

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. In the present review, an attempt has been made to pool all the available literature on the biodegradation of polythene under the following objectives: (1) to highlight the level of polythene pollution; (2) to enlist the cost effective methods; (3) to pool the source of polythene degrading microbes; (4) to brief the mechanism of polythene degradation; (5) to highlight the methods used for the biodegradation of the polythene; (6) to discuss the assessment of polythene degradation by efficient microbes; (7) to enlist the products of polythene under degradation process; (8) to test the toxicity level of the products of the degraded polythene, and (9) to discuss the future aspects of polythene degradation.

Keywords: Biodegradation, Polythene, Microbes, Waste, Biodegraded products, Toxicity

Introduction

The contamination of soil due to dispersal of industrial and urban wastes generated by the human activities is of great environmental concern [1]. Various plants possess the capacity to convert the toxic compounds into non-toxic forms and the process is known as phytoremediation. The concept of cleaning contaminated environment using plants is about 300 years old [2]. One of the major environmental threat is the slow/least rate of degradation or non-biodegradability of the organic materials under natural condition, e.g. plastics. The plastics of various forms such as nylon, polycarbonate, polyethylene-terephthalate, polyethylene, polypropylene, polystyrene, polytetrafluoroethylene, polyurethane, polyvinyl chloride are being continuously used in our day-to-day life [3]. Among the synthetic plastics waste produced, polythene shares about 64% [4]. 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 C_nH_{2n} , where 'n' is the number of carbon atoms [5]. Polythene is made from the cheap petrochemical stocks extracted from oil or gas through efficient catalytic polymerization of ethylene monomers [6]. 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 [5]. Various polymers such as lignin and paraffins were reported to be degraded by various microorganisms [6,7]. Jen-hou and Schwartz [8] carried out the comparative degradation study of paraffins and polythene for the first time and recorded utilization of polythene in terms of the growth of various bacteria on these alkenes. They concluded that microbes can degrade only low molecular weight polythene (MW up to 4800). Nineteen years later, degradation of high density polythene (HDPE) film (Mw 93000) was performed and it was documented that the main degraded component contained in HDPE film is the short-chain oligomer [9]. There is no such structural similarity between polythene and lignin except to have carbon-carbon bonding which is being broken by these microbes and using the polymers as a carbon source.

In the literature, various reviews had been written on biodegradation of the plastic [10-18]. Only a few review [19,20] deals with polythene but a comprehensive review on the polythene is lacking, so we tried to

highlight the glimpses of the polythene biodegradation. We also tried to discuss, how to encounter the polythene pollution in future.

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 [4-21]. 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 [4]. Similarly, in the marine environment alone, out of total marine waste, plastic shares about 60-80% by mass [10]. 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 [22]. 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 [23]. 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 [23-25]. As per report [26] 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 [27]. The polythene leads to

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Received July 07, 2012; Accepted August 28, 2012; Published August 30, 2012

Citation: Sangale MK, Shahnawaz M, Ade AB (2012) A Review on Biodegradation of Polythene: The Microbial Approach. J Bioremed Biodeg 3:164. doi:10.4172/2155-6199.1000164

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blockage of their digestive tract. It is also found that the 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 [27]. The undigested polythene was found to be responsible for various problems in the animals such as (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 track 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 septicemia [27]. The widely used packaging plastic (mainly polythene) constitutes about 10% of the total municipal waste generated around the globe [28]. As per literature, every year hundred thousand tons of plastics have been degraded in the marine environment resulting death [29]. 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 [30]. 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 [28-31].

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 [32]. 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 [13]. The biodegradation is a natural process of degrading materials through microbes such as bacteria, fungi and algae [29]. 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 CO₂ and anaerobic biodegradation results in the formation of water, CO₂ and methane as end products [33]. Generally, the conversion of the long chain polymer into CO₂ 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 environment friendly. This method is cheap and widely accepted [13]. Depending upon the formulation of the biodegradable polythene carry bags, three types along with one standard polythene, were studied for their degradation potential in the marine water. It was reported that after 40 weeks of exposure period the surfaces of the biodegradable polythene carry bags degraded less than 2% whereas the degradation of standard polythene was negligible [34]. The major consequences in the bio-degradation of polythene are enlisted briefly in the Table 1.

Sources of The Polythene Degrading Microbes

Following sites (Table 1) 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

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 [35,36,7]. In wood degrading fungi, the extracellular enzymatic complex (ligninolytic system) contains peroxidases, laccases and oxidases which leads to the production of extracellular hydrogen peroxide [37]. Depending upon the type of the organism or strain and culture condition, the characteristics of this enzyme system varies [38]. For degradation of lignin, three enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and phenoloxidase containing copper also known as laccase [7,39]. 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 [39]. During lignin degradation, phenolic compounds are being oxidized in the presence of H₂O₂ and manganese by manganese peroxidase (MnP). MnP oxidizes Mn-II to Mn-III and monomeric phenols [40], phenolic lignin dimmers [41] and synthetic lignin [42] are in turn oxidized by Mn-III via the formation of phenoxy radicals [36]. There is no such report in case of polythene degradation but a similar trend is predicted. The byproducts of the polythene varied depending upon the conditions of degradation. Under aerobic conditions, CO₂, water and microbial biomass are the final degradation products whereas in case of anaerobic/ methanogenic condition CO₂, water, methane and microbial biomass are the end products and under sulfidogenic condition H₂S, CO₂ and H₂O and microbial biomass are reported to be the end products [5].

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) are 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 [43]. 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 [44]. 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).

Sr. No.	Title of the paper	Type of the polythene used	Techniques used to assess polythene degradation	Source of the microbes used	Major findings/ conclusions/inferences	Level of Identification	Name of the microbes / enzymes responsible	Reference
1.	Assessment of the biodegradation of polythene	Polythene carry bags	Percentage of weight, surface corrosion, tensile strength	Plastic dumping sites	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.	Morphological keys and Biochemical tests	<i>Bacillus cerues</i> and <i>Pseudomonas sp.</i>	[56]
2.	Biodegradation of degradable plastic polyethylene by <i>Phanerochaete</i> and <i>Streptomyces species</i>	degradable plastic contained pro-oxidant and 6% starch	Weight loss, changes in tensile strength, percent elongation and molecular weight distribution	The lignocellulose degrading microorganisms (not specified the site of collection)	50% reduction in tensile strength (<i>S. viridosporus</i> T7A).	Not specified	<i>Streptomyces viridosporus</i> T7A, <i>S. badius</i> 252, and <i>S. setonii</i> 75Vi2 (bacteria) and <i>Phanerochaete chrysosporium</i> (fungus)	[4]
3.	Biodegradability of polythene and plastic by the help of microorganism: a way for brighter future	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	After one month of incubation in both bacterial and fungal isolates the maximum degradation by fungi (<i>Aspergillus niger</i>) and bacteria (<i>Streptococcus lactis</i>) was found as 12.25% and 12.5 % respectively	Morphological keys and biochemical tests	B1(<i>Pseudomonas</i>), B2(<i>Bacillus subtilis</i>), B3(<i>Staphylococcus aureus</i>), B4(<i>Streptococcus lactis</i>), B5(<i>Proteus vulgaris</i>), B6 (<i>Micrococcus luteus</i>), F1(<i>Aspergillus niger</i>), F2(<i>Aspergillus nidulance</i>), F3(<i>Aspergillus flavus</i>), F4 (<i>Aspergillus glaucus</i>), F5(<i>Penicillium</i>)	[57]
4.	Biodegradation of polyethylene by the thermophilic bacterium <i>Brevibacillus borstelensis</i> .	Branched low-density (0.92 g cm ⁻³) polyethylene	Gravimetric and molecular weight loss, FTIR	Soil	11% (gravimetric) and 30% (molecular) weights loss was reported at 50°C after 30 days	Molecular level (Using 16S rDNA)	<i>Brevibacillus borstelensis</i> strain 707	[58]
5.	Biodegradability of polyethylene starch blends in sea water	Pure polyethylene (5% starch) and modified polyethylene films (8% starch) and polyethylene with pro-degradant additives (master batch in amount of 20%)	Changes in weight, tensile strength and morphology of polymer	Microbes of the Baltic sea as the incubation of polymer samples was carried out in Baltic Sea water	For polyethylene blends in the sea water very little microbial degradation was observed in winter but in summer months the weight loss of polyethylene with the MB additive after 20 months reached 26%	Not specified	Not applicable	[29]
6.	Biodegradation of low density polyethylene (LDPE) by fungi isolated from marine water– a SEM analysis	LDPE in the powdered form	Sturm test where the degradation was attributed to the amount of carbon dioxide evolved and SEM analysis.	Sea water	Per week maximum 4.1594 g/L of CO ₂ was released after degradation of the polythene	Morphological keys	<i>Aspergillus versicolor</i> and <i>Aspergillus sp.</i>	[51]
7.	Biodegradation of low density polythene (LDPE) by <i>Pseudomonas species</i>	LDPE films	Weight measurements, tensile strength testing, FTIR-ATR spectrophotometer analyses, Scanning Electron Microscope based analyses and GC-MS analyses.	Known cultures but source was not specified	The highest level of polythene degradation (weight loss) out of the four bacteria was found as 20% by <i>Pseudomonas aeruginosa</i> after 120 days	Not applicable	<i>Pseudomonas aeruginosa</i> PAO1 (ATCC 15729), <i>Pseudomonas aeruginosa</i> (ATCC 15692), <i>Pseudomonas putida</i> (KT2440 ATCC 47054) and <i>Pseudomonas syringae</i> (DC3000 ATCC 10862)	[55]
8.	Biodegradation of maleated linear low-density polyethylene and starch blends	linear low-density polyethylene torque blended with starch	FTIR spectroscopy, weight loss, SEM, DSC, TGA.	Source of the microbes not specified but known cultures were used	The starch content in the blend was found directly proportional to the rate of degradation. Thus, higher the content of starch, higher will be the degree of degradation.	Not applicable	<i>Aspergillus niger</i> , <i>Penicillium funiculosum</i> , <i>Chaetomium globosum</i> , <i>Glucadium virens</i> and <i>Pullularia pullulans</i>	[59]

9.	Biodegradation of photo-degraded mulching films based on polyethylenes and stearates of calcium and iron as pro-oxidant additives	LDPE and LLDPE	Chemiluminescence, ATR-FTIR and GC-product analysis	Polythene films were scattered in agricultural vegetable field and after 30 days were used for the isolation of microbes	Polythene films 75-85% (containing Fe stearate) and 31-67% (containing Ca stearate) at 45°C leads to reduction in carbonyl index	Molecular level (16S rRNA gene sequencing)	<i>Bacillus cereus</i> , <i>B. megaterium</i> , <i>B. subtilis</i> and <i>Brevibacillus borstelensis</i>	[53]
10.	Biofilm development of the polyethylene-degrading bacterium <i>Rhodococcus ruber</i>	Branched low-density (0.92 g cm ⁻³) polyethylene with an average molecular weight of 191,000	Weight loss, SEM analysis and formation of extracellular protein and polysaccharide in biofilm of <i>R. ruber</i> strain C208 on polyethylene	Not specified	7.5% of polythene weight loss after eight weeks	Not specified	<i>Rhodococcus ruber</i> (C208)	[54]
11.	Colonization, biofilm formation and biodegradation of polyethylene by a strain of <i>Rhodococcus ruber</i>	Branched low-density (0.92 g cm ⁻³) polyethylene	Average Weight loss, Scanning electron microscopy ATR and FTIR	15 sites at which polyethylene waste from agricultural use (mainly films for soil mulching) had been buried	8% of polyethylene degradation in 4 weeks	Molecular level (16S rDNA sequencing)	<i>Rhodococcus ruber</i> C208	[60]
12.	Comparison of the biodegradability of various polyethylene films containing prooxidant additives	HDPE, LDPE and LLDPE with a balanced content of antioxidants and pro-oxidants	FTIR, SEC measurements, H NMR spectroscopy and SEM	American Type Culture	They concluded that the biodegradation is mainly controlled by nature of the pro-oxidant additive and to a lesser extent that of the matrix	Known microbe was used	<i>Rhodococcus rhodochrous</i> ATCC 29672	[61]
13.	Degradation assessment of low density polythene (LDP) and polythene (PP) by an indigenous isolates of <i>Pseudomonas stutzeri</i>	Low density polythene and polythene	Tensile strength, elongation and percent of extension	Plastics and soil from the plastic dumping site	After 45 days maximum change in percent extension (73.38% reduction), tensile strength (0.01 N/cm ² and it was similar even after 15 and 30 days) and elongation (1.8cm) of the polythene was recorded	Morphological keys and biochemical tests	<i>Pseudomonas stutzeri</i>	[62]
14.	Diversity and effectiveness of tropical mangrove soil microflora on the degradation of polythene carry bags	HDPE and LDPE	Mean weight	Mangrove soil sample from Suva, Fiji Islands	Nearly 5 % of weight loss after a period of eight weeks	Morphological keys and biochemical tests	<i>Bacillus</i> , <i>Micrococcus</i> , <i>Listeria</i> and <i>Vibrio</i>	[63]
15.	Diversity of cellulolytic microbes and the biodegradation of municipal solid waste by a potential strain	Municipal solid waste	Weight loss and cellulose enzyme production	Municipal solid waste, soil and compost	With the potential strain (<i>Trichoderma viride</i>) out of the 250 isolates (49 cellulolytic) after 60 days, the average weight loss was 20.10% in the plates and 33.35% in the piles	Morphological keys and biochemical tests	Total 250 isolates (165 belongs to fungi and 85 bacteria)	[64]
16.	Effect of pH on biodegradation of polythene by <i>Serratia marscence</i>	Polythene carry bags	Weight loss	Polythene dumping site	22.22 % of polythene degradation per month was recorded at pH 4, room temperature with regular shaking	Morphological keys and biochemical tests	<i>Serratia marscence</i>	[65]
17.	Effect of pro-oxidants on biodegradation of polyethylene (LDPE) by indigenous fungal isolate, <i>Aspergillus oryzae</i>	LDPE with average molecular weight of 1,80,000 Daltons and 8.7 PDI	Weight loss, tensile strength and percentage of elongation, FTIR spectroscopy, SEM analyses	Previously reported fungi [59]	Maximum 47.2% weight loss, 51% reduction in tensile strength and 62% reduction in percentage of elongation of LDPE (treated with manganese stearate followed by UV irradiation and incubation with <i>A. oryzae</i> for 3 months).	Known isolates was used	<i>Aspergillus oryzae</i>	[46]

18.	Environmental biodegradation of polyethylene	Commercially environmentally degradable polythene	Epifluorescence microscopy, Scanning Electron Microscopy and FTIR spectroscopy	American Type culture collection and one was their own isolate	After 243 days cross linking and chain scission was observed at higher temperatures leads to reduction in the molecular weight	Known cultures were used	<i>Rhodococcus rhodocorou</i> ATCC 29672, <i>Cladosporium cladosporides</i> ATCC 20251 and <i>Nocardia steroids</i> GK 911	[66]
19.	Enzyme-mediated biodegradation of heat treated commercial polyethylene by <i>Staphylococcus</i> species	Extruded low-density polyethylene (LDPE) with 20-micron thickness	SEM and FT-IR	Not specified	Organism BP/ SU1 degrading the polyethylene layer and creating holes in it. Different extracellular enzymes were responsible for the degradation of shredded polyethylene	Known cultures were used	<i>Staphylococcus epidermis</i>	[67]
20.	High-density polyethylene (HDPE)-degrading potential bacteria from marine ecosystem of Gulf of Mannar, India	High-density polyethylene (HDPE) (Commercially available HDPE)	Weight loss, percentage of crystallinity and Fourier transform infrared (FT-IR) spectrum	Partially degraded polyethylene along with soil samples adhering and adjacent to it was collected from 15 plastic waste dumped sites	After 30 days of incubation was nearly 12% (<i>Arthrobacter</i> sp.) and 15% (<i>Pseudomonas</i> sp)	Not specified	<i>Arthrobacter</i> and <i>Pseudomonas</i> sp.	[68]
21.	Impact of soil composting using municipal solid waste on biodegradation of plastics	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 of municipality corporation	In compost culture highest percentage of weight loss (11.54%) was recorded in LDPE1 after 12 months whereas highest percent loss in tensile strength was reported with HDPE1 in same time of incubation	Both morphological keys and biochemical tests were used	Following were predominant bacteria (<i>Bacillus</i> sp., <i>Staphylococcus</i> sp., <i>Streptococcus</i> sp., <i>Diplococcus</i> sp., <i>Micrococcus</i> sp., <i>Pseudomonas</i> sp. and <i>Moraxella</i> sp) and fungi (<i>Aspergillus niger</i> , <i>A. ornatus</i> , <i>A. nidulans</i> , <i>A. cremeus</i> , <i>A. flavus</i> , <i>A. candidus</i> and <i>A. glaucus</i>) found to be associated with degraded polythene bags and cups after 12 month	[69]
22.	Investigation on biodegradability of polyethylene by <i>Bacillus cereus</i> strain Ma-Su isolated from compost soil	LDPE and BPE 10 (10 % oxo-biodegradable additive)	Change in tensile strength, percent elongation, FT-IR spectroscopy, Contact angle and surface energy and SEM analyses	Municipal compost yard	Pre-treated BPE10 after 3 month of incubation with the <i>B. cereus</i> (C1) changes its tensile strength up to 17.036% and 17.4o reduction in Contact angl.	Morphological keys, biochemical tests and molecular markers	<i>Bacillus cereus</i> (C1)	[70]
23.	Occurrence and recalcitrance of polyethylene bag waste in Nigerian soils	Polyethylene bag wastes (pure water sachets)	Percentage of weight loss	Soil samples in a refuse dumping site	After 8 weeks, only 1.19% weight loss was recorded when treated with 0.5 M HNO ₃ followed by slight change in the colour	Not specified	<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas putida</i> , <i>Bacillus subtilis</i> and <i>Aspergillus niger</i>	[71]
24.	Polymer Biodegradation of disposable polyethylene by fungi and <i>Streptomyces</i> species	Disposable plastic films	Average weight loss, change in tensile strength and percent elongation	Nile River Delta (<i>Streptomyces</i>), Northern Regional Research Laboratory USDA (fungi <i>Mucor rouxii</i> 1835) their own culture collection (<i>Aspergillus flavus</i>)	The average reduction in the percent elongation with bacterial and fungal cultures were recorded as 28.5% and 46.5% respectively. This was preliminary report of extracellular enzyme(s) responsible for degrading of attacking degradable polythene (ten days heat treated)	Morphological keys	Eight <i>Streptomyces</i> strains and two fungi, <i>M. rouxii</i> NRRL 1835 and <i>Aspergillus flavus</i>	[48]

25.	Polythene and plastics-degrading microbes from the mangrove soil	Polythene bags and plastic cups	Percentage of weight loss	Mangroves rhizosphere soil	20.54 ± 0.13 (<i>Pseudomonas</i> sp.) 28.80 ± 2.40 (<i>Aspergillus glaucus</i>) percent of weight loss per month in shaker culture	Morphological keys were used	<i>Streptococcus</i> , <i>Staphylococcus</i> , <i>Micrococcus</i> (Gram +ve), <i>Moraxella</i> , and <i>Pseudomonas</i> (Gram –ve) and two species of fungi (<i>Aspergillus glaucus</i> and <i>A. niger</i>)	[72]
26.	Polyethylene degradation by lignin-degrading fungi and manganese peroxidase	High-molecular-weight polyethylene	Changes in relative elongation and relative tensile strength (Strograph-R3) and polyethylene molecular weight distribution (Waters model 150 -C)	Not specified	Relative elongation (91.2 ± 9.0 %) Relative tensile strength (100.0 ± 1.3 %) were recorded using MnP treated with 0.2mM MnSO ₄ and 50mM acetate. MnP is the key enzyme in polyethylene degradation by lignin-degrading fungi	Not specified	<i>Phanerochaete chrysosporium</i> ME-446, <i>Trametes versicolor</i> IFO 7043, and IZU-15413	[7]
27.	Polyethylene biodegradation by a developed <i>Penicillium</i> – <i>Bacillus</i> biofilm	Degradable polyethylene	Percent weight loss and emission of CO ₂ gas chromatography (GC)	Different types of polythene were dumped under soil were used for isolation of microbes after 2–4 years	When <i>P. frequentans</i> and <i>B. mycoides</i> were used together Weight loss 7.150 % (pre-heated at 70°C) and 6.657% (unheated) after 60 days	Morphological keys and biochemical tests	The most effective fungi and bacteria were <i>Penicillium frequentans</i> and <i>Bacillus mycoides</i>	[50]
28.	Polythene degradation potential of <i>Aspergillus niger</i>	Polythene carry bags	Weight loss	Polythene dumping site	25% of weight was observed after 8 months with regular shaking	Morphological keys	<i>Aspergillus niger</i>	[73]
29.	Production of an extracellular polyethylene-degrading enzyme(s) by <i>Streptomyces species</i>	Starch-polyethylene-prooxidant degradable plastics	FTIR spectra, mechanical properties, and polyethylene molecular weight distributions	Lignocellulose-degrading microbes but source was not specified	All three bacterial extracellular enzyme concentrates leads to detectable changes in the degradable plastic as determined by the FT-IR spectrometer and tensile strength (kg/mm ²) % elongation strain energy (Kg mm)	Known cultures were used	Extracellular enzymes of the following microbes such as <i>Streptomyces badius</i> 252, <i>Streptomyces setonii</i> 75Vi2, and <i>Streptomyces viridosporus</i> T7A	[35]
30.	Screening of polyethylene degrading microorganisms from garbage soil	Low density polyethylene powder	Weight loss	Garbage soil samples (waste disposable site dumped with polythene bag and plastic cup	<i>Actinomycetes</i> (<i>Streptomyces</i> KU8) leads to 46.16% weight loss of the polythene whereas bacteria (<i>Pseudomonas</i> sp) and fungi (<i>Aspergillus flavus</i>) degraded only 37.09% and 20.63 % after six months	Morphological keys and biochemical tests	<i>Streptomyces</i> KU8, <i>Streptomyces</i> KU5, <i>Streptomyces</i> KU1, <i>Streptomyces</i> KU6, <i>Pseudomonas</i> sp., <i>Bacillus</i> sp., <i>Staphylococcus</i> sp., <i>Aspergillus nidulans</i> and <i>A. flavus</i>	[74]
31.	Studies on biodegradation of polythene	Polythene carry bags	Weight loss, TLC, GC-MS and FTIR analyses	Plastic dumping sites, ARI, Pune and NCL Pune	After eight months of regular shaking maximum percentage of weight loss was recorded at room temperature with pH 4 i.e., 50% with fungi (<i>Phanerochaete chrysosporium</i>) and 35% with bacteria (<i>Pseudomonas aeruginosa</i>)	Morphological keys and Biochemical tests	<i>Serratia marcescens</i> 724, <i>Bacillus cereus</i> , <i>Pseudomonas aeruginosa</i> , <i>Streptococcus aureus</i> B-324, <i>Micrococcus lylae</i> B-429, <i>Phanerochaete chrysosporium</i> , <i>Pleurotus ostreatus</i> , <i>Aspergillus niger</i> and <i>Aspergillus glaucus</i>	[47]
32.	Studies on the biodegradation of natural and synthetic polyethylene by <i>Pseudomonas spp</i>	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	The highest weight loss percentage of natural polythene (46.2%) and synthetic polythene (29.1%) was reported with <i>Pseudomonas</i> sp. collected from sewage sludge dumping site	Morphological keys and biochemical tests	<i>Pseudomonas</i> spp. (P1, P2, and P3)	[75]

33.	Synergistic effect of chemical and photo treatment on the rate of biodegradation of high density polyethylene by indigenous fungal isolates	High density polyethylene films of 0.1µm thickness	Tensile strength, percentage of elongation, elongation break and FTIR analysis	High density polyethylene (HDPE) film buried in soil 3 months and then used as a sources of microbes	<i>Aspergillus oryzae</i> leads 72% reduction in percentage of elongation and abiotically treated HDPE film clearly showed generation of carbonyl peak at 1718.32 cm as compare to control	Molecular level (16S rDNA sequencing)	<i>Aspergillus niger</i> , <i>Aspergillus flavus</i> and <i>Aspergillus oryzae</i>	[76]
34.	Thermally treated low density polyethylene biodegradation by <i>Penicillium pinophilum</i> and <i>Aspergillus niger</i>	Powdered LDPE	DSC, X-ray diffraction XRD, FTIR and SEM	Not specified	After 31 months maximum 5% reduction in crystallinity (<i>Aspergillus niger</i>), 11.07% change in crystalline thickness (<i>Penicillium pinophilum</i>), <i>P. pinophilum</i> incubated with and without ethanol showed a higher TO-LDPE biodegradation efficiency than did <i>A. niger</i> . Mineralization was also higher for <i>P. pinophilum</i> with the addition of ethanol	Not specified	<i>Penicillium pinophilum</i> and <i>Aspergillus niger</i>	[52]

Table 1: The major consequences in the biodegradation of polythene.

Maximum Biodegradation of Polythene both *In Vitro* and *In Vivo*

The maximum 61.0% (*Microbacterium paraoxydans*) and 50.5% (*Pseudomonas aeruginosa*) of polythene degradation in terms of Fourier Transform Infrared coupled Attenuated Total Reflectance (FTIR-ATR) was recorded [45] within two months. But in terms of weight loss was the degradation of polythene was recorded as 47.2% after 3 months of incubation with the *A. oryzae* [46] followed by 50% weight loss of the polythene discs using fungus, *Phanerochaete chrysosporium* after 8 month of regular shaking with pH= 4.00 at room temperature [47]. But due to biodegradation, weight loss of the polythene is not always reported. Some workers [48] reported gain in the polythene weight after cultivation of the microbes on the polythene, incubated at regular shaking for one month at 30°C. Only three out of 10 microbes lead to weight loss. The maximum weight gain (2.02%) was reported with *Streptomyces humidus*. The possible reason for gaining of the polythene weight after the cultivation of the microbes on the strips is accumulation of cell mass on the polythene surface [48]. In case of *in vivo* study after 32 years of polythene dumping in the soil only partial degradation was reported [49].

Polythene Biodegradation Products

During polythene biodegradation, CO₂ gas emission was recorded [50-53]. As per report [54] *Rhodococcus rubber* (C208) uses polythene as a carbon source and produces polysaccharides and proteins. Another worker [47] also reported a number of polythene biodegraded products such as Ergosta-5, 22-dien-3-ol, acetate (3, 22 E), 1-Monanalinoeglycerol trimethylsilyl ether, Betamethasone acetate, Azafrin, 9, 12, 15-Octadecatrienoic acid, 2, 3-bis [(trimethylsilyl) oxy] propyl ester, (Z, Z, Z)-C₂₇H₅₂O₄Si₂). A group of workers [55] reported 22 different biodegraded products from the polythene but identified only 18 compounds as Benzene, methyl, Tetrachloroethylene, Benzene, 1,3-dimethyl, Octadecane, 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione, Hexadecanoic acid, Hexadecanoic acid, Ethyl ester, Eicosane, Octadecanoic acid, Docosane, 3-Chloropropionic acid, Heptadecyl ester, Tricosane, Octadecanoic acid, Butyl ester, 1-Nonadecene, Tetracosane, Pentacosane, 1, 2-Benxenedicarboxylic acid, Di-iso-ostyl ester and Hexacosane.

Toxicity Level of the Biodegraded Polythene Products

To the best of our knowledge there is no report on this aspect except Aswale [47]. 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 *Chironomus* 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 [56]. 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 in the literature 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. It was also been known that microbes from various sources are responsible for the degradation of polythene. But efficient polythene degrading microbe is still needed to screen from all the sources. The characterization of efficient polythene degrading microbes at molecular level is still not available up to the mark, which can be multiplied at large scale to commercialize the polythene biodegradation.

Acknowledgement

We are thankful to authorities of Jaykar Library, University of Pune for providing free access of the paid Journals. Authors are thankful to Board of Colleges and university Development (BCUD), University of Pune, Pune for providing financial support for publication. The second author is also thankful to the authorities of University of Pune, Pune-07, for providing research stipend.

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