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Microbes mediated polyethylene biodegradation for environmental sustainability

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Abstract

The increased use of polyethylene in various aspects of our lives has undeniably brought convenience. Tons of polyethylene waste accumulates in landfills due to inadequate recycling processes posing threats to both environmental sustainability and human health. Addressing this issue requires effective methods to reduce polyethylene waste, and among these methods, biological degradation stands out as one of the most promising approaches. Microorganisms have ability to utilize polyethylene as carbon source and release various microbial enzymes that break down polymeric unit into small monomers and oligomers. Due to which recent researchers has focused on understanding biodegradation of polyethylene by microbes. In this study diverse group of microorganisms has been analyzed for their potential to degrade polyethylene, understanding the mechanisms of polyethylene biodegradation and enzymatic processes involved in PE degradation and potentially develop effective strategies for addressing polyethylene pollution and its environmental impacts.

Keywords: Polyethylene, environment, biodegradation, plastic pollution, polymerization

Introduction

With advancement in applied sciences, plastic has increasingly taken the place of traditional materials such as glass, metal and wood in numerous applications due to their cost-effective production, long lasting properties with high strength as compared to the traditional material (Ahmed *et al.* 2018) [1]. Plastics, including polypropylene, nylon, polycarbonate, polyethylene, polystyrene, polytetrafluoroethylene, polyvinyl chloride and polyurethane are part of our life (Smith 1964) [56]. Polyethylene accounts for 64% of synthetic plastics waste generated in todays world (Lee 1991) [32]. In 1898, an accident has resulted in the synthesis of polyethylene by a German chemist Hans von Pechmann and that marked initial human discovery of polyethylene. In 1935 Michael Perrin from ICI Chemicals developed controlled high-pressure technology for polyethylene synthesis (Yao *et al.* 2022) [67]. Polyethylene is an linear hydrocarbon polymer that comprises of extended chains of ethylene monomers (C_2H_4). It has an general formula CnH_2n, where 'n' stands for carbon atom count in the chain (Arutchelvi *et al.* 2008) [5]. Polyethylene is product of petrochemical stocks obtained from oil or gas, that is produced by efficient catalytic polymerization of ethylene monomers (Fuhs 1961) [14]. Polyethylene is categorized into various types, including low-density polyethylene (LDPE), high-density polyethylene (HDPE), medium-density polyethylene (MDPE), and very low-density polyethylene (VLDPE) (Rivard *et al.* 1995) [46]. Polyethylene exhibits relatively weak mechanical properties, characterized by low tensile strength and poor creep resistance. However, it does offer good impact resistance (Koriem *et al.* 2021) [30]. At 60 °C, polyethylene is insoluble in most of the common solvents. It has been studied that prolonged exposure to aromatic hydrocarbons, halogenated hydrocarbons or some aliphatic hydrocarbons leads to its degraded properties. At temperatures above 60 °C, polyethylene partially dissolves in few organic solvents such as trichloroethylene, mineral oil and toluene (Lu 2017) [34]. Polyethylene (PE) is recalcitrant and inert material, that makes it an extremely resistant material that is not easy to be degraded in nature. even after being buried in landfills for several years. Under moist soil conditions for periods ranging from 12 to 32 years, a polyethylene sheet has indicated only limited degradation and negligible weight loss (Otake *et al.* 1995) [39]. The recalcitrance of PE to degradation can be attributed to several factors, including its insolubility in water, its hydrophobic nature because of the linear carbon atom chains, its degree of crystallinity, and its high molecular weight (Webb 2013) [64]. As per reports of German statistics company Statista, global production of polyethylene has reached approximately 104.4 million tons (2020) and it is projected to achieve 121.4 million tons target by the year

2026 (Tiseo 2021) [61]. Polyethylene is used by many industries that includes transportation, construction and packaging etc. (Geyer 2020) [19]. Polythene has remarkable strength and durability, that makes it non degradable up to 1000 years under natural environment (Denuncio *et al.* 2011) [11]. Considering the widespread use of polyethylene, it is crucial to focus on recycling and employing biological methods to degrade plastic waste (Ahmed *et al.* 2018) [1]. Environmentally friendly methods of plastic degradation are generally preferred over chemical and physical methods due to less impact on environment. Microorganisms have consistently played a vital role in natural ecosystems as they have capacity to break down a wide range of organic and inorganic substances. Several microorganisms, such as bacteria, fungi, and actinomycetes have ability to degrade polymers up to large extent (Swift 1997) [60].

This review article is an effort to provide a comprehensive review of polyethylene biodegradation using various microbes in ecofriendly manner. Numerous reviews have been published describing biodegradation of plastics in general, barring few that has specifically focused on polyethylene biodegradtion.

Impact of polyethylene pollution on environment

The utilization of polyethylene in large quantities has resulted in imposing significant challenge in its final disposal in nature. Huge dumps of various types of plastics have created problems in its ecofriendly disposal. (Ritchie and Roser 2018) [45]. LDPE and HDPE use by various industries pose significant environmental threats to ecosystem. Instances of polyethylene obstructions in gut of animals, birds, fish and marine inhabitants are burning examples of problems due to plastics (Shimao 2001) [52]. Ingestion of plastic waste has pushed many species belonging to different ecosystems on the brink of endangerment (Spear *et al.* 1995) [58]. Floating polyethylene, owing to its colour and odour, frequently attracts aquatic animals, potentially leading to entanglement or ingestion by marine life. Organ damage, genotoxicity, apoptosis and death of affected individual may result due to toxicity due to plastic wastes (Tiwari *et al.* 2020) [62]. It is estimated that millions of marine animals lose their lives with time as a result of plastic pollution (Mateos-C´ardenas *et al.* 2020) [36]. Smaller-sized plastic particles can indeed be more ecotoxic, as they are more easily ingested by a wider range of organisms and can have detrimental effects on ecosystems at various trophic levels (Prata *et al.* 2019) [42]. The studies indicate that direct toxic effects of microplastics on living beings results in significant histopathological damage and increased levels of antioxidant markers in muscles (Magara *et al.* 2018) [35]. Large marine animals, including seabirds, dolphins, whales, turtles and polar bears face mortality risks either by ingesting polyethylene plastic bags or by consuming fish with ingested polyethylene (Singh *et al.* 2020) [54]. The unavoidable reliance on polyethylene (PE) to enhance agricultural productivity within limited areas has given rise to a substantial social issue (Liu *et al.* 2014) [33]. Polyethylene pollution in agricultural farms is also taking place due to use of polyethylene for mulch, structural material and packaging. Polyethylene microplastic presence in soil adversely effect soil adsorption capability, changes in particle size aggregates, and reduction in microbial biodiversity (Hou *et al.* 2021) [23]. Therefore, polyethylene particles present in soil lead to the increased mobility of hydrophobic organic pollutants, that ultimately contribute to pollution related issues (Huffer *et al.* 2019) [24]. Balzani and co-workers have reported that there is rise in honeybee population mortality when exposed to elevated concentrations of polyethylene microparticles (Balzani *et al.* 2022) [7]. Reports have documented cases of terrestrial animals, like cows, succumbing to death because of ingesting polythene carry bags (Singh 2005) [53]. Packaging plastic, specifically polythene, is responsible for around 10% of the total municipal waste generated globally (Barnes *et al.* 2009) [8]. Small lots of these polythene wastes are subjected to recycling, whereas majority of it ends up in landfills, where degrades slowly producing toxic xenobiotics for hundred of years (Moore 2008) [37].

Mechanism of polyethylene biodegradation

Polyethylene biodegradation is a process where living organisms, such as invertebrates, bacteria, fungi, algae and several others, unwind complex polymers of polyethylene for utilizing them as carbon source. Microbial populations are considered potential candidates for polyethylene biodegradation as they have excellent environmental adaptability and have capacity to utilize range of chemicals as substrates needed for survival and multiplication (Yao *et al.* 2022) [67]. In the process of biodegradation, the strong carbon bonds within polyethylene are broken down by microbial activity. This breakdown reduces the strength of polyethylene, leading to a decrease in its molecular weight and ultimately resulting in its degradation (Pruter 1987) [43]. Colonization by microbes on substrates is the very first step of the process of polyethylene biodegradation. Studies have indicated that the biofilm formation has a crucial role in enhancement of interaction between the polyethylene surface and microbial population (Schwibbert *et al.* 2019) [49]. Fungal cells have the remarkable ability to adhere to the surface of polyethylene due to hyphal growth and they can attach to any substrate due to this feature (S´anchez 2020) [47]. After adhering to the polyethylene surface, microorganism feed on the polymers as their primary carbon source for life sustainability. Depolymerization is the next step, where extracellular enzymes and microbes work together and produce free radicals that finally break down the polyethylene into several smaller units (Jenkins *et al.* 2019) [25]. Enzymes produced by microbial cells perform primary degradation, that can be either extracellular (outside the cell) or intracellular (inside the cell). Hence enzymes play a important role in breaking the polymer chains of polyethylene (Amobonye *et al.* 2021) [3]. The third step assimilation stage, involves transportation of the low-molecular-weight compounds generated during the fragmentation process into the cytoplasm of the microorganisms. Research by Shahnawaz et. al. indicated that octadecane, a product of polymer degradation, is absorbed by bacteria *Pseudomonas*, indicating the assimilation process (Shahnawaz *et al.* 2019) [51]. The fourth step is the mineralization process during which the polyethylene degradation products transported into the cell undergo are exposed to many complex enzymatic reactions. These enzymes release several metabolites like CO_2, H_2O, CH_4, and N_2 after the break down polymer chains by them. This process finally leads to the harmless treatment or reuse of polyethylene. Finally mineralization occurs under aerobic or anaerobic conditions, that finally relies on the activity of various enzymes such as peroxidase, esterase, lipase and laccase etc. (Alshehrei 2017) [2].

Role of microbial enzymes in polyethylene degradation

The biodegradation of plastics is indeed a complex process influenced by a variety of abiotic (non-living) and biotic (living organisms) factors (Eubeler *et al.* 2010) [12]. The cooperative interaction between abiotic factors and microorganisms plays a crucial role in breaking down the bulk polymer, leading to increased accessible surfaces for biodegradation (Sivan 2011) [55]. The byproducts of polyethylene degradation can vary depending on the specific conditions of degradation. Under aerobic conditions, the final degradation products typically include CO_2, water, and microbial biomass. In anaerobic or methanogenic conditions, the end products often consist of CO_2, water, methane, and microbial biomass. In sulfidogenic conditions, the reported end products include H_2S, CO_2, water, and microbial biomass. These variations highlight the influence of environmental conditions on the outcomes of polythene degradation (Arutchelvi *et al.* 2008) [5]. Bacteria, such as *Streptomyces badius* 252 *Streptomyces viridosporus* T7A, *Streptomyces setonii* 75Vi2 and several wood-degrading fungi, have been reported to produce extracellular enzymes responsible for degradation of polyethylene in nature (Kim *et al.* 2005) [29]. Microbial enzymes that possess the capability to degrade lignin polymers, which contain oxidizable C-C bonds, have been implicated in the biodegradation of polyethylene (PE) (Suhas *et al.* 2007) [59] all these enzymes are reported to be responsible for polyethylene degradation and unwinding of carbon-carbon bonds during the degradation process (Krueger *et al.* 2015) [31]. These microbial enzymes include manganese peroxidase (MnP, EC 1.11.1.13), lignin peroxidases (LiP, EC 1.11.1.14), and laccases (EC 1.10.3.2.) (Wei and Zimmermann 2017) [65]. Enzymes present in the liquid phase participate in heterogeneous reactions taking place on the interface between solid and liquid phases and degrade large polyethylene molecules located on the surface of the solid material. (Kaushal *et al.* 2021) [28]. A copper-dependent laccase, derived from *R. ruber* strain C208, has the capability to degrade UV-pretreated polyethylene (PE) films (Santo *et al.* 2013) [48]. There is considerable reduction in molecular weight of polyethylene membranes when treated with laccase from *Trametes versicolor* in presence of 1-hydroxybenzotriazole as a mediator (Fujisawa *et al.* 2001) [15]. An increase in the extracellular secretion of laccases and MnP (Manganese Peroxidase) from *B. cereus* was observed when the strain was cultured with UV-irradiated polyethylene (PE) (Sowmya *et al.* 2014) [57]. Furthermore, laccases from *Aspergillus flavus* and *P. ostreatus* were found to display significant activity in degrading polyethylene (PE) (Zhang *et al.* 2020b) [66]. Degradation of heat-treated polyethylene has been reported by lignin peroxidase (LiP) activity found in concentrated culture supernatants of lignocellulose-degrading *Streptomyces species* (Pometto *et al.* 1992) [41]. Jeon and Kim in 2015 reported that low molecular weight polyethylene can be degraded by the enzymes produced by *Pseudomonas*. The degradation of polyethylene by *Pseudomonas aeruginosa*, involving enzymes such as alkane hydroxylase and reductase, was explored. It's worth noting that these studies often used crude or partially purified enzymes and required extended treatment times (Jeon and Kim 2015) [26]. Interestingly, recent research has shown that utilizing customized microbial consortia (a combination of different microorganisms) can be more effective in degrading materials like polystyrene (PS) and polyethylene (PE) compared to relying on single microorganisms (Esmaeili *et al.* 2013) [13]. It's important to note that while the use of microbial consortia has shown promise in plastic degradation, the detailed mechanisms of how these enzymes work together and their interactions have not been thoroughly investigated in many cases. Further research is needed to gain a deeper understanding of these mechanisms, which can potentially lead to more effective and efficient plastic degradation strategies.

Microbes involved in polyethylene degradation

Around 20 bacterial groups such as, *Ralstonia, Pseudomonas, Stenotrophomonas, Acinetobacter, Klebsiella, Rhodococcus, Staphylococcus, Streptomyces, Bacillus, Streptococcus* etc. have shown the potential for degrading several types of polyethylene (Danso *et al.* 2019) [9]. *Lysinibacillus*, originated from forest soils, decreased weight of polyethylene around 9% within 26 days of treatment (Jeon *et al.* 2021) [27]. Many of these bacterial strains exhibit the capability to degrade the surface of polyethylene and/or create a biofilm on it. Notably, complete degradation of polyethylene in a water-based environment was observed when treated with *Pseudomonas fluorescens* in the presence of both a surfactant and a biosurfactant, underscoring their significant roles in polymer oxidation and biodegradation (Arkatkar *et al.* 2010) [4]. A thermophilic bacterium *Brevibacillus borstelensis*, obtained from soil, has been reported to have the capacity to exclusively use low-density polyethylene as its carbon and energy source. During a 30-day incubation period, this bacterium was able to reduce 30% of the molecular weight of the PE film (Hadad *et al.* 2005) [22]. In a landfill environment, researchers isolated a mixed microbial community primarily composed of *Bacillus* and *Paenibacillus* species. When this microbial community was cultivated in an aqueous medium with polyethylene microplastics as the sole carbon source, it successfully reduced the weight of the polyethylene by approximately 14.7% and simultaneously decreased the average particle size by about 22.8% (Park and Kim 2019) [40]. The bacterial community isolated from a marine environment, with a specific focus on polyethylene degradation, was primarily comprised of *Idiomarina* (50%), *Marinobacter* (28%), *Exiguobacterium* (18%), and other minor constituents (4%) (Gao *et al.* 2021) [17]. A biofilm-producing strain of *Rhodococcus ruber* (C208) was successfully isolated, and this strain exhibited a polyethylene (PE) degradation rate of approximately 0.86% per week (Gilan *et al.* 2004) [21]. It has been reported that when subjected to thermal treatment, high-density polyethylene (HDPE) was degraded by *Klebsiella pneumonia*, resulting in a reduction of both the weight and tensile strength of the HDPE film by 18.4% and 60%, respectively, over a period of 60 days (Awasthi *et al.* 2017) [6].

Aside from bacteria, several fungal genera, including *Aspergillus, Cladosporium, Fusarium, Penicillium*, and *Phanerochaete*, have been reported for their involvement in polyethylene (PE) degradation processes (Glaser 2019) [20]. In general, fungi are considered to be more efficient than bacteria in the degradation of polyethylene (PE) because they possess the ability to adhere to the hydrophobic surface of the polymer (Krueger *et al.* 2015) [31]. Fungi are known for their capacity to produce extracellular enzymes that specifically target insoluble fibers. They have adapted to survive in challenging growth conditions, which further enhances their ability to break down complex and resistant materials like insoluble fibers (Shah *et al.* 2008) [50]. In laboratory conditions, the biodegradation of low-density polyethylene (LDPE) by both *A. niger* and *A. japonicas* has been observed to result in a decrease in dry weight of approximately 5.8% per month for *A. niger* and 11.1% per month for *A. japonicas* (Raaman *et al.* 2012) [44]. A study on the microbial degradation of LDPE by *Aspergillus* and *Fusarium* species revealed that *Fusarium sp*. FSM-10 and *Aspergillus sp*. FSM-3 exhibited the highest weight reduction, approximately 8-9%, following 60 days of incubation. In contrast, *Aspergillus sp*. FSM-5 showed a lower weight loss of only 5% during the same incubation period (Das and Kumar 2014) [10]. The degradation of low-density polyethylene (LDPE) was investigated using fungal strains isolated from landfills. The results indicate that *Aspergillus clavatus* was able to degrade approximately 35% of LDPE within a 90-day period (Gajendiran *et al.* 2016) [16]. Strains belonging to *Aspergillus flavus* and *A. nidulans* were isolated through enrichment culture, and these strains exhibited a clearing zone around their colonies on polyethylene (PE) agar plates (Usha *et al.* 2011) [63]. *A. niger* was successfully isolated from soils found in polyethylene (PE) waste landfills by utilizing a mineral medium with PE powder as the sole carbon source for cultivation (Esmaeili *et al.* 2013) [13]. Analysis of polyethylene biodegradation can be studied using tools like AFM (Atomic Force Microscopy) and SEM (Scanning Electron Microscopy). Many microbial strains of Chrysonilia, *Penicillium* and *Aspergillus* have shown potential for biodegradation that were isolated on synthetic medium and are being studied (Mishra *et al.* 2014) [36].

The microbial degradation of polyethylene can be ascertained by measuring sample weight loss its changing tensile strength, by using fourier transform infrared spectroscopy (FTIR). AFM (Atomic Force Microscopy) and SEM (Scanning Electron Microscopy) analyses can be used for confirmation of biodegradation of polyethylene by microbes, that often results in the formation of biofilms and several morphological changes on surfaces of high-density polyethylene (HDPE) and low-density polyethylene (LDPE). These changes may include the development of pits, holes, cracks, scions, and sometimes undulations on surfaces.

Conclusion

Polyethylene is an incredibly valuable material in our everyday lives, serving a wide range of purposes in day-to-day life such as packaging goods, food, disposables and scientific instruments. Popularity of polyethylene is ever growing as it is cheap and has high-quality properties such as strength and inert to most of the products. However, its persistence and accumulation due to large production and consumption in the environment poses a significant threat. In spite of several physical and chemical methods available for polyethylene degradation, microorganisms provide most cheaper and environmentally safe tool for getting rid of pollution due to plastic wastes. Microbes play a crucial role in polyethylene degradation by releasing certain extracellular enzymes. Extensive studies on these enzymes in relation to polyethylene degradation needs to be done using modern available techniques. Additionally, it has been observed that microbes from various municipal waste sites and landfills have the potential to break down polyethylene. Therefore, it is essential to continue screening for more microorganisms from waste dumping grounds that have the capability of polyethylene degradation. This research can help us better understand the exact mechanisms of degradation and identify the responsible genes involved in this process.

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