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Review Article

DEGRADATION OF PLASTIC BY FUNGI : A REVIEW

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Plastics are man-made, non-biodegradable polymers which do not degrade very easily or degrade only after a long period of time. They accumulatein the environment and pose a serious problem and ecological threat. The environmental concern includes soil and water pollution. Some plastics 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 article.

Plastics are polymers made up of monomer units which are derived from petrochemicals. Plastic is 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. Fathima *et al*. (2016) reported that 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. 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 is to be studied. To fulfill this goal, researchers need greater knowledge of how compounds are metabolized by existing and new organisms 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.

Polyethylene

Polyethylene (PE) is a long-chain synthetic resin obtained through the polymerization of ethylene (C_2H_4) monomers. Peacock (2000) reported that in its simplest form a polyethylene 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 (–CH_3).Rosato (2004) reported that polyethylene is characterized by toughness, low moisture absorption, good chemical resistance, good electrical resistance, a low coefficient of friction and ease of processing. Piringer and Baner (2008) reported that polyethylene is the most widely used plastic, with an annual production of approximately 80 million tonnes. Depending on their density, these compounds are classified as high-density polyethylene (HDPE), low-density polyethylene (LDPE) and linear low-density polyethylene (LLDPE).

High Density Polyethene (HDPE)

This is a high-density version of PE (0.941–0.965 gcc^{-1}), with a molecular weight ranging from 5,000 to 250,000 Da (Aaron *et al.,* 2010).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 (Carraher, 2003). 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 (La Mantia, 2002). Like the other PEs, HDPE is resistant to biodegradation.

Low Density Polyethene (LDPE)

This is the low-density version of PE (0.919–0.955 gcc^{-1}) (Hilado, 1998). 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 (Peacock, 2000). 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 (Knight, 2013). 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.

Linear Low Density Polythene (LLDPE)

LLDPE is a linear polymer, with significant numbers

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of short branches (Scheirs, 2009). 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 (Robertson, 2012). 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, polyethylene 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 (Islam, 2008). Along with this, Global demand for LDPE is expected to grow at around 2.6 % (Sagel and Remex, 2012). Adding to this, developing countries such as India and China are expected to consume more LDPE in the future (Platts, 2014).Australia produces around 13×105 tonnes of plastic annum^{-1}, mostly in the form of plastic bags, while it consumes 7 billion plastic bags annually (Brown, 2003). The United States of America (USA) uses approximately one billion plastic bags annually, resulting in 300,000 tons of landfill waste (Anonymous, 2012). After consumption, plastic bags are generally discarded, creating an ecological menace. The discarded plastic bags either enter landfills or marine ecosystems. Light weight 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 (Norton, 2005). 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 (Majumdar, 2007). 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 becomes 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 (Jana and Banerjee, 1999). As plastics break down, the microscopic fragments (microplastics) generated can be consumed by fish and thus enter the food chain (Meenakshi, 2012). In addition, marine debris can harm important components of the economy, including marine tourism, fishing and navigation.

Further the LDPE is used in agriculture for soil mulching. Soil mulching stimulates the microbial activity in soil through improvement of soil agro physical properties (Kanthaswamy and Venkadeswaran, 2020). Mulching films are used to suppress weeds, reduce the loss of moisture from soil, decrease the use of chemicals in weed control, reduces water consumption and to speed up crop development (Schettini *et al.,*2012).The greatest benefit of plastic mulch is that the soil temperature in the planting bed is raised promoting faster crop development and earlier harvest and more uniform soil moisture is maintained and irrigation frequency is reduced (Kanthaswamy and Venkadeswaran, 2020). It has been estimated that the global consumption of LDPE mulching films in horticulture is around 700,000 tons year^{-1} (Espi *et al.,*2006).Moreover, the use of LDPE for mulching contributes to the pollution problem. This is in general known as 'white pollution' (Hardwick and Gullino,2010). 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 (Stevens, 2002). In 2004, in US, about 143,000 tons of plastic mulches were disposed of, either in landfill or by being burned on site, releasing carcinogens into the air (Shogren and Hochmuth, 2004). In addition, LDPE is also used in other agricultural operations as silo bunker covers, silage bags, haulage 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 (Lambert *et al.,*2001).

Process of 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 (Ren, 2011). 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 CO_2, CH_4, water, inorganic compounds and biomass (Anonymous, 2006).

Biodegradation of LDPE can be classified as macrobiological and microbiological. Some reports suggest that certain insects can secrete fluids that degrade polyethene films (Anderson *et al.,* 1995). This is an example of macro (passive) biodegradation that leads to the deterioration of polyethylene to particle size. However, it is a rare process and its impact is negligible. Active biodegradation by microbes is comparatively faster; here bacteria and fungi are 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 (Ratner *et al.,* 2012). 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 (Kumbar *et al.,*2014.). 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 (Ratner *et al.,* 2012). Usually bulk erosion follows first order kinetics (Bader and Putnam, 2014).

Plastic degradation potential of fungal species

Khan *et al.* (2022) used 3% LDPE as sole carbon source in the screening medium and found that *Penicillium citrinum* showed fastest colony growth from among 16 isolates. It showed 38.82±1.08% weight loss of untreated LDPE. The degradation capacity of *P. citrinum* was improved from38.82±1.08% to 47.22±2.04% after its pre-treatment with nitric acid. They confirmed the role of enzymes such as laccase, lipase, esterase and manganese peroxidase in depolymerization process.

Mohamed *et al.*(2022) reported poly vinyl alcohol (PVA) biodegradated by four species of fungi. These strains showed varied PVA removal rates of 81% by *Penicillium brevicompactum*, 67% by *Talaromyces verruculosus,* 52% by *Penicillium polonicum* and 41% by *Aspergillus tubingensis*. Of these the most promising PVA biodegradation isolate was *P. brevicompactum* which produced enzymes such as lipase, manganese peroxidase and laccase at a pH 7 and temperature of 30^0C. These enzymes degrade the poly vinyl alcohol into monomer units.

Khruengsai *et al.* (2021) were able to grow four species *Diaporthe italiana,Thyrostroma jaczewaskii*, *Colletotrichum fructicola* and *Stagnosporosis citrulli* with LDPE film as the only sole carbon source. The biodegradability of these fungi was evaluated from the amount of CO_2 and enzyme production. These fungi produced CO_2 ranging from 0.45 to 1.45, 0.36 to 1.22, 0.45 to 1.45 and 0.33 to 1.26 gl^{-1} respectively. These fungi were also able to secrete large amount of laccase. Weight loss was 43.90% in case of *D. italiana*, 46.34% in case of *T. jaczewaskii*, 48.78% in case of *C. fructicola* and 45.12% in case of *S. citrulli*. Tensile strength was also reduced significantly by 1.56, 1.78, 0.43 and 1.86 MPa respectively.

Munir *et al.* (2018) isolated *Trichoderma viride* and *Aspergillus nomius* 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 2.64 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 on LDPE film.

Rani and Singh (2017) grown 15 isolates from dump site on polyethylene supplemented medium where polyethylene was the sole carbon source. They found that only 5 species showed highest colony diameter. These were *Fusarium solani* 91.44 mm, *Aspergillus flavus* 83.82 mm, *Aspergillus fumigatus* 78.74 mm, *Aspergillus niger* 63.50 mm and *Aspergillus terrus* 50.80 mm. *In situ* polyethylene degradation, it was found that weight loss was highest in case of *Fusarium solani*. Thus, *F. solani* have the highest potential to grow on polyethylene supplemented medium and highest degradation capacity of HDPE and LDPE.

Vignesh *et al.* (2016) isolated bacteria and fungi from various plastic dump soils. The bacteria were *Streptococcus* sp., *Pseudomonas* sp. and *Bacillus* sp. and fungi were *Aspergillus* and *Fusarium*. The biodegradative ability was determined by weight loss after a period of 30 days. Bacterial species degraded the plastic up to 23% and fungal species up to 44%. This shows fungi have better ability to degrade plastics than bacteria.

Indumathi and Gayathri (2016) buried 1 g weighed plastic strips to study degradation process. They prepared sterile soil pits and strips were placed in layers with soil alternatively. Then the pits were inoculated with *Aspergillu soryzae* at intervals. The strips were allowed to degrade for three months. They found significant weight loss, formation of microcracks and embrittlment in the plastic strips.

Sumathi *et al.* (2016) isolated *Cochliobolus* sp. from plastic dump soils, which secrete the enzyme laccase. This enzyme degrades poly vinyl chloride (PVC). They observed significant difference in FTIR, GC-MS and SEM results in between control and *Cochliobolus* sp. treated PVC.

Kanchi (2015) found that *Fusarium oxysporum* isolated from plastic dump site has the ability to degrade the LDPE.This fungus produces a ligninolytic enzyme called laccase. This enzyme is responsible for LDPE degradation.

Mahalakshmi and Andrew (2012) worked on *Rhizopus arrhizus* and *Penicillium* sp. and found that physico-chemically treated PE films were more efficiently degraded by the fungal isolates than untreated films. A physico-chemical treatment of the polymer leads to its oxidation and subsequent breakdown helping in easy assimilation by the fungus. The oxidized polymer helps in adhesion of microorganisms due to changes in hydrophobicity of the polymer surface. They also found higher biomass accumulationon pretreated plastic samples. Carbohydrate is the main energy source in the medium and non-availability of readily assimilating carbon source, force microorganisms to adhere to the polymeric surface resulting

information of biofilm which is essential for plastic degradation. They found production of large amount of reducing sugars which revealed that polyethylene was used as carbon source. Fungi are unable to transport polymeric material directly into the cells due to the lack of solubility in water and its size. Therefore, they secrete extra cellular enzymes which aid in the degradation of polymers outside the cells and then their absorption.

Raaman *et al.* (2012) isolated *Aspergillus niger* and *Aspergillus japonicus* from the polyethylene polluted sites around Chennai. They studied their effectiveness on the degradation of commercial polyethylene carry bags of low density over a period of 2 to 4 weeks. They measured the biodegradation in term of mean weight loss which was nearly 8 to 12 % after a period of 4 weeks. Through SEM analysis, they confirmed the degradation by the presence of porosity and fragility of the fungal degraded polyethylene surface. *Aspergillus japonicus* showed 12% degradation potential when compared to *Aspergillus niger* of 8% degradation in one month period.

Russell *et al.* (2011) found in a study that fungus *Pestalotiopsis microspora* was uniquely able to grow on 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 responsible for 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.

Yamada *et al.* (2001) reported that *Penicillium simplicissimum* degrade the polyethylene (PE) by secreting the extracellular enzymes. Kim and Rhee (2003) isolated the poly hydroxyl alkanoates (PHA) degrading fungi from the aquatic and marine environment. He found that most of these belong to Ascomycetes, Basidiomycetes and Deuteromycetes. Murphy *et al.* (1996) reported that poly capro lactone (PCL) is synthetic polyester and is easily degraded by *Fusarium*. Ghosh *et al.* (2013) reported that poly lactic acid (PLA) is a polymer frequently used in biodegradable plastics, is degraded by *Fusarium moniliforme* and *Penicillium roqueforti*. Polyurithane is degraded by fungi such as *Fusarium solani* and *Aureobasidium pullulans,* although its biodegradation is incomplete (Shimao, 2001; Nakajima-Kambe *et al.,* 1999).

Aspergillus glaucus (Kathiresan, 2003), *Aspergillus niger* and *Penicillium pinophilum* (Volke-Sepulveda *et al.,* 2002), *Aspergillus oryzae* (Konduri *et al.,* 2010), *Aspergillus versicolor* (Pramila and Ramesh, 2011a), *Chaetomium* sp. and *Aspergillus flavus* (Sowmya *et al.,* 2012) were reported to degrade low density and high density polyethenes upto different extents. *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.

Many fungal strains have been reported for plastic degradation such as *Aspergillus versicolar* (Pramila and Ramesh, 2011b), *Aspergillus flavus* (Swift, 1998), *Chaetomium* spp (Chee *et al.,* 2010). The polythene bags were degraded by some fungal species identified as *Aspergillus niger*, *A. ornatus*, *A. nidulans*, *A. cremeus*, *A. flavus*, *A. candidus* and *A. glaucus*. *Aspergillus niger* and *Aspergillus glaucus* were found associated with the degrading materials (Swift, 1998; Chee *et al.,* 2010). Sanchez *et al.* (2000) has reported that the poly capro lactone (PCL) is degraded by *Aspergill* sp.

Many studies on fungal degradation of the bioplastic have also been performed including *Paecilomyces lilacinus* (Oda *et al.,* 1995), *Fusarium moniliforme* (Torres *et al.,* 1996), *Aspergillus flavus* (Benedict *et al.,* 1983), *Thermoascus aurantiacus* (Sanchez *et al.,* 2000), *Tritirachium album*, *Paecilomyces verrucosum* (Jarerat and Tokiwa, 2001.) and *Aspergillus* sp. (Li *et al.,* 2011). Two genera of fungi *Penicillium roqueforti* and *Tritirachium album* degraded the poly lactic acid (PLA) (Mogilnitskii *et al.,* 1987). *Aspergillus niger* van Tieghem had the ability to degrade poly vinyl chloride (PVC). Fungi such as *Acremonium*, *Cladosporium*, *Debaryomyces*, *Emericellopsis*, *Eupenicillium*, *Fusarium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Pullularia*, *Rhodosporidium*, and *Verticillium* degraded the poly hydroxyl burate (PHB) and polyesters. *Aspergillus*, *Aureobasidium*, *Chaetomium*, *Cryptococcus*, *Fusarium*, *Rhizopus*, *Penicillium*, and *Thermoascus* degraded the plastic called poly capro lactone (PCL). *Aspergillus*, *Aureobasidium*, *Penicillium*, *Pullularia* degraded the plastic called poly ethylene adipate (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 polystyrene (PS) or polyurethane (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* (Ibrahim *et al.,* 2013).

Laccases, an enzyme secreted by several species of fungi, 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 (Lee *et al.,*2002) by an electron transfer mechanism (Sakurai, 1992.). The molecular mass of laccase ranges between 50 and 130 kDa (Morozova *et al.,*2007) . More than 100 forms of laccase have been purified and several have been characterized (Kunamneni *et al.,*2008). 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. Laccasescatalyse electron transfer between these phenolic propanoid groups and molecular oxygen. These enzymes can also degrade plastic wastes with olefin units (Xu, 2005). In conjunction with mediators of electron transfer, laccase can oxidize biphenol and alkyl phenol derivatives. They can also degrade organic pollutants (Dehghanifard *et al.,* 2013.) and recalcitrant pollutants (Shraddha *et al.*, 2011.). Laccases have been reported to oxidise alkenes (Niku-Paavola *et al.,*2000), carbazole and flourene (Bressler *et al.,*2000).

In the last it can be concluded that plastics are posing a great environmental challenge as they are thermo-elastic, water-insoluble polymers. Microbial degradation is better than chemical and physical methods as the biodegradation 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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