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BIODEGRADATION OF LOW DENSITY POLYETHYLENE BY FUNGI

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Abstract - Plastic is a recalcitrant polymer, once it enters into the environment it does not degrade easily. It accumulates into the environment. It takes about 1000 years to degrade completely. Accumulation of plastic waste in environment poses a serious problem and ecological threat. The environmental concern includes soil and water pollution. Some plastics such as Poly Vinyl chloride are carcinogenic. Furans and dioxins are gases that are produced on burning plastics. These gases are dangerous greenhouse gases and play an important role in ozone layer depletion. One way to meet the challenges of plastic pollution is to shifting the production from non-biodegradable plastics to biodegradable plastics which can easily be degraded in the environment. Another strategy to meet this challenge is to search for such microorganisms which have tools in the form of enzymes to degrade these polymers into monomers so that these monomers can be used as main energy source by these microorganisms. Fungi are the main decomposer in the environment. They produce diverse array of enzymes for degradation of different substrates in the environment. Many works have been done on fungi having capability to degrade the plastics. A brief review of different works has been done in this paper.

Keywords: Recalcitrant, Poly Vinyl Chloride (PVC), Biodegradable etc.

1 INTRODUCTION

Plastics are polymers made up of monomer units which are derived from petrochemicals. Plastic is recalcitrant, non-biodegradable organic material. Under natural environmental condition non-biodegradable organic materials are considered as the major environmental problem and plastic is one of them. Global plastic demand has increased several folds because plastics are easy to manufacture, light weight, low cost, highly durable and are of high tensile strength [1]. The production of plastic which was 1.5 million tons in 1950 has increased to 245 million tons in 2006. Despite recognition of the persistent pollution problems posed by plastic, global production is still increasing, with the largest increase expected in developing nations. The huge volume of plastics produced each year presents a problem for waste disposal systems. The scale of this problem and the recalcitrance of some polymers to degradation necessitate investigation into effective methods for biodegradation of plastics. To fulfill this goal, researchers need greater knowledge of how compounds are metabolized by existing organisms, an investigation of new microorganisms which have bioremediation potential, and the characterization of new metabolic capabilities. Further the understanding of the biological processes that lead to biochemical degradation will put forward the development of new plastics bioremediation techniques.

2 POLYETHYLENE

Polyethene (PE) is a long-chain synthetic resin obtained through the polymerisation of ethylene (C2H4) monomers. In its simplest form a PE molecule consists of chains of covalently linked carbon atoms with a pair of hydrogen atoms attached to each carbon atom (–CH2–). These chain ends are terminated by methyl groups (–CH3) [2]. PE plastic is characterized by toughness, low moisture absorption, good chemical resistance, good electrical resistance, a low coefficient of friction and ease of processing [3]. It is the most widely used plastic, with an annual production of approximately 80 million tones [4]. Depending on their density, these compounds are classified as high-density polyethylene (HDPE), LDPE and linear low-density polyethylene (LLDPE).

2.1 HDPE

This is a high-density version of PE (0.941–0.965 g/cc), with a molecular weight ranging from 5,000 to 250,000 Da [5]. In HDPE there is limited number of branches in its structure. This allows the polymer chains to pack closely together. This results in a dense, highly crystalline material [6]. As HDPE exhibits low swelling characteristics it is commonly used to pack juices, soft drinks and other food materials. HDPE is comparatively easier to recycle than LDPE [7]. Like the other PEs, HDPE is resistant to biodegradation.

2.2 LDPE

This is the low-density version of PE (0.919–0.955 g/cc) [8]. Though its chemical structure is similar to that of HDPE, unlike it, LDPE possesses high frequency of branching with more tertiary carbon atoms in its structure. This branching prevents the close approach of polymer molecules and results in decreased crystallinity [2]. Therefore LDPE is relatively soft, flexible and yet tough. The most popular application of LDPE is foil, from which carrier bags, packaging material and agricultural plastic are made. It is estimated that 500 billion tons of LDPE are produced in the form of plastic bags annually [9]. Another important use of LDPE is in soil mulching, where it is used as a covering material to prevent the evaporation of water from the soil and maintain the moisture level during cultivation.

2.3 LLDPE

LLDPE is a linear polymer, with significant numbers of short branches [10]. The tensile strength of LLDPE is high and also it has high puncture and impact resistance in comparison to LDPE. It is very flexible and elongates under stress [11]. LLDPE is resistant to chemicals and to ultraviolet (UV) radiation. LLDPE possesses a narrow heat sealing range, making its processing difficult. LLDPE is very cheap compared to other types of plastic such as nylon, poly (ethylene terephthalate) (PET) and polystyrene (PS). It can be used in manufacturing plastic wrap, stretch wrap and pouches.

Among the PE varieties mentioned above, LDPE is the most useful and widely used variety, in the form of plastic bags. It has been estimated that somewhere between 500 billion to a trillion plastic shopping bags are used every year [12]. Along with this, Global demand for LDPE is expected to grow at around 2.6 % [13]. Adding to this, developing countries such as India and China are expected to consume more LDPE in the future [14].

Australia produces around 13×105 tonnes of plastic per annum, mostly in the form of plastic bags, while it consumes 7 billion plastic bags annually [15]. The United States of America (USA) uses approximately one billion plastic bags annually, resulting in 300,000 tons of landfill waste [16].

Further the LDPE is used in agriculture for soil mulching. Mulching films are used to suppress weeds, reduce the loss of moisture from soil, decrease the use of chemicals in weed control, reduce water consumption and to speed up crop development [17]. It has been estimated that the global consumption of LDPE mulching films in horticulture is around 700,000 tons per year [18].

After consumption, plastic bags are generally discarded, creating an ecological menace. The discarded plastic bags either enter landfills or marine ecosystems. Lightweight plastic grocery bags are more harmful due to their propensity to be carried away by wind and cause aesthetic damage to their surroundings. Moreover, removing these bags from the streets is expensive and time-consuming. Discarded plastic bags are often eaten by birds and cattle, resulting in their death [19].Discarded plastic bags also end up in the oceans and cause severe damage to marine ecosystems. The obvious adverse effect associated with plastic bag debris in the oceans is aesthetic.

As LDPE has a lower density than water, it floats on the ocean surface, creating a visual menace [20]. Marine wildlife often consumes plastic bags, either inadvertently in the process of feeding, or deliberately because they mistake the plastic bags for food. For example, whales and sea turtles often mistake plastic bags for squid or jellyfish and ingest them. This ingested plastic may lead to starvation or malnutrition as marine debris collects in the animal's stomach. Marine life become ensnared as they get entangled in plastic debris. This leads to suffocation, starvation and drowning, and increased susceptibility to predators or other injury.

Plastic bags are generally made with a variety of additives such as plasticisers, fillers and antioxidant pigments, some of which prove toxic [21]. As plastics break down, the microscopic fragments (microplastics) generated can be consumed by fish and thus enter the food chain [22]. In addition, marine debris can harm important components of the economy, including marine tourism, fishing and navigation.

Moreover, the use of LDPE for mulching contributes to the pollution problem. This is in general known as ‘white pollution’ [23]. Most of the mulching film degrades within one year after usage, but the rest of it accumulates in arable land and pollutes both the ecological environment and the landscape [24]. In 2004, in US, about 143,000 tons of plastic mulch were disposed of, either in landfill or by being burned on site, releasing carcinogens in to the air [25]. In addition, LDPE is also used in other agricultural operations as silo bunker covers, silage bags, haylage covers, greenhouse covers, bale wrap and row covers which all contributing to the soil pollution problem. Furthermore, in recent years, LDPE use has extended to many industries, ranging from the manufacturing of common household goods to medical devices [26].

3 BIODEGRADATION

Biodegradation is a broad term. It does not have a precise definition. In simple terms, biodegradation can be defined as a natural process by which organic chemicals are converted to simpler compounds and mineralized [27].The latest definition was provided by ASTM in standard D-5488-94d. According to this document, biodegradation is defined as a process in which the decomposition of a material occurs predominantly by the enzymatic action of microorganisms that convert these materials into CO2, CH4, water, inorganic compounds and biomass [28].

Biodegradation of LDPE can be classified as macrobiological and microbiological. Some reports suggest that certain insects can secrete fluids that degrade PE films [29]. This is an example of macro (passive) biodegradation that leads to the deterioration of PE to particle size. However, it is a rare process and its impact is negligible. Active biodegradation by microbes is comparatively faster, with bacteria and fungi commonly used. Bacteria prefer simple carbon sources (i.e., glucose) for metabolic purposes. In a selective environment in which carbon sources are restricted, bacteria and fungi can consume polymers. This process is made more efficient by the isolation, enrichment and study of microbes that degrade polyolefins such as LDPE.

Biodegradation of solid polymers like LDPE occurs generally by two methods i.e. surface erosion method or bulk degradation method [30]. In surface erosion method, the polymer starts to degrade from the exterior portion to interior leading to the thinning of it with time. In this type of biodegradation, molecular weight of polymer remains constant. Rate of surface biodegradation is generally follows zero order kinetics and depends on the available surface area of polymer [31]. In bulk erosion, polymer biodegradation occurs throughout the polymer matrix at the same time. Molecular weight decreases with the increasing time and the matrix dimensions remains constant till the total mechanical failure. In this type of biodegradation polymer allows penetration of water into the bulk of material [30]. Usually bulk erosion follows first order kinetics [32].

3.1 Fungal Species Capable of Plastic Degradation

Penicillium simplicissimum degrade the polyethylene (PE) by secreting the extracellular enzymes [33]. Polyhydroxyalkanoates (PHA) degrading fungi have been isolated from aquatic and marine environment and belong mostly to Deuteromycetes (*Aspergillus* and *Penicillium*), Basidiomycetes and Ascomycetes [34]. Polycaprolactone (PCL) is synthetic polyester easily degraded by *Fusarium*[35].Polylactic acid (PLA) is a polymer frequently used in biodegradable plastics is degraded by *Fusarium moniliforme* and *Penicillium roqueforti* [36]. Polyurithane is degraded by several fungal species such as *Fusariumsolani, Aureobasidium pullulans,* although its biodegradation is incomplete [37, 38].

Trichoderma viride and Aspergillus nomius isolated from local landfill soil in Medan have capability to degrade low density polyethylene (LDEP). To check biodegradation of LDPE, weight loss and reduction of tensile strength of the treated film compared to the untreated one was evaluated. Trichoderma viride reduced the weight of LDPE film to a total loss of 5.13% and Aspergillus nomius by 6.3%. Both the fungus reduced the tensile strength of LDPE film compared to control film (5.292 MPa). The strength was reduced from 2.45 MPa to 1.96 MPa in Trichoderma viride culture with the average reduction of 58% and from 3.92 MPa to 2.646 MPa in Aspergillus nomius culture or 40% reduction. This shows that LDPE film treated with above fungi became fragile. Electron micrograph of treated film showed the formation of the crack, grove, and uneven surface of LDPE film [39].

Aspergillus glaucus [40], Aspergillus niger and Penicillium pinophilum [41], Aspergillus oryzae [42], Aspergillus versicolor [43], Chaetomium sp. and Aspergillus flavus [44] are reported to degrade low density and high density polythenes upto different extents. A. glaucus degraded the polythene and plastics upto 20.80% and 7.26% respectively. A. niger and P. pinophilum degraded the powdered LDPE from 5% and 11.07% respectively. A. oryzae degraded high density polyethylene films upto 72%. A. versicolor was found to degrade the LDPE in the powdered form. Chaetomium and A. flavus was also found to degrade the polyethylene.

Jonathan et. al. [45] found in a study that fungus Pestalotiopsis microspora was uniquely able to grow on a plastic called Polyester Polyurethane (PUR). This fungus uses PUR as the sole carbon source under both aerobic and anaerobic conditions. They did molecular characterization of this activity and suggested that a serine hydrolase is responsiblefor degradation of PUR. They observed the broad distribution of this activity and the unprecedented case of anaerobic growth using PUR as the sole carbon source. They suggested that this fungus is a promising source of biodiversity that can be used for biodegradation of plastics.

The growth of several fungi into the polymer solid leads to small scale scale swelling and bursting into the plastic. In recent years fungal strains have been reported for plastic degradation such as Aspergillus versicolar [46], Aspergillus flavus [47], Chaetomium spp [46] Mucor circinellodies species etc. The polythene bags were degraded by some fungal species identified such as Aspergillus niger, A. ornatus, A. nidulans, A. cremeus, A. flavus, A. candidus and A. glaucus were the predominant species. The microbial species are associated with the degrading materials were identified fungi (Aspergillus niger, Aspergillus glaucus), [47, 48]. Sanchez et al., [49] has reported that the PCL-degrading fungi, Aspergillus sp is effective in biodegradation as plastics studies.

Many studies on fungal degradation of the bioplastic have also been performed including Paecilomyces lilacinus [50], Fusarium moniliforme [51], Aspergillus flavus [52], Thermoascus aurantiacus [49], Tritirachium album [53], Paecilomyces verrucosum [53] and Aspergillus sp. [54]. Two genera of fungi Penicillium roqueforti and Tritirachium album degrade the polylactic acid (PLA) [55]. Aspergillus niger van Tieghem had the ability to degrade PVC. Fungi such as Acremonium, Cladosporium, Debaryomyces, Emericellopsis, Eupenicillium, Fusarium, Mucor, Paecilomyces, Penicillium, Pullularia, Rhodosporidium, and Verticillium degrade the PHB and polyesters. Aspergillus, Aureobasidium, Chaetomium, Cryptococcus, Fusarium, Rhizopus, Penicillium, and Thermoascus degraded the plastic called PCL. Aspergillus, Aureobasidium, Penicillium, Pullularia degraded the plastic called PEA. Fungus like Alternaria solani, Spicaria sp., Aspergillus terreus, Aspergillus fumigatus, Aspergillus flavus were isolated from soil where plastic have been dumped. In the shaken cultures, when PS PUR blocks were mixed with above fungi there was a significant loss in the weight of these blocks, the weight loss was 100% in case of the isolates of Fusarium solani [56].

Laccaseis an enzyme secreted by *Fusariumoxysporum*was shown to play an important role in LDPE biodegradation. Laccase encourages LDPE oxidation, and thus results in increased biodegradation by fungi.

The laccases are copper-containing enzymes capable of oxidising a wide range of substrates, including phenolic compounds, non-phenolic compounds, lignin and environmental pollutants. These oxido-reductases can also oxidise molecular oxygen to water

[57]) by an electron transfer mechanism [58]. The molecular mass of laccase ranges between 50 and 130 kDa[59].

More than 100 forms of laccase have been purified and several have been characterised[60]. In general, laccase holoenzymes are dimers or tetramers, and are covalently linked with carbohydrate moieties. They contain four copper ions in three different ionic states, with these ions playing an important role in the oxidation of substrates.

Laccases, particularly those from Basidiomycetes, were identified as having depolymerising capacity for lignin. Lignin contains phenyl propanoid units linked by C–C and C–O bonds. Laccases catalyse electron transfer between these phenolic propanoid groups and molecular oxygen. These enzymes can also degrade plastic wastes with olefin units [61]. In conjunction with mediators of electron transfer, laccase can oxidisebiphenol and alkyl phenol derivatives. They can also degrade organic pollutants [62] and recalcitrant pollutants [63]. Laccases have been reported to oxidise alkenes [64], carbazole and flourene [65].

4 CONCLUSION

Plastics are posing a great environmental challenge as plastics are thermo-elastic, water-insoluble polymers. Microbial degradation is better than chemical and physical methods as the degradation pathway leads to complete degradation and mineralization of polymer. However, biodegradability depends upon the microbial community adhered in it. Microbial community plays a significant role in modifying the physicochemical properties and degradation of plastics. Hence, better understanding of the microbial community would help in better development of plastic remediation.

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