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Fractionation and utilisation of corn fibre carbohydrates

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Abstract

Corn fibre contains starch, cellulose, hemicellulose, lignin, oil, protein, etc. The separation and the utilisation of its carbohydrate components were studied. A 99.8% of the starch was removed by a two-step enzymtic hydrolysis. The remaining solid fraction was pretreated with alkali (2.5% NaOH) and alkaline peroxide (2.5% NaOH, 0.6% H_2O_2), in order to isolate corn fibre gum (CFG). The highest yield could be obtained under the most severe conditions (alkaline peroxide, 120 C, 120 min). Following the pretreatment 51.3% of the corn fibre gum could be isolated by alcoholic precipitation from the soluble fraction. The solid fraction was used as carbon source for cellulase enzyme fermentation and as substrate for enzymatic hydrolysis. A 30% higher filter paper activity was obtained using pre-treated corn fibre as carbon source compared to the activities reached on original and destarched corn fibre. However, 15% higher filter paper activities were obtained on Solka Floc than on the pre-treated corn fibre samples. The enzymes produced were used for the hydrolysis of the pre-treated corn fibre substrate. The results of hydrolysis were compared to the performance of industrial enzymes. After 24 h of hydrolysis, the carbohydrate yields showed that the enzyme produced on pre-treated corn fibre could degrade the corn fibre substrate approximately 15% more efficiently.

Keywords: Biorefinery; Cellulose hydrolysis; Corn fibre; Corn fibre gum; Cellulase production; Hemicellulose-B; *Trichoderma reesei*

1. Introduction

Corn fibre is utilised mainly as animal feed however, as the market of animal feed is limited if the production increases there may not be an available market for the excess [1]. With fractionation valuable products and chemicals could be obtained including starch and cellulose. These are the main raw materials for bulk production of modified polysaccharides and their derivatives which are used wide spread in the paper and cosmetics industry [2].

The hydrolysis of corn fibre cellulose to glucose by cellulase has great potential, since glucose can be used as raw material for the production of various products, such as fuel ethanol [3]. However, a few reports have demonstrated that the enzymic conversion of cellulose to glucose is not yet economically feasible [4,5]. Enzyme production is the crucial step in the process and in order to lower the costs, cheap cellulase enzyme is needed [6]. *Trichoderma reesei* RUT C30 has proved to be one of the best cellulase producers [7] and extensive research has been carried out to find a suitable but inexpensive carbon source. Soluble carbon sources, such as glucose [8] and lactose [8,9] and insoluble carbon sources like Solka Floc [10], waste paper [11,12], wheat bran [13], wheat straw [14], corn stover [15], wood [16] and corn fibre [17] have been tested. The results showed that pure cellulose is the best carbon source among the insoluble materials. However, Solka Floc is quite expensive and in order to obtain a good enzyme yield, lignocellulosics should be pretreated prior to enzyme fermentation. This is a time and energy consuming process. A possible solution to this problem is to perform the pretreatment in a way that not only the lignocellulosic structure will be removed but a valuable product will also be produced.

Hemicellulose is an arabinoxylan polymer having five and six carbon atoms in its sugar structure [18]. Corn fibre hemicellulose (CFH) is usually referred as corn fibre gum (CFG) [19]. CFG is a quite sticky polymer; therefore it could serve as adhesive, thickener, or additive in plastics, as it increases their stretch, their breaking resistance and makes them more susceptible to biodegradation. On the other hand CFG is a good stabiliser [20] and is used as a film former and emulsifier [21]. Extraction with alkali is the most extensively studied method for hemicellulose isolation

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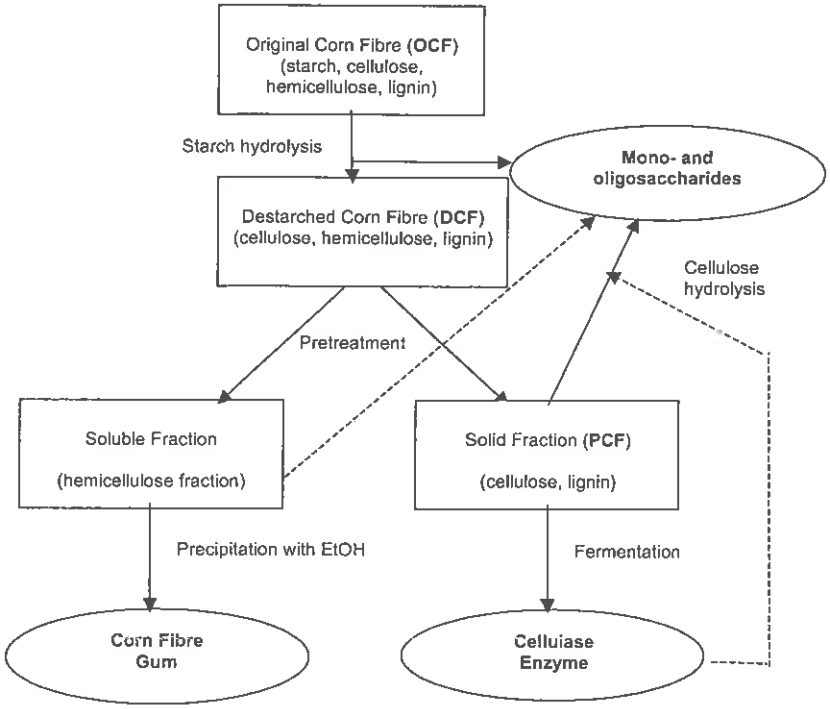

Fig. 1. Fractionation of corn fibre carbohydrates.

[19,22]. The hemicellulose fraction can also be removed easily from fibres by steam explosion and acidic hydrolysis. In these cases water-soluble mono- and oligosaccharides obtained from the hemicellulose polymer are present in the supernatant. Extensive studies have been conducted on the use of this high xylose containing syrup as raw material for the production of ethanol [23–26] or xylitol [27].

In this study the complex utilisation of the carbohydrate fraction of corn fibre was investigated (Fig. 1). Corn fibre starch was converted to glucose, hemicellulose was isolated, cellulase enzyme was produced on the remaining insoluble fraction and the performance of the locally produced and the industrial cellulase enzymes was compared in the hydrolysis of pre-treated material.

2. Materials and methods

2.1. Corn fibre

Corn fibre was obtained as a by-product of corn wet milling process from a Hungarian starch manufacturing company the maximum size of particles was up to 8 mm and the material was not ground before use. The same batch was used during the whole experiment. Analysis was performed as indicated later in this paper. Composition based on dry weight was the following: 23.1% starch, 18.7% cellulose, 28.7% hemicellulose, 7.5% lignin, 7.7% ash and 14.4% other insoluble materials (oil, protein, exractives, etc).

2.2. Starch hydrolysis

The starch fraction of the original corn fibre (OCF) was saccharified in a two-step process using thermostable α-amylase. Hydrolysis was performed in a 30 L bioreactor with a working volume of 20 L (B. Braun Biotech AG, Germany) with continuous stirring at 250 rpm. In the first step 18 L of 0.05 M acetate-buffer (pH 4.8) and 19.4 mL of thermamyl supra (Novozymes) were added at room temperature to 1.58 kg (dw) air-dried corn fibre. The bioreactor was heated up to 120 °C in 15 min and the mixture was stirred further at 120 °C for 20 min. In the second step, the slurry was cooled to 90 °C and supplemented with an additional 19.4 mL Thermamyl Supra and was kept with continuous stirring at this lower temperature for 1 h. After cooling the reaction mixture to room temperature the liquified material was filtrated on a 150 μm mesh nylon filter by vacuum and washed with hot distilled water. The supernatant and the wash water were combined and the volume of it was measured and then analysed for maltose, glucose, arabinose and xylose by HPLC.

2.3. Hemicellulose isolation

Destarched corn fibre (DCF) was pretreated either with alkali or alkali and hydrogen peroxide. To 20 g (dw) of air-dried DCF either 320 mL 2.5% NaOH solution and 6.66 mL distilled water or 320 mL NaOH solution and 6.66 mL 30% H_2O_2 were added. The suspension was heated

Table 1
Pre-treatment conditions and compositions of original corn fibre, destarched corn fibre and solid fractions remaining after pretreatments

Pre-treatment Conditions	DCF Destarched corn fibre	A 60 min NaOH	B 60 min NaOH + H_2O_2	C 90 min NaOH	D 90 min NaOH + H_2O_2	E 120 min NaOH	F 120 min NaOH + H_2O_2
Cellulose (%)	20.4	34.8	48.4	36.8	46.4	38.0	49.4
Hemicellulose (%)	41.0	20.9	11.9	21.2	13.3	21.7	13.8
Lignin (%)	12.2	4.1	3.7	4.1	5.0	3.4	3.0
Ash (%)	8.6	10.1	10.8	11.8	8.2	11.9	8.3
Other[a] (%)	17.8	30.1	25.2	26.1	27.1	25.0	25.5

[a] Other insoluble materials could be protein, oil, extractives, etc.

to 120 °C and kept for three different periods (60, 90, 120 min) as shown in Table 1. After cooling, the suspension was filtered on a 150 μm mesh nylon filter via vacuum and washed with 600 mL hot distilled water. The filtered solid was cellulosic residue i.e. pre-treated corn fibre (PCF). The CFG containing soluble fraction and the washing water were combined and used for the isolation of hemicellulose-B by the method of Doner [19]. The pH of the mixture was adjusted to 4.5 with 35% HCl. The insoluble hemicellulose-A was left to settle for 2 h and then centrifuged at 10,000 rpm for 5 min. Only a small amount of precipitate was produced and only the hemicellulose-A free supernatant was further processed. The hemicellulose-A free supernatant was supplemented with a double volume of 95% ethanol and the white flocculent precipitate of hemicellulose-B was allowed to settle out for a day. The remaining supernatant was then removed by vacuum filtration through a 150 μm mesh nylon filter. The CFG was rinsed with a small amount of ethanol, dried at 105 °C then the yield determined.

2.4. Analysis of substrate composition

The amount of removed starch was determined by the hydrolysis (120 °C; 10 min) of the liquid containing the starch degradation products, to which 8% H_2SO_4 was added in the volumetric ratio of 1:1. As a result of this hydrolysis, the oligosaccharides were degraded and the produced monosaccharides were measured by HPLC. The cellulose, hemicellulose and lignin content of OCF, DCF and PCF samples were measured using a modified Hägglund method [28]. One gram of dried material was mixed with 10 mL 72% H_2SO_4. After holding it for 4 h at room temperature, it was diluted with 25 mL of distilled water and kept overnight at room temperature. After the addition of 260 mL distilled water, it was boiled with reflux for 6 h. The insoluble fraction i.e. lignin was separated by filtration through G3 glass filter, washed thoroughly with distilled water, dried and weighed. The supernatant was analysed by HPLC for glucose, arabinose and xylose to determine the cellulose and hemicellulose content. The starch content of the samples was determined as follows: 3.0 g of dried material was dissolved in 100 mL, 1.125 g/mL HCl and boiled with reflux for 2.5 h. After centrifugation at 10,000 rpm for 5 min, the supernatant was analysed for glucose by HPLC. HPLC analysis was performed using an Aminex HPX-87H column at 65 °C. The eluent was 5 mM H_2SO_4 at a flow rate of 0.5 mL/min. Mono-and disaccharides were detected by their refractive index. The ash content of the samples was measured by gravimetric analysis at 600 °C.

2.5. Inoculum preparation

T. reesei RUT C30 (ATCC#: 56765) stock cultures were maintained on agar slants containing 20 g/L malt extract, 5 g/L glucose, 1 g/L peptone and 20 g/L agar. After 14 days at 30 °C the conidia were suspended in 5 mL of sterile water then 1 mL of this spore suspension was transferred aseptically to a 750 mL Erlenmeyer flask (E-flask) containing 150 mL of the sterile and pH adjusted (pH 5.5) medium prepared according to Mandels and Weber [29]. In this medium the concentration of nutrients were: 0.3 g/L urea, 1.4 g/L $(NH_4)_2SO_4$, 2.0 g/L KH_2PO_4, 0.3 g/L $CaCl_2$, 0.3 g/L $MgSO_4$, 0.25 g/L yeast extract, 0.75 g/L proteose peptone together with 7.5 g/L Solka Floc. The medium was also supplemented with the following trace elements: 5 mg/L $FeSO_4 \cdot 7H_2O$, 20 mg/L $CoCl_2$, 1.6 $MnSO_4$, 1.4 $ZnSO_4$. After 4 days at 30 °C and 350 rpm, the inoculum was ready.

2.6. Enzyme production/enzyme assays

A total volume of 15 mL mycelium suspension, obtained from the inoculum cultures, was used to initiate growth in a 750 mL E-flask containing 150 mL of Mandels' medium at pH 5.5 in which the concentration of the carbohydrates was equivalent to 5 g/L glucose. After inoculation the E-flasks were incubated in an orbitary shaker at 30 °C and 350 rpm for 5 days. Every day 3–3 mL of samples were withdrawn and at the same time the pH was determined and if necessary, adjusted to 6.0 by adding 10% sterile NaOH or H_2SO_4 solutions. The samples were centrifuged at 10,000 rpm for 5 min and the supernatants were collected for the determination of filter paper and β-glucosidase activities. The filter paper activity (FPA) of the samples was determined using filter paper substrate in accordance with the Mandels' procedure [30], while β-glucosidase activity was assayed using *p*-nitrophenyl-β-D-glucopyranoside substrate in accordance with Berghem and Petterson method [31].

2.7. Cellulose hydrolysis

The hydrolysis was performed in a 100 mL E-flasks with a working volume of 50 mL at 50 °C. A 2.5 g (dw) air-dried PCF obtained in experiment F was suspended in 0.05 M acetate buffer (pH 4.8) and the slurry was supplemented with produced or with industrial cellulase enzymes (15.0 FPU/g substrate and 7.5 IU/g substrate, filter paper and β-glucosidase activities, respectively). The industrial enzymes were Celluclast 1.5 L (Novozymes) and Novozyme 188 (Novozymes). Samples were withdrawn five times, centrifuged and the supernatants were analysed by HPLC to determine the sugars yields.

Table 2
Mass balance of starch hydrolysis and pretreatments

Components	DCF	A	B	C	D	E	F
Soluble fraction							
Glucan (g)	21.6	9.6	8.0	9.4	8.9	9.2	8.8
Hemicellulose (g)	–	27.8	28.6	29.2	31.0	32.4	34.2
Lignin (g)	–	10.9	11.2	10.9	10.9	11.2	11.5
Solid residue							
Cellulose (g)	15.0	10.7	12.3	10.9	11.5	11.2	11.6
Hemicellulose (g)	30.2	6.4	3.0	6.3	3.3	6.4	3.2
Lignin (g)	9.0	1.3	1.0	1.2	1.2	1.0	0.7
Ash (g)	2.4	3.1	2.8	3.5	2.0	3.5	2.0
Other (g)	21.8	30.2	33.1	28.6	31.2	25.1	28.0

The values in mass balance are calculated on the basis of 100 g dried original corn fibre as raw material.

3. Results and discussion

All experiments were performed in triplicate. The average values and the relative standard deviations of the concentrations, the yields and the enzyme activities were calculated and were always lower than 5% and in the case of cellulose hydrolysis lower than 4%.

3.1. Starch hydrolysis and corn fibre gum isolation

The two-step enzymtic process removed 99.8% of the starch, 25.36 g glucose could be obtained from 100 g dried OCF. The DCF was pretreated under six different conditions. The pre-treatment conditions, the composition of the solid fractions obtained as a result of the pretreatments and the composition of DCF samples are shown in Table 1. The mass balance of starch hydrolysis and the different pretreatments are calculated on the basis of 100 g (dw) original corn fibre (Table 2). After destarching OCF with enzyme most of the hydrolysed starch (99.8%) was present in the supernatant. A negligible amount of it (0.2%) remained in the DCF. After pretreatments there was a small amount of glucan in the soluble fraction; which could originate from the side chains of hemicellulose. Under alkaline treatment in all cases most of the lignin fraction was dissolved in the supernatant, however a part of it still remained in PCF samples. The aim of the pretreatments was firstly to isolate hemicellulose-B with a good yield and secondly to modify the structure of lignocellulosic materials to enhance cellulase enzyme production and cellulose hydrolysis. The results of CFG isolation are plotted in Fig. 2. The addition of peroxide increased the hemicellulose yield and enhanced the purity of the isolated gum in all cases. The highest yield could be obtained under the most severe condition i.e. at 120 °C, 90 min by alkaline peroxide pretreatment. Following alcoholic precipitation, 51.6% of the hemicellulose could be isolated. The trend could be seen clearly: the more severe the conditions of pretreatments are, the higher the hemicellulose yields are.

3.2. Cellulase fermentation

The six PCF samples were used as carbon sources for the production of cellulase with the *T. reesei* RUT C30 strain. Three additional experiments were performed with OCF, DCF and Solka Floc as carbon sources. Solka Floc was

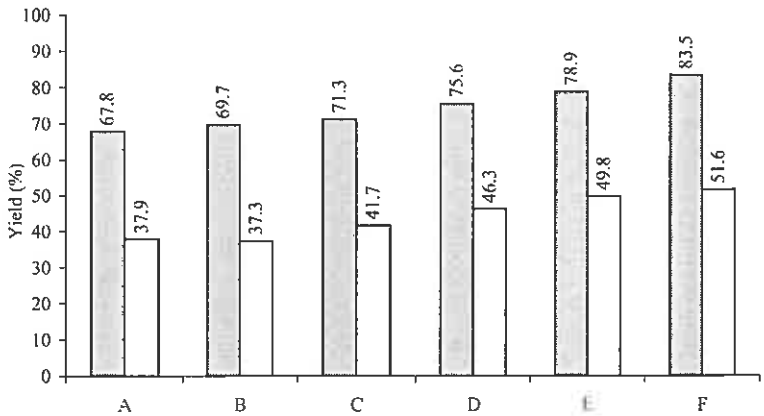

Fig. 2. CFG contents of the soluble fractions after pretreatment, precipitated CFG. The yields are calculated on the basis of hemicellulose content of DCF. (Legends as defined in Table 1).

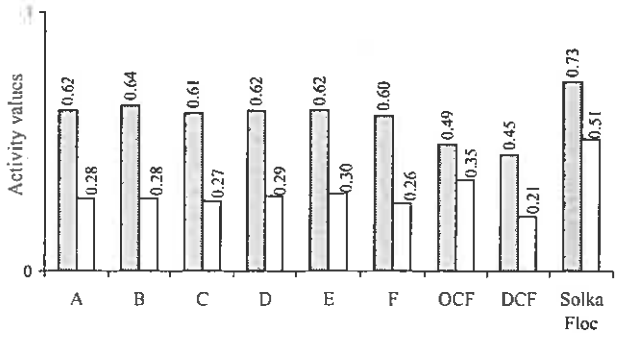

Fig. 3. Filter paper (FPU/mL) and β-glucosidase (IU/mL) activities obtained on Solka Floc, OCF, DCF and six different PCF samples after 5 days of incubation.

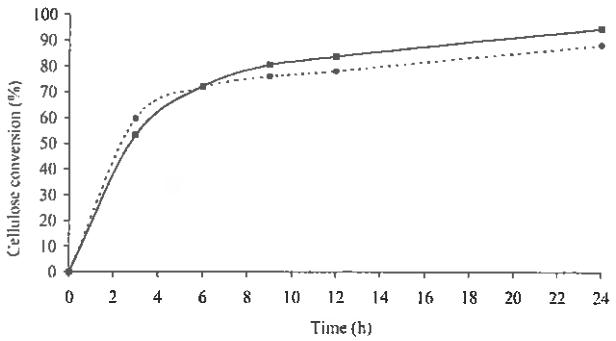

Fig. 4. Cellulose conversions obtained with enzymes produced on PCF (experiment F) carbon source (-■-) and with industrial enzymes (--●--).

used as a reference, since it is one of the most widely used insoluble carbon sources for cellulase enzyme production.

The results summarised in Fig. 3 show that there were no significant differences in the activities of cultures with PCF, but the pretreatment significantly (30%) increased the cellulase production compared to OCF. The average filter paper activity obtained on PCF substrate was 0.61 FPU/mL, whereas on Solka Floc 19.7% higher FPA was observed. The filter paper activities achieved on OCF and DCF were 0.49 FPU/mL and 0.45 FPU/mL, respectively. The average β-glucosidase activity obtained on the 6 different PCF samples was 0.28 IU/mL. This result is 20 and 45% lower than those achieved on OCF and Solca Floc respectively and 20% higher than those obtained on DCF. FPA and β-glucosidase yields obtained in this study were compared with reference data in Table 3. A detailed analysis of enzyme production in shake flasks by *T. reesei* RUT C30 using steam pre-treated willow (SPW), steam pre-treated spruce (SPS) and paper sludge (PS) were carried out by Bollók et al. [32]. FPA yields achieved on PCF are similar to the values obtained on Solka Floc and SPW. Moreover, β-glucosidase yield obtained on PCF is higher than obtained on the other substrates except Solka Floc.

3.3. Cellulose hydrolysis

Two sets of enzymic hydrolysis were performed on pre-treated (experiment F: 120 °C, 90 min, 2.5% NaOH, 0.6% H_2O_2) corn fibre. The first experiment was performed with the enzyme obtained on PCF in experiment F. The second experiment was carried out with industrial enzymes i.e. Celluclast 1.5 L and Novozyme 188. The time profile of cellulose conversions obtained are shown in Fig. 4. An average cellulose conversion of 89% was reached with industrial enzymes after 24 h. However, 16% higher conversion was achieved with enzymes produced on PCF substrate. Fig. 4 indicates that after a 24 h hydrolysis the curve has not reached a maximum but has shown a good trend.

Table 3
Comparison of enzyme yields produced on pre-treated corn fibre with other lignocellulosic material (PCF: pre-treated corn fibre, SPW: steam pre-treated willow, SPS: steam pre-treated spruce, PS: paper sludge) and Solka Floc

Substrate	FPA yield (FPU/g substrate)	β-Glucosidase yield (IU/g substrate)	Reference
PCF	122	56	This study
SPW	139	51	[32]
SPS	64	30	[32]
PS	75	30	[32]
Solka Floc	146	102	This study

4. Conclusions

Corn fibre consists of starch, cellulose, hemicellulose, lignin, oil, protein, etc. The separation and to some extent the application of the carbohydrate components were studied. A 25.36 g glucose could be obtained from 100 g dried OCF by starch hydrolysis. The remaining solid fractions were treated under six different conditions with alkali and alkaline peroxide. Corn fibre gum was isolated from the soluble fraction. The highest hemicellulose-B yield was obtained under the most severe pre-treatment conditions. When the destarched corn fibre was pretreated with alkaline peroxide at 120 °C for 120 min, 51.6% of the hemicellulose could be isolated as hemicellulose-B. The insoluble fractions mostly consisted of cellulose and lignin and were used for cellulase enzyme production. After 5 days of cultivation, the average filter paper and β-glucosidase activities obtained were 0.61 FPU/mL and 0.28 IU/mL, respectively when the carbohydrate concentration of carbon source was equivalent to 5 g/L glucose. The produced enzyme of experiment F was used for the hydrolysis of the same substrate. The results of hydrolysis were compared to the performance of industrial enzymes. After a 24 h hydrolysis, the carbohydrate yields showed that the enzyme produced on PCF substrate could degrade the corn fibre substrate approximately 15% more efficiently than commercial enzymes.

These results show that the complex utilisation of corn fibre has great potential, since the hemicellulose-B obtained is a highly valuable product and high yields of cellulase

enzymes could also be obtained on the residue. The filter paper activity of the enzyme complexes obtained were only 15% lower on pre-treated corn fibre than on pure cellulose. This technique could be improved by utilising the lignin, oil, protein, etc. components of corn fibre to develop a novel biorefining strategy for corn fibre.

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