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Sunflower seed hull based compost for *Agaricus blazei* Murrill cultivation

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ABSTRACT

Agaricus blazei Murrill is actually one of the most promising mushrooms species. An adaptation from the traditional biphasic compost fermentation method for *Agaricus bisporus* cultivation has been used for its cultivation. To make mushroom production profitable, the selection of compost materials from each region is essential. Sunflower seed hulls are an abundant lignocellulosic waste from the edible oil industry. It has been successfully used in the cultivation of other specialty mushrooms; however, there are no published reports on its use as part of *Agaricus* spp. compost. There is still no agreement about the usage of lignin by *A. bisporus*, and in the case of *A. blazei* there is no published data. This work presents a substrate formulation (50.0% sunflower seed hulls, 41.0% wheat straw, 4.5% wheat bran, supplements and additives) which after composting was assayed to evaluate the performance of *A. blazei* cultivation. Different types of containers, *i.e.* polyethylene bags (2.5 and 4.0 kg substrate, 0.08 m^2) and plastic trays (3.5 and 4.5 kg substrate, 0.12 m^2), in two independent trials, were also evaluated. It was demonstrated that the obtained compost was appropriate for the cultivation of *A. blazei* yielding BE ranging from 30% to 47%, depending on the container and substrate mass, being highest with polyethylene bags containing 2.5 kg substrate. In this case study, lignin accumulated during the composting process, but an important reduction was observed during the cultivation (58% on average), confirming the ability of this mushroom to degrade lignin; thus making it possible the access to nutrient sources of cellulose and hemicellulose.

1. Introduction

Agaricus blazei Murrill ss. Heinemann or *Agaricus brasiliensis* ss. Wasser (Wasser et al., 2002) is one of the most promising and studied mushroom species due to its nutritional and medicinal value (Mizuno, 2002). As with *Agaricus bisporus* (white button and portobello), *A. blazei* can also be cultivated on previously degraded materials obtained through a two-phase fermentation process, making materials selective for the mushroom cultivation (Rinker, 1986).

Many agro-industrial wastes are lignocellulosic materials and are able to be employed in *Agaricus* compost formulations. For *A. bisporus*, the most widely employed materials are wheat straw, stable bedding and poultry bedding. Generally, a compost consists of a voluminous lignocellulosic component with high carbon content and low nitrogen content, in addition to other concentrated components (usually brans and meals) in appropriate quantities to reach C:N rates of 30:1 in the case of *A. bisporus*, and 37:1 for *A. brasiliensis* (Eira, 2003).

Selection and management of starting ingredients and proper composting conditions make growing *Agaricus* species so demanding (Sánchez, 2004). A cost efficient use of lignocellulosic residuals as substrate for mushroom cultivation requires that they are abundant and available in the mushroom production region. Sunflower seed hulls (SSH), an important edible oil industry waste, is high in lignin content (25.2%) (Conghos et al., 2006); thus, making it difficult to be degraded by microorganisms and not useful for animal feed. Mushrooms secrete a multienzyme complex that is able to attack the lignin with the collaboration of free radicals (Boominathan and Reddy, 1992). Cultivation of different edible mushrooms, *i.e. Pleurotus* spp., *Lentinula edodes*, *Ganoderma lucidum* and *Hericium erinaceus*, using SSH as main substrate was previously demonstrated (Curvetto et al., 1998, 2002a,b; González Matute et al., 2002; Figlas et al., 2007); however, there are no published reports on the use of SSH as part of *Agaricus* spp. compost. This waste has as its main advantage a content of 5% lipids, which were reported as good growing factors (Guerin-Laguette et al., 2003), and an appropriate particle size enabling substrate porosity for the development of an adequate aerobic fermentation during the composting.

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Substrate mass and container size can affect mushroom yield. Braga and Eira (1999) increased *A. blazei* yield by 48% by doubling the substrate mass (30 kg/m^2–60 kg/m^2) in plastic trays (0.35 cm × 0.48 cm, 0.40 cm height). Bisaria et al. (1989) have shown the importance of an optimum size of containers in terms of area per unit volume for obtaining maximum yield of *Pleurotus pulmonarius* on different cylindrical wire mesh structure system diameters, containing different substrate volumes.

Similarly to white-rot mushrooms, *Agaricus* spp. can also degrade the lignin contained in compost lignocellulosic materials by activating the production and release of ligninases. It has been argued that the main purpose of the lignin degradation is to increase bioavailability of the underlying holocellulose (Ten Have and Teunissen, 2001). *A. blazei* was informed to have a high lacasse activity during vegetative phase (Ulrich et al., 2005).

In general, there is agreement that either the cellulosic or non-cellulosic polysaccharides of the wheat straw are used for the growth of *A. bisporus*. Nevertheless, the situation for lignin, the second more abundant component of the organic compost, is not clear. Iiyama et al. (1994) informed that the relative content of compost lignin increased as much during the composting as in the growth of *A. bisporus*, and that the chemical structure of the lignin was altered by condensation reactions and oxidation. However, several researchers (Waksman and Nissen, 1932; Muller, 1967; Gerrits, 1968; Wood and Leatham, 1983; Durrant et al., 1991) obtained evidence that indicates a compost lignin loss during *A. bisporus* growth.

The purpose of this study was to evaluate a substrate formulation containing SSH to obtain a compost capable to support a complete growth cycle of *A. blazei* with acceptable yield performance; also examine the use of different types of substrate containers and substrate masses and deep into the lignocellulose biodegradation in order to unequivocally decide if lignin is used efficiently as a nutrient by *A. blazei*, as well as to know the evolution and interrelation of the cellulose and hemicellulose. The results show an adequate compost for the cultivation of this mushroom with similar yields to those obtained with other commonly employed materials, and with yields higher when using polyethylene bags rather than trays as containers. It is also confirmed the lignin degradation by this mushroom during its cultivation.

2. Materials and methods

2.1. Mushroom strain

A. blazei Murrill was graciously supplied by Edison De Souza of Brasmicel, SP, Brazil. In commercial practice, this strain is a fast growing mushroom with good marketable size.

2.2. Preparation and conservation of the mushroom spawn

The mycelium culture was maintained in glass tubes with MYPA (20 g malt extract, 2 g yeast extract, 1 g peptone and 20 g agar, per liter) medium and covered with sterile liquid vaseline® at room temperature until use. A nutrient medium (CDYA) was prepared using the filtrate resulting from boiling 500 g of Phase II SSH based compost in 1 L of water and adding 2 g yeast extract, 20 g glucose and 20 g agar, per liter (González Matute, 2009). The medium was adjusted to pH 6.5 with HCl and sterilized at 1 atm for 30 min. *A. blazei* mycelium was inoculated on Petri dishes containing the nutrient medium and incubated in darkness at 25 °C for 10–15 days, at which time mycelium cultures were ready to be used to prepare spawn.

Wheat grain (250 g) was put into a 1 L glass bottle and 1.3% of $CaCO_3$ and 190 mL of water were added and allowed to stand overnight. The excess liquid was drained and the resulting mixture was autoclaved at 1 atm for 1.5 h. After mycelium inoculation (16 cm^2 of CDYA colonized), the spawn was incubated at 25 °C in darkness for 30 days, with occasional inspections and shakings, every 4 days until complete grain separation.

2.3. Trials

Two consecutive trials under similar environmental conditions, during the same time of the year (spring-summer) were carried out. Biological materials, compost formulation and containers used were the same.

2.4. Composter

Substrate was prepared in a 600 L plastic tank (97 cm diameter and 112 cm of height). Top 27 cm of the tank consisted of a 55 cm diameter neck (Fig. 1). The tank was cut lengthwise in the middle, hinged on one side and with three quick locking snaps on the other one. Six wheels were located under its base to facilitate its movement. Inside, the material was placed on a wire mesh located 10 cm from the bottom of the tank. The bottom had 3 cm diameter opening to permit air to enter. On top, a cover with holes allowed an outlet for vapor and hot air exhaust.

2.5. Compost preparation

The substrate formulation for composting consisted of: sunflower (*Hellianthus annus* L.) seed hulls (50.0%) (supplied by Cargill S.A.), wheat (*Triticum aestivum* L.) straw (*ca.* 5 cm length) (41.0%), wheat (*T. aestivum* L.) bran (4.5%), ammonium sulfate and urea (0.35% each) and gypsum and calcium carbonate (1.9% each). The initial N concentration of the formula, analyzed by Kjeldahl method, was 1.2% and the C/N ratio was 37, as recommended by Kopytowski Filho and Minhoni (2004).

Ingredients were homogeneously mixed and moistened (65%–70%) during an hour with half of the ammonium sulfate and urea supplements dissolved in water. Then, the composter was filled with approximately 180 kg of wet materials and placed in a chamber with isolated walls and controlled temperature, which varied, according to the composting phase requirements, between 25 °C and 60 °C. Whenever needed, temperature was adjusted using two 1600 W halogenous stoves (SIAM 1600, Argentina) connected to a thermostat.

Fig. 1. Plastic tank (600 L) adapted to compost materials for *Agaricus* spp. mushrooms cultivation on a small scale, before the first turn.

During Phase I, on days 4 and 5 for the first and second trial respectively, the composter was opened (first turn) and its content manually mixed with forks, and moistened (to restore humidity lost) with the other half of the nitrogen supplements dissolved in the water. Two more turns were carried out, on days 7 and 9, and at days 11 and 13, for the first and second trial respectively (water was added if needed). Afterwards, the pasteurization and conditioning (Phase II) was initiated. During this phase, the temperature was slowly increased (5 °C/h) until 55 °C–58 °C was reached. This temperature was maintained for 48 h; then, it was lowered to 45 °C–50 °C and kept until an ammonia scent was no longer perceptible. Phase II lasted 16 days in both trials.

2.6. Inoculation and incubation

Plastic trays (32 cm wide, 39 cm length and 24 cm height) and polyethylene bags (100 μm; 32 cm diameter) were used as experimental units. Thirteen and 12 containers of each type – for the first and second trial, respectively – were filled and inoculated with spawn at a 5% rate (on a fresh weight basis). During the first and second trial, trays were filled with 3.5 kg and 4.5 kg of Phase II compost, respectively, and covered with a plastic sheet; bags were filled with 2.5 kg and 4.0 kg, respectively, and closed with a cotton neck. Both, trays and bags were incubated at 25 °C in darkness.

2.7. Casing

When substrate was completely colonized (12 days post inoculation), a 3–4 cm layer of *Sphagnum* Peat Moss and $CaCO_3$ 1:1 (v/v) with addition of 65% of water were applied on top of the substrate. Final moisture and pH of the mixture was 79% and 7.25, respectively. Containers were placed back in the incubation room.

2.8. Fruiting

Once the surface of casing material was completely covered with the mushroom mycelium (9–13 days post casing), experimental units were exposed to a fruiting inducing environment,

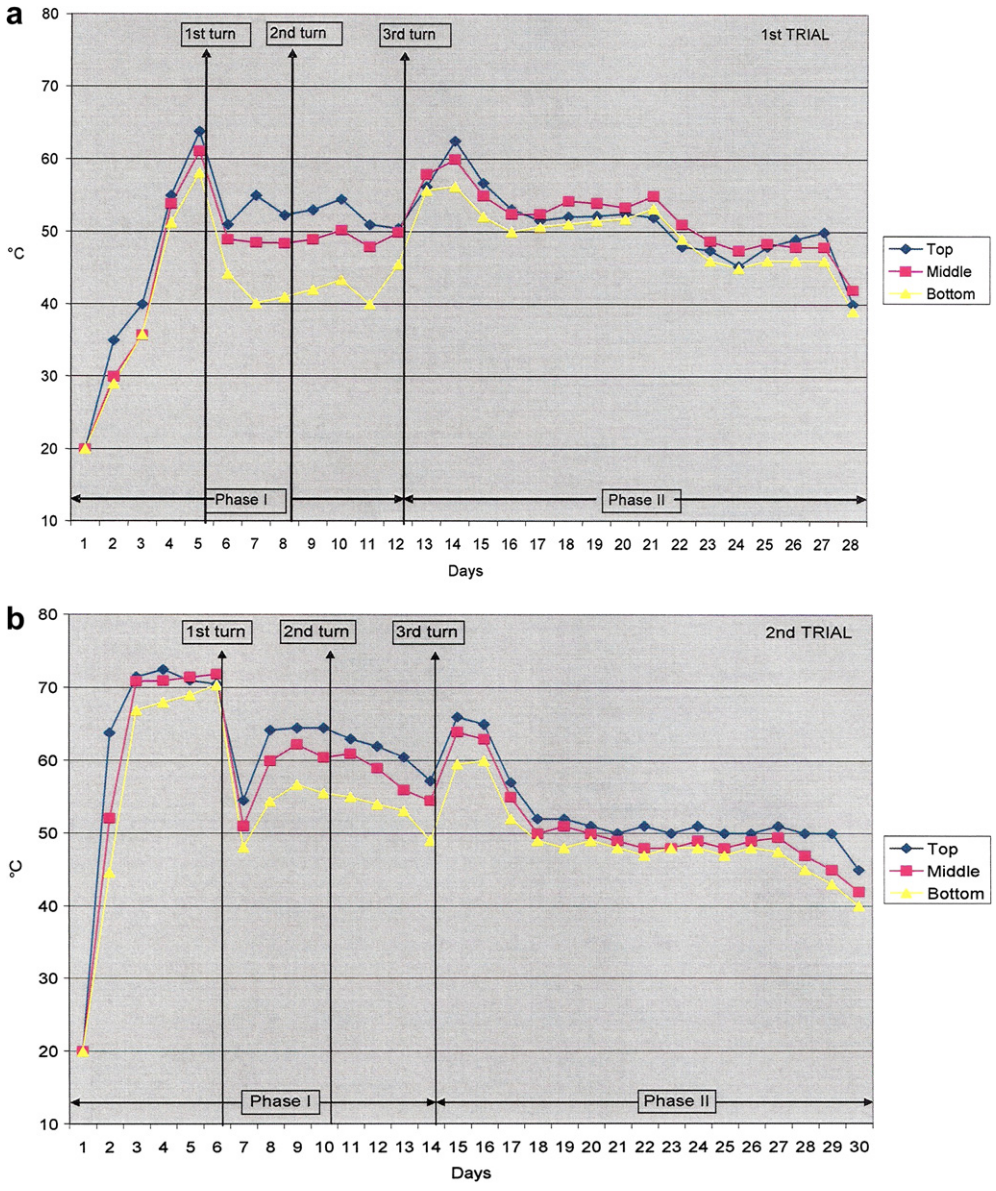

Fig. 2. Compost temperatures (°C) measured at different compost depths during composting for mushroom cultivation in two independent trials, first (A) and second (B).

Table 1
Evolution of compost in a sunflower hulls based substrate in an adapted plastic tank. Average values for pH, variation of electrical conductivity (ΔEC), moisture content (% H_2O, measured prior to water addition), and total nitrogen (%N total) are given for the different stages and for the two independent trials.

Stage	Trial	pH	ΔEC*	%H_2O	%N total
Start	1	6.4 ± 0.16	–	67 ± 2.0	1.2 ± 0.32
	2	6.4 ± 0.33	–	69 ± 2.2	1.2 ± 0.32
1st turn	1	7.8 ± 0.32	1.1 ± 0.14	66 ± 1.8	–
	2	8.0 ± 0.44	1.2 ± 0.10	72 ± 3.1	–
2nd turn	1	7.9 ± 0.25	1.6 ± 0.12	70 ± 2.3	–
	2	8.0 ± 0.31	1.6 ± 0.09	72 ± 1.7	–
3rd turn	1	7.8 ± 0.36	1.3 ± 0.32	71 ± 1.4	–
	2	7.9 ± 0.25	1.7 ± 0.27	71 ± 1.8	–
End of phase II	1	7.4 ± 0.28	1.4 ± 0.26	75 ± 2.6	1.9 ± 0.26
	2	7.5 ± 0.30	1.8 ± 0.22	70 ± 2.4	1.8 ± 0.16

*EC variation referred to the initial value (2350 and 2570 μS/cm, first and second trial, respectively).

i.e. 85%–90% R.H., a thermal amplitude of 20 °C–30 °C (Eira, 2003) and light (300 lux, measured with a Exposure Photometer, mod. 200-M, Photovolt Corporation, NY, U.S.A.), in an environmental automatically controlled plastic growing house. Experimental units were completely randomized distributed along the room (6.7 m × 10.0 m × 3.0 height, 200 m^3) on shelves.

After 5–7 days, watering began and it was applied whenever necessary (usually once a day). Mushrooms were manually harvested at maturity and before cap opening, and were cleaned, counted and individually weighed. Yields from first flush and at the end of two flushes were calculated in terms of percentage of biological efficiency (%BE) ([weight of fresh mushrooms/weight of dry substrate at spawn] × 100) and percentage mushroom production (%MP) (kg of fresh mushrooms/100 kg of fresh substrate at spawn).

2.9. Compost characterization

During compost turnings and at the beginning and end of each phase, three compost samples were randomly taken to determine moisture content, and measure pH and electrical conductivity (EC) values. Total N concentration was also analyzed at the end of Phase II by Kjeldahl method. The pH and the EC values were obtained from the liquid resulting after the extraction of compost with water (1:2 v/v). Resulted values for each trial were expressed. During the composting process, the compost temperatures were daily and manually recorded, with a spear type thermometer, at different compost depths (40 cm, 25 cm and 5 cm from top surface). Lignin, cellulose and hemicellulose on the compost/substrate were determined (×3) by the Van Soest acid detergent fiber method (Van Soest, 1973), employing a digester (Ankom 220 Fiber Analyzer, Ankom Technology, NY, USA) with filter bags F57, at each step of the composting (same samples abovementioned) and in the spent mushroom substrate (SMC) remained at the end of the second flush of mushrooms, randomly obtained from 3 experimental units of each treatment. Compost and substrate samples were dried in stove (50 °C for 48 h), homogenized and then a sufficient portion was randomly separated and milled (18 mesh) in a grinder Butt (model am-48, 60177, Ionomex, Argentina). Results were expressed as a mass balance of two trials.

2.10. Statistical analysis

Average yields (BE and Mushroom Production) of each trial were separately analyzed using Statistica 6.0 (StatSoft, Inc., OK, USA). In both cases, a one way ANOVA was used and the average yields obtained from the different containers were compared with Tukey's HSD at a significance level of 5%.

3. Results

Composting times were quite similar in both trials (27 and 29 days for the first and second trial, respectively). The composting average temperature of the whole process was higher in the second trial, 54 °C, than in the first, 48 °C. In Trial 1, during Phase I and just before the first turn the temperature steadily increased to a maximum of 64 °C; however, for the second trial, the temperature increase was faster, and reached a higher temperature of 72 °C (Fig. 2). In both cases, temperatures before first turn were uniform at different depths of the compost. However, between first turn and Phase II, temperatures within depths presented more variation, ranged between 5 °C and 15 °C. Top surface temperature measurements have always been the highest. In addition, laboratory data obtained during the composting process in both trials were similar (Table 1). Variation of electrical conductivity was higher in the second trial.

Considering both trials, average content of cellulose and hemicellulose were reduced during the whole process, 40% and 68%, respectively, being the major part of this reduction observed during *A. blazei* cultivation, 32% and 54%, respectively. The two trials average lignin content increased during the composting process (72%) and decreased during mushroom production (69%) (Table 2), being the final whole process reduction of 47%.

Total BE obtained in both trials was in the range of 25.2–47.5%, and the MP between 7.6 and 14.2% (Table 3). In the first trial, both BE and MP obtained from polyethylene bags were significantly higher ($p = 0.00003$) than that obtained using plastic trays, at first flush and total accumulated at the end of a second one. During the second trial, first flush and total accumulated yields obtained using polyethylene bags were also higher than with trays, although they were not significant.

An augment of 1 kg fresh substrate in the plastic trays, produced an increase in both the BE and MP, *i.e. ca.* 6 and 11%, respectively, only in the case of the total accumulated yield. However, in the case of polyethylene bags, the opposite was found for both mushroom flushes. In fact, when adding 1.5 kg substrate to the bags, total accumulated BE and MP decreased 36% and 35%, respectively.

During the fruiting period, no pest problems were observed and the harvested mushrooms showed a normal appearance and were of high quality (Fig. 3).

Average number and fresh weight of mushrooms per experimental unit obtained in both trials was *ca.* 10 and 40 g, respectively. Significant differences in the mushroom number were not

Table 2
Percentage of cellulose, hemicellulose and lignin average contents (two trials) during composting, at inoculation and end of two flushes of *A. blazei* (SMC).

	Composting				Mushroom cultivation	
	Start	1st turn	2nd turn	3rd turn	Inoculation	SMC
Cellulose	37.9 ± 1.73	40.4 ± 0.77	38.1 ± 0.59	34.5 ± 0.62	33.5 ± 1.23	22.8 ± 0.29
Hemicellulose	13.2 ± 0.49	11.8 ± 0.37	10.4 ± 0.42	9.3 ± 0.39	9.2 ± 0.99	4.2 ± 0.21
Lignin	13.8 ± 0.58	16.5 ± 1.06	17.8 ± 0.26	18.8 ± 0.69	23.7 ± 1.08	7.3 ± 0.09

Table 3
Mushroom yields (g), expressed as percentage of biological efficiency (% BE, [weight of fresh mushrooms/weight of dry substrate at spawn] × 100) and percentage mushroom production (%MP, kg of fresh mushrooms/100 kg of fresh substrate at spawn), and number of mushrooms (NM) of two flushes of *A. blazei* cultivated on sunflower hulls composted substrate, in two independent trials and in different kinds of containers and substrate mass.

Trial	Container	1st flush				2nd flush			
		yield (g)	%BE	%MP	NM	yield (g)	%BE	%MP	NM
1st*	3.5 kg tray	208.5 (87.08)	19.8b (8.29)	8.3 (3.48)	6 (3.1)	269.3b (101.19)	25.2b (10.00)	7.6b (3.01)	8 (3.3)
	2.5 kg bag	277.0 (106.22)	36.9a (14.16)	11.1 (4.25)	7 (3.6)	356.0a (90.61)	47.5a (12.07)	14.2a (3.62)	9 (4.5)
2nd**	4.5 kg tray	145.6 (60.17)	11.6 (4.80)	3.6 (1.50)	4 (2.1)	338.6 (74.33)	26.9 (6.00)	8.5 (1.86)	12a (5.0)
	4.0 kg bag	155.8 (58.58)	12.8 (4.80)	3.9 (1.46)	3 (1.6)	369.6 (110.03)	30.3 (9.01)	9.2 (2.75)	8b (3.4)

*$n = 13$, **$n = 12$, (Standard deviation). Different letters within same trial and column represent significant differences according to Tukey's HSD ($\alpha = 0.05$).

observed among containers types during the first trial in spite of the significantly higher mass of mushrooms obtained from the polyethylene bags. On the other hand, during the second trial, number of mushrooms was significantly different ($p = 0.016$) among the container types.

4. Discussion

Compost temperatures and final parameters values were within the acceptable ones (Rinker, 1986). Higher variation of electrical conductivity during second trial could probably be due to the higher temperatures achieved. Higher temperatures reflect a higher metabolic activity with a greater compost mineralization.

Lignin accumulation in the compost during composting phases agrees with that informed by Gonçalves de Siqueira (2006) who, for a composting process of 30 days and for an initial N level of 0.99% and 1.50%, informed a lignin increase of 94% and 86%, respectively. Lignin content increment is not cause of a biosynthesis process, but rather of a preferential reduction of the other components (Iiyama et al., 1994).

Fig. 3. *Agaricus blazei* mushrooms cultivated in sunflower hulls based composted substrate in two different container types – trays (0.12 m^2) (A) and bags (0.08 m^2) (B).

It was confirmed the reduction in the lignin levels of the substrate after two flushes of *A. blazei*, in good agreement with that informed for *A. bisporus* by most of the authors. The lack of available carbohydrates leads to the activation of a lignolytic enzymatic pathway (Jeffries, 1994). Thus in this organism the lignolytic system becomes activated as part of its secondary metabolism.

Yields for both trials and containers types were comparable, and in some cases even better, to those found by other authors. As an example, Braga and Eira (1999) evaluated three casing thickness (3 cm, 5 cm, and 8 cm), two substrate (based on sugarcane, coast-cross grass and soybean meal) masses (30 kg/m^2 and 60 kg/m^2) and two cultivation environments (plastic glasshouse and bamboo shelter). When using the plastic glasshouse, best yields were obtained with 5 cm casing thickness and a substrate mass of 60 kg/m^2; being the BE and MP they obtained around 32% and 12%, respectively. Nogueira de Andrade et al. (2007) and Kopytowski Filho and Minhoni (2004), also reported BE's around 30% with similar based materials substrate to the abovementioned study.

Hitherto known, there is not a method that allows the determination of the potential crop yield for a certain substrate. In the case of *A. blazei* cultivated on a plastic glasshouse environment, when substrate mass per area unit increases, number and weight of mushrooms and MP, in terms of mass or area, obtained also increased proportionally (Braga and Eira, 1999). However, same authors reported under a bamboo shelter cultivation that both BE and MP in terms of mass decreased when the substrate mass was doubled.

Regarding the mushrooms average number per experimental unit, it was *ca.* three times lower than the corresponding value reported by Braga and Eira (1999). However, mushrooms average fresh weight reported in the present work was higher. Larger mushrooms are more easily and rapidly harvested than smaller ones, which otherwise hinder the harvesting and process; thus increasing the production cost.

It is concluded that it is possible to obtain acceptable yields of good quality *A. blazei* using sunflower seed hulls as one of the substrate formula lignocellulosic components used to obtain compost.

Additionally, under the conditions and process here reported, polyethylene bags containing 2.5 kg compost produced the best yield performance viz-a-viz those obtained using either 4.0 kg or 4.5 kg compost in polyethylene bags or plastic trays, respectively.

According to the results obtained from sunflower seed hull based composted substrates and under the conditions described on this study, it can be concluded that the major degradation of the lignocellulosic components occurred during the *A. blazei* cultivation compared to that occurring in the biphasic composting process.

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