



## **POSSIBLE DEGRADATION OF LDPE AND BIODEGRADABLE POLYTHENE BY NATURAL FUNGAL FLORA**

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Issue of polythene pollution has evolved to become a major threat to global ecology because of polythene has strong resistance against degradation thus they remain in nature for very long time. A thinkable method to overcome this problem is bio-degradation by saprophytic fungi. Fungi from polythene decaying sites can initiate the de-polymerization of many polymers. In present study 10 saprophytic fungal isolates were grow on polythene by submerge fermentation technique under laboratory conditions and biodegradation was determine in terms of weight loss in both low density polythene (LDPE) and biodegradable polythene.

**Keyword:** Biodegradable polythene; Biodegradation; Fungi; LDPE.

### **Introduction**

A verity of heterotrophic fungi associated with polythene as a good biodegrading agent, reported from decaying sites near water bodies<sup>1</sup>. Saprophytic fungi are quickly germinated and vital for the conservation of natural ecosystem by breaking down of decomposes into small organic raw materials, which can be absorbed back by decomposers or nearby plants and other microbial flora<sup>2</sup>. Polythene substances which having active bonding sites are called biodegradable. Usually, Biodegradable substances contain atoms like carbon, oxygen, nitrogen, phosphorus and sulfur which make some charge imbalance so that enzymes can exploit it certainly<sup>3</sup>. Carbon source is necessary growth element for fungi. In lack of carbon source, fungi started utilizing carbon substrate of polythene if growing on or around polythene.

Biodegradable polythene contains more organic substrate in comparison of low density polythene (LDPE), so comparatively biodegradable polymers are easy for consumption by fungi. In fact, fungi secrete a variety of enzymes into soil, water and then these enzymes activate the breakdown of long chain polymers into monomers. Submerge fermentation is one of the best technique for enzyme production and secondary metabolites production. Fungi reported to secrete a variety of enzymes like-Laccases, Peroxidase and Oxidases enzyme<sup>4</sup> and these enzymes activate the breakdown of long chain polymers into monomers. Both oxidative and reductive reactions required for degradation of polythene while microorganisms produced enzymes and disrupted the polythene<sup>5</sup>. Biodegradation executed by the microorganisms has environmental-friendly

benefit and this method is less expensive and alternative of decaying (Physical and chemical changes) organic pollutant<sup>6</sup>.

The current study was carried out on possible degradation of low density polythene (LDPE) and biodegradable polythene by fungal flora from polythenes at natural decaying sites, in terms of determination of physical changes in tensile strength, weight loss and bond breakdown of polymers.

### Methodology

Samples were collected from different decaying sites near water bodies (dumping sites) of Jhansi city. These samples were further treated for fungal isolation. Samples were inoculated and plated on PDA plates and sub cultured for pure fungal isolates. Fungi were identified by microscopic examination<sup>7</sup>. These pure fungal isolates were named as PE-1, PE-2, PE-3 to PE-10 and tested on LDPE (Low density polythene) and biodegradable polythene for bio-degradation activity. Submerged fermentation (SF) method<sup>8</sup> was performed to examine polythene degradation. 100 ml of modified Sabaroud's Dextrose broth<sup>9</sup> was taken in 250 ml of Erlenmeyer flask and pH was set to 5.6. Each flask was inoculated accordingly with a fungal isolates and Pre-weighed 1x1 cm sterilized segments of LDPE and Biodegradable polythene, incubated for 15 days and 30 days at 27° C in a rotary shaker at 250 rpm. After incubation polythene segments were removed and cleaned well and then their weight loss was calculated by subtracting pre weight. Degradation was determined in terms of percentage of weight loss.

### Results and discussion

Fungi are vigorously found and easily grown, evolved to adapt for almost every environment. Fungi are widely used in biodegradation studies because of its robust

nature and for their great source of enzyme<sup>10</sup>, this property drives fungi to grown on polythene even in adverse environment. 10 fungal isolates namely *Penicillium chrysogenum*, *Rhizopus nigricans*, *Chaetomium murorum*, *Memnoniella echinata*, *Aspergillus fumigatus*, *Stachybotrys chartarum*, *Aspergillus niger*, *Chaetomium globosum*, *Aspergillus flavus* and *Fusarium oxysporum* were isolated from decaying polythene around water bodies of Jhansi and tested for biodegradation of polythene.

Modified Sabaroud's Dextrose broth<sup>9</sup> was inoculated with a fungal isolates and polythene segments. Modified media was used because in lack of carbon source, fungal strains able to use polythene as carbon source. Polythene segments were aseptically placed in broth for 15 days and 30 days. Same process was repeated with every fungal isolates. At the end of the fermentation period, cultures were centrifuged at 5000 rpm for 15 minutes at 4° C and polythene segments were removed from broth and cleaned well, then their weight loss was calculated after incubation. The results of the degradation were demonstrated by visual observation like thickness; roughness etc. some studies were done on polythene degradation by fungi and also observed the similar changes in roughness and thickness<sup>9</sup>.

The degree of biodegradation depends on carbon source present in culture medium and composition of polymeric material. Polythene weighed before incubation and after incubation period.

The percentage of weight reduction in polythene was measured after 15 and 30 days incubation, and was compared to the mass before incubation (Table 1). For comparison, polythene segments in control were also measured.

The percentage weight reduction was calculated with this formula:

$$\text{Weight reduction (\%)} = (W_1 - W_2) / W_1 \times 10$$

Where;  $W_1$  is the weight of polythene piece before incubation.

$W_2$  is the weight of polythene piece after incubation.

Some studies was also described that *Aspergillus* spp. is most dominantly contributing almost 58.8% and the other dominant species are *Fusarium* spp., *Curvularia* spp., *Chaetomium* spp. and *Cladosporium* spp. in environment<sup>12</sup>.

**Table 1. Percentage of degradation in 15 days incubation and 30 days incubation**

Sample no.	Fungal Isolate	LDPE Weight loss in 15 days	Biodegradable Weight loss 15 days	LDPE Weight loss in 30 days	Biodegradable Weight loss 30 days
PE-1	<i>P. chrysogenum</i>	2%	5%	8%	23%
PE-2	<i>R. nigricans</i>	0.5%	3%	6%	14%
PE-3	<i>C.murorum</i>	.01%	1%	2%	5%
PE-4	<i>M.echinata</i>	.02%	2%	3%	8%
PE-5	<i>A. fumigatum</i>	.4%	4%	7%	15%
PE-6	<i>S.chartarum</i>	.04	2%	2%	7%
PE-7	<i>A. niger</i>	4%	10%	9%	28%
PE-8	<i>C.globosum</i>	1%	4%	5%	10%
PE-9	<i>A. flavus</i>	1%	5%	7%	18%
PE-10	<i>F.oxysporum</i>	0%	1%	3%	8%

Initially in 15 days incubation, there was not much visible changes could see in evacuated LDPE and bio-degradable polythene but after completing one month incubation, more visible changes were noted in polythene segments. Segments were week, thin and lighter in color and weight.

*Penicillium chrysogenum* (PE-1) and *Aspergillus flavus* (PE-9) showed 5% reduction in biodegradable polythene after 15 days and that was followed 4% reduction by *Aspergillus fumigatus* and *Chaetomium globosum*. Only *Aspergillus niger* indicated the very good reduction after 15 days incubation period i.e. 4% reduction in LDPE and 10% reduction in biodegradable polythene. *Penicillium chrysogenum* (PE-1) showed 2% weight reduction in LDPE and 5% in biodegradable polythene over the 15 days incubation but later on after 30 days of

fermentation; fungus (PE-1) may be started utilizing both polythene and degrades it up to 8% LDPE and 23% biodegradable. This weight loss is in accordance with the finding of studies those were carried out degradation of low density polythene (LDPE) by using *Aspergillus fumigatus* and *Penicillium* species<sup>13-17</sup>.

Maximum weight loss was calculated in by *Aspergillus niger* in both LDPE and biodegradable polythene after 30 days incubation. Some scientist also worked on LDPE with *Rhizopus* species and observed around 8.4% weight reduction and 60% reduction in tensile strength of polymers<sup>17</sup>. In present finding, *Rhizopus nigricans* shows almost similar results to previous research 6% reduction was noted in weight loss of LDPE and 14% reduction noted in biodegradable polythene in

control environment. Some similar studies were also conducted on a comparative analysis between different time intervals after incubating them with *Rhizopus oryzae*<sup>18</sup>. *Aspergillus flavus* and *Aspergillus fumigatus* also showed a good weight reduction in LDPE 7% and in biodegradable polythene it was 15% and 18%. Reduction observed in low density polythene in 15 and 30 days' time as given in table 1. In initial 15 days biodegradable polythene was degraded but in very less percentage. But from 30<sup>th</sup> days onwards, maximum weight loss was observed in biodegradable polythene by fungal strain PE-7 (*Aspergillus niger*) as 28%. *Penicillium chrysogenum* was also degrading stipulated 23% of biodegradable polythene. This strain assured a great potential to degrade polythene. Biodegradable polythene indicated the maximum weight loss in comparison of low density polythene. *Aspergillus niger* perfectly distinguished for further study. Weight lost in samples of other fungi was negligible.

However, weight loss was not observed in control experiment. So there is a possibility that weight reduction might be because of consumption of consumption of LDPE film and Biodegradable polythene as carbon source by fungus which confirms the potential capability of fungi to degrade LDPE and biodegradable polythene. Biodegradable polythene is more prompt to degradation.

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