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Influence of Nutrient Amendment on the Biodegradation of Wheat Straw during Solid State Fermentation with *Trametes versicolor*

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The present study proposes a system whereby lignin biodegradation in lignocellulosic units may be optimised with the minimum loss of cellulose and other organic matter. Lignin, cellulose and organic matter losses were followed at 7-day intervals during 35-day solid state fermentation of wheat straw with *Trametes versicolor* with and without amendment carbon (C) and nitrogen (N) sources. Substrate supplementation with a C source favoured degradation of lignin; glucose as the C source was better than beet pulp molasses. Ammonium N slowed lignin removal. Lignin loss during 35-day fermentation in the presence of glucose was 42% as compared with 17.1% in the unamended wheat straw. The highest ratios of lignin loss to cellulose loss (9.55:1) and to organic matter loss (3.34:1) occurred in the first 14 days of fermentation when the straw was amended with glucose. These observations indicate that for efficient ligninolysis, it is appropriate to terminate fermentation of the glucose-amended straw at the 14-day stage at which 36.3% lignin degradation occurred at the cost of only 3.8% cellulose and 10.7% organic matter. Copyright © Published by 1996 Elsevier Science Limited.

INTRODUCTION

Fifty to sixty percent of the photosynthetically-produced biomass is incorporated in tissues of higher land plants as lignocellulosic substances (Kirk, 1983). Crop residues therefore represent an important by-product of the agricultural industry. The cellulose present in the lignocellulosic complex has the potential to be used as an energy source for ruminants or in industrial biotechnology processes (Reddy, 1978). Due to the intimate association of cellulose with lignin, however, it is not readily available as a C source unless the lignin component is chemically and/or biologically modified or removed (Freer & Detroy, 1982). Whereas chemical delignification is possible, it is expensive and poses a pollution problem (Kirk *et al.*, 1978). Biological degradation of lignin is an alternative, which is possible through the ligninolytic activities of white rot basidiomycetes (Ander & Eriksson, 1978; Kirk, 1983; Zadrazil, 1977). Efficiency of lignin removal depends upon the fungal species and varies from substrate to substrate, and on carbon and nitrogen supplements (Levonen-Munoz *et al.*, 1983; Bone & Levonen-Munoz, 1984).

In view of the relative abundance of wheat straw as an agricultural waste, its biodegradation by a number of basidiomycete species has been studied (Zadrazil & Brunnert, 1980). The white rot basidiomycete *Trametes versicolor* was found to be a potent degrader of wheat straw lignin (Kamra & Zadrazil, 1988; Valmaseda *et al.*, 1991). Though *T. versicolor* causes simultaneous degradation of lignin and polysaccharides (Valmaseda *et al.*, 1991), the preferential metabolism can be controlled by changing the incubation conditions (Yadav & Tripathi, 1991). It has been shown that the ligninolytic process is faster under nitrogen-starved conditions (Zafar *et al.*, 1989). The present study investigated the effect of C and N sources supplementation. Glucose, ammonium sulphate and beet pulp molasses were supplied at various stages of the wheat straw–*T. versicolor* solid state fermentation and the comparative removal of

lignin, cellulose and organic matter was assessed. The objective was to develop cultural conditions well-suited for maximum delignification at the minimal loss of other energy-rich components of the straw so as to identify an efficient solid state fermentation system.

MATERIALS AND METHODS

Fungus and inoculum preparation

Trametes versicolor PRL 2276, obtained from National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada, was maintained on standard potato dextrose agar medium in test tube slants at 5–7°C. Inoculum preparation and substrate inoculations were done as previously described (Zafar *et al.*, 1989).

Substrate preparation and incubation

Wheat straw var. Mexi-Pak, chopped into 30–50 mm pieces, was soaked in boiling water for 15 min, and excess water drained. Water content of this straw ranged between 65–70%. Moistened wheat straw (25 g) was transferred to 250 ml Erlenmeyer flask. The wheat straw was amended with 10 ml of the nutrient medium consisting of 2.0 g l^{-1} KH_2PO_4, 0.3 g l^{-1} $MgSO_4.7H_2O$, 0.4 g l^{-1} $CaCl_2.2H_2O$ and 0.1 g l^{-1} yeast extract in distilled water supplemented with: (G) 50.0 g l^{-1} glucose; or (M) 100 g l^{-1} beet pulp molasses (sugar approx 54%, N 0.65%); or (G + N) 50.0 g l^{-1} glucose + 2.1 g l^{-1} $(NH_4)_2SO_4$. Ten millilitres of distilled water instead of the supplemented nutrient medium served as the control (NA). Flasks and substrates were sterilized, inoculated with *T. versicolor* and incubated at 25°C for 7, 14, 21, 28 and 35 days. Four flasks of each straw-amendment treatment were harvested for the different incubation periods.

Analytical methods

The fermented wheat straw, after oven drying at 100–105°C, was ground to less than 1 mm. Loss of organic matter was calculated according to Zadrazil (1977). Cellulose and lignin were determined using the procedure of van Soest & Wine (1969). All calculations were done on dry weight basis.

RESULTS AND DISCUSSION

Cellulose biodegradation

Cellulose loss of 6.6% in wheat straw amended with molasses was significantly less (at $P = 0.05$; Duncan's multiple range test) than 10.2 and 9.2% noted, respectively, when the substrate was amended with glucose alone or with glucose + $(NH_4)_2SO_4$ during 35 days of fermentation with *Trametes versicolor* (Table 1, Fig. 1a). These observations indicate that extraneous supply of glucose and molasses as the C energy source exerted less demand on cellulose compared with the significantly greater degradation (14.2%) in the control substrate (NA). This is in agreement with the preferential consumption of reducing sugars during oat straw fermentation reported by Levonen-Munoz *et al.* (1983). Significantly low cellulose degradation on amendment with molasses may be further attributed, either singly or in combination, to the presence therein of 4–7% reducing sugars, phenolic compounds reported to inhibit cellulose-degrading enzymes (Chang, 1993), and the freely accessible 93–96% non-reducing sugars compared with the substrate-cellulose not being readily available due to the lignin barrier (Freer & Detroy, 1982). No statistically significant difference in cellulose degradation was observed throughout the fermentation period, except at the 14-day stage, when the substrate was amended with G or G + N indicating that N-supplementation had little or no influence on the biodegradation of cellulose.

Lignin biodegradation

Lignin loss during 35 days of fermentation was 42.0% when wheat straw was supplemented with only glucose, which was significantly higher in comparison with 17.1% loss in the control (Table 1, Fig. 1b). This is in agreement with the reported kraft lignin solubilization by *Phanerochaete chrysosporium* (Leisola *et al.*, 1983) and lignin removal from oat straw by *Polyporus sp.* A-336 (Bone & Levonen-Munoz, 1984) being linked with excess availability of glucose. Since the substrate cellulose was, nevertheless, degraded throughout the fermentation period (Table 1), the additional glucose was apparently utilized as a co-energy source to enhance lignin degradation. This also explains the significantly high cellulose loss associated with the lowest lignin loss in the control.

Table 1. Percentage Cellulose, Lignin and Organic Matter in Wheat Straw$^{\Omega}$ Biodegradation by *Trametes versicolor* at Various Periods at 25°C. Figures within Parenthesis indicate Percentage Losses due to Biodegradation

Period (Days)	Substrate amendment			
	NA	G	M	G+N
CELLULOSE				
0	42.3 ± 0.17	42.3 ± 0.17	42.3 ± 0.17	42.3 ± 0.17
7	40.6 ± 0.25 (4.1)^{d}	41.0 ± 0.23 (3.1)^{b}	41.6 ± 0.24 (1.6)^{a}	41.0 ± 0.34 (3.1)^{bc}
14	38.0 ± 0.29* (10.1)^{d}	40.7 ± 0.30 (3.8)^{b}	41.2 ± 0.14 (2.6)^{a}	39.3 ± 0.24* (7.2)^{c}
21	37.7 ± 0.16 (11.0)^{d}	39.0 ± 0.19* (7.8)^{b}	39.8 ± 0.18* (5.9)^{a}	39.0 ± 0.25 (7.8)^{bc}
28	36.8 ± 0.35 (13.0)^{d}	38.8 ± 0.16 (8.3)^{b}	39.6 ± 0.30 (6.4)^{a}	38.7 ± 0.28 (8.6)^{bc}
35	36.3 ± 0.31 (14.1)^{d}	38.0 ± 0.23 (10.2)^{bc}	39.5 ± 0.26 (6.6)^{a}	38.4 ± 0.27 (9.2)^{b}
LIGNIN				
0	12.4 ± 0.19	12.4 ± 0.19	12.4 ± 0.19	12.4 ± 0.19
7	11.9 ± 0.23 (4.4)^{a}	10.3 ± 0.26 (17.1)^{c}	11.0 ± 0.42 (11.3)^{b}	10.2 ± 0.23 (17.7)^{cd}
14	10.9 ± 0.17* (11.8)^{a}	7.9 ± 0.28* (35.9)^{d}	10.2 ± 0.18 (17.7)^{b}	9.8 ± 0.19 (20.9)^{c}
21	10.8 ± 0.15 (12.8)^{a}	7.8 ± 0.23 (37.3)^{d}	9.2 ± 0.16* (25.8)^{b}	8.7 ± 0.25* (30.2)^{c}
28	10.7 ± 0.15 (13.5)^{a}	7.6 ± 0.24 (38.7)^{d}	8.6 ± 0.28 (30.9)^{b}	8.4 ± 0.16 (32.0)^{bc}
35	10.3 ± 0.27 (17.1)^{a}	7.2 ± 0.33 (42.0)^{d}	8.1 ± 0.19 (34.8)^{bc}	8.1 ± 0.13 (34.4)^{b}
ORGANIC MATTER				
0	100.0	100.0	100.0	100.0
7	91.6 ± 0.37 (8.4)^{c}	93.8 ± 0.35 (6.1)^{a}	93.5 ± 0.34 (6.5)^{ab}	86.1 ± 0.32 (14.1)^{d}
14	84.2 ± 0.17 (15.8)^{bc}	89.2 ± 0.57 (10.7)^{a}	85.2 ± 0.26 (14.8)^{b}	81.0 ± 0.41 (19.0)^{d}
21	79.4 ± 0.34 (20.6)^{b}	84.9 ± 0.39 (15.1)^{a}	78.5 ± 0.31 (21.5)^{bc}	77.5 ± 0.24 (22.5)^{cd}
28	75.1 ± 0.16 (24.9)^{b}	80.2 ± 0.14 (19.8)^{a}	75.7 ± 0.32 (25.7)^{bc}	73.5 ± 0.42 (26.5)^{cd}
35	74.8 ± 0.32 (25.2)^{b}	79.9 ± 0.51 (20.2)^{a}	75.1 ± 0.23 (25.9)^{bc}	72.6 ± 0.34 (27.4)^{d}

Ω amended with 5% glucose (G); 10% beet pulp molasses (M); 5% glucose + 0.21% $(NH_4)_2SO_4$ (G+N); unamended (NA).
a.b.c.d. = values with same alphabets are not significantly different at $P = 0.05$ (Duncan's multiple range test).
* no statistically significant degradation beyond this period.
± standard error.

For determining the effect of added N on delignification, the amendment containing molasses (having 0.65% N in the mother liquor) can be considered together with G+N supplementation. Additional N in the fermenting straws on account of the two amendments was, respectively, equivalent to 0.026 and 0.023%. The corresponding delignification during 35 days of biodegradation with these treatments was 34.8 and 34.4%, which like glucose supplementation were also significantly greater than the control (Table 1, Fig. 1b). It may be noted, however, that more lignin was degraded with glucose alone than with the two amendments containing additional N. This agrees with the reported higher lignin losses in N-starved conditions on fermentation of oat straw with *Polyporus* tulipifereae (Levonen-Munoz *et al.*, 1983) and repression of lignin degradation by *Phanerochaete chryosporium* in the presence of N (Fenn & Kirk, 1981). Delignification in G plateaued within 14 days (Table 1, Fig. 1b). The corresponding stage with M and G+N was achieved in 21 days. Evidently, lignin degradation in N-starved conditions was not only greater, but also faster as compared with that in the N-supplemented substrates. These observations gain support from the ability of ligninolytic basidiomycetes to establish new cycles of growth at 10–15 day intervals, under N-starved conditions, by recycling the available proteinous N in the fermentation medium (Merril & Cowling, 1966; Ulmer *et al.*, 1983).

Ligninolytic efficiency

To develop an efficient ligninolytic system in white rot basidiomycete-mediated lignocellulosic biodegradation, it is essential to strike a balance between maximum lignin loss with a low removal level of cellulose and other organic matter within the shortest incubation period. It was observed that during the 35-day fermentation, the organic matter loss of 20.1% was the lowest when the straw was amended with G, increasing to 24.9, 25.2 and 27.4%, respectively, with M, NA and G+N (Table 1, Fig. 1c). Loss at the 14-day fermentation stage with G, however, was only 10.8% associated with the high degree of 36.3% lignin removal. Lignin loss per unit organic matter degraded at this stage (3.34:1) was the highest (Table 2). Corresponding with this observation was the highest ratio of lignin degradation per

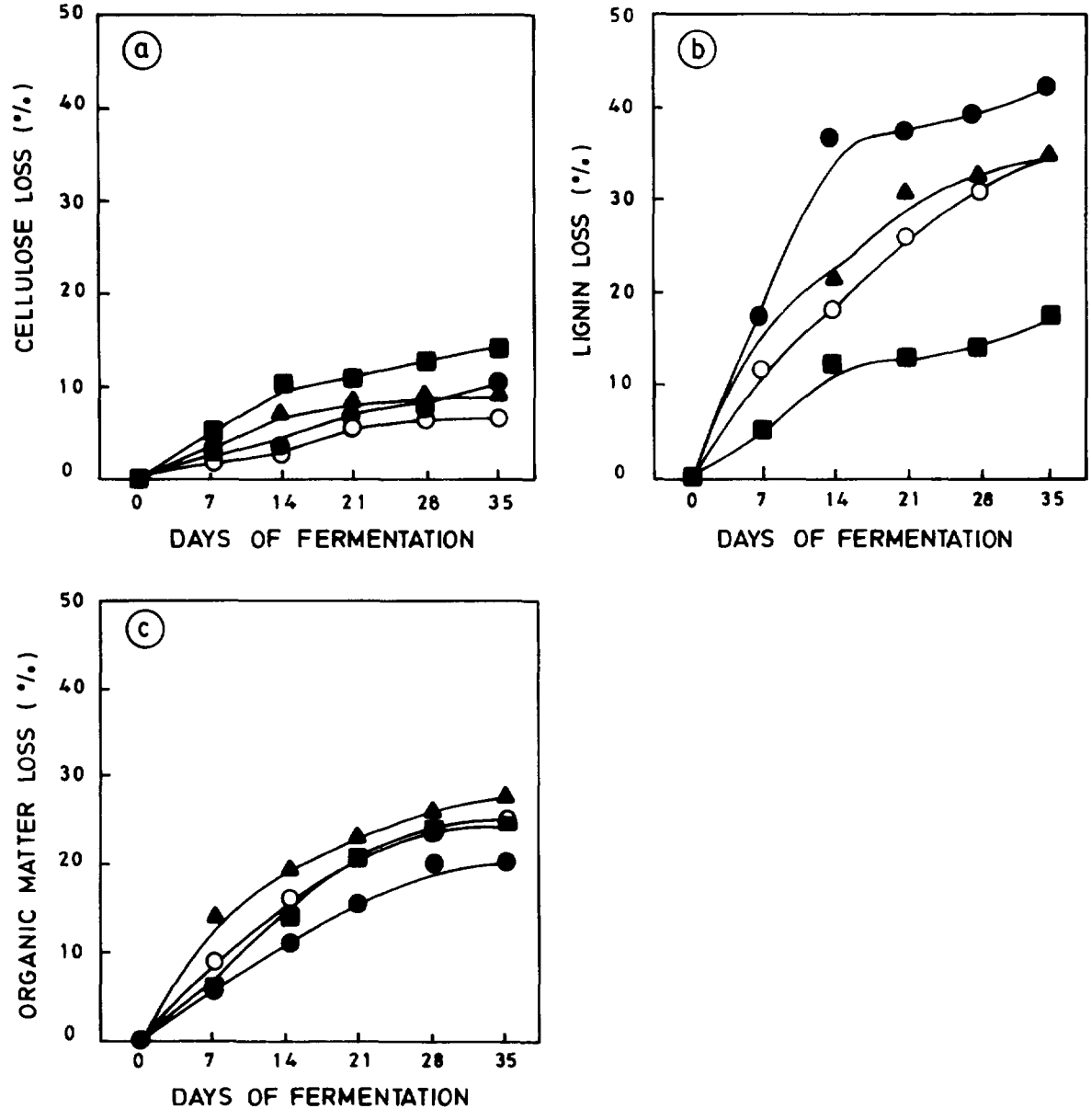

Fig. 1. Percentage degradation in the contents of (a) cellulose, (b) lignin, and (c) organic matter in wheat straw on fermentation with *Trametes versicolor* at 25°C when the wheat straw substrate was not amended (NA ■) and amended with supplementation medium containing: 5% glucose (G ●), 10% beet pulp molasses (M ○), or 5% glucose +0.21% $(NH_4)_2SO_4$ (G+N ▲).

Table 2. Lignin Loss Ratios per Unit Cellulose and Dry Matter lost During Wheat Straw$^{\Omega}$ Fermentation with *Trametes versicolor* at Various Periods at 25°C

Period (Days)	LIGNIN LOSS PER UNIT CELLULOSE LOSS Substrate amendment				LIGNIN LOSS PER UNIT ORGANIC MATTER LOSS Substrate amendment			
	NA	G	0 M	G+N	NA	G	M	G+N
7	1.07^{a}	5.58^{b}	6.85^{d}	5.71^{bc}	0.52^{a}	2.79^{d}	1.73^{c}	1.26^{b}
14	1.16^{a}	9.55^{d}	6.81^{c}	2.96^{b}	0.74^{a}	3.34^{d}	1.19^{bc}	1.10^{b}
21	1.17^{a}	4.75^{d}	4.37^{c}	3.87^{b}	0.62^{a}	2.45^{d}	1.20^{b}	1.34^{bc}
28	1.04^{a}	4.66^{c}	4.78^{cd}	3.79^{b}	0.54^{a}	1.95^{d}	1.20^{b}	1.22^{bc}
35	1.20^{a}	4.12^{c}	5.26^{d}	3.73^{b}	0.68^{a}	2.08^{d}	1.39^{bc}	1.25^{b}

Ω amended with 5% glucose (G); 10% beet pulp molasses (M); 5% glucose + 0.21% $(NH_4)_2SO_4$ (G+N); unamended (NA).
a.b.c.d. = values with same alphabets are not significantly different at $P = 0.05$ (Duncan's multiple range test).

unit cellulose consumed (9.55:1) at the 14-day fermentation period (Table 2). These observations agree well with no statistically significant delignification achieved beyond 14 days of incubation (Table 1). It is of interest to mention further that during all the successive 7-day periods of the 35-day biodegradation studies, the ratio of lignin to cellulose loss remained greater than 4:1. Biodegradation in the presence of molasses also remained significantly in favour of lignin, in comparison with cellulose, being 6.85:1 and 6.81:1 at 7- and 14-day intervals, and at no stage of the 35-day period dropping below 4:1 as was noted with glucose.

The study indicates that for efficient ligninolysis, it is appropriate to terminate fermentation at the 14-day stage at which 36.3% lignin degradation occurred with the loss of 3.8% cellulose when the straw was amended with glucose (42% lignin was degraded at the cost of 10.2% cellulose during 35 days). It was also observed that greater ligninolytic activity was induced in the presence of extraneous C supply, particularly glucose in the absence of N. Though not as efficient as glucose, supplementation with molasses was also found to be a suitable amendment in a *T. versicolor*–wheat straw fermentation system. Considering the low cost of molasses compared with glucose, this observation may aid in the eventual development of an economical large-scale delignification system based entirely on agricultural and agro-industrial wastes.

REFERENCES

Ander, P. & Eriksson, K.-E. (1978). Lignin degradation and utilization by microorganisms. In *Progress in Industrial Microbiology*, ed. M. J. Bull, Vol. 14, pp. 1–58. Elsevier, Amsterdam.

Bone, D.H. & Levonen-Munoz, E. (1984). Solid state fermentation of oat straw by *Polyporus sp.* A-336 and the effect of added sugars. *Biotechnol. Lett.*, **6**, 657–662.

Chang, S.-T. (1993). Biology and cultivation technology of *Volvariella volvacea*. In *Mushroom Biology and Mushroom Products*, eds S.-T. Chang, J.A. Buswell & S.-W. Chiu, pp. 73–83. Chinese Univ. Press, Hong Kong.

Fenn, P. & Kirk, T.K. (1981). Relationship of nitrogen to the onset and suppression of ligninolytic activity and secondary metabolism in *Phanerochaete chrysosporium*. *Arch. Microbiol.*, **130**, 59–65.

Freer, S.N. & Detroy, R.W. (1982). Biological delignification of ^{14}C-labelled lignocelluloses by basidiomycetes: degradation and solubilization of the lignin and cellulose components. *Mycologia*, **74**, 943–951.

Kamra, D.N. & Zadrazil, F. (1988). Microbiological improvement of lignocellulosics in animal feed production—a review. In *Treatment of Lignocellulosics with White Rot Fungi*, eds F. Zadrazil and P. Reiniger, pp. 56–63. Elsevier Applied Science, London.

Kirk, T.K. (1983). Degradation and conversion of lignocelluloses. In *The Filamentous Fungi*, eds J.E. Smith, D.R. Berry & B. Kristiansen. Vol. IV, pp. 266–295. Fungal Technology, London, Edward Arnold.

Kirk, T.K., Yang, H.H. & Keyser, P. (1978). The chemistry and physiology of the fungal degradation of lignin. *Dev. Ind. Microbiol.*, **19**, 51–61.

Leisola, M., Ulmer, D., Haltmeier, T. & Fiechter, A. (1983). Rapid solubilization and depolymerization of purified kraft lignin by thin layers of *Phanerochaete chrysosporium*. *Eur. J. Appl. Microbiol. Biotechnol.*, **17**, 117–120.

Levonen-Munoz, E., Bone, D.H. & Daugulis, A.J. (1983). Studies on Fractionation of Lignocellulosics of Oat Straw by Basidiomycetes, *Tech. Rep.*, 83-1, Deptt. Chem. Eng., Queen's Univ., Kingston, Ont., K7L 3NG, Canada, pp. 1–25.

Merrill, W. & Cowling, E.B. (1966). Role of nitrogen in wood deterioration: amounts and distribution of nitrogen in tree stems. *Canad. J. Bot.*, **44**, 1555–1580.

Reddy, C.A. (1978). Introduction to microbial degradation of lignin. *Dev. Ind. Microbiol.*, **19**, 23–26.

Ulmer, D., Leisola, M., Puhakka, J. & Fiechter, A. (1983). *Phanerochaete chrysosporium*: growth pattern and lignin degradation. *Eur. J. Appl. Microbiol. Biotechnol.*, **18**, 153–157.

Valmaseda, M., Martinez, M.J. & Martinez, A.T. (1991). Kinetics of wheat straw solid-state fermentation with *Trametes versicolor* and *Pleurotus ostreatus*—lignin and polysaccharide alteration and production of related enzymatic activities. *Appl. Microbiol. Biotechnol.*, **35**, 617–823.

van Soest, P.J. & Wine, R.H. (1967). Use of detergents in the analysis of fibrous feeds, IV, Determination of plant cell wall constituents. *J. Assoc. Anal. Chem.*, **50**, 50–55.

Yadav, J.S. & Tripathi, J.P. (1991). Optimization of cultivation and nutrition conditions and substrate pretreatment for solid state fermentation of wheat straw by *Coriolus versilcolor*.. *Folia Microbiol.*, **36**, 294–301.

Zadrazil, F. (1977). Conversion of straw into feed by basidiomycetes. *Eur. J. Appl. Microbiol.*, **4**, 273–281.

Zadrazil, F. & Brunnert, H. (1980). The influence of ammonium nitrate supplementation on digestion and *in vitro* digestibility of straw colonized by higher fungi. *Eur. J. Appl. Microbiol. Biotechnol.*, **9**, 37–44.

Zafar, S.I., Sheeraz, Q. & Abdullah, N. (1989). Degradation of the lignocellulosic component on wheat straw–*Coriolus versicolor* solid state fermentation under nitrogen-starved conditions. *Biol. Wastes*, **27**, 67–70.