

Waste Management and Environment Protection – A Study with Isolation and Characterisation of Low Density Polyethylene Degrading Microbes

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Abstract: In today's world most challenging task is protecting our earth environment from industrial hazards and other wastes. The most popular waste which is very difficult to manage should be the plastics that are used and thrown to the earth. Waste management helps to manage such waste and prevents the pollution that are caused by the things like plastics. Low density polyethylene is a vital source for environmental pollution. It happens by chocking sewer line through improper disposal thus posting everlasting ecological threats. Biodegradation of LDPE by active microbes which converts the polymer into monomer for disposal. The main objective of the study is to isolate and identify the microbes from polyethylene dumped garbage. The bacterial and fungal species were identified. A fungus shows maximum degradation than bacteria. The physic chemical and visual changes were observed in degradation. CO₂ evolution was also calculated to show the degradation of polyethylene strips. This paper examines the evils of plastics and the way to degrade the low density polyethylene.

Keywords: LDPE, bacteria, fungi, biodegradation, garbage.

1. Introduction

Waste Management is the 'generation, prevention, characterization, monitoring treatment, handling reuse and residual disposition of wastes [1]. Problems in waste management have become more and more complex during recent decades. The increasing volumes of waste produced and social environmental consciousness present prominent drivers for environmental managers towards the achievement of a sustainable waste management scheme. However, in practice, there are many factors and influences – often mutually conflicting – criteria for finding solutions in real-life applications. This paper presents a review of the literature on multi-criteria decision aiding in waste management problems for all reported waste streams.

Environmental protection is a practice of protecting the natural environment on individual, organizational or governmental levels, for the benefit of both the natural environment and humans. Due to the pressures of population and technology, the biophysical environment is being degraded, sometimes permanently. This has been recognized, and governments have begun placing restraints on activities that cause environmental degradation. Since the 1960s, activity of environmental movements has created awareness of the various environmental issues. There is no agreement on the extent of the environmental impact of human activity, and protection measures are occasionally criticized.

1.1 Evils from Plastics

Plastic has become an unavoidable material in our modern world. Plastics are versatile and durable, but this durability can make disposal problematic. Managing plastic waste is a major challenge in terms of environmental protection, but it's also a huge opportunity for resource efficiency. In a circular economy where high recycling rates offer solutions to material scarcity, I believe plastic has a future. I invite all stakeholders to

participate in this process of reflection on how to make plastic part of the solution rather than the problem.” Once in the environment, particularly in the marine environment, plastic waste can persist for hundreds of years. Up to 10 million tons of litter, mostly plastic, end up in the world's oceans and seas annually, turning them into the world's biggest plastic dump.

Plastic is often perceived as a cheap and disposable material in our "throw-away" society, and recycling rates are low. Half of all plastic waste generated in Europe goes to landfill, which should be avoided as plastic can contain hazardous components and disposal can result in undesirable emissions and concentrated, polluting residues. plastic plays in many industrial processes and applications, and the potential economic gains of higher recycling rates. As the world population grows and natural resources become scarcer, recycling plastics will be an alternative to the exploitation of virgin resources. To speed this change, better framework conditions are needed to support eco-design and environmental innovation, with waste prevention and recycling factored in to the design of plastic products.

If the plastics are not disposed properly it will affects a lot to the society. Some of them are listed:

- Accumulation of waste plastics in Earth's atmosphere as a result of human activities, causing global mean surface air temperature and subsurface ocean temperature to rise.
- Rising global temperatures are expected to raise sea levels and change precipitation and other local climate conditions.
- Changing regional climates could alter forests, crop yields, and water supplies.
- This could also affect human health, animals, and many types of ecosystems.
- Deserts might expand into existing rangelands, and features of some of our national parks might be permanently altered.

1.3 Extraction of Plastics

Plastics are synthetic polymers which extracted from oil, coal and natural gas. Polyethylene is a thermoplastic polymer produced by monomers of ethylene. Low density polyethylene is made from petroleum. LDPE materials are strong, light weight elastic and durable. LDPE is defined by density range of 0.910-0.940g/cm³. It is not re act at 37°C except by oxidizing agent. It can withstand temperature at 80°C continuously and 95°C for a limited period. It shows translucent or opaque in nature [2].

Low density polyethylene contains carbon and hydrogen. It brings excellent forms of acids, alcohols, bases, esters, aldehydes and ketones etc .It shows little resistance or inhibitory use of halogenated hydrocarbons [3]. Plastic bags imply a lot of convenient to people's life but at the same time it also produces harmful effects in terms of decomposition and burning. Inhaling of gases released from plastics causes lung cancer. The decomposition of LDPE bags takes about 1000 years. A lot of research work has been carried out to reduce the environmental burden by improving the degradability of waste. Abiotic treatment of uv radiation and thermal activity were subjected to reduce the hydrophilicity of polyethylene by introducing polar groups such as carbonyl groups to polyethylene chain [4]. The biodegradation of polyethylene by microbes like bacteria, fungi and actinomycetes were studied in both natural and synthetic plastics. The degradation of polyethylene has been studied extensively [5] earlier have reported the biodegradation of LDPE blended by fungi, *Streptomyces* species.

2. Biodegradation of Polyethylene

The major drawback in biodegradation of polyethylene is due to resistance to biological attack because of its hydrophobicity, high molecular weight and its lack of functional groups recognized by microbial enzymatic action [6]. Several analytical methods have been used to test biodegradability which shoes visual observation, molar mass variation, weight loss measurement, CO₂ evolution and clear zone formation etc. In aerobic conditions, microbes use oxygen to oxidize carbon into carbon dioxide as end product [7]. The aim of this study was to investigate the ability of bacteria isolated from polyethylene dumped garbage to degrade LDPE.

3. Materials and Methods

3.1 Extraction of polyethylene powder

Low Density Poly Ethylene (LDPE) sheets were collected from plastic industry. LDPE sheets were cut into bits and soaked in xylene and heated for 15 minutes as the xylene dissolves the LDPE film. The LDPE

powder so obtained was washed with ethanol to remove the residual xylene and allowed to remove the ethanol. The extracted powder was dried in a hot air oven at 60°C overnight.

3.2 Isolation of polyethylene degrading bacteria

1gm of sample was collected from polyethylene dumped garbage was suspended in 10 ml of sterile water and vortexed for 15 minutes. 1 ml of suspension was added to Erlenmeyer flasks containing 100ml of mineral salt medium, 1 gm of untreated thin LDPE strip was also added as sole source of carbon and energy. The isolated bacterial strains were identified macroscopically by colony morphology, surface pigment, shape and size on nutrient agar plates. Gram staining was used to study the behavior, shape and cell arrangement. Motile nature of bacteria was also observed by motility test.

Isolation of fungi from polyethylene dumped garbage

5gm of LDPE sample was inoculated in sterile synthetic medium and incubated at room temperature for a week. The isolated fungal strains were identified by both macroscopically and microscopic observation. The macroscopic identification was done by surface pigment formation on Sabouraud Dextrose Agar media. The shape, color and structure of conidia and hyphae was also observed.

3.3 Determination of dry weight of residual polyethylene

To achieve the accurate measurement of residual polyethylene weight, the bacterial and fungal biofilms were washed off the polyethylene surface with 2% sodium dodecyl sulfate and kept overnight and calculate the weight loss.

3.4 Colonization study

The colonizing capacity of the isolated fungi and bacteria were studied by microbes in petriplates. The mineral salt media and synthetic media can be used for bacteria and fungi. The media was poured into petriplates. LDPE sheets were cut into small pieces, disinfected with 70% ethanol for 30 minutes and transferred to sterile distilled water for 20 minutes. LDPE strips were added to the medium. The petriplates were incubated at room temperature and results were observed after 1 week to 28 days. The zone of clearance was observed [8].

3.5 CO₂ evolution analysis

The synthetic media supplemented fungal strains and mineral salts medium of bacterial colonies were used. Sterile air was allowed to flow through 1M potassium hydroxide solution containing bottles. The CO₂ free air was passed in test bottles. The CO₂ free air was utilized by inoculums that released carbon dioxide in absorption bottle. The test was carried out in a week. The amount of CO₂ produced in absorption bottle was calculated by adding barium chloride which forms a white precipitate of barium carbonate after a week [9].

The dissolved carbon dioxide in both medium were also measured volumetrically by titration. 25 ml of medium was taken in a conical flask and 0.05 ml of 0.1 N sodium thiosulphate solution was added, 2 drops of methyl orange was used as indicator. This solution was titrated against 0.02 M sodium hydroxide. The color changed from red to yellow as endpoint. Then added 2 drops of phenolphthalein, titration was continued till pink color was developed. The amount of CO₂ evolution was calculated by formula $A \times B \times 50 \times 1000 / V$ where A=ml of sodium hydroxide; B=normality of sodium hydroxide; V=Volume of sample.

4. Results and Discussion

Low density polyethylene degrading bacterial and fungal strains were isolated from polyethylene dumped garbage. These microorganisms were capable of growing on a synthetic and mineral salt medium containing polyethylene. The LDPE degrading bacteria were identified as *Streptococcus*, *E.coli*, *Klebsella*, *Bacillus* and *Pseudomonas* [10]. *Bacillus* has less capacity to degrade plastics. Fungal species were identified as *Saccharomyces*, *Aspergillus niger*, *Aspergillus flavus* and *Streptomyces*. After 30 days of incubation period, the percentage of weight reduction was reported in table 1 and 2. *Staphylococcus* and *Pseudomonas* showed maximum weight loss when compared with other bacterial strains. *Aspergillus niger* found to be higher in weight loss than other fungal species. *Aspergillus flavus* and *Klebsella* showed minimum weight loss of 11% and 21% respectively.

Table 1: Weight loss determination in LDPE degrading Bacteria

Sample	Initial weight	Final weight	Weight loss	Percentage loss
<i>E.coli</i>	0.25 grams	0.14 grams	0.11 grams	45%
<i>Staphylococcus</i>	0.23 grams	0.11 grams	0.12 grams	52%
<i>Pseudomonas</i>	0.28 grams	0.25 grams	0.13 grams	61%
<i>Klebsiella</i>	0.24 grams	0.19 grams	0.05 grams	21%
<i>Bacillus</i>	0.22 grams	0.13 grams	0.09 grams	40%

Table 2: Weight loss determination in LDPE degradation fungi

Sample	Initial weight	Final weight	Weight loss	Percentage loss
<i>Yeast</i>	0.25 grams	0.15 grams	0.10 grams	43%
<i>Aspergillus niger</i>	0.23 grams	0.09 grams	0.14 grams	72%
<i>Aspergillus flavus</i>	0.28 grams	0.25 grams	0.03 grams	11%
<i>Streptomyces</i>	0.22 grams	0.13 grams	0.09 grams	40%

The petri plates inoculated with different strains kept at room temperature were observed after 10-14 days from the day of inoculation. Zone of clearance was observed on the some of the colonies, of the total colonies, indicating the degradation of polythene strips(Fig 1) .

**Fig 1: Zone of clearance indicates the degradation of polythene strips**

The total amount of CO₂ evolved as calculated for bacterial and fungal cultures. We obtained good results from the carbondioxide evolution test for a period of week. *Aspergillus niger* evolved 4.2 g/L and *Pseudomonas* released 3.6 g/L. This also implies the degradation of polyethylene strips. Priyanka et.al,2011 reported that fungal strains showed maximum degradation in the form of weight loss, changes in tensile strength, percent elongation and molecular weight distribution.

Conclusion

From this study, we have proved that the hydrophobic LDPE films can act as a substrate for some group of microbes which formed a biofilm on LDPE. The bacterial and fungal species were isolated from polyethylene dumped garbage which grow well in a medium containing LDPE as a carbon source. These microbes degrade polyethylene strips and release carbondioxide. These ways help in the environment to reduce the pollution. It gives the advantages of water quality protection, reduce heat building, reduce soil erosion, improved air quality and natural resource conservation.

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