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Study on Low Density Polyethene Degrading Bacteria from Polyethene Garbage.

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ABSTRACT

Accumulation of plastics in the environment is creating a threat to the mankind. Some plastics are degradable which do not cause threat to the environment, and are being used for several purposes such as making of containers, use for packaging, water pipes etc. In this study, the isolation and characterization of polyethylene degrading bacteria from polyethylene garbage was done. Identification of the isolated strains was performed on the basis of colony morphology, grams nature, and several biochemical tests. Polyethylene strips were subjected to biodegradation by isolated bacteria using mineral salt medium. The degradation was observed by changes in physical and optical characteristics. The maximum degradation was observed in *staphylococcus* sp. *pseudomonas* sp showed the minimum degradation. The maximum amount of polyethylene degradation by weight loss method was observed by *staphylococcus* sp (52%) and 11% of degradation was found by *pseudomonas* sp.

Keywords: Biodegradation, plastics.bacteria, *pseudomonas* sp.

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INTRODUCTION

Plastics are the polymers which on heating become mobile and can be cast into moulds. Plastics are non-metallic compounds that can be moulded into any desired shapes and sizes. Plastics are used for packaging, making diapers, fishing nets, agricultural films and also used for many more purposes. Plastics and their use has become a part in all sectors of economy[1]. Present demand of plastics is increasing in the areas of agriculture, consumer goods, health and medicine

Plastics can be categorized into some of the basics classes such as: natural plastics, semi synthetic plastics, synthetic plastics, thermoplastics, thermosetting plastics. Amongst these plastics comes the Low density polyethylene (LDPE), Medium density polyethylene (MDPE) and High density polyethylene (HDPE) products. The LDPE, MDPE, and HDPE forms can be classified into polyethylene terephthalate, polyvinyl chloride, polypropylene, polystyrene etc[2].

Various mediums or environment as a whole is used for biodegrading polymers. Due to various physical as well as biological forces depolymerisation can be observed. The physical forces such as temperature, moisture, pressure cause mechanical damage to the polymers[3]. A large number of microorganisms have found to produce enzymes which degrade plastics and many more metallic as well as nonmetallic compounds.

Microbial degradation by enzymatic activities result in the cleavage of polymer into oligomers and monomers respectively. Aerobic metabolism results in the production of carbon dioxide and water, and anaerobic metabolism leads in formation of carbon dioxide, water and methane. Two types of degradation have been observed: oxidative degradation which results from oxidation and hydrolytic degradation which results from hydrolysis [4]. The physical as well as the chemical properties of the plastic determine the extent of degradation. The surface area, hydrophobicity, hydrophilic nature, chemical structure, molecular weight, crystal structure, elasticity, transition state, temperature etc play an important role in carrying out the degradation process [5].

One of the major factors in determining the degradability is the melting temperature(T_m). Melting temperature(T_m) is inversely proportional to biodegradability [6]. A large number of parameters such as roughening of surface, formation of holes, cracks, defragmentation, change in colour, formation of biofilms, CO_2 evolution, oxygen consumption, molar mass, formation of clear zones and weight loss of the compound help in measuring the extent of biodegradability[7,8,9].

MATERIALS AND METHODS

Three different grades of polyethylene bags obtained from plastic Industry.

Sample collection: Garbage soil samples (waste disposal sites dumped with plastic bags and cups) were collected in sterile containers from garbage dump in Selaiyur, Chennai.

Isolation of polyethylene degrading micro-organisms:

99ml of sterile distilled water was taken into a conical flask and One gram of soil sample was added to it and mixed well. Serial dilution of the sample was done. Pour plate method was adopted to isolate the microorganisms, and nutrient agar was used for the culture of bacteria. For each dilution, three replicates were made. For 2-7 days the plates were incubated at 30^oc. Subculturing of the developed colonies was done to obtain pure colonies and then the colonies were stored at 4^oc. The developed colonies were isolated and sub cultured repeatedly to get pure colonies and then preserved in slant at 4^oc [10].

Identification of microorganisms:

Gram staining, Colony morphology, Biochemical tests(catalase test, gelatin hydrolysis test, indole test, methyl red and voges proskauer test, starch hydrolysis test, simon's citrate test, and triple sugar iron agar test), and motility tests were performed on the isolated strains of microorganisms by bergey's manual and five types of microbial species were identified.

Degradation by clear zone method:

To the mineral salt medium, different three grades of polyethylene strips were added at concentration of 0.1 % (W/V) and the mixture was kept in shaker for one hour. The medium was sterilized at 121^oc and a pressure of 15 lbs/inch² for 20 minutes. The medium was poured into sterilized petriplates and isolated microbes were inoculated into the medium. The petriplates were incubated at 25-30^oc for 2-4 weeks and the organisms producing zone of clearance around colonies were selected for further tests [11].

Degradation by Broth culture method:

Prewighed polyethylene strips were added to the flask containing 50 ml of mineral salt medium and the medium was inoculated with the isolated microorganisms. The control flask was maintained with the mineral salt medium containing plastic strips but free of microorganisms. The test and the control samples were left in a shaker at 30^oc for 2 month period. After the incubation period the strips were collected from the medium, washed with distilled water, dried and weighed. The weight loss in the polyethylene strips was calculated finally.

RESULTS AND DISCUSSION

Identification of microorganisms: The identified bacterial species were E.Coli, Staphylococcus, Pseudomonas, Klebsiella and Bacillus. It is also based on the reports of Kathersan that *Pseudomonas* sp degraded the plastic upto 8.16% and 20.5% of degradation was observed anaerobically[12]. The 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. The colonies on which zone of clearance was observed can be further subjected to different tests. The colonies of *staphylococcus* sp showed maximum zone of clearance and the minimum zone of clearance was observed by *pseudomonas* sp. The degradation by broth culture was observed after 1 and 2 months of incubation on shaker at 150 rpm.

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	0.03 grams	11%
<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%

From the above table it can be interpreted that the degradation of plastic strips has taken place. Confirmation of the degradation can be done by observing the weight loss in the plastic strips. Maximum degradation was found to be by *staphylococcus* species and the minimum degradation was found to be by *pseudomonas* species. *Staphylococcus* showed 52% degradation and *pseudomonas* showed 11% degradation(Table1).

The major area of interest of this study is biodegradation of plastics by clear zone method and broth culture method. Microbial degradation of a polymer like polyethylene requires the formation of a biofilm on the polymer surface to enable the microbes to efficiently utilize the non-soluble substrates by enzymatic degradation activities [13]. Developments of multicellular microbial communities known as biofilm, attached to the surface of synthetic wastes have been found to be powerful degrading agents in nature [14].

The identification of the isolated strains was also done by gram staining which resulted in two gram negative and three gram positive strains of bacteria. Five different types of bacterial strains were identified. They are as follows:-*E.coli*, *Staphylococcus* spp, *Pseudomonas* spp, *Klebsiella* spp, and *Bacillus* spp.

The efficacy of microbes to degrade polythene strips was compared with the strains which showed the maximum and minimum degradation, and best quality plastics can be designed which can be easily degraded.

CONCLUSION

Biodegradation is a novel procedure to degrade different kinds of artificial substances in a biological manner. This helps to maintain balance in the surrounding. Bacterial strains were successfully isolated from polythene garbage dumps. Isolated bacterial strains were identified as *E.coli*, *Staphylococcus* sp, *Pseudomonas* sp, *Klebsiella* sp, and *Bacillus* sp. The isolated microbes are mutually native to LDPE, MDPE and HDPE. Most of the plastics are degraded naturally in 2years of decomposition. But LDPE degradation is carried out with microbes to reduce the environmental pollution.



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