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POLYETHYLENE DEGRADING FUNGI ISOLATED FROM LOCAL DUMPSITE- OF SHIVAMOGGA DISTRICT

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ABSTRACT

Aspergillus fumigatus was isolated from a local dumpsite of Shivamogga district for use in the biodegradation of polyethylene. Soil sample of that dumpsite was used as source for isolation of the *Aspergillus fumigatus*. Degradation was carried out using surface sterilized polyethylene. Degradation was monitored by observing weight loss and changes in physical structure by Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) Spectroscopy. *Aspergillus fumigatus* was able to degrade surface sterilized polyethylene (1.6%). SEM and FTIR results also showed changes in the surface of polyethylene. Enzymes responsible for polyethylene degradation were screened from *Aspergillus fumigatus*. 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 fumigatus*, Laccase, Manganese peroxidase, SEM, FTIR Biodegradation,

INTRODUCTION

Plastics are man-made long chain polymeric molecules. The word plastic comes from the Greek word “Plastikos”, which means “able to be molded into different shapes. The plastics which we use every day is made of inorganic and organic raw materials such as, carbon, silicon, hydrogen, nitrogen, oxygen and chloride. The basic materials used for plastic making are extracted from oil, coal and natural gas (Shah *et al.*, 2008). The most widely used plastic in packaging is polyethylene. Polyethylene, a stable and common commercial plastic, presents a costly and persistent environmental problem. Polyethylene or polythene is the most common plastic. The annual production is approximately 80 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 $(C_2H_4)_n$.

Plastic are advantageous as they are strong, light weight and durable. But, lack of degradability and the closing of landfill sites, as well as growing water and land pollution problems have led to concern about plastics. 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). 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). The degradation of polyethylene can occur by different molecular mechanisms such as chemical, thermal, photo and

biodegradation (Gu, 2003). 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.

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.

1. 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. Enrichment medium composed of 0.3g of NH_4NO_3, 0.5g of K_2HPO_4, 0.1g of NaCl, 0.02g of $MgSO_4.7H_2O$, 0.01g of yeast extract and 100ml distilled water, pH 6 (Medium A). Soil was added to conical flasks containing 100ml of sterilized enrichment medium. Flasks were incubated at 30°C for 4 weeks on rotary shaker at 200rpm. After incubation 1ml of suspension was added into 4ml of fresh enrichment medium. After 1 week of shaking, 5µl of the culture was spread on a 2% agar plates of medium A (0.1 g polyethylene, 0.3g of NH_4NO_3, 0.5g of K_2HPO_4, 0.1g of NaCl, 0.02g of $MgSO_4.7H_2O$, 0.01 g of yeast extract, 10 µg of thiamine. HCl, 20 µg of riboflavin, 20 µg of nicotinic acid, 20 µg of Ca-pantothenate, 20 µg of pyridoxine-HCl, 1 µg of biotin, 10 µg of p-aminobenzoic acid, 1 µg of folic acid and distilled water, pH 7±0.2) and the plates were incubated for several days. 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). The colonies were preserved at 4°C in 2% agar slants of malt and yeast extract medium (5% malt extract, 0.3% yeast extract and distilled water; pH 5-6) (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 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).

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 both control and treated polyethylene and left on shaker. After three months of incubation, the plastic discs were collected, washed thoroughly using distilled water, dried and then weighed for final weight (Kathiresan, 2003).

4. CONFIRMATION OF POLYETHYLENE DEGRADATION.

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

5. SCREENING OF ENZYMES RESPONSIBLE FOR POLYETHYLENE DEGRADATION

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

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). 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).

5.3. Enzyme assay

1 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).

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.

RESULTS

1. Isolation and Identification of Fungus

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

2. SCREENING OF FUNGI FOR POLYETHYLENE DEGRADATION

2.1. To check ability of fungus to grow on medium containing polyethylene

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

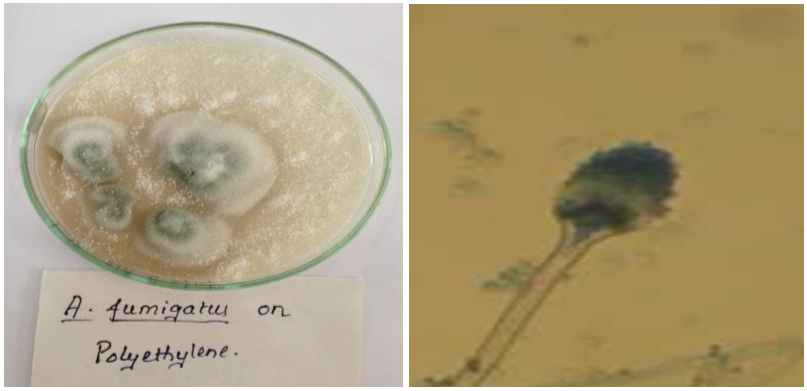

Fig. 1: *A. fumigatus* on medium containing polyethylene and microscopic view.

2.2. Degradation of surface sterilized polyethylene

Aspergillus fumigatus was able to degrade surface sterilized polyethylene. The weight loss percentage for surface sterilized polyethylene was 1.6% (Table 1).

Table 1: Weight loss of surface sterilized polyethylene.

Sl. No.	Initial weight	Final weight (mg)*	Weight loss (mg)	Weight loss (%)
1.	0.10	0.0984	0.0016 ± 0.00015	1.6

± = Standard Deviation, * = Mean

3. CONFIRMATION OF POLYETHYLENE DEGRADATION

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 fumigatus* (Fig. 2).

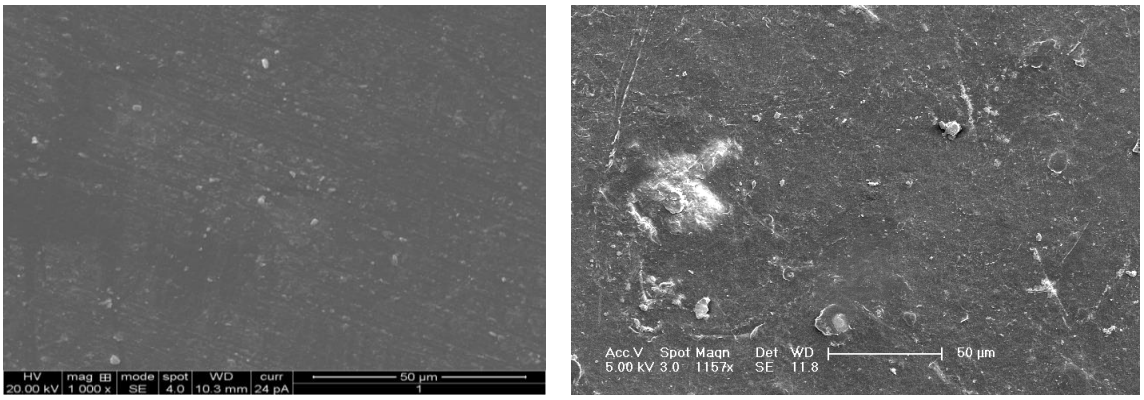

Fig. 2: SEM photographs of control and *A. fumigatus* treated surface sterilized polyethylene.

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.

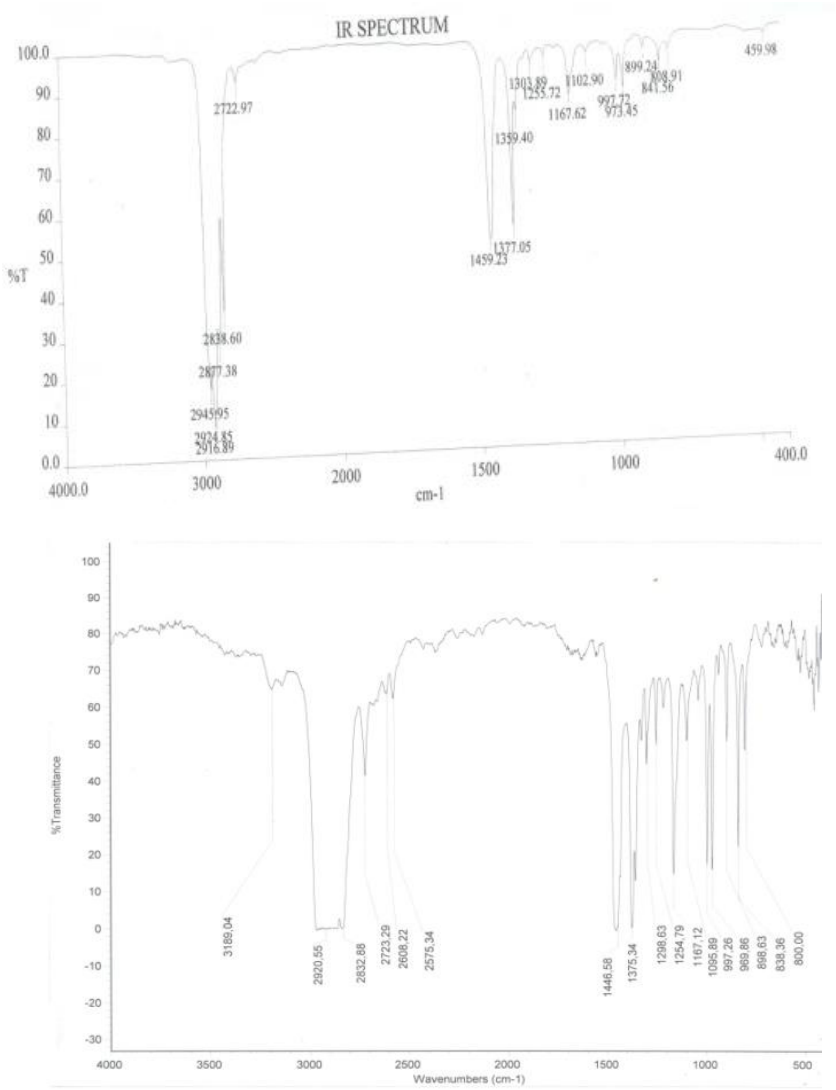

Fig. 3: FTIR spectrum of control and surface sterilized polyethylene

FTIR spectrum of control autoclaved polyethylene did not show formation of any degradation products.

FTIR spectrum of control polyethylene did not show formation of any degradation products. In surface sterilized polyethylene, Carboxylic acids (3189,04 cm^{-1}), Aldehydes (2723,29 cm^{-1}), alcohols, esters, ethers (1298,63 cm^{-1}) and alkyl halides (1167,12 cm^{-1}) were formed at different frequencies indicating degradation capacity of *Aspergillus fumigatus* (Fig. 3).

4. SCREENING AND CHARACTERIZATION OF POLYETHYLENE DEGRADING ENZYMES

Aspergillus fumigatus showed positive result for both laccase and manganese peroxidase enzyme.

4.1. Mass production of enzymes.

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

4.2. Enzyme assay

Activity of manganese peroxidase (0. 0.00683 IU/ml) was more compared to laccase activity (0.00712 IU/ml) after tenth week of incubation (Table 2 and Fig. 4).

Table 2: Enzyme activity of Laccase and Manganese peroxidase.

Enzyme/Weeks	4	5	6	7	8	9	10	11	12
Laccase	0.00025	0.00064	0.00101	0.00203	0.00309	0.00501	0.00683	0.00505	0.00331
Manganese peroxidase	0.00030	0.00072	0.00110	0.00213	0.00320	0.00521	0.00712	0.00539	0.00331

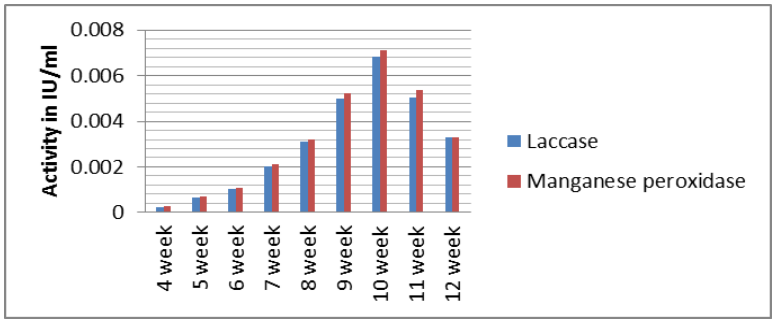

Fig. 4: Enzyme activity of Laccase and Manganese peroxidase.

4.3. Protein estimation

Specific activity of manganese peroxidase (0.0062µmol/ml/mg/min) was more compared to laccase (0.0160 µmol/ml/mg/min).

DISCUSSION

Aspergillus fumigatus was isolated from local dumpsite of Shivamogga district. Fungus was identified based on both macroscopic and microscopic observations. *Aspergillus fumigatus* was grown on medium containing polyethylene and agar. After the growth of *Aspergillus fumigatus* on polyethylene containing medium, it was screened for degradation of surface sterilized polyethylene. *Aspergillus fumigatus* was able to degrade surface sterilized polyethylene (1.6%).

Singh *et al*., 2012 have carried out degradation of LDPE using *Aspergillus fumigatus* and *Penicillium* sp. According to their work, *Aspergillus fumigatus* was able to degrade 4.65% of polyethylene and *Penicillium* sp. degraded 6.58% of polyethylene.

Mahalakshmi *et al*., 2012 have 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. SEM results showed formation of holes and erosion of polyethylene.

Pramila and Ramesh, 2011 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. LDPE degradation by the fungal strains was further evaluated by measuring the CO_2 evolved which was calculated gravimetrically and volumetrically by Sturm test. Fungi were identified as *Mucor circinilloides* and *Aspergillus flavus*. In present work also degradation was confirmed by using SEM.

Shimao *et al*., 2001, have 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. In present work screening for manganese peroxidase was carried out from *Aspergillus fumigatus*. So, this enzyme may play a important role in polyethylene degradation.

Iiyoshi *et al*., 1998, have 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, have investigated role of laccase-mediator system for biodegradation of polyethylene in presence of 1-hydroxybenzotriazole (HBT). They used laccase of *Trametes versicolor.* 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 fumigatus* has also given positive result for laccase enzyme.

According to earlier literature available laccase and manganese peroxidase are involved in polyethylene degradation. *Aspergillus fumigatus* has also shown positive result for these two enzymes indicating their role in polyethylene degradation.

CONCLUSION

Degradation of polyethylene was carried out with *Aspergillus fumigatus.* This organism was isolated from dumpsite soil. This organism was able to degrade polyethylene. Degradation was monitored by weight loss, SEM, FTIR and NMR studies. Weight loss of surface sterilized was 1.6%. SEM results showed formation of holes and erosion of polyethylene. FTIR results showed formation of aldehyde, alcohol, carboxylic acid, aromatic and ether group formation indicating degradation of polyethylene by isolated fungus. All these results confirmed polyethylene degradation. Enzymes responsible for polyethylene degradation were identified as laccase and manganese peroxidase. In future studies may be carried about

purification of enzymes and optimized conditions for maximum polyethylene.

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