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A REVIEW ON BIODEGRADATION OF POLYTHENE: THE MICROBIAL APPROACH

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Abstract

The use of polythene is increasing day by day and its degradation is becoming a great challenge. Annually about 500 billion to 1 trillion polythene carry bags are being consumed around the globe. Polythene is durable and needs up to 1000 years for natural degradation in the environment. This present review focuses on the level of polythene pollution, cost effective methodsof polythene degradation,, the source of polythene degrading microbes, brief mechanism of polythene degradation, the methods used for the biodegradation of the polythene, the assessment of polythene degradation by efficient microbes,the products of polythene under degradation process and the future aspects of polythene degradation.A brief survey is presented on various individual groups 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.

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Introduction

Plastics are one of the synthetic polymers or man-made polymers. The accumulation of the plastic is responsible for the most unique and long lasting changes to the environment (Himani, 2012). The composition ofthe plastics consist carbon, hydrogen, silicon, oxygen, chloride and Nitrogen. Oil, coal and natural gas are used for extraction of the basic materials of plastics. Because of its stable and durable characteristic,plastics are widely used. Mostly used plastics are polyethylene(LDPE, MDPE, HDPE and LLDPE), Poly Ethylene rephthalatePET),Polybutylene Terephthalate (PBT), nylons, Poly-Propylene(PP), Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyurethane (PUR) . These are the synthetic polymers which accumulate in theenvironment due to the absence of efficient methods for safe disposal and posing an ever increasing ecological threat to flora and fauna (Bhardwaj, 2012).

Biodegradation

Biodegradation is the process in which microorganisms likefungi and bacteria degrade the natural polymers (lignin, cellulose)and synthetic polymers (polyethylene, polystyrene) (Ford, 2000). As themicroorganisms possess different characteristics, so the degradationvaries from one microorganism to another. Microorganisms degradethe polymers like polyethylene, polyurethane by using it as a substrate for their growth (Glass 1989). Various factors which are responsible forbiodegradation includes:kind of polymers, organism characteristics, andthe type of treatment required. Discoloration, phase separation,cracking, erosion and delimitation are some of the characteristicswhich indicate the degradation of polymers. Breakage of bonds,transformation due to chemicals, and synthesis of new functional groups are responsible for the variations (Pospisil, 1997). Characteristics of microorganisms represent the type of enzymes

Which are produced for biodegradation like extracellular or intracellularenzymes which helps in the degradation of polymers (Ford,2000).The cellularmembranes of the microorganisms accumulate the substrate which isthen degraded by cellular enzymes. Microbes can easily degrade thesmall subunits of polymeric molecules found in the form of monomersor oligomers because high molecular weight causes insolubility whichis not suitable for the degradation of plastics by microbial flora (Fariha,2008).

Plastics

Plastics are defined as the polymers (solid materials) which on heating become mobile and can be cast into moulds. They are non-metallic moldable compounds and the materials that are made from them can be pushed into any desired shape and sizes (Saymour, 1989).Commonly plastics are used in many purposes including **packaging**, **disposable diaper backing**, **agricultural films** and **fishing nets**. Plastics and their use has become a part in all sectors of economy. Infrastructure such as **agriculture**, **telecommunication**, **building** and **construction**, **consumer goods**, **packaging**, **health** and **medical** are all high growth areas that ensures present demand for plastics. Plastic is the mother industry to hundreds of components and products that are manufactured and used in our daily life like **automobiles parts**, **electrical goods**, **plastic furniture, defense materials**, **agriculture pipes, packages and sanitary wares**, **pipes** and **fittings**, tiles and flooring, artificial **leathers**, **bottles** and **jars**, PVC **shoes** and **sleepers** hundreds of **household items** (Mueller, 2006).

Plastics are used in packaging of products such as food, pharmaceuticals, cosmetics, detergents and chemicals. Approximately 30% of plastics are used worldwide for packaging applications and the most widely used plastics used for packaging are polyethylene (LDPE, MDPE, HDPE, LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polybutylene terephthalate (PBT), nylons. At present the industry is split into organized and unorganised sectors. The organized sector produce quality products whereas unorganized sector is not capable of producing quality products, it produces low quality, cheap products through excessive use of plastic scrap. (Mueller, 2006).

Types of plastics

There are two types of plastics: Thermoplastics and Thermosetting polymers.

Thermoplastics: are plastics that do not undergo chemical change in their composition when heated and can be moulded again and again.

Thermosets: are assumed to have infinite molecular weight. These chains are made of many repeating molecular units, known as repeating units, derived from monomers; each polymer chain will have several thousand repeating units. Thermosets can melt and can be molded into various shapes. After they are solidified, they remain solid. In the thermosetting process, a chemical reaction occurs which is irreversible. Vulcanization of rubber is good example of thermosetting process. The polyisoprene is a tacky, slightly runny material, before heating with sulfur, but after vulcanization the product is rigid and non-tacky.

Other classifications are based on qualities that are relevant for manufacturing or product design. Plastics can also be classified depending on various physical properties, such as density, high tensile strength, and resistance to various chemical products.

Biodegradation

Any physical or chemical change in polymer as a result of environmental factors such as light, heat, moisture, chemical conditions and biological activity is termed as degradation of plastic. Biodegradable polymers are designed to degrade upon disposal by the action of living organisms. Microbial degradation of plastics is caused by enzymatic activities that lead to a chain cleavage of the polymer into monomers. Microorganisms utilize polythene film as a sole source of carbon resulting in partial degradation of plastics. They colonize on the surface of the polyethylene films forming a biofilm. Cell surface hydrophobicity of these organisms was found to be an important factor in the formation of biofilm on the polythene surface, which consequently enhances biodegradation of the polymers. Once the organisms get attached to the surface, starts growing by using the polymer as the carbon source. In the primary degradation, the main chain cleaves leading to the formation of low-molecular weight fragments (oligomers), dimers or monomers. The degradation is due to the extra cellular enzyme secreted by the organism. These low molecular weight compounds are further utilized by the microbes as carbon and energy sources. The resultant breakdown fragments must be completely used by the microorganisms, otherwise there is the potential for environmental and health consequences. (Fuhs,1961)

Polythene

Among the synthetic plastics waste produced, polythene shares about 64% (Lee,1991).As per the reports the most commonly used non-degradable solid waste is polythene which is a linear hydrocarbon polymers consisting of long chains of the ethylene monomers (C_2H_4). The general formula of polyethylene is CnH2n, where ‘n’ is the number of carbon atoms (Arutchelvi, 2008). Polythene is made from the cheap petrochemical stocks extracted from oil or gas through efficient catalytic polymerization of ethylene monomers (Jen-hou, 1998). Polythene finds a wide range of applications in human’s daily use because of its easy processing for various products used for carrying food articles, for packaging textiles, for manufacturing laboratory instruments and automotive components (Arutchelvi,2008).

Status of Polythene Pollution

The use of plastic, especially polythene is growing day by day. Every year 25 million tons of synthetic plastics are being accumulated in the sea coasts and terrestrial environment (Lee,1991) Polythene constitutes 64% of the total synthetic plastic as it is being used in huge quantity for the **manufacture of bottles, carry bags**, **disposable articles**, **garbage containers**, **margarine tubs,milk jugs**, and **water pipes** (Lee,1991). Similarly, in the marine environment alone, out of total marine waste, plastic shares about 60-80% by mass (Derraik,2002) All the polythene waste along with other plastic wastes generated by the human activity finally enters into marine water through rivers, canals/channels and municipal drainages. Therefore, the beaches were reported to be the excellent depository sites for the polythene (plastic) wastes. At dumping sites, polythene waste degraded with both chemical and mechanical weathering but it takes long time for mineralization and may remain in the microscopic form for long time (Corcoran, 2010) Annually 500 billion to 1 trillion polythene bags are being used routinely all over the world. Polythene is strong and highly durable and takes up to 1000 years for natural degradation in the environment. Furthermore, plastic degrades by sunlight into smaller toxic parts contaminating soil and water where they can be accidentally ingested by animals and thereby enter the food chain especially in the marine biota (Denuncio,2011)

Effect of polythene

To the marine life polythene waste is recognized as a major threat. Sometimes, it could cause **intestinal blockage in the fishes**, **birds and marine mammals**(Denuncio,2011).As per report (Coe,1997) due to plastic pollution in the marine environment minimum 267 species are being affected which includes all mammals, sea turtles (86%) and seabirds (44%). **The death of terrestrial animals such as cow was reported due to consumption of polythene carry bags (**Singh,2005). **The polythene leads to blockage of their digestive tract.** It is also found that polythene remains undigested in the stomach of the animals, after the death of the animals the polythene is again being eaten by some other animal and the cycle continues **(**Singh, 2005)The undigested polythene was found to be responsible for various problems in the animals**(**Singh, 2005).This includes: (1) during the digestion the fermentation process and mixing of the other contents were hampered due to ingested polythene and leads to **indigestion**; (2) the ingested polythene **blocks the opening between omasum and reticulum which leads to death of the animal if the polythene will not be removed,** (3) impaction: due to accumulation of large quantity of polythene bags rumen becomes impact which leads to **remenatony**; (4) **tympany**: due to blockage of the reticulum and omasum with polythene, accumulation of gases takes place in rumen, which leads to death of the animal if not removed properly; (5) **polybezoars**: In the digestive tract around the polythene deposition of salt takes place that leads to formation of stone like structure which hampers the food passages and leads to **pain and inflammation of rumen;** (10) immunosuppression: the accumulation of polythene in the stomach of the animals (cow) leads to increased sensitivity to infections such as **haemorrhagic (**Singh, 2005). The widely used packaging plastic (mainly polythene) constitutes about 10% of the total municipal waste generated around the globe (Barnes, 2009) As per literature, every year hundred thousand tons of plastics have been degraded in the marine environment resulting death (Barnes, 2009)The use of polythene is increasing every day and its degradation is becoming a great challenge. In the year 2000 about 57 million tons of plastic waste was generated around the world annually (Lederberg,2000). Only a fraction of this polythene waste is recycled whereas most of the wastes enter into the landfills and take hundreds of years to degrade (Rutkowska, 2002).

Cost Effective Methods of Polythene Degradation

The process which leads to any physical or chemical change in polymer properties as a result of environmental factors (such as light, heat and moisture etc.), chemical condition or biological activity is said to be polymer degradation (Pospisil, 1997) Based on the factors responsible for the degradation of the polymers, three types of polymer degradation methods are cited in the literature such as **photodegradation**, **thermo-oxidative** degradation and **biodegradation**(Shah,2008) The biodegradation is a natural process of degrading materials through microbes such as **Bacteria**, **Fungi**and **Algae**(Rutkowska,2002).The biodegradation involves microbial agents and does not require heat. Organic material can be degraded in two ways either aerobically or anaerobically. In landfills and sediments, plastics are degraded anaerobically while in composite and soil, aerobic biodegradation takes place. Aerobic biodegradation leads to the production of **water and CO_2**and anaerobic biodegradation results in the formation of **water, $CO_2$and methane** as end products (Barnes,2009). Generally, the conversion of the long chain polymer into $CO_2$and water is complex process. In this process, various different types of microorganisms are needed, with one leads to breakdown of the polymer into smaller constituents, one utilizes the monomers and excrete simple waste compounds as by products and one uses the excreted waste. **The efficiency of this method is moderate but is cheap,** widely accepted and **environment friendly** (Shah,2008).

Sources of the Polythene Degrading Microbes

Following sites were reported to be rich source of polythene degrading microbes:

a. Rhizosphere soil of mangroves.

b. Polythene buried in the soil.

c. Plastic and soil at the dumping sites.

d. Marine water.

Mechanism of Polythene Biodegradation

Microorganisms

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Secretion of extracellular enzymes

↓

Adherence of enzymes to the plastic surface

↓

Cleavage of polymer chains

↓

Erosion of plastic surface i.e. Biodegradation

↓

End products like CO_2, H_2O and CH_4 are produced

Figure 1: Mechanism of Enzymatic Biodegradation of Plastic.

Mechanism of Polythene Biodegradation

The degradation of polythene begins with the attachment of microbes to its surface. Various bacteria (*Streptomyces viridosporus* T7A, *Streptomyces badius* 252, and *Streptomyces setonii* 75Vi2) and wood degrading fungi produced some extracellular enzymes which leads of degradation of polythene(Kim,2005).In wood degrading fungi, the extracellular enzymatic complex (ligninolytic system) contains **peroxidases**, **laccases** and **oxidases** which lead to the production of extracellular hydrogen peroxide (Ruiz,2009). Depending upon the type of the organism or strain and culture condition, the characteristics of this enzyme system varies (Kirk,1987).For degradation of lignin, three enzymes such as **lignin peroxidase** (LiP), **manganese peroxidase** (MnP) and **phenoloxidase** containing copper also known as laccase(Maciel,2010) are involved in the degradation process.Based on the capabilities of these lingolytic enzymes, they are being used in various industries such as **agricultural, chemical**, **cosmetic**, **food,fuel,paper**, **textile**, and more interesting point is that they are also reported to be involved in the degradation of **xenobiotic compounds and dyes**(Maciel,2010).During lignin degradation, phenolic compounds are being oxidized in the presence of $H_2O_2$and manganese by manganese peroxidase (MnP). MnP oxidizes Mn-II to Mn-III and monomeric phenols (Wariishi, 1988).phenolic lignin dimmers (Wariishi, 1989).and synthetic lignin (Wariishi, 1991).are in turn oxidized by Mn-III via the formation of phenoxy radicals (Kim,2005). There is no such report in case of polythene degradation but a similar trend is predicted. The by products of the polythene varied depending upon the conditions of degradation. Under **aerobic conditions**, CO_2, water and microbial biomass are the final degradation products whereas in case of **anaerobic/ methanogenic** condition CO_2, water, methane and microbial biomass are the end products and under **sulfidogenic condition** H_2S, $CO_2$and H_2O and microbial biomass are reported to be the end products (rutchelvi,2008).

Determination of Polythene Degradation

The level of polythene degradation can be determined by the various methods as well as analytical techniques and the detail is given in Table 1. At topographical level, **the Scanning Electron Microscopy (SEM)**is being used to see the level of scission and attachment of the microbes on the surface of the polythene before and after the microbial attack (Sivan,2011). The microdestruction of the small samples is widely analyzed by an important tool such **as Fourier Transform Infrared spectroscopy (FT-IR),** and due to the recent up-gradation of this instrument the map of the identified compounds on the surface of the sample can be documented via collection of large number of FT-IR spectra (Prati,2010).**To measure the physical changes of the polythene** after the microbial attack various parameters are usually used to determine the **weight loss,percentage of elongation** and **change in tensile strength** (Table 1). The products from polythene degradation are also characterized using various techniques such as **Thin Layer Chromatography (TLC),High Performance Liquid Chromatography (HPLC)** and **Gas Chromatography-Mass Spectrometry (GC-MS)** (Table 1).

S.NO	Type of polythene used.	Techniques used to assess polythene degradation	Source of the microbes used	Level of Identification	Name of the microbes / enzymes responsible	Major findings/ conclusions
1	Polythene carry bags	Percentage of weight, surface corrosion, tensile strength	Plastic dumping sites	Morphological keys and Biochemical tests	Bacilliuscerues and Psedomonas sp.	After 3 months of regular shaking the polythene discs were corroded on the surface and tensile strength decreases and maximum 12.5% weight loss was recorded.
2	Polythene bags and plastic cups	Weight loss	Five sources: Medicinal Garden soil, (B) Sewage Water Soil, (C) Energy Park soil, (D) Sludge Area soil, (E) Agricultural Soil	Morphological keys and biochemical tests	B2(Bacillus subtilis), B3(Staphylococcus aureus), B4(Streptococcus lactis), B5(Proteus vulgaris),B6 (Micrococcus luteus), F1(Aspergillusniger) , F2(Aspergillusnidul ance), F3(Aspergillus flavus), F4 (Aspergillusglaucus) , F5(Penicillium	After one month of incubation in both bacterial and fungal isolates the maximum degradation by fungi (Aspergillusniger) and bacteria (Streptococcus lactis) was found as 12.25% and 12.5 % respectively
3.	Branched low-density (0.92 g cm−3) polyethylen e	Gravimetric and molecular weight loss, FTIR	Soil	Molecular level (Using 16S rDNA)	Brevibaccillusborste lensis strain 707	11% (gravimetric) and 30% (molecular) weights loss was reported at 50oC after 30 days
4	Branched low-density (0.92 g cm−3) polyethylen e with an average molecular weight of 191,000	Weight loss, SEM analysis and formation of extracellular protein and polysaccharide in biofilm of R. ruber strain C208 on polyethylene	Not specified	Not specified	Rhodococcusruber (C208)	7.5% of polythene weight loss after eight weeks
5	Branched low-density (0.92 g cm−3) polyethylen e ruber	Weight loss, SEM analysis and formation of extracellular protein and polysaccharide in	15 sites at which polyethylene waste from agricultural use (mainly films for soil	Molecular level (16S rDNA sequencing	*Rhodococcusruber*C 208	8% of polyethylene degradation in 4 weeks

		biofilm of *R. ruber*strain C208 on polyethylene	mulching) had been buried			
6	Low density polythene and polythene	Tensile strength, elongation and percent of extension	Plastics and soil from the plastic dumping site	Morphological keys and biochemical tests	Pseudomonas stutzeri	After 45 days maximum change in percent extension (73.38% reduction), tensile strength (0.01 N/cm2 and it was similar even after 15 and 30 days) and elongation (1.8cm) of the polythene was recorded
7	Polythene carry bags	Weight loss	Polythene dumping site	Morphological keys and biochemical tests	Serretiamarscence	22.22 % of polythene degradation per month was recorded at pH 4, room temperature with regular shaking
8	Commercially environmentally degradable polythene	Epifluorescence microscopy, Scanning Electron Microscopy and FTIR spectroscopy	American Type culture collection and one was their own isolate	Known cultures were used	*Rhodococusrhodocorous*ATCC 29672, *Cladosporiumcladosporides*ATCC 20251 and *Nocardia steroids* GK 911	After 243 days cross linking and chain scission was observed at higher temperatures leads to reduction in the molecular weigh
9	Extruded low-density polyethylene (LDPE) with 20-micron thickness	SEM and FT-IR	Not specified	Known cultures were used	Staphylococcus epidermis	After 30 days of incubation was nearly 12% (*Arthrobacter*sp.) and 15% (*Pseudomonas* sp)
10	Polythene carry bags and cups	Weight loss and reduction in tensile strength	Two types of sources: naturally buried polythene carry bags and cups in municipal composite and polythene strips were intentionally buried in the composite soil along with the solid waste	Morphological keys, biochemical tests and molecular markers	*Bacillus cereus* (C1)	Pre-treated BPE10 after 3 month of incubation with the *B. cereus* (C1) changes its tensile strength up to 17.036% and 17.4o reduction in Contact angl.

			of municipality corpora			
11	Polyethylene bag wastes (pure water sachets)	Percentage of weight loss	Soil samples in a refuse dumping site	Not specified	Pseudomonas aeruginosa, Pseudomonas putida, Bacillus subtilis*and* Aspergillusniger	After 8 weeks, only 1.19% weight loss was recorded when treated with 0.5 M HNO3 followed by slight change in the colour
12	Polythene bags and plastic cups	Percentage of weight loss	Mangroves rhizosphere soil	Morphological keys were used	*Streptococcus, Staphylococcus, Micrococcus* (*Gram +ve*)*, Moraxella, and Pseudomonas* (*Gram –ve*) and two species of fungi (*Aspergillusglaucus*and *A. niger*)	20.54 ± 0.13 (*Psedumonas*sp.) 28.80 ± 2.40 (*Aspergillusglaucus*) percent of weight loss per month
13	Polythene carry bags	Weight loss, TLC, GC-MS and FTIR analyses	Plastic dumping sites, ARI, Pune and NCL Pune	Morphological keys and Biochemical tests	Serratiamarcescens*724*, Bacillus cereus, Pseudomonas aeruginosa , StreptococusaureusB*-324*, Micrococcus lylae*B-429*, Phanerochaetechrysosporiu, Pleurotusostretus, Aspergillusniger*and* Aspergillusglaucus	After eight months of regular shaking maximum percentage of weight loss was recorded at room temperature with pH 4 i.e., 50% with fungi (*Phanerochaetechrysosporium*) and 35% with bacteria (*Pseudomonas aeruginosa*)
14	Natural polyethylene (6% vegetable starch) and synthetic polyethylene	Percentage of weight loss	Three sites: 1. Soil from domestic waste disposal site. 2. Soil from textile effluents drainage site and 3. Soil dumped with sewage sludge	Morphological keys and biochemical tests	*Pseudomonas spp.* (P1, P2, and P3)	The highest weight loss percentage of natural polythene (46.2%) and synthetic polythene (29.1%) was reported with *Pseudomonas* sp. collected from sewage sludge dumping site
15	High density polyethylene films of 0.1µm thickness	DSC, X-ray diffraction XRD, FTIR and SEM	Not specified	Not specified	Penicilliumpinophilum*and* Aspergillusniger	After 31 months maximum 5% reduction in crystallinity (*Aspergillusniger*), 11.07% change in crystalline thickness

						(*Pencilliumpinophilum*), *P. pinophilum*incubated with and without ethanol showed a higher TO-LDPE biodegradation efficiency than did *A. niger*. Mineralization was also higher for *P. pinophilum*with the addition of ethanol

Toxicity Level of the Biodegraded Polythene Products

Based on the literatures consulted there was no report on this aspect except (Aswale,2010).She tested the toxicity level of all the polythene biodegraded products on both the animal and plant systems. Among the plant systems, she tested the toxicity level of the degraded polythene products along with culture filtrate on the seed germination rate of the *Arachis hypogaea* (groundnut), *Glycine max*. (soybean), *Sesamum laciniatum* (oil seed, sesame), *Helianthus annuus* (sunflower) and *Carthamus tinctorius* (safflower). Moderate decrease in the germination of the seeds was recorded. For the animal system, she calculated the mortality rate of *Chironomous* larvae, and had not reported any significant difference in the mortality rates as compare to control.

Future Needs

The status of **polythene pollution should be updated area wise**. The awareness campaign of the polythene pollution should be promoted at mass level among the public. **The idea of using starch based polythene or biodegradable polythene** should be encouraged. **The microbes responsible for the degradation of polythene should be isolated from all the sources, screened to know the efficient isolates.** The efficient microbes are needed to characterize at molecular level. Some extracellular enzymes are responsible for the biodegradations of the polythene (Aswale, 2008). **These enzymes needed to be characterized and the genes responsible for those enzymes should be worked out**. **Once the genes responsible for the degradation of polythene would be known, the genes would be used to enhance the polythene degrading capacity of the other easily available microbes**. After field trials, the most efficient polythene degrading microbes should be multiplied at large scale to decompose the polythene at commercial level.

Conclusions

Based on the literature survey, it can be concluded that polythene is very useful in our day to day life to meet our desired needs. It can be used for wrapping the goods, food material, medicine, scientific instruments etc. Due to its good quality its use is increasing day by day and its degradation is becoming a great threat. Only in the marine biota annually almost one million marine animals are dying due to their intestinal blockage. Various polythene degradation methods are available but the cheapest, eco-friendly and acceptable method is degradation using microbes. The microbes release the extracellular enzymes such as lignin peroxidase, manganese peroxidase to degrade the polythene but the detailed characterization of these enzymes in relation to polythene degradation is still needed to be carried out, soas**the genes responsible for the degradation of polythene would be known, the genes would be used to enhance the polythene degrading capacity of the other easily available microbes**.

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