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Biodegradation Potential of Soil Fungus on Low Density Polyethylene

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Abstract

Aspergillus candidus was isolated from a local dumpsite of Shivamogga district for use in the biodegradation of polyethylene. Degradation was carried out using surface sterilized polyethylene. Degradation was confirmed by observing weight loss and changes in physical structure by Scanning Electron Microscopy and Fourier Transform Infrared Spectroscopy. *Aspergillus candidus* was able to degrade surface sterilized polyethylene (1.7%). Enzymes responsible for polyethylene degradation were screened from *Aspergillus candidus*. Enzymes were identified as laccase and manganese peroxidase. By observing these results we can conclude that, this organism may act as solution for the problem caused by polyethylene in nature.

Keywords: Polyethylene, *Aspergillus Candidus*, Laccase, Manganese Peroxidase, SEM, FTIR Biodegradation

1. Introduction

Polyethylene, particularly as thin films are used as packaging material because of its excellent mechanical properties, barrier properties against water borne microorganisms, low-cost and high energy effectiveness. Polyethylene, a stable and common commercial plastic, presents a costly and persistent environmental problem. The annual production is approximately 110 million metric tons. During past three decades, plastic materials are increasingly used in transportation, food – clothing, shelter construction, medical and recreation industries. Many kinds of polyethylene are known, with most having the chemical formula $(C_2H_4)_nH_2$.

With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic has assumed increasing importance in the last few years. Biodegradation is necessary for water soluble or water immiscible polymers, because they eventually enter water streams which can neither be recycled nor incinerated (Shah *et al*., 2008) [20]. The polyethylene is the most commonly found solid waste that has been recently recognized as a major threat to marine life. The polyethylene could sometimes cause blockage in intestine of fish, birds and marine mammals (Spear *et al*., 1995; Seechi and Zarur, 1999) [24, 19]. The degradation of polyethylene can occur by different molecular mechanisms such as chemical, thermal, photo and biodegradation (Gu, 2003) [7]. Biodegradability is evaluated by weight loss, tensile strength loss, changes in percent elongation and changes in polyethylene molecular weight distribution.

Degradation of polyethylene is a great challenge as the materials are increasingly used. The solid waste related problems pose threat to mega cities. So, an attempt has been made in this paper to isolate the potent fungus that degrades polyethylene from the soil of dumpsite area.

2. Materials and Methods

1. **Collection of soil sample:** Soil sample was collected from a local dumpsite of Shivamogga district and brought to the laboratory, preserved under laboratory conditions for further use.
2. **Isolation and identification of fungus from soil:** Enrichment procedure was used for the isolation of fungus where polyethylene was used as sole source of carbon. Isolated fungus was identified based on its microscopic and macroscopic appearance using standard manuals (Ellis, 1971 and 1976: Pitt, 1979: Domsch *et al*., 1980: Subramanian, 1983: Ellis and Ellis, 1997: Gilman, 2001 and Nagamani *et al*., 2006) [4, 2, 17, 1, 25, 3, 6, 13]. The colonies were preserved at 4°C in 2% agar slants of malt and yeast extract medium (Yamada-onodera *et al*., 2001).

3. Screening of fungus for polyethylene degradation-

3.1 Plate assay

The isolated fungus was inoculated to medium which contained 0.3g of NH_4NO_3, 0.5g of K_2HPO_4. 0.1g of NaCl, 0.02g of $MgSO_4.7H_2O$, 2g of agar, 0.5g of polyethylene and 100ml distilled water (Yamada-onodera *et al*., 2001). This agar plate test is also a simple semi- quantitative method to know depolymerization of polymer by the organism. After inoculation with the fungus into the medium containing fine particles of polyethylene, the formation of a clear hallow around the colony indicates the first step of fungal biodegradation (Nishida and Tokiwa, 1993) [14].

3.2 Degradation of Polyethylene

The pre-weighed discs of surface sterilized polyethylene of 1cm diameter prepared from polyethylene bags were aseptically transferred to the conical flask containing 50ml of Mineral Salt Medium. Loop full of organism was added to medium. Control was maintained with polyethylene discs in the microbe free medium. Triplicates were maintained for each type of polyethylene and left on shaker. After three months of incubation, the plastic discs were collected, washed thoroughly using distilled water, dried in hot air oven at 50°C, overnight and then weighed for final weight (Kathiresan, 2003) [10].

4. Confirmation of polyethylene degradation

Polyethylene degradation was confirmed by using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) Spectroscopy (Shah *et al*., 2008) [20].

5. Screening of enzymes responsible for polyethylene degradation

Earlier studies revealed that, laccase and manganese peroxidase are responsible for polyethylene degradation. Hence, screening, mass production and calculation of activity of these enzymes was carried out.

5.1 Screening of laccase and manganese peroxidase enzyme

The isolated fungus was screened for the laccase production using laccase screening medium (LSM) with following composition (g/l): 3.0 peptone, 10.0 glucose, 0.6 KH_2PO_4, 0.001 $ZnSO_4$, 0.4 K_2HPO_4, 0.0005 $FeSO_4$, 0.05 $MnSO_4$, 0.5 $MgSO_4$, 20.0 Agar (pH-6) supplemented with 0.02% guaiacol. Fungus was inoculated in LSM agar plate and the plate was incubated for 7 days in dark condition. Formation of reddish brown color in screening medium indicated the positive strain for laccase (Viswanth *et al*., 2008) [26]. For manganese peroxidase, H_2O_2 was used to the same medium.

5.2 Mass production by sub-merged fermentation

The mass level production of the enzyme was carried out in mineral salt medium under suitable environmental conditions (Shradda *et al*., 2011) [22].

5.3 Enzyme assay

One ml of the culture supernatant was added with one ml of 2mM guaiacol and 3ml 10mM Sodium acetate buffer (pH 4.6). The reaction mixture was incubated at 30°C for 15 mins. The color change was measured using spectroscope at 450 nm. One unit of laccase activity was defined as amount of enzyme required to hydrolyze guaiacol during incubation period**.** For the enzyme activity calculation of manganese peroxidase same procedure was used but for the reaction mixture 1 ml of H_2O_2 was added and incubated (Papinutti *et al*., 2006) [16].

5.4 Protein estimation

Protein estimation was done to calculate specific activity of enzymes. The protein concentration was determined by the Lowry's method, as described by Lowry's (1951) using Bovine Serum Albumin (BSA) as a standard.

3. Results

3.1 Isolation and Identification of Fungus

Aspergillus candidus was isolated and identified based on its morphological characters. *Aspergillus candidus* was selected for the study, because of its predominant presence in soil contaminated with waste polyethylene plastic bags.

3.2 Screening of fungus for polyethylene degradation

3.2.1 To check ability of fungus to grow on medium containing polyethylene

Aspergillus candidus was able to grow on agar medium containing polyethylene as sole carbon source (Fig 1). This showed its capacity to utilize polyethylene as carbon source and its capacity to degrade polyethylene.

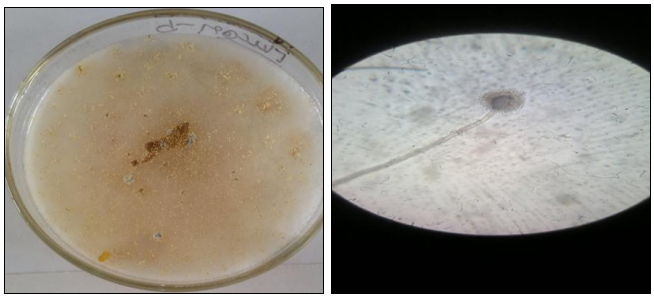

Fig 1.A: Candidus on polyethylene containing media and its microscopic view

3.2.2 Degradation of surface sterilized polyethylene

Aspergillus candidus was able to degrade surface sterilized polyethylene. This method confirmed that this organism can utilize polyethylene without any pre-treatment like, heat, UV light and acid. The weight loss for surface sterilized polyethylene was 1.7% (Table 1).

Table 1: Weight loss of surface sterilized polyethylene

S. No.	Initial weight	Final weight (mg)*	Weight loss (mg)	Weight loss (%)
1.	0.10	0.0983	0.0017 ± 0.00015	1.7

± = Standard Deviation, * = Mean

3.3 Confirmation of polyethylene degradation

3.3.1 Observation of discs using SEM

Surface sterilized polyethylene showed morphological changes when observed through SEM. Formation of holes, disruption of polyethylene structure confirmed degradation capacity of *Aspergillus candidus.* SEM photograph of control polyethylene did not show any structural changes (Fig 2).

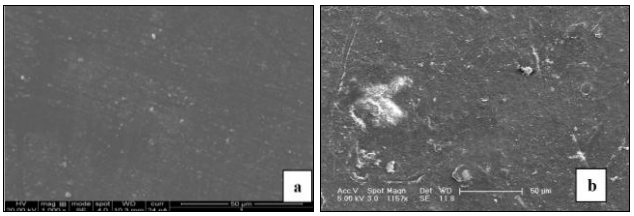

Fig 2: SEM photographs of (a) control and (b) surface sterilized polyethylene

3.3.2 Observation of discs using FTIR

FTIR results confirmed polyethylene degradation. Following are the figures showing FTIR spectrum of control and surface sterilized polyethylene.

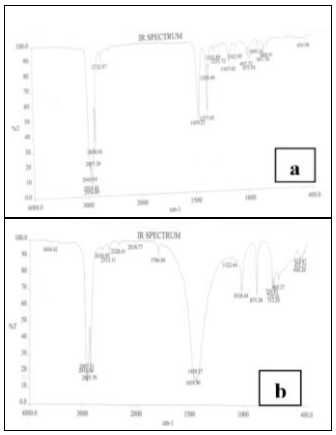

Fig 3: FTIR spectrum of (a) control and (b) surface sterilized polyethylene

Aldehyde, ketone, alcohol, carboxylic acid groups were not formed in control polyethylene. In surface sterilized polyethylene, Carboxylic acids (3300-2500 cm^{-1}), Alkynes (2260-2100 cm^{-1}) and alcohols, esters, ethers (1018, 44 cm^{1}) were formed at different frequencies indicating degradation capacity of *Aspergillus candidus* (Fig 3).

3.4 Screening and characterization of polyethylene degrading enzymes

Aspergillus candidus showed positive result for both laccase and manganese peroxidase enzymes.

3.4.1 Mass production of enzymes

Laccase and manganese peroxidase enzymes were produced in large amount using submerged fermentation.

3.4.2 Enzyme assay

Activity of manganese peroxidase (0. 0.00405 IU/ml) was more compared to laccase activity (0.00409 IU/ml) after tenth week of incubation (table 2).

Table 2: Enzyme activity of Laccase and Manganese peroxidase

Enzyme/Weeks	4	5	6	7	8	9	10	11	12
Laccase	0.00027	0.00061	0.00092	0.00116	0.00128	0.00183	0.00405	0.00222	0.00130
Manganese peroxidase	0.00033	0.00069	0.00102	0.00120	0.00136	0.00192	0.00409	0.00226	0.00136

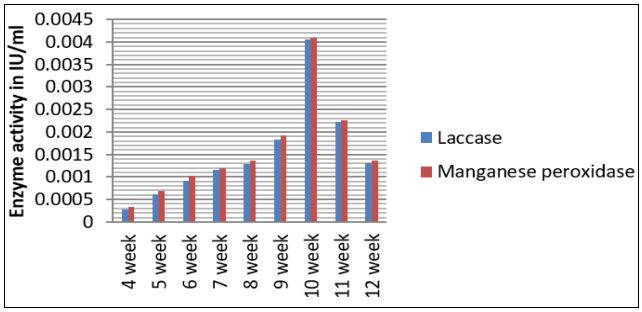

Fig 4: Enzyme activity of Laccase and Manganese peroxidase

3.4.3 Protein estimation

Specific activity of manganese peroxidase (0.0094 µmol/ml/mg/min) was more compared to laccase (0.00100 µmol/ml/mg/min).

4. Discussion

Aspergillus candidus was isolated from local dumpsite of Shivamogga district. It was identified based on both macroscopic and microscopic observations. *Aspergillus candidus* was grown on medium containing polyethylene and agar. After the growth of *Aspergillus candidus* on polyethylene containing medium, it was screened for degradation of surface sterilized polyethylene. *Aspergillus candidus* was able to degrade surface sterilized (1.7%).

Singh *et al*., (2012) [23] carried out degradation of LDPE using *Aspergillus candidus* and *Penicillium* sp. According to their work, *Aspergillus candidus* was able to degrade 4.65% of polyethylene and *Penicillium* sp. degraded 6.58% of polyethylene.

Mahalakshmi *et al*., (2012) [12] studied degradation of polyethylene using microorganisms isolated from compost soil. They studied degradation by inoculating isolated organisms into Mineral salt medium containing 1 gram of polyethylene films as sole carbon source. Degradation was studied using SEM and FTIR. They analyzed degraded products by Gas Chromatography. SEM and FTIR were also used in our study to evaluate biodegradation. SEM results showed formation of cavities and erosions. FTIR results confirmed degradation by showing formation of polyethylene degradation products like aldehydes, esters, alcohols, ethers etc.

Pramila and Ramesh, (2011) [18] studied the biodegradation of low density polyethylene by two fungal strains isolated from municipal landfill area. The degrading ability of the two fungal strains was evaluated by performing colonization studies, SEM and Sturm test analysis. Colonization studies on LDPE film was performed over a period of one month by measuring the fresh weight of the fungus. LDPE films colonized by fungus were analyzed by SEM for any structural changes caused in the LDPE films. Fungi were identified as *Mucor circinilloides* and *Aspergillus flavus*. In present work, degradation of polyethylene was evaluated by using SEM.

Jeon and Kim, (2013) [9] worked on low-molecular-weight polyethylene (LMWPE) degrading thermophilic bacterium *Chelatococcus* sp. They studied degradation by using FTIR.

The FTIR peaks corresponding to alkenes also were more intense, indicating that dehydrogenations occurred concomitantly with microbial induced oxidation. Our FTIR results also showed formation of alkenes.

Shimao *et al*., (2001) [21] studied degradation of high molecular weight polyethylene with partially purified manganese peroxidase from *Phanerochaete chrysosporium*. They carried out this experiment under nitrogen limited and carbon limited conditions. Even in our experiment we carried out screening of peroxidase from *Aspergillus candidus*, proving its role in polyethylene degradation.

Iiyoshi *et al*., (1998) [8] carried out degradation of polyethylene in the presence of Tween 80, Mn(II) and Mn(III) chelator. They confirmed that manganese peroxidase is key enzyme in biodegradation of polyethylene.

Fujisiawa *et al*., (2001) [5] investigated role of laccase-mediator system for biodegradation of polyethylene in presence of 1-hydroxybenzotriazole (HBT). Laccase of *Trametes versicolor* was used for evaluation. Degradation of polyethylene was confirmed by changes in relative elongation, relative tensile strength and molecular weight distribution. All these results confirmed degradation of polyethylene by laccase mediator system. *Aspergillus candidus* has also given positive result for laccase enzyme.

According to earlier literature available laccase and manganese peroxidase are involved in polyethylene degradation. In present work, *Aspergillus candidus* showed positive result for both laccase and manganese peroxidase indicating their possible role in polyethylene degradation.

5. Conclusion

Degradation of polyethylene was carried out with *Aspergillus candidus* which was isolated from dumpsite soil. This organism was able to degrade polyethylene. Degradation was monitored by weight loss, SEM and FTIR. Weight loss of surface sterilized polyethylene was 1.7%. FTIR results showed formation of aldehyde, alcohol, carboxylic acid, aromatic and ether group formation indicating degradation of polyethylene by *Aspergillus candidus*. All these results confirmed polyethylene degradation. Enzymes responsible for polyethylene degradation were identified as laccase and manganese peroxidase. Optimum conditions for increased polyethylene degradation can be studied in future.

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