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Bioconversion of hemicelluloses into fungal protein*

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SUMMARY

The utilization of cellulose from one ton of lignocellulose for ethanol production would yield 150–250 kg of hemicelluloses. The total soluble solids in the hemicellulose fraction (HF) obtained with the Université de Sherbrooke (UdeS) process contained about 56% carbohydrates. These carbohydrates were present in the form of oligomers of various sugars, predominantly xylose. All the test fungi, *Chaetomium cellulolyticum, C. cellulolyticum* (asporogenous mutant) and *Pleurotus sajor-caju,* were capable of utilizing all the carbohydrates present in HF. *C. cellulolyticum* gave the highest amount of protein (7 g/l) from 19 g carbohydrates/l. The yield of protein was higher than expected, indicating that carbon compounds other than reducing sugars present in HF might have been consumed for fungal growth. The inhibitory effect of toxic compounds on protein production increased with an increase in concentration of soluble solids in HF. The inhibitory effect was overcome by increasing the pH of the medium to 6.0 or 7.0. Fungal protein production from hemicelluloses will give extra revenue in our integrated approach for ethanol production from lignocelluloses.

INTRODUCTION

Extensive utilization of cellulose from lignocelluloses for ethanol production is envisioned in the near future [4,13]. The lignocelluloses (forest biomass) contain 45–56% cellulose, 10–25% hemicelluloses, and 18–30% lignin [30]; the other lignocelluloses (agricultural residues) contain 30–45% cellulose, 16–29% hemicelluloses, and 3–13% lignin [26]. Therefore, the utilization of cellulose from one ton of lignocelluloses for ethanol production would yield 150–250 kg of hemicelluloses. Various treatments [2,19,20,23,27] are known to isolate cellulose from lignocelluloses. During most of the treatments some toxic compounds (furfural, hydroxylmethylfurfural and their precursors, and phenolic compounds) are produced by dehydration of pentoses and hexoses [3,8,18]. When the treated lignocelluloses are fractionated into their individual components (cellulose, hemicelluloses and lignin), the tox-

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ic compounds are retained in the hemicellulose fraction.

There are many problems in the utilization of hemicellulose sugars because of the presence of toxic compounds – furfural and phenolic compounds [4,5]. Hemicelluloses are composed mainly of xylose and other sugars like mannose, galactose, arabinose, and glucose in various amounts, depending on the origin of the lignocelluloses. Very little is known about the conversion of hemicellulose fraction into ethanol or other useful products because of the presence of toxic compounds produced during treatments and also due to the fact that common yeasts cannot convert xylose, the major sugar, into ethanol. There are several reports on the conversion of xylose into ethanol [14,16,24,25,28,29]. In all these cases, however, the yield of ethanol from the xylose is very low, making these processes economically unfeasible.

Our preliminary studies showed that various fungi can utilize hemicelluloses obtained from wheat straw by acid (0.5 N H_2SO_4) hydrolysis. However, all the sugars present in this hydrolysate were in monomeric form [5]. The hemicelluloses used in the present study were obtained from aspen wood by the Université de Sherbrooke (UdeS) process. The filtrate obtained in this process contained hemicellulose sugars still in oligomeric form and some lignin. Various fungi were used for the conversion of this hemicellulose fraction into fungal protein to be used as animal feed supplement.

MATERIALS AND METHODS

Substrate. Hemicellulose fraction used in this study was obtained from two aspen species (*Populus tremuloides* and *P. deltoides*).

Fractionation of hemicelluloses. Hemicellulose fraction from *Populus deltoides* and *P. tremuloides* by the Université de Sherbrooke (UdeS) process was obtained as follows:

Aspen wood suspension containing 12–20% solids is prepared from ground wood (20.5 mm) and water. The suspension is subjected to repeated passages through a homogenizing valve. In this valve strong shearing force causes disintegration and defibration resulting in a homogeneous suspension. The homogenized suspension is rapidly heated by saturated steam and rapidly pumped through a homogenizing valve under a very high pressure (1000–6000 psi) for only a few fractions of a second. Then it is confined in a plug-flow reactor up to 120 s, between 180 and 240°C. The resulting suspension after cooling (40–60°C) is centrifuged to separate liquid and solid fractions. The liquid fraction contained solubilized hemicelluloses and some lignin. For the present study wood suspension was treated at 220°C for 88 s at 4500 psi to obtain solubilized hemicellulose fraction.

Estimation of total sugars. Total sugars from hemicellulose fraction were determined by the phenol-sulfuric acid method [21]. Reducing sugars were estimated by using a Beckman 344 gradient high-pressure liquid chromatograph (HPLC) with an Altex 156 refractive index detector and a Spherogel 7.5% Carbohydrate Column with a flow rate of 0.5 ml/min in the mobile phase of water at 80°C. The samples were appropriately diluted before injection.

Microorganisms. Chaetomium cellulolyticum (ATCC 32319), a well known fungus for fungal protein production from cellulose, lignocelluloses and manures [7,9–12], an asporogenous mutant of *C. cellulolyticum* developed by D.S. Chahal and *Pleurotus sajor-caju* (from H.S. Garcha, India), an edible mushroom [15,22], were used in this study.

CULTURE CONDITIONS

Medium. The following quantities of various chemicals from Chahal and Gray medium [6] (g/l): $(NH_4)_2SO_4$, 2.357; KH_2PO_4, 2.5; $MGSO_4 \cdot 7H_2O$, 1.25; $FeSO_4 \cdot 7H_2O$, 0.005; $ZnSO_4$, 0.044, and trace element solution, 1 ml (the trace element solution contained (mg/l): boric acid, 114; ammonium molybdate, 480; cupric sulfate, 780; and manganese chloride, 144) were added to the hemicellulose fraction containing total carbohydrates equivalent to 10 g of glucose. The pH of the medium was adjusted to 6.0 with the addition of NaOH solution.

One hundred ml of medium were dispensed in each Erlenmeyer flask of 250 ml capacity and sterilized at 121°C for 20 min.

pH of the medium. It is known that toxic compounds (furfural, hydroxymethylfurfural and their precursors, and phenolic compounds) are produced during various pretreatments of lignocelluloses [3,18]. Toxic compounds had been identified and quantified in the hydrolysate obtained from red oak (*Quercus fulcata*) wood chips treated with distilled water at 170°C for 1 h [28]. The most toxic compounds were furfural, syringaldehyde and syringic acid, and C_6–C_9 acids among the fatty acids found in the hydrolysate. In our previous work [5] it was noticed that the inhibitory effect of such compounds was overcome by growing the fungi in a medium maintained at pH around 6. Therefore, in the present study the pH of the medium was maintained around 6 during the entire growth period of the test fungi. The toxic compounds produced in the hydrolysate used in this study were not identified.

Inocula. To obtain inocula in the logarithmic phase, *C. cellulolyticum* and *C. cellulolyticum* asporogenous mutant were grown for 24 h while *P. sajor-caju* was grown for 48 h. Ten ml of inoculum were used to inoculate each flask. Inoculated flasks were incubated at 37°C on a shaker at 200 rpm and duplicate flasks were removed at various time intervals. The pH was measured and adjusted back to the initial value by adding acid or base.

Dry weight of mycelium. Dry weight of mycelium was determined by filtering the whole content of each flask through tared Whatman filter paper No. 1. The filtrate was used to estimate residual carbohydrates. The mycelia on the filter paper were washed three times with water and dried at 80°C overnight to determine the total weight of mycelia.

Table 1

Chemical composition of hemicellulose fraction (HF)[a]

Treatment	Monosaccharides (%)						Total monosaccharides (%)	Total carbohydrates[b] (%)
	xylose	glucose	galactose	mannose	arabinose	rhamnose		
A. HF of *P. deltoides*								
(i) as obtained (no treatment)	ND[c]	ND	ND	ND	ND	ND	–	56.0
(ii) acid hydrolysis[d] (0.8 N H_2SO_4)	36.9	3.6	2.3	3.6	0.1	0.4	46.9	
(iii) acid hydrolysis[e] (1.6 N H_2SO_4)	42.9	2.9	1.8	5.2	ND	ND	52.8	
B. HF of *P. tremuloides*								
(i) as obtained (no treatment)	ND	ND	ND	ND	ND	ND	ND	58.0
(ii) acid hydrolysis[d] (0.8 N H_2SO_4)	36.8	3.1	3.1	2.6	0.2	ND	45.8	

[a] Treatment conditions: 220°C at 4500 psi for 88 s.

[b] Estimated with phenol-sulfuric acid method [21].

[c] ND = not detectable.

[d] Acid hydrolysis with 0.8 N H_2SO_4 at 25°C for 1 h then at 120°C for 0.5 h.

[e] Acid hydrolysis with 1.6 N H_2SO_4 refluxed at 100°C for 1 h.

Crude protein. Total nitrogen was determined by the micro-Kjeldahl method of AOAC [1] and crude protein was calculated as 6.25 × N.

RESULTS AND DISCUSSION

Chemical composition of hemicellulose fraction (HF)

The HPLC analysis of the hemicellulose fraction (HF) did not show the presence of any monosaccharides. However, samples hydrolyzed with H_2SO_4 yielded about 46% monosaccharides based on total soluble solids present in HF. The predominant sugar was xylose. Glucose, mannose and galactose formed the second major group of monomer sugars. Arabinose and rhamnose were present in a very small quantity (Table 1). The phenol-sulfuric acid method gave 56–58% carbohydrates of the total soluble solids in HF. These results indicated that the HPLC method failed to determine the exact amount of reducing sugars, which may be due to incomplete hydrolysis. The analysis indicated that HF contained about 35–40% other materials such as lignin and non-carbohydrates.

Although the HF could be easily hydrolyzed into monomeric sugars, it was used as obtained, i.e. in oligomeric form, since all the test fungi can utilize hemicelluloses in situ (as in wood) as well as in solubilized form (as found in this fraction obtained with UdeS process).

Fungal protein production

a. Fungal protein production on HF

Three fungi were grown on HF (obtained from *P. tremuloides*) containing 6.9 g total carbohydrates/l (Table 2). The initial dry weight at 0 h included the weight of mycelial inoculum (about 400 mg/l) plus the weight of part of the precipitated HF and non-carbohydrates. All the test fungi grew very well on HF and consumption of carbohydrates varied from 82 to 90%. Maximum mycelial dry weights of 6.0, 5.6 and 4.9 g/l of *C. cellulolyticum, P. sajor-caju* and *C. cellulolyticum* (asporogenous mutant) were recorded, respectively. Total protein production (2.8 g/l) by *C. cellulolyticum* was higher than that of the other two test fungi. Although mycelial dry weight of *P. sajor-caju* was higher, total protein production (1.8 g/l) was lower than *C. cellulolyticum* asporogenous mutant (2.3 g/l) because the percentage of protein in the mycelia of *P. sajor-caju* was only 32.8%.

The protein content of dried mushrooms of *P. sajor-caju* reported by Mueller and Gawley [22] was only 17–22%. However, Khanna and Garcha [17] reported the protein content of another species of *Pleurotus (P. florida)* as 37.2% on a dry weight

Table 2

Fungal protein production with different fungi on hemicellulose fraction[a]

Fungus	Source of hemicelluloses	Total soluble solids in HF (g/l)	Total carbohydrates in HF (g/l)	Time for max. mycelial dry weight (h)	Max. mycelial dry weight[b] (g/l)	Protein (%)	Total protein (g/l)	Percent of carbohydrates consumed
C. cellulolyticum	*P. tremuloides*	12	6.9	51	6.0	47.0	2.8	82
C. cellulolyticum (asporogenous mutant)	*P. tremuloides*	12	6.9	36	4.9	48.0	2.3	90
Pleurotus sajor-caju	*P. tremuloides*	12	6.9	48	5.6	32.8	1.8	85
C. cellulolyticum	*P. deltoides*	10	5.8	30	4.7	46.7	2.2	87

[a] In Chahal and Gray medium [6] at pH 6.

[b] Mycelial weight also contained precipitated carbohydrates and non-carbohydrates present in HF.

basis of mushrooms. Therefore, to increase the protein content of *P. sajor-caju*, used in these studies, some improvements in cultural conditions are needed.

The protein production of 2.5 g/l (i.e. 2.8 g final wt. −0.3 g initial wt.) per unit of carbohydrates supplied (6.9 g/l), when calculated with a normal conversion factor, is higher than the expected theoretical values. For example, 6.9 g of carbohydrates should have yielded about 3.45 g of mycelial biomass based on 50% conversion, and subsequently it should have given 1.72 g protein based on about 50% protein content of *C. cellulolyticum* when grown on glucose. Therefore, it is assumed that extra protein might have been produced by the growth of *C. cellulolyticum* on carbon compounds other than reducing sugars present in HF. Such carbon compounds have not been identified as yet.

Similarly, *C. cellulolyticum* also grew well on HF of *P. deltoides* (Table 2).

b. Effect of different concentrations of HF

C. cellulolyticum was grown in different concentrations of HF from *P. deltoides* to find out the optimum concentration of total carbohydrates for production of fungal protein. Very good growth and protein production was recorded at 2.9 g total carbohydrates/l. There was an almost 2-fold increase in protein production when the concentration of carbohydrates was doubled (Table 3). However, when the concentration was increased 4-fold (11.6 g total carbohydrates/l), a long lag phase of about 24 h was observed and the growth as well as the protein production decreased considerably. Long lag phase and low protein yield might be due to the high concentration of toxic materials present in concentrated HF, as it is known that some toxic materials are produced during the pretreatment of wood at high temperature and high pressure [3,18,28].

c. Effect of pH and concentrations of HF

Another experiment with different concentrations of total soluble solids from HF obtained by treating *P. deltoides* at 220°C and 6000 psi for 88 s was carried out (Table 4). The inoculum obtained by growing *C. cellulolyticum* in a medium containing 1% glucose was used when it was still in logarithmic phase. The media were maintained at two different pH values (6 and 7) because previous results indicated that at these pH values the test fungi were able to withstand the toxic compounds present in the medium [5].

The use of an inoculum in the logarithmic phase reduced the lag phase considerably compared to that obtained in previous experiments (b) (Table 3). However, the lag phase increased with an increase in the concentration of total carbohydrates; 3, 6 and 20–30 h in the case of 5, 10 and 19 g of total carbohydrates per liter, respectively (Table 4). It is assumed that toxic compounds produced during pretreatment [3,8,18,28] were responsible for the

Table 3

Effect of different concentrations of hemicellulose fraction on the fungal protein production with *C. cellulolyticum*[a,b]

Total soluble solids in HF (g/l)	Total carbohydrates in HF (g/l)	Lag phase (h)	Time for max. mycelial dry weight (h)	Max. mycelial dry weight[c] (g/l)	Protein (%)	Total protein (g/l)
5	2.9	6	24	2.6	51.4	1.3
10	5.8	6	30	4.7	46.7	2.2
20	11.6	24	52	3.8	46.3	1.8

[a] Hemicellulose fraction of *P. deltoides*.

[b] Chahal and Gray medium [6] at pH 6.

[c] Mycelial weight also contained precipitated carbohydrates and non-carbohydrates present in HF.

Table 4

Effect of pH and concentrations of hemicellulose fraction (HF) from *P. deltoides* on protein production

Concentration of HF		pH of medium	Lag phase (h)	Time for max. mycelial dry weight (h)	Max. mycelial dry weight[a] (g/l)	Protein (%)	Total protein (g/l)
total soluble solids (g/l)	total carbohydrates (g/l)						
11.5	5	6	3	39	4.0	45.0	1.7
11.5	5	7	3	39	5.1	45.4	2.3
23.0	10	6	6	83	9.1	43.7	4.0
23.0	10	7	6	83	9.1	44.6	4.1
43.8	19	6	30	83	16.8	43.3	7.3
43.8	19	7	20	72	17.9	40.8	7.3

[a] Mycelial weight also contained precipitated carbohydrates and non-carbohydrates present in HF.

long lag phase in the original concentration of HF (containing 19.0 g total carbohydrates/l). As the carbohydrates were diluted to 10 and 5 g/l, toxic compounds were also diluted and reduced lag phases were observed at low concentrations of HF. However, the dry weight of mycelium and total protein increased correspondingly with an increase in concentration of total carbohydrates/total soluble solids. There seems to be no inhibition of growth even up to 19 g carbohydrates (43.8 g total soluble solids) per liter except that a long lag phase (20–30 h) was observed (Table 4). It has been noticed that the growth was slightly faster and greater at pH 7 than pH 6 at the highest concentration of substrate (43.8% soluble solids). Consumption of total carbohydrates was also greater (92.7%) at pH 7 than at pH 6 (84.1%). The present results confirmed our previous findings [5] that the test fungi can convert hemicelluloses into fungal protein if the pH of the medium is maintained at 6 or 7.

The maximum protein produced was about 7.3 g/l from 19 g carbohydrates or from 43.8 g total solids/l at pH 6 and 7 (Table 4). Production of 3–4 g protein/l in any process is considered to be commercially economical. Therefore, it seems that production of 7 g of protein/l within 72 h of fermentation of HF would contribute to generate a considerable extra revenue in our integrated process for the production of food, feed and fuel from biomass to make it economically feasible.

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REFERENCES

1 AOAC. 1970. Association of Official Agricultural Chemists. 11th Edn. Washington, DC.
2 Bender, R. 1979. Method of treating lignocellulose materials to produce ruminants feed. U.S. Patent No. 4, 136, 207.
3 Casebier, R.L., J.K. Hamilton and H.L. Hergert. 1969. Chemistry and mechanism of water prehydrolysis on southern pine wood. Tappi 52: 2369–2377.
4 Chahal, D.S. 1982. Enzymatic hydrolysis of cellulose: State-of-the-Art. Division of Energy Research and Development, National Research Council of Canada, Report No. 20283, 74 pp.
5 Chahal, D.S. 1984. Bioconversion of hemicelluloses into useful products in an integrated process for food/feed and fuel (ethanol) production from biomass. Biotechnol. Bioeng. Symp. 14: 425–433.
6 Chahal, D.S. and W.D. Gray. 1968. Growth of selected cellulolytic fungi on wood pulp. In: Biodeterioration of Mate-

rials – Microbiological and Allied Aspects (Walter, A.H. and J.S. Elphic, eds.), pp. 584–593, Elsevier Publ. Co., Barking, England.

7 Chahal, D.S. and D.L. Hawksworth. 1976. *Chaetomium cellulolyticum,* a new thermotolerant and cellulolytic *Chaetomium*. Mycologia 68: 600–610.

8 Chahal, D.S., S. McGuire, H. Pikor and G. Noble. 1982. Production of cellulase-complex by *Trichoderma reesei* Rut-C30 on lignocellulose and its hydrolytic potential. Biomass 2: 127–138.

9 Chahal, D.S. and M. Moo-Young. 1981. Bioconversion of lignocellulosics into animal feed with *Chaetomium cellulolyticum*. Dev. Ind. Microbiol. 22: 143–159.

10 Chahal, D.S., J.E. Swan and M. Moo-Young. 1977. Protein and cellulase production by *Chaetomium cellulolyticum* grown on wheat straw. Dev. Ind. Microbiol. 18: 433–442.

11 Chahal, D.S., D. Vlach, B. Stickney and M. Moo-Young. 1980. Pollution control of swine manure and straw conversion to *Chaetomium cellulolyticum* SCP stuff. Presented at the VIth International Fermentation Symposium, London, Ontario, Canada, July 20–25, 1980.

12 Chahal, D.S. and D.I.C. Wang. 1978. *Chaetomium cellulolyticum* growth behavior on cellulose and protein production. Mycologia 70: 160–170.

13 Flickinger, M.C. 1980. Current biological research in conversion of cellulosic carbohydrates into liquid fuels: How far have we come? Biotechnol. Bioeng. 22 (Suppl. 1): 27–48.

14 Gong, C.-S., L.-F. Chen, M.C. Flickinger, L.-C- Chiang and G.T. Tsao. 1981. Production of ethanol from D-xylose by using D-xylose isomerase and yeasts. Appl. Environ. Microbiol. 41: 430–436.

15 Jandaik, C.L. and J.N. Kapoor. 1976. Effect of carbon and nitrogen nutrition on growth of *Pleurotus sajor-caju*. Ind. Phytopathol. 29: 326–327.

16 Jeffries, T.W. 1981. Conversion of xylose to ethanol under aerobic conditions by *Candida tropicalis*. Biotechnol. Lett. 3: 213–218.

17 Khanna, P. and H.S.Garcha. 1981. Nutritive value of mushroom *Pleurotus florida*. Mushroom Sci. XI: 561–572.

18 Leonard, R.H. and G.J. Hajny. 1945. Fermentation of wood sugars to ethyl alcohol. Ind. Eng. Chem. 37: 390–395.

19 Lora, J.H. and M. Wayman. 1978. Delignification of hard woods by autohydrolysis and extraction. Tappi 61 (6): 47–50.

20 Millet, M.A., A.J. Baker and L.D. Satter. 1975. Pretreatments to enhance chemical enzymatic and microbial attack of cellulosic materials. Biotechnol. Bioeng. Symp. 5: 193–219.

21 Montgomery, R. 1961. Further studies of the phenol-sulfuric acid reagent for carbohydrates. Biochim. Biophys. Acta 48: 591–593.

22 Mueller, J.C. and J.R. Gawley. 1983. Cultivation of phoenix mushrooms on pulp mill sludges. Mushroom News. Trop. 4(1): 3–12.

23 Noble, G. 1980. Optimization of steam explosion pretreatment. Final Report submitted to U.S. Department of Energy, Fuels from Biomass program, Iotech Corporation Limited, Ottawa, Ontario, Canada, 356 pp.

24 Schneider, H., P.Y. Wang, Y.K. Chan and R. Maleszka. 1981. Conversion of xylose into ethanol by the yeast *Pachysolen tannophilus*. Biotechnol. Lett. 3: 89–92.

25 Slininger, P.J., R.J. Bothast, J.E. Van Cauwenberge and C.P. Kurtzman. 1982. Conversion of D-xylose to ethanol by yeast *Pachysolen tannophilus*. Biotechnol. Bioeng. 24: 371–384.

26 Sloneker, J.H. 1976. Agricultural residues including feed lot wastes. Biotechnol. Bioeng. Symp. 6: 235–250.

27 Tarkow, H. and W.D. Feist. 1969. A mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid ammonia. Adv. Chem. Ser. 95: 197–218.

28 Tran, Ai.V. and R.P. Chambers. 1985. Red oak wood derived inhibitors in the ethanol fermentation of xylose by *Pichia stipitis* CBS 5776. Biotechnol. Lett. 7: 841–846.

29 Wang, P.V., C. Shopsis and H. Schneider. 1980. Fermentation of a pentose by yeasts. Biochem. Biophys. Res. Commun. 94: 248–254.

30 Wenzel, H.F.J. 1970. The Chemical Technology of Wood. Academic Press, New York, NY.