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COMPARITIVE BIODEGRADATION OF SYNTHETIC POLYMERS (LDPE, HIPS AND NYLON 6) USING PLEUROTUS OSTREATUS

ARUN KUMAR. J*, GOPINATH. L, APRAJEETHA. J, DECCARAMAN. M AND SHANTHA PREMA RAJ

Department of biotechnology, Dr MGR Educational and Research Institute, Maduravoyal Chennai 600095, India

ABSTRACT

In the present study degradative ability of Pleurotus ostreatus was tested against LDPE, NYLON 6 and HIPS. Growth and enzyme activity were optimized to attain maximum degradability with parameters like pH, temperature and initial carbon source. Enumeration of degradation with loss of weight was further analysed by FTIR spectroscopy and SEM analysis. It has been observed based on results that Pleurotus ostreatus degrades Nylon 6 by much higher level (45.7%) followed by LDPE (36.9%) and HIPS (27%) in optimised MSM medium with lignin as initial carbon source. Changes in functional groups and surface degradation were observed in NYLON 6.

KEYWORDS: ***Pleurotus ostreatus*, LDPE, HIPS and NYLON 6.**

*Corresponding author

ARUN KUMAR. J
Department of biotechnology, Dr MGR Educational and Research Institute, Maduravoyal Chennai 600095, India

INTRODUCTION

A broad range of petroleum - based synthetic polymers are manufactured worldwide approximately 140 million tonnes per year and striking amounts of these polymers are introduced in the ecosystem as litter waste and industrial waste[1]. Synthetic polymers are used upto 30% in packaging of various industrial products. Their accumulation as waste in environment has triggered research to develop more readily degradable materials and to identify new methods for eliminating existing polymer waste[2]. Aliphatic polyamides, such as nylon-6 have been produced industrially since the late 1930s and continue to be important materials[3]. White-rot fungi are characterized by their unique set of enzymes that enables them to effectively degrade lignin, one of the principal components of wood. The fact that ligninolytic enzymes can also degrade a wide range of organic compounds structurally similar to lignin that includes synthetic polymers. Many reports on the synthetic polymers in the environment have been reported. However, reports on the fungal degradation of these polymers and the related hydrolytic enzymes are quite rare and not well documented. Considering that white rot fungi play a noteworthy role in degrading natural organic substances in the ecosystem, such as cellulose, hemicellulose, and lignin. The fungal contribution to the biodegradation of polyesters in the environment should be recognized along with the bacterial bioremediation of xenobiotic compounds. White-rot fungi break down the lignin in wood, leaving the lighter colored cellulose behind which results in bleaching of the wood substrate thus called as white rot fungi. Because white-rot fungi are able to produce enzymes, such as laccase, lignin peroxidise and manganese peroxidase needed to break down lignin and other complex organic molecules, they have been investigated for use in bio remedial applications. The aim of this study was to determine the possible effects of degradative capability on selected synthetic polymers like Low density polyethylene (LDPE), Nylon 6 and High Impact Polystyrene (HIPS) by *Pleurotus ostreatus*, a model white-rot fungus[4]. In our present study, we have investigated the better ability to degrade the above selected polymers and to enumerate the biodegradation of synthetic polymers.

MATERIALS AND METHODS

(i) Materials

LDPE, HIPS and NYLON 6 samples were commercially available in local supermarkets as carrier bags, cups and net were manually cut into 10cm×5 cm strips and weighed. Samples retrieved after treated with *Pleurotus ostreatus* for 3 months were washed three times, dried at 45ºC and equilibrated at room temperature before further analysis.

(ii) Isolation of Pleurotus ostreatus

Pleurotus ostreatus used in the present 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) Preparation of spore suspension

Pleurotus ostreatus was grown in potato dextrose agar plates for 7 days at 30°C. Then the plates were flooded with sterile distilled water and brushed with a 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[5].

(iv) Optimiasation of carbon source on enzyme production

The rate of enzyme production for various concentrations was studied with glucose, starch and lignin as the carbon source. Glucose, starch and lignin was weighed and added to the mineral salt medium (MSM) to make concentrations of 1%, 1.5%, 2%, 2.5%, 3%, 3.5 and 4% (w/w) along with 100 ml of

nutrient solution containing (g/litre): KH_2PO_4.2g; $MgSO_4.7H_2O$, $CaCl_2.2H_2O$ and NH_4H_2- PO_4 each 0.5 g; NH_4Cl, $FeSO_4.5H_2O$ and $MnSO_4$ each 0.1 g; $CoSO_4$, $ZnSO_4$, $CuSO_4.5H_2O$ and Na_2HPO_4 each 0.05 g. The medium was sterilized at 121ºC for 20 min. The solution was added with 10^5 spore suspension and incubated. 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 an enzyme necessary to oxidize 1.0 µmol Mn (II) to Mn (III) per minute.

(iv) Optimiasation of pH for enzyme production

Concentrations of pH from 3, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 were used to enumerate the optimum production of enzymes from the above formulated MSM medium.

(v) Optimiasation of temperature for enzyme production

Concentrations of temperature from 30ºC, 35 ºC, 40 ºC, 45 ºC, 50 ºC, 55 ºC, 60 ºC, 65 ºC and 70 ºC were used to optimise physical factor contributing the production of enzymes. Based on weight loss measurement the selected polymer was subjected to FTIR and SEM analysis.

(vii) Enumeration of biodegradation by FTIR analysis

The functional groups of Nylon 6 treated with *Pleurotus ostreatus* and Nylon 6 control were determined 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}.

(vii) Microscopic examination

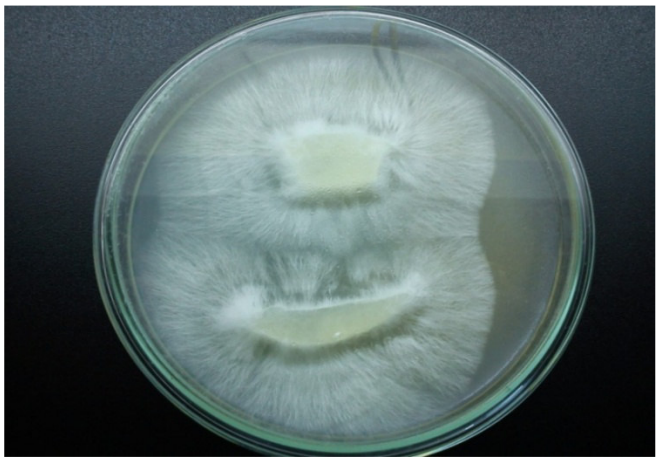
Scanning electron microscopy (SEM) analysis was used to examine changes in the surface of Nylon 6 sample during their degradation. Both untreated and treated samples were cut into 10mm×10mm pieces, fixed on the top of the specimen holder and were coated with gold grade. Samples were examined at 20 kV and 2000× magnification under a JEOL Carl Zeiss MA15 / EVO 18 Scanning Electron Microscope, Central workshop, Anna University, Chennai, Tamil Nadu, India.

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

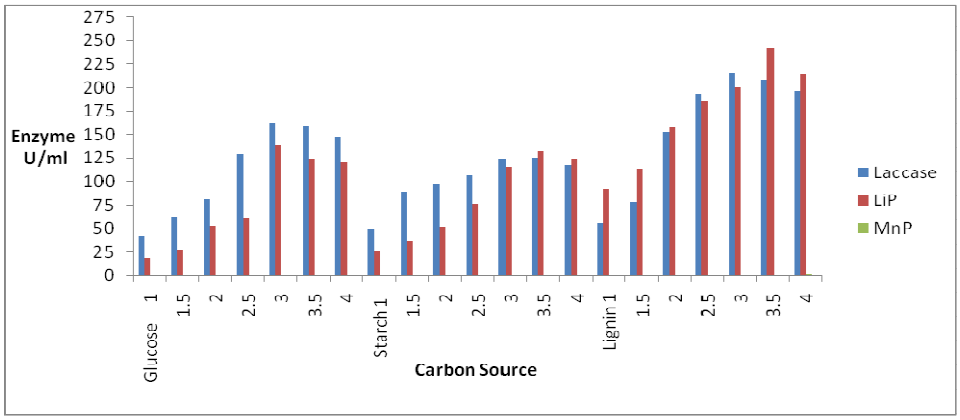


(ii) Optimisation of carbon source for enzyme production

The result of the carbon source optimization suggests that lignin was ideal initial carbon source for *Pleurotus ostreatus* when compared to glucose and starch. Higher production of laccase and lignin peroxidise was reported at 3.5% of lignin as optimised carbon source.

Graph 1

Optimiasation of carbon source for enzyme production

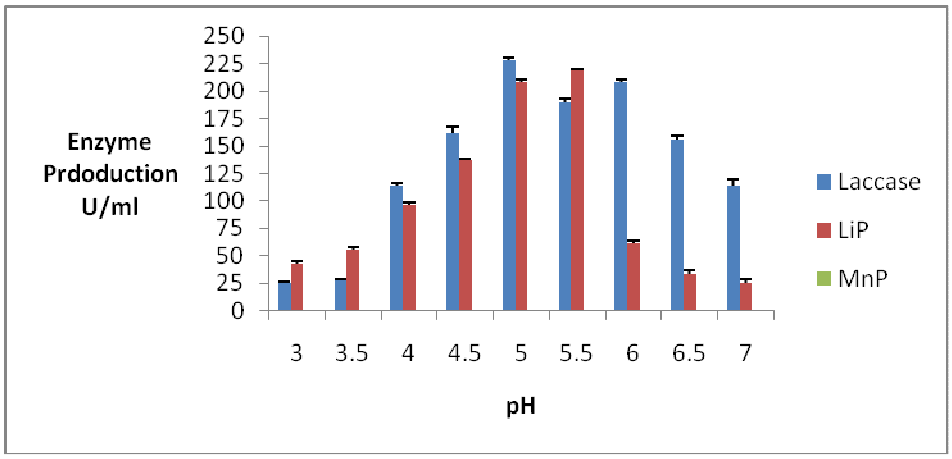


(ii) Optimisation of pH for enzyme production

The result of the pH optimization suggested that among the varying pH tested, maximum enzyme production was reported in pH 5, 5.5 and 5 for laccase, Lignin peroxidase and manganese peroxidase respectively were revealed in Graph 2. Manganese peroxide was reported in trace amounts (<0.1U/ml).

Graph 2

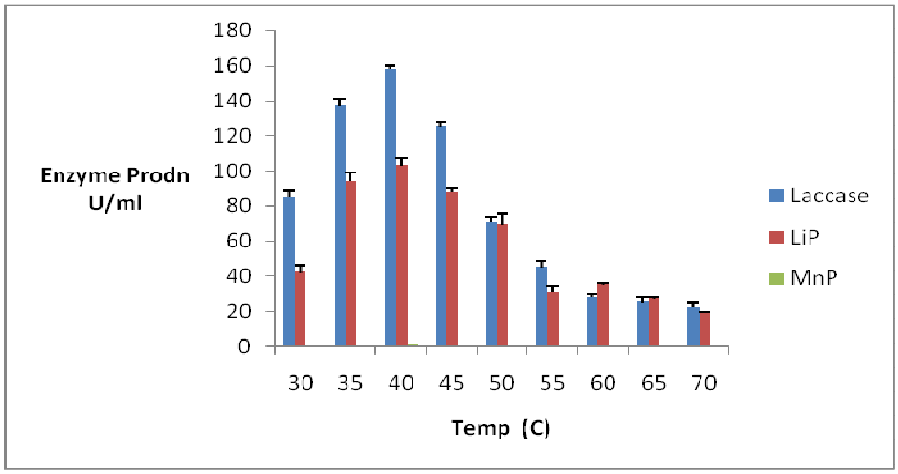
Optimiasation of pH for enzyme production



(v) Optimisation of temperature for enzyme production

Result of laccase and lignin peroxidise production was approximately two fold high as found to be at 40°C when compared to 30ºC. In the varying temperatures tested, the fungal growth was found to be moderately similar from 35ºC to 45ºC and the growth gradually reduced at temperatures higher than 45°C. Manganese peroxide was reported in trace amounts (<0.1U/ml).

Graph 3
Optimisation of temperature for enzyme production

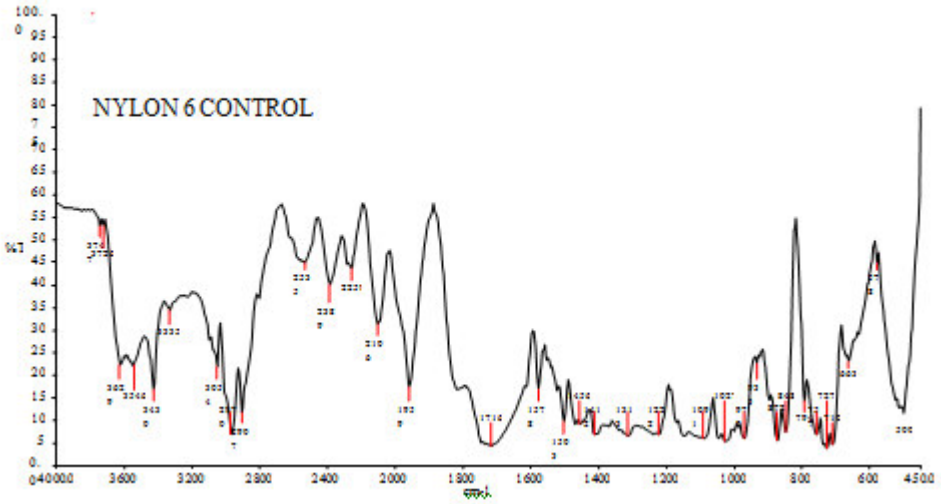


(vi) Enumeration of biodegradation by FTIR analysis

Degradation of Nylon 6 and the structural changes induced by degradation was analyzed by FT-IR. Fig. 2 shows FT-IR spectra of Nylon 6 films before and after degradation for 90 days from 450 to 4000 wave numbers cm^{-1}. Deletion of 710 (Nylon 6 control) and 3553 (Nylon 6 control) wave number represents - CH2 group and - NH2 group respectively[6], which indicated that the fungi oxidation of carbon and utilisation of Nylon 6 as nitrogen source also. Fig 3 shows newly formed peaks in treated samples 1405 and 1370 (Nylon 6 test) represented –OH group that was formation of alcoholic or phenolic compounds by oxidation, this proved the end product of degradation

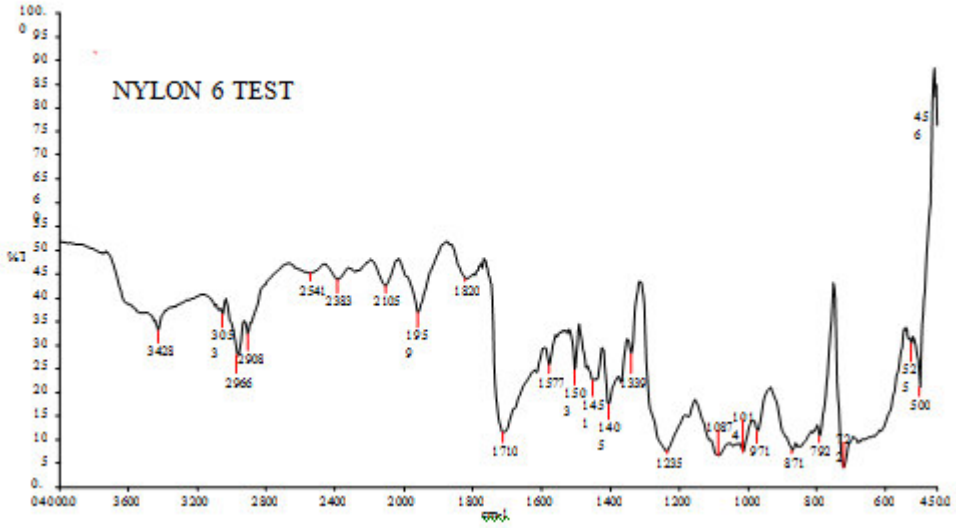
FT IR graph of Untreated Nylon 6 control sample

Figure 2
Functional groups of untreated Nylon 6 sample



FT IR graph of treated Nylon 6 sample

Figure 3
Functional groups of treated Nylon 6 sample

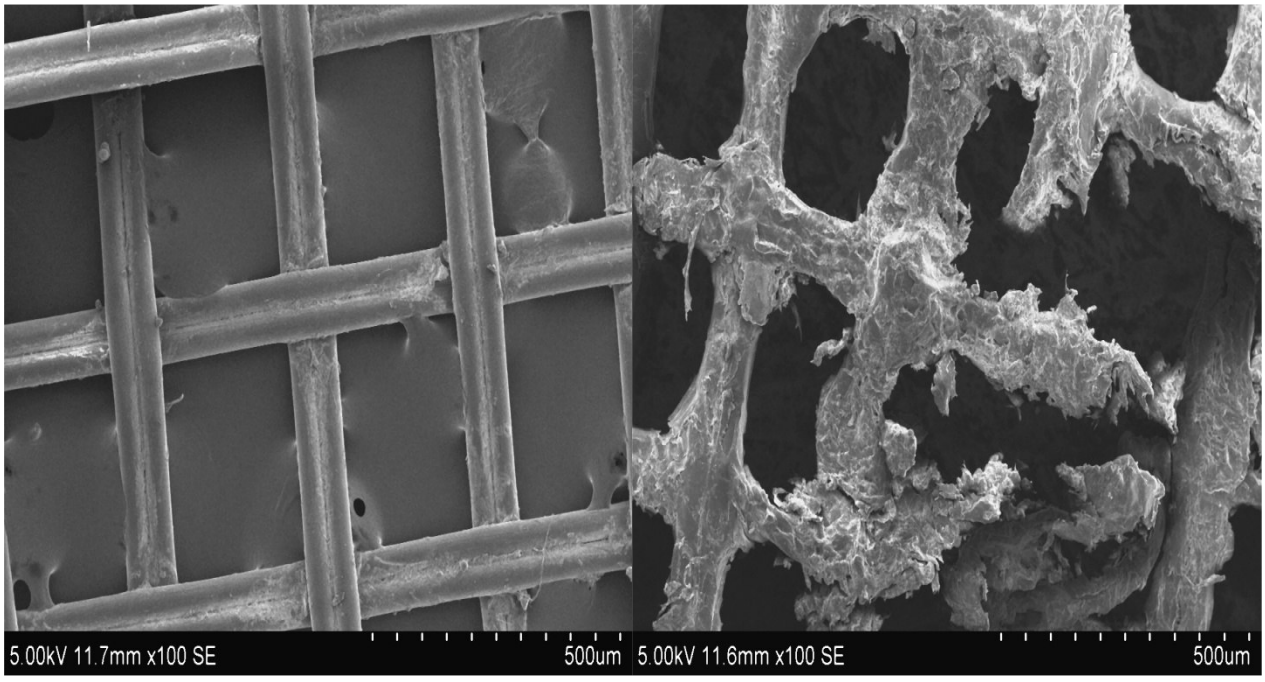


(vii) Microscopic examination

Scanning electron microscopy (SEM) was used to monitor changes in the surface of Nylon 6 sample. Fig. 3 show control and treated samples of Nylon 6. The pits were observed on the surface, suggesting that the fungi penetrated into the Nylon 6 during degradation. The surface of the polymer after biological attack was physically weak and readily disintegrated under mild pressure. Nylon 6 degradation by *Pleurotus ostreatus* was identified by structural changes (Fig 3).

SEM images of control Nylon 6 (untreated) and treated Nylon 6 sample

Figure 4
Nylon 6 control doesn't show any significant damage and Nylon 6 treated sample shows eroded fibres fragmented outer layers which will eventually break down.



DISCUSSIONS

From the present study it was observed that the fungal strain *Pleurotus ostreatus* showed the optimum temperature for enzyme production was 40°C and optimum pH was found to be pH 5. The pH and temperature of culture medium were considered as important factors for production fungal enzymes. 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[7] 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[8]. Basidiomycetes were able to degrade nylon-6 when grown on nylon as the only N-source. Presumably, MnP was the responsible enzyme due to its non-specific oxidative action[9], even though MnP was produced in trace amount (<0.1U/ml) in our study with the degradative effects of laccase and lignin peroxidase was effectively analysed. There is great potential for the development of a process of degrading 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 nylon 6. The results from FT-IR and SEM analyses show that the white rot fungi utilized Nylon 6 as a carbon source and nitrogen source. *Pleurotus ostreatus* shows better enzyme production with lignin as an initial carbon source. SEM images of this research show that the fungi have great potential for nylon 6 biodegradation in optimised MSM (Mineral Salt Medium). Weight loss results confirms biodegradation of Nylon - 6 is much higher (48%) followed by LDPE (34%) and HIPS (28%). SEM analysis shows that Nylon-6 is more degradable than LDPE followed by HIPS this may be because Nylon - 6 serves as both C-sole source and N-sole source, thus it can be concluded Nylon-6 is more susceptible to biodegradation by *Pleurotus ostreatus*.

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