BIODEGRADATION OF LOW DENSITY POLYETHYLENE BY MICROORGANISMS FROM GARBAGE SOIL

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ABSTRACT

Plastics have been widely used as a packing material in the form of low density polyethylene (LDPE). Continuous accumulation of plastic in the environment can cause threat to humanity and environment. In order to stop the accumulation of plastic and to make the surroundings free from plastic, microbes are isolated from Andhra Pradesh and Telangana areas garbage soil. These microbes are screened by clear zone technique using polythene powder to confirm the degradation activity. Biodegradation of polymer granules by the isolated organisms and it makes physical and structural changes over a period of time after microbial adhesion to the granules. To check the efficiency of biodegradation, weight method was performed under laboratory conditions for 2, 4 and 6 months. Experimental data revealed that Streptomyces spp have highest plastic degradation capacity and it degrades up to 46.7%, this degradation was followed by the Aspergillus niger (26.17%), bacterial species Pseudomonas spp (24.22%) and A. flavus (16.45%) for the period of 6 months. This work revealed that Streptomyces spp plays a vital role in degrading polythene powder and polymer granules.

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1 Introduction

Plastics are the synthetic polymers of carbon, hydrogen and oxygen which are derived from petrochemicals. About 140 million tons of synthetic polymers are produced worldwide annually with their utility escalating at a rate of 12% per annum (Shimao, 2001). Each year, an estimated 500 billion to 1 trillion plastic bags are consumed worldwide (Roy et al., 2008). After their usage these packing materials are dumped in landfills leading to pollution since they are non-biodegradable under natural environmental conditions (Burd, 2008). LDPE is a thermoplastic polymer with significant short branches, commonly made by copolymerization of ethylene with longer-chain olefins.

There are different methods of disposal of plastics such as incineration, recycling and landfills (Sharma & Sharma, 2004). However, biodegradation plays a key role in reducing the molecular weight of the polymer by naturally occurring microbes like bacteria, fungi and actinomycetes isolated from different environments (Gu, 2003; Sivan et al 2006). Although literature exists on degradation of many types of plastics, all of them are starch blended. Reports on microbes able to degrade pure form of polyethylene are not much highlighted, especially on polymer granules. The ability to degrade polymers depends on the enzymes produced by the microbes to convert the polymers to oligomers and then to monomers. These water soluble enzymatically cleaved products are further absorbed by the microbial cells as carbon source where they are metabolized (Vasile, 1993). Hence the present manuscript aims to isolates and investigate on the different microbial species that are capable to degrade polymer granules and LDPE bags produced from polymer granules.

2 Materials and Methods

2.1 Materials

Low density polyethylene powder (LDPE) with 53-75µm particle size was obtained from Sigma Aldrich Chemical Co (Product of USA) with density 0.94g/ml at 25°C. Low density polyethylene granules from Pack worth polymers and Pack mates India Private Ltd (Hyderabad, INDIA).

2.2 Samples Collection

Garbage soil samples were collected from four different areas viz. Near Petrol Bunk area, Hyderabad; Quthbullapur Mandal, Hyderabad; Kalpana society, Ranga reddy District, Hyderabad and Fourth sample was collected from garbage dumped site near Padmavati Nagar, Tirupati, Andhra Pradesh. The samples were collected in sterile Nasco sampling bags. The soil samples associated with polythene waste bags were collected at a depth of 3-5cm and dried at room temperature for 2hrs.

2.3 Isolation and identification of microorganisms

Microorganisms were isolated from one gram of soil sample by serial dilution method. The nutrient agar media for bacteria, Czapek Dox Agar for fungi and Glycerol Asparagine agar for Actinomycetes was prepared and isolation was performed by spread plate technique. For each sample, three replicas were maintained and kept for incubation at 37°C for Bacteria (24-48 hrs) and Actinomycetes (2-7 days) and for fungi at room temperature (3-5days). The isolated colonies developed were sub cultured in agar slants and preserved under refrigeration temperature.

The identification of bacteria was performed on the basis of macroscopic and microscopic examination as per Bergey’s Manual of Determinative Bacteriology Holt et al. (1994). The fungus was identified after staining with cotton blue by following the keys of Raper & Fennell (1987). The phenotypic and chemotaxonomic characteristics of the actinomycetes were determined by the method described by Shirling & Gottlieb (1966).

2.4 Screening of isolated microorganisms by clear zone method

Polyethylene powder was added to Mineral Salt medium at a concentration of 0.1% (w/v) and sonicated for 1hr at 120rpm. After sonication, agar was added and autoclaved at 120°C, 15lbs pressure for 15 min. Sterilized media was cooled to 45°C and poured into sterile petriplates. Once solidified, the isolated colonies were inoculated and then incubated at 30-35°C for 2-4 weeks. The organisms producing zone of clearance were selected for further analysis (Usha et al., 2011) with minor modification in incubation temperature.

2.5 Microbial degradation of polythene and polymer granules under laboratory conditions

2.5.1 Biodegradation of polymer granules

Mineral Salt media prepared and autoclaved at 120°C, 15lbs pressure for 15 min. After sterilization the media was poured into petriplates. Biodegradation studies were performed by using polymer granules, a new approach not mentioned in earlier studies. Polymer granules were kept at three locations in the plate prior to solidification. The polymer granules become stacked to Agar media after solidification and the isolated organisms were inoculated and incubated at 30-35°C and for a period of 4-8 weeks to check the physical and structural changes of polymer granules.

2.5.2 Biodegradation of LDPE by weight method

Low density polyethylene bag was cut into equal pieces 5cm X 2cm (1 x b) and disinfected with ethanol and air dried. The disinfected strips were added into conical flask containing 100ml of Mineral salt medium and then inoculated with polythene degrading organism.
Biodegradation of low density polyethylene by micro-organisms from garbage soil.

Control has been maintained for further reference and to confirm the reduction of molecular weight of the polyethylene. The flasks were left in orbital shaker at 30°C, 120rpm for 6 months. After a period of time, the strips were washed in 70% ethanol, air dried and weighed to check the final weight. Finally the weight loss was calculated and compared based on the below formula.

\[
\text{Weight loss (\%)} = \left( \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \right) \times 100
\]

3 Results

3.1 Soil Microbial count

The bacteria, fungi and actinomycetes isolates were enumerated separately based on their incubation period. The microbial population increased tremendously from the beginning to the end of incubation period. The total counts of heterotrophic microbes isolated from garbage soil sample are calculated in colony forming units multiplied by dilution factor (Table 1, Figure 1). As results represented in Figure 2, total six bacterial isolates viz Bacillus sps, Staphylococcus sps, Streptococcus sps, Diplococcus sps (Gram positive); Moraxella sps, Pseudomonas, (Gram negative) and; one actinomycetes isolate i.e. Streptomyces sps were identified with the help of Bergey’s Manual of Determinative Bacteriology. These isolates are identified based on the phenotypic and chemotaxonomic characteristics described by Shirling & Gottlieb (1966). Furthermore five fungal isolates viz Aspergillus flavus, A. fumigatus, A. niger, A. brasiliensis and A. ornatius were identified based on the mycelial structure and cotton blue staining following the keys of Raper & Fennell (1987).

3.2 Clear Zone formation

Growth was initiated over a period of 4-5 days, attaching to the surface of polymer granule, extended around the colony in 10-15 days. Within 40-45 days, a center halo liquefaction was clearly observed compared to un inoculated control sample. Thus structural or morphological change in the polymer granules associated with the microbes indicating degradation of polymer was observed visually in the inoculated plates when compared to control (Figure 3).

3.3 Morphological changes in polymer granule

Microbial growth has been initiated over a period of 4-5 days, initially an opaque zone was observed around the colony, but slowly a clear transparent zone formed within 10-20 days at 30-35°C. The diameter of the zone is ranging from 0.25 to 0.75cm. Where as in fungal plates (3), initially growth has been initiated all over the plate but instead of zone formation clear transparency has been started from one end and slowly spreading to other end (Figure 3).

3.4 Biodegradation of LDPE by weight method

In screening by weight method, it has been observed that one bacterial isolate Pseudomonas sps, one actinomycetes member of Streptomyces sps and two fungal isolates i.e. A. niger and A. flavus showed positive result for polymer degradation. Hence the selected microbes were further tested by weight method.

Table 1 Soil microbial count in cfu /g.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Description</th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Actinomycetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample-1</td>
<td>400 x10⁷</td>
<td>2x10⁻⁴</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>Sample-2</td>
<td>110x10⁷</td>
<td>1x10⁻⁴</td>
<td>Absent</td>
</tr>
<tr>
<td>3</td>
<td>Sample-3</td>
<td>78x10⁶</td>
<td>4x10⁻⁴</td>
<td>Absent</td>
</tr>
<tr>
<td>4</td>
<td>Sample-4</td>
<td>98 x10⁶</td>
<td></td>
<td>12x10⁻⁸</td>
</tr>
</tbody>
</table>

Whereas Sample-1 - Near Petrol Bunk area, Hyderabad; Sample-2 - Quthbullapur Mandal, Hyderabad; Sample-3 - Kalpana society, Ranga reddy District, Hyderabad; Sample-4 - Padmavati Nagar, Tirupati.

Table 2 Comparative analysis of weight loss of Polyethylene film in shaker cultures under laboratory conditions.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Description</th>
<th>Weight loss of Polyethylene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2nd Month%</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas sps</td>
<td>4.34±0.02</td>
</tr>
<tr>
<td>3</td>
<td>A. niger</td>
<td>10.78±0.16</td>
</tr>
<tr>
<td>4</td>
<td>A. flavus</td>
<td>5.69±0.08</td>
</tr>
<tr>
<td>5</td>
<td>Streptomyces sps</td>
<td>12.42±0.05</td>
</tr>
</tbody>
</table>
Figure 1 Colony morphology of isolated colonies – (a) Black mold (b) Grey mold (c) Green mold (d) Bacterial isolate and (e) Actinomycete.

Figure 2 Gram staining and Fungal staining – (a.) Gram negative Bacteria  (b) Fungi.

Figure 3 Clear zone formation around the colony in polythene powder incorporated media (a-f).
After one week of incubation, a thin bio film was observed over the surface of polyethylene film and when the weight of the LDPE strip was checked there is slight increase in the weight due to biofilm formation (Figure 5). Hence forth the samples were kept for long time incubation and weight was calculated for every two months. After six months a significant difference in weight compared to initial weight was reported, *Pseudomonas* sps reduced the weight of LSPE strip up to 24.22±0.01%, in case of *A. niger* this reduction was 26.17±0.05% and for *A. flavus* it was 16.45±0.01% respectively. *Streptomyces* sps exhibit huge difference in reduction of molecular weight of LDPE strip i.e. 46.7±0.01% compared to other microbes. Thus it confirms that microbes utilized polyethylene as sole source of carbon. Based on results given in Table 2, findings of present study confirm that Actinomycetes exhibit high potency in degradation of low density polyethylene than other microorganisms.

**Discussion**

Total average count of identified heterotrophic bacteria during study were $171.5 \times 10^6$, while for fungi, this value was $3 \times 10^6$. Whereas for Actinomycetes this value was $12 \times 10^5$ and it was reported from only in one sample. The isolated colonies from heterotrophic group were sub cultured in agar slants to maintain pure culture and preserved under refrigeration temperature for further study. Thus six bacterial isolates viz *Bacillus* sps, *Staphylococcus* sps, *Streptococcus* sps, *Diplococcus* sps, *Moraxella* sps, *Pseudomonas*, one isolate of Actinomycetes *Streptomyces* sps and five fungal isolates viz *A. flavus*, *A. fumigatus*, *A. niger*, *A. brasiliensis* and *A. ornatus* were obtained and these were also recorded for polythene degradation. Among all the isolates the polyethylene has been degraded by two species of fungi (*A. niger* and *A. flavus*), one species of bacteria (*Pseudomonas* sps) and one species of Actinomycetes (*Streptomyces* sps).

The results were compared with earlier research studies done by Vijaya & Reddy (2008) in which they reported the average number of heterotrophic bacteria and fungi found in association with polythene film and plastic cups were 37.08 $\times 10^4$ and 38.04 $\times 10^4$, 26.94 $\times 10^2$ and 35.13 $\times 10^2$ respectively. Kathiresan (2003) reported that the plastic materials in mangrove soil are rich total heterotrophic bacterial counts ($79.67 \times 10^4$) and fungal counts ($55.33 \times 10^2$) and the plastic materials have been colonized commonly by five species of bacteria (*Pseudomonas* sps, *Staphylococcus* sps, *Moraxella* sps, *Micrococcus* sps and *Streptococcus* sps) and two species of fungi (*A. glaucus* and *A. niger*). In this manner results of the study are in conformity with these previous findings and similar microbes were reported during the study.
Ishigaka et al. (2000) reported that the abundance of polymer degrading microorganisms were in seabed solid waste disposal site. Similarly, Imam et al. (1999) observed that significant biodegradation of plastic can be occurred only after colonization by resident microbial populations and he concludes that an increase in the bacterial load has correlation with degradation of the polymer. The mechanism of biodegradation of polymer granules happened in three steps, in first step microorganism attached to the polymer granule, in second steps they grow around the granule and in last step theses microorganisms degradation the polymer and utilized it as carbon source.

Augusta et al. (1993), reported that the zone of clearance around the colony is due to extracellular hydrolyzing enzymes secreted by the target organism into suspended polyesters agar medium. All the microbes involved in forming a clear zone were selected for further studied weight method. All minerals were supplied along with polyethylene as carbon source for the growth of the organism. The Bacteria, Fungi and Actinomycetes were separately inoculated to check the degradation activity under aerobic condition in an orbital shaker. After a week to cross check the ability of inoculated microbe to degrade the LDPE strips were taken out, a slimy growth on the surface of polyethylene was reported; it is a type of biofilm formation.

Study of biofilm formed by Penicillium frequentans and Bacillus mycoides showed that Penicillium frequentans had polyethylene (DPE-chemical or photo initiator added polyethylene) degradation capacity and because of this it can colonized on polyethylene Arutchelvi et al. (2008). Microorganisms utilize polythene films as a sole source of carbon resulting in partial degradation of polythene and plastics. They colonize the surface of the polyethylene films or plastics forming biofilm (Vijaya & Reddy, 2008).

Though initially there is increase in the weight (0.02%) of the polyethylene due to biofilm formation but later on a drastic reduction in the weight of the of LDPE strip confirming the usability of polyethylene as carbon source. When weight was measured for every two months to six months bacterial isolate Pseudomonas spp caused biodegradation from 4.34% to 24.22%, A.niger caused 10.78% to 26.17%, A. flavus caused 5.69% to 16.45% and Streptomyces showed high degradability i.e12.42% to 46.7% as compared to other isolated species.

Kathiresan & Bingham (2001) reported that biodegradation of polythene by bacteria was 2.19 to 20.54% for polythene and 0.56 to 8.16% for plastics. Among all the species, Aspergillus glaucus was more active than A. niger in degrading 28.8% of polythene and 7.26% of plastics within a month. Singh et al. (2012) reported that Penicillium sp. was more active in reducing LDPE i.e up to 6.58% compared to A. fimigatus as it reduced the weight upto 4.65%.

Once the organism attached to the surface of the polymer it starts growing by using polymer as carbon source. In the primary degradation, the main chain cleaves to low molecular weight fragments (oligomers), dimers or monomers Vasile (1993). The degradation is due to extracellular enzymes release by the organisms. The breakdown products of polymer should be completely utilized by microorganisms as carbon source to control environmental pollution Narayan (2006). Furthermore it is clearly reported by Oskay et al. (2004) Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil.

The present work concludes that naturally growing soil microbes like bacteria, fungi and Actinomycetes shows great efficacy in degrading synthetic polymer granules and LDPE polyethylene bags produced from polymer granules. Among all the isolates, Streptomyces spp. exhibit high rate of degradation compared to Pseudomonas spp, A.niger and A. flavus.

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**References**


