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Original Research Article

"The Investigation of Bacteria and Microbes that Generate Enzyme that have been Responsible for Biodegradation of Polyethylene"

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ABSTRACT
Environmental polyethylene degradation is a collaborative process including photo- and thermo-oxidative processes and biological activity. Abiotic oxidation is the primary mechanism of breakdown for commercial high molecular weight polyethylene. crucial first step that sets the pace. Biodegradable additives, photo-initiators, and copolymerization are used to create ecologically preferable polyethylene. Knowing how degradation products interact with the natural world is a crucial issue for the safe and effective use of polymers that may be broken down in the environment. Fragments of polymers and their breakdown products should be harmless to the environment and not accumulate. The first stage in calculating the total environmental effect of a chemical is identifying its abiotic as well as biotic oxidation products. Polyethylene decomposes into almost 200 distinct compounds when subjected to high temperatures or light. Everything from alkanes as well as alkenes to ketones including aldehydes to alcohols as well as carboxylic acids and keto-acids as well as dicarboxylic acids but rather lactones and esters falls under this area. Microorganisms in either an abiotic or biotic setting may take up such abiotic oxidation products, including the oxidized low molecular weight polymer. New polyethylene's with customized structures to minimize environmental deterioration through non-conventional means are expected to be a major focus of future research. Understanding the environmental effects of biodegradable polymers and also the dynamic interactions between nature as well as breakdown products is crucial alongside the development of these cutting-edge technologies.

***Keywords*—**Biodegradable, Microbiology, Biotechnology, Immunology, Industrial microbiology, Sustainable polymer

Study Resign: Observational study

1. Introduction

Sustainable polymer product development relies heavily on proper waste disposal practices for plastics collected from consumers' trash. Source reduction, product reuse, product recycling, energy recycling, and indeed the development of ecologically degradable polymeric materials are all potential components of a waste management strategy that prioritizes the biological recycling of plastic trash. Environmentally degradable polymers may degrade in any number of ways, including chemical, mechanical, biological, and physical ones. Damage to a structure may be caused by a wide variety of environmental and

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anthropogenic factors, including but not limited to: rain, wind, sunlight, insects, mammals, bacteria, and so on. After DuPont achieved large production and exclusive sales of nylon in 1940, research into other synthetic polymers has progressed. Since the 1960s, synthetic materials like plastic have gradually replaced organic ones due to their cheap cost, non-degradability, widespread availability, and ease of use.(Jabir & Yass LaHood, 2021)Around 5 and 13 million metric tons of trash are poured into the oceans annually, causing the extinction of many marine species. Between 350 and 400 million metric tons of non-biodegradable plastic are produced annually across the world. A total of 80,000 metric tons of trash made up of plastic were found in the Pacific Ocean, and this includes Hawaii, according to a study of something like the Great Pacific Garbage Patch. This estimate is determined by the fact that North American and Asian countries are responsible for contributing 54% of the trash found in the Great Pacific Garbage Patch. More than 92% of all synthetic plastics are made of polyethylene and polypropylene, which are used for a broad range of things including plastic bags, disposable containers, bottles, packaging materials, and many more. More than 92% of all synthetic polymers are polyethylene and polypropylene. Every year, humans use over a trillion plastic bags, which has far-reaching consequences for the environment.(2022)

Objective

The research aimed to fulfill the following objectives:

- To study Recent advances in analytical approaches for accurately estimating PE deterioration
- Biodegradation of polyethylene by microbial enzymes
- The Biodegradation of polyethylene by bacteria

2. Methodology

Since plastic trash poses a threat to living things across the board, its management and recycling have emerged as one of the most pressing environmental concerns on a worldwide scale. Researchers investigated PE biodegradation by monitoring a number of physicochemical and structural parameters using tools including FTIR spectroscopy and SEM. Yet, these pieces of data are not sufficient to demonstrate conclusively that PE may biodegrade. In this study, we highlighted current advancements for the prospective microbial enzymes and their potential involvement in the breakdown of polyethylene (PE). Also, we provided a summary of the microbial biodegradation of polyethylene. In addition, we compared the modern methods that are currently being utilized for accurately detecting the degradation of PE by using isotope-labeled PE in order to determine its metabolism into the end products such as 13CO2.

Recent Advances in Analytical Approaches for Accurately Estimating PE Deterioration

Several studies have investigated the microorganisms responsible for PE breakdown using commercially available polymers, which may include a broad variety of chemical additives. Mass loss and shifts in the surface's functional group composition were analysed using Fourier transform infrared spectroscopy to determine the extent of degradation (FTIR). Nevertheless, it is not always evident that surface structural flaws and the breakdown of compounds, which often make up a significant portion of the polymer, are responsible for the apparent weight loss. The reason for this is because additives make up a disproportionately high percentage of the overall polymer. Substantial alterations are required to distinguish between the normal deterioration of PE and the less probable artefacts arising first from the

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breakdown of additives. In particular, gas chroma-zoography has been validated as an effective method for gauging PE's biodegradability by quantifying the amount of carbon dioxide (CO2) released during microbial decomposition and respiration. To wit: (Oluwole et al., 2022) Carbon dioxide (CO2) production during microbial breakdown may be quantified using this method. The rate of carbon dioxide production was found to be related to the rate at which bacteria mineralized carbon sources via respiration. For this experiment, researchers cultivated Rhoda ochrous, a kind of soil bacterium, in a unique aqueous medium containing just polyethyleneimine (PE) as a carbon source. As the cells attained stationary growth, there was no discernible difference in the total amount of carbon dioxide produced between those grown with LDPE and those grown without the need for a carbon source. A lack of bioavailable carbon during bacterial growth on LDPE has been hypothesized to account for its slow rate of biodegradation. In order to further investigate the effect of UV pretreatment on the biodegradability of LDPE, R. Rhod ochrous was cultured with both UV processed LDPE and native LDPE for 35 days. All CO2 emissions were tracked during the trial. UV treated LDPE biodegraded three times as fast as untreated LDPE. Carnivora burakumins, a marine bacterium that degrades alkanes, was shown to do very well in a medium containing low density polyethylene, as the authors of this research report (LDPE). Carbon dioxide production by A. burakumins was unaffected by the addition of LDPE to its diet, as compared to the addition of a control sample devoid of LDPE, demonstrating that this strain cannot use LDPE. Before and after biodegradation with rhod ochrous rhod ochrous, 1320 PE-derived oxidized oligomers were analysed by MS and NMR spectroscopy, respectively. This was performed so that a comparison could be made between the two approaches. (Talib, 2022) Maximum absorption capacity for the e strain (95% soluble oligomers) is reached after 240 days. This shows that intracellular oxidation processes and extracellular chain cleavage mechanisms are important in PE biodegradation, since larger molecules were biodegraded more quickly than smaller ones. Further insight on plastic's biodegradability might be gleaned via tracing carbon from polymers to CO2 and biomass. A biodegradable plastic was assumed for these analyses. Poly (butylene adipate-co-terephthalate) (PBAT) biodegradability in soil was studied by Murmelstein et al. utilizing a 13C-tagged version of the polymer. PBAT's monomer units may be broken down by microbes and filamentous fungus in the soil, which are then used by these organisms as a carbon and energy source. This was shown to be the case by the study's findings. The use of 13C-labeled polystyrene allowed researchers to examine the contribution of Tenebrio Molitor to the biodegradation and mineralization of polystyrene. Evaluating fecula that were egested from Styrofoam-feeding larvae was made possible with the use of 13C cross-polarization/magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectroscopy. The results indicated that just 0.5% of the carbon from the ingested Styrofoam was absorbed into the biomass of the organisms, while 47.7% was transformed into CO2 and 49.2% was ejected as fecula. Tagging experiments shown that lipids absorbed 13CO2 released during PS degradation. According to recent mass balance research, when given PE, mealworms of the species Tenebrio Molitor turned 49.0 1.4% of it into carbon dioxide. In a recent study, chemical alterations in the leftover polymer from the faces of PE-fed mealworms were analysed using 1H-NMR data (Harshvardhan & Jha, 2013). In order to reach a conclusion, scientists compared their results to those obtained from studying mealworms that had been fed bran. As a means towards this end, we examined and contrasted the two mealworm varieties.

Biodegradation of Polyethylene By Bacteria

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Twenty or more bacterial species have been identified as having the potential to degrade different types of polyethylene (PE). These pathogens comprise both Gram-negative and Gram-positive bacteria. Some more organisms that belong here include the Rhod coccus, Staphylococcus, Streptococcus, Streptomyces, and Bacillus species. Almost all of these bacterial species are capable of forming a biofilm on PE, and some of them may even accelerate the breakdown of the material's surface. microbes that aid in the breakdown of polyethylene in the natural world Several research have looked at the different activities of organisms of the genus Pseudomonas and their capacity to breakdown and metabolize various synthetic plastics polymers and the by-products. Extracellular enzyme activity, either oxidative or hydrolytic, gives Pseudomonas species the capacity to destroy and metabolize polymers. These processes not only control the interaction between biofilms and polymer surfaces, but also aid in the uptake and destruction of polymer fragments. It was shown that PE underwent full biodegradation in water after being treated with P. fluorescens and having both a surfactant and a biosurfactant present. Our results suggest that both surfactant and biosurfactant play critical roles in polymer oxidation and biodegradation. After 45 days of cultivation in an LDPE degradation medium, Trivedi and Sil found that the addition of mineral oil accelerated the hydrophobic interaction to form biofilms on polymer surfaces, leading to a 5.1% breakdown of the initial PE material. Nevertheless, Tween 80 was able to prevent biofilms from forming when added to the mix. Brevibacillus burstinesses, a thermophilic bacterium isolated from soil, was shown to be the case when biofilms were developed on it. This bacterium was shown to get all of its carbon and energy needs from BLDPE. After being incubated for 30 days, the molecular weight of the PE film decreased by 30%. Rhodococcus equip strain C208, a biofilm bacterium, was shown to be responsible for the weekly loss of 0.86 percent in PE. Klebsiella pneumoniae has been proven to degrade high-density polyethylene that has been subjected to heat treatment (HDPE). Water's slow uptake into the cell may be owing to the hydrophobic cell surface this strain displays, such as a coating consisting of mycolic acid. Because of how effectively this strain adheres to HDPE, the biofilm became thicker and the HDPE film's weight (18.4%) and tensile strength (60%) decreased. To wit: (Kundera et al., 2019) Scanning electron microscopy (SEM) in conjunction with atomic force microscopy may provide images of the underlying corrosion, fissures, and surface roughness of a potentially biologically deteriorating HDPE film, which are likely caused by bacteria (AFM).

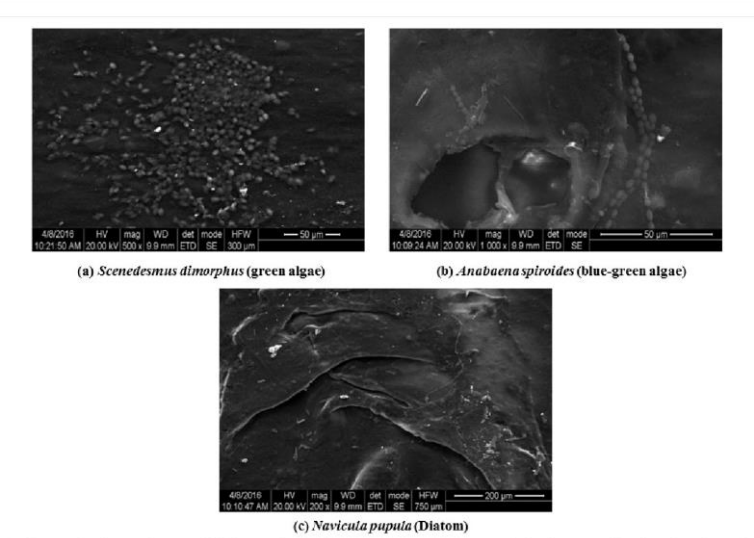

FIGURE 1. BIODEGRADATION OF POLYETHYLENE

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Biodegradation of Polyethylene by Microbial Enzymes

The biodegradation of plastic involves a series of steps that are affected by both biologic and environmental variables. The joint efforts of abiotic forces and microorganisms may break down the bulk polymer more quickly if it is fragmented first. Many extracellular enzymes catalyze a further step in the degradation of polymers. Several lignin-degrading enzymes are also required for the breakdown of PE thermoplastic. Oligomers, which are fragments of polymers, are capable of entering cells and being metabolized there. Typically, oligomers consist of 10 to 50 carbon atoms. PE is not readily biodegradable due to the absence of hydrolyzable organic compounds in its backbone. Pretreatment techniques, including thermo-UV irradiation and the addition of oxidizing agents, may be employed to create carbonyl and hydroxyl groups, which accelerate biodegradation. In the great majority of PE biodegradation research, substrate peroxidation has been used. Microbial enzymes that may oxidize C-C bonds in lignin polymers have accelerated the biodegradation of PE. Laccases (EC 1.10.3.2), manganese peroxidases (MN, EC 1.11.1.13), and lignin peroxidases are all examples of peroxidases (EC 1.11.1.14). (Lip, EC 1.11.1.14) UV light may destroy polyethylene (PE) film, with copper-dependent laccase from R. rubber strain C208 as a likely culprit. To wit: (Capri et al., 2010) By cleaving the amorphous portion of polyethylene (PE) films oxidatively with laccase, easily accessible carbonyl groups are generated, and the film weight is significantly reduced. Fujisawa et al. demonstrated that treatment with laccase from Taramites versicolor decreased the molecular weight of a PE membrane in the presence of 1-hydroxybenzotriazole as a mediator. For P. chrysophobia ME-446 and isolate IZU-154, the MN was the main enzyme in the lysis of a high-molecular-weight PE membrane. There was an increased rate of PE degradation when partially purified MN was combined with various surfactants. We have identified the IZU-154 MN that is most effective in degrading nylon-66 and oxidizing 2,6-dimethoxyphenol. UV irradiation of PE caused a rise in the extracellular concentrations of laccases as well as MN secreted by B. cereus. Nevertheless, when the same PE film was treated with a partially purified laccase and an MN from P. simplicissimum, there was hardly any weight loss at all. Lip activity has been connected to the degradation of heat-treated PE, and many lignocellulose-degrading Streptomyces species have already been implicated in this process. A high molecular weight PE sample that has been pre-oxidized has been reported to be degraded by as much as 70% following 15 days of treatment with P. chrysophobia strain MTCC-787. When it comes to biodegrading PE, this strain's extracellular peroxidases are important. As reported by (Jeon & Kim, 2016)

For the oligomeric hydrocarbon breakdown by the Alb family of alkane hydroxylases (AH), there are terminal and sub-terminal oxidation pathways (EC 1.14.15.3).

An 80-day incubation at 37 degrees Celsius using a recombinant AH of Pseudomonas sp. E4 converted 20% of a low-molecular-weight PE sample into CO2. (A, 2021) The expression of the whole AH system from P. aeruginosa strain E7 in E. coli led to a 30% decline in the quality of a PE sample. Nevertheless, these tests took much longer when raw catalysts or enzymes that had been only partially purified were utilised. Customized microbial consortiums have been found to be more effective than isolated microorganisms in the breakdown of PS and PE. Recent transcriptome research of G. Mellon Ella fed beeswax found upregulation of genes generating carboxylesterase, lipase, and many other enzymes associated in fatty acid metabolism. It has been determined that it is on par with physical

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education. However, the mechanisms that aid these enzymes in carrying out their roles have only been scratched at the surface. (2022)

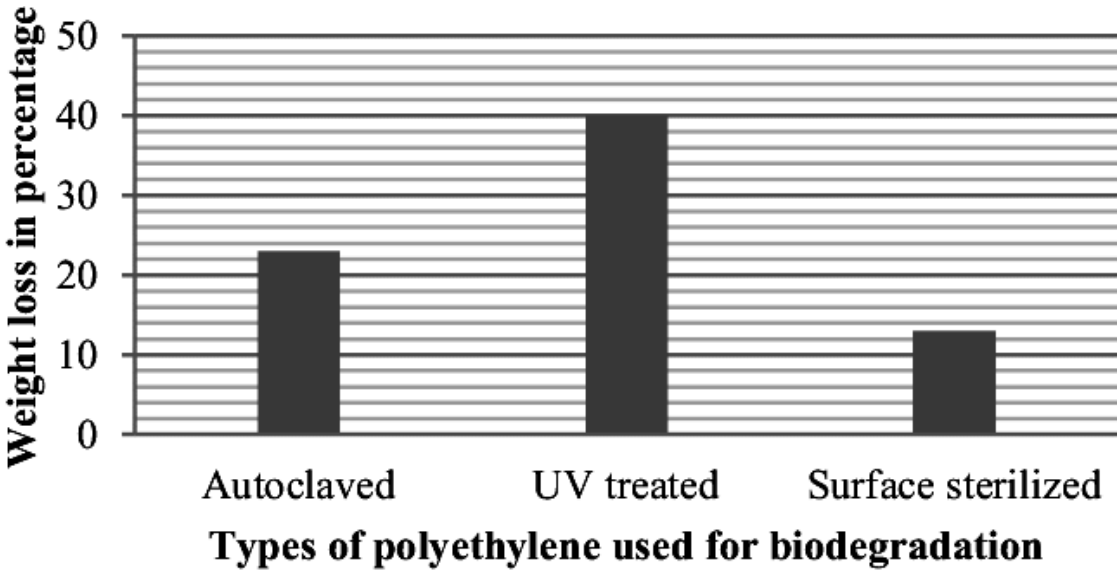

FIGURE 2: - BIODEGRADATION OF POLYETHYLENE

3. Conclusion

Analysis of the findings and projections for the future Until recently, a lot of studies on PE biodegradation have looked on how degradation of the material's structure and physicochemical properties (using methods like FTIR, DSC, XRD, SEM, AFM, and so on). The research for these investigations has been carried out in several labs all around the globe. Most of the observed decreases in mass, in combination to the measurable physicochemical shifts, were insufficient to infer genuine polyethylene biodegradation (PE). In order to reduce the quantity of artefacts that arise from the breakdown of compounds rather than PE, there is an immediate need to provide proof that is both tactile and dependable of the biodegradation of polyethylene. This is so because there will be less of an overall cultural output as a result (PE). Thus, it is suggested that more studies be conducted utilizing pure PE. It has also been hypothesized that throughout the course of a lengthy incubation period, 13C-polyethylene breakdown would show increasing synthesis of 13C-labeled metabolites, which could lead to CO2 emissions. This theory has not been confirmed as of yet. To learn more about the molecular route for the effective biodegradation of PE, research into the enzymatic degradation process is required.

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