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BIODEGRADATION OF POLYETHYLENE

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Abstract

Polyethylene (PE) is considered to be non-degradable for a long time. The classification of PE biodegradation by bacterial cultures has been identified, at different times, the validity of PE biodegradation remains limited in the literature. The current study deals with the separation of bacteria in the intestinal tract, 'Galleria mellonella' which is able to degrade PE films. Of the ten isolates found, five isolates showed a positive sign of degradation PE. PD8 has been identified as bacteria that degrade PE. It was found that the highest percentage of weight loss was obtained by PD8 at 40 microns of 11.93%, at 30 microns of 9.95% and at 40 microns of 8.83% of PE after completion of the day -60 incubation of PE. More than 60 days of incubation. The percentage of weight loss of the three PE films was determined and colour change was adjusted. Changes in colour from light blue to light white were also observed. There is promising evidence of natural environmental separation of PE.

Keywords: Bio degradation, *Galleria mellonella*, Isolation, Pollution, Polyethylene, waxworms,

1. Introduction

Polyethylene is a lightweight, long-lasting thermoplastic with a versatile crystal structure. It's done by polymerizing ethylene (olefin) monomers together or over polymerizing them. For polymerization of polyethylene, Ziegler-Natta and Metallocene catalysts are used. The chemical formula for polyethylene is $(C_2H_4)_n$ It is one of the world's most commonly used plastic. Plastic is a polymeric material that can be moulded by applying heat and pressure. This plastic material, which is often used in conjunction with other unique characteristics such as low volume, less power output, clarity, and hardness, is a good example, enables the manufacture of a wide range of goods from plastics. Plastics are an important part of our society and the demand for land and the production of plastics is much higher than before [1].

Polymers are plastics that are divided into two categories: thermoplastics and thermosets. Polyethylene and polystyrene, for example, are thermoplastics that can be moulded and repaired repeatedly. When it comes to thermosets, though, they cannot be renewed. The thermosetting structures undergo a chemical reaction during the initial processing that results in an uninstalled, insoluble network. In the last decade, global thermoplastics production has risen from less than two million tons in 1950 to between 230 and 245 million tons. Global plastic

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production is growing at about 5% every year, because plastics show benefits such as clarity, toughness, processefficiency and lightness and competitive price.

Plastic pollution is defined as the collection of various types of plastic material in the earth and in bodies of water such as rivers, seas, canals, lakes, etc. It has become an integral part of the the global polymer industry, including its production and disposal, posing serious risk to all forms of life on Earth. Plastic impurities, from marine and terrestrial sources, migrate to tropical gyres where they form small plastic particles that separate from the surrounding water without plastic impurities. The importation of marine debris by wildlife, as well as plastic for seabirds in particular, is widely documented. Futmar Fulmarus glacialis in the north was among the first marine species to be reported to absorb marine plastic waste. Plastic usually degrades for about 500 - 1000 years, it maynot even know its true time of destruction. Toxic chemicals in plastic include ethylene oxide, xylene, and benzene, all of which are harmful to the environment. It is difficult to get rid of, and it causes permanent harm to living things. PE is a gasoline-based plastic that is described as "[CH2 - CH2] n" and is widely used in everyday life, with a global annual production of approximately 140 million tons. The obvious difference between the significant durability and short service life of PE products, on the other hand, leads to the accumulation of PE waste in the environment, which has caused global issue.

There are various treatments available to degrade plastics. PE waste is treated with several bio, physio, and chemical methods. Depletion of PE chains occurred when PE samples were previously treated with UV light or thermo-oxidation, resulting in the formation of low-weight products such as alkanes, alkenes, ketones, aldehydes, various alcohols, and fatty acids, and then further degraded by selected bacteria. Pre-treatment reduces polymer hydrophobicity, makes it more sensitive to microorganisms, or introduces groups prone to microbial damage, such as CO or -OH. Severe biodegradation differentiation the absence of certain plastic-damaging microorganisms with a high potential for carbon chain oxidization and depolymerization has resulted in the degradation of virgin PE. Some researchers believe that the life span of small organisms, which is necessary for the survival of plastics, may be much longer than the history of PE applications. Researchers are still attempting to identify PE microorganisms by experimenting with plastic waste soils, landfills, manure, and marine life, and have identified different types of bacteria. (Kwadha, Charles A.; Ong'amo2017). As with other methods, biological tools are gradually being replaced by pollution control programs. Researchers are studying small pollutants that are contaminating polluted and contaminated areas. PE degradation is thought to be observed by

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photo or thermo oxidation and carried out by microorganisms' biological activity., a therapeutic process used tobreak down or reduce harmful ingredients into minimum toxic or non-toxic substances.

Biodegradation is a natural degradation that occurs naturally with microorganisms such as bacteria and fungi or other biological activity (Warren, L.O; Huddleston Paul1962). Composting is a man-made process in which biodegradation occurs under certain conditions. This involves the conversion of carbon atoms into active plastics into harmless biological elements in the environment. Macro degradation methods of biological and micro-organisms have been considered in plastic disposal (Anderson et al. 1995). Extensive ecological diversity involves the destruction of plastic by insects, birds, and other animals. Microbiological damage involves LDPE biodegradation by microbes, including fungi and bacteria (Hasan et al. 2007; Pramila et al. 2012). Products made from biodegradation (e.g., humus, carbon dioxide and water) are usually visually appealing and can be easily synthesized by organic matter (Prem Raj & Doble 2005). Therefore, biodegradation provides an alternative to LDPE disposal. Antimicrobial strategies often include time to enrich liquid cultures or solid media where they are allowed to grow as colonies. Pre-treatment decreases the polymer's hydrophobicity, makes it more susceptible to microorganisms, or adds groups like CO or OH, which are less susceptible to bacterial damage. Other issues have demonstrated the moderate potential for using Based on the formation of biofilm formations in PE films, weight loss of PE materials, and degradation and changes in mechanical and thermal properties of PE, PE can be used as a carbon source. It has been stated, for example, Pseudomonas causes a 20% weight loss in PE tested after 120 days. Furthermore, the Canadian Website announced the rapid separation of dual new strains, which resulted in a reduction of 22 percent or more of the checked PE in just six weeks. As a result, numerous environmental factors such as the breakdown of biofilm formation in PE films, weight loss of PE materials, erosion, and changes in mechanical and thermal properties of PE were considered appropriate.,

PE biodegradation by wax worm gut is an important and latest effective method. It shows that Bacteria derived from these plastic-eating worms have been identified as a promising source of harmful plastic insects. (Hanumanthaswamy, BC Rajagopal, 2017). It is able to degrade PE in a limited incubation period in terms of changes in PE as a result of biofilm formation (Dolbe, Arkatkar), physiological (strength and earth structure), chemical composition, molecular weight, and weight loss. Biodegradation of Polyethylene chemicals is compounded by a variety of ortho- or meta-cleavage methods. Information about enzymes, and factors involved

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in polyethylene degradation will improve and make the process of decomposition faster in PE degradation techniques (Albertsson, AC Erlandsson).

2.MATERIALS AND METHODS

Collection and Pre-treatment of PE films:

LDPE films with different strengths such as 15, 30, 40 microns are purchased at the local Visakhapatnam market. LDPE films are cut in a 5×5 cm^2 square area. Prior to the use of these degrading films, LDPE films were washed with sterile water and soaked in ethanol for 30 minutes. After that they were washed with distilled water and allowed to dry.

Collection of larvae of wax moths, Galleria mellonella

The honeycomb was purchased from Araku valley, Visakhapatnam. They wax moths were separated from the comb under aseptic conditions. These worms were placed in a container, which contains oat meal as their feed for better growth.

Figure 1 Growth of waxworms

Materials used in the media

Mineral Salt Media (MSM) was prepared with distilled water containing (per1,000 mL) 0.7 g of KH_2PO_4, 0.7 g of K_2HPO_4, 0.7 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of NH_4NO_3, 0.005 g of NaCl, 0.002 g of $FeSO_4 \cdot 7H_2O$, 0.002 g of $ZnSO_4 \cdot 7H_2O$ and 0.001 g of $MnSO_4 \cdot H_2O$, depending on the ASTM standard. This is sterilized by autoclave at 121°C for 20 minutes. Potato Dextrose Agar (PDA) and Tryptone Soy Agar (TSA) media have been used to isolatebacteria.

PE-Degrading Microorganisms Enrichment and Isolation

About 500 worms were collected in PE films. To obtain the intestinal content of the degrading PE bacteria, The larvae were sterilized by immersing them in 75% ethanol for 1 minute and then washing them twice with sterile Saline Water (SW). The worms' guts are then removed and inserted in a 50 mL centrifuge tube with 40 ml of SW. The intestinal tissue is carefully collected with a hose after 5 minutes of stirring in the vortex mixture. 1.5 mL of the suspension was transferred to a 250 mL Erlenmeyer flask containing 1g PE films and 80

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mL of MSM as a microbial inoculum. The flask was shaken at 150 rpm at 30°C in a rotating shaker. The remaining PE films were removed after 2 days of incubation, and the culture was still distributed on PDA and TSA plates. The colonies grew well in the TSA after a 24-hour incubation period compared to the PDA [7]. The various colonies that formed were transferred to TSA plates and kept there until clean pure colonies were discovered.

Degradation of PE under laboratory conditions

Liquid culture Method

Pre-treated PE films weighing 5 × 5 cm^2 were transferred into conical flasks containing 100 mL of MSM and inoculate with the isolated strains. These conical flasks bottles are placed in an orbital shaking incubator and stored at 30°C, 150 rpm for a period of 60 days. This was done to further the study of biodegradation

Figure 2 Conical flasks containing PE films in MSM

PE film Biodegradation Study

Spectrophotometer assay

The percentage of degradation in LDPE groups is estimated at 600 nm, the optical density is measured.1ml of media from incubated flasks was taken and Optical Density was noted at 600 nm. Microbial isolates were grown in MSM until the cultures received a log phase (0.6 absorption at 600 nm). 10% of the log phase cultures were placed in a 250 ml Erlenmeyer flask containing MSM medium and PE films. Biodegradation tests are performed triplicate, as controls of MSM are supplemented with LDPE films without inoculum which are also stored under similar conditions. Tests were performed three times for each isolate. Cultural broths were then analyzed by spectrophotometer analysis.

Morphology Study

Morphology changes e.g., colour and surface changes were observed.

Determination of pH changes

The study of pH changes to determine the metabolic activity of bacterial isolates in MSM was accepted, as metabolism expressed by microbial cells could significantly support the evidence for a degradation approach. This medium's initial pH was determined to be 7.0. Control is maintained.

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Determination of PE weight loss

The degradation of PE was tested with15,30,40 microns sizes of LDPE. Isolates are allowed to degrade PE in conical flasks under shaking conditions for a period of 60 days. PE films were collected period after the incubation period, thoroughly washed using sterile distilled water, dried in the shade, weighed in order to determine the final weight The weight loss of polyethylene was calculated in comparison to controls using the following equation: (Ariba et al., 2015).

$$Weight\ loss\% = \frac{wt1 - wt2}{wt1} \times 100$$

Wt1 (initial weight) = weight of PE film before incubation.Wt2 (final weight) = weight of PE film after incubation.

Scanning Electron Microscopy of Polyethylene

Scanning Electron Microscopy was used to examine the surface morphology of the PE films in order to look forany structural alterations.

3.RESULTS AND DISCUSSION

Microbes that can degrade LDPE were isolated from the gut of wax moths, *Galleria mellonella.* These isolatesare grown in MSM, which contains PE films as the only carbon source was studied.

Isolation of PE-Degrading Isolates

PD1, PD2, PD3, PD4, PD5, PD6, PD7, PD8, PD9, and PD10 were isolated during the enrichment of the gut contents of PE-eating waxworms when using PE as a single source of carbon. Following that, MSM with PE filmswas examined for PE degrading microorganisms among these isolates.

PE film Biodegradation Study

Spectrophotometer assay

The growth of the isolates was measured with Spectrophotometer assay. Of the ten isolates, five PD2, PD4, PD6, PD8, PD9 isolates were most active and their ODs were shown in Table1. The growth of microorganisms was confirmed by increased cultural resilience.

Table 1 Optical density of bacterial isolates

S.No	Isolates	Optical density 440nm

1	PD 1	0.021
2	PD 2	0.219
3	PD 3	0.04
4	PD 4	0.285
5	PD 5	0.061
6	PD 6	0.234
7	PD 7	0.065
8	PD 8	0.271
9	PD 9	0.256
10	PD 10	0.074
11	Control	0.030

Morphological changes

Morphological changes also show a major part in determining the degradation of PE. Various surface changes in PE films were observed after 60 days of incubation period. The surface of the PE films has turned smooth to coarse with cracks. Colour changes from blue to white for PE films are also shown. Sowmya, H.V (2014) reported morphological changes in the autoclaved and sterilized sample [9].

Figure 3 Colour change

Determination of dry weight of PE residuals

The efficacy of LDPE degradation is determined by the percentage of weight loss of PE films. The decline in PE has been demonstrated with the growth of PD2, PD4, PD6, PD8 and

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PD9 in MSM where PE is the only carbon source supported not only by survival but also by growth of isolate as reflected in dry weight loss in degradation of PE. The highest degradation capability of isolates was reported by PD8 for 15 microns is 8.83%, for 30 microns is 9.95 % and for 40 microns is 11.93% of PE after completion of 60 days incubation period as shown in the Table2. PE film is used as a single carbon source by these microorganisms, resulting in partial PE degradation. They colonize the PE film's surface and produce a bio film. The hydrophobicity of these organisms' cell surfaces was discovered to be important in the formation of biofilm on the surface of PE, which improved polymer biodegradation. Results shown in Table 2.

Table2 Weight loss percentage of PE films

Isolates	Weight loss%(15µ)	weight loss %(30µ)	weight loss%(40µ)
PD2	7.88	8.15	8.62
PD4	6.11	7.12	9.31
PD6	7.21	7.51	6.42
PD8	8.83	9.95	11.93
PD9	6.53	8.23	7.88
Control	0.00	0.00	0.00

Determination of pH changes

Of all the five, isolate PD8 15, 30 and 40 microns of PE it seemed to decline with a pH medium of values 6.684,

6.362 and 6.121. It was found that the media pH value of PD8 was 15.30, with 40 microns significantly lower than other media included with other isolates. All isolates showed an increase in pH except for PD-8 at 15, 30 and40 microns of PE containing media. pH changes have been adopted to confirm that any metabolic activity of bacterial isolates in a different environment, as well as metabolism exhibited by microbial cells, may help to support evidence of degradation. Similar results for Pseudomonas fluorescens, Pseudomonas aeruginosa have also been reported by [6]. The pH of the inoculated media was divided and measured and the results are shown in Table 3.

Table3 Effect of pH

Isolates	15Microns	30Microns	40 Microns

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PD2	7.631	7.412	7.910
PD4	7 .912	7.811	6.532
PD6	7.760	8.931	8.421
PD8	6.684	6.362	6.121
PD9	7.908	8.521	9.907
Control	7.00	7.00	7.00

Scanning Electron Microscopy of Polyethylene

The surface morphology of the PE films was examined using scanning electron microscopy to search for any structural alterations on the low-density polyethylene film. Figures 4&5 show Scanning Electron Microscopy micrographs of low-density polyethylene film before and after incubation with the isolates. The structural alterations and erosions on the surface of the polyethylene films were discovered as a result of this. On the polyethylene surface, there were also cavities.

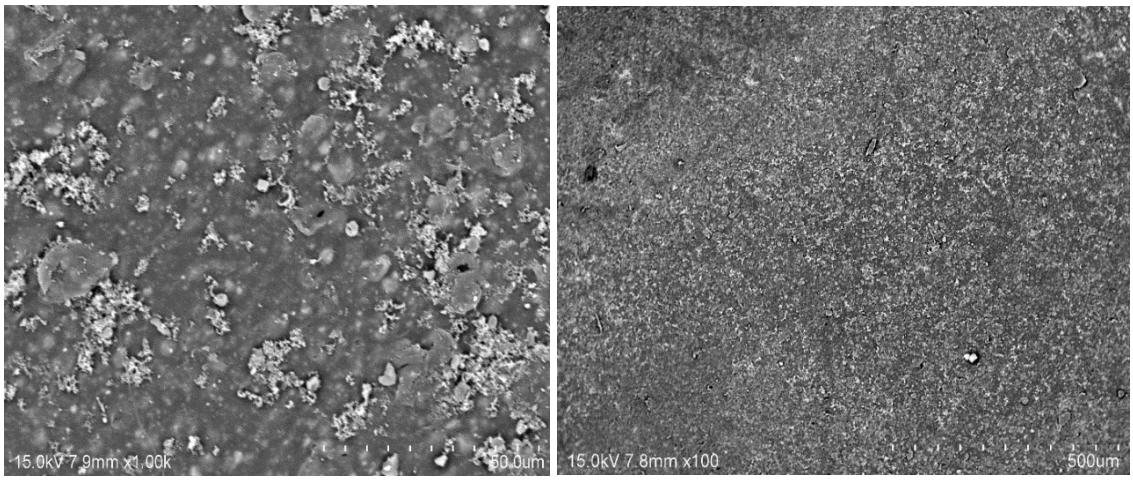

Figure 4 SEM Photograph of polyethylene samples treated with bacterial isolates

4.Conclusion

In this study it is shown that the microorganisms that were isolated from the gut of wax moths *Galleria mellonella* have the ability to degrade polyethylene. Microorganisms were able to use LDPE as the sole source of carbon, significant weight loss was observed by PD8 isolate at 40 microns at 11.93%, PD8 at 30 microns at 9.95% and at 40 microns is 8.83% of PE after completion of 60 days of incubation. Biodegradation is shown not only by biofilm formation and weight loss, but also by physical and chemical changes in the films. such as colour change, morphological changes.

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6.Conflict of Interest

This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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