Int J Pharm Bio Sci 2015 April; 6(2): (B) 1454 - 1461

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

BIODEGRDATION OF A NATURAL POLYMER (COIR) ALONG WITH SYNTHETIC POLYMER (NYLON) USING PLEUROTUS OSTREATUS

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ABSTRACT

In this study the biodegradative activity of *Pleurotus ostreatus* was tested against a natural waste (Coir) along with a synthetic polymer waste (Nylon 6). Lignin modifying enzymes such as laccase, lignin peroxidase and manganese peroxidase activity were tested for coir and nylon 6 degradation in solid substrate fermentation. Enumeration of degradation with loss of weight, lignin degradation assay (Klason lignin method ASTM D- 1106) and functional group changes monitored by FTIR spectroscopy. It has been observed as *Pleurotus ostreatus* degrades lignin present in coir from 30.6% to 6.2% within 30 days. Nylon 6 also reported 36% degradation. FT IR analysis of treated coir concludes changes in functional groups and IR bands converted from strong to weak represented degradation.

KEYWORDS: ***Pleurotus ostreatus*, Coir, Lignin, Laccase, Lignin peroxidase and Nylon 6.**

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INTRODUCTION

Coir is produced from the fibrous of coconut (*Cocos nucifera L*). Coir pith is a lignocellulosic waste material consists of lignin 20 - 40%, cellulose 40 - 50%, hemicellulose 15 - 35% and protein 2.04%[1]. More than 1423 million coconuts were produced in Tamilnadu with an average of 10,000 nuts/ha from which one ton of coir fiber and another one ton of coir pith became available. The estimated annual production of coir pith in coir industries of India is about 7.5 million tons[2]. and accumulates every year which leads to pollution of the environment. White-rot fungi, which have lignocelluloses degrading enzymes, play important roles in carbon recycling in nature, because lignin, next to cellulose, is the second most abundant organic carbon compound on earth. The white-rot fungi degrade lignin not only to use them as carbon sources, but also to remove a physical barrier against cellulose utilization. Due to their powerful degrading capabilities towards various recalcitrant chemicals, white-rot fungi and their lignin degrading enzymes have long been studied for biotechnical applications such as biobleaching. White rot fungi are well known for their out-standing ability to produce extracellular oxidative enzymes, which initiate lignin polymerization[3]. This capability extend their use to a series of biotechnological applications, all of them related to the degradation of structurally diverse aromatic compounds. At present, white rot fungi are the best known and most effective lignin degrading microorganisms[4]. Solid state fermentation is an attractive process to produce fungal microbial enzymes[5]. Solid state fermentation is one of the most economically viable processes for the bioconversion of lignocellulosic coir waste is represented by *Phanerochaete chrysosporium* and *Rhizopus stolonifer*[6]. The aim of this study was to determine the possible effects of degradative capability on Nylon 6 (Synthetic polymer) and Coir (Natural polymer) by Pleurotus ostreatus, a model white-rot fungus in solid substrate fermentation. In our present study, we have investigated the better ability to degrade the above selected natural and synthetic polymers and to enumerate the biodegradation of synthetic polymers.

MATERIALS AND METHODS

(i) Materials

Coir waste samples required for this study were collected from coconut retailers, Koyambedu, Chennai, Tamil nadu and Nylon 6 samples was commercially available in local supermarkets as net were manually cut into 10 cm×5 cm strips and weighed. Samples retrieved after treated with Pleurotus ostreatus for 30 days in solid substrate fermentation were washed three times, dried at 45ºC and equilibrated at room temperature before further analysis.

(ii) Isolation of Pleurotus ostreatus

Pleurotus ostreatus used in this study was procured from Tamil Nadu Agricultural University, Coimbatore. Potato dextrose agar with pH adjusted to 5.5 was autoclaved at 121ºC for 20 min and then poured into sterile Petri dishes. After inoculation the plates were incubated at 30ºC for one week. Fungal colonies were purified by multiple transfers of mycelium to fresh potato dextrose agar plates. Isolate was preserved on PDA slant in refrigerator.

(iii) Solid state fermentation

Solid state fermentation (SSF) of Coir along with Nylon 6 was carried out in 1000 ml Erlenmeyer flasks. 100 g of coir along with Nylon 6 was placed in individual flasks and 60 ml of distilled water was added to give a moisture content of 70%.The flasks were plugged with cotton and autoclaved at 121°C for 15 min. *Pleurotus ostreatus* was grown on potato dextrose agar plates for 7 days at 30°C. Then the plates were flooded with sterile distilled water and brushed with sterilised camel hair brush smoothly without disturbing the mycelial growth. The suspension was filtered over a sterile glass wool filter to remove the mycelia fragments and the concentration of the filtered spore suspension was adjusted to

10^5 spores/ml and inoculum is used for bioremediation studies. Biodegraded coir samples were withdrawn at intervals of 10 days, oven-dried at 60°C and analyzed their lignin content and for enzyme assays[7].

(iv) Enzyme assays

Laccase activity was measured by one unit (U) of Laccase was defined as the amount of enzyme necessary to oxidize 1.0 µmol ABTS per minute. One unit of lignin peroxidase enzyme activity was defined as the amount of enzyme oxidizing one mole of veratryl alcohol in 1 min. Manganese peroxidase (MnP) activity was measured by one unit (U) of MnP was defined as the amount of enzyme necessary to oxidize 1.0 µmol Mn (II) to Mn (III) per minute.

(v) Lignin degradation assay

150mg of fibre were added with 3ml of 72% Sulphuric acid and placed in a water bath with a control temperature at 30ºC for 1hour, 84ml of distilled water was added to the sample and the mixture was placed in an autoclave at 125 for 1 hour. Sample was cooled and lignin was filtered. Insoluble lignin was washed with distilled water until neutral pH and dried in an oven at 103ºC until constant weight.

(vi) Enumeration of biodegradation by FTIR analysis

The functional groups of Coir treated with *Pleurotus ostreatus* and Coir control were determined along with Nylon 6 treated and Nylon 6 control using FT-IR spectroscopy (BRUKER RFS 27: Stand alone FT-Raman Spectrometer, SAIF laboratory, IIT Madras) at room temperature in the transmission mode. Structural changes in the Nylon 6 surface were investigated using FT-IR spectrometer. A spectrum was taken in a range from 400 to 4000 wave numbers cm^{-1}.

RESULTS

(i) Isolation of Pleurotus ostreatus

The *Pleurotus ostreatus* strain (Fig 1) thus obtained was stored on PDA at 4°C for further spore suspension preparation

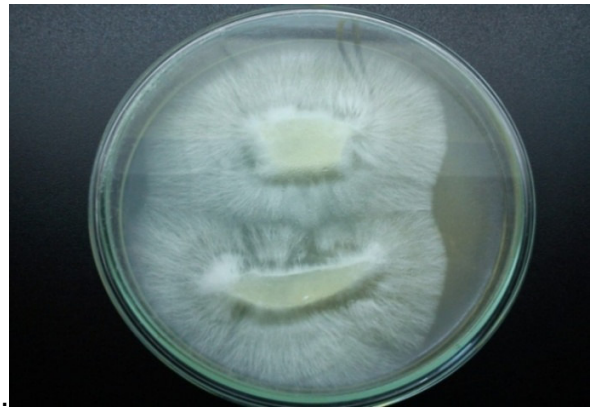


Figure 1
Pure culture of Pleurotus ostreatus strain in PDA plate

(iii) Solid state fermentation

Mycelium was observed in conical flask containing coir and Nylon 6 after 20 days of incubation at 28°c in 60% humidity.

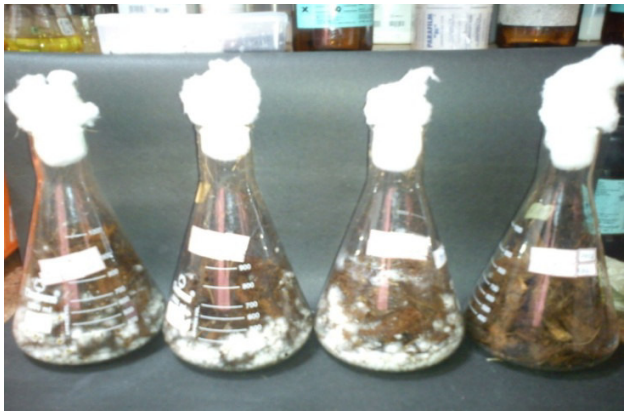
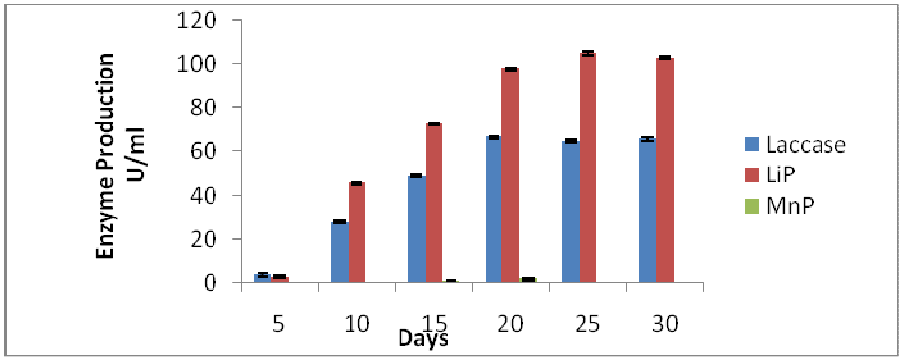


Figure 2
Mycelial growth of Pleurotus ostreatus in Coir at 5th day

(iv) Enzyme assays

Lignin modifying enzymes as laccase and lignin peroxidase were produced above 20U/ml from 10th day. Result of laccase and lignin peroxidise production was found to be higher in 20th day. Manganese peroxide was reported in trace amounts (<2U/ml).

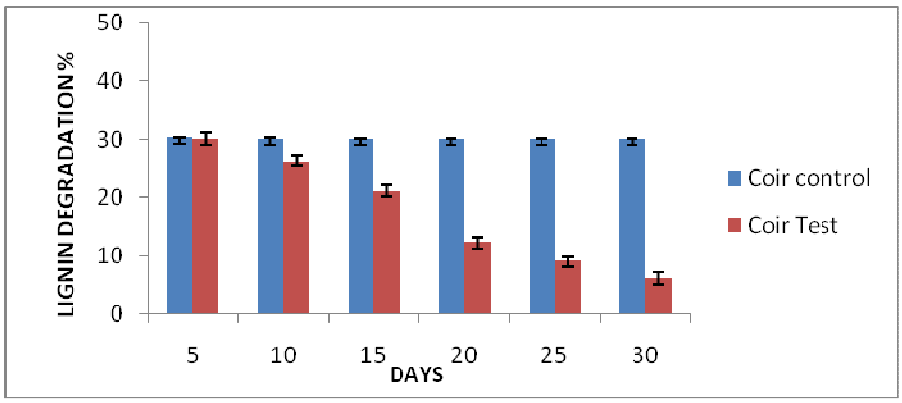
Graph 1
Enzyme assays



(v) Lignin degradation assay

It has been observed that the periodical analysis of samples of coir drawn at regular intervals of 5 days upto 30 days shows the rate of decomposition on lignin in coir pith (30.2% to 6.2%) from the initial value of 30.6%, in the control (Uninoculated coir). *Pleurotus ostreatus* degrades lignin present in coir from 30.6% to 6.2% within 30 days. No significant reduction of lignin content in control sample (Uninoculated). Klason lignin or sulphuric acid lignin is filtered, dried, and weighed.

Graph 2
Lignin degradation assay



(vi) Enumeration of Coir biodegradation by FTIR analysis

Structural and functional group changes were was analyzed after degradation for 30 days by *Pleurotus ostreatus* by FT-IR. Fig. 3 and 4 shows FT-IR spectra of Coir before and after degradation for 90 days from 450 to 4000 wave numbers cm-1. IR bands can be classified as strong (s), medium (m), or weak (w), depending on their relative intensities in the infrared spectrum. A strong band covers most of the y-axis. Based on fig 3 and fig 4 it has been identified all the functional groups IR bands converted from strong to weak. Inverted peak 3430 in control was converted to 3400 indicates functional group change.

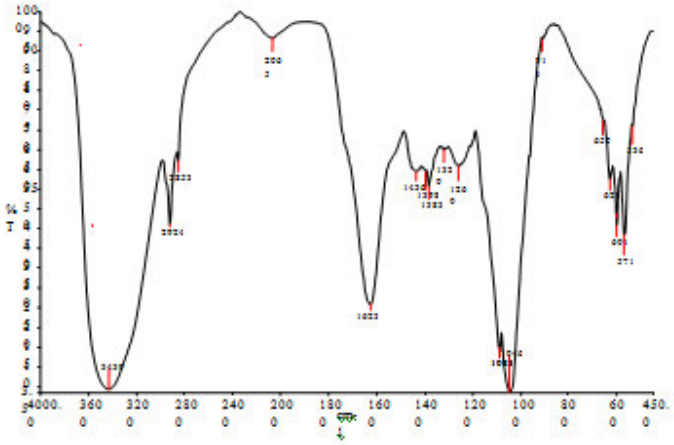


Figure 3
Functional groups of untreated Coir sample

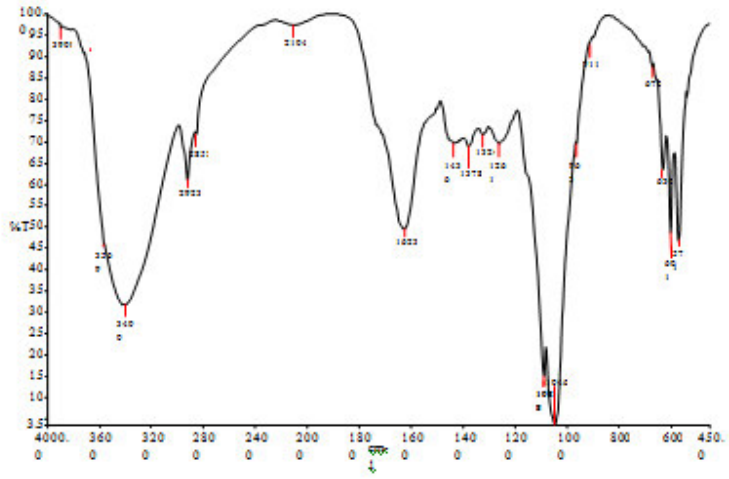


Figure 4
Functional groups of treated Coir sample

(vi) Enumeration of Nylon 6 biodegradation by FTIR analysis

Degradation of Nylon 6 and the structural changes induced by degradation was analyzed by FT-IR. Fig. 5 and fig 6 shows FT-IR spectra of Nylon 6 films before and after degradation for 30 days from 450 to 4000 wave numbers cm-1. Deletion of wave numbers 3346, 2807 and 1312 represented amines. This proved the degradation.

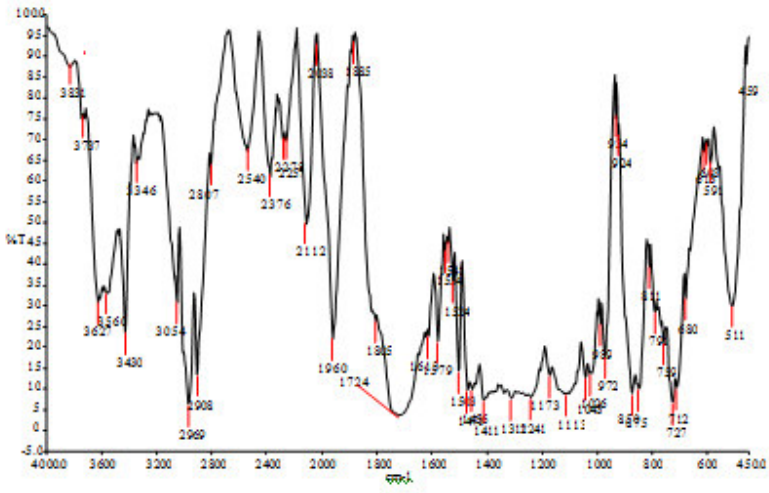


Figure 5
Functional groups of untreated Nylon 6 sample

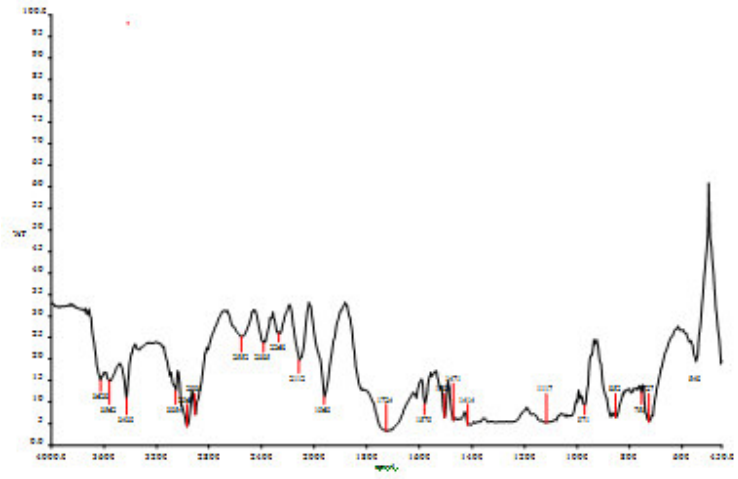


Figure 6
Functional groups of untreated Nylon 6 sample

DISCUSSIONS

From the present study it was observed that the fungal strain *Pleurotus ostreatus* showed the effective degradation of coir by reducing the lignin content from 30.6% to 6.2%. Nylon 6 degradation also reported 43% based on weight loss measurement. Due to its strong intermolecular cohesive force caused by hydrogen bonds between molecular chains of nylon, the rate of degradation is less compared to polyesters[8] and also due to scarce information on nylon-6 biodegradability, we believe that the potential of microorganisms to degrade it has not been adequately investigated. There is a surprising lack of studies using fungi in spite of the fact that they are known as a source of the greatest variety of enzymes[9]. Basidiomycetes were able to degrade coir which has a complex natural polymer lignin and synthetic polymer nylon-6 in solid substrate fermentation. Presumably, MnP was the responsible enzyme due to its non-specific oxidative action[9], even though MnP was produced in trace amount (<2U/ml) in our study with the degradative effects of laccase and lignin peroxidase was effectively analysed. Inverted peak 3430 in control was converted to 3400 indicates from strong amines (Control) to alcohols (Treated). Deletion of wave numbers 3346, 2807 and 1312 in Nylon treated represented amines. This proved the degradation. There is great potential for the development of a process of degrading coir and nylon6 in a composting environment using *Pleurotus ostreatus* in the near future.

CONCLUSION

The results obtained from observing the degradation process using *Pleurotus ostreatus* under sterile conditions indicate that ability of fungi to utilise both coir and nylon 6. The results from Lignin degradation assay and FT-IR shows that the white rot fungi utilized Coir and Nylon 6 as a carbon source and nitrogen source. Lignin present in coir and Nylon 6 degradation in this research shows that the fungi have great potential for compost method of remediation. A weight loss results confirms biodegradation of Nylon 6 by 36% and Lignin reduction in coir from 30.6% to 6.6%. FT IR results also supporting the degradation of Coir and Nylon 6.

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