. The bacteria includes actinomycetes, fungi were separately inoculated to check the degradation activity under aerobic condition in an orbital shaker. After a week, a slimy growth on the surface of polyethylene strips was reported; it is a type of biofilm formation. Study of biofilm formed by *Penicilliumfrequentans* and *Bacillus mycoides* showed that *P.frequentans*had polyethylene degradation capacity, since it colonized on polyethylene surface. Microorganisms utilize polythene films as a sole source of carbon resulting in partial degradation of polythene and plastics. They colonize the surface of the polyethylene films or plastics forming biofilm (Vijaya and Reddy, 2008). Though initially there is increase in the weight (0.02%) of the polyethylene due to biofilm formation but later on a drastic reduction in the weight of LDPE strip confirming the usage of polyethylene as carbon source. The weight of LDPE strip was measured for every two months to six months, bacterial isolate *Pseudomonas* spp.caused biodegradation from 4.34% to 24.22%, *A. niger* showed 10.78% to 26.17%, *A. flavus*showed 5.69% to 16.45% and *Streptomyces* showed high degradability from 12.42% to 46.7% as compared to other isolated species. Kathiresan and Bingham (2001) reported that biodegradation by bacteria was 2.19 to 20.54% for polythene and 0.56 to 8.16% for plastics. Among all fungus, *A.glaucus*was more active than *A. niger*in degrading 28.8% of polythene and 7.26% of plastics within a month. Singh *et al*. (2012) reported that *Penicillium*spp. was more active in reducing LDPE weight upto 6.58% compared to *A. fumigatus* with 4.65%.

Once the organism attached to the surface of the polymer, primary degradation happen where the main chain of the polymer cleaves to form low molecular weight fragments (oligomers), dimers or monomers (Vasile, 1993). Initial degradation is due to extracellular enzymes release by the microorganisms and the breakdown products of polymer are completely utilized by them as carbon source incontrolling environmental pollution (Narayan, 2006).

7. Bacterial biodegradation of polythene both in vitro and in vivo

Rajandas*et al*. (2012)observed the maximumof 61.0% polythene degradation by *Microbacteriumparaoxydans* and 50.5% by *Pseudomonas aeruginosa* within two months recorded using Fourier Transform Infrared spectrometer coupled Attenuated Total Reflectance (FTIR-ATR). But in terms of weight loss, the degradation of polythene was recorded 47.2% after 3 months of incubation. In most of the cases,weight loss of the polythene due to biodegradation is not always reported. Shafei*et al*. (1998) reported increase in polythene weight after inoculation ofmicrobes on the polythene, incubated at regular shaking for one month at 30°C and only three out of 10 microbes reported weight loss. The maximum weight gain (2.02%) was reported with *Streptomyces humidus*. The possible reason for increase in polythene weight after the cultivation of the microbes on the strips is due to the accumulation of cell mass on the polythene surface. In case of *in vivo* study, after 32 years of polythene dumping in the soil only partial degradation was reported by Otake*et al*. (1995).

8. Toxicity level of the biodegraded polythene products

Aswale (2010) tested the toxicity level of all the polythene biodegraded products in both the animal and plant systems. It was found that in plant systems, toxicity of the degraded polythene products with culture filtrate moderately decreased the seed germination rate of the *Arachishypogaea*(groundnut), *Glycine max* (soy bean), *Sesamumlaciniatum*(oil seed, sesame), *Helianthus annuus*(sunflower) and *Carthamustinctorius*(safflower). For the animal system, the mortality rate of *Chironomous*larvaewas calculated, and found no significant difference in the mortality rates as compare to controlSiddiquee*et al*. (2014).

The polythene and plastic degradation site in the soil can serve as a dumping groundfor these materials. It was found that polythenebiodegradation is relatively faster than that of the plastics. Initially, these materials surface turns from smooth to rough with cracking, due to the extra cellular enzymes secreted by the microbes that may break the complex molecular structure of plastics. Priyanka and Archana, (2011) isolated bacterial and fungal species from 5 different locations of soil and characterized basing on their morphological and biochemical characteristics. These microbial species were tested in the laboratory for their ability to degrade the polythene and plastics. The species tested were *Pseudomonas*spp., *Bacillus subtilis*, *Staphylococcusaureus*,*Streptococcus lactis*,*Proteus vulgaris*, *Micrococcusluteus*, *Aspergillus niger*, *Aspergillus nidulance*,*Aspergillus flavus*, *Aspergillus glaucus*, *Penicillium*spp.Each of these microbes was allowed to degrade the polythene and plastics inside and outside the laboratory. The degradation efficiency of polythene and plastic films was compared by the lossof weight while kept inside the soil (outside the environment) and in the culture media (inside the laboratory). This study provides evidence that the degradation rate is faster in the laboratory condition by the individual microorganism inoculated with sample which aid in the degradation of polythene and plastics.

9. Conclusions

Based on the literature survey, the uses of polyethylene is almost unavoidable commercially as well as in day today life, thus producing a great environmental threat. Most of the biodegradation studies done with microbes are found to be eco-friendly, cheap and widely acceptable. Thepolythene degrading microbes release the extracellular enzymes such as lignin peroxidase, manganese peroxidase, but the detailed characterization of these enzymes is still needed to be carried out. Even though, various polythene degradation methods are available in the literature,microbialdegradation is cheapest, eco-friendly and acceptable method. The microbes available from various sources are accountable for the degradation of polythene. But screening of efficient polythene degrading microbe is still need of hour. Efficient characterization of polythene degrading microbes at molecular level is still not available up to the mark to multiply at large scale to commercialize the polythene biodegradation. Further research and related molecular works can improveits efficiency in future.

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