Plant Production, Irrigated Agriculture

CULTIVATION OF *Pleurotus ostreatus* AND *Lentinus edodes* ON LIGNOCELLULOSIC SUBSTRATES FOR HUMAN FOOD AND ANIMAL FEED PRODUCTION

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Abstract

Ligninolytic microorganisms, mainly white root fungi are able to colonize different plant residues and increase the digestibility. Lignocellulosic wastes can also be used for the cultivation of edible mushrooms. Therefore, this study was set up to assess the suitability of wheat straw and sugarcane bagasse supplemented with various levels of wheat bran, as substrates for 2 fungi species. Milled wheat straw and sugarcane bagasse were placed in jars and supplemented with 0%, 5%, 10% and 15% of wheat bran. Deionized water (75%) was added to the jars, which were then sterilized (121°C; 30 min). The substrates were inoculated with mycelium of Pleurotus ostreatus and Lentinus edodes, and then incubated (25°C; 45 days). During the colonization period, the pH, water soluble substances, organic matter (OM) loss, lignin content and in vitro digestibility (IVD) of the substrates were measured. After the colonization period, the jars were placed in an incubator (18°C; RH 80-90%) for fructification. The fruiting bodies were collected until 150 days. The IVD of spent substrate after mushroom cultivation was measured. Generally, the fungi colonized better wheat straw than sugarcane bagasse. P. ostreatus degraded OM of substrate and lignin faster than L. edodes. The highest loss of organic matter of wheat straw after incubation with P. ostreatus and L. edodes for 60 days were 28.9% and 23.7% respectively. The substrate pH was lower on substrate incubated by L. edodes than by P.ostreatus (3.8 vs 4.3). It was corresponding with the water soluble substances of substrates. The digestibility of both substrates was 3.8% - 24.2% higher than the control. The supplementation of wheat bran on the substrates increased the fruiting bodies yield. The yield of P. ostreatus growing on wheat straw with supplementation of 15% wheat bran was highest (8.3% in DM). The in vitro digestibility of spent substrate after cultivation of L. edodes was 68%. The upgrading of lignocellulosic wastes into food and animal feed is possible. However, the appropriate technology for this bioconversion is needed.

Key words: *lignocellulose, lignin, digestibility, animal feed, white rot fungi*

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Introduction

Ligninolytic microorganisms, mainly white root fungi are able to colonize different plant residues (Zadrazil, 1979) and increase the digestibility of the substrate. In addition, lignocellulosic wastes can also be used for the cultivation of edible mushrooms (Zadrazil and Grabbe, 1984). Therefore, the cultivation of white root fungi has beneficial not only for human food but also for animal feed production.

In the present study, the *in vitro* digestibility of wheat straw during fungal colonization and the influence of supplementation of wheat bran on wheat straw and sugarcane bagasse as substrate for fruiting bodies production of *Pleurotus ostreatus* and *Lentinus edodes* were determined.

Materials and Methods

Fungi

The edible fungi of *Pleurotus ostreatus* and *Lentinus edodes* were used in the present study. The fungi were grown on malt extract agar medium at 25°C.

Substrate degradation

Twenty five gram of milled wheat straw (particle size ± 1 mm) were placed in 500 ml erlenmeyer flasks and added with 75 ml of deionized water. The flasks were closed with a cotton stopper and sterilized at 121°C for 30 min. After cooling, three replicates were inoculated with two agar plugs (7-mm diam.) per flask, and incubated at 22, 25 and 30°C for 30 and 60 days. After each incubation period the substrate were dried at 105°C and milled to homogeneity. Loss of organic matter, loss of lignin, pH, water soluble substances and *in vitro* digestibility of the substrates (Tilley and Terry, 1963) were determined.

Fruiting bodies production

Fifty g of substrate (wheat straw and sugarcane bagasse) was placed in 1,500 ml jars. The substrates were supplemented with 5, 10 and 15% of wheat bran. Substrate without supplementation was used as control. Deionized water (150 ml) was added to the jars, which were then sterilized at 121°C for 30 min. Under aseptic condition, four replicates were inoculated with 3 agar plugs per jar, sealed with polypropylene and incubated in the dark at temperature 25°C for 45 days.

After the colonization period, the jars were opened and placed in a light incubator at temperature ±18°C and relative humidity 80-90%. The fruiting bodies were collected until 150 days. The yield of fruiting bodies was determined after drying at 105°C, and was calculated as percent dry matter mass at the original substrate.

Results and Discussion

Loss of organic matter, loss of lignin, pH and water soluble substances

The fermentation process with white root fungi is completed in two stages. At the first stage, the fungus colonizes the substrate and utilizes easily degradable carbohydrates (Zadrazil, 1977). At the second stage, lignin is degraded relatively faster than other components. In this experiment *P. ostreatus* completely colonized the substrate in 12 days, while *L. edodes* colonized the substrates in 14 days under standard aerobic conditions.

Table 1 shows the influence of temperature and incubation time on organic matter decomposition, lignin degradation, pH and water soluble substances. The decomposition of organic matter of wheat straw with *P. ostreatus* was optimal at 30°C, while with *L. edodes* at 25°C. The highest loss of organic matter of wheat straw after incubation with *P. ostreatus* and *L. edodes* for 60 days were 28.9 and 23.7% respectively.

The trend of lignin degradation is similar to that loss of organic matter. The highest lignin decomposition occurred in substrate fermented with *P. ostreatus* (55.9%) at 30°C for 60 days. At 30°C, lignin decomposition began earlier than at 22 or 25°C, therefore, at highest temperatures, more lignin was decomposed than at lower temperature.

Table 1. Loss of organic matter (LOM), lignin degradation (LD), pH and water soluble substances (WSS) of substrates after fermentation with *P. ostreatus* and *L. edodes* at 22, 25 and 30°C for 30 and 60 days.

Fungi	Days	Temp. °C	LOM (%)	LD (%)	pH	WSS (%)
P. ostreatus	30	22	11.8	17.8	4.4	14.1
		25	12:8	26.8	4.3	14.2
		30	15:3	33.1	4.5	13.8
	60	22	25.2	39.5	4.4	18.8
		25	28.0	49.5	4.5	17.1
		30	28.9	55.9	4.7	17.5
L. edodes	30	22	10.1	12.3	3.8	17.6
		25	10.5	21.3	4.3	19.0
		30	13.4	29.9	4.5	19.4
	60	22	21.2	39.6	3.9	25.5
		25	23.7	46.4	4.1	26.4
		30	20.4	44.5	4.1	19.7

During fermentation the pH of substrate decreased until 4.3 (*P. ostreatus*) and 3.8 (*L. edodes*). After 30 days incubation with *P. ostreatus*, the concentration of water soluble substances in the substrate had decreased, due to mycelium production, but the concentration increased after 60 days of incubation. With *L. edodes*, the water soluble substances had increased after fermentation for 30 and 60 days.

In vitro digestibility

In vitro digestibility is the important parameter to determine the feed quality. The *in vitro* digestibility of wheat straw without fermentation was 45.8%. After fermentation with the both fungi, the *in vitro* digestibility increased (Table 2).

Table 2. Change of *in vitro* digestibility (ΔIVD) and process efficiency (PE) of substrate after fermentation with *P. ostreatus* and *L. edodes* at 22, 25 and 30°C for 30 and 60 days

Fungi	Days	Temperature °C	ΔIVD (%)	PE*
P. ostreatus		22	+ 4.6	0.39
		25	+ 5.2	0.34
		30	+ 5.6	0.20
		22	+ 10.6	0.90
		25	+ 11.1	0.73
		30	+ 3.8	0.14
L. edodes	30	22	+ 13.4	1.32
		25	+ 9.1	0.68
		30	+ 22.3	0.94
	60	22	+ 24.6	2.44
		25	+ 23.6	1.76
		30	+ 24.2	1.02

* PE is calculated from change of *in vitro* digestibility divided by loss of organic matter.

In general, the *in vitro* digestibility of the substrates after fermentation with *L. edodes* was higher than with *P. ostreatus*. The change of *in vitro* digestibility correlated with the increasing of the incubation time. But, there was no correlation between the temperature and the change of *in vitro* digestibility. It was also difficult to make correlation between the loss of lignin and the change of digestibility. The amount of lignin decomposed does not always correlate with a change of digestibility (Zadrazil, 1980).

The process efficiency for *in vitro* digestibility decreased with the increasing of the incubation time, but decreased with the increasing of the temperature. The highest process efficiency (2.44) occurred in fermentation with *L. edodes* at 22°C for 60 days.

Yield of fruiting bodies

The first fruiting bodies of *P. ostreatus* were formed after 57 days on sugarcane bagasse and 103 days on wheat straw The first fructification was earlier by the supplementation with wheat bran. The *L. edodes* grew well on wheat straw and produced the fruiting bodies after 86 days of inoculation, however the fungus did not form fruiting bodies on sugarcane bagasse.

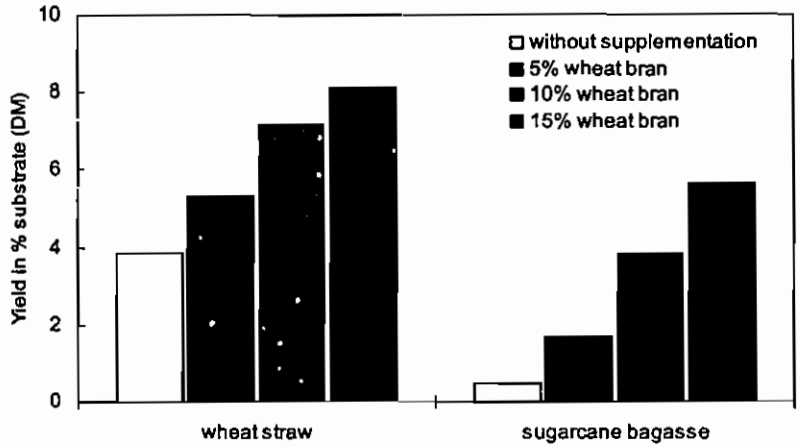

Figure 1. Effect of supplementation of wheat bran on fruiting bodies yield of *P. ostreatus* growing on wheat straw and sugarcane bagasse.

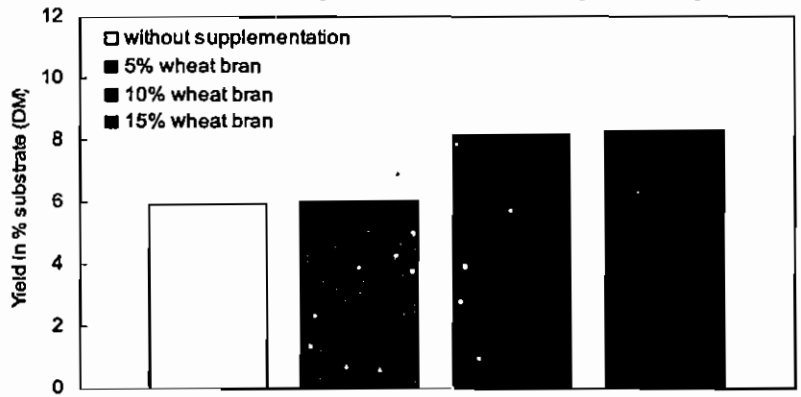

Figure 2. Effect of supplementation of wheat bran on fruiting bodies yield of *L. edodes* growing on wheat straw.

The supplementation of wheat bran increased the yield of fruiting bodies of *P. ostreatus* and *L. edodes*. The lowest yield of *P. ostreatus* (0.5%) was obtained using sugarcane bagasse and increased to 5.7% after supplementation with 15% wheat bran. The yield of *P. ostreatus* growing on wheat straw with supplementation of 15% wheat bran was higher (8.2%), this result related with Permana *et al.* (2000). The yield of *L. edodes* growing on wheat straw supplemented with 15% wheat bran was 8.3%, as compared with substrates not supplemented (5.9%).

In vitro digestibility of spent substrate

As shown in Table 3, the *in vitro* digestibility of substrate after cultivation of *L. edodes* was significantly higher compared to the digestibility of substrate after cultivation of *P. ostreatus*. The best value of digestibility was 68.0% in used wheat straw with addition of 10% wheat bran. The addition of wheat bran increased the *in vitro* digestibility of spent substrate.

Table 3. *In vitro* digestibility of spent substrate after mushroom cultivation.

Mushroom	Level of Wheat bran (%)	Substrate	
		Wheat straw	Sugarcane bagasse
P. ostreatus	0	47.4^{a}	13.8^{a}
	5	50.2^{b}	21.5^{b}
	10	56.2^{d}	28.7^{d}
	15	54.1^{c}	25.1^{c}
L. edodes	0	65.4^{b}	48.9^{a}
	5	65.4^{b}	51.2^{b}
	10	68.0^{a}	57.4^{c}
	15	62.0^{c}	55.7^{c}

Values with different subscripts in the same column are significantly different ($p<0.05$).

Conclusions

The fermentation of wheat straw with *P. ostreatus* and *L. edodes* increased the *in vitro* digestibility. The supplementation of wheat bran on wheat straw and sugarcane bagasse increased the yield of the mushroom.

References

Permana, I.G., G. Flachowsky, U. ter Meulen and F. Zadrazil. 2000. Use of Sugarcane Bagasse for Mushroom and Animal Feed Production. Proceeding of the 15th International Congress of the Science and Cultivation of Edible Fungi. Maastricht, the Netherlands. 15-19 May 2000. 385-390.

Tilley, J.M.A. and R.A. Terry. 1963. A Two Stage Technique for *in vitro* Digestion of Forage Crops. J. Br. Grassl. Soc. 18: 104-111.

Zadrazil, F. 1977. The Conversion of Straw into Feed by Basidiomycetes. Eur. J. Appl. Microbiol. 4: 291-294.

Zadrazil, F. 1979. Umwandlung von Pflanzenabfall in Tierfutter durch höhere Pilze. Mushroom Sci. X (Part I): 231-241.

Zadrazil, F. 1980. Conversion of Different Plant Waste into Feed by Basidiomycetes. Eur. J. Appl. Microbiol. Biotechnol. 9: 243-248.

Zadrazil, F. and H. Grabbe. 1984. Solid State Fermentation of Lignocellulose Containing Plant Residues with *Sporotrichum pulverulentum* Nov. and *Dichomitus squalens* (Karst). Eur. J. Appl. Microbiol. Biotechnol. 16: 45-51.

The Solubilization of Macrominerals and Ruminal Degradation of Selected Tropical Tree Legumes

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Abstract

A research to study about macromineral solubilities and biadegradation of some tree legumes by rumen microbes has been conducted. The legumes of Pteracarpus indicus (PI), Sesbania grodiflora (SG), Gliricidia sepium (GS), Callyandra callatyrsus (CC) and Leucoena leucocepholo (LL) were used in this experiment. The oven dried (60°C) and ground samples of the legumes were measured of their in vitro macrominerals solubilities, biodegradation, bioavailability, and fermentation products. The macrominerals (Calcium (Ca), phasphorus (P), magnesium (Mg) and sulfur (S)) solubilities were determined using atomic absorption spectraphotometer (AAS). The gas production was measured using Hohenheim method. The ruminal DM degradation and gas productions rates were calculated using formula $y = a + b(1-e^{-ct})$ according to Ørskov and McDonald (1979). The results showed that biodegrodation and cumulative gas production of selected tree legume were relatively the same. However, the gas production rate of SG and GS were significantly higher. There was no difference on VFA production, but SG produced more NH_3 than other tree legumes. Co was more soluble than other macrominerals. The Ca and Mg solubility of LL were siginificantly higher, while PI was a good soluble P source. GS is a good protein source and can be mixed with other legume as mineral supplement.

Key Words: tree legume, solubility, macrominerals, degradation,

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