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CELLULASE AND PROTEIN PRODUCTION BY *CHAETOMIUM CELLULOLYTICUM* GROWN ON RICE STRAW

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Abstract. Cellulase and protein production by *Chaetomium cellulolyticum* grown on rice straw has been reported in this study. The crude protein content of biomass increased by the growth of fungus on medium supplemented with pretreated rice straw. The pretreatment includes reaction with 0, 2, 4, 6, 8 and 10% (w/w) NaOH levels followed by steam pressure treatment (SPT) at 1.5 kg/cm² for 15, 30 and 60 min. Extracellular CMCase activity also increased significantly in medium suplemented with prelreated rice straw samples. Pretreatment of NaOH accompanied by steam pressure had very little effect on total sugar, reducing sugar and total lipid content of mycelium. Hence pretreated rice straw can be safely used by organism without having any adverse effect on its biochemical composition.

INTRODUCTION

Large quantities of agricultural residues with low feed values exist throughout the world. These materials are not only wastage of natural resources but are important sources of environmental pollution. These residues high in cellulose and hemicellulose can enzymatically be dehydrolyzed to sugars, which can subsequently be converted to fuel alcohol, chemicals or single cell protein (SCP). Single cell protein can be used as an additive to fodder. For SCP production, cellulose has not been as widely used as have hydrocarbons or other substrates. The main disadvantage in using cellulose is the difficulty of growing microorganism because of its micro-crystalline structure and lignin 'seal' surrounding it for which a pretreatment is usually necessary. The different pretreatments studied by different workers include mechanical (Tassinari *et al.*, 1982; Caulfield and Moore, 1974) and chemical (Saddler *et al.*, 1982; Knappert *et al.*, 1980; Wilke *et al.*, 1981; Joseleau and Martini, 1981) methods. Mycelial fungal organisms have certain advantages for SCP production compared to other type of organisms in that the harvesting techniques are relatively inexpensive. Keeping in view the availability of rice straw as unutilized biomass in India, the present investigation has been carried out to convert this cellulosic material into microbial biomass rich in protein.

MATERIALS AND METHODS

Microorganism: The culture of *Chaetomium cellulolyticum* was procured from the Department of Microbiology, Punjab Agricultural University, Ludhiana, India. The culture was maintained by subculturing fortnightly on potato dextrose agar medium.

Type of straw: Samples (100 g each) of straw were treated with NaOH at 0, 2, 4, 6, 8 and 10% (w/w) levels by adjusting the moisture to 50% with water. The treated straw was allowed to react at room temperature for 5 days and then the samples were treated with steam under pressure (1.5 kg/cm²) for 15, 30 and 60 min in the autoclave. These samples were then dried and ground before use.

Media: The basal synthetic medium was the same as used by Chahal and Gray (1968) with slight modification. The modification includes the replacement of the vitamin solution by 0.18 yeast extract. The detailed composition of the medium is

given as 0.4 g KNO_3, 2.5 g KH_2PO_4, 1 g dextrose, 1.25 g $MgSO_4$, 1 g yeast extract, 1 ml trace element solution, 1 ml $ZnSO_4.7H_2O$ (44 g/l), 1 ml $FeCl_3$ (1.92 g/1), distilled water to 1 litre. Trace element solution consists of 0.48 g $(NH_4)_6MO_7O_{24}.4H_2O$, 0.78 g $CuSO_4.5H_2O$, 0.11 g $MnCl_2.4H_2O$, distilled water to 1 litre.

Procedure: Half gram of the alkali and steam treated straw was added to 50 ml of the liquid synthetic medium in each Erlenmeyer flask and pH was adjusted to 4.5 with 1 *N* HC1. The flasks were autoclaved at 1 kg/cm^2 for 20 minutes. The flasks were inoculated with *Chaetomium cellulolyticum* and kept at 37° C on a rotary shaker. After 5 days of growth, the content of flasks was filtered through Whatman No.1 filter paper to determine the combined weight of fungal mycelium and undigested rice straw. The filtrate was used to measure cellulase activity. The dried mycelium along with undigested straw was used for chemical analysis.

Chemical analysis: Cell solubles, cell wall components, acid detergent fibre (ADF), hemicellulose and lignin were determined by the method of Goering and Van Soest (1970). Cellulose was determined by the method of Crampton and Maynard (1938). The ash was determined according to methods of AOAC (1970). Crude protein was determined by microkjeldahl technique with slight modification of McKenzie and Wallace (1954). The method described by Goran and Porath (1966) was used with slight modification for measuring cellulase activity. Total reducing sugars were determined by the method of Nelson (1944). Total sugars were determined by the method of Dubois *et al.* (1956). The total lipids was determined by the method of Folch *et al.* (1957).

RESULTS AND DISCUSSION

The chemical composition of untreated rice straw is shown in Table 1. Crude protein content of biomass was increased by the growth of fungus on a medium supplemented with pretreated rice straw (Table 2). The per cent protein content increased by an increase in time of steam pressure treatment up to 30 min. Beyond this time interval of steam pressure treatment the per cent increase in crude protein content of biomass was again found to decline. This effect was more pronounced at higher concentration of NaOH. For example, per cent increase in crude protein content was 80% in biomass obtained from rice straw treated between 4-8% NaOH accompanied by steam pressure treatment for 30 min. The adverse effect on per cent increase in crude protein content of biomass beyond 30 min may possibly be due to the severity of the heat treatment effect. Similar adverse effect of steam on crude protein production by *Cellulomonas* species on pretreated sample has also been reported by Han and Callihan (1974). The results of present findings with *C. cellulolyticum* grown on pretreated rice straw also

Table 1. Chemical constituents in untreated rice straw

Constituents	% (dry weight)
Cell solubles	8.34
Cell wall components	91.66
Acid detergent fibre	75.20
Hemicellulose	16.83
Cellulose	40.22
Lignin	5.22
Total ash	18.40
Crude protein	5.70

Table 2. Effect of supplementation of rice straw treated with NaOH and steam pressure treatment (1.5 kg/cm^2) on CMCase and protein production by C. *cellulolyticum*

Particulars	Period (min)	NaOH treatment levels (%)					
		0	2	4	6	8	10
Final pH	15	5.0	5.0	5.5	5.0	6.0	6.0
	30	5.5	5.5	5.5	6.0	6.0	6.0
	60	5.0	5.0	6.0	6.0	6.5	6.5
Increase in C.P. content of biomass, %	13	31.5	27.4	31.2	23.6	25.6	19.3
	30	34.5	26.8	80.5	79.1	82.3	42.0
	60	33.3	29.8	25.8	47.3	63.0	40.3
*CMCase activity (units/ml)	15	26	30	37	37	37	37
	30	20	20	26	30	30	42
	60	20	20	25	26	34	39
Dry matter (mg/flask)	15	450	382	381	360	283	296
	30	412	362	360	284	304	360
	60	360	340	421	346	357	425

* One unit CMCase activity is that activity which produces amount of reducing sugars equivalent to 0.1 mg of glucose with 1 ml of enzyme under assay condition

Table 3. Effect of supplementation of treated straw with NaOH and steam pressure treatment (1.5 kg/cm^2) on the total surgars and total lipid content of biomass obtained after 5 days of growth C *cellulolyticum* on DM basis (%)

Particulars	Period (min)	NaOH treatment levels (%)					
		0	2	4	6	8	10
Total sugars (g)	15	1.04	1.73	1.36	1.50	1.11	1.98
	30	1.14	2.26	2.11	1.80	1.98	1.65
	60	2.35	1.63	1.41	2.42	1.06	1.20
Reducing sugars (g)	15	0.46	0.41	0.13	0.26	0.18	0.56
	30	0.30	0.23	0.52	0.46	0.51	0.37
	60	0.36	0.15	0.04	0.04	0.02	0.01
Total lipids (g)	15	1.70	1.01	1.30	0.71	1.32	1.36
	30	1.23	1.41	1.72	2.21	1.70	1.74
	60	1.41	2.22	1.50	1.12	1.18	1.20

Table 4. Essential amino acid composition of protein of C. *cellulolyticum* and FAO reference protien, %

Amino acid	*C. cellulolyticum*	FAO reference
Threonine	6.14	2,8
Valine	5.76	4.2
Cystein	0.31	2.0
Methionine	2.33	2.2
Isoleucine	4.70	4.2
Leucine	7.54	4.2
Tyrosine	3.26	2.8
Phenylalanine	3.77	2.8
Lysine	6.80	4.2

support these observations. Extracellular carboxymethyl cellulase (CMCase) activity increased significantly in medium supplemented with pretreated rice straw samples. However, no correlation could be observed between CMCase and crude protein production. The progressive decline in dry matter per flask may possibly be due to the conversion of complex carbonhydrates of rice straw into soluble forms which ultimately come to aqueous fraction. On the other hand, the pretreatment of NaOH accompanied by steam pressure had very little effect on total sugar, reducing sugar and total lipid content of mycelium (Table 3). Moo-Young *et al.* (1971) compared amino acid composition of the protein in C. *cellulolyticum* with FAO recommendations (Table 4). Hence it can be concluded that pretreated rice straw can be safely used by the organism without having any adverse effect on its biochemical composition.

REFERENCES

AOAC 1970. *Official Methods of Analysis.* 11th edn. Association of Official Agricultural Chemists, Washington, D.C.

Caulfield, D.F. and Moore, W.E. 1974. Effect of varying crystallinity of cellulose on enzyme hydrolysis. *WoodSci. 6* : 375-9

Chahal, D.S. and Gray, W.D. 1968. Growth of selected cellulotytic fungi on wood pulp. *Biodeterioration of Materials.* Ed. Walter, A.H and Elphic, V.S. Elsevier Pub. Co. Ltd., England, p. 583-93

Crampton, E.W. and Maynard, I.A. 1938. The relation of cellulose and lignin content to the nutrition value of animal feeds. *J. Nutr.* 15 : 383-386

Dubois, M., Gills, K.A., Hamilton, J.K., Robers, P.A. and Smith, F. 1956. Colorimetric determination of sugars and related substances. *Anal. Chem.* 28 : 350

Folch, J., Lees, M. and Slounestanley, G.H. 1957. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226 : 497-506

Goering, H.K. and Van Soest, P.J. 1970. *Forage Fibre Analysis* Agricultural Research Service, United States Dept. of Agriculture, Agricultural Handbook 379: 1-12

Goran, P. and Porath, J. 1966. A cellulolytic enzyme from *Penicillium notatum. Methods on Enzymology,* ed. Colivick, S.P. and Kaplan, N.O., Vol. 8, Academic Press Inc., New York, p. 603-607

Han, Y.W. and Callihan, C.D. 1974. Cellulose fermentation : effect of substrate pretreatment on microbial growth. *Appl. Microbiol.* 16 : 1140-1145

Joseleau, J.P. and Martini, E. 1981. Effect of ozone

treatment on the *in vitro* digestibility of straw. *Cellul. Chem. Tech.* 15 : 473-478

Knappert, D., Grethlein, H.E. and Converse, A.O. 1980. Partial acid hydrolysis of cellulosic materials as a pretreatment for enzymic hydrolysis. *Biotechnol. Bioeng* 22 : 1449-1463

McKenzie, H.A. and Wallace, H.S. 1954. The Kjeldahl determination of nitrogen. *Aust. J. Chem.* 17 : 55-79

Moo-Young. M., Chahal, D.S., Swan, J.E. and Robinson, C.W. 1977. SCP production by *Chaetomium cellulolyticum*, a new thermotolerant cellulytic fungus. *Biotechnol. Bioeng.* 19 : 527-538

Nelson, N. 1944. A photometric adaptation of the Somogyi method for determination of glucose. /. *Biol. Chem.* 153 : 375-380

Saddler, J.N., Brownell, H.H., Clermont, L.P. and Levitin, N. 1982. Enzymic hydrolysis of cellulose and various pretreated wood fractions. *Biotechnol. Bioeng.* 24: 1389-1402

Tassinari, T.H., Macy, C.F. and Spano, L.A. 1982. Technology advances for continuous compression milling pretreatment of lignocellulosics for enzymic hydrolysis. *Biotechnol. Bioeng.* 24: 1495-1505

Wilke, C.R., Yang, R.D., Sciamanna, A.F. and Freitas, R.P 1981. Raw materials evaluation and process development studies for conversion of biomass to sugars and ethanol. *Biotechnol. Boieng.* 23 : 163-183

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