ENZYME PRODUCTION AND COMPOSTING POTENTIAL OF OYSTER MUSHROOM (*Pleurotus* spp.)

By

P. OWSEPH ANSU

THESIS
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DECLARATION

I hereby declare that this thesis entitled "**Enzyme production and composting potential of oyster mushroom (*Pleurotus* spp.)**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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P. OWSEPH ANSU

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Certified that this thesis entitled "**Enzyme production and composting potential of oyster mushroom (*Pleurotus* spp.)**" is a record of research work done independently by Miss. P. Owseph Ansu under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellayani,
13.8 -1999

Dr. D. Geetha
Chairman, Advisory Committee
Associate Professor (Plant Pathology)
Instructional Farm, Vellayani

APPROVED BY:

CHAIRMAN

DR. D. Geetha
Associate Professor (Plant Pathology),
Instructional Farm,
College of Agriculture, Vellayani

MEMBERS

1. Dr. S. Balakrishnan
Professor and Head,
Department of Plant Pathology,
College of Agriculture, Vellayani

2. Dr. M. Suharban
Associate Professor (Plant Pathology),
Instructional Farm,
College of Agriculture, Vellayani

3. Dr. P. Rajendran
Associate Professor,
Department of Soil Science and
Agricultural Chemistry,
College of Agriculture, Vellayani

EXTERNAL EXAMINER

13/8/89

(G. MARIMUTHU)

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INTRODUCTION

INTRODUCTION

Kerala is the leading state in the production of coconuts, the production of which during the year 1995-96 was 5905.7 million nuts (Thampan, 1997). Coir pith, a highly lignocellulosic material, is available in large quantities as a byproduct of coir industry. One tonne of coir pith accumulates for every 10,000 husks used in the coir industry (Nagarajan *et al.*, 1985).

Coir pith is usually considered as a waste and is dumped in mounds in increasing proportion every year. The tannins that ooze from the dump yards during monsoon are considered to create environmental problems. In Kerala there are about 84, 000 retting and coir extracting units (Dhamodharan and Arumughan, 1993).

Due to its high water holding capacity in the range of 400 to 600 per cent, rich potash content and the ability to absorb heavy metals, it can be used to improve the physiochemical properties of the soil (Savithri and Khan, 1994). Meerow (1994) has suggested the use of coir pith as a substitute for sphagnum or sedge peat in soil less container grown plants.

However, its high lignin concentration coupled with wide C / N ratio does not permit its direct application to soil. Biodegradation of coir pith for converting it into a nutrient rich material for plant growth not only reduces environmental pollution but it is also an ecofriendly approach.

A number of fungi like *Pleurotus sajor-caju*, *Trichoderma* sp. and *Aspergillus* sp. were found to be potent degraders of coir pith (Savithri and Khan, 1994).

The present study was undertaken with the view to evaluate the comparative ability of four *Pleurotus* species viz., Vellayani P_2 isolate of *Pleurotus* sp., *P. sajor-caju*, *P. platypus* and *P. djamor* in degrading coir pith (retted and non retted), in comparison with its effect on paddy straw.

The degradative enzymes produced by the *Pleurotus* spp. and the effect of these enzymes on lignin and cellulose content at different periods of incubation were evaluated.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Oyster mushrooms, *Pleurotus* spp. are edible fungi popularly known as wood fungi. The genus *Pleurotus* was established by Fries in 1821.

There are a number of species under this genus. According to Pegler (1976) and Singer (1986) this genus is known to contain 50 species, of these about 25 species are known to occur in India. These species are suitable for cultivation under varied climatic conditions.

The different species used in the present study were Vellayani P_2 isolate of *Pleurotus* sp., *P. sajor-caju* (Fr.) Singer, *P. djamor* (Fr.) Boedijin and *P. platypus* (Cooke and Masse) Sacc.

Vellayani P_2 isolate is an interstock hybrid developed at the College of Agriculture, Vellayani, Kerala Agricultural University, by crossing *P. petaloides* and a wild mutant of *Pleurotus* sp. (Balakrishnan, 1994). *P. sajor-caju* was reported by Lloyd (Butler and Bisby, 1931). Devi (1982) reported *P. platypus* from Kerala. *P. djamor* was reported by Geetha (1994) from oil palm bunch waste of Kerala.

1.1 Spawn

The mycelium of mushroom growing in its substrate and prepared for the purpose of mushroom production is called spawn (Chang, 1982).

Sinden (1932, 1934) was the first to introduce grain spawn in the cultivation of mushrooms. Different kinds of grains viz., wheat, rye, millets etc. can be used as spawn substrates (Kotwaliwale *et al.*, 1991). They are first half cooked, shade dried and mixed with 5-6 per cent calcium carbonate. The

addition of calcium carbonate prevents the grains from clogging (Stroller, 1962). Sivaprakasam and Kandaswamy (1981a) reported sorghum, bajra and maize grains as ideal spawn bases. Suharban (1987) reported maximum mycelial growth in bengal gram and wheat grains. Kotwaliwale *et al.* (1991) standardized the spawn production techniques on grains and reported a marked preference for cereal substrates over the pulse substrates for *Pleurotus* spawn production.

The slow growing nature of *P. sajor-caju* in spawn bottles has been reported (Kotwaliwale *et al.*, 1991). According to Geetha (1994) *P. djamor*, a fast growing mushroom took 14 days for complete colonisation in spawn bottles compared to *P. sajor-caju* which took 18 days. Anita (1998) studied the growth rate of various *Pleurotus* species in spawn bottles and reported that Vellayani P_2 isolate required the shortest period (11.33 days) for completion of mycelial run in spawn bottles.

1.2 Cultivation of oyster mushroom

Cultivation of *Pleurotus* spp. on their natural habitat like tree stumps and logs was first described at the beginning of twentieth century (Falck, 1917) and on a sawdust-cereal mixture by Kaufert (1935). The foundation for the industrial production of *Pleurotus* on different substrates was laid by several workers (Kalberer and Vogel, 1974 ; Zadrazil, 1974 and Kurtzman, 1979).

Pleurotus spp. have been successfully cultivated on different agricultural wastes such as mixture of coconut fibre and coffee pulp (Bernabe *et al.*, 1993), kidney bean and broad bean stubbles (Sobal *et al.*, 1993), sugarcane bagasse (Shi, 1994) and cotton wastes (Haq *et al.*, 1994).

Pleurotus spp. have also been successfully cultivated on aquatic weeds such as water hyacinth (Murugesan *et al.*, 1995) and *Eliocharis pantogena* (Suharban *et al.*, 1993).

Deka *et al.* (1994) studied the feasibility of cultivation of *P. florida*, *P. sajor-caju* and *P. ostreatus* on paddy straw and bamboo leaves. Paddy straw was the best substrate for all the *Pleurotus* spp. tested when taking into account the spawn run period and yield characteristics.

Sangwan (1995) cultivated *P. sajor-caju* on various agro-industrial wastes and found that addition of sugarcane baggasse to wheat or paddy straw was most effective in increasing the biological efficiency from 98.5 to 117.5 and 118.5, respectively.

Sivaprakasam and Kandaswamy (1981 b) and Geetha (1994) have reported that the yield of sporophores was positively correlated with cellulose content and also with the cellulose lignin ratio.

1.3 Enzymology

Extracellular enzymes are those enzymes produced within the cells and then liberated into the external environment to carry out the function of utilization of the nutrients in the substrate. The role of extracellular enzymes is pivotal to the production of the mushrooms, since, only by their production and activity can the mycelium grow and produce mushroom fruit bodies. The extracellular enzymes are produced to degrade the large insoluble molecules of the substrates into small soluble molecules which the mycelium can utilize (Wood, 1990).

The extracellular enzymes of *Pleurotus* spp. play a major role in the degradation of structural elements such as cellulose, hemicellulose, lignin and pectin present in the natural substrates. The enzymes most frequently associated with cellulose degradation are cellulases and those associated with lignin degradation are laccase (Wood, 1990).

1.3.1 Cellulases

Cellulases play a major role in the degradation and recycling of cellulose, the most abundant carbohydrate produced by plants in the biosphere (Beguin and Aubert, 1985). Cellulose is a simple polymer of β-1,4 linked glucose units but it forms insoluble crystalline microfibrils (Eriksson, 1978).

The degradation of cellulose is brought about by an enzyme complex, cellulases composed of three major enzymes, viz., Endo β-1,4 glucanase (Cx), Exo β-1,4 glucanase (C_1) and β glucosidase. Cx acts randomly on native cellulose chain while C_1 attack the non reducing ends of the polymer producing mainly cellobiose. The cellobiose generated is acted upon by β-glucosidase converting it to glucose (Eriksson, 1978).

Extracellular enzymes like cellulase, hemicellulase, amylase, pectinase and protease could be detected during the degradation of lignocellulosic medium by *Lentinus edodes* (Leatham, 1985). Nigam *et al.* (1987) reported that *Polyporus* spp. in solid state fermentation system was able to decompose sugarcane bagasse due to their cellulolytic activity.

Cellulolytic enzyme production by *Pleurotus* spp. is well established. The quantitative changes in the constituents of rice straw during different

stages of growth of *P. flabellatus* were observed by Rajarathnam *et al.* (1979). The progressive breakdown of constituents was correlated to an appropriate increase in the activity of cellulases and hemicellulases. Thayumanavan (1982) reported cellulase production *in vitro* by *P. sajor-caju* which could degrade farm and paper wastes. *Pleurotus* spp. have been reported to produce endoglucanase and β-glucosidase (Rai *et al.*, 1990) but extracellular, exo β-1, 4 glucanase activity was doubtful (Wood, 1988 ; Rajarathnam *et al.*, 1979 and Rai and Saxena, 1990).

Among various *Pleurotus* spp., *P. sajor-caju* was reported to be the most potent producer of cellulolytic enzymes (Hong *et al.*, 1985). Theradimani and Marimuthu (1991) have also reported the high level of cellulase production by *P. sajor-caju* on coir pith. Joseph *et al.* (1991) compared the cellulolytic enzyme production of three oyster mushrooms. *P. florida* recorded the maximum cellulolytic enzyme production, followed by *P. citrinopileatus* and *P. sajor-caju*.

The level of cellulase production has been reported to increase during fruiting. Claydon *et al.* (1988) and Natarajan and Kaviyarasan (1991) obtained a correlation between enzyme production and biomass of sporophores during cultivation.

1.3.2 Laccase

Most white rot fungi produce extracellular laccase (Kirk and Farrell, 1987). Saxena and Rai (1992) have reported significant activities of laccase during the growth of *P. sajor-caju* on wheat straw. *P. eringii*, the most

efficient lignin degrading oyster mushroom (Zadrazil and Dube, 1992) has been reported to produce high level of laccase and aryl alcohol oxidase on their substrates (Kirk and Farrell, 1987).

Thayumanavan (1982) reported that the enzymes of *Pleurotus* spp. which degrade lignin are of phenol oxidase in nature and include tyrosinase and laccase. Reddy (1985) reported the capacity of *Pleurotus* spp. to produce laccase and degrade part of lignin and cellulose present in their substrate. Kirk (1983) reported that laccase catalyses the electron oxidation of phenolics to phenoxy radicals and plays a role in lignin degradation.

Jablonsky (1984) cultivated *P. ostreatus* and *P. florida* on ground maize rachises and observed high laccase activity during the period from primodia formation to complete development of fruit body. Many workers have reported the role of laccase in sexual fruiting and yield of fruiting bodies (Wood *et al.*, 1991 and Dhaliwal *et al.*, 1992).

Laccases could also be involved in the detoxification of phenolic compounds or could be coupled via phenol oxidation to the cellulolytic system (Wood *et al.*, 1991).

Turner *et al.* (1975) showed that laccase is not elaborated by all lignin degrading fungi and that certain fungi showing laccase activity failed to degrade lignin. Evans (1985) reported that lignin degradation was unaffected by laccase activity and that lignin degradation was not essentially the function of laccase *in vivo* in the case of *Coriolus versicolor*. Assay of laccase during different stages of growth of *Pleurotus* spp. on paddy straw showed that laccase production reached the maximum after 24 days of incubation and

declined thereafter. However, the lignin degradation did not show any corresponding increase or decrease indicating the absence of any direct role played by laccase in lignin degradation (Dhanda *et al.*, 1996).

1.4 Cellulose degradation

White rot fungi including *Pleurotus* spp. are known to degrade cellulose during the colonization of lignocellulosic substrates (Zadrazil and Dube, 1992). Due to association with lignin and hemicellulose degrading ability, the cellulase in *Pleurotus* spp., though low as compared to established cellulolytic microbes, would have greater access to the cellulose in the native lignocelluloses (Saxena and Rai, 1992).

Ramaswamy *et al.* (1985) reported the association of glucose oxidase (acting on glucose) with H_2O_2 generation and the involvement of H_2O_2 in lignin degradation. Boominathan and Reddy (1992) reported that glucose obtained from cellulose degradation serves as a co-substrate for lignin degradation.

There are reports illustrating the reduction of cellulose content of the substrate following the growth of *Pleurotus* spp. (Zadrazil, 1978 ; Rajarathanam *et al.*, 1979 ; Moorthy, 1981 and Geetha, 1994).

Sivaprakasam and Kandaswamy (1981b) reported that *P. sajor-caju* during the growth period of 40 days utilized 15-25 per cent of cellulose present in rice straw. Substrates colonized with *P. sajor-caju, P. sapidus* and *P. cornucopiae* reduced the cellulose content of wheat straw by 20 per cent (Tsang *et al.*, 1987).

Dhanda *et al.* (1996) reported reduction in cellulose content from 32.8 per cent to 28.7 and 25.85 per cent after the growth of *P. florida* and *P. sajor-caju*, respectively, on paddy straw for 30 days.

Cellulose degradation in coir pith on inoculation with *Pleurotus* spp. has been reported (Nagarajan *et al.*, 1985). Theradimani and Marimuthu (1991) reported 53 per cent reduction in the cellulose content of coir pith after the growth of *P. platypus* and *P. sajor-caju* for 30 days.

1.5 Lignin degradation

Lignin is a very complex structure formed by the oxidative polymerization of coumaril, coniferyl and synapyl alcohol (Kirk and Farrell, 1987 and Boominathan and Reddy, 1992). Lignin degradation is important in the global recycling of carbon because of the great abundance of lignin in the biosphere and also because it is an important factor delimiting the degradation of cellulose and other polysaccharides (Kirk and Farrell, 1987).

Several workers have suspected that lignin degradation may play an important role in the formation of humus (Dordick *et al.*, 1986 ; Garcia *et al.*, 1987 and Kirk and Farrell, 1987). Lignin is also one of the most important factors which influences the physical constituent of soil through its ability to reduce and concentrate aromatic rings, which as they become humic acid represent the very basis of soil structure (Lemieux, 1996).

All fungi capable of degrading lignin are known as white rot fungi. The most rapid and extensive degradation of lignin described to date is caused by certain fungi particularly the white rot, in highly aerobic condition

(Kirk and Farrell, 1987). Kawase (1962) have reported that wood decay fungi of the white rot type are mostly responsible for the degradation of lignocellulosics in forest litter.

Lignin degradation is reported to occur by non specific enzyme catalysed burning or oxidation and is induced by carbohydrate, nitrogen and sulphur limitation (Kirk and Farrell, 1987). Boominathan and Reddy (1992) has reported that lignin degradation occurs during secondary metabolism when the essential nutrients are exhausted and growth ceases.

Pleurotus is a white rot fungus capable of degrading lignin (Zadrazil and Dube, 1992). Several workers have reported the lignin degrading ability of *Pleurotus* spp. (Ibrahim and Pearce, 1980 and Nizkovskaya *et al.*, 1984). Bisaria and Madan. (1984) reported 97 per cent loss in lignin during the cultivation of *P. sajor-caju* on paddy straw for 40 days. Nicolini *et al.* (1987) reported lignin degradation to a level of 44.4, 21.6 and 55 per cent on wheat straw, grape stalks and orange peel respectively. Increase in the digestibility of substrates due to lignin degradation by *Pleurotus* spp. has been reported (Beg *et al.*, 1986 ; Moyson and Verachtert, 1991 and Akin *et al.*, 1993). *Pleurotus sajor-caju* was reported to have degraded paper mill sludge at later stage of growth by elaborating lignin degrading enzymes (Kannan and Oblisami, 1990). *P. djamor* and *P.citrinopileatus* during their growth, degraded lignin in all the substrates tested (Geetha, 1994).

Coir pith, a lignocellulosic substrate, with high lignin concentration and wide C / N ratio, can be composted with *Pleurotus* (Nagarajan *et al.*, 1985). He has reported a reduction in lignin concentration of coir pith to 4.8 per cent

from initial 30 per cent after the growth of 30 days by *Pleurotus* spp. Theradimani and Marimuthu (1991) reported 82 and 78 per cent reduction in lignin content of coir pith inoculated with *P. sajor-caju* and *P. platypus* respectively.

1.6 Weight reduction of substrates on SSF with white rot fungi

There are several reports of weight reduction caused by white rot fungi on solid state fermentation (SSF) (Merrill *et al.*, 1964 ; Seal and Eggins, 1976 and Abraham *et al.*, 1992).

SSF of aspen (*Populus tremuloides*) wood with *Merulius tremellosus* (white rot fungus) for eight weeks caused 12 per cent reduction in weight (Reid, 1985). Arora and Sandhu (1985) reported 7-8 per cent loss in weight of saw dust on SSF with *Polyporus versicolor*.

Kirk (1983) reported 17, 30 and 27 per cent reduction in weight of beech wood, reed straw and sunflower stalk, respectively, on SSF of the substrates with *P. florida* for 60 days. Azizi *et al.* (1990) recorded 25.5 per cent reduction in weight of sugarcane bagasse on cultivation of *P. sajor-caju*. Savithri and Khan. (1994) reported reduction in particle size of coir pith on composting with *P. sajor-caju*.

Relation between enzyme production, yield and biodegradation

High cellulolytic activity was observed in spawn bottles after 26 days of growth by *Pleurotus* and the high enzyme activity is believed to have involved in the production of fruit primodia of the fungus in spawn bottle itself

(Rawal *et al.*, 1981). Wood *et al.* (1991) reported the direct correlation between cellulase production and biomass increase in the case of *Agaricus bisporus*. Natarajan and Kaviyarasan (1991) reported the correlation between cellulase production and biomass in synthetic medium as well as during cultivation.

Kochuthressiamma *et al.* (1991) observed higher yields of *P. florida* than the other *Pleurotus* species tested. *P. florida* also recorded the maximum liberation of all the major fractions of the cellulolytic enzyme, thus, leading them to the conclusion that the activity of cellulolytic enzymes of mushroom fungi can be considered as an indicator for their yield potential. *P. sajor-caju* during the growth period of 40 days on paddy straw utilized 15 - 25 per cent cellulose in paddy straw (Sivaprakasam and Kandaswamy, 1981 b). Thayumanavan (1982) reported that cellulases produced by *Pleurotus* were responsible for degradation of cellulose in farm wastes. Norkrans (1961) reported that cellulase production is directly correlated with cellulose utilization.

Dhaliwal *et al.* (1992) suggested that laccase may play a role in determining the induction pattern of fruiting. Their experiments showed early fruiting and higher biological efficiency by hyper laccase mutants of *P. florida*, while delayed appearance of fruiting and poor yield by laccase depressed mutants.

Kirk *et al.* (1968) found a correlation between laccase production by fungi and lignin decomposition. However, there are conflicting opinions as to the direct relationship between laccase and lignin degradation.

Theradimani and Marimuthu (1992) could not find any correlation between laccase production and lignin degradation. Dhanda *et al.* (1996) observed an increase in laccase production during the growth of *Pleurotus*, reaching the maximum on the 24th day of incubation and decreasing thereafter. However, the lignin degradation in the substrate did not show any corresponding increase or decrease.

MATERIALS AND METHODS

MATERIALS AND METHODS

2.1 Isolation and maintenance of pure cultures of *Pleurotus* spp

Isolation and maintenance of pure cultures of *Pleurotus* spp. *viz.*, *Pleurotus sajor-caju* (Fr.) Singer, *Pleurotus djamor* (Fr.) Boedijin, *Pleurotus platypus* (Cooke and Masse) Sacc. and Vellayani P_2 isolate of *Pleurotus* (an interstock hybrid developed at the Department of Plant Pathology, College of Agriculture, Vellayani) was carried out adopting tissue culture method (Scrase, 1995).

Tissue from the junction of pileus and stipe of sporocarp was scooped out and surface sterilized by placing in 95 per cent ethyl alcohol for one minute. This was then transferred to Potato Dextrose Agar (PDA) plated Petri dishes under aseptic conditions and incubated at room temperature (28 ± 4°C) for four days. Following, subculturing it was maintained on PDA slants. (Plate 1&2)

2.2 Spawn preparation

Wheat grain spawn of the four *Pleurotus* spp. was prepared adopting the method described by Sivaprakasam (1980).

Wheat grains were boiled in water till half cooked. After draining excess water, it was mixed with calcium carbonate at the rate of 50 g per kg of wheat grains to prevent adhesion of grains and for optimising the pH for spawn run. Glucose drip bottles of 750 ml capacity were filled with the grains to two third of its capacity, plugged with cotton and autoclaved at 1.05 kg cm^{-2} for 2 h.

Plate 1. Pure cultures of *Pleurotus* spp. in PDA slants in test tube

1. *P. platypus*

2. *P. sajor-caju*

3. V P_2 isolate of *Pleurotus*

4. *P. djamor*

Plate 2. Pure culture of *Pleurotus* spp. in conical flasks

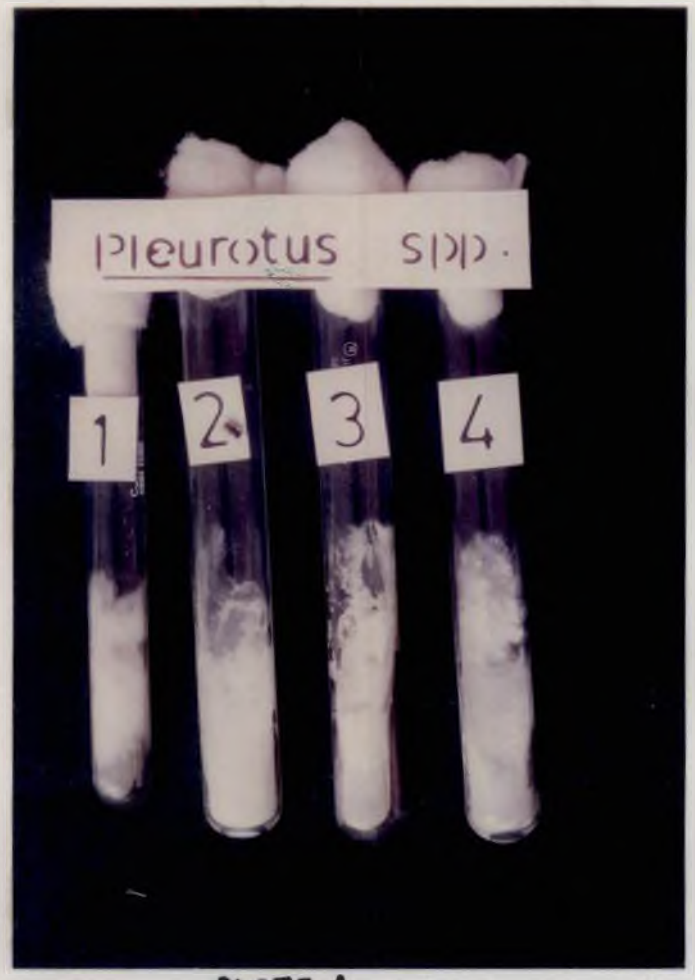


PLATE 1

PLATE 2

Inoculation of the grains with pure cultures of *Pleurotus* spp. was carried out and incubated at room temperature 28 ± 4°C. The nature of growth and time taken for completing mycelial colonisation of the grains was recorded. The spawn thus prepared was utilized for laying out mushroom beds. (Plate 3).

2.3 Preparation of mushroom beds

Three different substrates were tried for mushroom production viz., paddy straw, non retted coir pith (obtained by manually extracting the mesocarp) and retted coir pith.

Mushroom beds were prepared following the method described by Bhaskaran *et al.* (1978) in the case of paddy straw or non retted coir pith as the substrate and the method described by Theradinani and Marimuthu (1991) was adopted for the preparation of beds using retted coir pith as the substrate.

In the case of paddy straw and non retted coir pith, substrates were soaked in water over night, boiled for half an hour, drained and dried in shade to retain about 60 to 70 per cent moisture. In perforated poly bags of size 60 x 30 cm, 5 layers of the substrate (each of 5 to 8 cm thickness) was placed with spawning of each layer. Hundred gram spawn per mushroom bed (of 0.5 kg weight) was used. The bags were then tied and incubated in the dark for 15 days after which they were opened and transferred to cropping room where adequate ventilation and moisture was maintained.

In the case of retted coir pith, well perforated polythene bags of size 30 x 60 cm was first filled with 0.5 kg of the substrate and inoculated with *Pleurotus* spawn (at the rate of 100 g per kg of the substrate) uniformly over

Plate 3. Grain spawn of *Pleurotus* spp.

PLATE 3

surface and covered with another layer of 0.5 kg coir pith. This process was repeated till the bag was filled. The bag was tied and incubated in a room where adequate moisture and ventilation were present.

The nature and rate of growth and yield characteristics in all the substrates were recorded.

Samples were drawn at 10, 20 and 30 days interval to study cellulase and laccase production and also to estimate lignin and cellulose content. The reduction in weight and height of beds after 30 days of incubation and the yield characteristics were also recorded.

2.4 Enzyme assay

2.4.1 Extraction of enzymes

Five grams of the sample was ground in a precooled pestle and mortar with 20 ml cold distilled water and filtered through muslin cloth. The filtrate was centrifuged at 12000 rpm for 20 minutes at 6^0C. The supernatant was collected and used as the enzyme source (Maxwell and Bateman, 1967).

2.4.2 Assay of cellulase

Endoglucanase production by *Pleurotus* spp. in different substrates at different time intervals was estimated. The production of reducing sugar (glucose) due to cellulolytic activity was measured by Dinitrosalicylic acid (DNS) method (Miller, 1972).

0.15 ml of one per cent carboxy methyl cellulose in sodium citrate buffer at pH 5 was added to 0.35 ml of enzyme source. The mixture was

incubated at 55^0 C for 15 minute in a water bath. Immediately after removing the enzyme substrate mixture, 0.5 ml of DNS reagent was added. The mixture was kept in boiling water bath for 5 minutes. While still warm, 1 ml potassium sodium tartarate solution was added to the mixture. It was then cooled to room temperature, volume made upto 5 ml using distilled water and transmission measured in a Spectrophotometer at 540 nm. Boiled enzyme served as control. A standard curve was prepared with glucose in the concentration range of 100 to 1000 µg / ml.

2.4.3 Assay of laccase

Laccase production by *Pleurotus* spp. in different substrates at different time intervals was assayed using the method described by Frochner and Eriksson (1974). Five milli litre sodium phosphate buffer, pH 6, containing 10 mM guaiacol was added to the test tubes. Enzyme source of 0.1 ml was added to this and the mixture incubated for 5 minutes. The absorbance was determined at 470 nm in a Spectrophotometer. Boiled enzyme served as control. The activity of laccase was expressed in terms of enzyme units (1 unit = the change in absorbance of 0.01 per minute).

2.5 Estimation of cellulose

Cellulose content of the different substrates at different periods of SSF was estimated by adopting the method described by Updegraff (1969).

One hundred mg of oven dried sample was mixed with three ml acetic / nitric reagent (150 ml (80 %) : 15 ml) in a test tube. The tube was placed in a

water bath at 100^{0}C for 30 minutes. The contents of the tube was centrifuged at 8000 rpm for 15 minutes. The supernatant was discarded and residue washed in distilled water. Ten milli litre of 67 per cent H_2SO_4 was added to the residue and allowed to stand for one hour. From this 1 ml was taken and diluted to 100 ml. Five milli litre of anthrone reagent was added to 0.5 ml of this diluted solution and kept in a boiling water bath for 10 minutes. After cooling, the transmission was measured in a spectrophotometer at 630 nm. Anthrone reagent with distilled water served as control.

A standard curve with glucose was prepared in the concentration range of 40 to 200 μ g glucose / ml.

Estimation of lignin

Lignin content of different substrates at different periods of SSF was estimated following the method of Chesson (1978). One gram of the sample was added to 5 ml of concentrated sulphuric acid (98 %) and thoroughly shaken. It was transferred into a 1000 ml conical flask containing 450 ml of distilled water and boiled for 10 minutes. The contents of the flask were filtered through Geena G_3 glass filter. The acidic residues were washed to neutrality with distilled water, dried at 70^{0}C for 4 hours and weighed. The results were expressed in terms of per cent lignin content, on dry weight basis, of the substrates.

RESULTS

RESULTS

3.1 Isolation and maintenance of pure cultures of *Pleurotus* spp.

Pure cultures of four *Pleurotus* spp. viz., *P. sajor-caju, P. djamor, P. platypus* and Vellayani P_2 isolate of *Pleurotus* sp. were obtained from the sporocarps by tissue culture method. The cultures were maintained on PDA slants.

3.2 Spawn preparation

Wheat grain spawn of the four *Pleurotus* spp. was prepared and their nature and rate of growth on wheat grain was evaluated.

The four species showed statistically significant difference in the growth rates in spawn bottles (Table 1).

Vellayani P_2 isolate recorded the shortest period (12.33 d) for completion of mycelial run on wheat grains in spawn bottles, while *P. sajor-caju* required the maximum period (18.33 d) for completion of mycelial run. The rate of growth of *P. platypus* and *P.djamor* in spawn bottles were on par (15 and 14.66 days respectively).

Vellayani P_2 isolate showed vein like growth forming a network, while cottony growth was observed in the case of *P. sajor-caju* and *P. platypus*.

3.3 Nature and rate of growth of *Pleurotus* spp. on different substrates

Solid substrate fermentation (SSF) of paddy straw and coir pith (retted and non retted) using *Pleurotus* spp. was carried out. The nature and rate of growth of *Pleurotus* spp. on the three substrates differed significantly (Table 2).

Table 1 Mycelial growth in spawn bottles

Pleurotus spp.	Nature of growth	Period required for completion of growth (d)
V P2 isolate	Vein like growth forming network	12.33
P. sajor-caju	Cottony growth	18.33
P. platypus	Cottony growth	15.00
P. djamor	Fluffy growth	14.66
CD (0.05)		0.74

Table 2 Nature and rate of growth of *Pleurotus* spp. on different substrates

Pleurotus spp.	Paddy straw		Non retted coir pith		Retted coir pith		Mean
	Nature of growth	Period required for mycelial run (d)	Nature of growth	Period required for mycelial run (d)	Nature of growth	Period required for mycelial run (d)	
VP_2 isolate	Profuse	5.0	Moderate	6.6	Feeble	8.6	6.7
P. sajor-caju	Profuse	6.0	Moderate	5.6	Profuse	7.0	6.2
P. platypus	Profuse	5.6	Moderate	5.3	Profuse	6.3	5.7
P. djamor	Profuse	5.3	Moderate	4.6	Feeble	9.6	6.5
Mean		5.47		5.52		7.87	

CD - (0.05) Fungus - NS
Substrate - NS Fungus x substrate - 1.58

Table 3 Yield characteristics of *Pleurotus* spp. on different substrates

Pleurotus species	Paddy straw		Non retted coir pith		Retted coir pith		Mean
	Days for appearence of pin heads	Yield (g) / 500 g substrate	Days for appearence of pin heads	Yield (g) / 500 g substrate	Days for appearence of pin heads	Yield (g) / 500 g substrate	
VP_2 isolate of *Pleurotus*	15	369	15	137	-	-	168.8
P. sajor-caju	18	331	19	81	-	-	137.3
P. platypus	16	301	16	70	-	-	123.6
P. djamor	15	361	16	128	-	-	163
Mean		340.5		104			

CD (0.05) Substrate interaction Interaction - 187.25
Fungi - NS Substrate - 93.62

Vellayani P_2 isolate and *P.djamor* in retted coir pith took the maximum period for mycelial run.

The following treatments recorded the shortest period : Vellayani P_2 isolate of *Pleurotus* on paddy straw, *P. djamor* on non retted coir pith and *P. platypus* on retted coir pith.

P. platypus and *P. sajor-caju* showed profuse mycelial growth in all the three substrates, while the growth of Vellayani P_2 isolate and *P. djamor* on retted coir pith was very feeble. (Plate 4).

3.4 Yield characteristics of *Pleurotus* spp. on different substrates

There was significant difference in the sporophore production on different substrates. In the present study, paddy straw was found to be the best substrate for sporophore production (Table 3). Sporophore production was not observed on retted coir pith.

Maximum yield was recorded by Vellayani P_2 isolate (369 g), followed by *P. djamor* (361 g). The yield recorded by *P. platypus* (301 g) was the lowest. (Plate 5 & 6).

3.5 Changes in weight and height of *Pleurotus* inoculated substrates

The reduction in weight and height of the substrates after 30 days of SSF with *Pleurotus* spp. was recorded. In case of retted coir pith there was considerable change in colour from dark brown to coffee brown on incubation with *Pleurotus* species (Plate 7).

Plate 4. Growth of *Pleurotus* spp. on retted coir pith in conical flask

T_1 - V P_2 isolate of *Pleurotus*

T_2 - *P. sajor-caju*

T_3 - *P. platypus*

T_4 - *P. djamor*

Plate 5. Growth of *Pleurotus* spp. on non retted coir pith

Plate 4.

Plate 5.

Plate 6. Growth of *Pleurotus* spp. on paddy straw


VP_2 isolate


PLEUROTUS DJAMOR


PLEUROTUS SAJOR-CAJU


Pleurotus platypus

PLATE 6

Plate 7. Composted coir pith (retted) in comparison with uncomposted coir pith (retted)

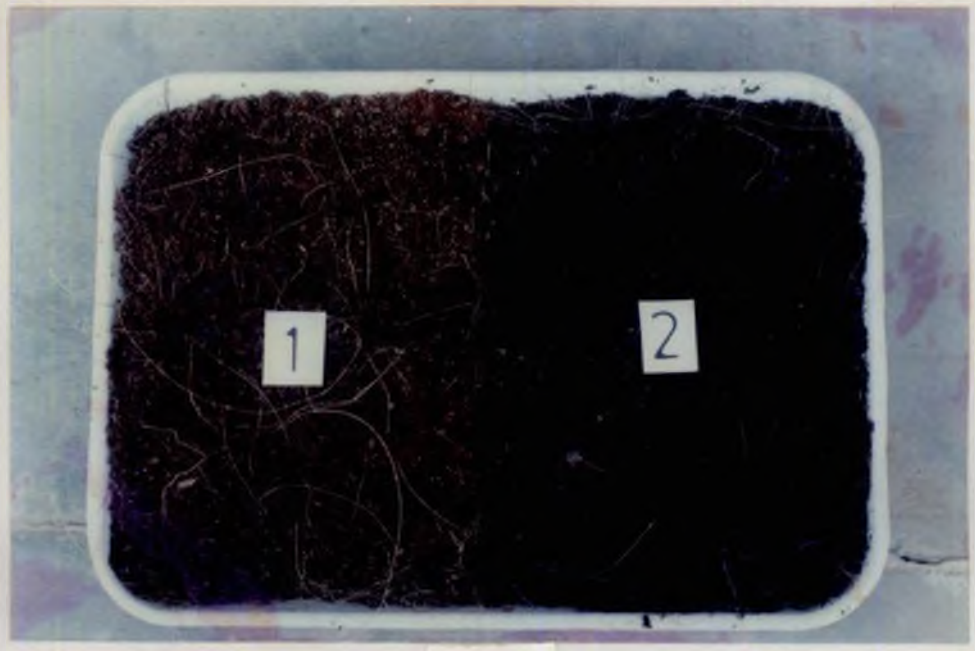


Plate 7.

3.5.1 Reduction in weight of substrates

There was significant difference in the weight reduction of different substrates on SSF with *Pleurotus* spp. (Table 4).

The maximum reduction was recorded in paddy straw (22.74 %) followed by that in non retted coir pith (9.07 %) and retted coir pith (3.09 %).

Among the different *Pleurotus* spp. tested, maximum weight reduction was caused by *P. platypus* on paddy straw (23.83 %) followed by the same in non retted coir pith (9.91 %) and retted coir pith (3.38 %). The lowest per cent reduction in weight (2.26) was caused by Vellayani P_2 isolate in retted coir pith (Fig. 1).

3.5.2 Reduction in height of substrates

There was significant difference in the height reduction of different substrates on SSF with *Pleurotus* spp. (Plate 8).

Maximum reduction in height was recorded in paddy straw (38.2 %) followed by non retted coir pith (27.54 %) and retted coir pith (23.46 %) (Table 5).

P. sajor-caju, among the four species tested, caused the maximum height reduction (31.63 %). The lowest per cent reduction in height was caused by Vellayani P_2 isolate (28.14 %). (Fig. 2).

3.6 *In vivo* production of cellulase by *Pleurotus* spp.

There was significant difference in the cellulase production by different *Pleurotus* species in different substrates at different time intervals (Table 6).

Plate 8. Retted coir pith composted with *Pleurotus* species in polybags

PLATE 8

Table 4 Reduction in weight of different substrates after inoculation with *Pleurotus* spp.

Pleurotus spp.	Paddy straw (Per cent reduction)	Non retted coir pith (Per cent reduction)	Retted coir pith (Per cent reduction)	Mean (Per cent reduction)
VP_2 isolate	22.16	7.79	2.26	10.73
P. sajor-caju	22.50	9.51	3.32	11.77
P. platypus	23.83	9.91	3.88	12.54
P. djamor	22.50	9.07	2.93	11.50
Mean	22.74	9.07	3.09	

CD (0.05) Substrate - 2.46
Fungus - NS Fungus x substrate - 4.90

Table 5 Reduction in height of different substrates after inoculaiton with *Pleurotus* spp.

Pleurotus spp.	Paddy straw (Per cent reduction)	Non retted coir pith (Per cent reduction)	Retted coir pith (Per cent reduction)	Mean (Per cent reduction)
VP_2 isolate	36.16	26.50	22.83	28.49
P. sajor-caju	40.50	29.33	25.06	31.63
P. platypus	39.16	27.00	22.74	29.63
P. djamor	37.00	27.33	23.23	29.18
Mean	38.20	27.54	23.46	

CD (0.05) Substrate - 2.83
Fungus - NS Fungus x substrate - 4.9

Fig. 1 Reduction in weight of different substrates on SSF with Pleurotus spp.

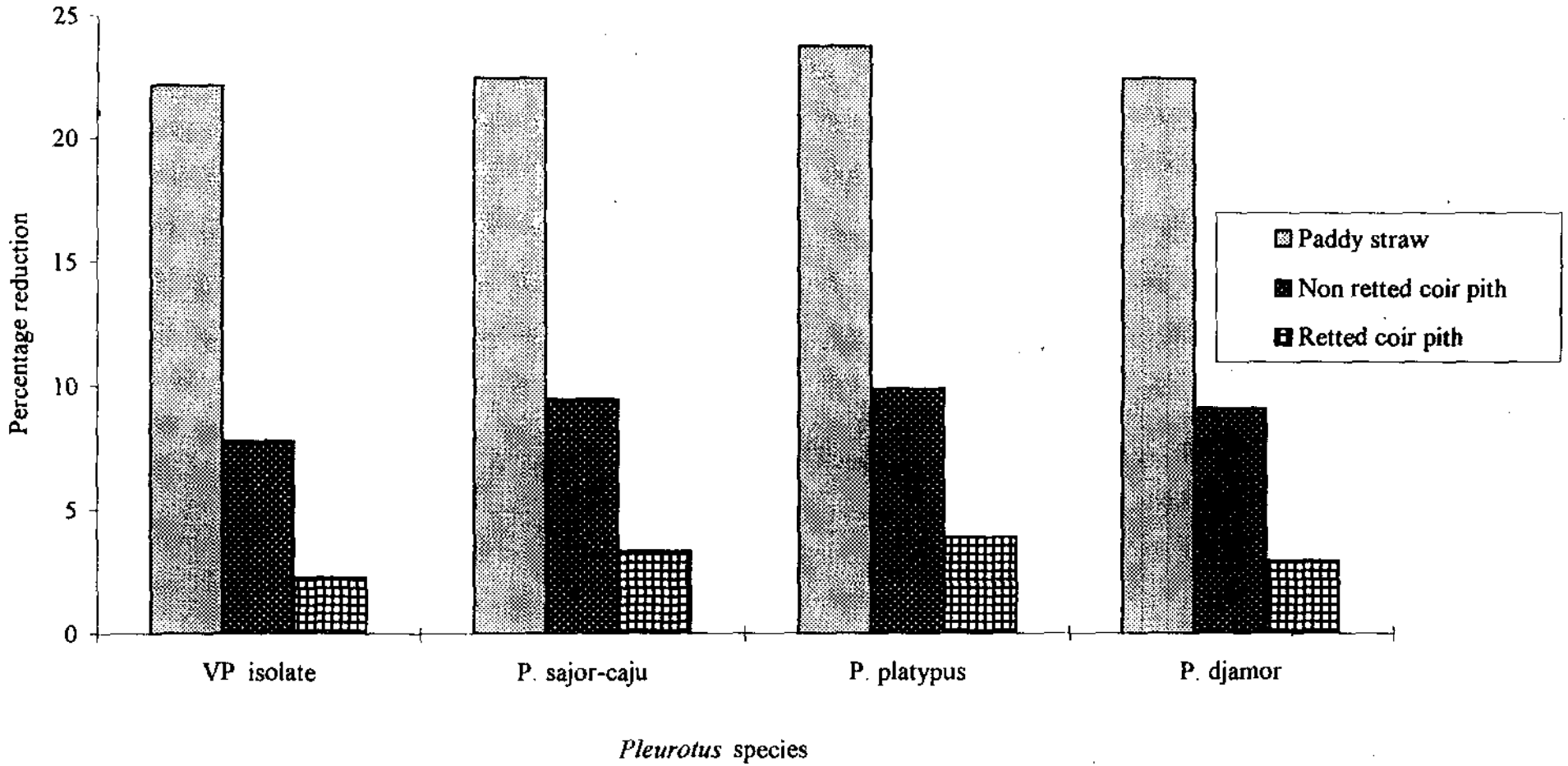


Fig. 2 Reduction in height of different substrates on SSF with *Pleurotus spp.*

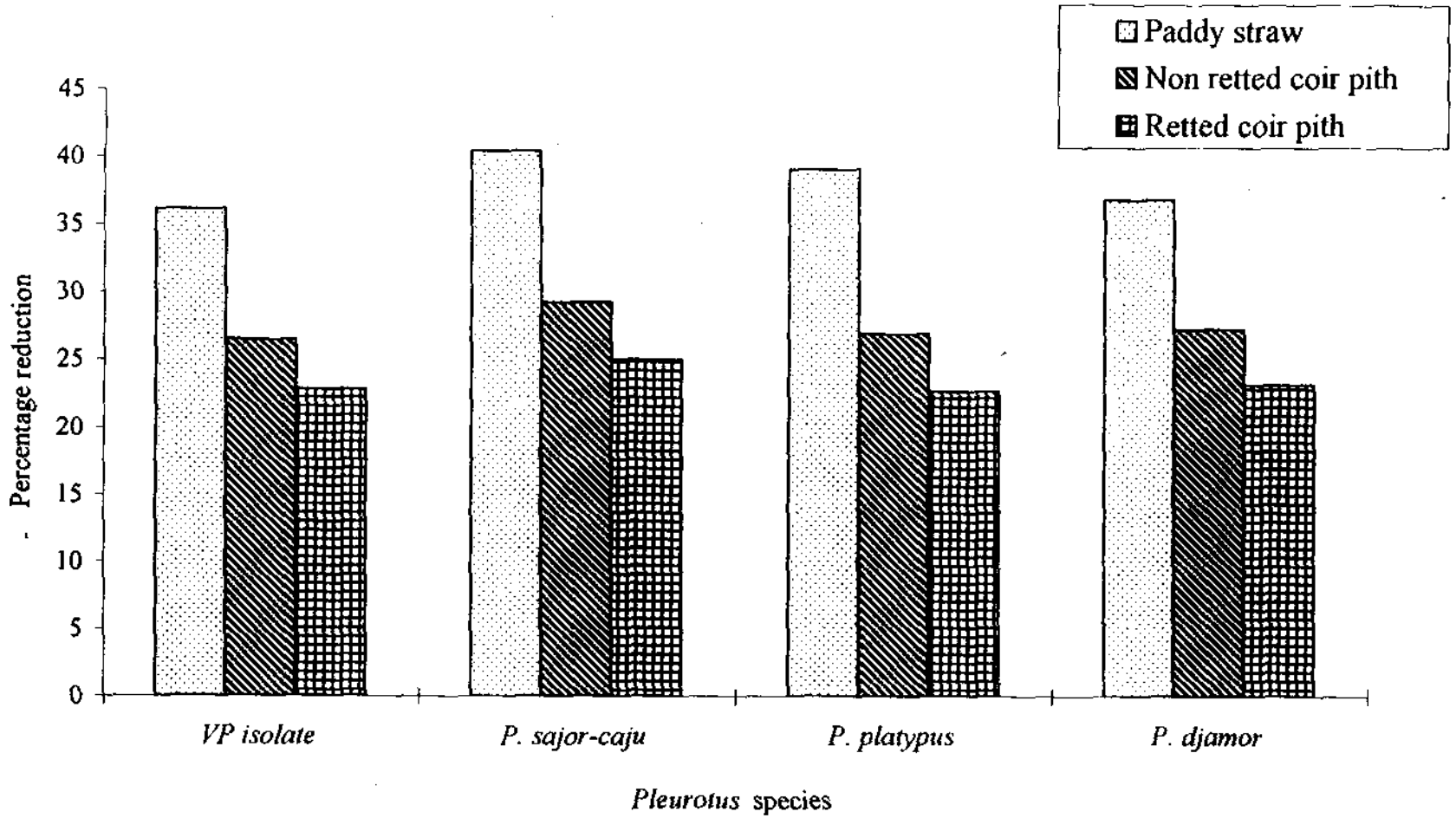


Table 6 ***In vivo*** **production of cellulase by** ***Pleurotus*** **spp. on different substrates at different time intervals**

Pleurotus spp.	Paddy straw			Non retted coir pith			Retted coir pith			Mean
	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)	
VP_2 isolate	0.23	0.32	0.26	0.23	0.32	0.24	0.17	0.23	0.19	0.24
	(0.48)	(0.57)	(0.51)	(0.48)	(0.56)	(0.49)	(0.41)	(0.48)	(0.43)	(0.49)
P. sajor-caju	0.57	0.77	0.60	0.55	0.74	0.58	0.39	0.52	0.44	0.57
	(0.75)	(0.88)	(0.77)	(0.74)	(0.86)	(0.76)	(0.62)	(0.72)	(0.66)	(0.75)
P. platypus	0.13	0.15	0.11	0.10	0.13	0.11	0.08	0.10	0.07	0.11
	(0.36)	(0.39)	(0.32)	(0.32)	(0.36)	(0.33)	(0.29)	(0.32)	(0.27)	(0.33)
P. djamor	0.30	0.42	0.33	0.29	0.40	0.32	0.24	0.28	0.24	0.31
	(0.55)	(0.65)	(0.57)	(0.55)	(0.63)	(0.57)	(0.49)	(0.53)	(0.49)	(0.56)
Mean	0.29	0.38	0.30	0.27	0.36	0.29	0.21	0.26	0.22	
	(0.54)	(0.62)	(0.55)	(0.52)	(0.60)	(0.54)	(0.45)	(0.51)	(0.46)	

CD (0.05) - 0.053
Values in parentheses are transformed values
C x - mg of sugar released per 5 g substrate

The maximum level of cellulase production was recorded by *P. sajor-caju* in paddy straw on the 20th day of incubation.

Among the different *Pleurotus* species tested *P. sajor-caju* followed by *P. djamor* recorded highest level of cellulase production.

3.6.1 Cellulase production on different substrates

There was significant difference in the cellulase production by *Pleurotus* spp. on different substrates (Fig. 3).

Among the different treatments, the maximum cellulase production was recorded by *P. sajor-caju* on paddy straw and non retted coir pith, followed by retted coir pith. Cellulase production by Vellayani P_2 isolate on paddy straw and non retted coir pith, and that by *P. djamor* on coir pith (retted and non retted) was on par. The lowest level of cellulase production was recorded by *P. platypus* on retted coir pith (Table 6a).

Cellulase production by *Pleurotus* spp. on the three substrates varied significantly, the maximum being recorded on paddy straw followed by non retted coir pith and retted coir pith.

3.6.2 Cellulase production at different time intervals

There was significant difference in the cellulase production by *Pleurotus* spp. at different periods of incubation (Fig. 4).

Among the different treatments, the maximum level of cellulase production was recorded by *P. sajor-caju* after 20th day of incubation. *P. platypus* on the 30th and 10th day of incubation recorded the lowest level of cellulase production (Table 6b).

Fig. 3 *In vivo* production of cellulase by *Pleurotus* spp. on different substrates

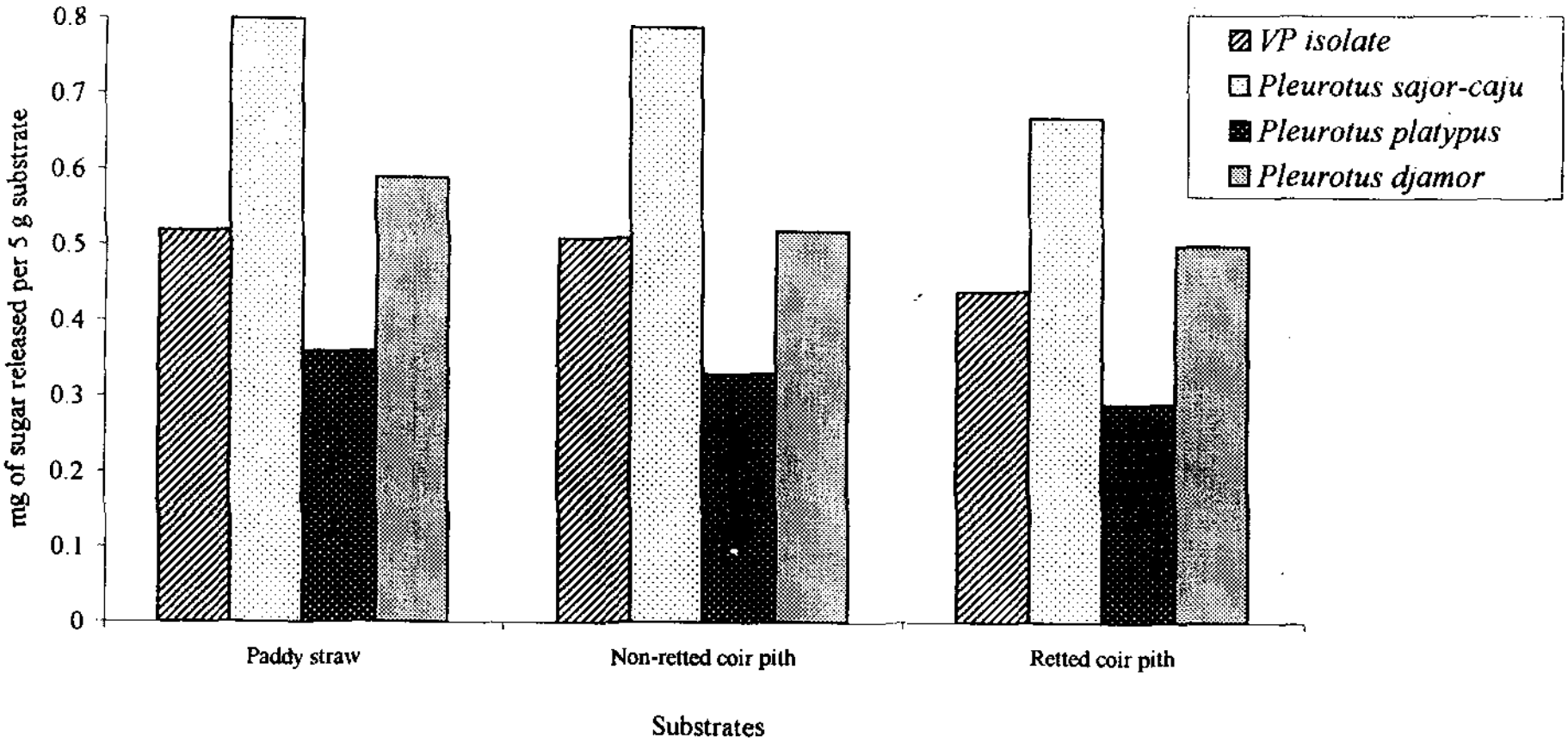


Table 6a *In vivo* production of cellulase by *Pleurotus* spp. on different substrates

Pleurotus spp.	Substrate			
	Paddy straw	Non-retted coir pith	Retted coir pith	Mean
VP_2 isolate	0.27 (0.52)	0.26 (0.51)	0.19 (0.44)	0.24 (0.49)
Pleurotus sajor-caju	0.64 (0.80)	0.62 (0.79)	0.45 (0.67)	0.57 (0.75)
Pleurotus platypus	0.13 (0.36)	0.11 (0.33)	0.09 (0.29)	0.11 (0.33)
Pleurotus djamor	0.35 (0.59)	0.34 (0.52)	0.25 (0.50)	0.31 (0.56)
Mean	0.34 (0.56)	0.33 (0.53)	0.24 (0.47)	

CD (0.05)

Fungus - 0.018
Substrate - 0.015
Fungus x substrate - 0.030

C x - mg of sugar released per 5 g substrate
Values in parentheses are transformed values

Fig. 4 *In vivo* production of cellulase by *Pleurotus* spp. at different time intervals

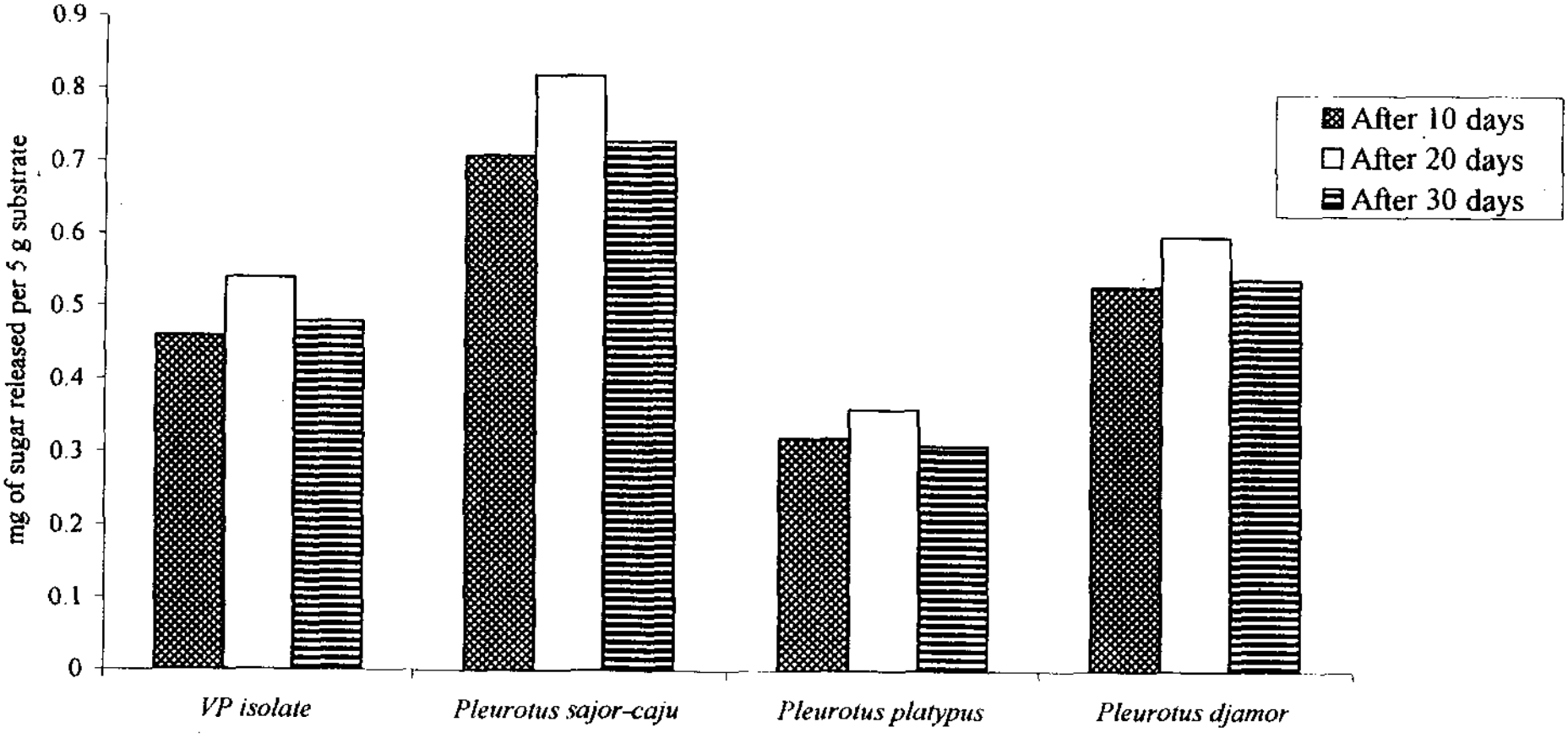


Table 6b *In vivo* production of cellulase by *Pleurotus* spp. at different time intervals

Treatment	After 10 days	After 20 days	After 30 days	Mean
VP_2 isolate	0.21 (0.46)	0.29 (0.54)	0.23 (0.48)	0.24 (0.49)
Pleurotus sajor-caju	0.50 (0.71)	0.67 (0.82)	0.53 (0.73)	0.57 (0.75)
Pleurotus platypus	0.10 (0.32)	0.13 (0.36)	0.10 (0.31)	0.11 (0.33)
Pleurotus djamor	0.28 (0.53)	0.36 (0.60)	0.30 (0.54)	0.31 (0.56)
Mean	0.25 (0.50)	0.33 (0.58)	0.27 (0.52)	

CD (0.05)

Fungus - 0.018
Time interval - 0.015
Fungus x time interval - 0.030

Cx - mg of sugar released per 5 g substrate
Values in parentheses are transformed values

Cellulase production by *Pleurotus* spp. on the 20th day was significantly higher than that on the 30th and 10th day of incubation.

3.7 *In vivo* production of laccase by *Pleurotus* spp.

There was significant difference in the level of laccase production by the different *Pleurotus* spp. at different time intervals (Table 7).

The maximum level of laccase production was recorded by Vellayani P_2 isolate in paddy straw on the 20th day of incubation.

Among the different *Pleurotus* species tested maximum level of laccase production was recorded by *Pleurotus sajor-caju* followed by Vellayani P_2 isolate. *P. djamor* recorded the lowest level of laccase production.

3.7.1 Laccase production on different substrates

There was significant difference in the level of laccase production on three different substrates by *Pleurotus* spp. (Fig. 5).

The maximum level of laccase production was recorded by *P. sajor-caju* and Vellayani P_2 isolate on paddy straw. *P. djamor* on retted coir pith recorded the lowest level of laccase production (Table 7a).

The level of laccase production on the three substrates was significantly different, the maximum level being recorded on paddy straw followed by retted and non retted coir pith.

3.7.2 Laccase production at different time intervals

Significant difference in the levels of laccase production was observed

Table 7 ***In vivo*** **production of laccase by** ***Pleurotus*** **spp. on different substrates at different time intervals**

Pleurotus spp.	Paddy straw			Non retted coir pith			Retted coir pith			Mean
	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)	
VP_2 isolate	0.17	0.27	1.65	0.11	0.14	0.09	0.02	0.03	0.02	0.24
	(1.08)	(1.13)	(1.63)	(1.05)	(1.07)	(1.05)	(1.01)	(1.01)	(1.01)	(1.11)
P. sajor-caju	0.81	1.12	0.57	0.17	0.04	0.00	0.19	0.19	0.17	0.34
	(1.34)	(1.46)	(1.25)	(1.08)	(1.02)	(1.00)	(1.09)	(1.09)	(1.08)	(1.16)
P. platypus	0.17	0.63	0.43	0.03	0.03	0.11	0.18	0.18	0.18	0.22
	(1.08)	(1.28)	(1.20)	(1.01)	(1.02)	(1.05)	(1.09)	(1.09)	(1.09)	(1.10)
P. djamor	0.25	0.88	0.22	0.07	0.08	0.00	0.24	0.00	0.00	0.15
	(1.12)	(1.37)	(1.10)	(1.04)	(1.04)	(1.00)	(1.12)	(1.00)	(1.00)	(1.07)
Mean	0.34	0.71	0.68	0.09	0.07	0.05	0.10	0.10	0.11	
	(1.16)	(1.31)	(1.29)	(1.05)	(1.04)	(1.03)	(1.05)	(1.05)	(1.05)	

CD (0.05) - 0.006
Values in parentheses are transformed values
Enzyme production expressed as change in absorbance of 0.01 per minute

Fig. 5 *In vivo* production of laccase by *Pleurotus* spp. on different substrates

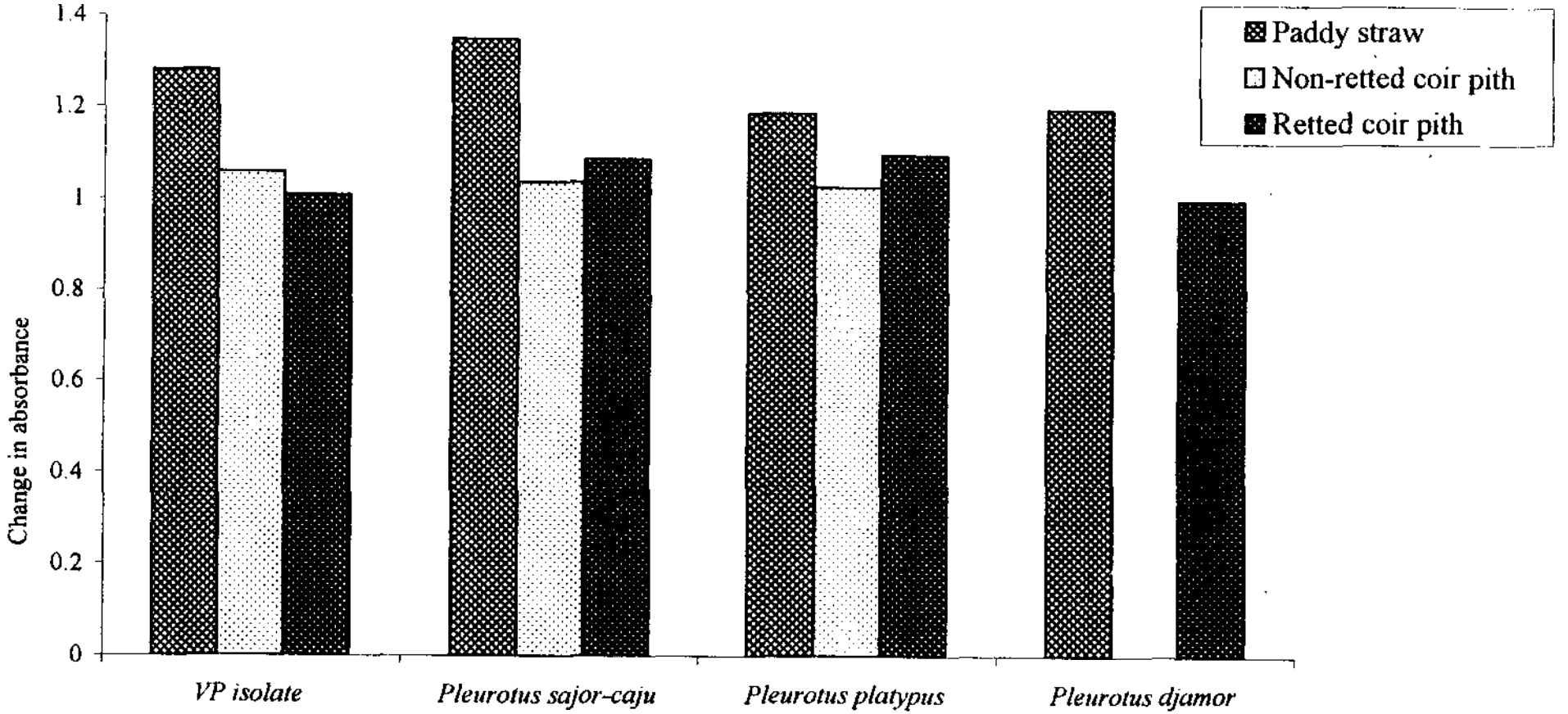


Table 7a *In vivo* production of laccase by *Pleurotus* spp. on different substrates

Treatment	Substrate			
	Paddy straw	Non-retted coir pith	Retted coir pith	Mean
VP_2 isolate	0.63 (1.28)	0.11 (1.06)	0.02 (1.01)	0.24 (1.11)
Pleurotus sajor-caju	0.83 (1.35)	0.07 (1.04)	0.18 (1.09)	0.34 (1.16)
Pleurotus platypus	0.40 (1.19)	0.05 (1.03)	0.20 (1.10)	0.22 (1.10)
Pleurotus djamor	0.43 (1.20)	0.05 (1.03)	0.00 (1.00)	0.15 (1.07)
	0.57 (1.25)	0.07 (1.04)	0.10 (1.05)	

CD (0.05)

Fungus - 0.002
Substrate - 0.002
Fungus x substrate - 0.003

Values in parentheses are transformed values
Enzyme production expressed as change in absorbance of 0.01 per minute

at different periods of incubation with *Pleurotus* spp. (Fig. 6).

The level of laccase production was highest after 20 days of incubation. The level of laccase production on the 30th day of incubation was low but higher than that on the 10th day of incubation (Table 7b).

3.8 Cellulose degradation by *Pleurotus* spp.

There was significant difference in the cellulose content of different substrates at different periods of incubation with *Pleurotus* spp. (Table 8).

The maximum percentage of cellulose reduction (55.8 per cent) was recorded in retted coir pith on the 30th day of incubation with *P. sajor-caju*. The lowest percentage of cellulose reduction (9.1 per cent) occurred in paddy straw on the 10th day of incubation with *P. platypus* (Table 8a).

Among the different *Pleurotus* species *P. sajor-caju* followed by *P. djamor* caused maximum level of cellulose degradation. *P. platypus* caused the lowest level of cellulose production.

3.8.1 Cellulose reduction by *Pleurotus* spp. in different substrates

Among the different substrates tested, the cellulose content was found to be maximum in paddy straw (40.5 %) followed non retted coir pith (36.5 %) and retted coir pith (25.5 %).

Significantly higher level of reduction in cellulose content (from 25.2 to 12.86 per cent) was recorded in retted coir pith by *P. sajor-caju* followed by the same fungus in non retted coir pith (from 36.5 to 24.35 %) and paddy straw (40.5 to 32.42 %) (Table 8 b).

Fig. 6 *In vivo* production of laccase by *Pleurotus* spp. at different time intervals

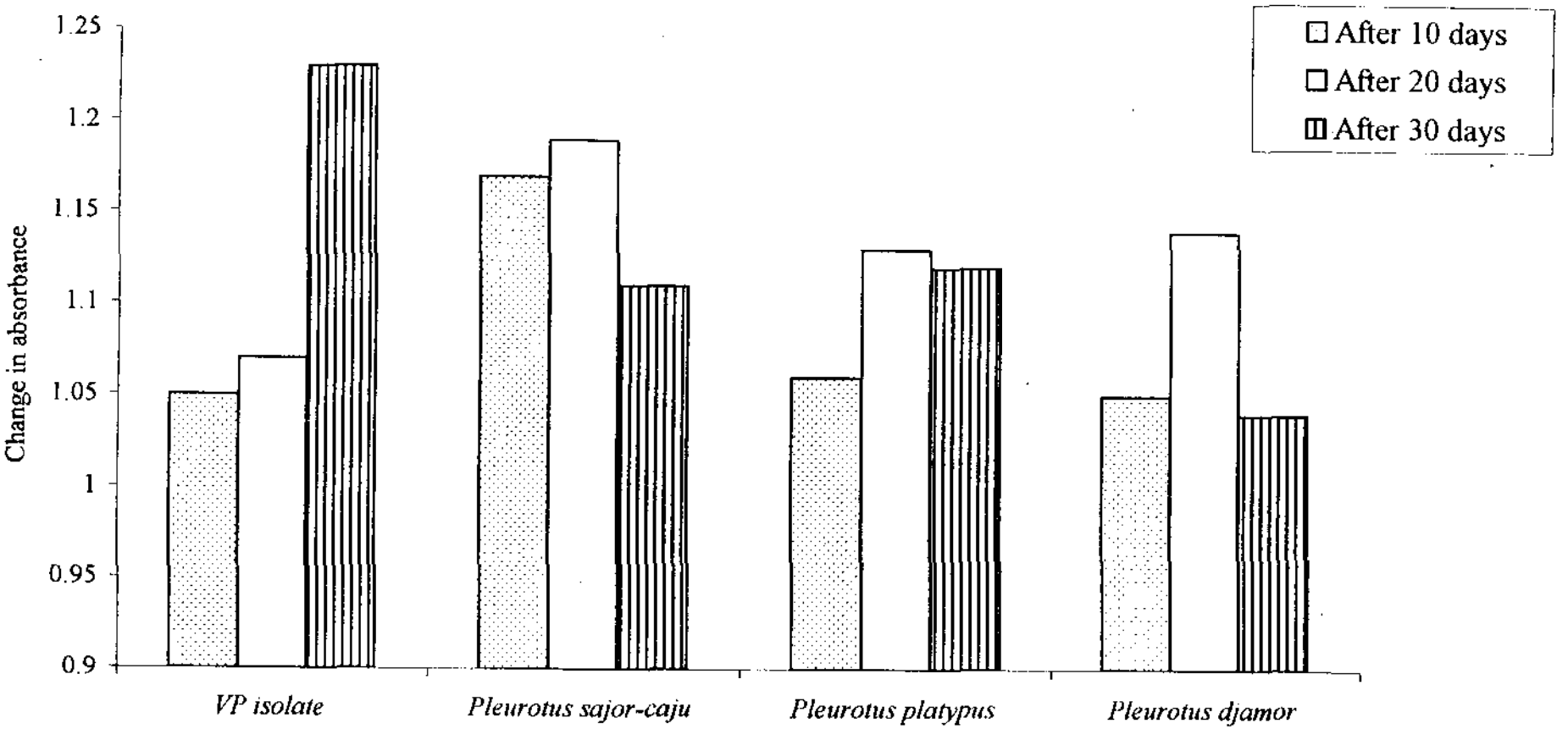


Table 7b *In vivo* production of laccase by *Pleurotus* spp. of different time intervals

Treatment	After 10 days	After 20 days	After 30 days	Mean
VP_2 isolate	0.10 (1.05)	0.14 (1.07)	0.51 (1.23)	0.24 (1.11)
Pleurotus sajor-caju	0.38 (1.17)	0.42 (1.19)	0.24 (1.11)	0.34 (1.16)
Pleurotus platypus	0.12 (1.06)	0.27 (1.13)	0.26 (1.12)	0.22 (1.10)
Pleurotus djamor	1.10 (1.05)	0.29 (1.14)	0.07 (1.04)	0.15 (1.07)
	0.17 (1.08)	0.28 (1.13)	0.26 (1.12)	

CD (0.05)

Fungus - 0.002
Time interval - 0.015
Fungus x time interval - 0.003

Values in parentheses are transformed values
Enzyme production expressed as change in absorbance of 0.01 per minute

Table 8 Cellulose content of different substrates at different time intervals of incubation with *Pleurotus* spp.

Pleurotus spp.	Paddy straw			Non retted coir pith			Retted coir pith			Mean
	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)	
Control	40.50	40.50	40.50	36.50	36.50	36.50	25.50	25.50	25.50	34.01
	(39.51)	(39.51)	(39.51)	(37.15)	(37.15)	(37.15)	(30.32)	(30.32)	(30.32)	(35.66)
VP_2 isolate	34.37	33.97	33.03	28.83	26.07	24.77	18.30	17.13	16.57	25.58
	(35.88)	(35.63)	(35.07)	(32.46)	(30.69)	(29.83)	(25.32)	(24.44)	(24.01)	(30.37)
P. sajor-caju	33.06	32.20	32.00	25.73	24.87	22.50	14.17	13.23	11.26	22.67
	(35.09)	(34.56)	(34.43)	(30.47)	(29.90)	(28.30)	(22.17)	(21.32)	(19.60)	(28.42)
P. platypus	36.80	36.63	34.33	29.97	27.87	27.13	18.97	18.43	17.57	27.20
	(37.33)	(37.23)	(35.85)	(33.18)	(31.85)	(31.38)	(25.81)	(25.42)	(24.77)	(31.42
P. djamor	34.29	32.70	32.90	26.27	24.00	22.97	15.40	14.80	13.73	23.67
	(35.83)	(34.86)	(34.99)	(30.80)	(29.32)	(28.62)	(23.10)	(22.62)	(21.74)	(29.17)
Mean	35.78	35.17	34.52	29.39	27.76	26.64	18.31	17.64	16.67	
	(36.73)	(36.36)	(35.97)	(32.82)	(31.78)	(31.06)	(25.32)	(24.82)	(24.09)	

CD (0.05) - 0.642
Values in parentheses are transformed values

Cellulose content - in per cent (on dry weight basis)

Table 8a Per cent cellulose reduction caused by *Pleurotus* spp. on differnet substrates at different time intervals

Pleurotus spp.	Paddy straw			Non retted coir pith			Retted coir pith		
	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)
VP_2 isolate	15.1	16.1	18.1	21.1	28.7	32.3	28.2	32.9	35.0
P. sajor-caju	18.3	20.4	22.2	29.5	32.0	38.1	44.7	48.2	55.8
P. platypus	9.1	9.5	15.3	18.0	23.8	25.7	25.8	27.6	31.0
P. djamor	15.3	17.5	18.7	28.2	34.2	37.2	39.6	41.9	46.1
Mean	15.4	15.8	18.5	24.4	29.6	33.3	34.5	37.6	42.0

Table 8b Cellulose content of different substrates after inoculation with *Pleurotus* spp.

Treatment	Paddy straw		Non retted coir pith		Retted coir pith		Mean	
	Cellulose content (%) on dry weight basis	% Cellulose reduction	Cellulose content (%) on dry weight basis	% Cellulose reduction	Cellulose content (%) on dry weight basis	% Cellulose reduction	Cellulose content	% Cellulose reduction
Control	40.50 (39.51)	-	36.50 (37.15)	-	25.50 (30.32)	-	34.01 (35.66)	
VP_2 isolate	33.79 (35.53)	16.56	26.54 (30.99)	27.28	17.33 (24.59)	32.03	25.58 (30.37)	14.83
Pleurotus sajor-caju	32.42 (34.69)	19.95	24.35 (29.56)	33.28	12.86 (21.01)	49.56	22.67 (28.42)	20.30
Pleurotus platypus	35.92 (36.81)	11.3	28.31 (32.14)	22.43	18.32 (25.33)	28.15	27.20 (31.42)	11.89
Pleurotus djamor	33.29 (36.23)	17.8	24.40 (29.59)	33.15	14.64 (22.48)	42.58	23.67 (29.10)	18.40
Mean	35.16 (36.35)	16.4	27.92 (31.89)	29.03	17.53 (24.75)	38.08		

CD (0.05)

Substrate - 0.166
Fungus - 0.214
Fungus x substrate - 0.371
Values in parentheses are transformed values

Cellulose reduction in different substrates varied significantly, the maximum being recorded in retted coir pith (from 25.5 to 12.86 %), followed by that in non retted coir pith (from 36.5 to 27.92 %) and paddy straw (from 40.5 to 36.35 %) (Fig. 7).

3.8.2 Cellulose degradation at different periods of incubation

Significantly higher level of reduction in cellulose content (from 34.01 to 21.26 %) was caused by *P.sajor-caju* after 30 days of incubation. The lowest level of reduction in cellulose content (from 34.01 to 32.1 %) was recorded by *P. platypus* (Table 8 c).

Cellulose reduction at different periods of incubation varied significantly, the maximum reduction (34.01 to 25.58 %) occurred after 30 days of incubation. The cellulose content after the 20^{th} day of incubation (26.53 %) was significantly lower than that on the 10^{th} day (31.62 %) of incubation (Fig. 8).

3.9 Lignin degradation

There was significant difference in the lignin content of different substrates at different periods of incubation with *Pleurotus* spp. (Table 9).

The maximum per cent lignin reduction (76.7) was recorded in retted coir pith on 30th day of incubation with *P. sajor-caju*. Lowest per cent lignin reduction (9.4) occurred in paddy straw on 10th day of incubation with Vellayani P_2 isolate of *Pleurotus* (Table 9a).

Among different *Pleurotus* species tested *P. sajor-caju* followed by

Fig. 7 Cellulose content of different substrates after inoculation with *Pleurotus* spp.

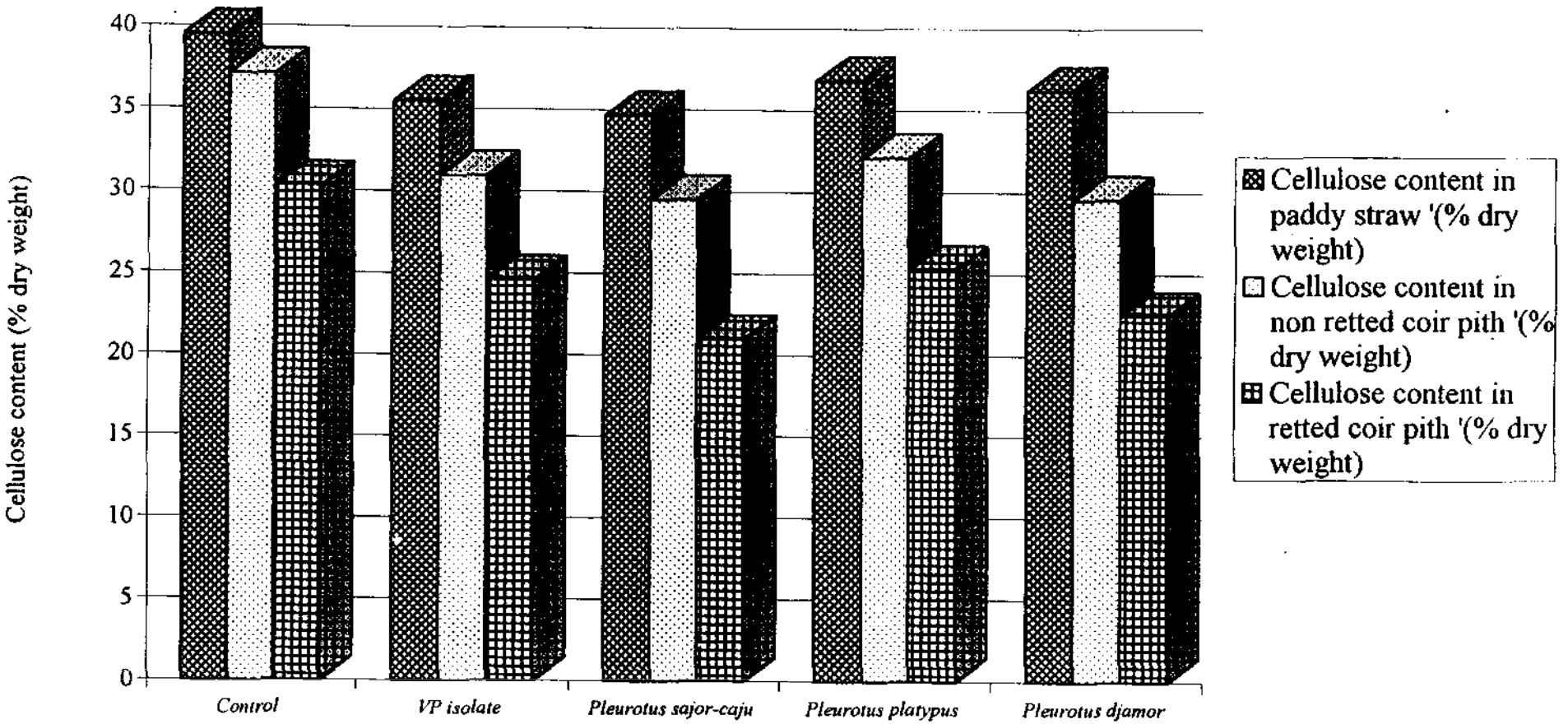


Table 8c Effect of duration of incubation on cellulose content of substrate treated with *Pleurotus* spp.

Treatment	Cellulose content after 10 days (% dry weight)	Cellulose content after 20 days (% dry weight)	Cellulose content after 30 days (% dry weight)	Mean
Control	34.01 (35.66)	34.01 (35.66)	34.01 (35.66)	34.01 (35.66)
VP_2 isolate	26.88 (31.22)	25.40 (30.25)	24.47 (29.64)	25.38 (30.37)
Pleurotus sajor-caju	23.85 (29.22)	22.92 (28.59)	21.26 (27.45)	22.67 (28.42)
Pleurotus platypus	28.27 (32.10)	27.32 (31.50)	26.03 (30.67)	27.20 (31.42)
Pleurotus djamor	24.89 (29.92)	23.42 (28.93)	22.71 (28.45)	23.67 (29.10)
Mean	27.51 (31.62)	26.53 (30.99)	25.58 (30.37)	

CD (0.0.5)

Fungus - 0.214
Time interval - 0.166
Time interval x Fungus - 0.371

Values in parentheses are transformed values

Fig. 8 Effect of duration of incubation on cellulose content of substrates inouclated with *Pleurotus* spp.

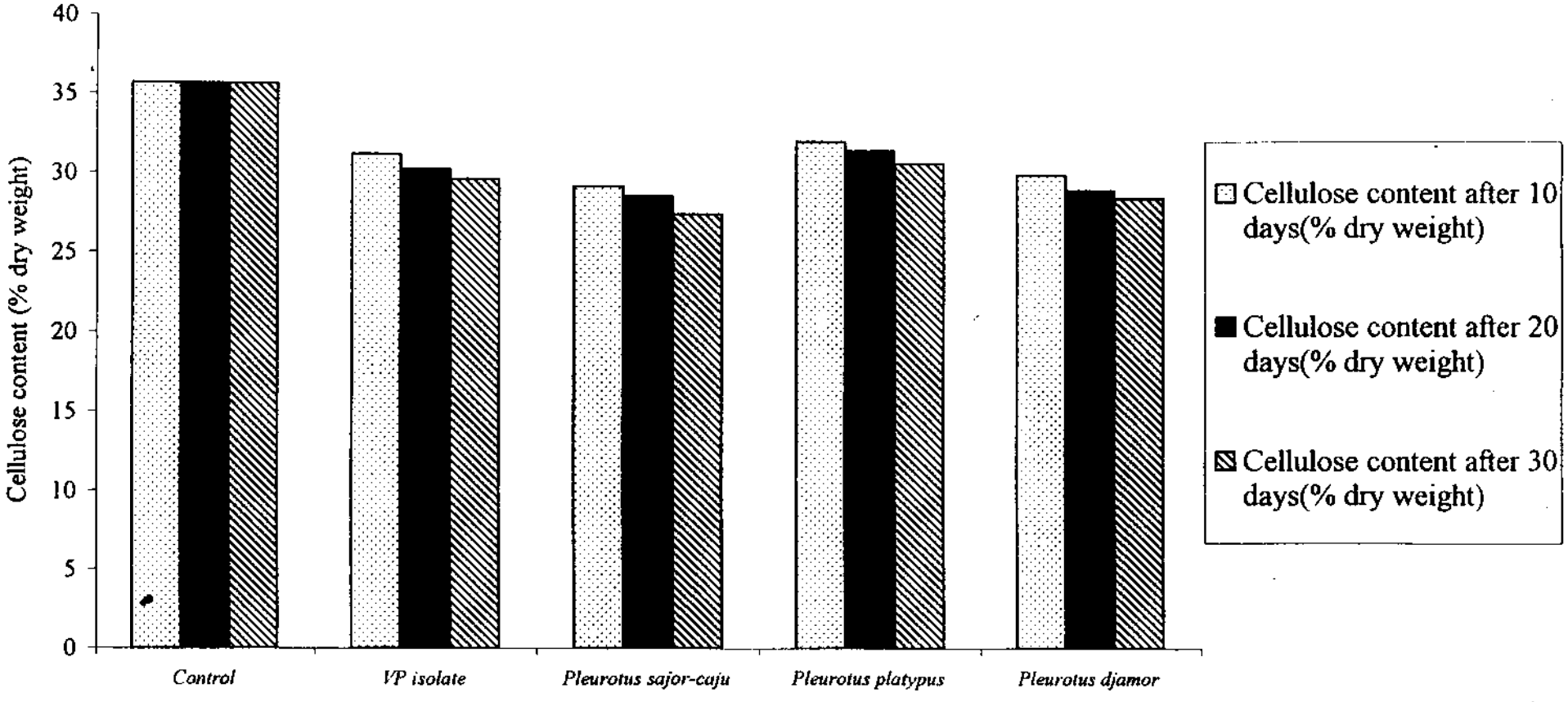


Table 9 Lignin content of different substrates at different periods of incubation with *Pleurotus* spp.

Pleurotus spp.	Paddy straw			Non retted coir pith			Retted coir pith			Mean
	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)	
Control	7.40	7.40	7.40	16.80	16.80	16.80	24.23	24.23	24.23	15.47
	(15.78)	(15.78)	(15.78)	(24.19)	(24.19)	(24.19)	(29.48)	(29.48)	(29.48)	(23.15)
VP_2 isolate	6.70	6.43	6.00	14.40	13.23	14.04	16.70	13.90	7.43	10.64
	(14.99)	(14.69)	(14.17)	(22.29)	(21.32)	(22.02)	(24.11)	(21.88)	(15.81)	(19.03)
P. sajor-caju	6.30	5.60	4.89	12.80	10.70	7.10	16.07	12.27	5.63	8.69
	(14.53)	(13.68)	(12.77)	(20.95)	(19.09)	(15.45)	(23.62)	(20.43)	(13.72)	(17.14)
P. platypus	6.40	5.80	5.10	13.40	11.60	9.20	16.30	21.07	6.60	9.29
	(14.64)	(13.93)	(13.04)	(21.46)	(19.90)	(17.65)	(23.87)	(30.32)	(14.88)	(17.74)
P. djamor	6.70	6.30	5.80	14.30	12.90	11.07	16.67	12.90	7.30	10.12
	(14.99)	(14.53)	(13.93)	(22.21)	(21.04)	(19.42)	(24.09)	(21.04)	(15.67)	(18.55)
Mean	6.69	6.29	5.81	14.31	12.98	11.42	17.90	14.82	9.47	
	(14.99)	(14.52)	(13.94)	(22.22)	(21.11)	(19.74)	(25.02)	(22.63)	(17.91)	

CD (0.0.5) - 0.391
Values in parentheses are transformed values
Lignin content - in per cent (on dry weight basis)

Table 9a Per cent lignin reduction caused by *Pleurotus* spp. on differnet substrates at different time intervals

Pleurotus spp.	Paddy straw			Non retted coir pith			Retted coir pith		
	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)	10 (d)	20 (d)	30 (d)
VP_2 isolate	9.4	13.1	18.9	14.2	21.1	16.2	30.7	42.6	69.3
P. sajor-caju	14.8	24.3	33.9	23.7	36.2	57.6	33.6	49.3	76.7
P. platypus	13.5	21.6	31.0	20.2	30.9	45.2	31.8	50.1	72.7
P. djamor	9.4	14.8	21.6	14.8	23.1	34.0	31.1	46.7	69.8
Mean	13.1	18.4	26.3	18.2	27.8	38.2	31.8	47.1	72.1

Values in parentheses are transformed values

P. platypus was the most efficient lignin degrader and Vellayani P_2 isolate was least efficient (Table 9a).

3.9.1 Lignin degradation by *Pleurotus* spp. in different substrates

Among the different substrates tested, the maximum lignin content was found in retted coir pith (24.23 %) followed by that in non retted coir pith (16.8 %) and paddy straw (7.4 %).

Significantly higher level of reduction in lignin content (from 24.23 to 10.89 per cent) was recorded in retted coir pith by *P. sajor-caju* followed by that of the same fungus in non retted coir pith (from 16.8 to 10.07 %) and paddy straw (form 7.4 to 5.58 %) (Table 9b).

Lignin degradation in different substrates varied significantly, the maximum being recorded in retted coir pith (from 24.23 to 13.87 %), followed by that in non retted coir pith (from 16.8 to 12.88 %) and paddy straw (from 7.4 to 6.26 %) (Fig. 9).

3.9.2 Lignin degradation by *Pleurotus* spp. at different periods of incubation

Significantly higher level of reduction in lignin content (from 15.47 to 5.84 %) was caused by *P. sajor-caju* after 30 days of incubation. The lowest recorded level of lignin reduction was that caused by Vellayani P_2 isolate on the 10^{th} day of incubation (Table 9 c).

Lignin reduction at different periods of incubation varied significantly, the maximum reduction (from initial 15.47 to 8.75 %) occurred after 30 days of incubation. The lignin concentration after the 20^{th} day of incubation (11.06 %) was significantly lower than that on the 10^{th} day (12.55 %) of incubation Fig. 10).

Table 9b Lignin content of different substrates after inoculation with *Pleurotus* spp.

Treatment	Paddy straw		Non retted coir pith		Retted coir pith		Mean	
	Lignin content (%) on dry weight basis	% Lignin reduction	Lignin content (%) on dry weight basis	% Lignin reduction	Lignin content (%) on dry weight basis	% Lignin reduction	Lignin content	% Lignin reduction
Control	7.40 (15.78)	-	16.80 (24.19)	-	24.23 (29.48)	-	15.47 (23.15)	
VP_2 isolate	6.37 (14.62)	13.91	13.90 (21.88)	17.26	12.39 (20.60)	48.86	10.64 (19.03)	17.80
Pleurotus sajor-caju	5.58 (13.66)	24.59	10.07 (18.50)	40.05	10.89 (19.26)	55.05	8.69 (17.14)	25.96
Pleurotus platypus	5.75 (13.87)	22.29	11.34 (19.67)	32.50	11.34 (19.67)	53.19	9.29 (17.74)	23.37
Pleurotus djamor	6.26 (14.48)	15.40	12.72 (20.89)	24.28	12.00 (20.26)	50.47	10.12 (18.55)	19.87
Mean	6.26 (14.48)	19.04	12.88 (21.02)	28.52	13.87 (21.85)	51.89		

CD (0.05)

Fungus - 0.130
Time interval - 0.101
Fungus x Time interval - 0.226

Values in parentheses are transformed values

Fig. 9 Lignin content of different substrates on treatment with *Pleurotus* spp.

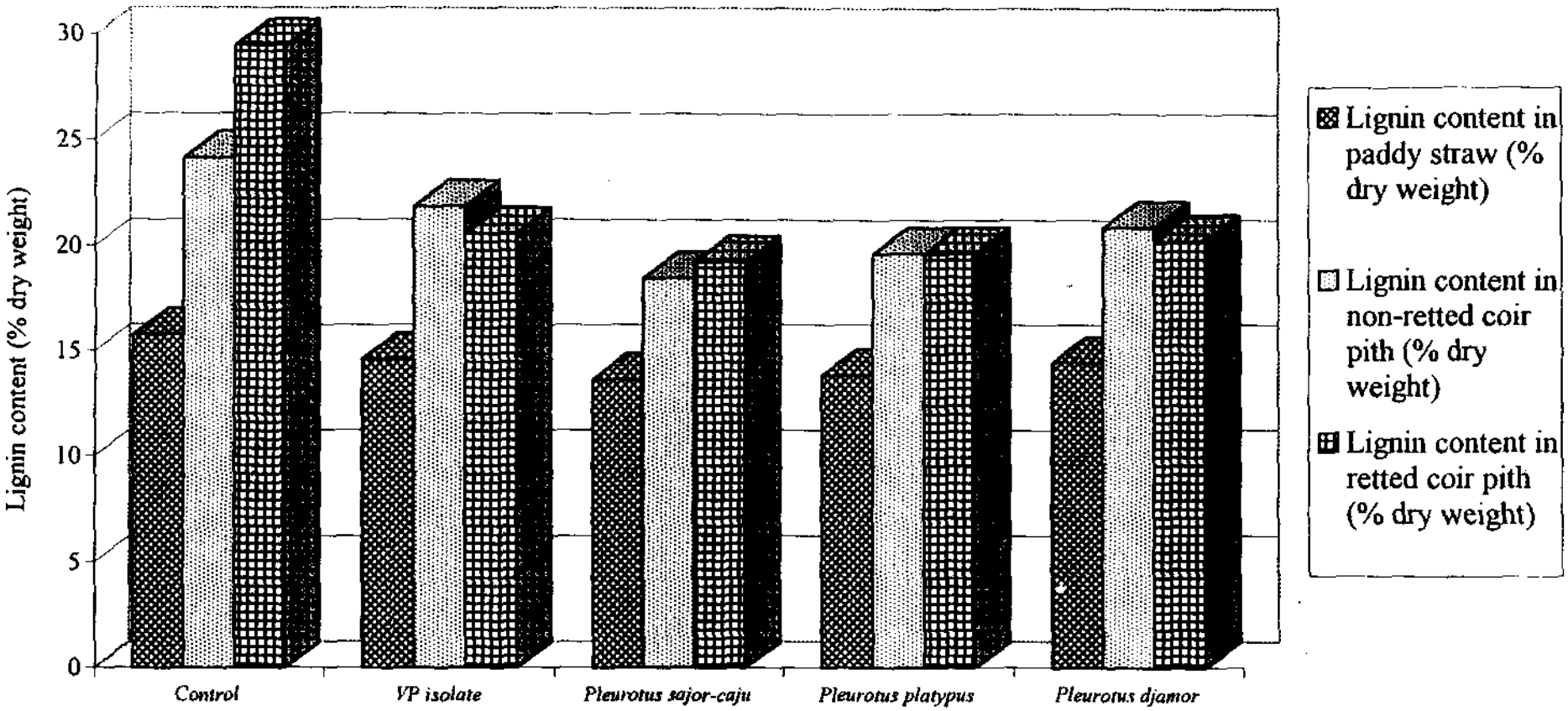


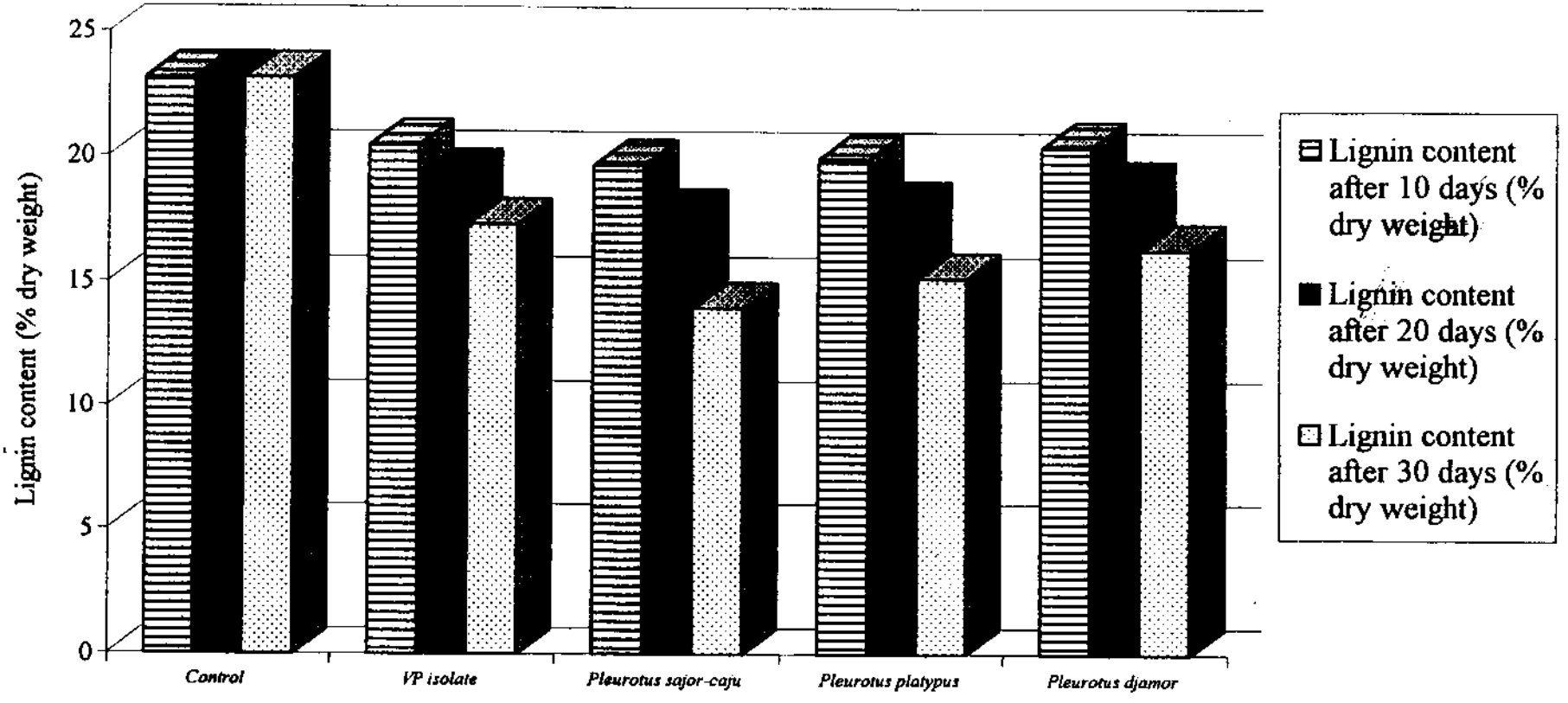
Table 9c Effect of duration of incubation on lignin content of substrates after inoculation with *Pleurotus* spp.

Treatment	Lignin content after 10 days (% dry weight)	Lignin content after 20 days (% dry weight)	Lignin content after 30 days (% dry weight)	Mean
Control	15.47 (23.15)	15.47 (23.15)	15.47 (23.15)	15.47 (23.15)
VP_2 isolate	12.23 (20.47)	10.93 (19.30)	8.89 (17.34)	10.64 (19.03)
Pleurotus sajor-caju	11.37 (19.70)	9.28 (17.73)	5.84 (13.98)	8.69 (17.14)
Pleurotus platypus	11.67 (19.97)	9.61 (18.05)	6.87 (15.19)	9.29 (17.74)
Pleurotus djamor	12.19 (20.43)	10.47 (18.87)	7.92 (16.34)	10.12 (18.55)
Mean	12.55 (20.74)	11.06 (19.42)	8.75 (17.20)	

CD (0.05)

Fungus - 0.13
Time interval - 0.101
Fungus x substrate - 0.226
Values in parentheses are transformed values

Fig. 10 Effect of duration of incubation on lignin content of substrates after inocultion with *Pleurotus* spp.



3.10 Correlation between enzyme production and degradation

Significantly positive correlation was found between cellulase production by the *Pleurotus* spp. and cellulose degradation in all the substrates tested. The maximum correlation was found in retted coir pith, followed by that in paddy straw and non retted coir pith. (Table 10a).

In the case of lignin degradation also there was positive correlation between laccase production and lignin degradation in paddy straw and retted coir pith. However the correlation was not significant. (Table 10b).

Table 10a Correlation between cellulase production and cellulose degradation

Pleurotus sp.	Days	Paddy straw		Non retted coir pith		Retted coir pith	
		Cellulase	% Cellulose reduction	Cellulase	% Cellulose reduction	Cellulase	% Cellulose reduction
Vellayani P_2	10	0.23	15.1	0.23	21.1	0.17	28.2
	20	0.32	16.1	0.31	28.7	0.23	32.9
	30	0.25	18.1	0.24	32.3	0.18	35.0
P. sajor-caju	10	0.57	18.3	0.55	29.5	0.39	44.7
	20	0.77	20.4	0.73	32.0	0.52	48.2
	30	0.60	22.2	0.57	38.1	0.43	55.8
P. platypus	10	0.13	9.1	0.10	18.0	0.08	25.8
	20	0.15	9.5	0.12	23.8	0.10	27.6
	30	0.11	15.3	0.11	25.7	0.07	31.0
P. djamor	10	0.30	15.3	0.29	28.2	0.24	39.6
	20	0.41	17.5	0. 4	34.2	0.28	41.9
	30	0.33	18.7	0.32	37.2	0.24	46.1
Correlation coefficient		0.78**		0.64**		0.87**	

** Significant at 1 % level

Table 10b Correlation between laccase production and lignin degradation

Pleurotus sp.	Days	Paddy straw		Non retted coir pith		Retted coir pith	
		Laccase	% Lignin reduction	Laccase	% Lignin reduction	Laccase	% Lignin reduction
Vellayani P_2	10	0.166	9.4	0.107	14.2	0.020	30.7
	20	0.266	13.1	0.142	21.1	0.027	42.6
	30	1.650	18.9	0.093	16.2	0.022	69.3
P. sajor-caju	10	0.806	14.8	0.174	23.7	0.193	33.6
	20	1.124	24.3	0.041	36.2	0.190	49.3
	30	0.568	33.9	0.004	57.6	0.168	76.7
P. platypus	10	0.171	13.5	0.027	20.2	0.180	31.8
	20	0.631	21.6	0.031	30.9	0.179	50.1
	30	0.431	31.0	0.106	45.2	0.245	72.7
P. djamor	10	0.247	9.4	0.071	14.8	0.001	31.1
	20	0.876	14.8	0.082	23.1	0.005	46.7
	30	0.216	21.6	0.005	34.0	0.002	69.8
Correlation coefficient		0.29 NS		-0.45 NS		0.15 NS	

DISCUSSION

DISCUSSION

More than half the total produce from land remains unused as waste in the form of straw, leaves, stems, roots etc. (Zadrazil, 1978).

Among the different agrowastes, lignocellulosics are the most difficult to be degraded due to their wide C/N ratio and its lignin fraction ; the degradation of which is mainly limited to the filamentous fungi of the white rot type (Kirk, 1983). Coir pith is one such lignocellulosic waste, the production of which during 1991-92 in India was estimated as one million tonnes (Savithri and Khan, 1994). In Kerala the production of coconuts during the year 1995-96 was 5950.7 million nuts (Thampan, 1997) ; each of ten thousand nuts contributing to the production of one tonne of coir pith (Nagarajan *et al.*, 1985).

Pleurotus spp. are white rot fungi capable of degrading lignocellulosics with their extracellular enzymes (Zadrazil, 1978 and Theradimani and Marimuthu, 1991).

In the present study, four *Pleurotus* species were tested for their enzyme production and lignocellulose degrading ability in coir pith (retted and non retted) in comparison with that in paddy straw. The four *Pleurotus* species included for the study were Vellayani P_2 isolate of *Pleurotus* sp., *P. sajor-caju, P. platypus* and *P. djamor*.

The superiority of wheat and sorghum grains for spawn production has been reported (Sivaprakasam and Kandaswamy, 1981a and Kotwaliwale *et al.*, 1991). Studies on the rate of mycelial growth in spawn bottles by the four *Pleurotus* species indicated the fast growing nature of Vellayani P_2, in spawn

bottles. This is in conformity with the report by Anita (1998). The shortest period (12.33 d) for completion of mycelial growth in spawn bottles was recorded by Vellayani P_2 isolate followed by *P. djamor* (14.66 d) and *P. platypus* (15 d), the growth rates of which were on par. *P. sajor-caju* recorded the maximum period (18.33 d). Suharban (1987) has also reported the slow growing nature of *P. sajor-caju* in spawn bottles.

The growth characteristics of *Pleurotus* spp. on different substrates indicated that paddy straw and non retted coir pith supported profuse mycelial growth of all the *Pleurotus* spp. tested. *P. sajor-caju* and *P. platypus* showed profuse mycelial growth in all the substrates tested, while the growth of *P. djamor* and Vellayani P_2 isolate on retted coir pith was very feeble. The feeble growth may be attributed to the low level of cellulase production by these two *Pleurotus* species. Urmila and Phutela (1991) reported the possible involvement of cellulolytic enzymes in the mycelial colonization of substrates. *P. platypus*, despite its low cellulase production showed profuse mycelial growth on retted coir pith. This may be due to the greater accessibility of its cellulolytic enzyme owing to its high lignin degrading ability (Saxena and Rai, 1992).

Many workers have reported the superiority of paddy straw for sporophore production (Jandaik, 1974 ; Sivaprakasam *et al.*, 1987 and Bano *et* al., 1978). The yield of sporophore production depends upon the nature of substrate used for bedding material as well as the chemical constituent of the substrate (Zadrazil, 1978). In the present study paddy straw turned out to be the best substrate for sporophore production, followed by non retted coir pith.

The cellulose content of the substrates also showed the same trend. This is in conformity with the report by Sivaprakasam *et al.* (1981 b) that the yield of sporophores is positively correlated with cellulose content and negatively correlated with the lignin content of the substrate. In retted coir pith there was high lignin content as compared to the other two substrates. The toxic phenolics produced during lignin degradation is reported to interfere with cellulase production. This may account for the absence of sporophore production on retted coir pith, in which the lignin degradation was the highest.

The reduction in volume of substrates on SSF has been reported by many workers (Seal and Eggins, 1976 ; Nagarajan *et al.*, 1985 and Arabham *et al.*, 1992). In the present study also there was significant reduction in the height and weight of all the substrates incubated with *Pleurotus* spp. The highest percentage of height and weight reduction was recorded in paddy straw followed by that in non retted coir pith, and retted coir pith, contrary to the fact that the lignocellulose degradation showed the reverse trend. This may be due to the absence of sporophore production in retted coir pith. In retted coir pith, with high lignin concentration and higher level of lignocellulose degradation, in addition to CO_2, many degradation products must have been formed. Reid *et al.* (1982) and Ulmer *et al.* (1983) have reported that, besides CO_2, many water soluble intermediaries are produced during lignin degradation. Formation of humic acid and degradation dependent binding of humic acid to the mycelium of white rot fungi during lignin degradation have been reported (Leisola and Garcia, 1989).

Among the various *Pleurotus* spp. tested *P. sajor-caju* recorded the maximum cellulase production, followed by *P. djamor*. *P. platypus* recorded the lowest cellulase production. The high cellulase production potential of *P. sajor-caju* has been reported by many workers (Hong *et al.*, 1985 and Theradimani and Marimuthu, 1991).

In the present study, the maximum cellulase production was recorded on paddy straw, followed by non retted coir pith and retted coir pith. The cellulose concentration and the sporophore production in these substrates also showed the same trend. This is in confirmation with the report that cellulase production and sporophore production depend upon the cellulose concentration of the substrates (Sivaprakasam and Kandaswamy, 1981 b).

Among the different *Pleurotus* species tested, *P. sajor-caju*, followed by *P. djamor* and Vellayani P_2 caused the maximum cellulose degradation. *P. sajor-caju* caused 22.2, 38.10 and 55.80 per cent reduction in cellulose concentration, in paddy straw, non retted coir pith and retted coir pith, respectively. The cellulose reduction caused by *P. platypus* was the lowest i.e. 15.3, 25.7 and 31.1 per cent in paddy straw non retted coir pith and retted coir pith respectively. The cellulase production by the different *Pleurotus* spp. tested also showed the same trend. Positive correlation was found between cellulase production and cellulose degradation. This is similar to the report by Norkrans (1967) that cellulase production is directly correlated with cellulose degradation.

The maximum recorded cellulase production was observed on the 20^{th} day of incubation, which is also the time for the formation of first flush of

fruiting bodies. This observation is in confirmation with the report by Wood *et al.* (1991) that the activity of endocellulase increases or decreases in parallel with the biomass of the fruit bodies, in each flush of the cropping cycle. Natarajan and Kaviyarasan (1991) have also reported the increase in cellulase production during fruiting.

The highest recorded laccase production was observed in the case of Vellayani P_2 isolate on the 30th day of incubation. However the lignin degradation caused by Vellayani P_2 was the lowest. Further, the positive correlation obtained between laccase production and lignin degradation was not significant. This finding indicates that though laccase may play a significant role in lignin degradation, as reported by many workers (Kirk *et al.*, 1968 and Reddy, 1985), the key role in lignin degradation may be played by many other enzymes. There are many conflicting reports on the direct involvement of laccase in lignin degradation (Turner, 1975 and Evans, 1985). Dhaliwal *et al.* (1992) have reported that laccase plays a role in early fruiting and biomass yield. This may be the reason for the high yielding character of Vellayani P_2 isolate, inspite of its low cellulase production. Further the high laccase production by Vellayani P_2 may be the reason for its low cellulase production. Dhaliwal *et al.* (1992) have also reported the antagonistic effect of laccase production on cellulase production.

The cellulose concentration of the substrates gradually decreased with the growth of *Pleurotus* spp. Among the different *Pleurotus* species tested *P. sajor-caju* followed by Vellayani P_2 isolate caused the maximum cellulose degradation in all the substrates tested i.e., 22.2, 38.1 and 55.8 per cent

cellulose reduction in paddy straw, non retted and retted coir pith respectively, after 30 days of incubation. The cellulose reduction caused by *P. platypus* was the lowest. In the present study the highest level of cellulose reduction (42.0 %) occurred in retted coir pith followed by that in non retted coir pith (33.32 %) and paddy straw (18.5 %) contrary to the reverse trend in the cellulase production on these substrates. This may be due to involvement of oxidative enzymes in cellulose degradation in retted coir pith. Eriksson (1974) reported the presence of enzymes, such as, glucose oxidase, cellobiose oxidase and 2 cellobiose ; quinone oxido reductase, involved in cellulose degradation. The high cellulose degradation rates observed in substrates wherein higher levels of lignin degradation occurred, may be due to the greater accessibility of the cellulolytic enzymes in these substrates. Saxena and Rai (1992) have reported that lignin degradation increases the accessibility of native celluloses to cellulolytic enzymes and hence greater the lignin degradation, more the cellulose degradation.

Lignin degrading ability of *Pleurotus* spp. has been reported by many workers (Thayumanuvan, 1982 and Dhanda *et al.*, 1996). In the present study also significant level of lignin degradation occurred on solid substrate fermentation with *Pleurotus* spp. *P. sajor-caju* followed by *P. platypus* showed the maximum lignin degrading ability. *P. sajor-caju* caused 33.9, 57.6 and 76.7 per cent lignin reduction while *P. platypus* caused 31, 45.2 and 72.6 per cent lignin reduction in paddy straw, non retted coir pith and retted coir pith respectively, after 30 days of incubation. The superiority of these two

species in their lignin degrading ability has been reported earlier (Theradinani and Marimuthu, 1991).

Among the different substrates tested, the maximum lignin degradation occurred in retted coir pith (72.1) per cent followed by non retted coir pith (38.2) and paddy straw (26.3). The lignin concentration of the substrates also showed the same trend. This is in confirmation with the report by Chua *et al.* (1983) that the rate of lignin degradation depends upon the initial concentration of lignin.

The rate of lignin degradation was independent of the laccase production confirming the involvement of other lignin degrading enzymes. Savithri and Khan (1994) have reported the production of three peroxidase positive proteins and two glucose oxidases by *Pleurotus sajor-caju* during lignin degradation. Lignin degradation is essentially peroxidative (Kirk and Farrell, 1987 and Boominathan and Reddy, 1992). Gutierrez *et al.* (1994) has reported the production of aryl alcohol oxidase (responsible for H_2O_2 generation) and Mn^2 oxidizing peroxidases by *Pleurotus eringii* during lignin degradation by the fungus.

SUMMARY

SUMMARY

The present study "Enzyme production and composting potential of oyster mushroom (*Pleurotus* spp.) was undertaken to evaluate the comparative ability of four *Pleurotus* spp. viz., Vellayani P_2 isolate, *P. sajor-caju*, *P. platypus* and *P. djamor* in their enzyme (cellulase and laccase) production and lignin and cellulose degrading ability on three different substrates viz., paddy straw, non retted coir pith and retted coir pith. Solid state fermentation (SSF) of the substrates was carried out using the four different *Pleurotus* species. The nature of growth and yield characters of the four *Pleurotus* species on different substrates and the height and weight reduction of the substrates on SSF with the *Pleurotus* spp. was recorded. Cellulose and lignin concentrations of the substrates before SSF as well as at 10, 20 and 30 days of incubation were determined. Assay of cellulase and laccase at different periods (10, 20 and 30 days) of incubation was also carried out. The salient findings of the study are presented below.

Vellayani P_2 isolate among the different *Pleurotus* spp. tested, recorded the fastest growth rate in spawn bottles, completing mycelial growth in 12.33 days.

Among the different substrates tested, paddy straw as well as non retted coir pith supported sporophore production, though the yield obtained on the latter was significantly low. No sporophore production was recorded on retted coir pith.

P. sajor-caju was the most potent cellulase producer as well as cellulose degrader, in the present study. The maximum cellulase production was recorded in paddy straw. Cellulase production on the 20th day of incubation was the highest recorded. The maximum cellulose reduction was recorded in retted coir pith. Cellulose content after 30 days of incubation with *Pleurotus* spp. was the lowest.

P. sajor-caju recorded the highest level of laccase production. Paddy straw, among the different substrates, supported the maximum laccase production. Laccase production on the 20th day of incubation was the highest recorded.

P. sajor-caju, among the different *Pleurotus* spp. tested, was the most efficient lignin degrader. Maximum lignin concentration as well as lignin reduction was recorded in retted coir pith. Highest recorded level of lignin degradation occurred on the 30th day of incubation.

Significantly positive correlation was observed between cellulase production and cellulose degradation, in all the substrates tested. Though positive correlation was observed between laccase production and lignin degradation in paddy straw and retted coir pith, it was not significant.

In the light of the above findings the following conclusions are drawn.

⇒ Cellulase production by *Pleurotus* spp. can be noted as an indication for sporophore production as well as cellulose degradation.

⇒ Laccase production does have a role in fruit body production.

⇒ Cellulose concentration has positive role while lignin concentration has a negative role in biomass production.

⇒ Laccase production cannot be taken as an indicator for lignin degradation rate.

⇒ Higher the lignin concentration of the substrate, higher the proportion of lignin degradation.

⇒ *P. sajor-caju*, among the four species tested in the present study, was the most potent degrader of lignocellulose in all the substrates.

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ENZYME PRODUCTION AND COMPOSTING POTENTIAL OF OYSTER MUSHROOM (*Pleurotus* spp.)

By

P. OWSEPH ANSU

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COLLEGE OF AGRICULTURE
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ABSTRACT

In the present study, solid state fermentation (SSF) of different substrates were carried out using *Pleurotus* spp. to evaluate the comparative efficiency of four *Pleurotus* spp. for their enzyme (cellulase and laccase) production potential and cellulose and lignin degrading ability.

The yield characteristics as well as reduction in height and weight caused by different *Pleurotus* spp. on paddy straw and coir pith (retted and non retted) were recorded. Samples drawn at 10, 20 and 30 days of SSF were subjected to chemical analysis for their enzyme (cellulase and laccase) production and cellulose and lignin reduction. The cellulose and lignin concentration of uninoculated substrates were also estimated.

Paddy straw supported the maximum fruit body production whereas there was no sporophore production in retted coir pith. The highest yield was recorded by Vellayani P_2 followed by *P. djamor*.

The height and weight of all the substrates reduced significantly on SSF, the maximum reduction being recorded in paddy straw followed by that in non retted coir pith and retted coir pith. However no significant difference was observed in the reduction caused by the different *Pleurotus* spp. tested.

The maximum cellulase production was recorded by *P. sajo-caju* on paddy straw on the 20th day of incubation. *P. sajor-caju* caused the highest level of cellulose degradation, the maximum percentage of degradation having occurred in retted coir pith on the 30th day of SSF.

The highest recorded level of laccase production occurred in paddy straw on the 30th day of incubation with Vellayani P_2 isolate of *Pleurotus*. However, among the different *Pleurotus* tested *P. sajor-caju* recorded the maximum level of laccase production.

The maximum percentage of lignin degradation was recorded by *P. sajor-caju* in retted coir pith on the 30th day of SSF.

Correlation worked out between cellulase production and cellulose degradation by different *Pleurotus* spp. was significant positive. However the positive correlation obtained between laccase production and lignin degradation by *Pleurotus* spp. was not significant.