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A COMPARATIVE STUDY OF LDPE DEGRADATING BACTERIA FROM POLYTHENE DUMPED GARBAGE

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Abstract

Plastics are used extensively in packaging and other industrial applications. Improper disposal of plastic material can cause environmental pollution and harmful to the life. The present article reveals the biodegradation of Low Density Polyethylene by bacteria and fungi strains isolated from polyethylene dumped garbage .The degrading activity of the bacterial strains were identified by performing colonization studies, clear zone formation SEM and XRD. Scanning Electron Microscope was used to study the structural changes in LDPE during degradation. The bacterial colonies of *E.Coli and Klebsella* were degraded rapidly than other bacterial colonies at pH 4 and pH9.

Keywords:

Plastics, SEM, bacteria and polyethene.

Introduction

Low Density Poly Ethylene is one of the major sources of environment pollution. LDPE is a thermoplastic which made from hydrocarbons of petroleum products. These materials are strong, light weight and durable. This widely used for making containers, dispensing bottles and various molded laboratory wares[1]. The disposal of polyethylene waste creates lot of ecological problems,lack of water holding capacity of the soil. It causes lung diseases and lung cancer[2].

Microbes play a important role in degradation of plastics. *Bacillus Megaterium*, *Pseudomonas,Azotobacter, Ralsonia eutropha, Halomonas* [3] and *Aspergillus flavus ,Mucor circinlloides*[4],*E.Coli,Stretococus*[5] and *Asergillus niger*[6]. All kinds of microbial strain could promote degradation for shorter period of time and degradation started only after a minimum period of 3-4 months [7].

Several analytical methods include visual observation, changes in molar mass, weight loss measurement, carbondioxide evolution,clear zone formation. Plasticsthat has been enzymatically broken and are easily absorbed by microbial cells and production of carbondioxide and water through anaerobic metabolism [8,9] . This study aims to isolate the polythene degrading bacteria and fungi from polythene dumped garbage and its degradation is determined by scanning Microscope.

Materials and methods

Soil samples were collected from polythene dumped garbage near camp road,Chennai, TamilNadu, during the month of June. 1gm of soil was suspended in 10ml of sterile water and vortexed for 15 minutes.

The Low Density Polyethylene (LDPE) film was used for this study was collected from local Market and its thickness is about 20 microns. LDPE films were cut into 3x3 cm strips and then washed with 70% ethanol for 30 minutes and air dried for 15 minutes and was added to the medium.To isolate microorganisms associated with materials (polythene bag and plastic cup) by pour plate method was adopted using, nutrient agar for bacteria. For each dilution, three replicates were made. The plates were then incubated at 30°C for 2-7 days. The developed colonies were isolated and sub cultured repeatedly to get pure colonies and then preserved in slant at 4°C.

The isolated bacterial strains were ranged as sample 1 to 5. The bacterial strains were identified macroscopically by examining colony morphology, surface pigment, shape and size on nutrient agar plates. Microscopic examination including Gram's staining is used to study the staining behavior, shape and cell arrangement.

Degradation of bacteria 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 121^0c and a pressure of 15 lbs/inch^2 for 20 minutes. The medium was poured into sterilized petriplates and isolated microbes were inoculated into the medium.The petri-plates were incubated at 25-30^0c for 2-4 weeks and the organisms producing zone of clearance around colonies[10].

Broth Culture Method: The pre weighted strips of 2cm diameter of different grades 5, 10 and 15 microns were added separately to 250ml of mineral salt medium and then inoculated with different polythene degrading microorganisms. Each flask was maintained at pH 4 and pH 9 inoculated with different microbial species containing different grade of polythene strip separately. Control was maintained with polythene and plastic strips in the microbe free medium and left in a shaker at 30°C, 150 rpm for 2, 4 and 6 month period. After the period of shaking the strips were collected, washed thoroughly

using distilled water, shade dried and then weighted to check the final weight. Finally the weight loss of the polyethene strips were calculated and compared with control.

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.

Colonization study: The colonizing capacity of the isolated fungi and bacteria were studied by microbes in petriplates. . The mineral salt 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 1week to 28 days. The zone of clearance was observed [8]

Scanning Electron Microscope and XRD Analysis

The surface morphology of the polyethene film was analyzed through scanning electron microscope. The structural change in the LDPE film when it was subjected to the degradation of polythene by bacteria was observed by SEM. A piece of film was placed on the sample holderand scanned at different magnification [11].

Results and discussion

The bacterial and fungal strains were isolated from polythene dumped garbage. This organisms are capable of multiplying on a carbon free synthetic and mineral salt medium containing polyethene [12]. The LDPE degrading bacteria were identified as *Streptococcus, E.coli, , klebsella,Bacillus* and *Pseudomonas. E.coli* and *Pseudomonas* showed maximum degradation. [13] .*E.coli* showed maximum zone of clearance(1.6 cm) in 5 micron thickness of polythene at pH 4 when compared with *klebsella* strains after 3months of incubation(Table1).

Table1: Zone of clearance (cm) in different thickness of low density polyethene treated with *E.coli and klebsella.*

LDPE thickness	***E.coli* at pH4**	***E.coli* at pH9**	***Klebsella* at pH 4**	***Klebsella* at pH 9**
5 micron	**1.6**	**1.5**	**1.5**	**1.2**
10 micron	**1.1**	**1.0**	**1.0**	**0.8**
15 micron	**0.6**	**0.3**	**0.5**	**0.3**

Figure-1: Zone of clearance of *E.coli*

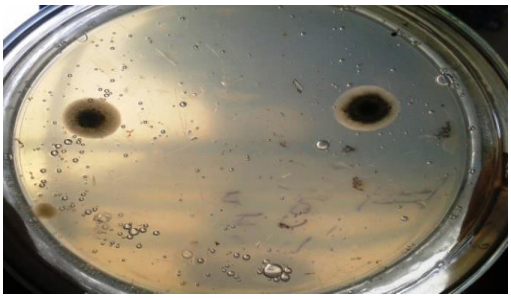

Figure: 2 Zone of clearance of *Klebsella.*

Zone of clearance observed on plate isolated with *E.coli,* containing 10 micron plastic strip maintained at pH 9 (Fig 1).Zone of clearance observed on plate isolated with *klebsiella,* containing 15 micron plastic strip maintained at pH 4 (Fig2) .The degradation by broth culture was observed after 2 and month of incubation on shaker at 150 rpm. And at the end of second month the weight loss in the samples were as follows.

Table-2: Weight loss in different ranges of low density polyethene.

LDPE thickness	***E.coli* at pH4 (weight loss/ %)**		***E.coli* at pH9 (weight loss/ %)**		***Klebsella* at pH 4 (weight loss/ %)**		***Klebsella* at pH 9 (weight loss/ %)**	
5 micron	**0.15**	**60**	**0.10**	**40**	**0.13**	**52**	**0.09**	**36**
10 micron	**0.08**	**32**	**0.07**	**28**	**0.08**	**32**	**0.8**	**24**
15 micron	**0.04**	**16**	**0.03**	**14**	**0.03**	**12**	**0.02**	**9**

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[14]. Maximum degradation was found to be by *E.coli* at pH 4 containing 5 micron plastics and the minimum degradation was found to be by *Klebsiella* at pH 9 containing 15 micron plastic strips.*E.coli* showed 60% degradation and conformed by SEM and *klebsiella* showed 9% degradation (Fig 3).

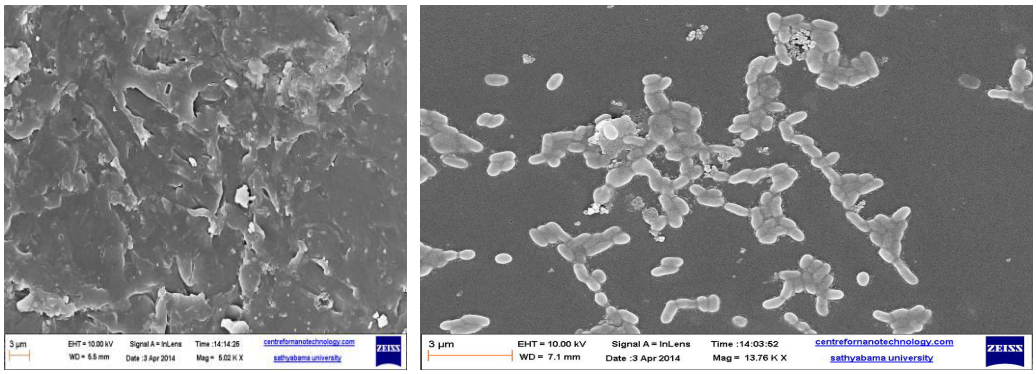

Figure-3: SEM of *Klebsella* and *E.coli* treated polythene strips.

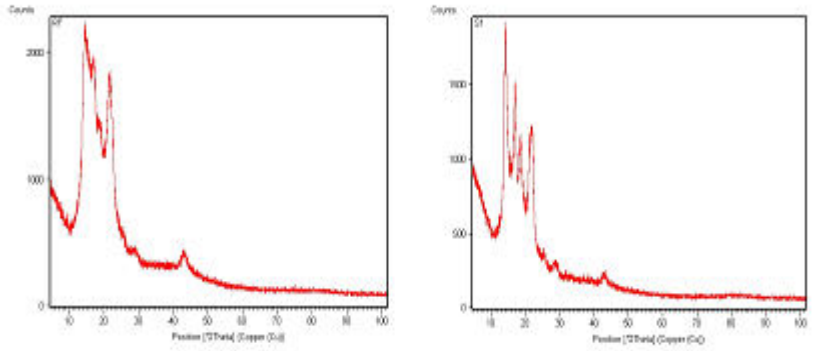

Figure-4: XRD analysis of untreated and Ecoli treated polythene strips.

The surface morphology of the treated polythene was observed through Scanning Electron Microscope. This helps to check the structural changes on low density polythene films. Pits and cavities were observed on the polythene surfaces (Fig 3). This is due to the absence of uniform distribution of branches or biodegradable products in polymer matrix[9].SEM showed the level of scission and attachment of the microbes on the surface of polythene[10,15]. XRD reflects the structural changes and bonds cleavage in degradation. The chemical degradation of polythene was proved by XRD (Fig 4).

Conclusion

In the present study, the bacterial strains of *E.coli* and *Klebsella* were isolated from polythene dumped garbage. Hydrophobic nature of LDPE films act as substrate for microorganism which colonize the surface of the film. The maximum degradation of polythene strips at pH4 .Thus polythene strips of 5-15 micron thickness were rapidly degraded and reduce the environmental pollution.

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