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# BIODEGRADATION OF PLASTIC BY MICRO ORGANISMS ISOLATED FROM GARBAGE SOIL

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## ABSTRACT :

*Plastic wastes accumulating in the environment are posing an ever increasing ecological threat. Plastics that are biodegradable can be considered eco-friendly; they have an increasing range of potential application and are driven by the growing use of plastics in packaging. In this study, the biodegradation of polythene bag was analyzed by varying period of incubation in liquid culture media. The microbial species associated with the polythene materials were identified as* ***Bacillus subtilis, Aspergillus niger and Aspergillus flavus.****The efficacy of microbes in the degradation of plastics was studied comparatively by bacteria (Bacillus subtilis) and fungal species (****Aspergillus niger and Aspergillus flavus).*** *The present study shows that the fungal species can degrade more as compare to bacterial species.*

## INTRODUCTION

Any physical or chemical change in polymer as a result of environmental factors such as light, heat, moisture, chemical conditions and biological activity is termed as degradation of plastic. Biodegradable polymers are designed to degrade upon disposal by the action of living organisms. Microbial degradation of plastics is caused by enzymatic activities that lead to a chain cleavage of the polymer into monomers. Microorganisms utilize polythene film as a sole source of carbon resulting in partial degradation of plastics. They colonize on the surface of the polyethylene films forming a biofilm. Cell surface hydrophobicity of these organisms was found to be an important factor in the formation of biofilm on the polythene surface, which consequently enhances biodegradation of the polymers. Once the organisms get attached to the surface, starts growing by using the polymer as the carbon source. In the primary degradation, the main chain cleaves leading to the formation of low-molecular weight fragments (oligomers), dimers or monomers. The degradation is due to the extra cellular enzyme secreted by the organism. These low molecular weight compounds are further utilized by the microbes as carbon and energy sources. The resultant breakdown fragments must be completely used by the microorganisms, otherwise there is the potential for environmental and health consequences. The purpose of this study was to isolate microorganism from dumped soil area and screening of the potential polyethylene degrading microorganisms and indentifying the high potential microorganism that degrade the plastics.

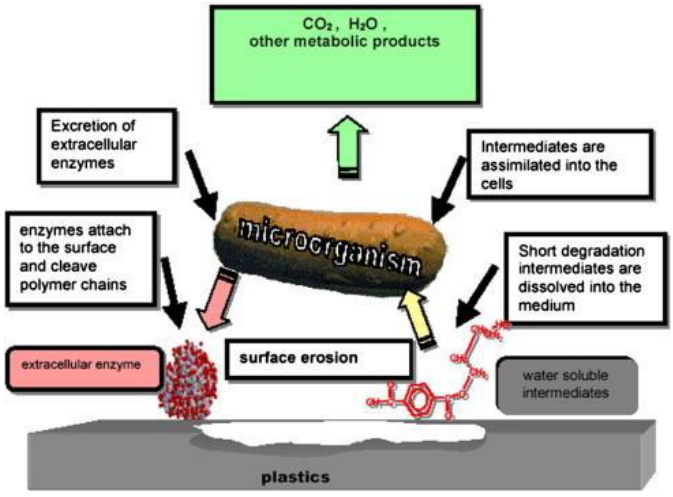

Fig.1: General mechanism of plastic biodegradation under aerobic conditions

Anaerobic consortia of microorganisms are responsible for polymer deterioration under anoxic conditions. The microbial biomass, Carbon dioxide, methane and water

are the primary products under methanogenic (anaerobic) conditions (e.g. landfills/compost) (Fig. 1).

## OBJECTIVES

This investigation proceeds with following different objectives

1. Isolation of microorganisms degrading plastics from plastic waste plastic polyethylene.
2. Determination of the percentage of degradation of polyethylene sample.
3. Comparative study of degradation of low density polyethylene/plastic by bacteria and fungus.
4. The final determination of low density polyethylene by measuring its final weight.

## MATERIALS AND METHODS

1) **Sample collection:**Plastic sample was collected from the MNC dumping ground located behind D.S.M. College, Parbhani
2) **Isolation of plastic degrading microorganism:**

a) **Serial dilution:**After the collection of plastic sample and 1gm of this sample was cut into pieces and added to 9 ml of sterile water to make 1:10 dilution, adding 1ml of the 1:10 dilution of 9ml of sterile water to obtain a 1:100 dilution and so on.

3) **Identification :**Identification of the isolates were performed on the basis of their morphological, cultural characters by following Bergey's Manual of Systematic Bacteriology. All the isolates were subjected to Gram staining.
4) **Enrichment:** The nutrient agar and Potato dextrose agar medium is used for bacterial and fungal enrichment.

## GRAM STAINING:

**METHOD:** A clean grease free slide was taken and a smear of the bacterial culture was made on it with a sterile loop. The smear was air-dried and heat fixed. Then it was subjected to the following staining reagents:

1. Flooded with Crystal violet for 1 min. followed by washing with running distilled water.
2. Again, flooded with Gram's Iodine for 1 min. followed by washing with running distilled water.
3. Then, the slide was flooded with Gram's Decolourizer for 30 seconds.
4. After that the slide was counter stained with Safranin for 30 seconds, followed by washing with running distilled water.
5. The slide was air dried and cell morphology was checked under microscope.

## COLONY MORPHOLOGY:

This was done to determine the morphology of selected strains on the basis of shape, size and colour.

**MOTILITY TEST:** The motility test was done to determine the motility of the organism. Bacterial cultures were stabbed into the motility test medium (Himedia) and were incubated at $37^0$ C for 48 hrs. Turbidity and observation of growth besides the stab line indicated a positive reaction whereas clear visibility with growth indicated a negative reaction.

**OXIDASE TEST:** The oxidase test was done with the help of commercially available disc coated with a dye N-tetramethyl paraphenylene diamine dihydrochloride (Himedia), to detect the presence of cytochrome 'c' oxidase which is responsible for the oxidation of the dye. Rubbing a small quantity of bacterial culture by means of a sterile toothpick on the disc causes formation of purple colour within 10-30 sec indicating positive reaction whereas no colour change indicates a negative reaction.

**CITRATE UTILISATION TEST:** This test determines the ability of bacteria to convert citrate (an intermediate of the Kreb's cycle) into oxaloacetate (another intermediate of the Kreb's cycle). Citrate is the only carbon source available to the bacteria in this media. If bacteria cannot use citrate, it will not grow. Positive result is seen if the bacteria grows and the media turns into bright blue colour as a result of an increase in the pH of the media.

**GAS PRODUCTION FROM GLUCOSE:** Gas production from glucose was assessed by inoculating the isolated strains in MRS broth containing glucose containing Durham tube in inverted condition and incubated at 37oC for 48-72 hrs. The upward movement of inverted Durham tube indicates positive reaction (gas production).

**CARBOHYDRATE UTILIZATION TEST:** For carbon utilization pattern HiCarbo Kit(Part A, Part B, and Part C) (Himedia catalog no. KB009) was used. Bacteria produce products that are acidic in nature when they ferment certain carbohydrates. The carbohydrate utilisation tests are designed to detect the change in pH that occurs if fermentation of the given carbohydrate occurred. Acids lower the pH of the medium which causes the pH indicator (phenol red) to turn yellow. If the given carbohydrate is not fermented by bacteria then the media remains red.

**Isolation of soil fungi, associated with materials (polyethylene bags and plastic bags) :**

-1g of dried soil sample was transferred into a conical flask containing 99ml of sterile distilled water.

-The contents were shaken and serially diluted. Fungi, associated with materials (polyethylene bags and plastic bags**)** were isolated by pour plate method using PDA.

-These plates were incubated at 28°C for 7 days.

-The fungal growth was isolated and sub-cultured to obtain pure colonies.

-Then, sub cultured colonies were preserved in slant at 5 $^{0}$C in refrigerator

**Microbial Degradation of Plastics in Laboratory Condition:**

**Determination of Weight Loss:**

Pre-weighed the piece of polythene bags were aseptically transferred to the conical flask containing 150 ml of culture broth medium, inoculated with different bacterial species. Control was maintained with the piece of polythene bags in the microbe-free medium. Different flasks were maintained for each treatment and left on the surface. After completion of incubation period the polyethylene piece were collected, washed thoroughly using distilled water, and ethanol air-dried and then weighed for final weight. The degradation of polyethylene was calculated by putting the collected data in the following formula.

$$\text{Degradation (\%)} = \frac{\text{1-Final weight}}{\text{Initial weight}}$$

**RESULT AND DISCUSSION**

The bacteria fungi were identified to be ***Bacillus Subtilis, A.niger***and***A.flavus*** fungi degrades plastic more than that of bacteria. Bacteria has less capacity to degrade plastic as compared to fungi. The isolated microbes were native to the site of polyethylene disposal and shown some degradability in natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media. This study has covered the major concerns about the natural and synthetic polymers, their types, uses and degradability also it has looked at the disposal methods and the standards used in assessing polymer degradation. Another area examined has been the biodegradation of plastics by the liquid culture method. It is clear that most recalcitrant polymers can be degraded to some extent in the appropriate environment at the right concentration.

The present study deals with the isolation, identification and degradative ability of plastic degrading microorganisms from soil. Different types of changes are produced by the microorganism during morphological and biochemical analysis. Synthetic plastic sample was

collected from the dumped soil of hostel garden was used in this study. This plastic was used to study their biodegradation by microorganisms isolated from them.

Microbial degradation of a solid 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. Development of multicellular microbial communities known as biofilm, attached to the surface of synthetic wastes have been found to be powerful degrading agents in nature. 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.

In the present study, plastics were inoculated in the liquid culture medium containing bacterial and fungal isolates and kept for varying days of period(30,40,50,60 respectively) to observe the percentage of weight loss by microbes. The result shows the degradative ability of the microorganisms after incubation. The percentage of weight loss due to degradation was found more by fungi. This shows it has the greater potential of degradation compared to other bacteria.

**Table no.1: Percentage degradation by bacteria (*B.subtilis*).**

| Incubation Period(Days) | Treatment | Initial weight(gm) | Final weight (gm) | % weight loss/days(%) |
|---|---|---|---|---|
| | Control | 0.036 | 0.036 | 00.00 |
| 30 | *B.subtilis* -Sample 1 | 0.031 | 0.025 | 31.45 |
| 40 | *B.subtilis* -Sample 2 | 0.029 | 0.016 | 33.93 |
| 50 | *B.subtilis* –Sample 3 | 0.029 | 0.012 | 34.06 |
| 60 | *B.subtilis* –Sample 4 | 0.029 | 0.008 | 34.20 |

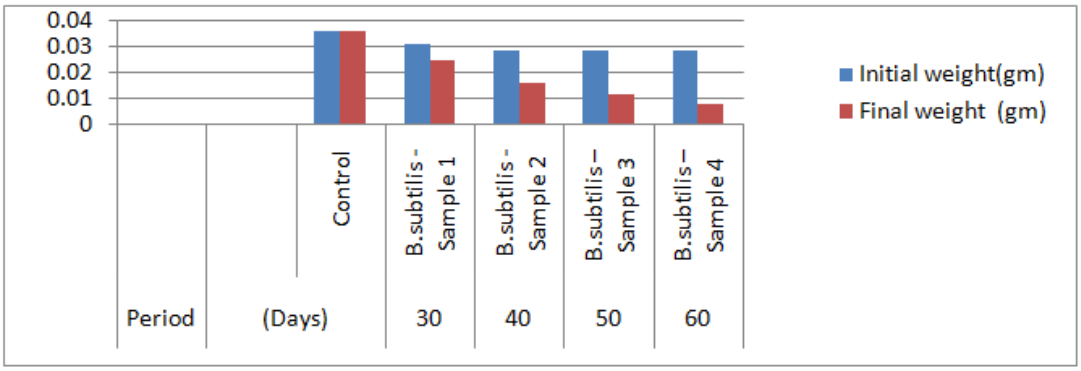


**Table no.2: Percentage degradation by *A.niger species.***

| Days | Treatment | Initial weight(gm) | Final weight (gm) | % Weight loss/days (%) |
|---|---|---|---|---|
| | Control | 0.036 | 0.036 | 00.00 |
| **30** | *A. niger* -Sample 1 | **0.042** | **0.034** | **23.00** |
| **40** | *A.niger* –Sample 2 | **0.021** | **0.015** | **46.90** |
| **50** | *A.niger* –Sample 3 | **0.021** | **0.010** | **47.14** |
| **60** | *A.niger* –Sample 3 | **0.21** | **0.015** | **46.90** |

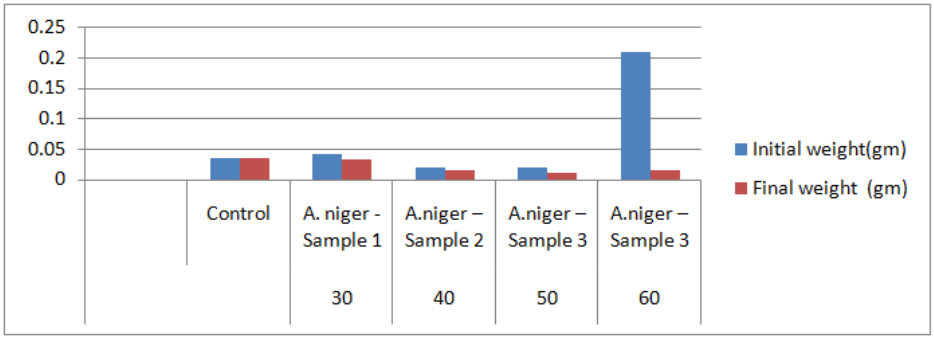


**Table no .3 Percentage of degradation *by A.flavus species.***

| Days | Treatment | Initial weight(gm) | Final weight (gm) | % weight loss/days (in %) |
|---|---|---|---|---|
| | Control | 0.036 | 0.036 | 00.00 |
| 30 | *A.flavus* -Sample 1 | 0.058 | 0.045 | 16.46 |
| 40 | *A.flavus* -Sample 2 | 0.036 | 0.026 | 37.07 |
| 50 | *A.flavus* -Sample 3 | 0.11 | 0.020 | 44.50 |
| 60 | *A.flavus* -Sample 4 | **0.13** | **0.013** | **66.92** |

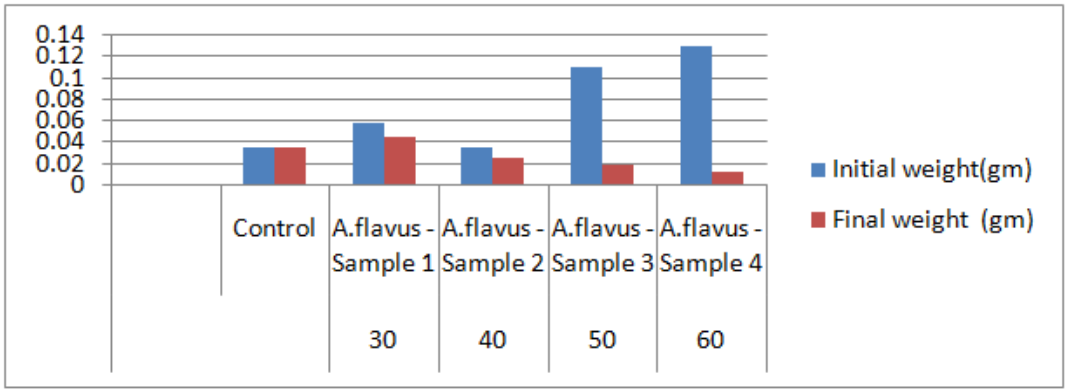


**Table no.4: Result of Biochemical Test**

| Species | Catalase test | Oxidase test | Motility test | Citrate utilization test | Gas production from glucose |
|---|---|---|---|---|---|
| *B.subtilis* | +ve | +ve | Non motile | +ve | -ve |
| *A.niger* | +ve | +ve | Non motile | +ve | -ve |
| *A.flavus* | +ve | +ve | Non motile | +ve | -ve |

## CONCLUSION

- The bacteria fungi were identified to be ***Bacillus Subtilis, A.niger*&*A.flavus***
- Fungi degrades plastic more than that of bacteria.
- Bacteria has less capacity to degrade plastic as compared to fungi.
- The isolated microbes were native to the site of polyethylene disposal and shown some degradability in natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media.

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