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Short Communication

Studies on Isolation and Identification of Active Microorganisms during Degradation of Polyethylene / Starch Film

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Abstract

Low density polyethylene is a vital cause of environmental pollution. It occurs by choking sewer line through mishandling thus posing an everlasting ecological threat. Biodegradable plastics are eco-friendly; they accumulate great potential applications in various industries. Biodegradable polymers degrade upon disposal by the action of active microorganisms in the soil. The result of degradation can be interpreted with physical changes through biological force. Microbial degradation of plastics convert polymer into oligomers and monomers. This microbial degradation may be based on aerobic and anaerobic metabolisms. The main objective of present study is to isolate and identify the microorganisms from soil during biodegradation testing of polyethylene/starch film. The isolation is been carried out through soil serial dilution method. An isolated microorganism is cultivated in culture media. After growth of microorganisms at 37 ^{0}C identification of microorganisms was carried out by macroscopic/microscopic examination. During identification it is found that bacteria (Pseudomonas spp, Streptococcus spp, Staphylococcus spp, Micrococcus spp and Moraxella spp etc), fungi (Aspergillus niger, Aspergillus glaucus etc), and Actinomycetes are present on the surface of polyethylene/starch film. Surface morphology of polyethylene/starch film has been analyzed by scanning electron microscopy (SEM) before and after degradation. Physico - mechanical properties has also been determined before and after degradation of film in order to understand the rate as well as the mechanism of degradation.

Keywords: Polyethylene, SEM, Biodegradation, Microorganisms, media.

Introduction

Plastics are man-made of long-chain polymeric molecules i. The initial breakdown of a polymer can result from a variety of physical, chemical, and biological forces ii. There is enormous data on the development of biodegradable plastics as well as the degradation of existing plastics using microorganisms since they are capable of degrading most of the organic and inorganic materials, including lignin, starch, cellulose, and hemicelluloses iii. There is a lot of interest in the microbial degradation of plastic and polythene waste material iv. The main objective of the present study is to isolate and identify microorganism from soil which utilizes polyester polyurethane as a sole carbon and nitrogen source v. Degradation has been reflected through changes of material properties such as mechanical, optical, electrical characteristics, crazing, cracking, erosion and discoloration phase separation[1,2].

The change includes bond scission, chemical transformation and formation of new functional groups vi. The disposal of plastics, especially those used in packaging, gives a serious challenge to the entire world such as land filling, recycling and incineration. Biodegradable plastics are now solving such environmental issues.

Material and Methods

Materials: Low density polyethylene grade from Reliance Polymer and starch from the Fizmerk India Chemicals (India) were used in present study. The chemicals used for biochemical tests were purchased from Bionic. Soil was used in biodegradation of polyethylene and starch film and film collected from sewage treatment plant, and films were washed and standardized as per ASTM D-5338 standard.

Preparation of LDPE/starch film: LDPE and starch were blended with twin screw extruder and the film was extruded in blown film plant of 60 micron thickness.

Soil burial biodegradation test: Square sample was taken in shape 5 cm where buried in about depth of 5 inch in soil. Biodegradability testing in the laboratory scale compost was conducted according to ASTM D - 5338. A series of twelve composting vessels (three test specimens, blank and negative and positive controls) were repeatedly tested twice times. Mixtures of testing soil (100 g, on dry basis) and testing specimen (10 g, on dry basis) were introduced and incubated at 58 ± 2^O C. The air flow rate was controlled 10 to 40 ml/min. the CO_2 evolved was absorbed by 0.024 N Ba (OH) $_2$ the amount of CO_2 was determined by titrated the solution with 0.05 NHCL.

(Frequency of every 3 to 4 days for first 2 to 3 weeks, and after then every 1 to 3 weeks).

Isolation of microorganisms: Soil burial degraded LDPE/starch film was taken and the microorganisms were isolated by the help of serial dilution technique. There are several studies are available for isolation and enumeration of microorganisms (bacteria, fungi, actinomycetes, protozoa, algae and viruses) from the soil. The isolation of microorganisms from the surface of the polymer pieces after soil burial was studied. Serial dilution technique was used for cultivation of the microorganisms. Finally media were used for isolation of microorganisms e.g. Nutrient agar for bacteria, Glycerol yeast agar for Actinomycetes and Czapek-Dox agar or Sabourad's dextrose agar for fungi. About 10^{-3} to 10^{-5} microorganisms were selected for enumeration of fungi, 10^{-3} to 10^{-7} for actinomycetes and 10^{-4} to 10^{-7} for bacteria relative to their proportion in soil.

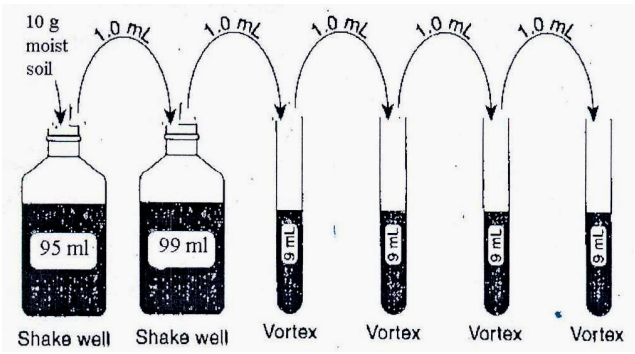


Figure-1
Soil dilution technique

Identification using biochemical tests: After the isolation, culture plates of specific microorganisms were used for gram staining to analysis the microorganism through the microscope. Identification of the isolates were performed according to their cultural and biochemical characteristics by following Bergey's Mannual of Systematic Bacteriology. All the isolates were subjected to gram staining and specific biochemical tests. Biochemical tests for identification of microorganisms were performed according to fungus and bacteria.

Fungal identification: The fungal identification were performed to fungal strains are isolated from soil with mixed sewage sludge. The fungus attached on the surface of specimen were purified on Saboraud agar plates and then identified by both macroscopic and microscopic examination. Macroscopic identification was done by visualizing surface pigment and reverse pigment on Sabouraud's agar and microscopic identification included shape, color, structure of conidia, hyphae, conidiophores and conidial head.

Bacterial identification: The bacterial strains isolated with the ability to degrade and performed on the basis of macroscopic and microscopic examination and biochemical test. The bacterial isolates were identified macroscopically by examining colony by examining colony morphology, surface pigment, size shape, margin, surface on media plates and microscopic examination including, grams staining to study the staining behavior, shape, and cell arrangement and granulation, spore staining. The motility test was also performed. Then second step performed biochemical test.

Figure-2
Bottle for biochemical test

Figure-3
Sabroud dextrose agar tube

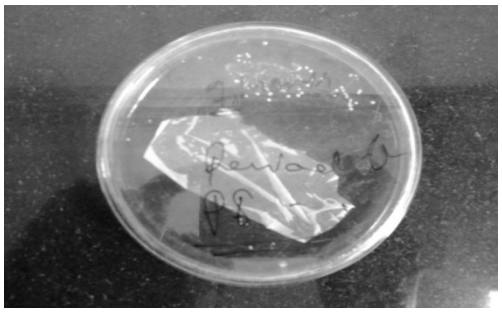
Results and Discussion

This study has covered the major concerns about the active microorganism during biodegradation. It is clear that most natural polymers can be degraded to some extent in the appropriate environment at the right concentration.

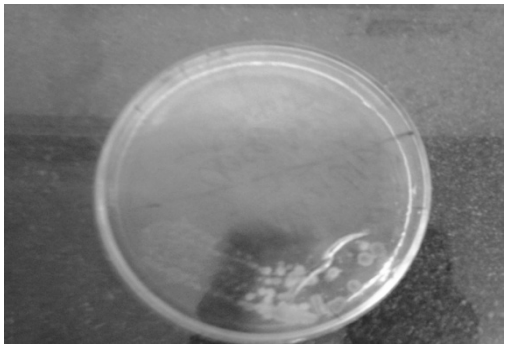
The present study deals with the isolation, identification, media and ability of plastic degrading microorganisms from soil. Different types of changes are produced by the microorganism during biochemical analysis. Synthetic plastic sample collected from the college campus was used in this study.

When the total biodegradation process of any organic substrate is considered the formation of microbial colony is critical to the initiation of biodegradation. Thus, the duration of the microbial colonization is an important factor that effects total degradation period.

Results of Biochemical Test: Biochemicals tests show that, catalaze and oxidize test result of all the strains were found to be positive and results were tabulated in table 1.



(a) Specific Microorganism growth



(b) Sample with blood agar media

Figure-4

Blood agar media plate for Bacterial cultivation and identification

i. Mannitol test of the strains were also found positive excluding strain Motility test. ii. Test show that all the strains are non-motile. iii. Citrate test of strain no 1 and 4 are found positive and rest of them show a negative result. iv. Nitrate reduction test of strain.4 is found positive and rest of them show a negative result. v. Malonate utilization test shows only strains 4 and 6 give a positive result. vi. The test named gas production from glucose show strains 2, 3 and 4 show a positive result.

Conclusion

The bacteria is identified to be Pseudomonas spp, Streptococcus spp, Staphylococcus spp, Micrococcus spp and Moraxella spp Bacillus subtilis, Bacillus amylolyticus and Arthobacter defluvii. Bacillus amylolyticus is more useful than other bacteria. Bacillus subtilis has less capacity to degrade plastic as compared to other bacteria. Fungi (Aspergillus niger, Aspergillus glaucus). The isolated microbes are native to the site of LDPE. Some degradability is present in natural conditions, yet they also exhibit biodegradation in laboratory conditions on culture media.

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Table-1

Biochemical test results

Sl. No.	Catalase test	Oxidase test	Mannitol test	Motility test	Citrate utilisation	Nitrate reduction	Malonate utilisation
1.	+ve	+ve	+ve	Non-motile	+ve	-ve	-ve
2.	+ve	+ve	+ve	Non-motile	-ve	-ve	+ve
3.	+ve	+ve	+ve	Non-motile	+ve	-ve	+ve
4.	+ve	+ve	+ve	Non-motile	-ve	+ve	+ve
5.	+ve	+ve	+ve	Non-motile	-ve	-ve	-ve
6.	+ve	+ve	- ve	Non-motile	-ve	+ve	-ve
7.	+ve	+ve	+ve	Non-motile	-ve	-ve	-ve