

## **Composted Cotton Straw Silage as a Substrate for *Pleurotus* sp. Cultivation**

N. Silanikove, O. Danai

MIGAL, Galilee Technological Centre, Kiryat Shmona 10-200, Israel

&

D. Levanon

Agricultural Research Organization, The Volcani Center,  
Bet Dagan 50-250, Israel

(Received 10 January 1988; accepted 3 February 1988)

### *ABSTRACT*

*The possibility of growing *Pleurotus* while utilizing cotton straw and a combination of cotton straw (50%) and wheat straw (50%) as substrates for the production of edible mushrooms was examined on a pilot-plant scale. It is possible to preserve cotton straw anaerobically, creating a 'silage-like' material with a typical pH of 5.5. Following composting, the pH of preserved cotton straw increased to 8.78. Organic matter losses after fungal growth on composted cotton straw substrates were considerably lower than on standard substrates (90% wheat straw and 10% alfalfa hay). The yield of edible mushrooms was maximal on the 50% wheat straw-50% cotton straw substrate, and the interval between inoculation and first harvesting of the mushrooms was shorter on cotton straw-based substrates than on the standard substrate. The increase in the ash content during the growing period was also lower in the cotton straw-based substrates than in the standard substrates. During the growth cycle, lignocellulose content of the substrates decreased and detergent-soluble content increased, indicating that the quality of the substrate as a feedstuff for ruminants had improved.*

## INTRODUCTION

Cotton and cereal crops generate large amounts of organic agricultural waste in many countries (Silanikove & Levanon, 1986). Cereal straws have an economic value and are utilized mainly in cattle production, as a feedstuff and as bedding. Moreover, the nutritional value of cereal straw for ruminants may be improved by various chemical treatments (Fan *et al.*, 1982). The response of cotton straw (CS) to conventional alkali and acidic treatments is small (Shefet & Ben-Ghedalia, 1982; Silanikove & Levanon, 1987), thereby preventing practical use of these processes.

In addition, before utilization of CS for any process a proper storage system must be developed because of the high moisture content of CS. We have recently managed to preserve CS under anaerobic conditions, which seems to be also the most economical way to do it (Silanikove & Levanon, 1986).

Many wood-decomposing fungi utilize lignocellulose efficiently and this characteristic is related to their ability to metabolize lignin (Kirk, 1971). *Pleurotus* species have been found to be one of the most efficient lignocellulose solid-state-decomposing types of the white-rot fungi (Zadrazil & Brunnert, 1981; Platt *et al.*, 1983, 1984). *Pleurotus* is cultivated for the production of edible mushrooms, utilizing lignocellulose waste as substrate.

Cotton straw was found in laboratory experiments to be an excellent substrate for *Pleurotus* (Platt *et al.*, 1983). Water extracts of CS encourage growth and lignin degradation by *Pleurotus florida* (Platt *et al.*, 1984).

The major obstacle to the application of a new process is its scaling-up. In the present experiment the possibility of growing *Pleurotus* utilizing CS or a combination of CS and wheat straw (WS) as substrates for the production of edible mushrooms was examined on a pilot-plant scale.

## METHODS

### Preservation of CS

Cotton straw was collected from approximately 100 acres of former lake bed soil in the Hula Valley, located in the north of Israel (Silanikove & Levanon, 1986). About 600 t of CS were harvested and chopped into particles of 2–3 cm with a forage harvester originally designed to cut maize. The material was taken by truck to a concrete silo with storage capacities of 150 and 450 t. The material was pressed with a heavy tractor and then covered with black plastic sheets. After one month of storage the pH of the preserved CS stabilized and the material was utilized every 14 days for 8–9 months.

## Substrate preparation

### *Types of substrates*

Three types of substrates were examined: (1) Substrate based on untreated WS (standard substrate). This was composed of 90% WS and 10% alfalfa hay, both chopped into pieces 2–4 cm long. (2) Substrate composed of 50% WS and 50% preserved CS (WS/CS) of equal moisture contents. (3) Substrate based on preserved CS only.

### *Composting stage*

Approximately 14 t (DM) of each type of substrate was arranged on a concrete floor as a flat stack, with the water content adjusted to 72% for 3 days. Each day the material was turned over with a pitch fork to prevent excess heat accumulation in the stack and sprayed with water to replace the quantity which had evaporated.

### *Pasteurization and inoculation*

The material was transferred into a pasteurization tunnel in which the temperature was raised to 60°C by injection of steam for 24 h. When the temperature dropped down to 25–30°C, the substrate was inoculated by mixing with 15 kg spawn of *Pleurotus ostreatus* per 1000 kg substrate. The inoculated substrate was then poured into plastic bags each containing approximately 20 kg substrate.

## Growing and fruiting conditions

Bags containing inoculated substrates were transferred to growth chambers. During the mycelial growth the temperature was kept at 25°C and relative humidity at 90% without ventilation. About 14 days were needed for mycelial growth. To induce fruiting, conditions were then changed to a temperature of 16–18°C and a relative humidity of 80%. CO<sub>2</sub> concentration was maintained below 0.08% by ventilation. Light intensity was 100 lux at the surface of the substrate for 12 h a day, supplied by daylight-type fluorescent tubes.

The mushrooms were picked in three flushes at intervals of 7–10 days.

## Substrate sampling and analysis

Substrates were sampled in triplicate from the following stages: raw materials, after composting, and after picking of the mushrooms. About 500 g were sampled each time, dried at 60°C overnight, and ground in a knife mill to pass through a 1-mm screen. The last material was used for chemical analysis.

Dry matter, ash, pH, nitrogen, cell wall (neutral-detergent fiber) and lignocellulose (acid-detergent fiber) were determined as described previously by Silanikove & Levanon (1986, 1987).

## RESULTS AND DISCUSSION

### Anaerobic preservation of CS

As shown previously on a small scale (2 t), it is possible to preserve CS under anaerobic conditions without special treatment (Silanikove & Levanon, 1986). In the present experiment we reconfirmed these results in a farm context (storage loads of 150 and 450 t). In the previous experiment CS was not ensiled during anaerobic preservation, even after water content was adjusted to the optimal level for ensilage. In the present experiment the larger amount of material treated allowed better compaction and a much smaller ratio between surface area and the mass stored. Consequently, the straw created a 'silage' with an average pH of 5.5. The pH of CS 'silage' did not reach the pH typical of grass or legume silage (3.8–4.2), apparently because of a deficiency of soluble carbohydrates (Silanikove & Levanon, 1986).

An alkaline pH (8.6) was recorded under the present preservation conditions only in the outer shell (about 1% of the total and 10 cm deep) of the preserved straw, apparently because of air trapped between the straw and the plastic cover. However, it was not contaminated with fungi. Cotton straw which was left uncovered spoiled rapidly within 24–48 h, due to fungal development.

In previous experiments (Silanikove & Levanon, 1986) two negative side effects were recorded: loss of some organic compounds, presumably soluble carbohydrates, as measured by increased ash content; and increase of lignin content to a larger extent than could be predicted from losses of organic matter *per se*.

These negative effects were reconfirmed in the present experiment only in the small fraction of the outer shell which became alkaline during preservation (Table 1). Fortunately, these two negative side effects were not recorded in the stored CS preserved as 'silage-like' material. We assume that lignification processes which are enzymatic in nature are deactivated by the anaerobic environment or by the acidic conditions, or both. Another advantage of anaerobic preservation over aerobic is that CO<sub>2</sub> production is considerably lower in the former. Consequently, losses of organic matter are considerably lower under anaerobic preservation than in aerobic preservation. This was reflected in a considerably higher ash content in the outer-shell material (Table 1).

**TABLE 1**  
The Chemical Composition (% of DM) of Anaerobically Preserved Cotton Straw Yielding Acidic ('Silage') and Alkaline ('Outer Shell') Materials

	<i>Original</i>	' <i>Silage</i> '	' <i>Outer shell</i> '
DM(%)	42.4	42.5	45.7*
pH	6.84	5.54*	8.59*
Crude protein	8.3	8.0	8.0
Cell wall	66.8	67.1	74.1*
Lignocellulose	56.3	56.4	62.0*
Cellulose	40.7	40.8	42.5
Hemicellulose	10.5	10.7	12.1
Lignin	15.4	15.6	19.5*

\* Values significantly different from the respective values in the original material by *t*-test ( $P < 0.05$ ).

### Changes in substrate composition

Composting lignocellulosic agricultural waste as a preliminary step before inoculation with *Pleurotus* spawn caused elevation of the pH to alkaline levels in CS- and WS-based substrates (Table 2). This procedure was associated with losses of organic matter as shown by the increase in the ash content of the substrates. In WS substrate, the increase was 17.6% compared

**TABLE 2**  
Composition (% of DM) of *Pleurotus* Substrates Based on Cotton (CS) and Wheat Straw (WS) at Three Stages: (a) Raw Material; (b) Post-Composting, Before Inoculation; (c) Post-Harvesting.

<i>Substrate and stage</i>	<i>DM(%)</i>	<i>pH</i>	<i>Crude protein</i>	<i>Cell wall</i>	<i>Lignocellulose</i>	<i>Ash</i>
Cotton straw						
(a)	42.5	5.54	8.0	67.1	56.4	7.3
(b)	33.2	8.78	6.9	74.1	62.0	7.9
(c)	28.9	5.16	8.4	64.0	52.5	11.9
Cotton straw/Wheat straw						
(a)	—	—	—	—	—	—
(b)	31.5	8.95	8.1	72.4	55.0	13.3
(c)	30.5	5.15	9.6	48.0	35.6	22.0
Wheat straw						
(a)	90.1	6.7	5.7	82.4	65.4	13.1
(b)	29.5	8.85	6.3	85.5	67.2	15.4
(c)	30.0	5.25	7.5	54.5	34.5	24.7

to 8.2% in CS. As ash content was also initially higher in WS, the ash contents at the start of mycelium development were in the following order (Table 2): WS (15.4%), WS/CS (13%), CS (7.9%).

Agosin & Odier (1985) found a linear inverse relationship between CO<sub>2</sub> gas production and weight loss during degradation by white-rot fungi, including *P. ostreatus*. Based on the above results and on the larger increase in ash content when *Pleurotus* was grown on WS compared to CS substrate, it seems that larger amounts of organic matter were fermented during growth on WS substrate.

Fermentation by *Pleurotus* resulted in accumulation of neutral-detergent (ND) solubles (reciprocal of cell-wall content) and reduction in lignocellulose content (Table 2).

In a preliminary report Danai *et al.* (1986) noted that there is a significant linear relationship between lignin content and dry matter digestibility, measured *in vitro* (IVDMD) with bovine ruminal fluid. However, even better statistical relationships were found between the increase in IVDMD and the increase in ND solubles. This indicates that the digestibility of CS spent-substrates as a feedstuff for ruminants would have been considerably improved at the end of the *Pleurotus* growth cycle.

Accumulation of water-soluble lignocellulose complexes, highly digestible, during the growth of white-rot fungus on solid lignocellulose substrates, was described by Agosin *et al.* (1985) and Reid *et al.* (1982). Our results indicate that the extent of such lignocellulose complex formations during fermentation is even larger than that found on the basis of water solubility.

The use of *Pleurotus* spent-substrate as a feedstuff would reduce the cost of growing mushrooms, as this would cover most of the cost of the raw substrate.

### **Mushroom yield on CS 'silage'**

Maximal yield was obtained with the WS/CS substrate, followed by CS and WS substrates (Table 3). Since more organic matter was fermented on WS substrates it seems that the gross conversion ratio (the substrate fermented to mushroom yield) was much more efficient (smaller) in CS substrates than in WS.

The better gross conversion ratio could be explained by the higher growth-rate, as judged by the shorter interval between inoculation and first harvesting, on CS and WS/CS in comparison to growth on WS alone (Table 3). Higher growth-rate and similar yield would result in lower apparent maintenance requirements of the mycelium so allowing transfer of more net energy for growth.

**TABLE 3**  
Mushroom Yield and Interval Between Inoculation and First Harvesting of  
*Pleurotus* Grown on Cotton (CS) and Wheat Straw (WS) Substrates\*

	Number of replications	Yield (g fresh weight per kg substrate)†	Intervals (days)
Cotton straw	5	166 <sup>ab</sup>	20.0 <sup>a</sup>
CS/WS	5	184 <sup>a</sup>	19.4 <sup>a</sup>
Wheat straw	5	164 <sup>b</sup>	23.0 <sup>b</sup>

\* Values marked by different letters differ significantly ( $P < 0.05$ ) by one-way analysis of variance ( $F$ -test).

† The three types of substrates had similar water contents of approximately 70%.

The higher growth-rate on CS than on WS probably reflects differences in their chemical composition. Cotton straw contains about 30% cell solubles in comparison to 15% in WS. The amount of soluble carbohydrates in CS (2–4%) is also higher than in WS (0.4–1.4%) (Silanikove & Levanon, 1986). Cotton straw contains considerable amounts of soluble phenolics (6–8%) which are found in insignificant amounts in WS.

Since white-rot fungi are efficient monomeric and polymeric phenolic degraders (Kirk, 1971), soluble phenolics in CS may be used as readily available nutrients. In accordance with the above findings, Platt *et al.* (1984) found that water-soluble, extracted substance from CS increased the growth rate of *Pleurotus* when added to WS culture.

The reduction in the length of mushroom growth cycle on CS substrate is also economically valuable. On an annual basis it may be utilized to add one or more growth cycles or, alternatively, to cut down the costs of growing by 17% in comparison to growing on WS substrate.

## REFERENCES

- Agosin, E. & Odier, E. (1985). Solid state fermentation, lignin degradation and resulting digestibility of wheat straw fermented by selected white-rot fungi. *Appl. Microbiol. Biotechnol.*, **21**, 397–403.
- Agosin, E., Monties, B. & Odier, E. (1985). Structural changes in wheat straw components during decay by lignin degrading white-rot fungi in relation to improvement of digestibility for ruminants. *J. Sci. Food Agric.*, **36**, 925–35.
- Danai, O., Levanon, D. & Silanikove, N. (1986). The efficiency of lignocellulose degradation by varieties of *Pleurotus* sp. *Proceedings of the International Symp. Sci. Tech. Aspects of Cult. Edible Fungi*, Pennsylvania State University, USA, p. 40 (abstract).

- Fan, L. T., Lee, H. M. & Chrpuray, M. N. (1982). The nature of lignocellulosics and their pretreatment for enzymatic treatment. *Adv. Biochem. Eng.*, **23**, 157–87.
- Kirk, T. K. (1971). Effects of microorganisms on lignin. *Ann. Rev. Phytopath.*, **9**, 185–210.
- Platt, M. W., Hadar, Y., Henis, Y. & Chet, I. (1983). Increased degradation of lignocellulose by *Pleurotus ostreatus* ssp. *florida*. *Eur. J. Appl. Microbiol. Biotechnol.*, **17**, 140–2.
- Platt, M. W., Hadar, Y. & Chet, I. (1984). Fungal activities involved in lignocellulose degradation by *Pleurotus*. *Appl. Microbiol. Biotechnol.*, **20**, 150–4.
- Reid, I. D., Abrams, G. D. and Peppers, J. M. (1982). Water soluble products from the degradation of aspen lignin by *phanerochaete chrysosporium*. *Can. J. Bot.*, **60**, 2357–64.
- Shefet, G. & Ben-Ghedalia, D. (1982). Effect of ozone and sodium hydroxide treatments on the degradation of cotton straw monosaccharides by rumen microorganisms. *Eur. J. Appl. Microbiol. Biotechnol.*, **15**, 47–51.
- Silanikove, N. & Levanon, D. (1986). Cotton straw: composition, variability and effect of anaerobic preservation. *Biomass*, **9**, 101–12.
- Silanikove, N. & Levanon, D. (1987). Inter-relationship between acidic alkali treatments of cotton straw and wheat straw and their fiber chemical properties. *J. Sci. Food Agric.*, **38**, 117–24.
- Zadrazil, F. & Brunnert, F. (1981). Investigation of physical parameters important for the solid state fermentation of straw by white rot fungi. *Eur. J. Appl. Microbiol. Biotechnol.*, **11**, 183–8.