ELSEVIER

Bioresource Technology 84 (2002) 171–176

BIORESOURCE TECHNOLOGY

Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls supplemented with N–NH_4^+ and/or Mn(II)

N.R. Curvetto *, D. Figlas, R. Devalis, S. Delmastro

Departamento de Agronomía, Universidad Nacional del Sur, San Andrés 800, 8000 Bahía Blanca, Buenos Aires, Argentina

Received 8 May 2001; received in revised form 20 December 2001; accepted 25 December 2001

Abstract

The mycelial growth rates in lineal growth assay, yield, and production rate of five *Pleurotus ostreatus* strains were evaluated in response to different levels of Mn(II) and/or NH_4^+ in a substrate containing sunflower seed hulls as a main energy and nutritional component. Each strain showed different basal values for mycelial growth rate and biological efficiency on sunflower seed-hull substrate. Adding growth limiting mineral nutrients increased the mycelial growth rate by 13–25%. Primordia initiation for the first flush appeared between day 24 and 28 and days to the second crop ranged from 39 to 51. Biological efficiency increased over control values and reached 60–112%, depending on the strain and the concentration of Mn(II) and NH_4^+. This study demonstrated the advantage of selecting the most productive *P. ostreatus* strains in a substrate formulated with sunflower seed hulls to provide the main energy and nutritional ingredients and supplemented with Mn(II) and/or NH_4^+. © 2002 Elsevier Science Ltd. All rights reserved.

Abbreviations: BE, biological efficiency; MP, mushroom production

Keywords: Mushroom cultivation; Nutrient supplementation; Ostra mushroom; *Pleurotus*; Strain selection; Mushroom yield

1. Introduction

Pleurotus ostreatus is a white-rot basidiomycete that can be easily cultivated on a variety of substrates, including agricultural wastes (Hadar et al., 1992). This fungus can degrade most components of wood and is considered as a primary agent of lignin degradation in nature (Zadrazil, 1985; Buswell and Oider, 1987). The ability of some *Pleurotus* strains to use and degrade different lignocellulosic residues via the preferential degradation of lignin has been investigated (Hadar et al., 1992; Martínez et al., 1994). Blanchette (1984) also suggested that Mn(II) is an important component in the selective delignification of wood by white-rot fungi. An increase in the Mn(II) content of substrates increases both the level of ligninolytic enzymes and lignin degradation by *P. ostreatus* (Kerem and Hadar, 1995).

P. ostreatus has been cultivated on substrates such as cereal straw, corn cobs, sawdust, bagasse, wood pulp, cotton and oil palm waste, banana leaves, coconut husks, poultry wastes, tree bark and leaves, and flax shive (Khan and Chaudhary, 1989). Growth and nutritional values of *Pleurotus* mushrooms mainly depend on substrate type and cultivation conditions (El Kattan et al., 1991).

Sunflower (*Helianthus annus*) seed hulls are a low cost, abundant residue of some edible oil industries and are suitable as a substrate for cultivating *P. ostreatus*. The particle size as it emerges from the oil industry (average 12 mm) is adequate for use as a media component (Darjania et al., 1997). Sunflower seed hulls contain proteins, lipids and carbohydrates similar to those in other substrates commonly used for *P. ostreatus* cultivation (Cancalon, 1971). The nutritional content of substrates can be improved by nitrogen supplementation (Lelley and Janßen, 1993a). Although N increases yields, above a certain level it inhibits fruiting (Zadrazil and Brunnert, 1979). Supplementing the substrate with controlled liberation of urea and Mn(II)Cl shortens the crop period from 35 to 28 days for *Pleurotus* spp. and also increases mushroom productivity (Lelley and Janßen, 1993a).

It is well known that different *P. ostreatus* strains exhibit different performances in their ability to colonize a substrate and their capacity to fructify with different efficiency and productivity (Lelley and Janßen, 1993b).

* Corresponding author. Fax: +54-291-459-5127.
E-mail address: ficurvet@criba.edu.ar (N.R. Curvetto).

0960-8524/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-8524(02)00013-5

The purpose of this work was to evaluate the growth performance, yield, and productivity of five *P. ostreatus* strains in response to formulations containing different levels of Mn(II) and/or NH_4^+ in a substrate whose main energy and nutritional source was provided via sunflower seed hulls.

2. Methods

2.1. Mushroom strains and culture conditions

Five *P. ostreatus* (Jacq:Fr.) P. Kumm. were used: Somycel 3001 (France), 38537 (the ATCC collection, Peoria, IL, USA), UAT PO3 (Popocatepetl Volcano, Mexico), UAT PO4 (California, USA) and UAT PO7 (Prague, Czech Republic). The last four strains were kindly provided by Dr. Carmen Sánchez from the collection of the Biotechnology Laboratory (Research Center for Biological Sciences of the Autonomous University of Tlaxcala, Tlaxcala, Mexico). All mushroom strains were cultivated in MYPA medium (Kinugawa et al., 1994): 20 g l^{-1} malt extract, 2 g l^{-1} yeast extract, 1 g l^{-1} peptone and 20 g l^{-1} agar, pH 5.5, autoclaved at 15 psi for 30 min. Cultures were incubated for 7 days in darkness at 25 °C.

2.2. Lineal growth test

The lineal growth assay (Duncan, 1997) was used to evaluate the mycelial growth rate of the five *P. ostreatus* strains on sunflower seed hull substrate supplemented with different amounts of Mn(II) and NH_4^+. This substrate contained 37.5% fresh sunflower seed hulls, 60% water, 0.5% $CaCO_3$, and 2% $CaSO_4$, on a weight basis. Taking into account the extent of the effective doses reported for different substrates, a range of Mn(II) and NH_4^+ concentrations was chosen using a factorial design to formulate the substrates (Table 1). For substrate treatments, $MnSO_4$ and $(NH_4)_2SO_4$ were dissolved in water and added to basal substrate to obtain the following concentrations on the basis of wet substrate: 0, 20, 100, and 200 ppm Mn(II); 0, 200, 500, and 750 ppm NH_4^+, and their combinations. Substrates were packed in 200 mm long and 16 mm diameter glass tubes to a density of approximately 0.5 g cm^{-3}. The substrates

Table 1
Mycelial lineal growth rate (mm day^{-1}) of five *Pleurotus ostreatus* strains grown in a sunflower seed hull substrate with different levels of Mn(II) and/or NH_4^+

Strain	NH_4^+ (ppm)	Mn(II) (ppm)			
		0	20	100	200
UAT PO4	0	4.4 c[A] (0.02)[B]	3.9 d (0.04)	5.5 a (0.04)	4.4 c (0.01)
	200	5.0 b (0.03)	4.8 c (0.04)	4.5 c (0.03)	4.9 c (0.05)
	500	4.9 c (0.03)	5.6 a (0.01)	4.6 c (0.03)	5.4 a (0.04)
	750	5.4 a (0.05)	4.2 c (0.01)	4.8 c (0.05)	4.7 c (0.02)
UAT PO7	0	4.7 f (0.02)	4.8 f (0.01)	4.7 f (0.02)	4.6 f (0.01)
	200	4.9 f (0.02)	5.2 e (0.01)	4.4 f (0.01)	4.8 f (0.01)
	500	5.0 f (0.01)	5.0 f (0.02)	5.3 e (0.02)	4.8 f (0.01)
	750	5.2 e (0.01)	5.2 e (0.02)	4.8 f (0.01)	4.9 f (0.03)
ATCC 38537	0	4.2 j (0.02)	4.6 i (0.01)	4.0 j (0.01)	4.3 j (0.01)
	200	4.4 j (0.01)	4.3 j (0.01)	4.5 j (0.01)	4.2 j (0.04)
	500	4.5 j (0.01)	4.7 i (0.03)	4.2 j (0.01)	4.3 j (0.01)
	750	4.7 i (0.02)	4.4 j (0.01)	4.4 j (0.01)	4.8 i (0.02)
Somycel 3001	0	4.1 o (0.01)	5.0 m (0.02)	4.6 n (0.01)	4.2 o (0.01)
	200	4.4 o (0.01)	4.9 m (0.01)	4.9 m (0.01)	4.9 m (0.02)
	500	5.2 m (0.02)	4.4 o (0.02)	4.2 o (0.03)	4.4 o (0.02)
	750	4.6 n (0.03)	5.1 m (0.04)	4.9 m (0.01)	5.2 m (0.01)
UAT PO3	0	4.3 r (0.01)	4.5 r (0.02)	4.5 r (0.01)	4.4 r (0.01)
	200	4.5 r (0.01)	4.6 r (0.02)	4.4 r (0.01)	4.2 r (0.06)
	500	4.9 q (0.02)	4.9 q (0.01)	4.5 r (0.02)	4.6 r (0.03)
	750	4.6 r (0.01)	4.9 q (0.02)	4.4 r (0.01)	4.9 q (0.02)

a–d UAT PO4 values with different letters are significantly different ($P \leqslant 0.05$).
e–h UAT PO7 values with different letters are significantly different ($P \leqslant 0.05$).
i–l ATCC 38537 values with different letters are significantly different ($P \leqslant 0.05$).
m–p Somycel 3001 values with different letters are significantly different ($P \leqslant 0.05$).
q–t UAT PO3 values with different letters are significantly different ($P \leqslant 0.05$).
[A] Mean values were separated by the Tukey test.
[B] Standard error values.

were inoculated on one end with a disk of mycelium-covered agar that practically covered the whole section of the tube, being pushed there with the help of a syringe piston. The ends of the tube were plugged with cotton and the tubes were incubated at 25 °C in darkness for 7 days. Ten tubes were cultured per treatment. The rate of mycelial lineal growth was determined after 7 days of spawn running.

2.3. Spawn production

Spawn were prepared in 1 l bottles, containing wheat (*Triticum durum*) grain mixed with 0.1% (w/w) $CaCO_3$, 0.8% (w/w) $CaSO_4$, and 40% water. The mixture was sterilized at 15 psi for 1.5 h and inoculated with mycelia of a *P. ostreatus* strain (two wedges/bottle). The spawn were incubated at 25 ± 1 °C in darkness for 15–20 days and were periodically shaken.

2.4. Substrate preparation and spawn running

From the results of the lineal growth test, supplemented substrates exhibiting the best mycelia responses to Mn(II), NH_4^+, or their combinations for each *P. ostreatus* strain were chosen. These substrates were formulated, placed in cylindrical glass flasks (100 mm high and 80 mm diameter) to a height of 80 mm, covered with a cap with a 35 mm centered hole plugged with cotton and autoclaved at 15 psi for 2 h. The sterilized substrates were surface inoculated with 10% (w/w) spawn and incubated in darkness at 24 ± 1 °C for 15 days.

2.5. Fruiting, cropping and production

After full substrate colonization by mycelium, flasks were uncovered and exposed to a controlled environment of 21 ± 3 °C with a 12 h photoperiod (1500–2000 lux), and 80–90% relative humidity. Adequate ventilation was provided to prevent mushrooms abnormalities due to increased concentrations of CO_2 during formation of fruiting bodies. Considering the production profitability of growers in the cultivation of these mushrooms under controlled environmental conditions, it is not economically advisable to cultivate them beyond the second flush. Hence, the accumulated biological efficiency (% BE, in kg fresh mushrooms/kg dry substrate), mushroom production (% MP, in kg fresh mushrooms/kg wet substrate) and productivity (% BE/day) of mushrooms at the end of the second harvest were calculated.

2.6. Statistical analysis

A factorial design for a wide range of Mn(II) and NH_4^+ concentrations, and their combinations was used for the lineal growth test. Growth rate from each treatment was measured in triplicate runs with 10 replicates per treatment in each run after 7 days running and expressed in mm day^{-1}. Mushroom cropping from the best formulations (in duplicate), 10 samples per treatment, were weighed and data analyzed. Results were evaluated by two-way ANOVA test, and the separation of mycelial rate mean values was done by the Tukey test (Ott, 1984). Analysis of variance and Tukey's studentized test for media comparison ($P \leqslant 0.05$) were done using the Media program (Mathematics Department, Universidad Nacional del Sur, Argentina).

3. Results and discussion

3.1. Lineal growth test

Mycelial lineal growth rate at day 7 of incubation for the *P. ostreatus* strains with different levels of Mn(II) and/or N–NH_4^+ is shown in Table 1. Each strain exhibited mycelial growth on sunflower seed hull substrate alone and addition of Mn(II) and/or NH_4^+ as growth limiting mineral nutrients increased mycelial growth rate to approximately 25%. Thus, for some nutrient combinations, the best formulations gave optimal lineal growth rate in the range 4.8–5.6 mm day^{-1} improving the mycelial growth rate by approximately 13% for UAT PO3, UAT PO7 and ATCC 38537, and approximately 25% for Somycel 3001 and UAT PO4. The addition of 500 ppm NH_4^+ alone produced the highest mycelial growth in Somycel 3001 and UAT PO3, while 750 ppm NH_4^+ was better for UAT PO4, UAT PO7 and ATCC 38537 when compared to other concentrations of NH_4^+ alone. Lower concentrations of NH_4^+ alone or combined with different levels of Mn(II) did not significantly improve mycelial growth rate (Table 1). Conversely, lower concentrations of Mn(II) alone also produced mycelial growth enhancement in three of the five strains (UAT PO4, ATCC 38537 and Somycel 3001). The highest dose of 200 ppm Mn(II) alone produced the same results as 0 ppm Mn(II). A preliminary study showed that high doses of Mn(II) (400–500 ppm) inhibited the growth of the oyster mushroom on a sunflower seed hull substrate (unpublished results). This inhibitory effect could be due to toxicity. The low Mn(II) doses at 20 and 100 ppm, alone or in combination with NH_4^+ in the sunflower seed hull substrate contrast with the wide range (150–620 ppm) reported for other substrates (Kerem and Hadar, 1995). Such differences could possibly depend on the activity of Mn(II)-dependent enzymes in each culture conditions including the composition of substrates.

The observed stimulatory concentrations of N–NH_4^+ are comparable to those reported by Kaal et al. (1995) intheir study related to the activity of ligninolytic enzymes with several basidiomycetes, including *P. ostreatus*.

These authors showed that mycelial growth in a medium with the high content of N–NH_4^+ (approximately 800 ppm) is generally improved compared to a substrate with the lower content (approximately 30 ppm). All the assayed basidiomycetes had manganese-peroxidase activity, which consistently increased with higher levels (56 mM) of N-peptone and N–NH_4^+. However, caution should be exercised with N substrate supplementation. It has been reported that N supplementation of decontaminated substrate that contained a high quantity of carbohydrates can stimulate dramatic growth of molds and competitive bacteria, producing a temperature increase (thermogenesis) sufficient to kill the mycelia of the oyster mushroom in less than one day (Lelley and Janßen, 1993b). This was not observed in our study because the substrate was sterilized, although this possibility should be considered when transferring our results to a production protocol where decontamination of the substrate is made instead of sterilization and the spawn is homogeneously inoculated (Curvetto et al., 1997).

3.2. Spawn running, fruiting, cropping and production parameters

As expected, the speed of colonization of substrate in flasks with different formulations showed a positive correlation with the values obtained using the lineal growth test (data not shown); treatments inducing the fastest mycelial growth in tubes also produced it in flasks.

Primordia initiation for the first flush occurred earlier in strains UAT PO7 and UAT PO4 (24 days). The remaining strains exhibited a later initiation (28 days). Days to the second crop were 39 for ATCC 38537 and UAT PO7, 41 for UAT PO4, 47 for UAT PO3 and 51 for SOMYCEL 3001.

Yield parameters % BE and % MP obtained on the best Mn(II) and/or NH_4^+ supplemented substrate formulations for each *P. ostreatus* strain, as well as their respective controls are shown in Table 2. Some of the Mn(II) and/or NH_4^+ supplemented substrate formulations produced significant increases in the biological

Table 2
Accumulated biological efficiency (% BE) and mushroom production (% MP) obtained on the best Mn(II) and/or NH_4^+ supplemented substrate formulations for five *Pleurotus ostreatus* strains

Strain	Treatment	BE (%)[A]	MP (%)
UAT PO7	0 ppm Mn(II)–0 ppm NH_4^+	73.6 b (6.28)[B]	29.5
	100 ppm Mn(II)–500 ppm NH_4^+	112.2 a (5.42)	44.7
	20 ppm Mn(II)–200 ppm NH_4^+	100.7 a (3.52)	23.3
	20 ppm Mn(II)–750 ppm NH_4^+	97.4 b (3.81)	39.0
	750 ppm NH_4^+	72.4 b (4.31)	28.8
UAT PO3	0 ppm Mn(II)–0 ppm NH_4^+	37.2 d (3.22)	14.9
	20 ppm Mn(II)–500 ppm NH_4^+	60.4 c (3.13)	24.3
	20 ppm Mn(II)–750 ppm NH_4^+	58.3 c (4.30)	23.4
	200 ppm Mn(II)–750 ppm NH_4^+	56.3 c (2.38)	23.2
	500 ppm NH_4^+	49.2 d (5.04)	19.9
UAT PO4	0 ppm Mn(II)–0 ppm NH_4^+	41.3 f (2.31)	16.3
	20 ppm Mn(II)–500 ppm NH_4^+	80.6 e (6.40)	32.1
	750 ppm NH_4^+	67.0 e (3.95)	26.5
	100 ppm Mn(II)	57.1 e (2.08)	22.8
	200 ppm Mn(II)–500 ppm NH_4^+	56.3 e (2.92)	22.6
ATCC 38537	0 ppm Mn(II)–0 ppm NH_4^+	65.9 h (2.35)	26.8
	200 ppm Mn(II)–750 ppm NH_4^+	105.3 g (4.40)	42.5
	20 ppm Mn(II)–500 ppm NH_4^+	86.9 g (4.37)	34.6
	20 ppm Mn(II)	82.0 g (5.42)	32.7
	750 ppm NH_4^+	58.8 h (2.84)	23.3
Somycel 3001	0 ppm Mn(II)–0 ppm NH_4^+	57.1 j (3.03)	23.3
	20 ppm Mn(II)	94.1 i (4.67)	38.3
	200 ppm Mn(II)–750 ppm NH_4^+	87.3 i (5.34)	34.9
	20 ppm Mn(II)–750 ppm NH_4^+	77.1 i (5.83)	30.1
	500 ppm NH_4^+	63.2 j (3.49)	25.5

a,b UAT PO7 values with different letters are significantly different ($P \leqslant 0.05$).
c,d UAT PO3 values with different letters are significantly different ($P \leqslant 0.05$).
e,f UAT PO4 values with different letters are significantly different ($P \leqslant 0.05$).
g,h ATCC 38537 values with different letters are significantly different ($P \leqslant 0.05$).
i,j Somycel 3001 values with different letters are significantly different ($P \leqslant 0.05$).
[A] Mean values were separated by the Tukey test.
[B] Standard error values.

Table 3
Productivity of five *Pleurotus ostreatus* strains grown in a sunflower seed hull substrate with optimized levels of Mn(II) and/or NH_4^+

Strain	Primordia initiation (days)	Time to second crop (days)	Mushroom production (%)	Biological efficiency (%)	Productivity (kg (100 kg day)$^{-1}$)	Production increase over control (%)
UAT PO4	24	41	32.1	80.6 (6.40)[A]	2.0	100
UAT PO7	24	39	44.7	112.2 (5.42)	3.0	58
ATCC 38537	28	39	42.5	105.3 (4.40)	2.7	59
Somycel 3001	28	51	38.3	94.1 (4.67)	1.8	50
UAT PO3	28	47	24.3	60.4 (3.13)	1.2	50

Substrates for growing UAT PO4, UAT PO7, ATCC 38537, Somycel 3001 and UAT PO3 strains were supplemented with 20 ppm Mn(II), 200 ppm Mn(II) + 750 ppm NH_4^+, 200 ppm Mn(II) + 750 ppm NH_4^+, 20 ppm Mn(II), and 20 ppm Mn(II) + 500 ppm NH_4^+, respectively. Primordia initiation, time elapsed until second flush, mushroom production and biological efficiencies are also shown.
[A] Standard error values.

efficiency; the composition of the best formulations was dependent on the strains. For example, UAT PO4 had a BE of 41% in the control substrate but supplementation with 20 ppm Mn(II) and 500 ppm NH_4^+ raised it to 80%; in the case of UAT PO7, BE was 112% compared to 74% in the control. Data for the best substrate formulation obtained for each of the strains, including the increase in productivity are shown in Table 3. Cumulative productivity (in kg mushroom/100 kg substrate per day) obtained in the two flushes was 2.0 for UAT PO4 (in substrate supplemented with 20 ppm Mn(II)), 3.0 for UAT PO7 (in substrate supplemented with 200 ppm Mn(II) and 750 ppm NH_4^+), 2.7 for ATCC 38537 (in substrate supplemented with 200 ppm Mn(II) and 750 ppm NH_4^+), 1.8 for Somycel 3001 (in substrate supplemented with 20 ppm Mn(II)) and 1.2 for UAT PO3 (in substrate supplemented with 20 ppm Mn(II) and 500 ppm NH_4^+).

Sunflower seed hull substrate supplemented with Mn(II) and/or NH_4^+ produced an increase in productivity of 50% or more, reaching a 100% increase for UAT PO4. From these results and those obtained for substrate mycelial colonization rates it is clear that fast mycelial spread need not necessarily lead to higher yields in terms of biological efficiency but it does when considering productivity.

4. Conclusions

This study demonstrates the production advantage obtained from selecting adequate *P. ostreatus* strains when using sunflower seed hulls as a substrate for providing the main energy and nutritional sources, and supplemented with Mn(II) and N–NH_4^+. Each strain presented significantly different rates for whole substrate colonization and different production levels depending on the formulation of the substrate. Under the culture conditions in this study, the productivity of different *P. ostreatus* strains was in the range of 1.2–3.0 kg fresh mushroom/100 kg dry substrate per day, with the Mexican strain UAT PO7 being the most productive.

Considering that in the production system proposed in a previous work (Curvetto et al., 1997) the spawn was homogeneously inoculated into the decontaminated substrate mass, it could be expected that productivity values markedly increased because of the improved rate of substrate colonization by the mushroom mycelium.

References

Blanchette, R.A., 1984. Manganese accumulation in wood decayed by white rot fungi. Phytopathol. 74, 725–730.

Buswell, J.A., Oider, O., 1987. Lignin biodegradation. Crit. Rev. Biotechnol. 6, 1–60.

Cancalon, P., 1971. Chemical composition of sunflower seed hulls. J. Am. Oil Chem. Soc. 48, 629–632.

Curvetto, N.R., Delmastro, S.E., Devalis, R.J., Figlas, D., 1997. A low cost method for decontaminating sunflower seed hull-based substrate in the cultivation of *Pleurotus* edible mushroom. Mushroom Res. 6, 25–28.

Darjania, L., Curvetto, N., Schapiro, M., Figlas, D., Curvetto, D., 1997. Sunflower seed hulls as a substrate for cultivation of *Pleurotus ostreatus*. Mushroom News 45, 6–10.

Duncan, K.W., 1997. An ecophysiological approach to the evaluation, modification and production of mushroom growing media. Mushroom News 45, 12–27.

El Kattan, M.H., Helmy, Z.A., El Leithy, M.A., Abdelkwai, K.A., 1991. Studies on cultivation techniques and chemical composition of oyster mushrooms. Mushroom J. Tropics 11, 59–66.

Hadar, Y., Kerem, Z., Gorodecki, B., Ardon, O., 1992. Utilization of lignocellulosic waste by edible mushroom *Pleurotus*. Biodegradation 3, 189–205.

Kaal, E.E.J., Field, J.A., Joyce, T.W., 1995. Increasing ligninolytic enzyme activities in several white-rot basidiomycetes by nitrogen-sufficient media. Bioresour. Technol. 53, 133–139.

Kerem, Z., Hadar, Y., 1995. Effect of manganese on preferential degradation of lignin by *Pleurotus ostreatus* during solid-state fermentation. Appl. Environ. Microbiol. 61, 3057–3062.

Khan, S.M., Chaudhary, Y.A., 1989. Some studies on oyster mushroom (*Pleurotus spp.*) on the waste material of corn industry in Pakistan. Mushroom Science, Part II. In: Proceedings of the 12th Intl. Cong. on the Science and Cultivation of Edible Fungi, pp. 23–29.

Kinugawa, K., Phusawang, W., Chinbenjaphol, S., Fukada, S., Tanesaka, E., Okada, M., Tsutsui, H., 1994. Progress Report (1991–1993) of joint research program of Kinki and Chiang Mai Universities on the promotion of mushroom research. Mem. Fac. Agr. Kinki Univ. 27, 93–113.

Lelley, J.I., Janßen, A., 1993a. Productivity improvement of oyster mushroom substrate with a controlled release of nutrient. Mushroom News 41, 6–13.

Lelley, J.I., Janßen, A., 1993b. Interactions between supplementation, fructification-surface and productivity of the substrate of *Pleurotus spp.*. In: Chang, S.-T., Buswell, J.A., Chiu, S.-W. (Eds.), Mushroom Biology and Mushroom Products. The Chinese University Press, Hong Kong (Chapter 9).

Martínez, A.T., Camarero, S., Guillen, F., Gutiérrez, A., Muñoz, C., Varela, E., Martínez, M.J., Barrasa, J.M., Ruel, K., Pelayo, M., 1994. Progress in biopulping of non-woody materials. Chemical, enzymatic and ultrastructural aspects of wheat straw delignification with ligninolytic fungi from the genus *Pleurotus*. FEMS Microbiol. Rev. 13, 265–274.

Ott, L., 1984. An introduction to statistical methods and data analysis, 2nd ed. PWS Publ., Boston, Massachusetts.

Zadrazil, F., 1985. Screening of fungi for lignin degradation and conversion of straw into feed. Angew. Bot. 59, 433–452.

Zadrazil, F., Brunnert, H., 1979. Influence of ammonium nitrate on the growth and straw decomposition of higher fungi. Zeit Pflanzenernaehr Bodenkd 142, 446–455.