



Biodegradation of low-density plastic by bacterial isolates of polluted sites

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Abstract

Polythene plays an important role in our day to day life, from carry bags to storage containers and also food packaging. It is the most consistent commodity that has penetrated its roots deep into our lives. Being so widely used, it also causes nuisance in the environment due to its complex structure, higher molecular weight and recalcitrant nature. Conventional methods of disposal cause further harm by releasing toxic chemicals, gases and leachates into the atmosphere, soil and water. Therefore, to minimize the addition of toxic by-products because of incineration and land filling, biological methods are being adopted to contain the explosion of plastic. In accordance to this, the present study was conducted to assess the effect of bacterial strains isolated from polluted sites on low-density plastics. Bacteria were isolated from two sites of Pantnagar that were almost similar to each other in terms of physicochemical properties but differed in the type of waste being dumped. Site A was dumped with biomedical waste from Veterinary College whereas Site B was dumped with laboratory waste like broken glassware, old stationary and other refuses. The isolated bacteria were tested for biochemical activity and then inoculated in flasks filled with minimal media and strips of low-density plastic in them. After incubating for a period of 30 days, it was observed that isolate Ah8 exhibited maximum degradation efficiency of 8.43% (weight loss percentage). The isolate showed high similarity of 99% with bacterial strain *Bacillus altitudinis*.

Keywords: Bio-degradation, isolates, low-density plastic, degradation efficiency, incubation, microplastic, inoculation

Introduction

Plastic is a synthetic polymer which constitutes of carbon, oxygen, hydrogen, chloride, silicon and nitrogen. Organic polymers such as polyethylene, PVC (polyvinyl chloride), nylon, etc. are used to synthesize it, which can be molded into shape while soft, and set into a rigid or slightly elastic form. Coal, oil and natural gases are used to extract basic material for plastic production (Seymour, 1989). General formula of polyethylene is C_nH_{2n} , where 'n' is the number of carbon atoms (Sangale *et al.*, 2012). The first known natural polymers were shellac, resin, cobweb, animal glue and tortoise shell whereas first synthetic material was invented by Baekeland in 1907, 'Bakelite'. The light weight, low cost, high durability, high strength, corrosion resistance with high thermal and electrical insulation properties of plastic material render them fit for daily use. Polythene plays an important role in various products like packaging material, carry bags, transportation, insulation, water proofing etc. Polyethylene [Low Density (LDPE), Moderate Density (MDPE), High Density (HDPE) and Linear Low Density (LLDPE)], Polybutylene terephthalate (PBT), Polypropylene (PP), Polyvinyl Chloride (PVC), Polystyrene (PS), Polyurethane (PUR) and Nylons are the most predominantly used plastics.

Plastics are a success story as the world plastic production totaled around 57 million tons (Shristi Kumar *et al.*, 2007) in the year 2007 and presently about 322 million metric tons (Statista, 2017). Synthetic polymers started to take over natural polymers in almost every area, about half a century ago. With such an increasing amount of production, there is accumulation in the environment which evokes issues that are difficult to deal with. Currently, the world faces an increased flow of waste, energy exploitation and climate change accompanied by dumping of non-biodegradable

plastic wastes. This ends up dumped at various sites around the globe leading to unmanageable conditions as it takes many years to degrade. Plastics are extensively used in a wide range of products from daily use containers, carry bags, bottles, cups and trays to construction material like tubing, insulation, seals and gaskets etc. It makes progress possible by making the electrical and electronic appliances around us a lot safer, light weight, attractive and less noisy. Plastic is versatile, sanitary, durable and light weight that renders it fit to be used for packaging applications that include baby products, vending packaging, drums and protection packaging. With the increasing advancements in the field of transporting, it has become the need of the hour for cost-effective and safe transportation of people as well as goods. Due to the non-corrosive, mouldable and low-maintenance nature, plastic has become an integral part of transportation industry, being used internally for fittings as in dashboards, seats, flooring etc. and externally to give a flexible design and smooth curves to locomotive bodies.

Table 1.1: Types of plastic and their applications

Plastic(s)	Applications
Polyurethane (PUR)	Coating, insulation, paints, packing
LDPE, LLDPE	Bags
Polystyrene (PS), polypropylene (PP), PVC	Tanks, jugs, containers
Polyethylene terephthalate (PET), PVC, high density polyethylene (HDPE)	Bottles, tubes, pipes, insulation molding
Low density polyethylene (LDPE), linear low density polyethylene (LLDPE), polyvinylchloride (PVC)	Films and Packaging

Recalcitrant nature of plastic is due to its high molecular weight, complex three-dimensional structure, and hydrophobic nature; all of them hamper its availability to microorganisms (Hadad *et al.*, 2005). Not just soil, but plastics pose a threat to the marine environment too (Spear *et al.*, 1995). Marine plants and animals are often found entangled and dead in plastic nets and strings. They also ingest microplastic particles that further block their digestive tracts and ultimately cause death. Several methods are used to reduce the waste accumulation like burning, dumping and land-filling but these measures contribute to other issues like leaching of harmful substances, release of poisonous gases like NO_x , SO_x , and unburnt hydrocarbons

leading to air pollution. Time has come to adopt alternative measures to either reduce the use of such material or to eradicate the problem, once and for all. For this purpose, the concept of degradation has been introduced. Degradation is a process that involves breaking down of components of a material by environmental factors like light, heat, climatic conditions (Pospisil and Nespurek, 1997). Most of the recent studies on degradation of plastic are carried out using living entities like bacteria, fungi, etc. called biodegradation. It is a process in which micro-organisms break down compounds made from organic substances (mostly aerobically) and convert them into simpler compounds like CO_2 , H_2O and NH_3 .

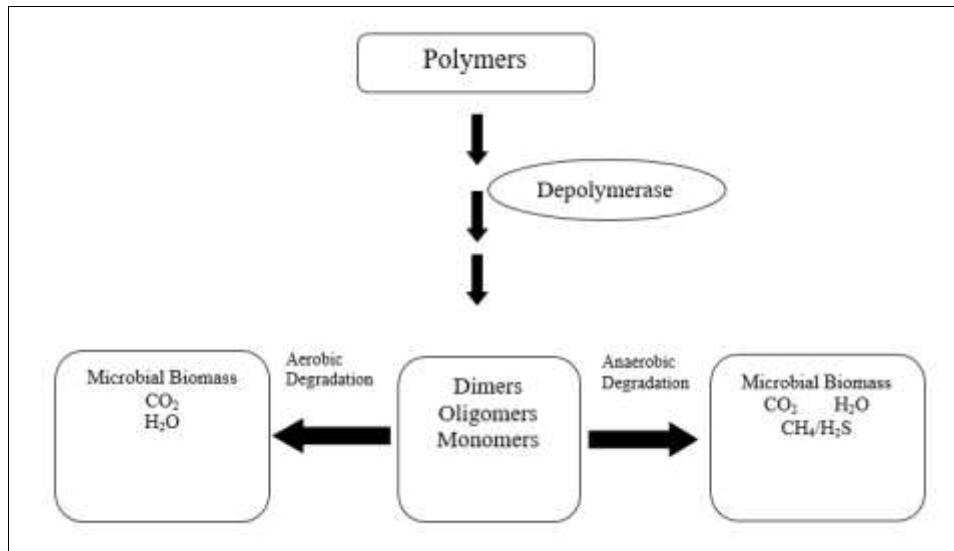


Fig 1: Polymer degradation process by aerobic and anaerobic microbes.

It is nature's way of dealing with waste accumulation and breaking down organic matter into intermediates that can further be used by other organisms. Bio-degradation is directed by various factors like polymer characteristics, type of organism and the nature of pre-treatment (Artham *et al.*, 2008; Gu *et al.*, 2000). Common sites for dumping plastic waste are the land-fills where soil microbial population plays a vital role in carrying out the degradation process. These microbes breakdown complex components of plastic material and enrich the soil by releasing the organic matter from the compound for which the first step required is attachment of microbes to the surface of plastic and form biofilm. At later stages, microbes release enzymes responsible for breaking down complex structures into smaller ones that are easier to absorb by the living entity. Recently, enzymatic degradation is most extensively being studied for plastic waste management. This method increases the rate of degradation without causing any harm to the environment (Bhardwaj *et al.*, 2012). Soil contains a wide range of microbes with different enzymatic activities assisting the biodegradation process. Most frequently reported bacteria and fungi degrading plastic material are *Pseudomonas*, *Acinetobacter*, *Arthrobacter*, *Brevibacillus* and *Aspergillus*, *Penicillin*, *Rhodococcus* respectively. The efficacy of these organisms to degrade polymers has been documented to increase using consortia instead of a single species. In the past decades, breakthrough advancement in technology like addition of commercially available oxo-biodegradable polymer additive has helped in the isolation and identification of microbial populations suitable for the

sole purpose of dealing with the proper disposal and management of plastic waste. Synthetic polymers like polyester, polyurethane, polyethylene with starch blend are found to degrade adroitly. Bioplastics (Biopolymers) obtained from plants or from growth of microorganisms; genetically-engineered to produce such polymers, are replacing currently used plastics at least in some of the fields (Lee, 1996). Many bacteria and archaea are being utilized to synthesize biodegradable plastics, polyhydroxyalkanoates is one of the biopolymers exhibiting properties like polyethylene and polypropylene (Kim and Lenz, 2001; Rehm, 2003).

Material and Methods

Sample collection: Soil sample was collected from sites dumped with plastic around Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Physicochemical analysis of soil

a. Texture: Soil is sieved using 2.0 mm (8 mesh), 1.0 mm (16 mesh) and 0.2 mm (72 mesh) sieve (BSS standards) one after the other.

- Weigh the soil taken initially on a weighing balance.
- Arrange the sieves in descending order of their size.
- Place the weighed soil sample on a sieve 1 and shake it.
- Weigh the sieved portion of soil and record the reading.
- Repeat the procedure for other sieves.
- Calculate the relative percent of sand, silt and clay in the soil sample as follows:

% Sand = mass of sand / total soil mass x 100

% Silt = mass of silt / total soil mass x 100

% Clay = mass of clay / total soil mass x 100

b. Soil moisture: Gravimetric method was employed to measure the moisture content of soil. Pre-weighed amount of soil was oven dried at 105°C for 24 hours. Final weight was taken and percentage was calculated based on the formula:

$$\text{Percentage moisture} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

c. pH and Electrical Conductivity: Potentiometrically determined using pH meter and EC meter. Soil and water are mixed in a ratio of 1:10 to carry out the measurement (Tan, 1996; Rhoades, 1996)

d. Organic Carbon: It was estimated by Walkley-Black titration method (1934) in terms of percent carbon in soil.

e. Nitrogen: Availability of nitrogen was estimated as described by Subbiah and Asija (1956).

f. Phosphorous: To determine phosphorous content in soil Dickman and Bray (1940) method was followed.

Isolation

a. Serial dilution: 1gm of sample was taken in test tubes and added with 9 ml of distilled water to make 1:10 dilution. Then adding 1ml of 1:10 dilution to 9ml of distilled water making 1:100 dilutions and so on till a dilution of 10⁻⁸ was reached.

b. Total Viable Count: Colony Forming Units/ g

$$\frac{\text{Number of Colonies}}{\text{Inoculum size (ml)} \times \text{Dilution Factor}}$$

Identification

Procedures mentioned in the Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986) were used to identify the isolates of bacteria. The basis of identification was difference in morphology, cultural and biochemical characteristics.

Morphology

Gram staining: Gram staining is used to classify bacteria into Gram positive and Gram negative by detecting peptidoglycan, also known as murein, which is found in the cell walls of Gram positive bacteria only. Gram negative bacteria also contain peptidoglycan but a very small amount that is dissolved by addition of alcohol.

Shake flask experiment

After the isolation and identification of bacteria, strips of commercially used polythene bags were set up for shake flask experiment.

1. Pre-treatment of polythene

1. Polythene bags were cut into small squares of 3x3 cm of size.
2. These were then transferred to a fresh solution used for surface sterilization containing 7ml Tween-20

(Polysorbate 20), 10ml bleach and 983ml distilled water then stirred for 30-60 minutes (El-Shafei *et al.*, 1998).

3. Strips were shifted to a beaker filled with distilled water and stirred for an hour.
4. Next, the pieces of polythene were placed in ethanol solution (70% v/v) for 30 minutes with the help of sterile forceps.
5. Finally the strips were placed on Petri plates to shade dry overnight.

Degradation of Pre-treated Polythene

- Initially weighed strips were placed in 200ml conical flasks containing 50 ml minimal media and inoculated with bacteria.
- Control was retained with microbe free media in the flasks.
- Flasks were kept in a shaking incubator for 30 days at 35°C, 120rpm
- After the incubation period, the strips were aseptically collected and rinsed with distilled water.
- Finally, strips were weighed and percentage weight loss was calculated using the formula mentioned below (Usha, et al. 2011):

Result and Conclusion

- The degradation of plastic was shake flask experiment where plastic strips were kept in minimal media, inoculated with different bacterial isolates in a shaker for one month.
- The plastic strips were the sole carbon source for microbial growth and percentage weight loss was calculated for each sample after one month.
- Maximum degradation rate was demonstrated by isolate Ah8 (8.43%) followed by 5.06% by isolate Bf7 and 2.87% by sample Ae1 after the incubation period of 30 days.
- A declining trend was observed in the weight of polythene strips even when the media was uninoculated.
- Data was analysed using statistical method: Two-way ANOVA which showed significant difference among the isolates but non-significant difference between same samples.
- The isolate exhibiting maximum degradation showed 99% similarity with *Bacillus altitudinis*.
- Physical rupturing of the polyethylene and chemical washing by ethanol might have added value to its degradability as some weight loss was observed in the control as well (0.07%).

Therefore, from the above findings we can conclude that the isolates were able to degrade plastic under laboratory conditions in a short span of time. Although, *B. altitudinis* hasn't been studied extensively and so its pathogenicity is still under question but can be revealed through more advanced study. The abovementioned results are indicative of the fact that if studied further, this microbe could be the missing piece of the puzzle that is plastic waste management.

References

1. Seymour RB. Polymer Science Before and After 1899: Notable Developments during the Lifetime of Maurtis Dekker. Journal of Macromolecular Science: Part A-Chemistry, 1989;26:1023-1032.

2. Sangale MK, Shahnawaz M, Ade AB. A Review on Biodegradation of Polythene: The Microbial Approach. *Journal of Bioremediation and Biodegradation*, doi:10.4172/2155-6199.1000164. 2012;3:164.
3. Hadad D, Geresh S, Sivan A. Biodegradation of Polyethylene by the Thermophilic Bacterium *Brevibacillus borstelensis*. *Journal of Applied Microbiology*, 2005;98:1093-1100.
4. Bhardwaj H., Gupta R and Tiwari A. Communities of Microbial Enzymes Associated with Biodegradation of Plastics. *Journal of Polymers and the Environment-Springer*. 2012;21(2):575–579.
5. Spear LB, Ainley DG, Ribic CA. Incidence of Plastic in Seabirds from the Tropical Pacific, 1984-91: Relation with Distribution of Species, Sex, Age, Season, Year and Body Weight. *Marine Environmental Research Journal*, 1995;40:123-141.
6. Usha R, Sangeetha T, Palaniswamy M. Screening of Polyethylene Degrading Microorganisms from Garbage Soil. *Libyan Agriculture Research Center Journal International*. 2011;2(4):200-204.
7. EI-Shafei H, EI-Nasser NHA, Kansoh AL, Ali AM. Biodegradation of disposable polyethylene by fungi *Streptomyces species*. *Polymer Degradation and Stability*, 1998;62:361-365.
8. Kandler O, Weiss N. Genus *Lactobacillus* Beijerinck 1901, 212 AL. In *Bergey's Manual of Systematic Bacteriology*, Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins, 1986;2:1209-1234.
9. Dickman SR, Bray R H. Colorimetric determination of phosphate. *Industrial & Engineering Chemistry Analytical Edition*, 1940;12(11):665-668.
10. Subbiah BV, Asija GL. A rapid method for the estimation of nitrogen in soils. *Current Science*, 1956;26:259-260
11. Tan KH. Infrared spectroscopy. Soil sampling, preparation and analysis. New York: Marcel Dekker, 1996, 278-298.
12. Kim YB, Lenz RW. Polyesters from microorganisms. *Advances in Biochemical Engineering/Biotechnology*, 2001;71:51-79.
13. Artham T, Doble M. Biodegradation of aliphatic and aromatic polycarbonates. *Macromolecular Bioscience Journal*, 2008;8(1):14-24.
14. Pospisil J, Nespurek S. Highlights in chemistry and physics of polymer. *Macromolecular Symposia*, 1997;115(1):143-163.