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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

Link to publication in University of Groningen/UMCG research database

Standard Assays Do Not Predict the Efficiency of Commercial Cellulase Preparations Towards Plant Materials

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Abstract: Commercial cellulase preparations are potentially effective for processing biomass feedstocks in order to obtain bioethanol. In plant cell walls, cellulose fibrils occur in close association with xylans (monocotyls) or xyloglucans (dicotyls). The enzymatic conversion of cellulose/xylans is a complex process involving the concerted action of exo/endocellulases and cellobiases yielding glucose and xylanases yielding xylooligomers and xylose. An overview of commonly measured cellulase-, cellobiase-, and xylanase-activity, using respectively filter paper, cellobiose, and AZCL-dyed xylan as a substrate of 14 commercially available enzyme preparations from several suppliers is presented. In addition to these standardized tests, the enzyme-efficiency of degrading native substrates was studied. Grass and wheat bran were fractionated into a water unsoluble fraction (WUS), which was free of oligosaccharides and starch. Additionally, cellulose- and xylan-rich fractions were prepared by alkaline extraction of the WUS and were enzymatically digested. Hereby, the capability of cellulose and xylan conversion of the commercial enzyme preparations tested was measured. The results obtained showed that there was a large difference in the performance of the fourteen enzyme samples. Comparing all results, it was concluded that the choice of an enzyme preparation is more dependent on the characteristics of the substrate rather than on standard enzyme-activities measured.
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Keywords: commercial cellulase; xylanase; wheat bran; grass; cellulose; xylan; bioethanol

INTRODUCTION

According to the Kyoto agreements, the total CO_2 emissions in the industrialised nations need to be reduced by 5% in 2010 as compared to the 1990 level (Programme, 2002). In the longer term, a reduction of more than 50% is required to stabilise the CO_2 level in the atmosphere. A major strategy to achieve these objectives is the large-scale substitution of petrochemical fuels and products by CO_2-neutral alternatives derived from biomass. In this respect, the production of fuel bio-ethanol from cellulose-containing biomass residues is studied worldwide. Such a process generally involves a certain type of biomass-pretreatment, which often makes use of heat (Garrote et al., 1999; Shimizu et al., 1998) and acid (Papatheofanous et al., 1995; Parajo and Santos, 1995) or alkali (Curreli et al., 1997; Sun et al., 2000). Biomass-pretreatment is needed to make cellulose embedded in the plant cell wall accessible for enzymatic hydrolysis (Lynd, 1996; Lynd et al., 2002). Such a hydrolysis, which yields fermentable sugars like glucose, precedes the final fermentation step in which bio-ethanol is obtained. For environmental reasons, enzymatic hydrolysis is preferred over chemical hydrolysis. The enzymatic conversion of cellulose involves the synergistic action of exo/endoglucanases, cellobiohydrolases (CBH), and cellobiases yields glucose (De Vries and Visser, 2001; Teeri, 1997; Voragen et al., 1992). Most of these enzymes are generally present in commercial cellulase preparations. This work focuses at such commercial cellulase preparations by studying both their activity towards standard substrates and towards more complex native plant materials.

The biomass samples studied for the production of bio-ethanol are generally plant-materials composed of cellulose, hemicellulose, and lignin. A few examples of such materials are wheat bran, grasses, wheat straw, corn, and many types of wood (Lynd, 1996). For the present study, the preferred biomass samples were wheat bran and grass, both containing xylan as main hemicellulose besides cellulose. Wheat bran, the outer parts of a wheat grain, is produced worldwide as a by-product of the milling of white wheat flour. Wheat bran consists mainly of cellulose (7%–11% w/w), xylan (22%–25% w/w), and β-glucan. Furthermore, in the bran rather high amounts of starch are present (11%–30% w/w),

as well as protein (14%–17% w/w) and lignin (3%–10% w/w) (Bataillon et al., 1998; Brillouet and Mercier, 1991; Schooneveld Bergmans et al., 1996; Shiiba et al., 1993). The chemical composition of grass is highly dependent on the species studied. However, compared to wheat bran, in general the content of cellulose is higher, whereas the content of hemicellulose, protein, and lignin is similar. Starch is not present in grass (Anger et al., 2002; Foster et al., 1996).

Cellulose is a linear polymer composed of β-(1-4)-linked glucose molecules. Each glucose residue is inverted (180°) compared to its neighboring residue, so that the basic repeating unit is actually cellobiose. Cellulose chain length can vary between 100 and 14,000 residues. Furthermore, the degree of crystallinity of cellulose is depending on origin and pretreatment. For example, cellulose from cotton has a crystallinity of about 70%. The degree of crystallinity of most commercial celluloses varies between 30% and 70% (Beguin and Aubert, 1994; Wood, 1988).

Xylan as present in the cell walls of monocotyles (grasses and cereals) consist of linear chains of β-D-(1,4)-linked D-xylopyranosyl residues, which can generally be substituted with α-L-arabinofuranosyl at the 2-*O* and/or 3-*O*-position(s) and α-D-glucopyranosyl uronic acid or its 4-*O*-methyl derivative at the 2-*O*-position (Brillouet et al., 1982; Carpita, 1996; Shibuya and Iwasaki, 1985). Some publications describe the presence of *O*-acetyl substituents (Ishii, 1991; Wende and Fry, 1997b). Furthermore, small amounts of ester-linked coumaric and ferulic acid are found to be linked to (some of) the arabinose residues (Ishii, 1997; Saulnier et al., 1995; Wende and Fry, 1997a). In spite of these general characteristics, the source from which the xylan is extracted strongly determines the specific features with regard to the type, the amount, position, and distribution of substituents over the xylan-backbone.

Within the plant cell wall architecture, hemicellulose is thought to coat the cellulose-fibrils resulting in a reduced accessibility of the cellulose-fibrils (McCann and Roberts, 1991). Therefore, enzymatic hydrolysis of the hemicelluloses is essential to facilitate complete cellulose degradation (Vincken et al., 1994, 1995). Knowing the diversity in xylan-structures a diversity of hemicellulases, for example endo/exoxylanases, arabinosidases, acetylesterases, glucuronosidases, should be needed to degrade xylans. Furthermore, a complete degradation of xylan to xylose will make the production of bio-ethanol from plant materials more profitable, aiming at the possibility to ferment both glucose and xylose to ethanol (Lawford and Rousseau, 1996; Rossi et al., 1998).

In the present study, 14 commercially available cellulase preparations were analyzed for their efficiency. First, these enzyme samples were analyzed for the amount of cellulase, cellobiase, and xylanase activity, using standard assays. Subsequently, both xylanase and cellulase activity were studied towards the more natural substrates grass and wheat bran. Finally, the commercial enzymes were evaluated for their use in degrading (pretreated) cellulose-rich material in order to produce bio-ethanol.

EXPERIMENTAL

Feedstock Materials

Wheat bran special grant 3607-1687 was supplied by Meneba (Wormerveer, The Netherlands, October 12, 2002). Grass was obtained from the nature reserve Weerribben in the Netherlands (October/November 2002). Both materials were dried and milled with a Culatti mill (≤1 mm).

Characterization and Fractionation of Feedstock Materials

To remove starch, first the feedstock material (100 g) was suspended in maleate-buffer (900 mL; 0.01 M maleic acid, 0.01 M sodium chloride, 0.001 M calcium chloride, 0.05% (w/v) sodium azide, adjusted to pH 6.5 with alkali) and stirred for 1.5 h at 85°C. The suspension was cooled to 30°C and porcine alpha-amylase (Merck, Whitehouse Station, NJ; 150 U/g feedstock) was added to degrade most of the starch of the feedstock material (20 h; 30°C). Residues were collected and the alpha-amylase treatment was repeated. Finally, residues were collected, washed with distilled water (65°C) and freeze-dried resulting in so-called water unextractable solids (WUS).

WUS (45 g) was suspended in 900 mL of 4 M potassium hydroxide (KOH)/0.26 M NaBH$_4$, for 16 h at 25°C under continuous stirring. After centrifugation (10,000g; 30 min) and filtering (G3 glass-filter), the residues were reextracted with 4 M KOH (+0.26 M NaBH$_4$). The final residues were neutralized with acetic acid, dialyzed against distilled water and freeze-dried (KOHres). The corresponding supernatants were collected, neutralized, dialyzed, and freeze-dried (KOHss).

Enzymatic Degradation of the Wheat Bran and Grass Fractions (WUS, KOHss, and KOHres)

A solution of substrate (13 mg WUS, KOHss or KOHres in 25 mM sodium acetate buffer pH 5 (1 mL) was incubated with commercial enzymes (0.1% w/w enzym/substrate) for 24 h at 40°C. Enzymes were dialyzed against 25 mM sodium acetate buffer pH 5 at 4°C by using Amicon Bioseparations centrifugal devices (regenerated cellulose; 10,000 MWCO; Millipore, Billerica, MA). Tubes were changed within 45 min to prevent degradation of the regenerated cellulose membranes. The commercial enzymes tested and their suppliers are presented in Table I. After inactivation of the enzyme (5 min, 100°C), the digests were analyzed by HPAEC and HPSEC (see below).

Enzyme Evaluation Essays

These assays are performed according to the International Union of Pure and Applied Chemistry (IUPAC).

To define filter paper units (FPU) of each of the commercial enzyme preparations (Table I) filter paper (type 1;

Table I. Cellulase, cellobiase, xylanase activities (U/mL), and protein concentration (mg protein/mL) of commercial enzyme preparations.

Enzyme sample	Supplier	FPU (U/mL)[a]	Cellobiase (U/mL)[b]	Xylanase (U/mL)[c]	Bradford[d]	Pierce[e]
					Protein (mg/mL)	
Cellubrix	Novozymes (Bagsvaerd, Denmark)	56	136	107	43	122
Novozymes 188	Novozymes	<5	1,116	31	57	168
Cellulase 2000L	Rhodia-Danisco (Vinay, France)	10	nd	568	7	48
Rohament CL	Rohm-AB Enzymes (Rajamäki, Finland)	51	28	261	44	152
Viscostar 150L	Dyadic (Jupiter, USA)	33	111	3,074	40	163
Bio-feed beta L	Novozymes	<5	12	184	8	29
Energex L	Novozymes	<5	19	5	28	222
Ultraflo L	Novozymes	<5	20	469	18	32
Viscozyme L	Novozymes	<5	23	5	27	219
Cellulyve 50L	Lyven (Colombelles, France)	24	nd	54	34	105
GC 440	Genencor-Danisco (Rochester, USA)	<5	70	272	29	118
GC 880	Genencor	<5	86	396	43	162
Spezyme CP	Genencor	49	nd	455	41	135
GC 220	Genencor	116	215	677	64	211

nd, not determined.
[a]Filter paper units determined according to IUPAC method; pH 5.0 and 50°C.
[b]Determined according to IUPAC method with cellobiose; pH 5.0 and 50°C.
[c]Determined with the Xylazyme AX method of Megazyme; pH 5.0, 50°C.
[d]Determined by using the Bradford-assay.
[e]Determined by using the Pierce-BCA-assay.

Whatman, Middlesex, UK) was incubated with an enzyme sample in a 0.05 M citrate-buffer of pH 5 at 50°C for 1 h. After inactivation (5 min, 100°C) and centrifugation the reducing ability was measured according to the Nelson–Somogyi assay (Somogyi, 1952).

To define the cellobiase activity (U/mL), a similar experiment was performed by using the same citrate buffer at 50°C. However, cellobiose was used as a substrate and the incubation time was 30 min. After inactivation (5 min, 100°C) and centrifugation, the amount of glucose formed was measured according to the K-gluc assay kit (Megazyme, Wicklow, Ireland).

To define the xylanase-activity (U/mL), the Xylazyme AX test kit (Megazyme) was used. One tablet of Xylazyme AX was incubated at pH 5 (0.05 M citrate buffer) and 50°C for 10 min. The reaction was stopped by addition of a 2% Na3PO4 (pH 11) solution. After centrifugation the amount of the chromogenic compound released was measured (590 nm) and related to the amount of reducing end groups formed.

Protein Content

The protein content was determined for all commercial cellulase preparations in triplicate using two widely accepted methods: the Bradford reagent assay (Sigma, St. Louis, MO; Bradford, 1976) and the BCA protein assay (Pierce kit: Pierce, Rockford, IL).

Neutral Sugar Composition

The neutral sugar composition was determined by gas chromatography according to Englyst and Cummings (1984), using inositol as an internal standard. The samples were treated with 72% w/w H_2SO_4 (1 h, 30°C) followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100°C and the constituent sugars released were analyzed as their alditol acetates.

Uronic Acid Content

The uronic acid content was determined as uronic acid (UA) by an automated *m*-hydroxydiphenyl assay (Blumenkrantz and Asboe-Hansen, 1973; Thibault, 1979) using an autoanalyzer (Skalar Analytical BV: Skalar, Breda, The Netherlands).

Acetic Acid Content

The degree of acetylation was determined on a Thermo Separation Products system HPLC, using an Aminex HPX column (Voragen et al., 1986). Detection was performed by uing a Shodex RI-71 detector. The level of acetyl substitution was corrected for the free acetic acid in the sample.

Starch Content

Starch was determined enzymatically using the test kit supplied by Boehringer (Ingelheim, Germany).

Capillary Electrophoresis (CE)

The commercial enzyme preparations were studied by using capillary electrophoresis (CE). CE experiments were performed using a MDQ (Beckman Coulter, Fullerton, CA) equipped with a diode array detector (UV/VIS; 280 nm and 200 nm), and according to Jorgensen et al. (2003). Separations were carried out in a fused silica capillary of 75 μm ID and 60 cm total length (detection 10 cm from capillary end) at 20°C using 15 kV. The separation buffer

(pH 7.25) was 50 mM potassium phosphate plus 10 mM 1,2-diaminopropane (Jorgensen et al., 2003). Enzyme preparations were dialyzed against distilled water and filtered (0.22-μ low-protein-binding filter). Samples were injected at a pressure of 0.5 psi for 5 s. The capillary was rinsed with 0.1 M NaOH, water, and buffer between each run. The cellobiohydrolases I (CBH I) analyzed was purified in our laboratory from Trichoderma viride (Beldman et al., 1985), previously named exoglucanase III.

HPSEC

High-performance size-exclusion chromatography was performed on a Thermo Separation Products HPLC system equipped with a membrane solvent-degasser, three TSKgel columns (7.8 mm ID × 30 cm per column) in series (G4000, G3000, G2500; Tosohaas, Stuttgart, Germany), in combination with a PWX-guard column (Tosohaas). Elution took place at 30°C with 0.2 M sodium nitrate at 0.8 mL/min. The eluate was monitored using a refractive index (RI) detector (Shodex RI-71). Calibration was performed using pullulans (Polymer Labs, Shropshire, UK).

HPAEC (pH 12)

High-performance anion-exchange was performed on a Thermo Separation Products system equipped with a Dionex (Sunnyvale, CA) CarboPac PA-1 column (4 mm ID × 250 mm) in combination with a Dionex CarboPac PA guard column (3 mm × 25 mm) and PAD detection (Dionex) (Lee, 1996). For the analysis of arabinose, xylose, and glucose in the hydrolysates, an isocratic elution (1 mL/min) of 20 min was carried out with a solution of 16 mM NaOH. After 20 min a gradient of 0–550 mM sodium acetate in 100 mM NaOH during 25 min was applied to elute oligomers. Each elution was followed by a washing and equilibration step.

RESULTS AND DISCUSSION

In general, reports on cellulase or xylanase activities from commercial enzymes are hard to compare. Methods to analyze enzyme activity differ in conditions, for example type of substrate (Johnston, 2003). An exception is the technical communication of Nieves et al. (1998), who surveyed some commercial cellulases for standard cellulase activities.

In this study, 14 recent commercial cellulase preparations were subjected to three standardized tests. These include one determining the overall cellulose activity (filter paper assay), one measuring the cellobiase activity, and a third one determining the xylanase activity using the Megazyme's Xylazyme assay (Table I). Additionally, of the commercial cellulases used the protein content was determined as well (Table I).

Comparing the cellulase activities, the developmental preparation GC 220 from Genencor presented the highest activity towards filter paper. Additionally, GC 220 contained a rather high cellobiase activity as well, just as Cellubrix. Cellubrix is sold as a renewed Celluclast (Novozymes, Bagsvaerd, Denmark; Nieves et al., 1998), which is especially enriched in cellobiase activity. An excess of cellobiase activity was found for Novozym 188, which is on the market as a specific cellobiase preparation. The protein content, determined by the Bradford- and Pierce-assay, varied from 7 to 64 mg and 29 to 222 mg protein/mL, respectively. The protein content measured depended on the method used, but in general, the same trends were observed when comparing the different enzymes. More importantly, no relationships between the amount of protein present and (total) enzyme activity could be found. The commonly used filter paper activity assay (IUPAC; Decker et al., 2003; Ghose, 1987) is a good measure of the hydrolytic potential of a cellulase preparation. Filter paper is seen as highly crystalline cellulose, of which the degradation is depending on the combination of activities of endoglucanases (EG) and CBH, whereby the EG create new chain ends for the CBH to split of cellobiose (Beldman et al., 1988a; Teeri, 1997; Vincken et al., 1994). In Trichoderma reesei, about 80% of the total cellulolytic protein is consisted of CBH I (60%) and CBH II (20%) (Teeri, 1997). Furthermore, cellobiase (actually β-glucosidase) activity is responsible for the formation of glucose from cellobiose and is thought to play an important role in cellulose degradation by relieving end product (cellobiose) inhibition.

Besides their individual activity, the synergism of EG, CBH, and cellobiase is important for the complete degradation of cellulose. Especially CBH I act synergistically with EG (Beldman et al., 1988a). The amount of CBH I in the commercial preparations was studied by CE. In the enzyme preparations Cellubrix, Rohament CL, Cellulyve 50L, Spezyme CP, and GC 220 a relatively large part of the protein present originated from CBH I. For example, the presence of CBH I in the Genencor preparations as analyzed by CE is presented in Figure 1. The commercial cellulases having a FPU lower than 5 (Table I), contained little CBH I. Surprisingly, Viscostar 150L did not contain CBH I as well. Cellulase 2000L, Bio-feed beta L and Energex L were not analyzed by CE, but since their activity towards filter paper was less than 5 FPU, CBH I is not expected to be present in these preparations.

The xylanase activity is also of interest in relation to a complete degradation of cellulose. Xylans are coating the cellulose fibrils and therefore hindering the accessibility of cellulose for cellulases. The standard xylanase activity of the commercial cellulase preparations is presented in Table I. The Cellulyve 50L preparation and the Novozymes preparations Novozymes 188, Energex L, and Viscozyme L contained a xylanase activity of less than 100 U/mL. All other preparations except Viscostar 150L were analyzed to contain a xylanase activity in a range of 100–700 U/mL. The highest xylanase activity (3,074 U/mL) was present in Viscostar 150L. Viscostar 150L was indeed specified as xylanase preparation.
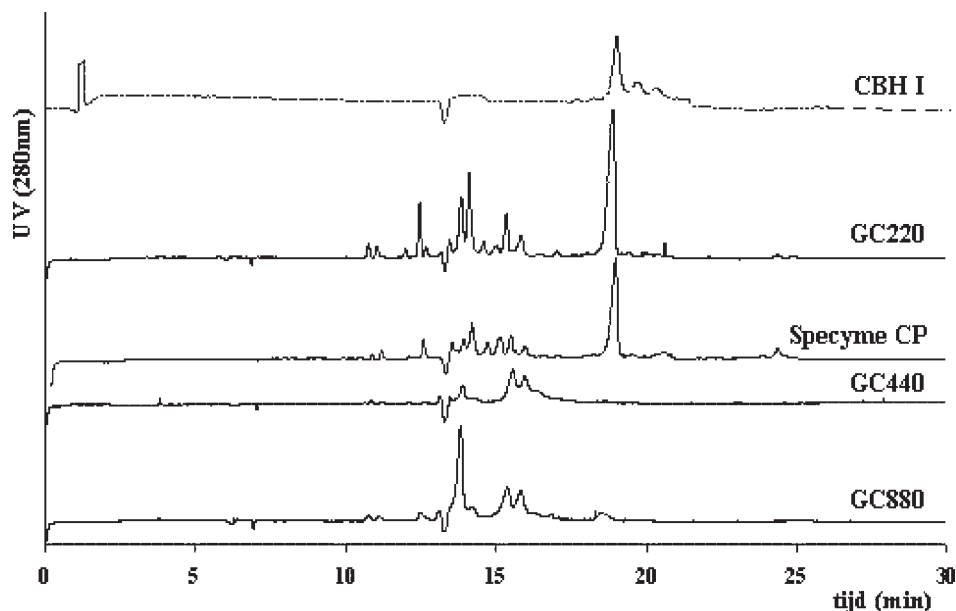
Figure 1. CE-electropherogram of the commercial preparations GC220, Spezyme CP, GC440 and GC880, and of the purified cellobiohydrolases I (CBH I).

Almost all enzymes tested originated from a strain of Trichoderma longibrachiatum, formerly known as Trichoderma reesei or Trichoderma viride, or a genetically modified strain of this species. Only Novozym 188 originated from a submerged fermentation of Aspergillus niger, while Cellubrix came from a separate fermentation of Trichoderma longibrachiatum and Aspergillus niger.

Besides the standard activities of commercial enzyme preparations, the activity of the commercial enzymes towards more natural and therefore more complex substrates was analyzed. This research was aimed at the enzymatic degradation of heterogenic substrates containing both cellulose and hemicelluloses. Hereto, wheat bran, and grass were selected and partially fractionated. First, starch was removed by using a rather mild treatment with amylases and buffer resulting in the so-called water unsoluble solids (WUS). During preparation of the WUS, the cellulose-hemicellulose network was not disturbed. The sugar composition of the prepared fractions is shown in Table II.

In wheat bran, 7% (w/w) of cellulose was present and 23% (w/w) of xylan. Grass contained a similar amount of xylan, but a much higher amount of cellulose (26% w/w). Wheat bran contained a rather high amount of starch (27% w/w), which was removed prior to the cellulase activity studies. In grass only small amounts of starch were present.

The destarched fractions (WUS) of both wheat bran and grass were fractionated further by using 4 M KOH, which is known to selectively dissolve hemicelluloses (Verbruggen, 1995; Vincken et al., 1994). A KOH-soluble fraction was obtained (KOHss) and the remaining solids were collected as KOHres.

In the KOHss fractions of both wheat bran and grass indeed mainly arabinoxylan was present (Table II). In addition, in the wheat bran KOHss some glucose was present. This

Table II. Sugar composition (% w/w) of wheat bran and grass and of their corresponding fractions.

	Total sugar (% w/w)	Ara (% w/w)	Xyl (% w/w)	Man (% w/w)	Gal (% w/w)	Glc (% w/w)	UA (% w/w)	Acetic acid[c] (% w/w)
Wheat bran								
Feedstock	59	8	13	1	1	34 (7[a], 27[b])	2	0.27
WUS	57	11	19	1	4	19	3	0.19
KOHss	68	15	34	0	1	16 (0[a,b])	2	—
KOHres	73	16	15	1	1	35	5	—
Grass								
Feedstock	54	3	15	1	2	27 (26[a])	4	1.47
WUS	64	4	19	1	2	33	4	1.26
KOHss	72	10	52	0	2	3	4	—
KOHres	79	3	10	1	2	57	4	—

Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acids.
[a]Cellulose (% w/w).
[b]Starch (% w/w).
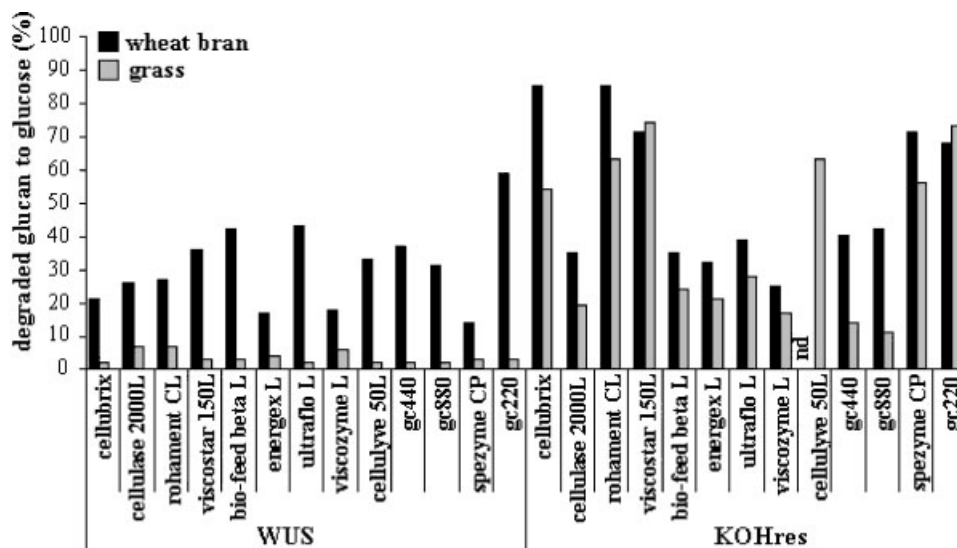[c]Bound acetyl groups, corrected for free acetic acid.

Figure 2. Degradation of wheat bran and grass cellulose present in the water non-soluble (WUS) and the cellulose enriched (KOHres) fractions by using commercial enzyme preparations (nd, not determined).

glucose was analyzed not to originate from starch, but most likely it originated from β-glucans. In general in wheat bran 6% (w/w) of the non-starch polysaccharides consists of β-glucans (Bacic and Stone, 1981). Both the KOHss of wheat bran and of grass were sufficiently enriched in xylans to be used as substrate in the evaluation of xylanase activity from commercial enzymes. The remaining residues (KOHres) were mainly consisting of cellulose, although some arabinoxylans were present as well. The xylans present in the KOHres fractions were much higher substituted with arabinose groups compared to the arabinoxylans in the KOHss fractions. The enriched cellulose fractions (KOHres) of both wheat bran and grass were used as substrate in the evaluation of cellulase activity from commercial enzymes.

All fractions prepared (Table II) were incubated with commercial enzyme preparations. The degradation of cel-

lulose into glucose was determined and these results are presented in Figure 2. For all preparations tested, the degradation of cellulose into cellobiose was less than 6% (w/w) of the original amount of cellulose present (not shown).

Wheat bran WUS was degraded much better compared to grass WUS (Fig. 2). Probably, the lignin-xylan-cellulose network was less rigid in wheat bran compared to the grass studied. Next, by using alkali the cellulose-xylan network was disturbed and this effect was seen in the much higher degradability of both wheat bran and grass KOHres compared to the corresponding WUS (Fig. 2). Furthermore, remarkable differences in degradation of the KOHres were seen for the different commercial enzymes. The Genencor GC 220 preparation resulted in the best degradation of the cellulose present. Surprisingly, the percentage of cellulose degradation in KOHres was not well correlated with the
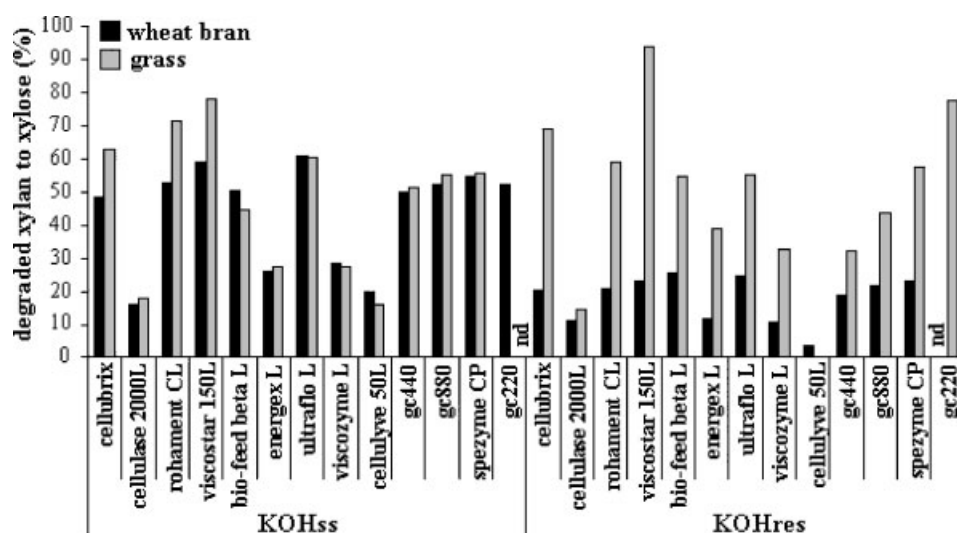

Figure 3. Degradation of wheat bran and grass xylan present in the xylan enriched (KOHss) and the cellulose enriched (KOHres) fractions by using commercial enzyme preparations (nd, not determined).

cellulase activity as measured in the standard activity test (Table I vs. Fig. 2). For example, the standard cellulase activity of Cellubrix, Rohament CL, Viscostar 150L, Cellulyve 50L, Spezyme CP, and GC 220 ranged from 24 to 116 FPU, while cellulose from the KOHres was degraded for approximately 60% by these enzymes. Obviously, the substrate characteristics are of vital importance for the efficiency of the complex of cellulases in commercial enzyme preparations. Crystallinity of cellulose is seen as one of the main characteristic effecting cellulase activity (Lynd et al., 2002).

By analyzing the degradation of the xylan present in wheat bran KOHss and grass KOHss into xylose, the xylanase activity of commercial enzymes towards natural substrates was studied. The results are presented in Figure 3. Some of the enzymes degraded the xylan in the KOHss fractions from both wheat bran and grass not only to xylose monomers (Fig. 3), but also (partly) to oligomeric fragments (results not shown). Most likely, the enzyme preparation is not optimal balanced for the complete degradation of substituted xylans completely to xylose resulting in the (substituted) oligomers analyzed. The efficiency of the commercial enzymes in degrading the xylans still present in the KOHres fraction of grass was comparable to the degradation in grass KOHss. However, the activity of the enzymes towards the xylans still present in wheat bran KOHres was relatively low (Fig. 3). This effect could again be explained by the substitution of the xylan. Namely, the xylans in grass KOHss, grass KOHres, and wheat bran KOHss were decorated with arabinose in a ratio of arabinose (mol) to xylose (100 mol) of 19, 31, and 45 respectively, while the Ara/Xyl-ratio of the xylans in the most resistant wheat bran KOHres was 108 (mol Ara/100 mol Xyl). The enzymes in the commercial mixtures were apparently highly hindered by the arabinose side chains in degrading the xylan-backbone in wheat straw KOHres, which was already suggested from the release of oligomers from the grass and wheat bran xylan fractions. Another conclusion from these results was that in the commercial mixtures tested almost no arabinose-releasing activity was present, which could increase the efficiency of complete degradation of xylans enormously.

Again the activity analyzed in the standard xylanase test did not present a high correlation with the degradation of xylans in the xylan-rich KOHss fractions of wheat bran and grass. A rather striking example is the comparison of the standard activity presented in Table I of Cellubrix (107 U/mL) and Cellulase 2000L (568 U/mL) with the degradation of xylan to xylose of Cellubrix (50%–60%) and Cellulase 2000L (18%–20%) presented in Figure 3.

A critical note for the determination of commercial enzyme preparations by studying their mode of catalytic action is the non-specific nature of some of the enzymes. Non-specific glucanases originating from Trichoderma ssp. are not only able to hydrolyse cellulose, cellobiose, and cello-oligomers, but are able to attack xylan as well. Therefore, recently the enzymes are classified based on structural properties (Beldman et al., 1988b; Lynd et al., 2002; Wong and Saddler, 1992, and references herein).

In conclusion, 14 commercial enzymes from different suppliers were tested for their cellulase and xylanase activity. Remarkable differences between enzyme activities were found towards both standard activity and in activity towards the cellulose- and xylan-enriched fractions from grass and wheat bran. The developmental enzyme preparations showed improved activity, both in standard activity (FPU) and in activity towards the cellulose-enriched fractions. A relatively low standard activity of enzymes was not always correlating with the degradability of the xylan- and cellulose-rich fractions from wheat bran and grass. In general, the choice of an enzyme preparation is dependent on the substrate characteristics rather than on standard enzyme-activities measured.

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