BIODEGRADATION OF PLASTIC BY BACILLUS SUBTILIS ISOLATED FROM POLYTHENE POLLUTED SOIL

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ABSTRACT

Polyethylene is a commonly used raw material in manufacturing plastics, which are non-biodegradable substances that stay in the environment for an infinite period of time. According to the Central Pollution Control Board (CPCB), India generates nearly 56 lakh tonnes of plastic waste each year with only 60% of it being recycled and reused. The remaining 40% causes not just littering but it is hazardous to freshwater, marine and terrestrial ecosystems. Bacillus subtilis is the most efficient soil microbes which degrade polyethylene without the need of applying any external treatments and may provide an eco-friendly solution for management of plastic waste. Plastic degradation by microbes due to the activity of enzymes that cause cleavage of the polymer chains into monomers and oligomers. The degradation of plastic by Bacillus subtilis is analysed using liquid (shaker) culture method. Microbes degrade plastic more in 1 month period.

KEYWORDS: Polyethylene, plastic, microbes, degradation, Bacillus subtilis, eco-friendly, ecosystem.

INTRODUCTION

Plastic is the most versatile synthetic ‘manmade’ substance created out of the fossil fuel resources that enable most of the industrial and technological revolutions of the 19th and 20th...
centuries. During the past 25 years, plastic materials have gained widespread use as they have been increasingly used in food, clothing, shelter, transportation, construction, medical and leisure industries. Plastics are composed of petroleum based materials called resins (e.g., polythene and polypropylene) materials that are resistant to biodegradation. Due to this resistance, plastics that are disposed in landfills remain in their original form in perpetuity. Plastics offer a number of advantages over alternative materials they are lightweight, low cost, extremely durable and relatively unbreakable.[1] Plastic is the general term for a wide range of synthetic or semi-synthetic polymerized products. The term biodegradable plastics normally refer to an attack by microorganisms on non-water soluble polymer-based materials (Plastics). The extracellular enzymes are too large to penetrate deeply into the polymer material, and so act only on the polymer surface; consequently, the biodegradation of plastics is usually a surface erosion process. A very general estimate of worldwide plastic waste generation is annually about 57 million tons.[2]

The polythene is the most commonly found non-degradable solid waste that has been recently recognized as a major threat to life. Low density polyethylene is one of the major sources of environmental pollution. Polyethylene is a polymer made of long chains of ethylene monomers.[3] Plastic degradation by microbes due to the activity of certain enzymes that cause cleavage of the polymer chains into monomers and oligomers. Aerobic metabolism produces carbon dioxide and water. Instead of anaerobic metabolism produces carbon dioxide, water, and methane as end product.[4] Plastics are defined as the polymers (solid materials) which on heating become mobile and can be cast into mould.

**Types of plastics:** There are two types of plastics: thermoplastics and thermosetting polymers. Thermoplastics are plastics that do not undergo chemical change in their composition when heated and can be moulded again and again. Thermosets are assumed to have infinite molecular weight. These chains are made of many repeating molecular units, known as repeating units, derived from monomers; each polymer chain will have several thousand repeating units. Thermosets can melt and can be molded into various shapes. After they are solidified, they remain solid. In the thermosetting process, a chemical reaction occurs which is irreversible. Vulcanization of rubber is a thermosetting process.[5]

**Biodegradation:** 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. Microbial degradation of plastics is caused by enzymatic activities that
lead to a chain cleavage of the polymer into monomers. 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 extracellular enzyme secreted by the organism. These low molecular weight compounds are further utilized by the microbes as carbon and energy sources. Exoenzymes from microorganisms break down complex polymers during degradation yielding smaller molecules of short chains, that are small enough to pass the semi-permeable outer bacterial membranes, and then utilized as carbon and energy sources. This process is called depolymerisation. The degradation is called mineralization when the end products are Carbon dioxide, water, or methane.\textsuperscript{[6]}

**MATERIALS AND METHODS**

**Sample collection:** Plastic sample was collected from the Pavendar Bharathidasan Institution, Ladies hostel, Trichy.

**Serial dilution:** Samples were placed in shaker at room temperature for 24-48 hrs. The serially diluted samples were plated on Trypticase Soy Agar (TSA) and incubated at 37\textdegree C for 24 hrs.

**Identification:** Identification of the isolates were performed according to their morphological, cultural and biochemical characteristics.\textsuperscript{[7]} All the isolates were subjected to Gram staining and specific biochemical tests.

**Morphology**

**Gram-staining method:** A clean grease free slide was taken and smear of the bacterial culture. The smear was air-dried and heat fixed.

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

**Biochemical test:** Biochemical Identification test kit is a standardized colorimetric identification system. The test is based on the principle of change in pH and substrate utilization.

**Catalase test:** The catalase test was performed to detect the presence of catalase enzyme by inoculating a loopful of culture into tubes containing 3% of hydrogen peroxide solution. Positive test was indicated by formation of effervescence or appearance of bubbles, due to the breaking down of hydrogen peroxide to O₂ and H₂O.

**Oxidase test:** The oxidase test was done with the help of commercially available disc coated with a dye N-tetramethyl paraphenylene diamine dihydrochloride, to detect the presence of cytochrome ‘c’ oxidase. 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.

**Motility test:** The motility test was done to determine the motility of the organism. Bacterial cultures were stabbed into the motility test medium and were incubated at 37°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.

**Mannitol test:** This experiment is generally performed to determine whether the bacteria are capable of fermenting mannitol sugar or not. Whenever organisms ferment mannitol agar, the pH of media becomes acidic due to production of acids. The fermentation of the media form red to yellow which shows positive test result.

**Nitrate reduction test:** This test was done to test if microorganisms are able to convert nitrate to nitrite or not by adding 1-2 drops of sulphanilic acid and 1-2 drops of N, N-Dimethyl-Naphthylanine reagent to the kit medium. Immediate development of pinkish red colour there on addition of reagent indicates positive reaction. Negative reaction could be observed if there is no change in the colour.

**Citrate utilisation test:** This test determines the ability of bacteria to convert citrate into oxaloacetate. 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 grow and the media turns into bright blue colour as a result of an increase in the pH of the media.

**Microbial degradation of plastic**

Pre-weighed discs of 0.5cm diameter prepared from polythene were aseptically transferred to the conical flask containing 50 ml of culture broth medium, inoculated with different bacterial species. Control was maintained with plastic discs in the microbe free medium. Different flasks were maintained for each treatment and left in a shaker. After one month of shaking, the plastic discs were collected, washed thoroughly using distilled water, shade dried and then weighed for final weight. From the data collection, weight loss of the plastics was calculated.

**RESULT**

Table no: 1 Gram staining

<table>
<thead>
<tr>
<th>Colour</th>
<th>Characteristic</th>
<th>Shape</th>
<th>Bacterial strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purple</td>
<td>Gram positive</td>
<td>Rod</td>
<td><em>Bacillus subtilis</em></td>
</tr>
</tbody>
</table>

Table no: 2 Biochemical test

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Catalase Test</th>
<th>Oxidase Test</th>
<th>Motility Test</th>
<th>Mannitol Test</th>
<th>Nitrate Reduction Test</th>
<th>Citrate Utilisation Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus Subtilis</em></td>
<td>+</td>
<td>-</td>
<td>motile</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

(+) Presence, (-) Absence

Table no: 3 Result of degradation of plastic sample by bacteria after 1 month

<table>
<thead>
<tr>
<th>Strain</th>
<th>Initial weight (mg)</th>
<th>Final weight (mg)</th>
<th>Difference (mg)</th>
<th>Weight loss/month (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>50</td>
<td>35</td>
<td>15</td>
<td>30</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Biodegradation of plastics take an active part in different soil conditions according to the nature of the bacteria, because the microorganisms responsible for the degradation. This study has covered the major concerns about the natural and synthetic polymers, their types, uses and degradability. The isolated strain was identified as *Bacillus subtilis* by performing appropriate identification tests. The surface changes of the plastic samples were observed after incubation with soil isolate. *Bacillus subtilis* has the ability to tolerate and degrade many toxic and hard polymers substances.
CONCLUSION
The surface of plastic materials has turned from smooth to rough with cracking. This may due to the compounds secreted by *B. subtilis* that may break the complex molecular structure of plastics. Hence, study on microbial enzymes in degradation of the polyethylene plastics will pave way for technology for degrading the plastic material. Degradation of plastic leads to decrease the molecular weight.

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REFERENCE