UTILISATION OF AGRO-RESIDUES AS ANIMAL FEED THROUGH BIOCONVERSION

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Abstract

Bioconversion of agro-residues, rice straw and wheat straw, was carried out with the edible mushroom Pleurotus sajor-caju, *with a view to increasing nutritive values and digestibilities for animal feed. Amongst the inorganic and organic nitrogen sources tested, soyabean meal and ficus fruits, in which nitrogen is present in complex form, were the best supplements in enhancing the* in vitro *dry matter digestibilities of the residues.*

Key words: Animal feed, *Pleurotus sajor-caju*, agro-residues, bioconversion.

INTRODUCTION

Agro-residues which contain three major structural polymers, cellulose, hemicellulose and lignin, are the main sources of feed for ruminants. However, their quality as feed, especially in terms of digestibility, depends not only on the amount and structure of these polymers but also on the amount of readily available substances such as sugars and amino acids. White-rot fungi which degrade cellulose and hemicellulose as well as lignin are widely used to increase the digestibility of agro-residues (Kurtzman, 1981; Neelakantan, 1987; Zadrazil, 1987). Considerable work has been done in the author's laboratory on the use of the white-rot fungus, *Pleurotus sajor-caju*, known as the oyster mushroom (Bisaria *et al.*, 1987a, 1987b, 1987c, 1989, 1990; Gujral *et al.*, 1987; Jain *et al.*, 1988) for the production of edible mushrooms. It was of interest to study the effectiveness of this fungus for enhancing the feed value of agro-residues.

The nitrogen contents of the residues were generally below the optimal levels for growth of fungi. Supplementation with nitrogenous compounds would, therefore, be expected to lead to better proliferation of fungi as increased nitrogen content with concomitant lower C:N ratio would tend to support fungal growth. However, since excess of nitrogen may also inhibit the synthesis of lignin-degrading enzymes by the fungus, and since lignin is the recalcitrant polymer, the degradability may decrease. A balance has, therefore, to be made so that the maximum digestibility of bioconverted residues is achieved on supplementation of these residues with nitrogen sources. This paper reports the findings, using various inorganic and organic nitrogen sources, on bioconversion of rice straw and wheat straw with *Pleurotus sajor-caju*.

METHODS

Solid-state bioconversion of straws

The solid-state bioconversion was carried out in 500 ml conical flasks with 15 g substrate. The substrate was mixed with 60 ml water. The flasks were autoclaved at 15 psi for 30 min. In all the studies, 20% inoculum (i.e. 3 g grain spawn) of *Pleurotus sajor-caju* was used. The flasks were incubated at 25°C for up to 25 days. During incubation, bioconverted straw samples were taken at intervals of 4 days, oven-dried at 60°C and analysed for nitrogen, cellulose, hemicellulose and lignin contents, and *in vitro* dry matter digestibility (IVDMD).

The effects of supplementation with nitrogen sources on solid-state bioconversion was studied using inorganic N and soyabean meal, corn steep liquor and wild ficus fruits, as these latter were nitrogen-rich wastes available near the Institute. The cultures were provided with different amounts of nitrogen sources to obtain C:N values of 30 or 60. Unsupplemented rice straw having a C:N ratio of 74 and wheat straw having a C:N ratio of 70 served as controls.

Analytical procedures

Cellulose, hemicellulose and lignin

These were estimated on materials before or after treatment by the method of Datta (1981).

Crude protein
Nitrogen was estimated by a micro-kjeldahl method (Lang, 1958). Crude protein was determined by multiplying the nitrogen content by 6·25. The samples of bioconverted residues were washed with water to remove soluble nitrogenous compounds, such as urea, before analysis. However, it was not possible to remove the complex nitrogen substances, soyabean meal or ficus fruits, from the bioconverted residues. The apparent crude protein levels of these residues were accordingly higher than were those of cultures supplemented with soluble nitrogen compounds.

In vitro *dry matter digestibility*
It was determined as described by Guggolz *et al.* (1971).

Enzyme assays
For estimation of enzyme activities, 1 g of residue undergoing bioconversion (without drying) was suspended in 20 ml citrate buffer (pH 4·8, 0·05 M) and shaken gently for 5 min to dislodge the adsorbed enzymes into the buffer. The enzyme activities were assayed in the supernatant obtained after centrifugation of the suspension. Filter paper activity, endoglucanase activity, β-glucosidase activity, and xylanase activities were measured as described by Ghose (1987).

Reproducibility
All experiments were carried out in triplicate. The results reported are the means of the three cultures, which differed by not more than 8%.

RESULTS AND DISCUSSION

Bioconversion of unsupplemented rice and wheat straws
To estimate the *in vitro* dry matter digestibility (IVDMD) of the straws without any supplementation with nitrogen sources, control experiments were performed with *P. sajor-caju* for 20 days as described above. Rice straw contained 11% ash and 10·8% hot-water solubles (pectin and oligosaccharides), besides cellulose, hemicellulose and lignin. These hot-water solubles were removed first as they are of high degradability compared to cellulose, hemicelluloses or lignin. The incubated rice straw after 20 days showed that 31·8% of organic matter was lost (LOM), which included degradation of cellulose, hemicellulose and lignin. Crude protein and IVDMD increased in the bioconverted residue (Table 1).

Bioconversion of wheat straw resulted in 33·5% loss of organic matter, while crude protein increased as did IVDMD (Table 1).

Bioconversion of rice and wheat straws after supplementation with inorganic and organic nitrogen sources
At each C:N ratio, incubation was for 20 days. The effect of nitrogen supplementation was assessed with respect to changes in the contents of cellulose, hemicellulose and lignin polymers, LOM, crude protein and IVDMD. The results of these experiments are summarized in Table 2 for rice straw as well as for wheat straw.

In the case of rice straw, supplementation with ammonium nitrate resulted in the maximum (34·7%) LOM at C:N of 60, probably because the salt provided a readily-assimilable source of nitrogen. The

Table 1. Bio-conversion of unsupplemented rice straw and wheat straw by *Pleurotus sajor-caju*: effect on degradation of cellulose, hemicellulose, and lignin, crude protein and IVDMD

Incubation period (day)	LOM (%)[a]	Composition of bioconverted residue (%)			Nitrogen content (%)	Crude protein (%)	IVDMD (%)
		Hemi-cellulose	Cellulose	Lignin			
Rice straw							
0	—	25·6	35·8	17·2	0·46	2·87	19·7
4	9·8	21·8	30·8	15·6	0·62	3·87	17·8
8	20·4	18·6	25·1	13·1	0·81	5·06	21·8
12	25·1	16·7	22·8	11·7	0·90	5·6	25·6
16	26·8	14·8	19·7	11·7	0·98	6·1	28·4
20	31·8	14·4	17·9	9·5	1·02	6·3	29·8
Wheat straw							
0	—	25·8	30·8	13·6	0·5	3·1	27·2
4	14·8	21·5	23·6	11·3	0·82	5·1	27·6
8	22·6	19·7	20·7	10·2	0·97	6·1	28·1
12	25·3	17·2	17·6	9·5	1·08	6·6	31·7
16	28·7	15·7	16·3	8·3	1·12	7·0	32·5
20	33·5	13·4	13·6	6·4	1·2	7·5	36·8

[a] The composition of macromolecules was computed on the basis of their content in the original residue after taking loss of organic matter (LOM) into account.

Table 2. Effect of C:N ratio on bioconversion of rice straw and wheat supplemented with various nitrogen sources in terms of loss of organic matter (LOM) content of macromolecules crude protein, and IVDMD, after 20 days fermentation

Supplementation with	LOM (%)[a]		Cellulose (%)		Hemicellulose (%)		Lignin (%)		Crude protein (%)		IVDMD (%)	
	Initial C:N = 60	Initial C:N = 30	Initial C:N = 60	Initial C:N = 30	Initial C:N = 60	Initial C:N = 30	Initial C:N = 60	Initial C:N = 30	Initial C:N = 60	Initial C:N = 30	Initial C:N = 60	Initial C:N = 30
(a) Rice straw												
Ammonium nitrate	34·7	41·4	16·8	13·1	13·2	11·3	8·7	9·1	7·2	7·6	29·6	23·7
Urea	33·8	41·7	16·2	14·8	13·7	12·2	8·5	9·2	7·4	7·9	19·5	25·1
Soyabean meal	33·0	39·2	16·7	13·4	11·3	9·8	7·1	8·4	9·0	9·2	31·4	9·0
Corn steep liquor	34·2	41·2	16·3	12·1	11·7	9·2	7·6	8·7	7·7	8·0	29·0	26·4
Ficus fruits	32·9	38·1	16·8	13·8	11·9	10·1	6·8	7·8	9·2	9·5	31·8	29·7
(b) Wheat straw												
Ammonium nitrate	34·2	42·4	13·2	12·0	13·2	12·4	7·2	8·3	7·7	8·1	30·3	28·8
Urea	35·2	41·7	13·4	13·0	13·2	11·7	7·7	8·7	7·9	8·4	31·2	29·2
Soyabean meal	33·7	38·2	13·1	12·4	13·7	12·0	6·8	7·4	9·5	9·8	34·3	31·8
Corn steep liquor	34·8	40·7	13·0	11·2	13·1	11·5	7·1	8·0	8·0	8·3	31·7	30·2
Ficus fruits	33·3	38·7	13·0	11·4	13·1	11·0	6·2	7·3	9·7	10·2	34·8	32·3

[a] The composition of macromolecules was computed on the basis of their content in the original residue after taking loss of organic matter (LOM) into account.

degradation of cellulose and hemicellulose polymers was, in general, highest with nitrogen-supplemented rice straw (lower C:N ratio), whereas the degradation of lignin was the highest with unsupplemented rice straw (higher C:N ratio). Crude protein was the highest (9·5%) in ficus fruit supplementation of rice straw (C:N = 30) and this supplementation gave the high IVDMD of 31·8% at C:N of 60. Supplementation with soyabean meal (C:N = 60) resulted in an equally high IVDMD of the bioconverted residue.

For wheat straw, supplemented with various nitrogen sources, urea supplementation caused the highest LOM at a C:N of 60. As with rice straw, the nitrogen supplementation increased the degradation of cellulose and hemicellulose but decreased the degradation of lignin. The crude protein content was maximum for wheat straw supplemented with ficus fruits. The increase in IVDMD of wheat straw was highest in the case of ficus fruits and soyabean meal supplementation at a C:N of 60.

It seems, therefore, that ficus fruits and soyabean meal, which contain nitrogen in more complex forms than do the other sources, urea, ammonium nitrate or corn steep liquor, are more suited as supplements to these straws. Slow release of nitrogen may keep synthesis of lignin-degrading enzymes under a derepressed state as lignin removal was the highest in these cases. The amount of soyabean meal or ficus fruits needed to supplement wheat straw is of the order of 20 g kg^{-1} wheat straw. Considering the cost of these nitrogen sources (less than \$0·5 kg^{-1}), they seem to be quite attractive candidates for use as supplements to enhance the digestibility of straws through fungal bioconversion.

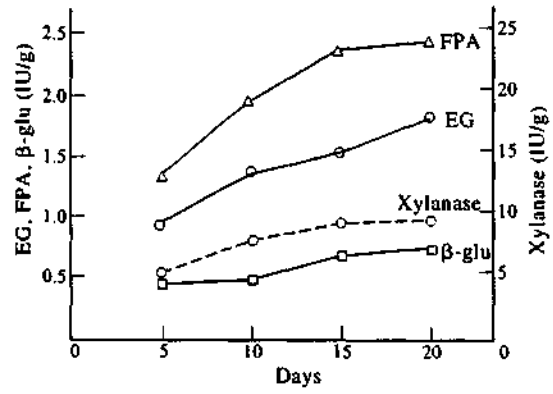

Fig. 1. Production of cellulase (EG, endogluconase; FPA, filter-paper activity), *β*-glucosidase and xylanase enzymes during solid-state bioconversion of rice straw supplemented with ficus fruits (C:N = 60) by *Pleurotus sajor-caju*.

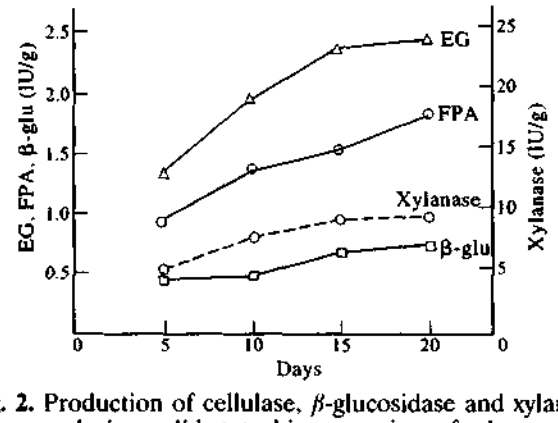

Fig. 2. Production of cellulase, *β*-glucosidase and xylanase enzymes during solid-state bioconversion of wheat straw supplemented with ficus fruits (C:N = 60) by *Pleurotus sajor-caju*.

Enzyme production during bioconversion

The production of cellulase, xylanase and *β*-glucosidase was monitored during the bioconversion of ficus fruits-supplemented rice straw and wheat straw

(C:N = 60). In the case of rice straw, the filter paper activity and the β-glucosidase activity attained maxima of 3·7 IU g^{-1} and 0·6 IU g^{-1}, respectively, after 15 days of culture, whereas endoglucanase attained a maximum activity of 1·7 IU g^{-1} after 20 days. Xylanase activity remained constant at 12 IU g^{-1} after 15 days of culture (Fig. 1). The enzyme production during bioconversion of wheat straw is shown in Fig. 2. The enzyme levels of filter paper activity, endoglucanase and β-glucosidase were maximum at 1·8 IU g^{-1}, 22·41 IU g^{-1} and 0·7 IU g^{-1}, respectively, after 20 days of bioconversion. Xylanase activity was also maximum after 20 days.

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