**ISSN 0974-3618 (Print)**
**0974-360X (Online)**

**www.rjptonline.org**

***RESEARCH ARTICLE***

# Instrumental Analysis on Optimization of Laccase Production from *Pleurotus ostreatus* strain PKN 04 in Different Substrate by Solid State Fermentation (SSF)

**T.V. Preethi[1*], A.K. Kathireshan[2], G. Narendrakumar[3]**

[1]Research Scholar, Department of Microbiology, School of Life Science, Vels Institute of Science,Technology and Amp; Advanced Studies, Chennai – 600 117, Tamilnadu, India

[2]Department of Microbiology, School of Life Science, Vels Institute of Science,Technology and Amp; Advanced Studies, Chennai – 600 117, Tamilnadu, India

[3]Department of Biotechnology, School of Bio and Chemical Engineering, Sathyabama Institute of Science and Technology, Chennai – 600 119, Tamilnadu, India

*Corresponding Author E-mail: **preethynaren@gmail.com**

## ABSTRACT:

Solid state fermentation (SSF) is a green process to produce enzymes from fungi. Different substrates such as rice husk, paddy straw, sugarcane bagasse and wheat husk taken from top soil were used to produce laccase by *Pleurotus ostreatus* strain PKN 04. The process of SSF was characterized by FTIR. Microbial fermentation resulted in conversion of complex polymeric lignocelluloses substrates into simpler monomers. Fourier transform infrared spectroscopy (FT-IR) analyses of metabolites confirmed the breakdown and complete degradation of lignin, cellulose and polysaccharides at different fermentation time intervals of 0, 15 and 30 days.

**KEYWORDS:** *Pleurotus ostreatus* strain PKN 04, SSF, FT-IR.

## INTRODUCTION:

The accessibility of enormous agro-industrial wastes from crop cultivation and food processing areas, offers a advantageous way to cultivate the bacteria and fungi that convert them into different Industrially important products. Production of lignocellulolytic enzymes using different plant raw materials by white rot fungi have successfully been performed in Submerged Fermentation[1-3] (SmF) and Solid-State Fermentation (SSF) [4-7]. Solid state fermentation is a process that commonly used to produce metabolites from the microbes especially from fungi[8-10]. Laccases are N-glycosylated multi copper oxidases belonging to the group of the blue copper proteins[11]. They can catalyze the oxidation of many substances coupled to the reduction of molecular oxygen to water[12,13].

Received on 21.07.2017 Modified on 11.09.2017
Accepted on 14.10.2017 
*Research J. Pharm. and Tech 2018; 11(2): 657-660.*
**DOI: 10.5958/0974-360X.2018.00123.3**

Laccases are widely found in fungi and higher plants and in a lower proportion in insects and bacteria. Laccase was first described by Yoshida in 1883 when the enzyme extracted from the exudates of the Japanese lacquer tree *Rhus vernicifera*, from which the name laccase was derived[14,15].

One of assuring sources for production of laccase has been white rot fungus, *Pleurotus ostreatus* and it could decolorize different textile dyes[16-18]. *Pleurotus ostreatus* is a commercially important edible mushroom, famous for its delicious taste and high quantities of protein, carbohydrates, minerals, and vitamins but low fat[19-,21].

## MATERIALS AND METHOD:

### Isolation of Fungi:

The isolation of fungi was done humus material containing soil extracted from the forest of Chennai. The materials were brought to the laboratory with the prescribed conditions.

**Identification of Fungi:**
**Molecular identification:**
The isolated organism was confirmation by 18s rRNA analysis[22,23] and the sequence was submitted in NCBI.

**Screening of Fungi for Laccase Production:**
Screening for laccase production was performed using ABTS assay (Bourbonnais and Paice, 1990)[24,25].

**Inoculum Preparation:**
Solid State fermentation (SSF)
In sterile (15 min, 15 PSI and 121°C), rice husk, paddy straw, sugarcane bagasse and wheat husk was used with no previous physical treatment. 50 g of substrate and 2.5 g inoculum were placed in a hermetically sealed bag. The bags were incubated at 25°C and removed from the incubator after 0, 3, 5 or 7 days of fermentation for analysis.

**Assay of Laccase:**
**Enzyme assays:**
Laccase activity was expressed by the oxidation of ABTS. The non-phenolic dye ABTS (2, 2´-azino-bis- [3 – ethyl benzothiazoline – 6 –sulphonic acid]) was oxidized by laccase produced by *Pleurotus ostreatus* strain PKN 04 to the more stable state of the cation radical. The intense blue-green colour formed was correlated to enzyme activity and read at 420nm.

The mixture contained 0.5mM ABTS, 0.1M sodium acetate (pH 4.5), and an appropriate amount of enzyme. Oxidation of ABTS was observed by determining the increase in A420 (ε420, 3.6 × 104 M-1cm-1). The reaction mixture contained 0.5mM substrate (ABTS), 2.8 mL of 0.1 M sodium acetate buffer of pH 4.5, and 100 µL of culture supernatant and incubated for 5 min. Absorbance was read at 420 nm in a spectrophotometer against a suitable blank.

One unit was specified as the amount of the laccase that oxidized 1 µmol of ABTS substrate per min. The absorbance was read after 10 min interval using UV/VIS spectrophotometer[26] (Varian Cary® 100 UV-Vis).

Protein estimation was performed using Lowry et al 1951[27].

**CHN Analysis:**
The different samples for the analysis were prepared in the powder form by sieving, and the fraction with particle sizes with pan size was separated after drying at 95 °C for 24 h. The elemental composition analysis of the substrate was carried out using a Perkin Elmer 2400 Series II CHN analyser [28-31].

## RESULT AND DISCUSSION:

**Isolation of Fungi:**
*Pleurotus ostreatus* strain PKN 04 is a white rot fungus isolated from forest of Chennai District, Tamilnadu, India (12.9261° N, 80.1722° E). It was cultivated at 37°C on potato dextrose agar (PDA) and stored at 4°C.

**Identification of Fungi:**
**Molecular identification:**
The culture has been deposited at Biotechnology Laboratory of Sathyabama Institute of Science and Technology, Chennai. The confirmation of the organism was done by 18s rRNA analysis[22,23] and the accession number from NCBI -KX151954 was procured.

**CHN Analysis:**
The Carbon, Hydrogen and Nitrogen, Lignin and Carbohydrate were estimated and tabulated in Table 1.

**FT-IR:**
The aromatic C-H Bending at 680 nm, Alkenyl C-H Stretch, Alkenyl C=C Stretch at 1680, Aldehyde C=O Stretch at 1780 were seen in all the substrates. (Fig-1,2,3,4)

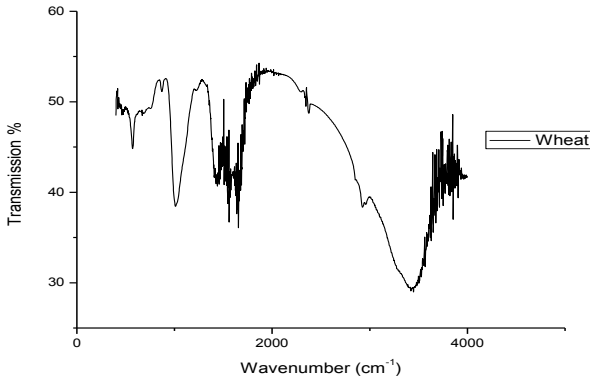


**Fig.1 FTIR results of Wheat husk.**

**Table 1 CHN composition of different substrates**

| S.No. | Sample | Carbon (%) | Hydrogen (%) | Nitrogen (%) | Lignin | Carbohydrate |
|---|---|---|---|---|---|---|
| 1 | Wheat husk | 47.26 | 4.32 | 0.26 | 25.4 | 9.5 |
| 2 | Sugarcane bagasse | 45.57 | 5.51 | 1.24 | 23.9 | 18.2 |
| 3 | Rice husk | 39.24 | 6.51 | 2.19 | 19.2 | 29.4 |
| 4 | Paddy straw | 41.39 | 5.13 | 2. 48 | 24.6 | 25.3 |

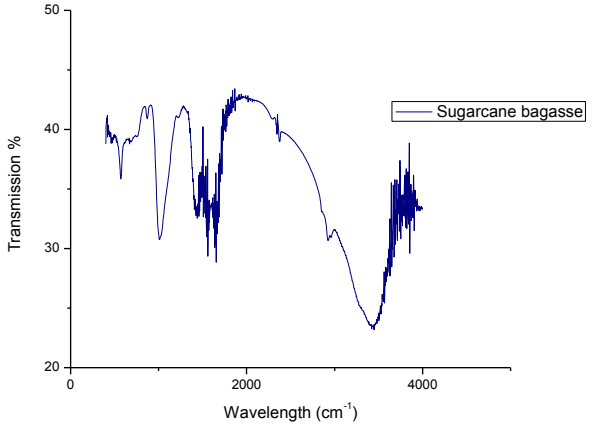


**Fig.2 FTIR results of Sugarcane bagasse.**

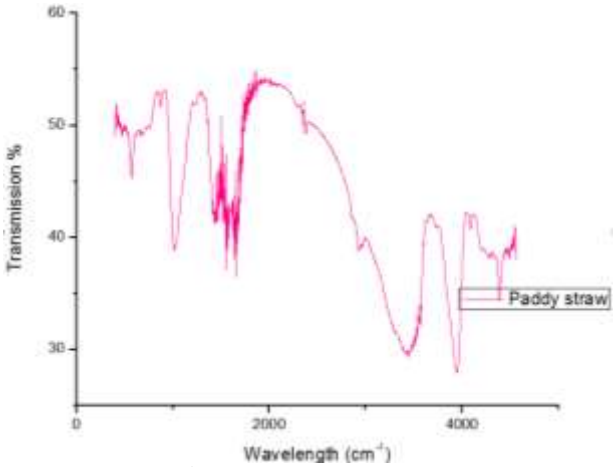


**Fig.3 FTIR results of Paddy straw.**

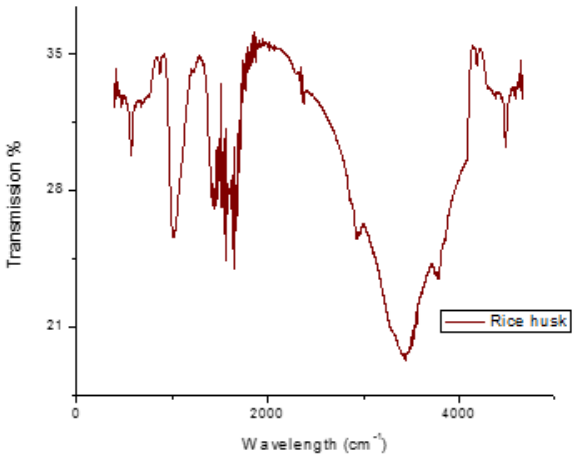


**Fig.4 FTIR results of Rice husk.**

The FTIR results shows that the Paddy straw has considerably many different side chains in it and may be contributing for the maximum production of the laccase. The experimental analysis clearly suggests that with Paddy straw or with the combination may have maximum productivity of the enzyme.

## REFERENCE


1. Govumoni SP, Gentela J, Koti S, Haragopal V, Venkateshwar S, Rao LV. Extracellular Lignocellulolytic Enzymes by Phanerochaetechrysosporium (MTCC 787) Under Solid-State Fermentation of Agro Wastes. Int. J. Curr. Microbiol. App. Sci. 2015;4(10):700-10.
2. Swartz CJ. On chemical kinetics. J. Chem. Educ. 1969; 46(5): 308.
3. Chawachart N, Khanongnuch C, Watanabe T, Lumyong S. Rice bran as an efficient substrate for laccase production from thermotolerant basidiomycete Coriolus versicolor strain RC3. Fungal Divers. 2004 1;15: 23-32.
4. Chenthamarakshan A, Parambayil N, Miziriya N, Soumya PS, Lakshmi MK, Ramgopal A, Dileep A, Nambisan P. Optimization of laccase production from Marasmielluspalmivorus LA1 by Taguchi method of Design of experiments. BMC biotechnology. 2017; 13;17(1):12.
5. Chhaya U, Gupte A. Optimization of media components for laccase production by litter dwelling fungal isolate Fusarium incarnatum LD3. Journal of Basic Microbiology. 2010 1;50(1):43-51.
6. Demir A, Aytar P, Gedikli S, Çabuk A, Arisoy M. Laccase production with submerged and solid state fermentation: benefit and cost analysis. Hacettepe J. Biol. Chem. 2011;39:305-13.
7. Devi VM, Inbathamizh L, Ponnu TM, Premalatha S, Divya M. Dye decolorization using fungal laccase. Bulletein of Environment, Pharmacology and Life Sciences. 2012;1(3):67-71.
8. Díaz R, Téllez-Téllez M, Sánchez C, Bibbins-Martínez MD, Díaz-Godínez G, Soriano-Santos J. Influence of initial pH of the growing medium on the activity, production and genes expression profiles of laccase of Pleurotus ostreatus in submerged fermentation. Electronic Journal of Biotechnology. 2013;16(4):6-.
9. Preethi TV, Kathireshan AK, Narendrakumar G. Journal of Chemical and Pharmaceutical Research. 2016;8(7):576-84.
10. Elisashvili V, Kachlishvili E. Physiological regulation of laccase and manganese peroxidase production by white-rot Basidiomycetes. Journal of Biotechnology. 2009;144(1):37-42.
11. Arunkumar T, Anand DA, Narendrakumar G. Phylogenetic analysis of the internal transcribed spacer (ITS) region in laccase producing Pseudomonas aeruginosa ADN04 by predicted rRNA secondary structure. BiosciBiotechnol Res Asia. 2014; 11(3): 1573-9.
12. Gorska EB, Jankiewicz U, Dobrzynski J, Galazka A, Sitarek M, Gozdowski DA, Russel S, Kowalczyk PA. Production of ligninolytic enzymes by cultures of white rot fungi. Pol J Microbiol. 2014; 63(4):461-5.
13. Govumoni SP, Gentela J, Koti S, Haragopal V, Venkateshwar S, Rao LV. Extracellular Lignocellulolytic Enzymes by Phanerochaetechrysosporium (MTCC 787) Under Solid-State Fermentation of Agro Wastes. Int. J. Curr. Microbiol. App. Sci. 2015; 4(10): 700-10.
14. Gregori A, Švagelj M, Pohleven J. Cultivation techniques and medicinal properties of Pleurotus spp. Food Technology & Biotechnology. 2007;45(3).
15. Arunkumar T, Anand DA, Narendrakumar G. Production and Partial Purification of Laccase from Pseudomonas aeruginosa ADN04. Journal of Pure & Applied Microbiology, 8, 727, 2014.
16. Hadri SH, Asad MJ, Gulfraz M, Asghar M, Minhas NM, Mahmood RT. International Journal of Advanced Research in Biological Sciences. Int. J. Adv. Res. Biol. Sci. 2014;1(9):33-44.
17. Preethi TV, Kathireshan AK, Narendrakumar G. Optimization of media components for the production of laccase from Pleurotus ostreatus PKN 04 using response surface methodology. Pharm Lett. 2016;8:33-40.
18. Iandolo D, Amore A, Birolo L, Leo G, Olivieri G, Faraco V. Fungal solid state fermentation on agro-industrial wastes for acid wastewater decolorization in a continuous flow packed-bed bioreactor. Bioresource technology. 2011;102(16):7603-7.
19. Karp SG, Faraco V, Amore A, Birolo L, Giangrande C, Soccol

VT, Pandey A, Soccol CR. Characterization of laccase isoforms produced by Pleurotus ostreatus in solid state fermentation of sugarcane bagasse. Bioresource Technology. 2012;114:735-9.
20. Kerem Z, Friesem D, Hadar Y. Lignocellulose degradation during solid-state fermentation: Pleurotus ostreatus versus Phanerochaetechrysosporium. Applied and Environmental Microbiology. 1992;58(4):1121-7.
21. Kumari J, Negi S. Development of bioprocess for the production of laccase by Pleurotus ostreatus MTCC 1802 using evolutionary optimization technique., *Indian J. Exp. Biol.*, 2014:52, 1106.
22. Masutti D, Borgognone A, Setti L. Production of enzymes from rice husk and wheat straw in solid-state fermentation. Chemical Engineering Transaction. 2012;27:133-8.
23. Aslam S, Asgher M, Hussain F, Khan NA. Exploration of optimum operating conditions for enhanced laccase enzyme production by Pleurotus nebrodensis WC 850 through Response Surface Methodology., Journal of Animal and Plant Sciences. 2016;26(3):794-804.
24. Parani K, Eyini M. Production of ligninolytic enzymes during solid state fermentation of coffee pulp by selected fungi. Sci Res Rep. 2012;2(3):202-9.
25. Patel H, Gupte A, Gupte S. Effect of different culture conditions and inducers on production of laccase by a basidiomycete fungal isolate Pleurotus ostreatus HP-1 under solid state fermentation. BioResources. 2009;4(1):268-84.
26. Radhika R, Jebapriya GR, Gnanadoss JJ. Production of cellulase and laccase using Pleurotus sp. under submerged and solid-state fermentation. International Journal of Current Science. 2013;6:7-13.
27. Aslam S, Asgher M. Partial purification and characterization of ligninolytic enzymes produced by Pleurotus ostreatus during solid state fermentation. African Journal of Biotechnology. 2011;10(77):17875-83.
28. Manwar Jagdish, KakasahebMahadik, Anant Paradkar. Plackett-Burman Design: A Statistical Method for the Optimization of fermentation process for the yeast Saccharomyces cerevisiae isolated from the flowers of Woodfordia fruticose. Ferment Technol. 2013; 2(1):1-6.
29. Valle JS, Vandenberghe LP, Santana TT, Almeida PH, Pereira AM, Linde GA, Colauto NB, Soccol CR. Optimum conditions for inducing laccase production in Lentinuscrinitus. Genetics and Molecular Research. 2014;13(4):8544-51.
30. Zhao XS, Huang XJ, Yao JT, Zhou Y, Jia R. Fungal growth and manganese peroxidase production in a deep tray solid-state bioreactor, and in vitro decolorization of poly R-478 by MnP. J Microbiol Biotech. 2014;25:803-13.
31. A. Skoog Douglas, Holler, F. James and Crouch, Stanley R. " Principles of Instrumental Analysis, 6th Ed." Thomson, Belmont, California (2007).