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Research Paper

Low density polyethylene degrading fungi isolated from local dumpsite of shivamogga district

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Abstract

Objective: To isolate polyethylene degrading fungi from dumpsite soil and to screen the enzymes responsible for polyethylene degradation.
Methods: Enrichment method was followed for isolation of fungi. Plate assay method was followed for screening of fungus for its capacity to degrade polyethylene. Degradation experiment was carried out using different types of polyethylene. Degradation was confirmed by weight loss, Scanning Electron Microscopy (SEM) and Fourier Transform Infra-Red (FTIR) studies. Enzymes responsible for polyethylene degradation were screened and their activity was measured by spectrophotometric method.
Results: Fungus isolated was identified as *Chaetomium globsum*. *Chaetomium globsum* was able to degrade differently treated polyethylene which was confirmed by weight loss, SEM and FTIR studies. Lactase and Manganese peroxidase enzymes were responsible for degradation.
Conclusion: From current investigation, it can be concluded that our isolated fungus *Chaetomium globosum* had the capacity to degrade polyethylene and it can be useful in solving the problem caused by polyethylene in the environment.

Keywords: *Polyethylene; Chaetomium globosum; Laccase; Manganese Peroxidase; Biodegradation.*

1. Introduction

Low density polyethylene (LDPE) is a hydrophobic synthetic polymer of high molecular weight. It is characterized by good toughness, Resistance to chemicals, Flexibility and clarity. These properties make LDPE an important plastic grade widely used for manufacturing various laboratory containers, dispensing bottles, tubing, plastic bags, food containers and corrosion-resistant work surfaces etc (Negi, et al., 2011). 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). Bio degradability 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 microorganism that degrades polyethylene from the soil of dumpsite area.

2. Materials and methods

2.1. 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.

2.2. 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). The colonies were pre-

served at 4^0C in 2% agar slants of medium malt and yeast extract (Yamada-onodera et al., 2001).

2.3. Screening of fungus for polyethylene degradation

2.3.1. 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 fungi 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).

2.3.2. Degradation of polyethylene

The pre-weighed discs of Autoclaved, Surface sterilized and UV treated polyethylene of 1cm diameter prepared from polyethylene bags were aseptically transferred to the conical flask containing 50ml of Mineral Salt Medium. Loop full of organisms 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 50^0C^over night and then weighed for final weight (Kathiresan, 2003).

2.4. Confirmation of polyethylene degradation.

Polyethylene degradation was confirmed by using Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FTIR) Spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy (Shah et al., 2008).

2.5. Screening of enzymes responsible for polyethylene degradation

Earlier studies revealed that, laccase and manganese peroxidase are responsible for polyethylene degradation. So, we carried out screening, mass production and crude extraction of these enzymes.

2.5.1. Screening of Laccase and manganese peroxidase enzyme

The isolated fungus was screened for the Laccase production using laccase screening medium (LSM). Fungus was inoculated in LSM agar plate and the plate was incubated for 7 days in dark condition. The substrate utilized 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.

2.5.2. 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).

2.5.3. 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 30^0C for 15 min. 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).

2.5.4. 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

Chaetomium globosum was isolated and identified based on its morphological characters. *Chaetomium globosum* 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

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.

3.2.2. Degradation of autoclaved polyethylene

Chaetomium globosum was able to degrade autoclaved polyethylene, indicating its capacity to use polyethylene as sole carbon source. The weight loss for autoclaved polyethylene was 7.5% (Table 1).

Table 1: Weight loss of Autoclaved polyethylene

Sl. No.	Initial weight	Final weight (mg)*	Weight loss (mg)	Weight loss (%)
1.	0.10	0.0925	0.0075± 0.0001	7.5

± = Standard Deviation, * = Mean

3.2.3. Degradation of UV treated polyethylene

Chaetomium globosum was able to degrade UV treated polyethylene more efficiently than autoclaved and surface sterilized (Figure 1). The weight loss for UV treated polyethylene was 21% (Table 2). Degradation was more here because of pre-treatment using UV light and acid. UV light is known as initiator of polyethylene oxidation and enhances the fungal degradation, when compared with its corresponding UV untreated control (Lee et al., 1991). Both UV and acid treatment causes pro-oxidant and photo-oxidant to produce free radicals on the long chain, causing the material to lose some of its physical properties to become oxidized and more accessible to microbial biodegradation (Cornell et al., 1984). The fungal attachment was found on the surface of the plastic and it indicates possible utilization of plastic as carbon source.

Table 2: Weight Loss of UV Treated Polyethylene

Sl. No.	Initial weight	Final weight (mg)*	Weight loss (mg)	Weight loss (%)
1.	0.10	0.079	0.021 ± 0.0001	21

± = Standard Deviation, * = Mean

3.2.4. Degradation of surface sterilized polyethylene

Chaetomium globosum 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 PE was 5.6% (Table 3).

Table 3: Weight Loss of Surface Sterilized Polyethylene

Sl. No.	Initial weight	Final weight (mg)*	Weight loss (mg)	Weight loss (%)
1.	0.10	0.0944	0.0056 ± 0.0002	5.6

± = Standard Deviation, * = Mean

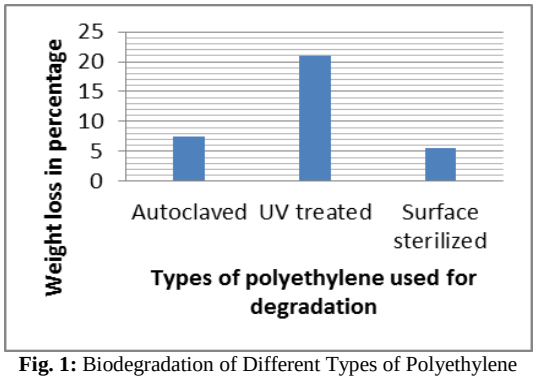

Fig. 1: Biodegradation of Different Types of Polyethylene

3.3. Confirmation of polyethylene degradation

3.3.1. Observation of discs using SEM

Autoclaved (Figure 2), UV treated and surface sterilized polyethylene (Figure 3) showed morphological changes when observed through SEM. Formation of holes, disruption of PE structure confirmed degradation capacity of *Chaetomium globosum*.

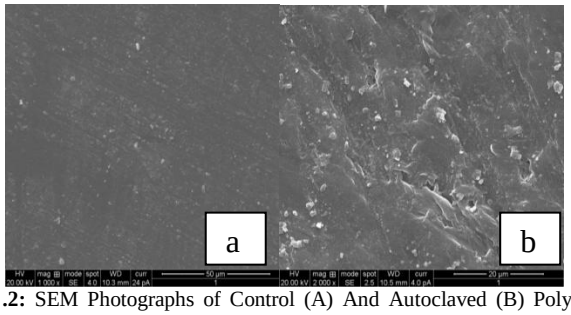

Fig .2: SEM Photographs of Control (A) And Autoclaved (B) Polyethylene

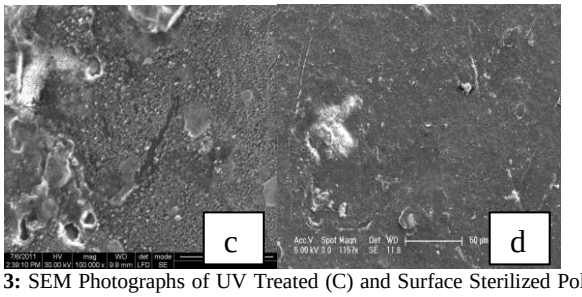

Fig. 3: SEM Photographs of UV Treated (C) and Surface Sterilized Polyethylene (D)

3.3.2. Observation of discs using FTIR

FTIR results confirmed polyethylene degradation. Following are the figures showing FTIR spectrum of control, autoclaved, UV treated and surface sterilized polyethylene. Aldehyde, ketone, alcohol, carboxylic acid groups were not formed in control polyethylene (Figure 4).

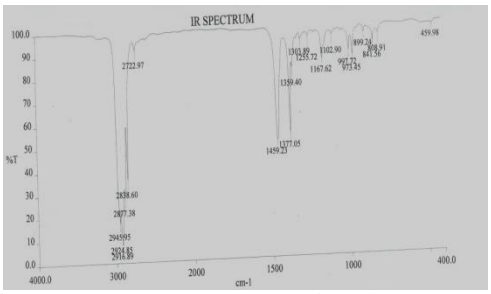

Fig. 4: FTIR Spectrum of Control

In autoclaved polyethylene, carboxylic acids (2931,39 cm^{-1}), Aldehydes (2717,70 cm^{-1}), Alcohols, esters, ethers (1298, 59 cm^{-1}) and Aromatics (898,61) were fromed at different frequencies (Figure 5).

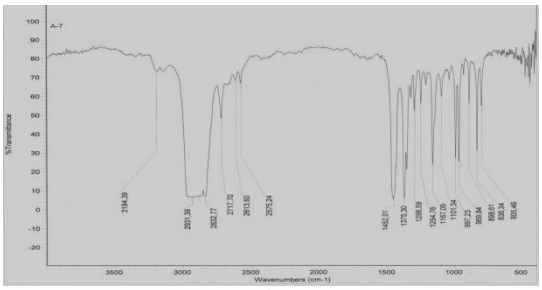

Fig. 4: FTIR Spectrum of Autoclaved Polyethylene

In UV treated polyethylene, carboxylic acids (3199, 87 cm^{-1}), aldehydes (2717, 70 cm^{-1}), aromatics (1462, 97 cm^{-1}), alcohols, esters. Ethers (1298, 59 cm^{-1}), alkyl halides (1161, 61 cm^{-1}) and alkenes (969, 84 cm^{-1}) were formed at different frequencies (Figure 6).

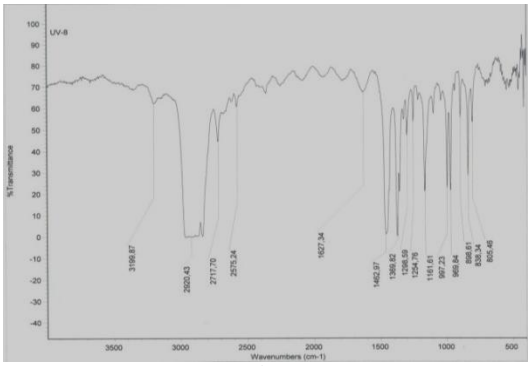

Fig. 6: FTIR Spectrum of UV Treated Polyethylene

In surface sterilized polyethylene Alcohols, phenols (3603, 49 cm^{-1}), carboxylic acids (3301, 62 cm^{-1}), aromatics (1454, 11 cm^{-1}), esters, ethers (1018, 45 cm^{-1}) were formed at different frequencies indicating its degradation by *Chaetomium globosum* (Figure 7).

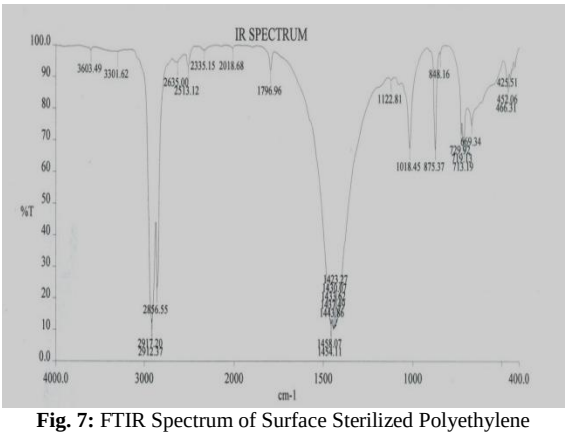

Fig. 7: FTIR Spectrum of Surface Sterilized Polyethylene

3.4. Screening and characterization of polyethylene degrading enzymes

Chaetomium globosum showed positive result for both laccase and manganese peroxidase enzyme.

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. 0.00710 IU/ml) was more compared to laccase activity (0.00705 IU/ml) after tenth week of incubation (Table 4) (Figure 6).

Table 4: Enzyme activity of Laccase and Manganese peroxidase

Weeks	Laccase	Manganese peroxidase
4	0.00026	0.00030
5	0.00052	0.00059
6	0.00115	0.00120
7	0.00209	0.00215
8	0.00326	0.00330
9	0.00509	0.00515
10	0.00705	0.00710
11	0.00535	0.00540
12	0.00300	0.00338

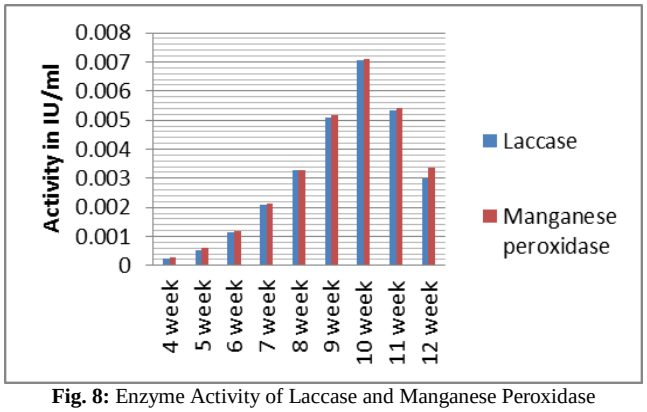

Fig. 8: Enzyme Activity of Laccase and Manganese Peroxidase

3.4.3. Protein estimation

Specific activity of manganese peroxidase (0.0082 ± 0.002 µmol/ml/mg/min) was more compared to laccase (0.0079 ± 0.114 µmol/ml/mg/min).

4. Discussion

Chaetomium globosum was isolated from local dumpsite of Shivamogga district. Fungus was identified based on both Macroscopic and Microscopic observations. *Chaetomium globosum* was grown on medium containing polyethylene and agar. After the growth of *Chaetomium globosum* on polyethylene containing medium, it was screened for degradation of autoclaved; UV treated and surface sterilized polyethylene. *Chaetomium globosum* was able to degrade UV treated (21%) polyethylene more efficiently than autoclaved (7.5%) and surface sterilized (5.6%).

After the treatment of polyethylene with UV light, *Chaetomium globosum* was able to degrade it more efficiently. Same results were concluded by Albertsson et al., (1987). He concluded that carbonyl groups are produced by UV light or oxidizing agents and these groups are the main factors at the beginning of the degradation, being attacked by microorganisms that degrade the shorter segments of polyethylene chains.

Singh et al., (2012) 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) 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 results showed formation erosions and cavities and same were recorded in our experiment. SEM and FTIR were also used in our study to evaluate biodegradation.

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 scanning electron microscope (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*. Even in our work we have evaluated degradation by using SEM.

Nwogu et al., (2012) studied degradation of polyethylene using mushrooms and degradation was confirmed by FTIR studies. FTIR analysis showed additional absorbance at regions corresponding to carbonyl groups in the spectra of polyethylene biodegraded by *P. tuber-regium* and *P. pulmonarius*, suggesting that polyethylene was degraded oxidatively.

Shimao et al., (2001) 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 *Chaetomium globosum*. So, this enzyme may play a important role in polyethylene degradation.

Iiyoshi et al., (1998) 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) 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. *Chaetomium globosum* has also given positive result for laccase enzyme.

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

5. Conclusion

Degradation of polyethylene was carried out with *Chaetomium globosum*, which was isolated from dumpsite soil. This organism was able to degrade polyethylene. Efficiency of *Chaetomium globosum* to use UV treated polyethylene as sole source of carbon was much better than autoclaved and surface sterilized. Degradation was monitored by weight loss, SEM, FTIR and NMR studies. Weight loss of UV treated polyethylene (21%) was more followed by autoclaved (7.5%) and surface sterilized (5.6%). FTIR results showed formation of aldehyde, alcohol, carboxylic acid, aromatic and ether group formation indicating degradation of polyethylene by *Chaetomium globosum*. All these results confirmed polyethylene degradation. Enzymes responsible for polyethylene degradation were identified as laccase and manganese peroxidase. Purifi-

cation of enzymes and their capacity to degrade polyethylene can be studied in future.

Acknowledgment

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