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Research Article

AN ATTEMPT TO STUDY THE BACTERIALDEGRADATION OF POLYTHENE BAG AND RUBBER BAND USING VELLORE (DUMPSITES) ENRICHED SOIL

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Abstract

Plastic materials pose a major problem despite being used in day to day lives. They cannot be degraded in a short span of time and thus plastic pollution is a matter of concern all over the world. A thin plastic bag itself requires 10-20 years to degrade, while a plastic bottle requires about 100 years for biodegradation (http://cmore.soest.hawaii.edu/cruises/super/biodegradation.htm). There is a need to understand in depth about the natural processes involved in breaking down of plastic so that new and better methods can be introduced based on the preexisting methods which speed up the process of degradation [1]. A thorough experimentation was carried out to find out if the microorganisms in soil can help in the process. The end result was the appearance of small pores on the plastic materials [2]. This proved that microorganisms play an important role in the degradation and breakdown of the bonds of plastic materials.

Keywords: Plastics, Degradation, Microorganisms.

Introduction

A plastic is a synthetic polymer made by linking together repeating units of small molecules called monomers. Commercially used plastic bags are made of polyethylene (-CH2-CH2-)[3]. Degradation of plastic is any change which has adverse effects on its properties and function. Plastics with high molecular weight are immune to microbial action. Plastics used in daily life such as polyethylene, polypropylene are difficult to degrade [4]. Low molecular weight hydrocarbons can be degraded by microbes. They are taken up by microbial cells, activated by attachment to coenzyme-A and converted to cellular metabolites within the microbial cell. However, these processes do not function in an

extracellular environment and the plastic molecules are too large to enter the cell. They are broken down by photochemical or chemical reactions to decrease their molecular weight so that microbial attack can successfully proceed. Microorganisms can degrade plastic over 90 genera, from bacteria and fungi, among them; *Bacillus megaterium*, *Pseudomonas* sp., *Azotobacter*, *Ralstoniaeutropha*, *Halomonas*sp., etc[5].

Plastic is used every day for all purposes. Today, life without plastic cannot be imagined as most of the things used by humans, from bottles to cups to rubber bands are made from plastic. Plastic is a material which cannot degrade in a small amount of time [6]. As a result, plastic pollution is a major concern today. India generates 56 lakh tonnes on plastic annually. 9,025 tonnes of plastic is recycled per day in the country and 6,137 tonnes remain uncollected and littered. In this way, about 40% of plastic waste is not recycled [7].The main motive behind studying this topic was to find out in detail about the natural ways to degrade plastic. Additional aims included the role of microbes in degrading plastic. Plastic pollution adversely affects wildlife, humans and plants. So, the need of the hour is to find out new and effective ways as well as improve the existing ways to dispose plastic without causing harm to the environment. The reason for choosing soil was solely because of the fact that soil is the natural abode of a variety of microorganisms, especially bacteria.

Materials and Methods:

Chemicals:

Nutrient agar, Agar powder, 1% glycerol, 70% ethanol (for surface sterilization) was used. They were procured from the store of Biochemistry lab of VIT University, Vellore.

Soil sample collection and dilution:

3 different samples of soil were collected from places in and around VIT, namely, VIT campus Woodstock, opposite All-mart and opposite Tom's diner, Vellore. Serial dilution of these samples was performed using Nutrient Broth up to 10^{-6}. 10^{-4} and 10^{-5} samples were chosen for further experimentation.

Pretreatment and preparation of broth:

The samples of plastic were cut and sterilized using 70% ethanol. Medium was prepared by adding 3.6g Nutrient Agar and 2g Agar powder to 120ml of distilled water. Broth was made by mixing 1.3g Nutrient Broth in 100ml distilled water. The petri pates, test tubes, media and broth were sterilized in an autoclave.

Spread plate using plastic sample as baits:

The prepared nutrient broth was poured in petri plates. The first petri plate was kept as control in each case. In the 2nd and 3rd plates, 10^{-4} and 10^{-5} were studied respectively. The diluted soil samples were spread on the plates by using a sterile L-shaped glass rod. Each sample of plastic was weighed and was then put in plates as baits. The plates were sealed using paraffin sealing tape and were kept in the incubator for about 10 days.

A few colonies of bacteria were seen growing after 10 days. Some samples showed colonies of organisms other than bacteria as soil is full of microorganisms.

Shaker Culture:

Fresh Nutrient broth was prepared and poured in 3 conical flasks. They were sterilized in autoclave. Keeping one as control, each sample of plastic was added to the flask after surface sterilization. The colonies formed in the petri plates were identified, and with the help of inoculation loop, transferred in each flask. The flasks were covered with cotton and were left in the shaker for 48 hours.

Result

Polythene and rubber band kept for incubation was observed under microscope. They were observed under 10X and images were taken.Small pores were observed on the surface of incubated polythene and the rubber band (Figure 2, Figure 3, and Figure 5). Significant difference in the physical structure of the original plastic and the plastic in shaker culture was observed. The appearance of pores over a time span of few days indicated that microorganisms which are present in soil can degrade and help in the process of natural degradation [8].

Table-1: Summary of previous work done in the field.

ORGANISM ISOLATED	SCIENTIST	COUNTRY	YEAR
Bacillus Subtilis, Bacillus Amylolyticus and Arthobacterdefluvii.	Pooja Thakur	India (Rourkela, Odhisha)	
***Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Diplococcus*sp., and**	K. Kathiresan	India (Mangrove	2003

***Micrococcus* sp. (belong to Gram- positive bacteria); *Moraxella* sp. and *Pseudomonas* sp. (belong to Gram-negative bacteria).**		forest soil)	
S. aureus, P. aeruginosa*, *A. niger, Rhizopussp* and *Streptomyces sp	M. Kannahi and P. Sudha	India (Muthupet mangrove soil)	2013

Images of Results:

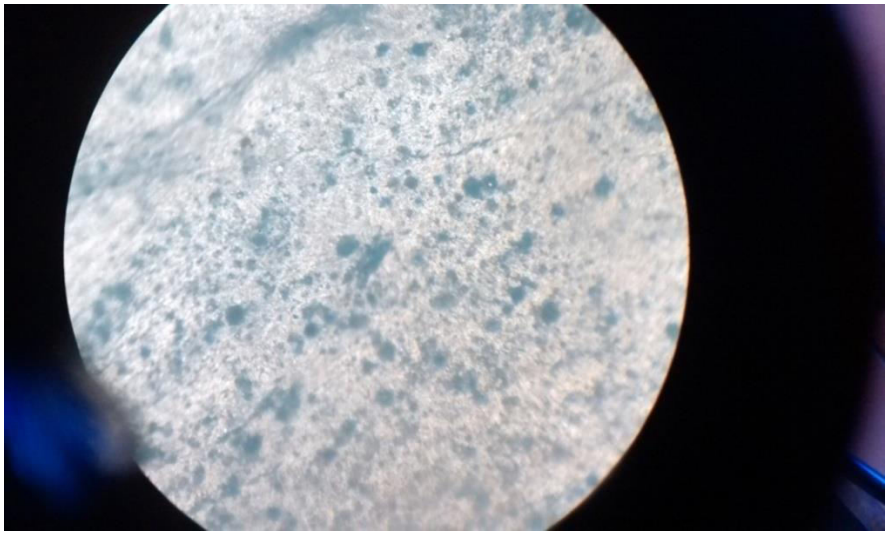

Figure-1: Polythene bag before degradation.

Microscopic image of original polythene bag before it was used in the experiment.

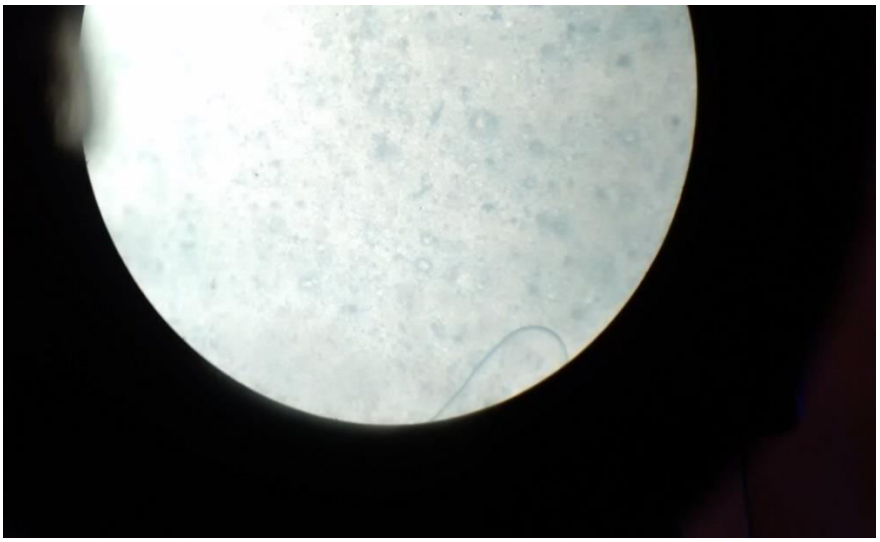

Figure-2: Polythene bag after degradation.

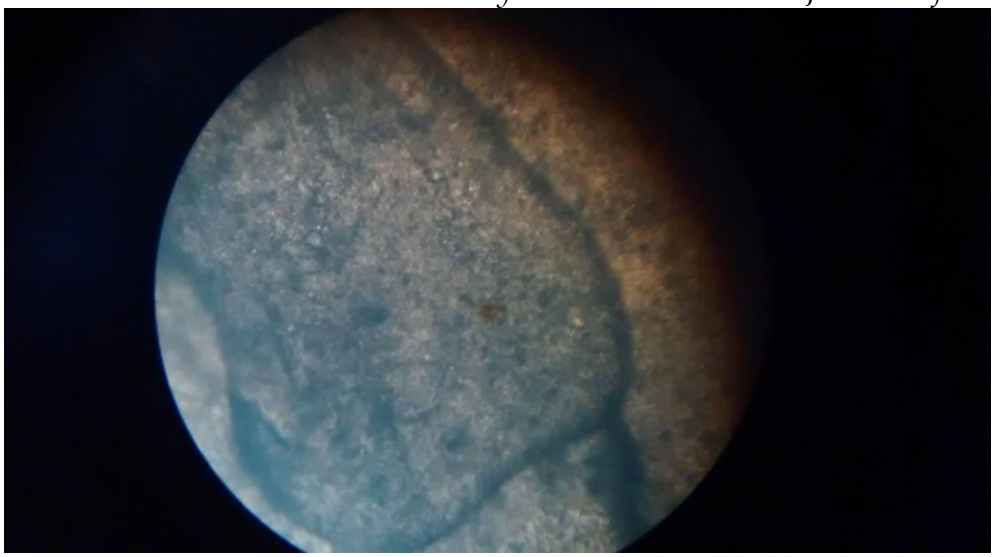

Figure-3: Polythene bag after degradation.

Microscopic image of the polythene bag after it was removed from the shaker culture.

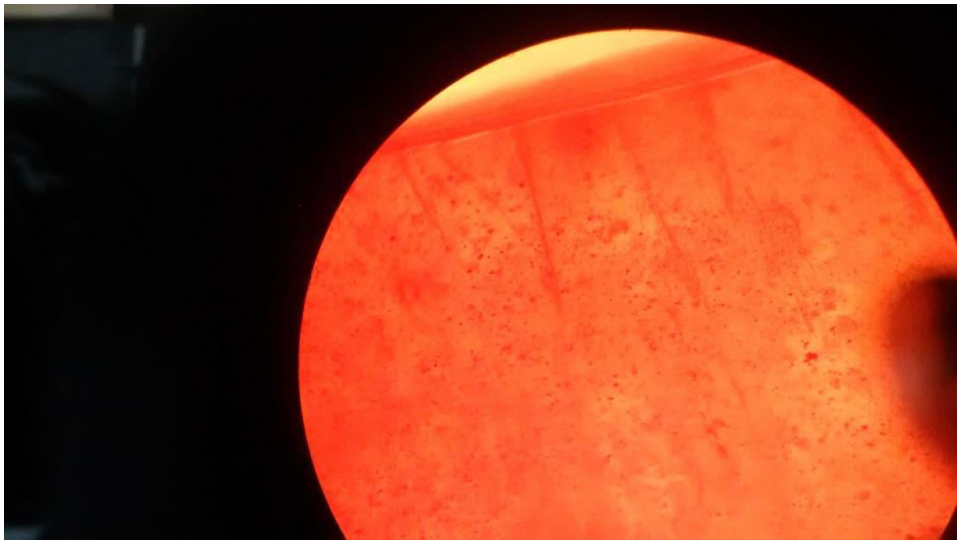

Figure-4: Rubber band before degradation.

Microscopic image of rubber band before it was used in the experiment.

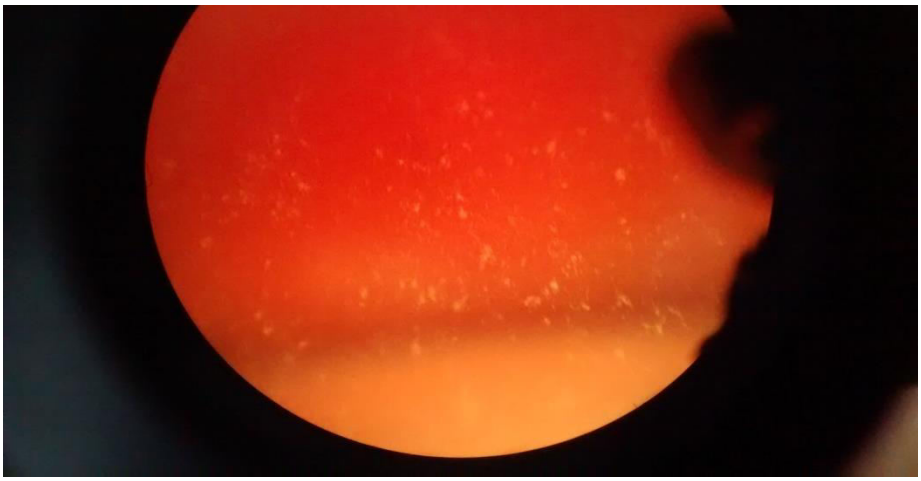

Figure-5: Rubber band after degradation.

Microscopic image of rubber band after it was removed from shaker culture.

Discussion

In the initial pictures of the bag and the rubber band (Figure1, Figure 4), clear bonds were seen but after leaving in shaker culture, definite bonds could not be seen in the rubber band while in the polythene bag, there were some structural differences [9]. The appearance of pores in the plastic materials gave evidence that degradation has occurred and suggested that microorganisms play a major role in plastic degradation. The nutrient broth provided the required nutrients for the growth of colonies [10]. The shaker culture ensured that the colonies didn't settle down and that the plastic samples received adequate aeration.

Conclusion

Soil contains various different species of microorganisms, especially bacteria, which help in the process of biodegradation of plastics. In a span of 10 days, the plastic developed pores which indicated that the biodegradation process had started. If left for a long time, further degradation will take place. Our future plans are to find out certain chemicals which can speed up this process of biodegradation so that the new method can be industrially useful as well as economic.

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