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Cellulase Production of *Trichoderma reesei* Rut C 30 Using Steam-Pretreated Spruce

Hydrolytic Potential of Cellulases on Different Substrates

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Abstract

Various techniques are available for the conversion of lignocellulosics to fuel ethanol. During the last decade processes based on enzymatic hydrolysis of cellulose have been investigated more extensively, showing good yield on both hardwood and softwood. The cellulase production of a filamentous fungi, *Trichoderma reesei* Rut C 30, was examined on carbon sources obtained after steam pretreatment of spruce. These materials were washed fibrous steam-pretreated spruce (SPS), and hemicellulose hydrolysate. The hemicellulose hydrolysate contained, besides water-soluble carbohydrates, lignin and sugar degradation products, which were formed during the pretreatment and proved to be inhibitory to microorganisms. Experiments were performed in a 4-L laboratory fermentor. The hydrolytic capacity of the produced enzyme solutions was compared with two commercially available enzyme preparations, Celluclast and Iogen Cellulase, on SPS, washed SPS, and Solka Floc cellulose powder. There was no significant difference among the different enzymes produced by *T. reesei* Rut C 30. However, the conversion of cellulose using these enzymes was higher than that obtained with Iogen or Celluclast cellulases using steam-pretreated spruce as substrate.

Index Entries: Cellulase production; steam-pretreated spruce; enzymatic capacity of cellulases; filter paper activity measurement; *Trichoderma*.

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Introduction

The conversion of biomass to liquid fuels, such as ethanol, has been the focus of interest over the past two decades owing to the oil crisis of the 1970s and the concern about environmental and climatic effects during the last few years. Besides producing ethanol from crops cultivated for this purpose, different sorts of wastes (agricultural, municipal, and forestry) can also be utilized. This can decrease the production cost greatly, since the cost of the feedstock is one of the major contributors to the relatively high production cost of, e.g., fuel ethanol *(1–4)*. A positive effect of burning fuel ethanol from so-called renewable raw materials is that they do not increase the carbon dioxide level of the atmosphere in contrast to when using fossil fuels *(4,5)*. Although the conversion of starch-containing materials to glucose, which can be further converted to ethanol, is well established *(6)*, the conversion of lignocellulosics is more problematic. However, the latter raw materials are important because they are available in large quantities from agriculture and forestry.

Different methods are available for converting lignocellulosic materials to monomeric sugars, e.g., one-step concentrated acid hydrolysis, two-step dilute acid hydrolysis, and enzymatic hydrolysis *(7–10)*. The main drawback of concentrated acid hydrolysis is that it requires high-quality stainless steel equipment, which increases the capital cost considerably. Typically, the hemicellulose fraction is hydrolyzed earlier than the cellulose fraction, and the resulting sugars from hemicellulose are exposed to degradation to byproducts. These byproducts decrease the sugar yield and can cause inhibition in the ethanol fermentation. The recovery of acid used, which is usually energy demanding, is important in order to make the process economically feasible. In contrast to concentrated acid hydrolysis, a low concentration of acids is applied in the dilute-acid process but at higher temperatures. The dilute-acid process usually consists of two separate hydrolysis steps. In the first step, the hemicellulose fraction is degraded to water-soluble sugars. In the second step, the cellulose is converted to glucose. This minimizes the formation of byproducts owing to unnecessarily long residence time. However, considerable amounts of toxic compounds can be found in these hydrolysates because of the higher temperature applied *(2,7,9,11,12)*.

In the enzymatic process, the hemicellulose fraction is removed in a similar way as in the dilute-acid process, but in the second step, the cellulose fraction is hydrolyzed by means of cellulases instead. Because of the milder conditions in the enzymatic hydrolysis, fewer byproducts are formed, resulting in a well-fermentable sugar solution with good sugar yields *(13–15)*. However, to obtain good yields in the enzymatic hydrolysis of cellulose, high enzyme loading is required, which will increase production costs considerably *(16)*.

A typical process configuration based on the enzymatic hydrolysis of cellulose consists of at least five main steps: pretreatment of raw material,

including chopping, sieving, and prehydrolysis of hemicellulose; cellulase production; enzymatic hydrolysis of cellulose; fermentation; and ethanol recovery *(9,10)*. An optional wastewater treatment section, such as evaporation or biological purification, can also be included to minimize freshwater demand and wastewater discharge *(17)*.

One way of performing the prehydrolysis of the hemicellulose fraction is the high-pressure steam pretreatment in the presence of acid catalyst, i.e., sulfuric acid or sulfur dioxide. This has been used for a large variety of woody materials showing good yields from both hardwoods and softwoods *(18)*. During the steam pretreatment of wood, the hemicellulose part of the material is hydrolyzed to monosaccharides (glucose, xylose, mannose, galactose, arabinose). The cellulose in the fibrous material, comprising cellulose and structurally modified lignin, is made more accessible to enzymatic attack.

The cellulose is further hydrolyzed to cellobiose and glucose by means of cellulolytic enzymes. There are three types of enzymes involved in the complete breakdown of cellulose to glucose: endoglucanases, cellobiohydrolases, and cellobiase/β-glucosidase *(19,20)*. The β-glucosidase itself is not considered to be a cellulase, since it does not act directly on the cellulose chain. However, it has an important role in converting cellobiose produced by endoglucanases and cellobiohydrolases to glucose, which is less inhibitory to endoglucanases and cellobiohydrolases than cellobiose *(21)*. As a consequence, by increasing the amount of β-glucosidase, the conversion rate and yield of hydrolysis can be increased considerably *(22)*. The lack or difference in amounts of β-glucosidase in different enzyme mixtures could also be a problem when the filter paper activity of these samples is measured *(23)*.

During the pretreatment, in addition to water-soluble sugars, lignin and sugar degradation products are formed. These compounds were found to be inhibitory to yeast and bacteria, as well as to cellulolytic enzymes, causing a decrease in productivity and yield *(24,25)*. One way of dealing with the problem is to separate the liquid fraction from the fibrous material after pretreatment. This sugar solution can then be utilized for cellulase enzyme production by *Trichoderma reesei* Rut 30. *Trichoderma* species are very good cellulase-excreting microorganisms, with the ability to utilize a large variety of monosaccharides *(26)*.

In the present study, the enzyme production of *T. reesei* Rut C 30 was examined using different mixtures of fibrous cellulose and hemicellulose hydrolysate from steam-pretreated spruce (SPS). The hydrolytic capacity of the produced enzymes was tested on different substrates and compared with two commercially available cellulase preparations. The hydrolysis experiments were intended to be performed with the same enzyme: substrate (FPU [filter paper units]/g of cellulose) ratio. To do this, the filter paper measurement was modified, and a suitable method for determining a β-glucosidase-independent cellulase activity was developed.

Table 1
Cellulase and β-Glucosidase Activities
of Celluclast 2L, Iogen Cellulase, and Novozym 188

Activity	Celluclast 2L	Iogen Cellulase	Novozym 188
FPA (FPU/g)[a]	97.0	140.6	—
FPA-M (FPU/g)[b]	147.1	170.8	—
β-Glucosidase (IU/g)	33.2	146.8	468.3

[a]Filter paper activity according to Mandels et al.'s *(31)* procedure.
[b]Modified Mandels et al.'s *(31)* procedure, using addition of β-glucosidase enzyme.

Materials and Methods

Enzymes

Commercially available enzyme solutions, Celluclast 2L and Novozym 188, were obtained from Novo Industri A/S (Bagsvaerd, Denmark). Iogen Cellulase was purchased from Iogen (Ottawa, Canada). Celluclast 2L and Iogen Cellulase were analyzed for cellulase and β-glucosidase activity, whereas only β-glucosidase activity was determined in Novozym 188. Table 1 summarizes the results of the activity measurements.

Pretreatment of Spruce

Fresh, bark-free spruce (*Picea abies*) chips were provided by a sawmill, Höörsågen AB (Höör, Sweden). The material had a dry wt of 43.3 wt%. The lignin content of the raw material was analyzed using Hägglund's method *(27)* with the modification that the acid hydrolysate obtained was analyzed for concentrations of various sugars. The untreated spruce contained 43.0 wt% glucan, 11.2 wt% mannan, 4.8 wt% xylan, 2.1 wt% galactan, 1.0 wt% arabinan, 26.7 wt% lignin, and 11.2 wt% other compounds based on oven-dried material.

The spruce chips were first rechopped and sieved. The fraction between 2.2 and 10 mm was collected and further processed. The wet, chopped, sieved wood chips, corresponding to 650 g of dry material, were impregnated with about 30 g of gaseous sulfur dioxide in a well-sealed plastic bag. After 20 min of residence time, the excess SO_2 was vented off, and the SO_2 uptake was determined by weighing. The SO_2 concentration was about 2.7 wt% based on water content. The impregnated material was then subjected to steam pretreatment with saturated steam at 215°C for 5 min *(28)*. The slurry of SPS was collected and analyzed for total dry wt and water-insoluble material, which were 16.4 and 11.2 wt%, respectively. The SPS was used as substrate in the hydrolysis tests.

A portion of the SPS was filtered on an ordinary vacuum filter unit. The filtrate (F) comprising the hydrolyzed hemicellulose fraction was saved and analyzed on high-performance liquid chromatography (HPLC) for sugars, acetic acid, and sugar degradation products (furfural and hydroxymethyl furfural [HMF]). Table 2 presents the composition of the filtrate.

Table 2
Composition of Filtrate

Compound	Concentration (g/L)
Glucose	19.5
Mannose	16.6
Cellobiose	0.9
Galactose	3.5
Xylose	6.7
Arabinose	1.3
Acetic acid	4.4
Furfural	1.2
HMF	2.8

The filter cake was washed thoroughly with hot tap water and analyzed for cellulose and lignin content using the modified Hägglund's *(27)* method. The washed SPS (SPS-W) contained 41.7 wt% cellulose and 55.6 wt% lignin. Both the fibrous SPS-W and F were used as carbon sources for enzyme production. The washed solid material, SPS-W, was also used as substrate in the enzymatic hydrolysis experiments.

Preparation of Inoculum

The fungus *T. reesei* Rut C 30 (ATCC no. 56765) was stored on agar slants containing 20 g/L of malt extract, 5 g/L of glucose, 1 g/L of proteose peptone, and 20 g/L of bacto agar, prior to usage. After 15 d at 30°C, the conidia were suspended in 5 mL of sterile water, and 1.5 mL of the suspension was used to initiate growth in a 1-L baffled Erlenmeyer flask containing 200 mL of Mandels and Weber's medium *(29)* in which the concentration of nutrients was 0.3 g/L of urea, 1.4 g/L of $(NH_4)_2SO_4$, 2.0 g/L of KH_2PO_4, 0.3 g/L of $CaCl_2$, 0.3 g/L of $MgSO_4$, 0.25 g/L of yeast extract, and 0.75 g/L of proteose peptone together with 7.5 g of Solka Floc 200 cellulose powder (FS&D, Urbana, IL). Trace elements were also added 5 mg/mL of $FeSO_4 \cdot 7H_2O$, 20 mg/mL of $CoCl_2$, 1.6 mg/mL of $MnSO_4$, and 1.4 mg/mL of $ZnSO_4$. The pH of the medium was adjusted to 5.4–5.6 before sterilization by the addition of 10 wt% of NaOH solution. After 4 d at 30°C and 300 rpm, the inoculum was ready.

Enzyme Production in Fermentor

The cellulase enzyme production was performed in a 4-L CMF Mini fermentor (Chemap AG, Volketswil, Switzerland) at 30°C and 300 rpm using a modified Mandels and Weber's *(29)* medium, in which the yeast extract and proteose peptone were replaced with 0.38 g/L of dry yeast *(30)*. The amount of nutrients added to the fermentor corresponded to that needed for 2.5 L of culture medium, in which the concentration of carbon source was 10 g/L of carbohydrates. The volume of mycelium suspension used to initiate growth in the fermentor constituted 10% of the total volume

after inoculation. The pH of cultivation was set once a day to 6.0 with the addition of either 10 wt% NH_4OH or 10 wt% H_2SO_4 solutions depending on the actual pH. Samples were withdrawn after every 10–12 h, centrifuged at 3400*g* for 10 min using an A.L.C. centrifuge (A.L.C., Cologno Monzese, Italy) and analyzed for filter paper and β-glucosidase activities, as well as concentrations of sugars.

After 92 h of cultivation, the fermentation broth was harvested and filtered. A glass-fiber filter was used to prevent enzyme losses owing to adsorption on normal filter paper. The culture filtrate was concentrated five to six times by vacuum evaporation at 40°C using a Büchi RE 121 Rotavapor (Büchi Labortechnik AG, Flawil, Switzerland) and was reanalyzed for enzymatic activities.

Enzymatic Hydrolysis

Hydrolysis of three different substrates—SPS, SPS-W, and Solka Floc 200 cellulose powder—was performed at 1.1 wt% cellulose concentration, corresponding to 2 wt% dry fibrous material in the cases of SPS and SPS-W, in a 0.1 *M* sodium acetate buffer solution (pH 4.8) supplemented with 41.8 FPU of cellulases and 1.1 g of Novozym 188/g of cellulose. The stirred hydrolysis vessel containing a total amount of 500 g of material was maintained at 40°C for 48 h. Samples were withdrawn after 0, 3, 6, 9, 12, 24, and 48 h. The sample suspensions were boiled for 5 min in sealed centrifuge tubes to stop enzyme activities and were centrifuged at 1700*g* for 5 min. The supernatant was collected for sugar analysis.

Analysis

The enzyme activity of samples was determined as filter paper activity (FPU) using Mandels et al.'s *(31)* procedure and β-glucosidase activity using Berghem and Petterson's method *(32)*. All samples were analyzed in triplicate, and the mean values were calculated. The relative standard deviation of enzyme activity measurements were always lower than 5%.

Samples for analysis of sugar and byproduct contents were first filtered through 0.2-μm membrane filters (Advantec MFS, Plesanton) and then analyzed on an HPLC unit (Shimadzu, Japan) equipped with a refractive index and an ultraviolet detector. Cellobiose, glucose, xylose, galactose, arabinose, and mannose were separated on an Aminex HPX-87P (Bio-Rad, Hercules, CA) column at 85°C using ultrapure water as mobile phase, at a flow rate of 0.5 mL/min. Acetic acid, HMF, and furfural were analyzed using an Aminex HPX-87H (Bio-Rad, Hercules, CA) column at a temperature of 65°C using a 5 m*M* H_2SO_4 solution as eluent, at a flow rate of 0.5 mL/min.

Results and Discussion

Measurement of Filter Paper Activity

For FPU measurement, there are basically two different methods in use: Mandels et al.'s *(31)* and the International Union of Pure and Applied

Chemistry (IUPAC) procedure *(33)*. In both methods, a 50-mg stripe of filter paper is used as a substrate. Mandels et al. *(31)* recommend a dilution of the enzyme solution to be measured in such a way that after 60 min of incubation time, the absolute amount of liberated reducing sugars (practically glucose) must not be more than 2 mg, which would mean a maximum conversion of 4%. Performing the analysis under these conditions, the rate of hydrolysis was supposed to be linear and not affected by end-product inhibition (cellobiose/glucose). However, according to the IUPAC method, the hydrolysis rate decreases over the incubation period. At least two dilutions must be made from each enzyme sample: one that would release slightly more and one that would release <2 mg of glucose in reaction conditions. From these two measurements, the enzyme concentration that would have released exactly 2 mg of glucose can be calculated with nonlinear interpolation. This information can then be used to calculate the filter paper activity. This method presumably would result in a more realistic enzyme activity, because it standardizes end-product inhibition and includes possible nonlinear behavior of the hydrolysis. The drawback of this procedure is that it cannot be applied in cases of very low enzyme concentrations. Unfortunately, none of these methods provides any solution for compensating the effect of different β-glucosidase concentrations in various enzyme preparations on the measurement of FPA.

Measurement of a β-glucosidase-independent cellulase activity was introduced based on the Mandels et al.'s *(31)* procedure. The method was developed using Celluclast 2L and Novozym 188 and was later applied to Iogen Cellulase and all enzyme preparations. Celluclast 2L was diluted to 0.1 wt% in citrate buffer (0.05 *M*, pH 4.8). Novozym 188 was applied in two different dilutions: 0.1 and 1 wt%. Different mixtures of these diluted enzymes were prepared in which the amount of Celluclast 2L was kept constant, whereas the amount of Novozym 188 was increased stepwise. Table 3 summarizes the mixtures prepared. Both filter paper and β-glucosidase activities were measured in all mixtures. The absolute amount of liberated reducing sugars never exceeded 1.5 mg of glucose.

Figure 1 shows the FPA activity recalculated to grams of original cellulase enzyme preparation vs measured β-glucosidase activity calculated to grams of original cellulase enzyme. The FPU activity of both cellulase enzymes first increased with increasing the amount of added Novozym 188 up to 1400 IU/g of original cellulase, from which point there was no further change in FPA activity. The original activity of 97 FPU/g was increased by 52% up to 147 FPU/g for the Celluclast 2L. The same activities for the Iogen Cellulase were found to be 141 and 171 FPU/g, respectively. The effect of the addition of external β-glucosidase on the FPU activity of Iogen Cellulase was less compared with that observed for Celluclast 2L, since the former had a 4.4-fold higher cellobiase activity from the beginning. Note that although in Iogen Cellulase the FPU:β-glucosidase ratio was found to be almost 1:1, a 21% increase was observed by further addition of β-glucosidase enzyme.

Table 3
Experimental Design of Cellulase Activity Measurement

0.1 wt% Celluclast 2L (mL)	0.1 wt% Novozym 188 (mL)	1 wt% Novozym 188 (mL)	Citrate buffer (mL)
0.50	0.00	0.00	1.00
0.50	0.05	0.00	0.95
0.50	0.10	0.00	0.90
0.50	0.20	0.00	0.80
0.50	0.50	0.00	0.50
0.50	1.00	0.00	0.00
0.50	0.00	0.20	0.80
0.50	0.00	0.30	0.70
0.50	0.00	0.50	0.50
0.50	0.00	1.00	0.00

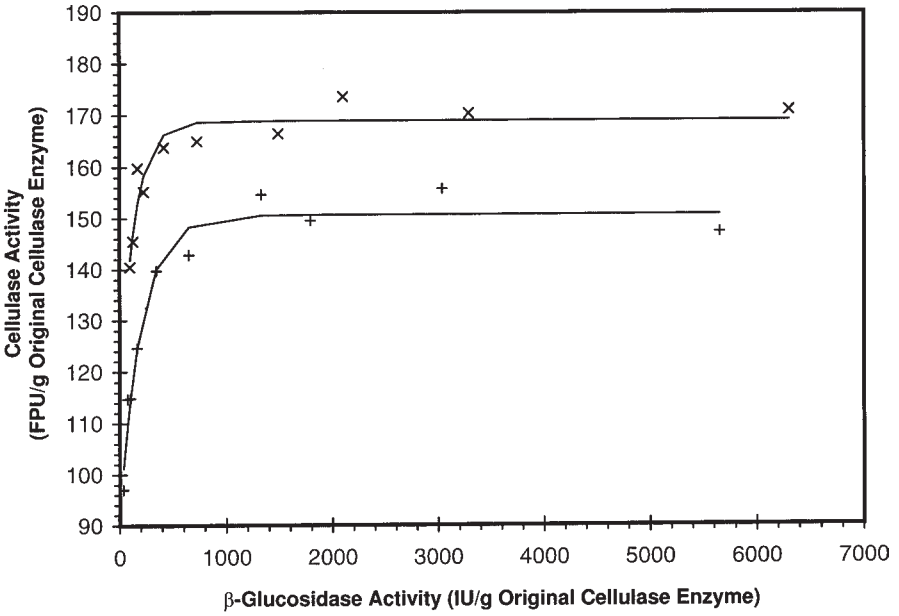

Fig. 1. β-Glucosidase dependency of filter paper activity measurement: FPU vs β-glucosidase activity for celluclast 2L (+) and Iogen Cellulase (×).

Enzyme Production on SPS

Three different fermentation runs were performed with SPS-W supplemented with F with a total carbon source concentration of 10 g/L. The amount of soluble carbon source (F) was set to 0, 20, and 50% of the total carbohydrate concentration. Table 4 summarizes the enzyme activities obtained after 92 h of residence time. The highest cellulase activity, 0.79 FPU/mL, was obtained using only SPS-W (E1) and the lowest,

Table 4
Cellulase and β-Glucosidase Activities Obtained
with *T. reesei* Cultivated on Different SPS-W and F Mixtures

	Before evaporation		After evaporation		
Enzyme	FPA (FPU/mL)[a]	β-Glucosidase (IU/mL)	FPA_{evap} (FPU/mL)[a]	$FPA\text{-}M_{evap}$ (FPU/mL)[b]	$\beta\text{-Glucosidase}_{evap}$ (IU/mL)
E1[c]	0.79	0.18	4.29	6.04	0.97
E2[d]	0.54	0.18	4.23	5.21	1.01
E3[e]	0.48	0.09	2.79	3.71	0.48

[a]Filter paper activity according to Mandels et al.'s *(31)* procedure.
[b]Modified Mandels et al.'s *(31)* procedure, using addition of β-glucosidase enzyme.
[c]Enzyme obtained using SPS-W alone.
[d]Enzyme obtained using SPS-W substituted with 20% F.
[e]Enzyme obtained using SPS-W substituted with 50% F.

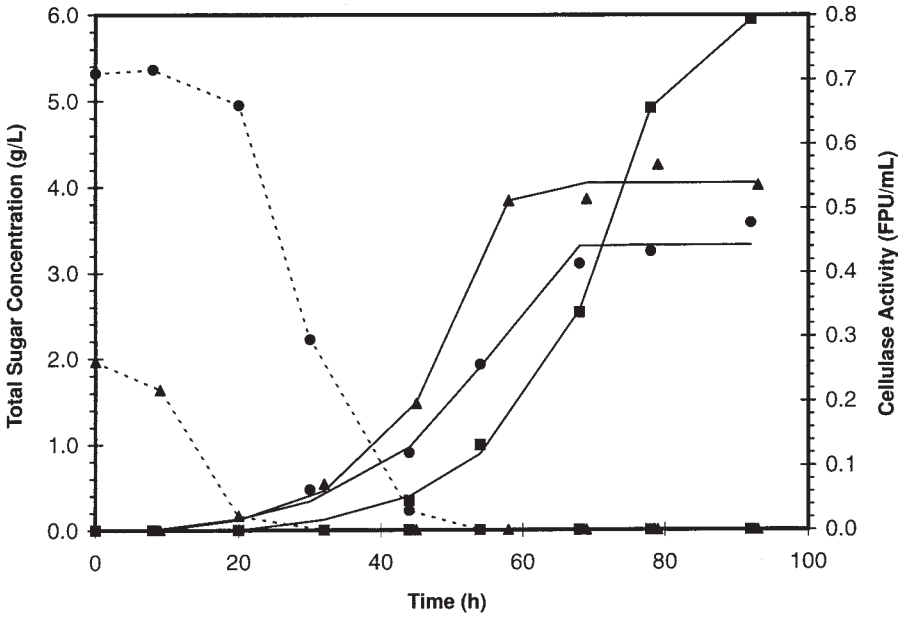

Fig. 2. Cellulase enzyme production of *T. reesei* Rut C 30 on SPS-W (■), SPS-W supplemented with 20% F (▲), and SPS-W supplemented with 50% F (●). (——), enzyme activities; (– – – –), total sugar concentrations.

0.48 FPU/mL, when half of the SPS-W was substituted with F (E3). Using 100% insoluble carbon source, a 32-h lag phase was observed before measurable cellulase activity appeared (Fig. 2). The length of the lag period decreased to 20 h when a part of the fibrous SPS-W was substituted with F, which contained easily utilizable sugars. Although *T. reesei* grew faster on the multiple carbon source, the obtained final cellulase activities were 30–40% lower. The most likely explanation for the lower release of cellu-

lases is inhibition by sugar degradation products such as furfural, HMF, and acetic acid, formed during pretreatment of spruce.

It is known that under anaerobic conditions, *Saccharomyces cerevisiae* is able to reduce furfural and HMF into less-inhibitory compounds *(34)*. It has also been shown that the inhibition of cellulases by different byproducts can be reduced by fermenting the hemicellulose fraction separately and using the fermentation broth as a diluting liquid in the hydrolysis step instead of water (Tengborg, C., personal communication). This detoxification method could be used to avoid the inhibition of *Trichoderma*. From a process point of view, it would also be advantageous, because the sugars, mostly pentoses, left after the yeast fermentation could be utilized as well.

Enzymatic Hydrolysis of Different Substrates

With all five enzyme preparations—Celluclast 2L, Iogen Cellulase, and three enzymes produced by *T. reesei* Rut C 30 (E1, E2, E3)—hydrolysis tests were run using three substrates: SPS, SPS-W, and Solka Floc 200. Table 5 summarizes the conversions of cellulose obtained after 48 h of hydrolysis. The highest conversion of cellulose, 78.4%, was obtained with enzyme produced on SPS-W (E1) using SPS-W as substrate, and the lowest, 55.6%, with enzyme obtained on 50% F containing medium (E3) using Solka Floc 200 as substrate in the hydrolysis experiment.

Celluclast 2L performed almost equally well on all three substrates. An average final cellulose conversion of 69.4% was obtained (*see* Table 5). Lager deviations in final yields were observed using Iogen Cellulase. The lowest conversion (59.4%) was obtained using SPS. By washing off the inhibitory compounds including soluble sugars, the conversion was increased considerably. However, the best result, 72.1%, was obtained for Solka Floc 200 as substrate. All enzymes produced on softwood showed better yields on SPS than the commercial cellulase preparations, with a maximum conversion of 78.4% for E1. However, considerably lower yields were obtained on Solka Floc 200 compared with Celluclast 2L or Iogen Cellulase. Of all three enzymes produced by *T. reesei* Rut C 30, the one obtained on SPS supplemented with 50% F (E3) showed the poorest yields on all three substrates. The effect of removing the water-soluble inhibitors from the SPS yielded different results for the different enzymes. The negative effect of inhibitory compounds on final yield can be seen only in the case of Iogen Cellulase. In the case of the other enzyme preparations, the differences in the final yields obtained using SPS and SPS-W were not significant.

Table 6 summarizes the integrated productivity (r_3, the slope of the line drawn through the data point from the origin) data calculated after 3 h of hydrolysis. Using SPS as a substrate, the highest hydrolysis rate of 0.86 g of glucose/(L·h) was obtained with E1. Enzymes E2 and E3 were much slower at the beginning of the hydrolysis compared with Celluclast 2L and Iogen Cellulase. By removing the water-soluble byproducts and

Table 5
Enzymatic Hydrolysis of SPS, SPS-W,
and Solka Floc 200 Using Different Cellulases

	Cellulose conversion (%)		
Enzyme	SPS	SPS-W	Solka Floc 200
Celluclast 2L	67.5	69.4	69.9
Iogen Cellulase	59.4	69.1	72.1
E1[a]	78.4	73.5	62.0
E2[b]	78.4	76.1	61.6
E3[c]	70.3	66.2	55.6

[a]Enzyme obtained using SPS-W alone.
[b]Enzyme obtained using SPS-W substituted with 20% F.
[c]Enzyme obtained using SPS-W substituted with 50% F.

Table 6
Enzymatic Hydrolysis of SPS, SPS-W,
and Solka Floc 200 Productivity Calculated After 3 h of Hydrolysis

	r_3 (g/[L·h])		
Enzyme	SPS	SPS-W	Solka Floc 200
Celluclast 2L	0.72	0.97	0.91
Iogen Cellulase	0.69	0.79	1.08
E1[a]	0.86	1.13	0.89
E2[b]	0.58	0.63	0.73
E3[c]	0.49	0.59	0.73

[a]Enzyme obtained using SPS-W alone.
[b]Enzyme obtained using SPS-W substituted with 20% F.
[c]Enzyme obtained using SPS-W substituted with 50% F.

sugars, 10–35% higher hydrolysis rates were obtained depending on the enzyme used. An increase of 30–35% in r_3 was observed when Celluclast 2L and E1 were used for hydrolysis. Using Solka Floc 200 as a substrate, the hydrolysis rates were in the range of 0.73–1.08 g of glucose/(L·h). Figure 3 shows the hydrolysis curves obtained with Celluclast, Iogen Cellulase, and E1 using SPS as substrate.

The enzyme produced on softwood, E1, proved to be better compared with the other enzymes regarding both final yield and initial hydrolysis rate.

Conclusion

The amount of β-glucosidase has a considerable effect on the FPU activity of cellulase enzyme solutions. The increase in FPU activity of different cellulases was in the range of 20–50% of original activity. This must be considered when comparing different cellulase preparations on the basis of filter paper activity.

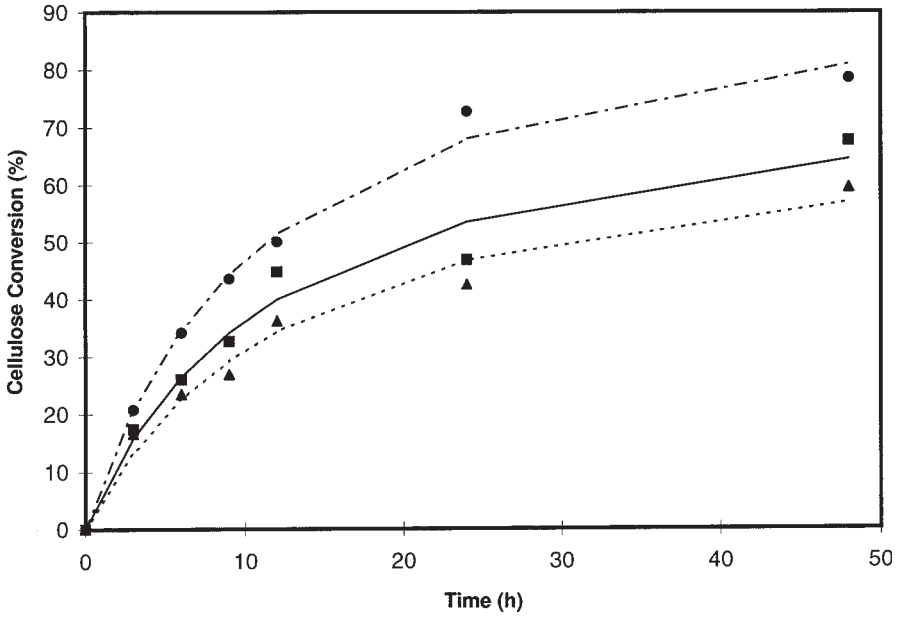

Fig. 3. Hydrolysis curves of SPS using Celluclast 2L (■, solid line), Iogen Cellulase (▲, broken line), and enzyme produced on SPS-W (E1) (●, dotted line).

A filter paper activity of 0.79 FPU/mL was obtained using *T. reesei* Rut C30 on SPS-W. Although the replacement of solid carbon source with water-soluble carbohydrates from hemicellulose hydrolysate (F) resulted in a faster growth rate of *T. reesei*, lower activities, 0.54 and 0.48 FPU/mL, were obtained. When 50% of the SPS-W cellulose was replaced with sugars from F, the growth rate was slightly decreased compared with that observed on SPS-W supplemented with 20% F, but it was still faster than on SPS-W alone. The decrease in FPU activity could be the result of inhibition owing to byproducts formed during the pretreatment, which is clearly reflected in a slower sugar consumption rate at a higher amount of F used (Fig. 2).

In general, the produced cellulases supplemented with a large excess of β-glucosidase, showed better yields on SPS than the commercially available cellulases, Celluclast 2L and Iogen Cellulase. This indicates less sensitivity to inhibition caused by sugars and byproducts. A maximum cellulose conversion of 78.4% was obtained using the enzyme produced on SPS-W (E1), which shows good agreement with the data in the literature *(28,35)*.

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