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Assessment of Low Density Polyethylene (LDPE) biodegradation potential of an edible mushroom, *Pleurotus florida*

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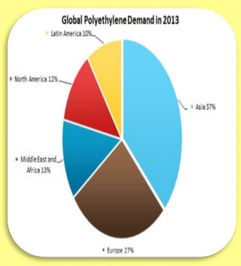
Abstract

Increasing amounts of synthetic polymers produced places pressure on capacities available for plastic waste disposal which results in increasing interest in polymer biodegradation. This study aims in investigating the low density polyethylene (LDPE) biodegradation potential of *Pleurotus florida* in various *invitro* conditions. The mushroom was grown on potato dextrose agar (PDA) medium and Mineral salt agar medium (MSM) and their mycelia growth on both the medium was recorded at periodic intervals. On observing the growth of the mushroom in the mineral salt agar medium, the biodegradation experiment was carried out in the liquid medium. The mushroom mycelia was inoculated in the liquid media supplemented with the pre-weighed low density polyethylene sheet as the only carbon source. The culture flasks were maintained at optimum temperature and the LDPE sheet after 30 days incubation with mushroom was analysed for various changes through Fourier Transform Infra Red spectroscopy and Scanning electron microscope imaging. The FTIR results of control and treated LDPE sheets showed different peaks based on the chemical groups present which indicated the oxidative degradation of the polymer. The LDPE sheets were weighed after a month of incubation and the weight loss percentage were calculated. The scanning electron microscope analysis of the LDPE sheet revealed the regions of fungal mass colonization and degradation patterns. Analysis of extracellular enzymes liberated in the liquid medium supported the biodegradation potential of *Pleurotus florida*.

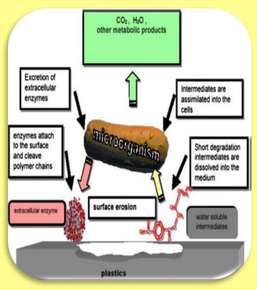
Background

Low density polyethylene is one of the major sources of environmental pollution. Polyethylene is a polymer made of long chain monomers of ethylene. The worldwide utility of polyethylene is expanding at a rate of 12% annum and approximately 140 million tones of synthetic polymers are produced worldwide each year (Shimao, M., 2001). With such huge amount of polyethylene getting accumulated in the environment, their disposal evokes a big ecological issue. It takes thousand years for their efficient degradation (Usha *et al.*, 2011).

Biological approaches based on industrial and environmental biotechnology is for the remediation of waste. One such biological method is mycoremediation which is based on the use of fungi and mushroom for the removal of waste from the environment. The mushrooms and other fungi possess enzymatic machinery for the degradation of waste/pollutants and therefore, can be applied for a wide variety of pollutants (Purnomo *et al*., 2013; Kulshreshtha *et al*., 2013).



Microbial degradation of plastics is caused by certain enzymatic activities that lead to a chain cleavage of the polymer into oligomers and monomers (Starnecker and Menner, 1996). The enzymes produced by mushroom which are lignin perioxidase, manganese perioxidase and laccase penetrate, break and digest or mineralizes harmful substances in waste (Stamets, 2005). These enzymes act singly or collectively in aiding mycelium to break down nature or human made resistant materials (Stamets, 2005).



White-rot *Basidiomycetes* are unique in their ability to degrade all components of lignocellulose due to their capability to synthesize the relevant hydrolytic and oxidative extracellular enzymes (Eriksson *et al*., 1990; Aro *et al*., 2005). *Pleurotus* species are commonly called Oyster mushrooms. Oyster mushrooms now rank second among the important cultivated mushrooms in the world. It is one of the most commonly used edible mushroom and is also used as a bioremediator (De Boer, E. and A. E. Heuvelink, 2000).

Materials and methods

Growth of *Pleurotus florida* culture in PDA medium and mineral salt agar medium

The culture of *Pleurotus florida* was grown in potato dextrose agar medium and maintained as slants (Shivaprakasam and Kandaswamy, 1983) and in mineral salt agar medium supplemented with polyethylene powder (Nwogu *et al*., 2012).

Laccase and manganese peroxidase enzyme production assay was done in liquid medium (Papinutti *et al*., 2006)

Analysis of polyethylene biodegradation

Polyethylene degradation was confirmed by Fourier transform infrared spectroscopy (FTIR) analysis and Scanning Electron Microscope (SEM) analysis.

Results and Discussion

Different growth stages of *Pleurotus florida* culture in PDA medium

Plate assay for laccase enzyme production

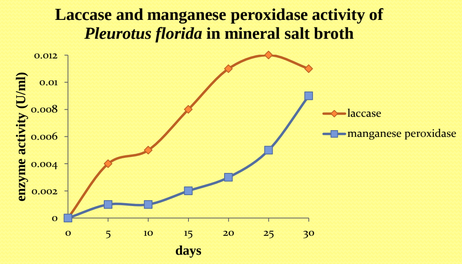
Mycelial growth in MS medium

Growth pattern of *Pleurotus florida* culture in potato dextrose agar medium (cm)

Day 2	Day 4	Day 6	Day 8	Day 10
0.87±0.058	2.57±0.12	4.53±0.15	6.87±0.21	8.33±0.15

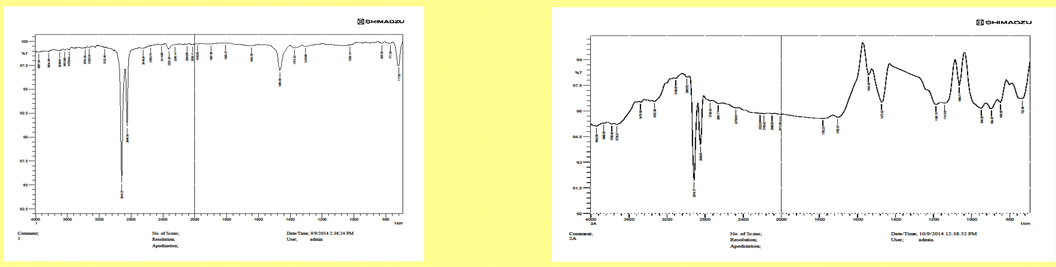
Growth pattern of *Pleurotus florida* culture in mineral salt agar medium (cm)

Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
0.97±0.21	1.87±0.15	2.87±0.25	4.17±0.61	5.67±0.25	6.13±0.21

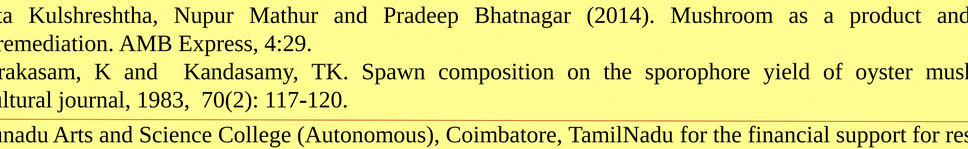


FTIR spectra of untreated LDPE sheet

FTIR spectra of LDPE sheet treated with *Pleurotus florida* culture



SEM images of LDPE film untreated and treated with *Pleurotus florida* culture



(a) untreated LDPE film

b) & (c) LDPE film treated with *Pleurotus florida* culture

The mycelium of *Pleurotus florida* was found to survive and grow in the mineral salt agar medium which shows the wide range of utilization of carbon sources. The levels of laccase and manganese peroxidase in mineral salt broth may be attributed to the enzymatic biodegradation of LDPE. 3.7% weight loss was observed in the LDPE sheet treated with culture of *Pleurotus florida.*

The FTIR analysis of the treated LDPE sheet showed intense peaks at 2916.37 cm $^{-1}$ and 2846.93 cm^{-1}. This region corresponds to the alkyl groups. The absorbance at 1782.23 and 1705.07 of the FTIR spectrum corresponds to carbonyl compounds. The FTIR spectra shows that oxidative biodegradation of polyethylene has occurred by the formation of functional groups. SEM examination shows presence of breaks on the polyethylene surface and mycelial colonization which may be due to the activity of extracellular enzymes secreted into the medium. This is an indicative response for utilization of the LDPE by the mushroom mycelia. The observations shows that the mycelium of *Pleurotus florida* was found to thrive in mineral salt agar medium and mineral salt broth medium. These results confirm the findings of Okwulehie *et al* (2006) that *Pleurotus* species are able to utilize different carbon sources for growth.

Conclusion

In recent years, deteriorative effects of plastics on environment has created public interest in plastic waste disposal and management. The degradation of polyethylene in a biological way is widely accepted and recommended. Reports support that mushrooms can play a pivotal role in bioremediation of polyethylene with the aid of the extracellular enzymes such as laccase and manganese peroxidase. From the present study it can be concluded that the mycelium of *Pleurotus florida* are able to utilize the polyethylene sheet as a carbon source for the growth and can play a role in biodegradation of low density polyethylene.

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