Cultivation of *Lentinula edodes* on mixture of cassava bagasse and sugarcane bagasse

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SUMMARY

Potential of the production of *Lentinula edodes* (shiitake) mushrooms on agro-industrial residues, such as cassava bagasse and sugarcane bagasse, is evaluated under solid state fermentation. Visual observations in flaskes and also plastic bags indicated good growth on these substrates. The fructification and biological efficiency of the process was found to be good. Best results for basiodiocarp production were evident in the medium containing 80 % cassava bagasse + 20 % sugarcane bagasse (w/w). Data on kinetics of starch consumption and protein synthesis showed the consumption of 77.42 % starch during the biotransformation process, with three times increase in protein content. Fructification was achieved after 60 days of inoculation and it amounts to decrease by 7 times, as compared to the conventional process of the production on oak tree logs. The results provide a novel alternate technology for shiitake production on the mixture of cassava bagasse and sugarcane bagasse.

Keywords: *Lentinula edodes* , shiitake , solid state fermentation, cassava bagasse, sugarcane bagasse, fructification, basidiocarp production, process cycle, kinetics, biological efficiency.

RESUME

Culture de *Lentinula edodes* sur un mélange de résidus fibreux de manioc et de bagasse de canne à sucre.

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La possibilité de produire des *Lentinula edodes* (shiitake) sur des résidus agro-industriels, comme la bagasse de manioc et la bagasse de canne à sucre, par fermentation en milieu solide, est explorée. Les observations morphologiques effectuées sur des cultures en flacon ou en sac en plastique permettent d'estimer une bonne croissance sur ce type de substrat. La fructification et l'efficacité biologique du procédé sont déclarés satisfaisants. Les meilleurs résultats pour la production de basidiocarpes sont obtenus en employant un milieu contenant 80% de bagasse de manioc et 20% de bagasse de canne à sucre. Des résultats concernent la cinétique de la consommation de l'amidon contenu dans la bagasse de manioc parallèlement à celle de la synthèse de protéines. Pendant la biotransformation, une consommation d'amidon, estimée à 77,42%, est associée à une synthèse de protéines trois fois plus abondante que celle couramment décrite. La fructification est achevée au bout de 60 jours d'incubation et a été diminué de 7 fois si l'on compare au procédé conventionel de production sur des troncs de chênes. Ce temps de fructification s'avère singulièrement réduit. Sa durée s'avère le septième de celle couramment décrite pour la culture faite sur résidus de chênes. Ces résultats ouvrent une nouvelle voie pour la production de *Lentinula* en développant une nouvelle stratégie de culture sur un milieu innovant, un mélange de bagasse de manioc et de bagasse de canne à sucre.

Mots-clés : Shiitake, *Lentinula edodes*, fermentation en milieu solide, bagasse de manioc, bagasse de canne à sucre, fructification, production de basidiocarpes, cycle de production, cinétique, efficacité biologique.

INTRODUCTION

The goal of this present paper will be the elaboration of a new alternative for the solid waste from raw cassava and sugarcane residues, agro-industrial residues produced in Paraná State, Brazil, to allow the edible mushroom *Lentinula edodes* (shiitake) cultivation.

In nature, the shiitake mushroom grows on trunks of the Fagaceae family, mainly *Quercus acutissima* and *Quercus errata* (oak tree) (Lee, 1980). This fungus is the most important edible mushroom cultivated in Japan (Tokimoto and Tomatsu, 1978). It is popular, for its excellent organoleptical properties and nutritional quality as well as presence of all the essential aminoacids. It also possesses therapeutical properties due to the production of specific polysacharides which have antitumoral activities (Chiyokichi, 1990). Some adenine derivatives that are able to reduce the cholesterol and some substances capable of stimulating the immunological system are also reported (Chiyokichi, 1990).

The traditional cultivation of this mushroom is on the trunks of the trees specified above and the fructification occurs after about sixteen months (Lee, 1980).

In Brazil, oak trees do not grow and, therefore, searching for an altenative substrates and also to reduce the cultivation period may constitute a good opportunity to upgrade some of the potential agro-industrial residues of Paraná State. It is estimated that the cassava crop increased by 55.86 % and the sugarcane crop increased 25.81 % during the 91/92 and 92/93 harvests, respectively, when compared with the late five harvests. Currently, 770,458 tons/year of solid wastes from raw cassava and 3,840,197 tons/year of sugarcane residue is generated in Brazil (Seab, 1993). The upgradation of these agro-industrial residues in to mushroom with economical, nutritional and therapeutical properties justifies development of process concerning large scale production of *Lentinula edodes*.

In this paper, the results concerning the inocula production, substrate preparation, fructification and the biological efficiency are reported.

MATERIALS AND METHODS

MICRORGANISM AND SUBSTRATES

Lentinula edodes LPB 031 from the Biotechnological Processing Laboratory of the University was used. In this paper, two by-products were i.e. cassava bagasse (solid fibrous waste) and sugarcane bagasse. Wheat grain was used for comparative purposes.

INOCULA PRODUCTION

It was carried out in plastic bags containing 5 g (or 15 g, when necessary) of wheat grain, moistened until 60 %. The plastic bags were autoclaved for 15 min. at 121° C (Tan and Chang, 1989) and, after cooling, these were inoculated with discs (6-mm diam) of wheat extract agar containing sufficient mycelium of *Lentinula edodes* LPB 031 in an advanced growth stage. The plastic bags were incubated at 23°C for 15 days.

SUBSTRATE PREPARATION

Before using, the cassava bagasse and the sugarcane bagasse were dried in an air oven at 65° C for 24 h. After drying, these were sieved and, the fraction between 2 and 0.8 mm was used (Soccol, 1994). For visual evaluation of the mycelium, the wet substrate (approximately with a moisture content of 70 %) was weighted to 50 g each, placed in glass flasks, and autoclaved at 121°C for 1 h.

PLASTIC BAG PREPARATION

To evaluate the biological efficiency, the cassava bagasse was used alone or in combination with sugarcane bagasse at a level of 20 to 80 % proportionally. It was placed (150 g) in each autoclavable plastic bag. They were moistened with distilled water until 70 % moisture and autoclaved for 1 h at 121° C.

INOCULATION, INCUBATION AND FRUCTIFICATION

Inoculation was at 10 % for each substrate type and was performed in a aseptic chamber. The inoculated flasks were incubated at 23°C for 40 days. The plastic bags containing different proportions of solid wastes were incubated at 23° C for 60 days and, after this period, these were transferred and opened in a cultivation chamber, maintained at 18°C and 80 and 90 % relative humidity. The basidiocarps were removed at the opening time and were weighted using a semi-analytical scale balance.

ANALYTICAL ASPECTS

Moisture content, ash, fats, starch, proteins, fibres and total caloric values of the mushroom were determined according to Nadial (1990).

RESULTS AND DISCUSSION

VISUAL EVALUATION OF THE MYCELIUM GROWTH IN FLASKS

The mycelium growth was followed during 40 days after the inoculation (Fig. 1). After 30 days, the mycelium had spread over the substrates totally. The mycelium mass formed on the cassava fibrous residue was more dense than that on the sugarcane bagasse. On the 40th day, the solid fibrous wastes from cassava began to change its color to brown, thereby reflecting the basidiocarpus development of *Lentinula edodes* (Olivier *et al*, 1991). Consequently, the ability of filamentous fungi to growth can be measured visualy and the degree of colour change (due to the growth of fungi) can be used as an indicator.

VISUAL EVALUATION OF THE MYCELIUM GROWTH IN PLASTIC BAGS

The exact moment to remove the plastic bags from the culture room was determined by the mycelium characteristics. This was found to vary with the substrate.

A

a b c d

B

Fig. 1. A comparison of the mycelial growth of *Lentinula edodes*, cultivated during 40 days, on different supports. A : cassava fibrous waste and B : sugar cane bagasse (a : 0 day; b : 10 days; c : 20 days and d : 40 days).

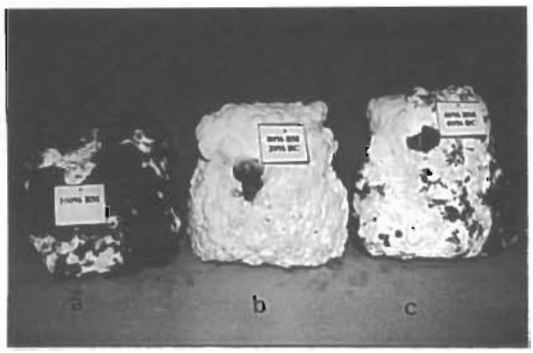

Fig. 2 - Mycelium of *Lentinula edodes* on the substrate surface of different solid support after 60 days inoculation (a : 100 % cassava bagasse; b : 80 % cassava bagasse + 20 % sugar cane bagasse; c : 60 % cassava bagasse + 40 % sugar cane bagasse).

After 60 days of inoculation and incubation, it was observed that the dense white mat on the substrate surface started turning progressively brown. Until today, this fact constituted the best indicator to remove the plastic bags from the culture room (Fig. 2).

FRUCTIFICATION AND BIOLOGICAL EFFICIENCY

The early basidiocarps appeared 65 days after the inoculation start (5 days after the plastic bags removal). But, the time varied between 70 to 90 days based on the substrate used and their combination. The composition of the substrates is given in Table 1.

Table 1. Chemical composition of cassava and sugar cane bagasse (g/100 g).

	Cassava bagasse	Sugarcane bagasse
Humidity	9.03	5.89
Ashes	1.70	1.80
Fats	0.21	0.62
proteins	1.26	0.70
Fibres	12.61	34.81
Carbohydrates	75.61	56.19
Total caloric value*	307.84	233.10

*Kcal/100 g

According to Soccol (1994), the substrate texture has an influence on the fructification. It was observed that the substrate prepared with cassava bagasse only was toot dense and thick, thereby resulting in a reduction in the yield of *Pleurotus ostreatus* 22 basidiocarps. In order to optimize the texture, it was mixed with different quantities of sugarcane bagasse as it has high fibre content and is available abundantly in the State of Parana.

Fig. 3. Basidiocarps of *Lentinula edodes* grown on 100 % cassava bagasse.

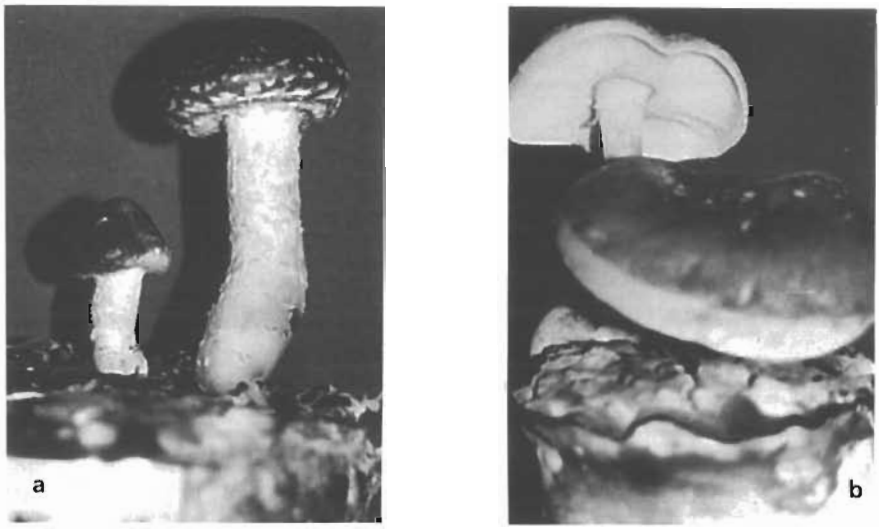

Fig. 4. Basidiocarps of *Lentinula edodes* developed on the medium containing 80 % cassava bagasse (a : first crop; b : second crop).

The fructification occurred in 100 % cassava bagasse medium (Fig. 3), medium containing 80 % cassava bagasse + 20 % sugarcane bagasse (Fig. 4) and, the medium containing 60 % cassava bagasse + 40 % sugarcane bagasse (Fig. 5). Contrastly, there was no trace of any basidiocarpus development in the medium containing 40 and 20 % of cassava bagasse (results not shown).

Fig. 5. Basidiocarps of *Lentinula edodes* grown on medium containing 60 % cassava bagasse.

Table 2. Biological efficiency of the process.

Dried substrate (g)					
Cassava bagasse (%)	Cassava bagasse (g)	Sugar cane bagasse (g)	Inoculum (g)	Total weight (g)	B.E. (g)
100	150	-	15	59.11	39
80	120	30	15	109.37	72
60	90	60	15	57.36	38
40	60	90	15	-	-
20	30	120	15	-	-

B.E. : Fresh weigth of the fructification bodies/Dry weigth of the substrate used

Better biological efficiency was obtained when the substrate used was composed of 80 % cassava bagasse and 20 % sugar cane bagasse (Table 2). Fig. 6 shows the kinetic evolution of starch consumption and protein synthesis before and during the 60 days incubation of the fructification substrate containing 80 % cassava bagasse and 20 % sugar cane bagasse.

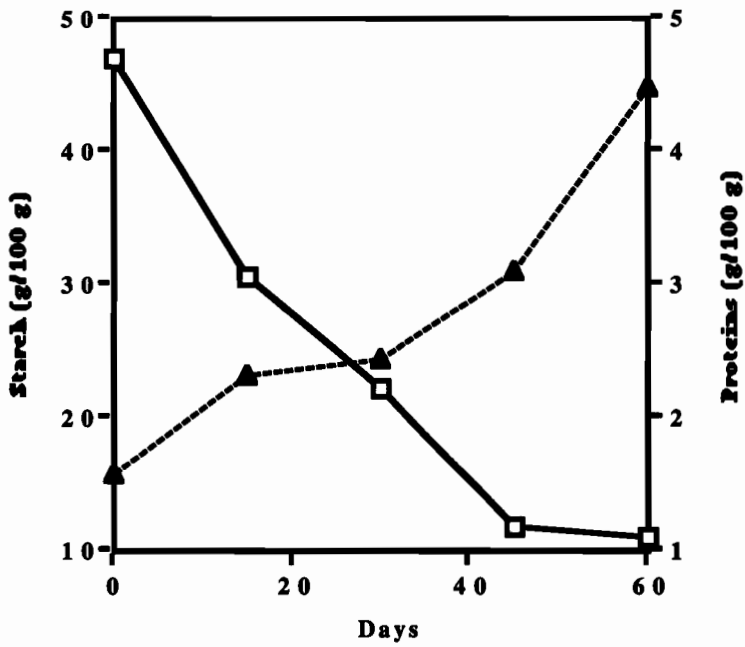

Fig. 6. Evolution of starch consumption and protein synthesis of the fructification substrate, containing 80 % cassava bagasse and 20 % sugar cane bagasse.

The results show that 77.42 % of the starch contained in the substrate has been consumed during the biotransformation. After 60 days, the protein content was nearly tripled.

According to Moyson and Verachter (1991), though shiitake grows on logs, the lignine degradation is considered as a secondary metabolism, beginning only on the 42nd day after the mycelium inoculation, when the fungus is inoculated into substrate containing a large quantity of polysaccharids. According to Leatham (1986), the enzymatic degradation of starch, through amylase liberation by the

mycelium, is stronger in the beginning of the incubation, and *Lentinula edodes* is an effective starch degrading agent. Data obtained confirmed the results of these authors.

After 60 days from inoculation, the fructification started. The fructification starts when a limit in nutrients has been reached, and it happens faster on substrates containing easily-degradable carbohydrates than on logs.

The fructification time on this substrate has been reduced by about 7 times as compared to the growth on logs.

CONCLUSION

It is possible to use cassava bagasse and sugarcane bagasse to efficiently cultivate *Lentinula edodes.* Change in colour during the fungal growth provides an interesting technological alternative to use these by-products. When compared with the traditional method, the most effective medium contained 80 % cassava + 20 % sugarcane bagasse. It reduced the cultivation cycle of *Lentinula edodes* by seven times. This method could be more economic than the presently followed method.

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