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A simple procedure for preparing substrate for *Pleurotus ostreatus* cultivation

Daniel Hernández [a], José E. Sánchez [b,*], Keiko Yamasaki [a]

[a] *Instituto Tecnológico de Tapachula, Km 2 Carretera a Puerto Madero, Tapachula, Chiapas, Mexico*
[b] *El Colegio de la Frontera Sur, Apdo Postal 36, Tapachula, Chiapas 30700, Mexico*

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Abstract

The use of wooden crates for composting a mixture of 70% grass, (*Digitaria decumbens*), and 30% coffee pulp, combined with 2% $Ca(OH)_2$, was studied as a method for preparing substrate for the cultivation of *Pleurotus ostreatus*. Crate composting considerably modified the temperature pattern of the substrate in process, as compared to pile composting, where lower temperatures and less homogeneous distributions were observed. Biological efficiencies varied between 59.79% and 93% in the two harvests. Based on statistical analysis significant differences were observed between the treatments, composting times and in the interactions between these two factors. We concluded that it is possible to produce *P. ostreatus* on a lignocellulosic, non-composted, non-pasteurized substrate with an initial pH of 8.7, and that composting for two to three days improves the biological efficiency.
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1. Introduction

Various techniques are used to prepare substrate for the cultivation of *Pleurotus* mushrooms (Geml et al., 2001; Villa-Cruz et al., 1999). One of these is composting, which, when carried out properly, reduces the level of competitive microorganisms in the material being prepared. Microorganisms indigenous to the by-products metabolize other compounds, including sugars and generate heat during metabolism. As a result, the substrate reaches temperatures ranging from 50 to 70 °C for several days (Laborde et al., 1993; Stamets and Chilton, 1983). Composting has been successfully applied in the preparation of a substrate selective for *Pleurotus* spp. with biological efficiencies of 70% achieved in three harvests (Villa-Cruz et al., 1999). In that study, the authors recommended making a pile on the ground of a 1:1 mixture of corncobs and coffee pulp with 2% $Ca(OH)_2$, then adding water to achieve a 70% moisture content, followed by composting for seven to nine days. This type of composting required no further thermal treatments, nor was the application of fungicides necessary to eliminate competitive microorganisms. Using a mixture of 70% pangola grass (*Digitaria decumbens*), 30% coffee pulp and 2% $Ca(OH)_2$ followed by composting for five to seven days, biological efficiencies of 121% in three *P. ostreatus* harvests were achieved (Huerta-Palacios and González, 2000).

The compost pile method, which is still used in *Agaricus bisporus* mushroom farming (Sinden and Hauser, 1953), was the first known composting method, but it has the disadvantages of not maintaining efficient temperature or moisture control, and of requiring manpower to oxygenate the pile.

Other composting methods developed mainly for the transformation of organic waste into fertilizer involve the use of wooden crates of varying shape and size, piles with passive ventilation or indoor tunnels based on forced ventilation (Mathur, 1991; Miller, 1991). These methods have been studied and successfully employed in commercial button mushroom cultivation (Nair and Price, 1993; Overtijns, 1993), but have been less utilized for *Pleurotus* species, where steam pasteurization has been the standard practice (Stamets and Chilton, 1983). The use of wooden crates could be adapted to Mexico's rural areas as a low input technology for the cultivation

* Corresponding author. Tel.: +52-962-628-1103/1104; fax: +52-962-628-1015.
E-mail address: esanchez@tap-ecosur.edu.mx (J.E. Sánchez).

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of *Pleurotus* spp. In this project we studied the effect of passive ventilation and mixing during composting in crates on biological efficiency and compared the results with the pile process. This study also takes a step towards the development of "indoor" technologies better adapted to higher-production-volume *Pleurotus* growing operations.

2. Methods

2.1. Biological material and composting containers

Pleurotus ostreatus ECS-0152 from the Tropical Mushrooms laboratory at ECOSUR was used. Crates measuring $75 \times 75 \times 75$ cm were constructed from tepemixtle (*Ocotea* sp.). Fifty holes 0.635 cm in diameter were drilled uniformly over the bottom of the crate to provide ventilation by natural convection. Four openings of the same diameter (Fig. 1) were also made at 10, 30, 50 and 70 cm from the top surface of the substrate along one of the sides. These openings were used to take temperature readings from different parts of the substrate. For composting, the crates were placed on the ground in a large roofed area, where environmental conditions were 28 ± 2 °C and $85 \pm 10\%$ relative humidity.

2.2. Substrate and treatments

Two percent lime ($Ca(OH)_2$) was added to a mixture of 30% coffee pulp and 70% dry pangola grass (*D. decumbens*). Moisture content was adjusted to 70%. Seventy five kilograms (wet weight) of substrate were used per crate. A total of five treatments were prepared as follows: For Pile method (T_1, control), a pile was made with 75 kg of substrate, and covered with plastic sheeting. The pile was mixed once daily for the five days of composting to provide the necessary aeration. For the crate without mixing treatment (C), 75 kg substrate were placed in a crate, and the top covered with a jute sack to maintain temperature and avoid evaporation. This was designated T_2. The preparation was not mixed during the entire composting period (five days). For the crate with chimney without mixing treatment (CCH) substrate was placed in a crate, and a wooden cover placed on top of the crate with a PVC chimney measuring 10 cm in diameter and 15 cm in height. The material was not mixed during the entire composting period. This was designated T_3. For the crate with mixing treatment (CM), substrate was put inside the crate, the top covered with a jute sack, and the preparation was mixed once every 24 h until the end of the process. This was designated T_4. For the crate with chimney and mixing treatment (CCHM), substrate was added, and a cover placed over it with a PVC chimney measuring 10 cm in diameter and 15 cm in height, and the preparation mixed every 24 h. This was designated T_5.

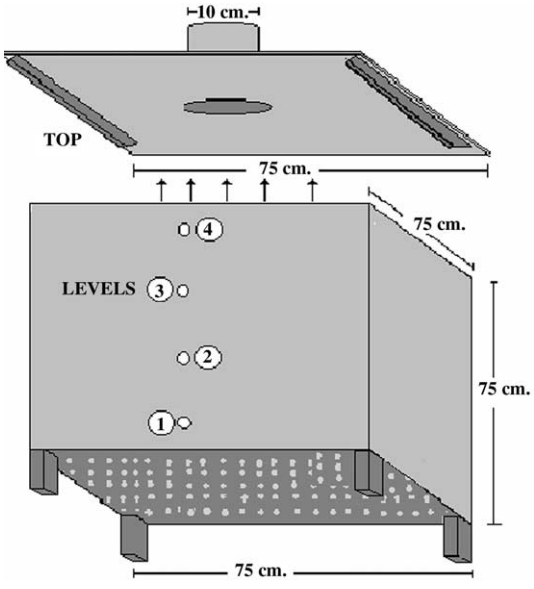

Fig. 1. Dimensions of crate used in treatments T_2–T_5.

2.3. Sampling and cultivation

Each day, three 0.15 kg samples were taken from the center of the compost material so that moisture and pH could be measured. Five additional 1 kg samples were used for the cultivation of *P. ostreatus* (five portions, 1 kg each). For cultivation, the substrate samples were left to cool to ambient temperature and were immediately inoculated with 5% sorghum grain spawn. The inoculated substrate was placed in polyethylene bags with 1 kg of substrate per bag and incubated at room temperature until colonization was complete (12–15 days). Later, the samples were subjected to conditions favoring fruiting: 26 °C, 85–90% relative humidity and $CO_2 \leqslant$ 800 ppm.

2.4. Analyses

Substrate temperature, pH and moisture were measured once a day during the composting process. Temperatures were taken at four different sites inside the pile and inside each crate with a mercury thermometer (Figs. 1 and 2). To determine moisture, substrate samples (50 g wet weight) were collected and dried in an oven (105 °C; 18 h). The pH was determined using a model 719 A S-003584 potentiometer (Orion Res. Inc., 529 Main Street, Boston, MA 02129, USA) by adding 10 g of sample to 100 ml recently boiled water as recommended by Williams (1984).

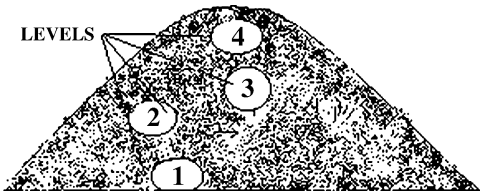

Fig. 2. Temperature sampling points in the pile.

2.5. Mushroom production

Production data were taken from the first two harvests. Once fruiting occurred, biological efficiency (BE) was calculated and expressed as a percentage of the fresh weight of the harvest over the dry weight of the substrate used.

2.6. Experimental design and statistical analysis

A randomized complete block design was used with five repetitions per block. Four blocks were completed at different times of the year (June, July and August–September). Data were analyzed using the analysis of variance procedure and means were separated using Tukey's test at a level $\alpha = 0.05$ (Steel and Torrie, 1988). For temperature, pH and moisture, a repeated measures analysis was performed (Schneier and Gurevitch, 1993). In both cases the ANOVA procedure by SAS was used (SAS Institute Inc. Cary, NC, USA, 1998).

3. Results

3.1. Compost temperature

Temperature change in the five treatments at different depths from the surface of the substrate is shown in Fig. 3. In general, compost temperature approximated the surrounding temperature (26–28 °C) at the outset, then increased slightly until days 2–3, stabilized on day 4 and decreased (rather slightly depending on the treatment), on day 5. Statistical analysis revealed significant differences between treatments, for depths ($p = 0.0001$) and time ($p = 0.0001$), and in the interaction between them ($p = 0.0001$). In the pile, the highest temperature of the mass in process was obtained at level 4 (upper surface) at approximately 60 °C. In the crates, the highest temperature was obtained at level 3 also around 60 °C. As depth within the substrate increased, the temperature decreased, but this varied among the treatments. In the pile method (T_1), differences between levels 1 and 4 had an average value of 22.4 °C, while this difference was 9.8, 9.3, 12.8 and 10.7 °C for treatments 2, 3, 4 and 5 respectively. However, in the pile, the temperature of the lowest level (1) never reached 40 °C, while in all the other treatments, this temperature was achieved on day 3 and surpassed considerably on day 4 (51, 49, 44 and 45 °C for T_2, T_3, T_4 and T_5 respectively). Also noteworthy is that in the pile, the temperature achieved on the upper surface of the substrate (level 4) was always the highest of all the temperatures observed in that pile. This was not, however, the case in any of the other treatments where the temperature on the upper surface of the substrate dropped beginning on day 3. In fact, level 4 on day 5 (120 h), exhibited temperatures below levels 2 and 3 and similar to level 1 (C and CCH); and in the case of the last two treatments (CM and CCHM) lower than levels 2 and 3 but higher that 1.

3.2. pH response

The variations in the substrate pH in the center of the mass for the five treatments under evaluation are shown in Fig. 4. The pH at t_0 (moment of spawning) was 8.7; the following day it was 8.2 in all treatments, and then slowly rose in each treatment, until it reached values between 8.38 and 8.5 for all treatments at the end of the composting period. There was no significant difference between the various treatments ($p = 0.735$), however, for time comparisons a significant decrease ($p = 0.0001$) between day zero and day one (0.5 units) was observed.

3.3. Moisture

The variation of moisture content of substrate in each treatment during composting is shown in Fig. 5. From a starting moisture of 70% all treatments decreased up to 58–61%, probably due to evaporation. Statistical analysis revealed no significant difference for the various treatment ($p = 0.744$); however at different times there was a linear decrease of moisture of approximately 3.1% daily ($p = 0.0001$; $Y = -0.0831x + 69.2$; $r^2 = 0.887$).

3.4. Biological efficiency

The biological efficiency obtained in two harvests with the substrate prepared under the different composting conditions under evaluation is shown in Table 1. These values ranged from 59.7% (CCH, day 5) and 93.8 (CCH, day 3). Statistical analysis revealed significant differences at level $p = 0.0001$, for each of factors A (treatments) and B (duration of composting), as well as the interaction between these factors. Analysis of the interaction of the treatments with days of processing, permitted a classification into five statistical groups. Group A, (with higher efficiencies) included treatments 3, 4 and 1 (CCH, CM and pile) at three days of composting, and treatments 5 and 1 (CCHM and pile) at four days (BE: 93.83%, 93.36%, 85.55%, 85.87%, 83.45% respectively). Included in the last group, (lower efficiency; BE values between 59.79% and 71.95%) are all

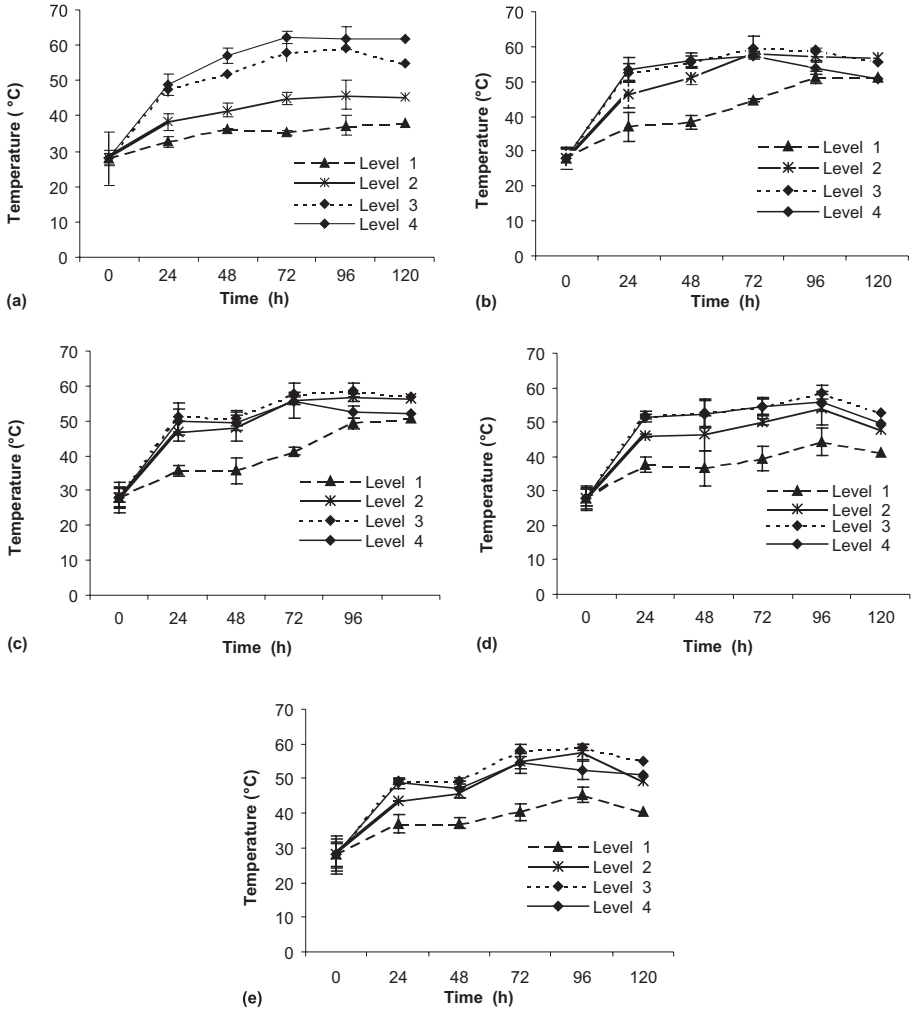

Fig. 3. Variation of temperature in each treatment, at different points from the substrate surface during the composting period. Level 1: 70 cm, level 2: 50 cm, level 3: 30 and level 4: 10 cm under the surface. (a) Pile composting (control), (b) crate without mixing, (c) crate with chimney without mixing, (d) crate with mixing, (e) crate with chimney and mixing.

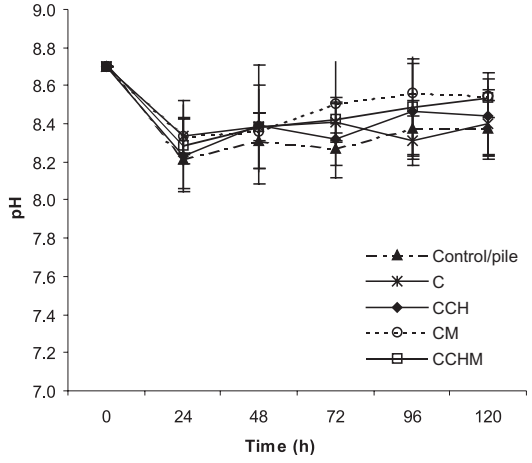

Fig. 4. Variation of pH in the substrate during composting.

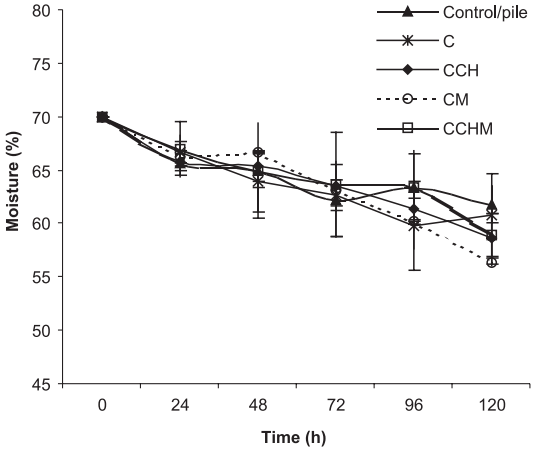

Fig. 5. Variation of moisture in substrate during composting.

the treatments composted for five days as well as treatment 2 (CM) at three days of composting.

The variation in biological efficiency with respect to the duration of pile method composting is shown in

Table 1
Biological efficiency (%) in two flushes of *P. ostreatus* ECS-0152 using a mixture 70% grass, 30% coffee pulp with 2% $Ca(OH)_2$ composted during 3–5 days

Treatment[a]		Day 3	Day 4	Day 5
T_1	P	85.55 ± 3.79ab	85.87 ± 2.83ab	71.95 ± 3.56cde
T_2	C	65.51 ± 5.14de	72.67 ± 1.87cd	63.19 ± 7.69de
T_3	CCH	93.83 ± 5.29a	78.11 ± 1.4bc	59.79 ± 2.33e
T_4	CM	93.36 ± 6.67a	81.06 ± 3.01bc	65.73 ± 6.04de
T_5	CCHM	79.01 ± 3.26bc	82.63 ± 1.39abc	71.62 ± 2.85cde

Values with different letter indicate statistical differences in the interaction between treatments and time of composting. $\alpha = 0.05$, $CV = 8.01\%$.
[a] T_1 = pile (control); T_2 = crate without mixing; T_3 = crate with chimney without mixing; T_4 = crate with mixing; T_5 = crate with chimney with mixing.

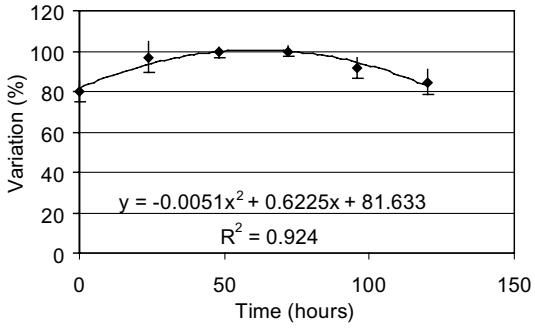

Fig. 6. Effect of composting time on biological efficiency. Substrate: mixture of grass 70% coffee pulp 30% with 2% $Ca(OH)_2$. Strain: *P. ostreatus* ECS-0152.

Fig. 6. The highest biological efficiencies were achieved with substrate composted for 24–72 h ($r^2 = 0.92$). Non-composted substrate also produced fruiting bodies, although biological efficiency obtained was less (80% of that obtained at 72 h of composting).

4. Discussion

In this study we compared the composting process in stationary and manually mixed piles with composting in wooden crates with four different forms of passive and/or manual ventilation. Composting in crates notably altered the temperature pattern of the substrate compared to the pile method. This modification was mainly due to the presence of holes in the bottom of the crates, which permitted better ventilation and thus potentially more efficient microbial metabolism. Treatments with crates led to less temperature variation between the bottom and the top surface of the substrate than using the pile method. The presence of chimneys in two of the treatments (T_3 and T_5) had no observable effect on the temperatures observed in the mass during the composting process. Conversely, the mixing of the substrate in the crates was a determining factor for the temperature pattern; indeed, when there was no mixing (T_2 and T_3), the temperature at the lowest level tended to increase proportionately to the highest substrate level of that crate or lot.

As to the biological efficiency obtained, it is particularly notable that the lack of mixing negatively affected the biological efficiency (T_2), most likely due to a lack of ventilation causing an inadequate breakdown of the substrate. This drop in the BE from lack of mixing was ameliorated by installing a chimney in the top of the crates (T_3), permitting a better ventilation by convection.

According to the data obtained the best treatment (for homogeneity of temperature, the pH of the composting mass, biological efficiency and the possibility of passive aeration of the substrate during the composting process) is T_3 (CCH—chimney without mixing). This conclusion should be considered preliminary until further studies are done, however the results obtained in this study do indicate that it is possible to produce carpophores of *P. ostreatus* when this fungus is spawned in a lignocellulosic substrate even without composting, at a pH of 8.7 (adjusted with $Ca(OH)_2$) and at 70% moisture. The use of any further thermal treatment for pasteurizing the substrate is rendered unnecessary, although a two to three day composting process improved biological efficiency. These results are in agreement with previous composting studies which indicate that a short three- to five-day fermentation period, depending on environmental temperatures, results in higher biological efficiencies than with a non-composted substrate (Martínez-Carrera et al., 1985); nevertheless, in those studies a substrate with an initial pH of 5 to 6 was used, so the substrate had to be pasteurized afterwards to impede contaminants. In addition, Philippoussis et al. (2001) found that by spawning two strains of *P. ostreatus* in sterile wheat straw, which had been previously submerged in water for 24 h and then mixed with 2% calcium carbonate, a BE of between 78% and 94% could be achieved. The authors also determined that composting the substrate for 12 days reduced the biological efficiency.

The use of an alkaline medium to reduce competing microorganisms has been previously suggested (Stölzer and Grabbe, 1991), and it was found that an alkaline pH had a greater effect on the mycelial growth of common

contaminating deuteromycetes than it did on *P. ostreatus* itself. In our study, no fungal or bacterial contaminants were found, but in a later study, we noticed that during the fruiting stage and under excessively moist conditions, some synthetic logs and even some carpophores were invaded by *Fusarium* sp. We corrected the problem by keeping the relative humidity of the fruiting room no higher than 85%.

The composting process and the quality of the product obtained depend on the characteristics of the material (chemical composition, pH, moisture and particle size) and on the conditions under which the process takes place (amount of material, temperature, container, environmental humidity, ventilation, light or the presence of any microorganism). Therefore, more studies are needed to address such factors as the total ventilation area and the location of this area on the bottom of the container, as well as the size of the substrate particles being composted.

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