IJIRR

International Journal of Information Research and Review
Vol. 04, Issue, 03, pp.3876-3884, March, 2017

Research Article

OPTIMIZATION OF PLEUROTUS PLATYPUS THROUGH CARBON UTILIZATION IN LIGNIN DEGRADATION

[1,*]Gayathiri, E., [1]Bharathi, B., [1]Natarajan, S., [2]Selvadhas, S. and [2]Kalaikandhan, S.

[1]Department of Plant Biology and Biotechnology, Gill Research Institute, Guru Nanak College, Chennai
[2]Department of Plant Biology and Plant Biotechnology, Loganatha Narayanasamy Govt. College, (autonomous), Ponneri

ARTICLE INFO

Article History:

Received 09th December, 2016
Received in revised form
21st January, 2017
Accepted 20th Febuary, 2017
Published online 30th March, 2017

Keywords:

Pleurotus platypus,
Lignin, Laccase,
Coir Pith, Sugarcane Leaves.

ABSTRACT

White rot fungus, *Pleurotus platypus* are a well known lignin degrader, was examined for its optimum growth conditions such as pH and temperature as well as carbon sources (glucose, sucrose, maltose and mannitol). For the maximum growth of *Pleurotus platypus* the 7 day incubation period was required at pH 5.0 *Pleurotus platypus* has an optimum temperature of 20-30°C with the maximum growth of 40.5 mm on the day 9 and the growth rate was 6 mm per day. The optimum pH for the growth of *Pleurotus platypus* was pH 6-7. The experiments on different carbon sources by *Pleurotus platypus* indicated that increasing concentration of carbon sources increased the fungal growth and induced the enzyme production. At 25mm concentration. of sources in basal medium induced the maximum production (35.1 U/L) of lignin peroxidase. Evidently the carbon source remarkably induced the lignin peroxidase and decreased the laccase, *Pleurotus platypus* was reported to be a better degrader of raw coconut coir pith with higher bioefficiency

INTRODUCTION

Lignin is a complex polymer and its degradation takes a number of years. It constitutes the second largest sink next to cellulose for fixed carbon therefore lignin biodegradation occupies a significant portion in the global carbon cycle (Eriksson *et al.,* 1990). The mechanism of lignin biodegradation is an oxidative process carried out mainly by white rot fungi, but our actual knowledge of the ligninolytic system still reveals large gaps. To date, three types of extracellular ligninolytic enzymes have involved in this process such as lignin peroxidase, manganese peroxidase and laccase (Kirk and Farrell, 1987; Higuchi, 1990). White rot fungi may degrade lignin and polysaccharide simultaneously but selective degradation of lignin can occur (Eriksson *et al.,* 1990). The degree of degradation of these components varies from species to species. The white rot fungi Phanerochaete chrysosporium and *Pleurotus platypus* are a well known lignin degrader (Tien and Kirk, 1983; Gold *et al.,* 1984; Kirk and Fareell, 1987; Eriksson *et al.,* 1990; Agosin *et al.,* 1985) and can produce various enzymes, such as extracellular peroxidase (Kang *et al.,* 1993), veretryl alcohol oxidase (Sannia *et al.,* 1991), glucose oxidase (Shin *et al.,* 1993) and laccase (Sannia *et al.,* 1986; Kim *et al.,* 1987) all of which are related to lignin degradation.

Corresponding author: Gayathiri, E.,
Department of Plant Biology and Biotechnology, Gill Research Institute, Guru Nanak College, Chennai.

Apart from the classical biotechnological sciences which use microorganism capable of producing molecules of interest for industry, degradative or environmental biotechnologies have recently emerged. Bioconversion of the vast quantities of lignocellulosic materials of agriculture, house-hold and industrial origin into useful products has received considerable attention but has led to any commercially viable process (Coughlan, 1985; Doelle, 1984). Cultivation of mushroom around the world represents the only commercial successful large scale bioconversion of lignocellulosic residues into food. Economic and biotechnological significance of mushroom production as a successful large scale microbial technology has been appreciated (Wood, 1989). Mushroom is one of the most efficient producer of food protein from worthless agrowaste and their efficiency lies in part, in their extensive degradation ability of lignocellulose. Among the various mushrooms *Pleurotus sp.* Are the most versatile, capable of colonizing and degrading a variety of lignocellulosic waste and have been considered suitable for bioconversion of agrowaste into food and feed on the developing countries (Chang and Miles, 1989., Lelley, 1987., Rajarathnam & Bano, 1989). *Pleurotus sajor caju* (Fr) Sing, is very high yielding and popular oyster mushroom. Crop residues after mushroom cultivation could be used as a better source of organic manure (Marimuthu & Krishnamoorthy, 1991). *Pleurotus platypus* was one of the most efficient species in decomposing the substrates (Nallathambi & Marimuthu 1993). Thus *Pleurotus platypus*

offers greater potential for mushroom production and recycling of substrates into useful animal feed and manure *Pleurotus platypus* degrades raw coconut and coir pith into valuable organic manure than other *pleurotus spp*. (TheradiMani & Marimuthu, 1992).*Pleurotus platypus* a potent oyster mushroom considered for organic recycling of agricultural wastes (Nallathambi & Marimuthu 1993). No work has been initiated on lignolytic enzymes such as lignin peroxidase, manganese dependent peroxidase and laccase of *Pleurotus platypus*. Since these enzymes are no intensive research, thus it was aimed to optimize the growth conditions of *Pleurotus platypus* in liquid cultures. An attempt was made to study the extracellular enzymes such as lignin peroxides and laccase on different carbon sources. The enzymatic degradation of coir pith and sugarcane leaves by *Pleurotus platypus* was also studied.

MATERIALS AND METHODS

Organism and Inoculum: The organism *Pleurotus platypus* (Cook and Massee) sacc. is a white rot fungus, belong to the class basidiomycetes. The organism was collected from the culture collection from G.S. Gill Research Institute, Guru Nanak College, Chennai and maintained on potato dextrose agar slants. Mycelial disc of 4 mm diameter from seven day old culture of the fungus maintained in CDB medium were used as inoculum. The *Pleurotus platypus* was maintained prior to enzymatic study on Czapek's Dox agar medium and the enzymatic study was carried out on Czapek's dox broth medium. Assay for lignin peroxidase was done by the modification of Kang *et al.,*1993; Perumal & Kalaichelvan, 1995 method in a Beckman spectronic DU-40 Spectrophotometer at an absorbance of 436nm. Pyrogallol was used as the substrate.

Assay Mixture

	Volume	Concentration
Buffer	2.00 ml	0.1M
Ph	7	
Pyrogallol	0.2 ml	0.1 M
Sample	1.0 ml	0.1 M
H_2O_2	0.1 ml	0.1 M

The assay mixture was added in the order given above and the readings were recorded for 3 minutes each at 30 sec. intervals. The readings were recorded against a blank which was glass distilled water instead of H_2O_2. The enzyme activity was calculated and expressed in units / liter. Laccase activity (or) production was measured polarographically using Yellos spring Model-5300 biological oxygen monitor by the method of (Wood & Goodenough, 1977). The protein estimation was determined according to the method of (Bradford, 1976) using Bovine Serum Albumin (BSA) as a standard protein.

$$\text{Unit/liter} = \frac{V \times \Delta E \times 1000}{\epsilon \times 1 \times t}$$

Where
V = Volume of assay mixture
ΔE = Extinction charge
€ = Extinction co-efficient
l = Light path
t = Time

The amount of protein present in the sample was estimated from a standard graph prepared with Bovine Serum Albumin (Fr IV Sigma).

Comparision of growth at different temperature

To study at which temperature the *Pleurotus platypus* has maximum growth, the *Pleurotus platypus* 4 mm disc were inoculated aseptically in the Petri-plates containing 20 ml of Czapek's Dox agar medium and plates were kept at different temperature such as 0, 5, 10, 20, 30 and 40°C. The growth was measured every day. The radial growth of the fungal mycelium was measured.

Comparision of growth on different pH

The Czapek's Dox broth medium was adjusted to a different pH range such as 4, 5, 6, 7 and 8 pH using in 0.1N NaOH and in 0.1N HCL. The 100 ml Erlenmeyer flask containing 30 ml of modified Czapek's Dox broth medium (pH 6.5) were aseptically inoculated with 4mm disc of *Pleurotus platypus* and incubated at 30°C at static condition. The mycelial growth was determined in terms of dry weight. The extracellular protein content was determined by the method of (Bradford, 1976). The H^+ ion concentration was measure in expandable ion analyzer Ea 940 Orion Research (U.S.A). Screening for lignolytic enzyme production of *Pleurotus platypus* was determined by the method of (Adaskaveg& Gilbertson, 1989).

Laccase

Malt extract medium (2%) was amended with 0.05, 0.10, 0.15 and 0.20 % each tannic acid (Sigma U.S.A) and gallic acid (Sigma U.S.A) were used to test for polyphenol oxidase (i.e) Laccase. The petriplates containing 20 ml of each concentration of MAE medium was aseptically inoculated with 4mm disc of *Pleurotus platypus*. The inoculated plates were incubated at 30°C and periodically observed. The oxidase reaction on gallic acid and tannic acid was tested.

Peroxidase

Malt extract medium (2%) was amended with a 1:1 mixture of 1% pyrogallol and 5% hydrogen peroxidase amended in different concentration such as 0.05, 0.10, 0.15 and 0.20% in MEA medium used to test peroxidase. The petriplates containing 20 ml of each respective medium were aseptically inoculated with 4mm disc of *Pleurotus platypus* and incubated at 30°C. The growth and the brown zone due to oxidase reaction were observed every day and the result were recorded.

Growth and lignolytic enzyme production on different carbon

Different carbon sources such as glucose, sucrose, maltose and mannitol were amended in CDB medium at different concentration. A 4 mm disc of *Pleurotus platypus* was inoculated to 100 ml Erlenmeyer flask containing 30 ml of respective media pH 6.5. The inoculated flasks were harvested at every 3 days intervals. The respective cultures were filtered through Whatman No.1 filter paper. The culture filtrate was used as a carbon source of extracellular enzyme.

The partial separation Fungal enzymes extraction was made from culture filtrate using 83% w/v of Ammonium sulphate saturation (Fay.L.Myers& Northcote, 1958). Dialysis and Lyophilisation was carried using standard procedure (Lee & Blackburn, 1974). The enzyme powder that collected from the flask was transferred in to a sterile vial and stored at 4^{O}C. The further protein profile and the presence of enzyme were confirmed by SDS PAGE analysis.

The enzymatic degradation of coir pith and sugarcane leaves

The coconut coir pith and sugarcane leaves were collected from a farm near Villupuram, Tamilnadu. The coir pith and sugar cane leaves was chopped into small bits (1-2 cm) and dried in an oven at 80^{o}C for an overnight. The sun dried sugar cane leaves were ground into fine powder. 2 grams of coir pith and sugar cane leaves separately amended in 250 ml of Erlenmeyer flask containing 50 ml of distilled water and autoclaved. The discs of *Pleurotus platypus* (9 mm dia) were aseptically inoculated and incubated for 9 days at stationary condition. The duplicate cultures were harvested and filtered through Whatman No.1 filter paper. The filtrate served as the enzyme source for lignin peroxidase and laccase.

RESULTS

Growth at different temperature

Pleurotus platypus was considerer moderate temperature fungus. *Pleurotus platypus* has an optimum temperature of 20-30°C with the maximum growth of 40.5 mm on the day 9 and the growth rate was 6 mm per day. At 20°C the maximum growth was 39 mm on the 9th day (4 mm/day). There was no growth at 0, 5, 10 and 40°C. The optimum temperature for the growth of *Pleurotus platypus was* 30°C (Fig.1).

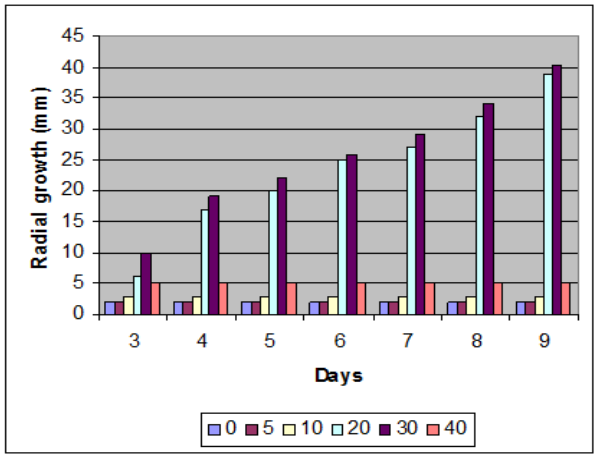
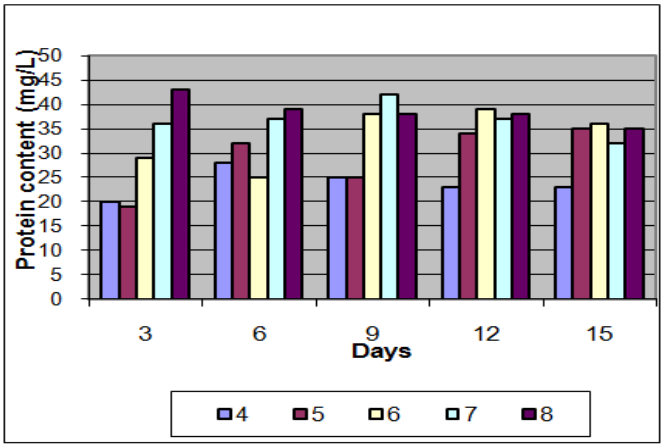


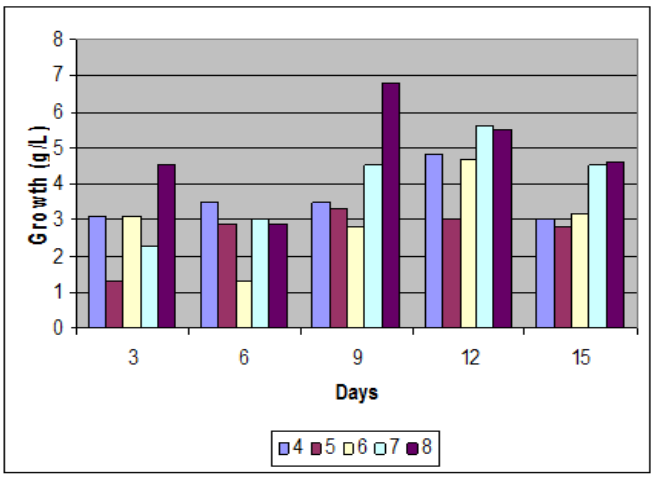
Fig.1. Mycelial growth of *Pleurotus platypus* at different temperature (°C)

Growth at different pH

Of the different pH media tested the highest amount of the protein content was 43, 42, 39 and 28 mg/L in the pH 7, 6, 8, 5 and 4 observed on the day 9, 9, 12, 12 and 9 respectively (Fig.2.B). The highest amount of mycelial growth was 10.8, 4.9, 4.7 and 3.2 g/l in the pH 8, 7, 4, 6 and 5 observed on the day 12, 12, 12, 9 and 12 respectively (Fig.2.A). The optimum pH for the growth of *Pleurotus platypus* was pH 6-7.



A



B

Fig.2. Mycelial growth (A) and Protein content (B) of *Pleurotus platypus* at different pH (30° C)

Screening for lignolytic enzyme production

Laccase

The mycelial growth and oxidase reaction on the different concentration of gallic acid and tannic acid varied considerably between different concentrations. Mycelial growth and oxidase reaction were negatively correlated at 0.15 and 0.20% concentration of gallic acid and tannic acid the oxidase reaction was very strong, but did not grow well. At higher concentration above 0.20% of gallic acid and tannic acid the mycelial growth was greatly inhibited. The oxidase reaction rate was very strong (+++) in both 0.15 and 0.20%, moderately strong (++) in 0.10% and week (+) in 0.05%. Overall the mycelial growth was more in 0.5% (17 mm) in tannic acid and 0.10% (11 mm) in gallic acid. At 0.15 and 0.20% of tannic acid produced very strong oxidase reaction which confirms the laccase production of *Pleurotus platypus* (Table.1 Plate 1 & 2).

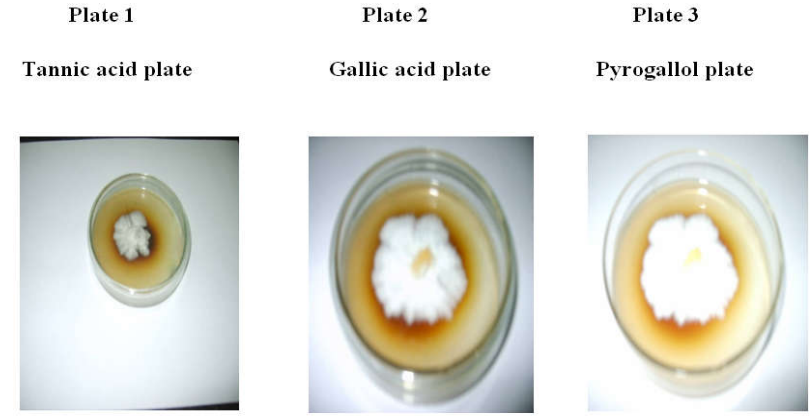
Peroxidase

The mycelial growth and oxidase reaction for lignin peroxidase were observed only on 0.05 and 0.10%. At 0.15 and 0.20% the growth was inhibited.

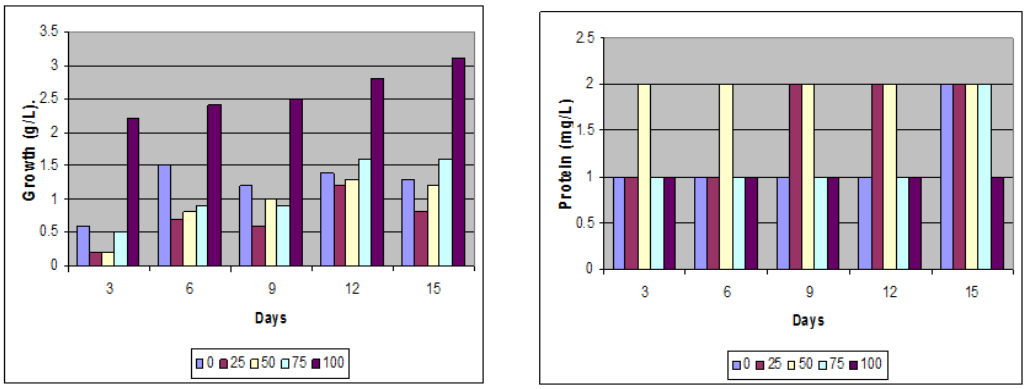
Table 1. Screening for lignin peroxidase and laccase on 2% mea medium

	GALLIC ACID		TANNIC ACID		PYROGALLOL AND HYDROGEN PEROXIDE	
	Mycelial growth (mm)	Oxidase reaction	Mycelial growth (mm)	Oxidase reaction	Mycelial growth (mm)	Oxidase reaction
Control	20	-	20	-	20	-
0.05	6	+	17	+	5	++
0.10	11	++	9	++	2	+++
0.15	7	+++	9	+++	N.G	-
0.20	6	-	6	+++	N.G	-
0.25	N.G	-	N.G	-	N.G	-
0.50	N.G	-	N.G	-	N.G	-
0.75	N.G	-	N.G	-	N.G	-
1.0	N.G	-	N.G	-	N.G	-

- : Negative reaction +: Weak reaction ++: Strong reaction +++ :Very strong reaction N.G: No growth

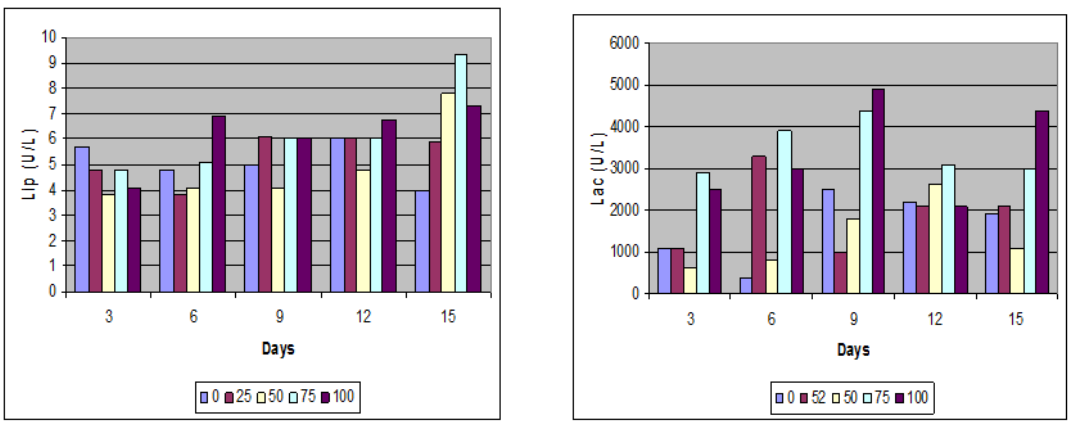


CARBON SOURCE
GLUCOSE (0, 25, 50, 75and 100 mM Conc.)



A B

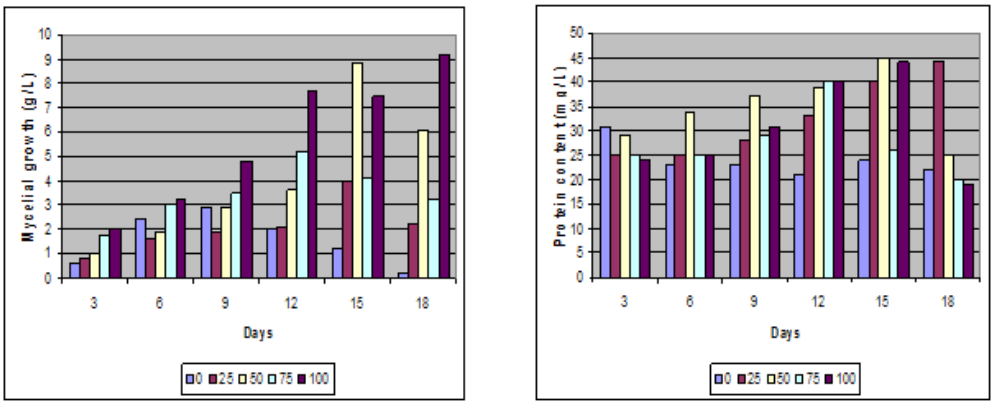
Fig.3. Mycelial growth (A) and Protein content (B) of *Pleurotus platypus* on different concentration (mM) of glucose in CDB media.



A B

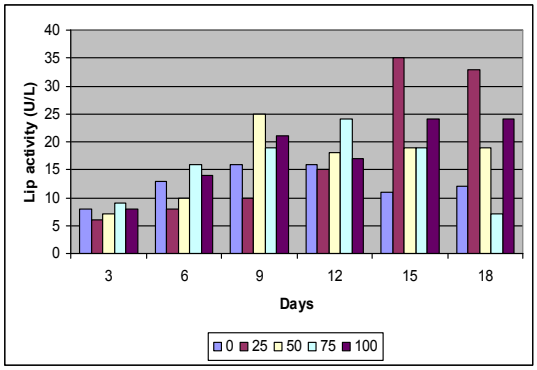
SUCROSE (0, 25, 50 and 100 mM conc.)

Fig.4. Lignin peroxidase (A) and Laccase activity (B) of *Pleurotus platypus* on different concentration (mM) of glucose in CBD media



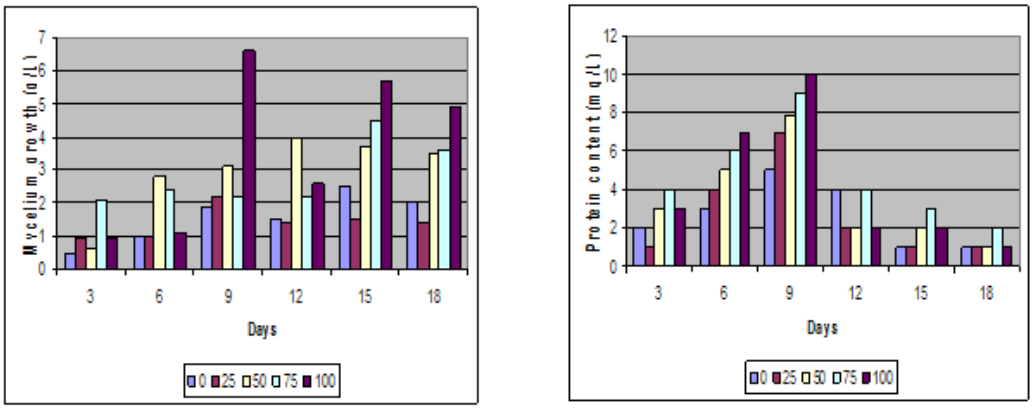
A B

Fig.5. Mycelial growth (A) and Protein content (B) of *Pleurotus platypus* on different concentration (mM) of sucrose in CDB media.



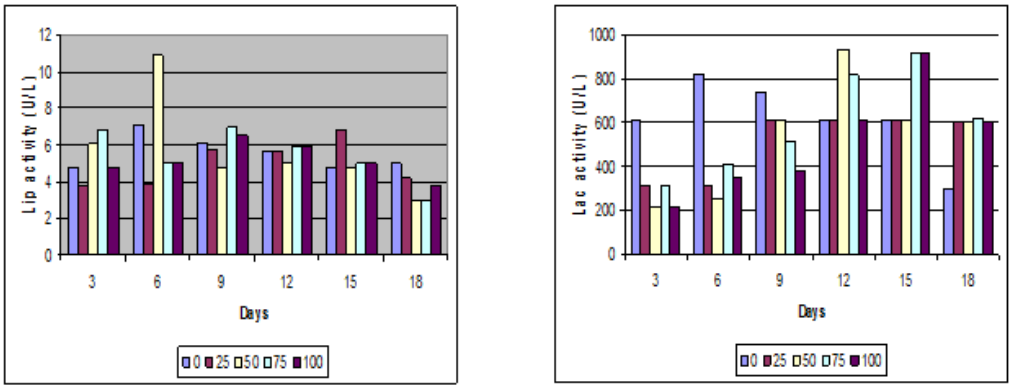
MALTOSE (0, 25, 50 and 100 mM conc.)

Fig.6. Lignin peroxidase activity of *Pleurotus platypus* on different concentration (mM) of sucrose in CDB media



A B

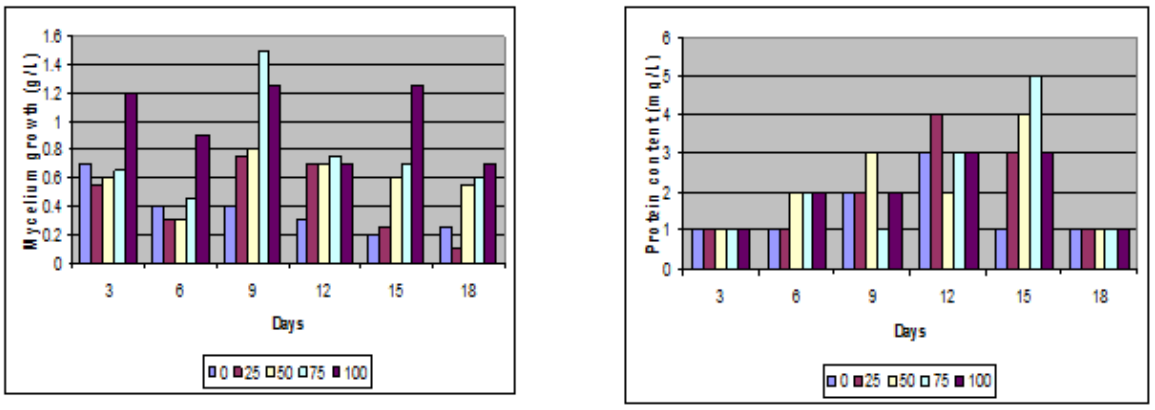
Fig.7. Mycelial growth (A) and Protein content (B) of *Pleurotus platypus* on different concentration (mM) of maltose in CDB media



A B

Mannitol (0, 25, 50 and 100 mM conc.)

Fig.8. Lignin peroxidase (A) and Laccase activity (B) of *Pleurotus platypus* on different concentration (mM) of maltose in CDB media



A B

Fig.9. Mycelial growth (A) and Protein content (B) of *Pleurotus platypus* on different concentration (mM) of mannitol in CDB media

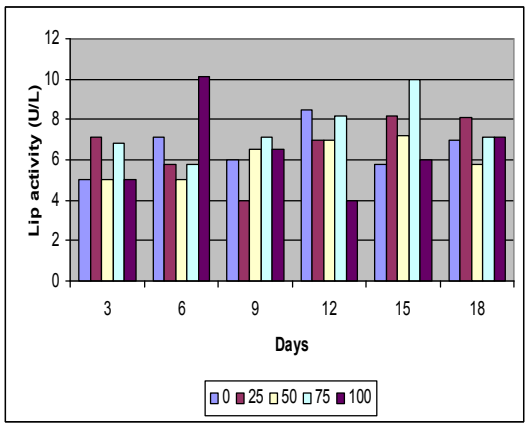


Fig.10. Lignin peroxidase activity of *Pleurotus platypus* on different (mM) of mannitol in CDB media

The oxidase reaction was very strong (+++) on 0.05% and moderately strong (++) in 0.10%. The mycelial growth was more in 0.05% than in 10% of pyrogallol, thus conforms the production of the lignin peroxidase by *Pleurotus platypus* (Table.1, Plate 3)

Carbon Source

GLUCOSE (0, 25, 50, 75and 100 mM Conc.)

The basal medium amended with 0, 25, 50, 75 and 100 mM Conc. of glucose produced the highest amount of the mycelial growth was 1.5, 1.1, 1.3, 1.7 and 3 g/L recorded on the day 12, 12, 12 and 15 respectively (Fig. 3.A). The highest amount of extracellular protein content was 2 mg/L recorded on the 15th day at 25, 50 and 75 mM conc. (Fig.3.B). The maximum production of lignin peroxidase was 6.1, 6.1, 7.7, 9.2 U/L recorded on the day 12, 15, 15, 15 and 15 (Fig.4.A). The maximum production of laccase was 2500, 2100, 1687, 4372 and 4750 U/L on the day 18, 12, 12, 9 and respectively (Fig.4.B).

SUCROSE (0, 25, 50 and 100 mM conc.)

The basal medium amended with 0, 25, 50 and 100 mM conc. of the sucrose produced the highest amount of mycelial growth was 2.7, 4, 8.7, 5.4 and 9.4 g/L recorded on the day 9,15,15,12 and 18 respectively (Fig.5.A). The highest amount of extracellular protein content was 23, 44, 45, 49 and 43 mg/L determined on the day 15, 18, 18, 18 and 15 respectively (Fig.6).

MALTOSE (0, 25, 50 and 100 mM conc.)

The basal medium amended with 0, 25, 50 and 100 mM conc. of the Maltose produced the highest amount of mycelial growth was 2.4, 1.5, 4.1, 4.6 and 6.5 g/L recorded on the day 15, 15, 12, 15 and 9 respectively (Fig. 7.A). The highest amount of protein content was 5, 7, 8, 9 and 10 mg/L on the 9th day for all media tested (Fig.7.B). The maximum production of lignin peroxidase was 6.1, 6.7, 10.8, 7.2 and 6.6 U/L recorded on the day 9, 15, 6, 9 and 9 respectively (Fig.8.A). The maximum production of laccase was 833, 625,937, 937 and 937 U/L recorded on the day 6, 9, 12, 15 and 15 respectively (Fig.8.B).

MANNITOL (0, 25, 50 and 100 mM conc.)

The basal medium amended with 0, 25, 50 and 100 mM conc. Of the mannitol produced the highest amount of mycelial growth was0.416, 0.733,0.833, 1.5 and 1.8 g/L recorded on the day 9, 9, 9, 9 and 6 respectively (Fig.9.A). The highest amount of extracellular protein content was 3, 4, 3, 5 and 6 mg/L

recorded on the day 12, 12, 9, 12 and 15 respectively (Fig.9.B). The maximum production of lignin peroxidase was 8.7, 8.2, 7.7 and 10.3 U/L recorded on the day 15, 15, 15, 15 and 18 respectively (Fig.10). Very low amount of laccase activity was determined in all the concentration of mannitol.

The enzymatic degradation of coir pith and sugarcane leaves

Pleurotus platypus grown on natural lignocellulosic such as coir pith sugarcane leaves produced extracellular lignonolytic enzymes in the culture media tested. The coir pith amended media produced 60.93 U/L of lignin peroxidase and 892 U/L of laccase. The sugarcane leaves amended media produced 6.2 U/L of lignin peroxidase and 155 U/L of laccase. The lignin model compound produced 12.3 U/L of lignin peroxidase and 288U/L of laccase. (Table 2 , Plate 4,5).

Table 2. Production Of Lignin Peroxidase And Laccase By *Pleurotus Platypus* On Natural Lignocellulosic Substrate

Substrates	Lignin peroxidase (U/L)	Laccase (U/L)	Protein (mg/L)
Coir pith	60.93	892	7
Sugarcane leaves	6.2	155	7
Indulin+Minimal Media	12.3	288	115
Minimal media	18.0	187	50

Plate 4. Degradation of coir pith

Plate 5. Degradation of sugarcane leaves

DISCUSSION

Pleurotus Sp. are white rot fungi having a wider growth temperature ranges from 15 C to 40°C of which P.ostreatus grew at 28°C (Youn*et al.,* 1995) and *P.sajor-caju*(Saxena& Rai 1992). *P.erynii* (Guillen & Evans 1994) grew well in the optimum temperature at 25 & 28°C. The test fungus *Pleurotus platypus* had a good growth at optimum temperature ranges from 20 to 30°C and had a growth rate of 6 mm/day. The *Pleurotus platypus* can be considered as moderate temperature loving fungus. The mycelial growth and the extracellular enzyme production of many white rot fungi were pH dependent (Jefferies *et al.,* 1981; Kall*et al.,* 1993). In many *Pleurotus Sp*. the enzyme production appears as constitutive nature and produced at different phases under different culture conditions (Guillen *et al.,* 1990; Valmaseda*et al.,* 1991).

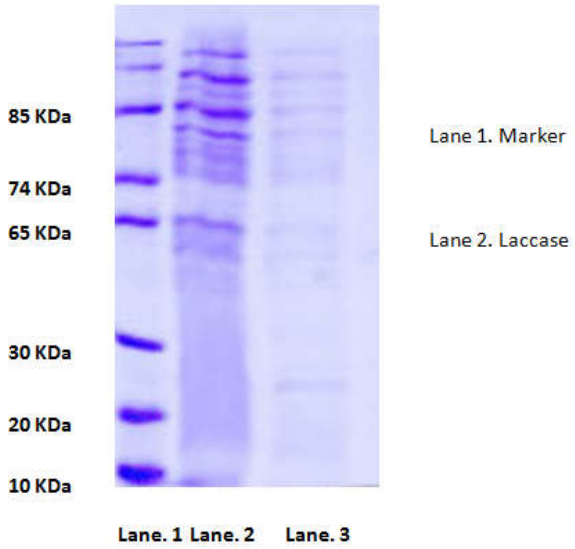


Plate 6. Protein profile in SDS – PAGE

The results on pH indicated that the alkaline pH favoured the mycelial growth. The extra cellular protein content was maximum at pH 7 and the fungal growth was maximum at pH 8. Thus the alkaline pH influenced the enzymatic degradation of lignolytic substrates by *Pleurotus platypus.* However these fungi capable of growing under alkaline conditions and extracellular industrially useful enzymes have been little investigated (Bansod *et al.,* 1993). Most of these white rot fungi produces lignolytic enzyme such as lignin peroxidase, manganese dependent eroxidase and laccase. The lignin peroxidase can directly oxidize nonphenolic lignin models, whereas manganese dependent peroxidase and laccase are suppose to degrade mainly phenolic units (Camarero *et al.,* 1994). Some of the white rot fungi produced lignin peroxidase but not laccase. In Phanerocheate chrysorium no detectable amount of laccase was recorded (Krik & Farrell 1987).

Among the white rot fungi the *Pleurotus Spp*. were not produced lignin peroxidase but produced laccase (Gutierrez *et al.,* 1991; Martinz *et al.,* 1994). In three species of the genus Pleurotus in which neither lignin peroxidase nor manganese dependent peroxidase has been found (Guillen *et al.,* 1990; Sannia *et al.,* 1991; Guillen *et al.,* 1992), but Kang *et al.,* 1993 reported that *Pleurotus ostreatus* produced peroxidases and characterized this enzyme from culture filtrate of *Pleurotus ostreatus.* The experimental results suggested that *Pleurotus platypus* produced extracellular lignin peroxidase under experimental conditions. Thus it may be concluded that *Pleurotus platypus* produced both lignin peroxidase and laccase which may be involved in degradation of phenolic and nonphenolic lignin models. According to present day paradigm most of the white rot fungi produce ligninolytic enzymes in

response to carbon, nitrogen and sulphur limitations (Kaal *et al.,* 1993). The most studied ligninolytic system was in Phanerochaete chrysosporium. The ligninolytic activity is an expression of secondary metabolism triggered not only by nitrogen limitation but also by carbon and sulphur limitation (Jefferies et al, 1981). However Kimura *et al.,* 1990 found that some wild type white rot produced lignin peroxidase only in organinc rich medium. Ligninolyticv activity of *Pleurotus platypus* is controlled by nitrogen and carbon limitation (Keyser *et al.,* 1978) but also by carbohydrate and sulphur limitation (Jefferies *et al.,* 1981). The experiments on different carbon sources by *Pleurotus platypus* indicated that increasing concentration of carbon sources increased the fungal growth and induced the enzyme production.

At 25mM concen. of sources in basal medium induced the maximum production (35.1 U/L) of lignin peroxidase. In *Phanerochaete chrysosporium* ligninolytic activity begins when the physiological equilibrium is reached. Decreasing mycelium growth induced the onset of ligninolytic enzyme (Jefferies *et al.,* 1981) but the results remarkably indicated the increasing the mycelial growth increased the production of lignin peroxidase. Thus the result evidently indicated that the ligninolytic enzymes production is expressed during the primary and secondary mycelial growth of *Pleurotus platypus*. The relationship between ligninolytic enzymes production and growth substrate is unclear (Fenn & Krik 1980).Evidently the carbon source remarkably induced the lignin peroxidase and decreased the laccase, *Pleurotus platypus* was reported to be a better degrader of raw coconut coir pith with higher bioefficiency (Nallathambi & Marimuthu, 1993).

The experimental result of *Pleurotus platypus* on coir pith and sugarcane leaves remarkably produced both laccase and lignin peroxidase. The production of lignin peroxidase and laccase is involved in the degradation of phenolic and non phenolic subunits (Camararo *et al.,* 1994). *Pleurotus platypus* was reported to degrade lignin 78.07% in coir pith (Theradimani & Marimuthuy 1992). The production of lignin peroxidase and laccase of *Pleurotus platypus* on the coir pith and sugarcane leaves might be in response to the lignin degradation. Four laccase isozymes synthesized by *Pleurotus ostreatus* have molecular masses of about 60 and 65kDa (Mariana Mansur, 2003), were lignin peroxidase having the molecular weight of about 35- 40 Kda. Where laccase and lignin peroxidase of our isolates showed bands nearing 60KDA and 25-30 Kda.

Conclusion

The resistance of the lignocellulose constituents in coir pith to biodegradation results in the accumulation of the same as huge hillock in the coir fibre production units. This causes problem of disposal and management of the coir pith. The aim of the present work is to study the efficacy of the *Pleurotus platypus* to degrade coir pith and sugarcane leaves. The Physico-chemical parameters such as pH, Temperature, Lignin, Organic carbon, decomposition were studied. Lignin degrading mushroom *Pleurotus platypus* has been proved to degrade coir pith and convert it into organic manure. The study could emphasize the fact that lignin degrading mushroom species could be used for finding a solution to the problem of accumulation of the biological waste 'coir pith' and 'sugarcane leaves' also convert it into a value added eco-friendly fertilizer for different type of plants.

REFERENCES

Adaskaveg, J.E. and Gilberston, R.L. 1989. Cultural studies of four north American species in the Ganoder maluicidum complex with comparision to G.lucidum and G.luicidum and G.tsugae. *Mycol.Res.* 92 : 182 – 191

Agosin, E., daudin, J.J and Odier, E. 1985. Screening of White-rot fungi on [14c] whole labeled wheat straw. *Appl.Microbiol.Biotechnol*.22 : 132-138.

Bansod, S.M., Dutta-Choudhary, M., Srinivasan, M.C. and Rele, M.V. 1993. Xylanase actice at high pH from an alkalotolerant Cephalosporium Sps. *Biotechnology Letters* 15(9): 965 – 970.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein – dye binding. *Anal. Biochem*. 72 : 248 – 254.

Camarero, S., Galletti, G.c. and Martinez, A.T 1994. Preferential degradation of phenolic lignin units by two white rot fungi. *Appl. Environ. Microbiol*. 60 : 4509 – 4516.

Chang, S.T. and Miles, P.G. 1989. Edible mushroom and their cultivation. CR Press, Boca-Raton.

Coughlan, M.P. 1985. Biotechnology and Genetic Engineering Reviews, Vol.3 (GE Russel ed) p.39 – 109. Interseot, Ponteland.

Doelle, H.W. 1984. *Biotechnol Adv* 2 : 1 – 5.

Eriksson, K.E.L., Blanchette, R.A., Ander, P. 1990. In: Microbial and enzymatic degradation of wood and wood components, Springer-Verlag, Berlin Heidelberg, 407.

Fenn, P., Choi, S and Kirk, T.K. 1980. Lignolytic activity of Phanerochaete chrysosporium Physiology of suppression by NH4 and L-glutamate. *Arch. Microbiol*. 130 : 66 – 71.

Gold, M.H., Kuwahara, M., Chiu, A.A. and Glenn, J.K. (1984). Purification and characterization of an extracellular H2O2 – requiring diarylpropane oxygenase from the White rot basidiomycetes, Phanerochaete chrysosoporium. *Arch. Biochem. Biophys*.234 : 353 – 362.

Guillen, F., and Evans, 1992. Anisaldehyde and veratradehyde acting as redox cycling agents for H2O2 production by Pleurotuseryngii. *Appl.Environ.Microbiol*. 60(8) : 2811 – 2917.

Guillen, F., Martinez, A.T. and Martinez, M.J. 1990. Production of hydrogen peroxide by aryl-alcohol oxidase from the lignolytic fungus Pleurotuseryngii. *Appl. Microbiol. Biotechnol*. 32 : 465 – 469.

Gutierrez, A., Caramelo, L., Prieto, A., Martineze, M.J. and Martineze, A.T. 1994. Anisaldehyde production and aryl alcohol; oxidase and dehydroginase activities in lignolytic fungi of the genus Pleurotus. *Appl. Environ. Microbol*. 60 : 1783 – 1788.

Higuchi, T. 1990. Lignin biosynthesis and biodegradation. *Wood Sci. Technol*. 24 : 23 – 63.

Jefferies, T.W., Chio, S. and Kirk, T.K. 1981. Nutritional regulation of lignin degradation by Phanerochaete chrysosporium. *Appl. Environ. Microbiol.* 42: 290 – 296.

Kaal, E.E.E.J., de Jong, E. and Field, J.A. 1993. Stimulation of lignolytic peroxidase activity by nitrogen nutrients in the white rot fungus Bjerkandra Sp. Strain BOS 55. *Appl. Environ. Microbiol.* 59 : 4031 – 4036.

Kang, S.O., Shin, K.S., Han, Y. H., Youn, H.O. and Hah, Y.C. 1993. Purification and characterization of an extracellular peroxidase from white-rot fungus Pleurotus ostratus. *Biochem. Biophys. Acta*. 1163 : 158 – 164.

Kim, K.J. Maeng, J.S. Shin, K.S., Kang, S.O. Hal, Y.C. and Hong, S.W. 1987. Characterization of laccaxe of laccase from Pleurotus ostreatus *Korean J. Microbial.* 25 : 148 – 156.

Kirk, T.K. and Ferrell, R.L. 1987. Enzymatic combustion : the microbial degradation of lignin *Annu. Rev. Microbiol*. 41 : 465 – 505.

Lelley, J. 1987. *Mushroom J.Tropics* 7: 135 – 140.

Marimuthu, T., Krishnamoorthy, A.S. 1991. In Glimpses of mushroom research. In: TNAU (T. Marimuthu, A.S.Krishnamoorthy and R.Jeyarajan eds.) pp 30 – 31.

Martinez, M.J. and Martineze, A.T. 1994. Study of manganese peroxidase of Pleurotuseryngii produced in peptone in peptone medium, Abstr. MS. 14/39, p.416. Abstr. 7th Symp. *Int. Union Microbiol. Soc.*

Nallathambi, P. and Marimuthu, T. 1993. *Pleurotus platypus* : A potent oyster mushroom for organic recycling of agriculrural wastes. Mushroom. Res 2: 75 – 78.

Perumal, K and Kalaichelvan P.T. 1995. Production of extracellular peroxidase and laccase by lignolytic white rot fungus Ganoder malucidium PTK3 and its degradation on extracted lignin from sugarcane bagasse I.*J. Exp. Biol.* Pp 443-447

Rajarathnam, S. and Bano, Z. 1989. CRc *Crit. Rev. Fd. Sci. Nutri.* 28 : 31 – 93.

Sannia, G., Limogi, P., Cocca, E., Buonocore, F., nitti, G. and Giardina, P. 1991. Purification and characterization of a veratryl alcohol oxidase enzyme from the lignin degrading basidiomycete Pleurotus ostreatus. *Biochem. Biophys. Acta.* 1073 : 114 – 119.

Saxena, S, and Rai, R.D. 1992. Effect of nitrogen on production of extracellular degradative enzymes by Pleurotus sajro – caju (fr). Sing. on wheat staw. Mushroom Res. 1 (1): 45 – 48.

TheradiMani, M and Marimuthu, T. 1992. Utilization of Pleurotus spp. for decomposting coconut coirpith. Mushroom Res. (1): 49 – 51.

Tien, M. and Kirk, T.K. 1983. Lignin degrading enzymes from the hymenomycete Phanerochaete chrysosporium Burds. Science 221: 661 – 663.

Valmaseda, M., Martinez, M.J. and Martinez, A.T. 1991. Kinetics of wheat straw solid-state fermentation with Trametes versicolor and Pleurotus ostreatus – lignin and polysaccharide alternation and production of related enzymatic activities. *Appl. Microbiol. Biotechnol*. 35 : 817 – 823.

Wood, D.A. and 1989. Internl. Indus. Biotechnol. 9 : 5 – 8.

Wood, D.A. and Goodenough, P.W. 1977. Fruiting of Agaricus bisporus. Changes in extracellular enzyme activities during growth and fruiting. *Arch. Microbiol*. 114 : 161 – 165.

Youn, H. O., Kim, K.J., Maeeng. J.-S., Han, Y.H., Jeong I.B. GajinJeong, Jang, S.O. and Yung Chil Hah. 1995. Single electron transfer by an extracellular laccase from the white – rot fungus Pleurotus ostreatus. *Microbiology* 141 : 393 – 398.

****