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Microbial Population Associated With Plastic Degradation

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Abstract

Promiscuous and frequently deliberate release of plastics is responsible for growing environmental pollution. Low cost, Low technology, eco-friendly treatments capable of reducing and even eliminating plastics, are developed by the researchers. Among biological agents, microbial enzymes are one of the most powerful tools for the biodegradation of plastics. Activity of biodegradation of most enzymes is higher in fungi than in bacteria. This review focuses on induced biodegradation rate of plastics by fungal and bacterial communities and the mode of action of biodegradation. Following a discussion of microbial population and the factors affecting the production of enzymes, a brief survey is presented on various individual group of enzymes such as laccase, cutinase, hydrolase, esterase, protease and urease etc. These enzymes are secreted by various predominant microbes like *Streptococcus, Bacillus, Pseudomonas, Staphylococcus, Aspergillus, Penicillium, Phanerochaete, Pestalotiopsis* etc.

Keywords: Biodegradation; Enzymes; Environment; Microbes; Plastics

Introduction

Plastics are one of the synthetic polymers or man-made polymers [1]. The accumulation of the plastic is responsible for the most unique and long lasting changes to the environment [2]. The composition of the plastics consist carbon, hydrogen, silicon, oxygen, chloride and nitrogen. Oil, coal and natural gas are used for extraction of the basic materials of plastics [3]. Because of its stable and durable characteristic, plastics are widely used. Mostly used plastics are polyethylene (LDPE, MDPE, HDPE and LLDPE), Poly Ethylene Terephthalate (PET), Polybutylene Terephthalate (PBT), nylons, Poly-Propylene (PP), Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyurethane (PUR) [4]. These are the synthetic polymers which accumulate in the environment due to the absence of efficient methods for safe disposal and posing an ever increasing ecological threat to flora and fauna [5,6].

Biodegradation is the process in which microorganisms like fungi and bacteria degrade the natural polymers (lignin, cellulose) and synthetic polymers (polyethylene, polystyrene) [7]. As the microorganisms possess different characteristics, so the degradation varies from one microorganism to another. Microorganisms degrade the polymers like polyethylene, polyurethane by using it as a substrate for their growth [8]. Various factors which are responsible for biodegradation are kind of polymers, organism characteristics, and the type of treatment required [9,10]. Discoloration, phase separation, cracking, erosion and delimitation are some of the characteristics which indicate the degradation of polymers. Breakage of bonds, transformation due to chemicals, and synthesis of new functional groups are responsible for the variations [11].

Characteristics of microorganisms represent the type of enzymes which are produced for biodegradation like extracellular or intracellular enzymes which helps in the degradation of polymers [9,12]. The cellular membranes of the microorganisms accumulate the substrate which is then degraded by cellular enzymes. Microbes can easily degrade the small subunits of polymeric molecules found in the form of monomers or oligomers because high molecular weight causes insolubility which is not suitable for the degradation of plastics by microbial flora [13].

Plastics

There are different mechanisms for the degradation of plastics: thermal, chemical, photo and biodegradation. Polyethylene is a synthetic polymer having high hydrophobic level and high molecular weight. It is widely used as plastic bags, packaging material, milk and water bottles, responsible for various environmental problems [14]. Polyurethanes (PU) represent the most common class of polymers which is used in the medical, automotive and industrial fields. They are found to be present in various products like furniture, coatings, fibers, synthetic skins, adhesives, elastomers, constructional materials, padding's and paints. And pre-polymer of polyurethane is poly (ethylene adipate) (PEA). This polymer is made up of a series of urethane linkages [15,16].

Some polymers are being used for the manufacture of biodegradable plastics like polyhydroxybutyrate (PHB) and copolymers containing other hydoxyalkanotes. These polymers are consumed by various microorganisms as carbon and energy sources and various enzymes like polyhydroxyalkanoates (PHA) depolymerases secreted by them help in the degradation of these types of plastics [5].

Bioplastics are biodegradable plastics. It means these types of plastics are either produced from fossil materials or can be synthesized from biomass or renewable resources. Some petroleum based plastics are polycaprolactone (PCL) and poly (butylenes succinate) (PBS) but they can be degraded by microorganisms. While the plastics which are produced from biomass or renewable resources are poly(hydroxybutyrate) (PHB), poly(lactide) (PLA) and starch blends [17].

Plastics can be biodegradable by improving the hydrophilic level, or polymer chain length can be reduced by oxidation which is to be accessible by microbial growth [17]. Biodegradability of these polymers can be assessed by measuring changes macroscopically or by observing the microbial growth after exposure to biological or enzymatic environment, but mostly by CO_2 evolution [8,18-20].

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Plastic Biodegrading Microbes

Surface of the soil buried polyester polyurethane showed the growth of both bacteria and fungi and they were tested in the laboratory for PUR degrading ability [21,22]. Fungi had been found more in comparison to bacteria for degrading the plastics. Some of those bacteria that can degrade polyester in vitro and which utilize the PUR as sole carbon source have been identified from the genera *Pseudomonas, Comamonas*, and *Bacillus* [23-26].

Brevibacillus borstelensis strain isolated from soil, a thermophillic bacterium, recovered for the degradation of branched low- density polyethylene by utilizing it as the sole carbon source and energy source. The incubation of polyethylene film with *B. borstelensis* revealed the reduction in molecular weight of polyethylene by 30% [27].

Number of fungi isolates were identified from the surface of polyester PU foam as a sole carbon source, buried for 28 days, from the genera *Emericella, Trichoderma, Aspergillus, Fusarium, Gliocladium* and *Penicillium* [28]. *Geomyces pannorum* was found to be the predominant fungi consisting 22-100% of the polyester PUR degrading fungi [29]. The other genera of the fungi including the majority of the organisms found in soil for the degradation of polyurethane like *Plectosphaerella, Nectria, Neonectria, Phoma* and *Alternaria*. The reported biodegradation activity with *Aspergillus niger* was observed to be quite slow with visible signs of deterioration occurring only after 30 days [30].

Mechanism of Enzymatic Biodegradation

The most attractive plastic waste treatment method is enzymatic degradation. Polyethylene degradation through microbial enzymes comprises two steps. Firstly enzyme adheres to the polyethylene substrate and then catalyzes a hydrolic cleavage. Intracellular and extracellular depolymerases in fungi and bacteria degrade the polyethylene. Endogenous carbon content by the accumulating bacteria themselves is hydrolyzed through intracellular degradation while the utilization of exogenous carbon source not necessarily by accumulating microorganisms is the extracellular degradation [31]. Complex polymers disintegrate into short chains of oligomers, dimers, and monomers which can pass through the bacterial membranes and act as a source of carbon and energy. This process is referred as depolymerisation. And mineralization is the degradation process in which the end products are carbon dioxide (CO_2), water (H_2O) or methane (CH_4) are produced [32]. Temperature, pressure and moisture are the physical parameters which mechanically damage the polymers due to which the biological forces like enzymes and other metabolites produced by microbes induce the process [33].The mechanism of the biodegradation of plastics can be easily understand by the following flowchart (Figure 1).

Enzymes Varies with Plastics

Enzymes exist in every living cell and hence in all microbes. Relative amounts of the various enzymes produced by the microorganisms vary with species and even between strains of the same species. Enzymes are very specific in their action on substrates, so the different enzymes help in the degradation of various types of enzymes [34].

Laccase can help in the oxidation of the hydro-carbon backbone of polyethylene. Gel permeation chromatography determine that cell-free laccase incubated with polyethylene helps in the reduction of average molecular weight and average molecular number of polyethylene by 20% and 15 % respectively [2]. Laccase produced by the actinomycete *R.ruber*, involved in biodegradation of polyethylene. Laccases are mostly present in lignin- biodegrading fungi, where they catalyze the oxidation of aromatic compounds. Laccase activity is known to act on non-aromatic substrates [35].

Papain and urease are the two proteolytic enzymes were found to degrade medical polyester polyurethane. Polymer degraded by papain was due to the hydrolysis of urethane and urea linkages producing free amine and hydroxyl groups [36].

Lignin and manganese-dependent peroxidases (LiP and MnP, respectively) and laccases are the three main enzymes of ligninolytic system [37].

The enzymes catalyze the hydrolysis of polylactic acid (PLA), which is the plastic obtained from renewable resources and the hydrolysate can be recycled as material for polymers. Lipase from *Rhizopus delemar* and polyurethane esterase from *Comamonas acidovorans* has been investigated for the degradation of low molecular weight PLA and high molecular weight PLA have been found to be degraded with the strains of *Amycalotopsis* sp. [38].

Some strains which are capable of degrading the polyethylene are *Brevibacillus* spp., *Bacillus* spp., where proteases are responsible for degradation [2]. The enzymes responsible for biodegradation by *Pseudomonas* spp. are serine hydrolases, esterases and lipases. PHA depolymerases are serine hydrolases which are able to attack on the branching chains and the cyclic components of the polymers. *R. delemar* lipase degraded 53% of the polyester type-polyurethanes (ES-PU) film after 24h reaction. *C. acidovorans* degrade the ES-PU made up of poly (diethylene adipate) contained a type of esterase [5,16].

Several organisms, including bacteria *Pseudomonas chlororaphis* and *Comomonas acidovorans* as well as the fungus *Candida rugosa* are the source of proteins and enzymes like putative polyurethanases which have been isolated and characterized. The active enzymes have been grouped as esterases, lipases, proteases and ureases which degrade the polyurethane substrate by cleaving the ester bonds. *Pestalotiopsis microspora*, the endophytic fungi has been isolated containing serine hydrolase utilize the polyurethane as a substrate, source of carbon and degrade it within a few number of days [30].

Lignin degrading fungi and manganese peroxidase, partially purified from the strain of *Phanerochaete chrysosporium* also helps in the degradation of high molecular weight polyethylene under nitrogen limited and carbon limited conditions [5]. Ligninolytic enzyme families include phenol oxidase (laccase), heme peroxidases {Lignin Peroxidase

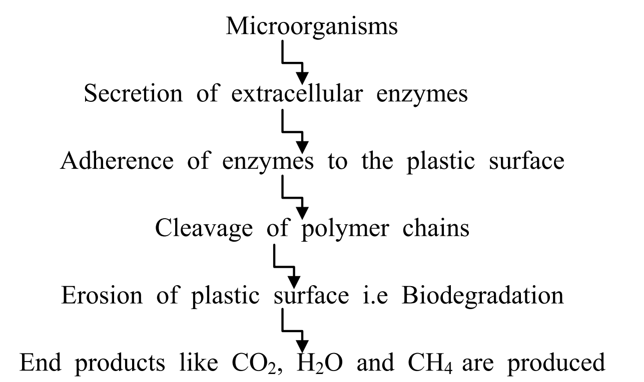

Figure 1: Mechanism of Enzymatic Biodegradation of Plastic [13].

Source	Enzyme	Microorganisms	Plastic act as substrate	Reference(s)
Fungal	Glucosidases	*Aspergillus flavus*	Polycaprolactone(PCL)	[16]
	Unknown	*Penicillium funiculosum*	Polyhydroxybutyrate(PHB)	[16]
	Cutinase	*Aspergillus oryzae*	Polybutylene succinate(PBS)	[40]
	Catalase, Protease	*Aspergillus niger*	PCL	[16]
	Unknown	*Streptomyces*	PHB, PCL	[16]
	Urease	*Trichoderma sp.*	Polyurethane	[41]
	Cutinase	*Fusarium*	PCL	[5]
	Unknown	*Amycolaptosis sp.*	Polylactic Acid(PLA)	[5]
	Serine hydrolase	*Pestalotiopsis microspora*	Polyurethane	[30]
	Manganese peroxidase	*Phanerochaete chrysosporium*	Polyethylene	[5]
Bacterial 1	Lipase	*Rhizopus delemar*	PCL	[16]
	Unknown	*Firmicutes*	PHB, PCL, and PBS	[16]
	Unknown	*Protobacteria*	PHB, PCL, and PBS	[16]
	Lipase	*Penicillium, Rhizopus arrizus*	Polyethylene Adipate(PEA), PBS, PCL	[16]
	Serine hydrolase	*Pseudomonas stutzeri*	Polyhydroxyalkanoate(PHA)	[5]

Table 1: Some bacterial and fungal strains depicting the biodegradation of plastics.

[LiP], Manganese Peroxidase [MnP], and Versatile Peroxidase [VP]} [39].

Some other enzymes which are responsible for the degradation of various types of plastics (Table 1), which depicts the substrates that utilize the plastics as carbon and energy sources and helps in biodegradation.

Significance

Biodegradation process is very eco-friendly. The growth of the microbes responsible for biodegradation must be optimized by controlling the temperature, humidity, incubation time and the substrate like polyethylene, polyurethane which are consumed as a carbon and energy source. This helps in the production of large amount of enzyme. These microbial enzymes induce the rate of biodegradation of plastics very effectively without causing any harm to the environment.

Conclusion

Safe disposal of plastic waste by biodegradation should focus on the most consumed polymers like polyethylene, polypropylene, polyurethane and polystyrene. Unfortunately, these polymers are also long lasting as well as the most durable plastics. In view of these problems, several tasks should be addressed in order to get safe waste disposal. One of the most successful method to handle these problems is enzymatic biodegradation of plastic that will enhance the biodegradation rate.

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