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Preliminary Results on Optimization of Pilot Scale Pretreatment of Wheat Straw Used in Coproduction of Bioethanol and Electricity

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Abstract

The overall objective in this European Union-project is to develop cost and energy effective production systems for coproduction of bioethanol and electricity based on integrated biomass utilization. A pilot plan reactor for hydrothermal pretreatment (including weak acid hydrolysis, wet oxidation, and steam pretreatment) with a capacity of 100 kg/h was constructed and tested for pretreatment of wheat straw for ethanol production. Highest hemicellulose (C5 sugar) recovery and extraction of hemicellulose sugars was obtained at 190°C whereas highest C6 sugar yield was obtained at 200°C. Lowest toxicity of hydrolysates was observed at 190°C; however, addition of H_2O_2 improved the fermentability and sugar recoveries at the higher temperatures. The estimated total ethanol production was 223 kg/t straw assuming utilisation of both C6 and C5 during fermentation, and 0.5 g ethanol/g sugar.

Index Entries: Lignocellulose; hydrothermal; pretreatment; pilot plant; SSF; bioethanol.

Introduction

The recent years extensive research in bioethanol processes from lignocellulosic materials has brought the process closer to commercialization. A number of pretreatment methods are yet available—dilute acid pretreatment *(1)*, H_2SO_4 or SO_2-catalyzed steam explosion *(2,3)*,

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and wet oxidation *(4)*. Furthermore, the cost of employing enzymatic hydrolysis has during the past 3 yr been significantly reduced owing to the research grants from the US Department of Energy to Novozymes Biotech. and Genencore International. However, much of the research is still performed at laboratory scale and many of the promising results needs to be validated in pilot and commercial scale. Larger scale equipment and equipment for running in continuous mode also need to be tested.

Since the year 2000, it has been mandatory for Danish power plants to utilize a total of 1.2×10^6 t of biomass yearly, mainly from straw, for heat and power production. However, owing to the high content of especially potassium in straw, this type of biomass is not well suited for combustion. In a joint EU-project "coproduction biofuels," the largest Danish power producer Elsam A/S, The energy research centre—Risø National Laboratory, The Royal Veterinary and Agricultural University, and two small enter-prices—Sicco K/S and TMO Biotec (Ltd.), are exploring the possibility from combinations of lignocellulosic and starch/sugar feedstocks of merging the production of ethanol with the production of heat and power at combined heat and power plants. The objective is to develop a system that removes the potassium salts and a substantial part of the carbohydrates (mainly hemicellulose) from the straw, to be used as feedstock for ethanol fermentation with TMO's C5 and C6 fermenting thermophiles under development. The solid fraction is essentially free of potassium and therefore well suited for combustion. This cellulose-rich fraction containing the lignin can be burned for electricity or used for ethanol production. Byproducts from fermentation processes will be concentrated and used for animal feed or fertilizer (Fig. 1).

The bioethanol process will be placed at the power plants, giving advantages such as easy access to cheap supply of electricity and steam from the power plants, and to efficient electricity production from residual cellulose and lignin.

The main body of the project is the construction and testing of a pilot scale two-step pretreatment reactor system with a planned capacity of 1000 kg of biomass/h (Fig. 2). The reactor system is designed to be flexible, facilitating the handling of high concentrations of dry matter (exceeding 50%), large particles (e.g., straw more than 5 cm in length), temperatures up to 230°C and different pretreatment methods, e.g., counter-current extraction, diluted acid pretreatment, steam pretreatment, and wet oxidation. It will thus be possible to verify the many results reported from laboratory scale experiments in pilot scale.

This article describes the concept of the pilot plant and some results from the optimisation trials obtained during 2004 and 2005 with a one-step pretreatment reactor (Fig. 3) that will be integrated into the final two-step pretreatment system. The reactor has been tested with a

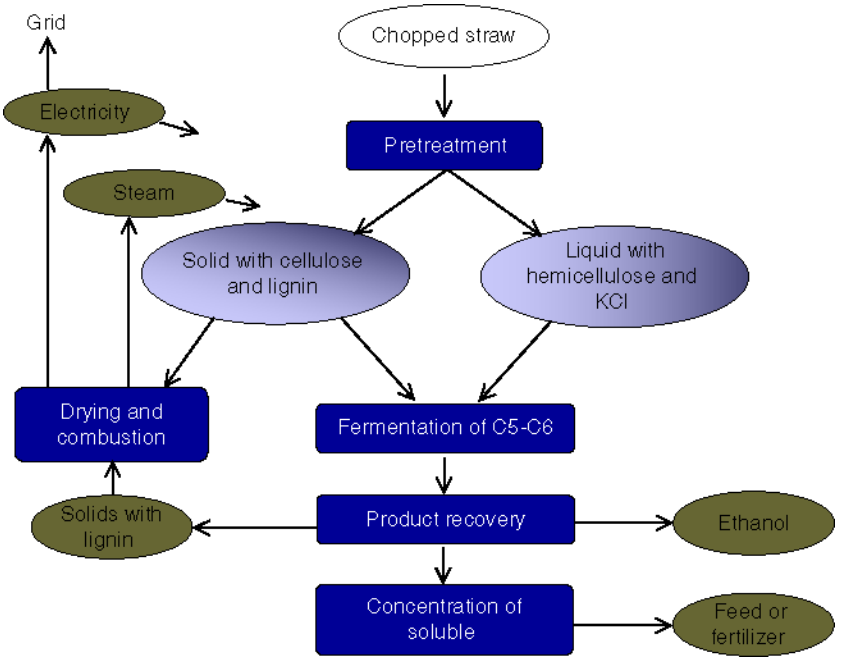


Fig. 1. Flow sheet of the straw-to-ethanol process.

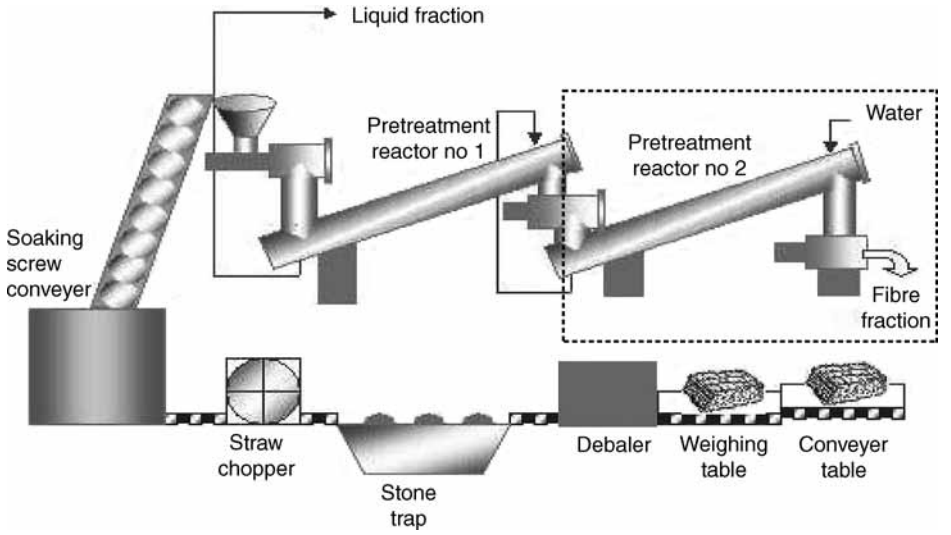
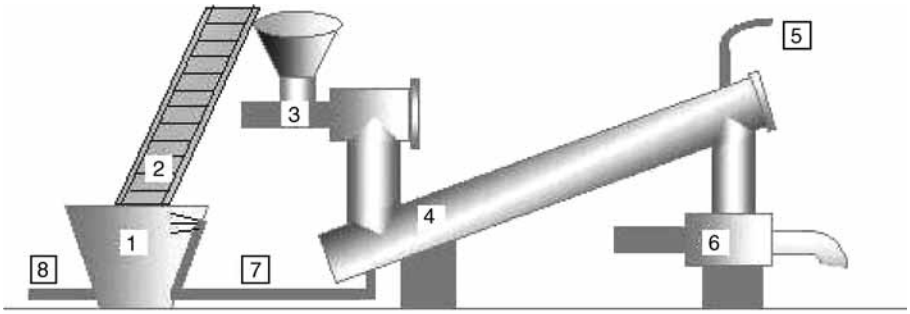


Fig. 2. Flow sheet of the process, as it will appear when the second pretreatment step has been added. The existing plant is a one step pretreatment plant. The liquid fraction from the reactors might either be mixed with the fiber fraction for enzymatic hydrolysis (SHF) or go directly to the fermentation step (SSF).

continuous flow of straw of 50 kg/h (6-min residence time). Several trials were made with varying parameters of water level, chemical addition and flow in the reactor.



1. Intake of biomass and soaking tank with hot water recycled from reactor
2. Dosing belt conveyor with dewatering screen bottom
3. Particle pumb no.1 for introduction of biomass into the reactor
4. Reactor zone with double screw conveyor system
5. Water inlet into reactor
6. Particle pumb no. 2 for the extraction of biomass out of the reactor
7. Recycling system for process water
8. Drain

Fig. 3. Flow sheet of one-step test reactor.

Description of Pilot Plant

The final pilot plant is based on Sicco's proprietary design and will consist of a complete system for pretreating up to 1000 kg/h of straw and testing of this plant started September 2005 (Fig. 2).

A total of 3–4 big bales (approx 500 kg each) can be placed on the up front conveyer table thereby enabling automatic feeding of straw for up to 2 h. The flow of straw to the plant is controlled by the speed of the dosing belt into the de-baler unit. After the de-baler unit the straw goes through a stone trap to remove sand, stones, and metal parts before the chopping into approx 5 cm pieces in a commercial straw chopper. The cut straw goes through a presoaking step in a 10 m long screw conveyer. The temperature in this step can be up to 100°C and residence time can be up to 30 min. The straw is fed into the first reactor by means of a particle pump designed especially for introduction of biomass into the reactor against high pressure and temperature *(5)*. The first reactor will be a screw conveyor system operated under pressure and temperatures up to 200°C. The conveyor moves the straw up through the reactor and water from the reactor 2 is passed counter currently to obtain leaching of the straw. From the first reactor the straw is let into reactor 2 also by the use of a particle pump. Reactor 2 is similar to reactor 1 but is able to operate at higher pressure and temperatures (up to 32 bar and 230°C). Biomass is removed from reactor 2 by another particle pump and can be used for production of boiler fuel or ethanol. The liquid extracted from reactor 1, which contains compounds such as monomeric sugars, salts, hemicellulose, lignin, and degradation products, can be used for bio-ethanol production by enzymatic hydrolysis and subsequent fermentation.

Table 1
Chemical Composition of Untreated Wheat Straw

	Composition straw [g/100 g DM]	
	Straw batch 1	Straw batch 2
Cellulose	30.4	33.9
Xylan	18.4	20.3
Arabinan	2.9	2.7
Total hemicellulose	21.3	23
Klason lignin	19.4	19.1
Ash	5.9	5.3
Residual	23	18.7

Two different batches of straw was used in the experiments.

Materials and Methods

Raw Materials

Wheat straw (*Triticum aestivum* L.) was grown and harvested after a drying period in Denmark during summer 2003. The straw was cut into 1–6 cm pieces on the field by a New Holland FX375 forage harvester and stored in containers at ambient temperature. The dry matter content was 90–92%. Table 1 shows the chemical composition of the untreated wheat straw.

Pretreatment of Wheat Straw

For these preliminary experiments a pretreatment pilot plant reactor with a capacity of 100 kg/h was used (Fig. 3). The straw was fed to a presoaking vessel continuously at a rate of 50 kg straw/h. The temperature in the presoaking vessel was 80–90°C, and the residence time was around 6 min. After presoaking the biomass was transported to the reactor inlet by a dosing belt conveyor. This conveyor would drain much of the free water from the biomass thereby securing a counter current flow of water against the biomass inside the reactor. Water was introduced at the top of the reactor giving a counter current flow. The pretreated biomass was transported out of the reactor by particle pump no. 2 following the same principle as particle pump no. 1. The pretreated biomass was weighed and collected in containers. Samples were collected and kept at –5°C until analysis. Processed water was recycled in the presoaking vessel and let to a drain. Samples of the liquid fraction were collected from the drain in the bottom of the reactor. Liquid samples were kept at –5°C until analysis. In this series of optimization trials on the pilot plant several experiments were run with varying parameters of water flow (0 L/h (steam), 100 L/h, 250 L/h, and 500 L/h), chemical addition (H_2SO_4, Na_2CO_3, NH_3, and H_2O_2 (wet oxidation), and temperature in the reactor (190°C, 195°C, and 200°C).

Analysis Methods

Analysis of Ash and Dry Matter Content

The ash content in the solid fraction was determined by incineration of around 0.5 g of dried sample at 550°C for 3 h. The dry matter content in the liquid extract was determined by drying 5 mL of sample overnight at 105°C. Dry matter content of the raw material and solid fractions was determined by drying and weighing in a mettler Toledo moisture analyzer HR83, Halogen.

Analysis of Carbohydrates in Solid Fractions

The composition of the raw and pretreated straw fibers was measured by strong acid hydrolysis of the carbohydrates. Dried and milled samples (160 mg) were treated with 72% (w/w) H_2SO_4 (1.5 mL) at 30°C for 1 h. The solutions were diluted with 42 mL of water and autoclaved at 121°C for 1 h. The hydrolysates were filtered, and the Klason lignin content was determined as the weight of the filter cake subtracted the ash content. The filtrates (5 mL) were mixed with 0.5 g $Ba(OH)_2{\cdot}8H_2O$ and after 5 min, the samples were centrifuged at approx 3000*g* for 5 min. The supernatant was analysed for sugars on high-performance liquid chromatography (HPLC). The recovery of D-glucose, D-xylose, and L-arabinose was determined by standard addition of sugars to samples before autoclavation. The sugars were determined after separation on an HPLC-system (Shimadzu) with a Rezex ROA column (Phenomenex) at 63°C using 4 m*M* H_2SO_4 as eluent and a flow rate of 0.6 mL/min. Detection was done by a refractive index detector (Shimadzu Corp., Kyoto, Japan). Conversion factors for dehydration on polymerization was 162/180 for glucose and was 132/150 for xylose and arabinose.

Analysis of Carbohydrates in Liquid Fractions

Carbohydrates in the liquid (filtrate) after pretreatment were both polymers and monomers, thus the samples were hydrolyzed using 4% (w/w) H_2SO_4 at 121°C for 10 min to determine the total glucose, xylose, and arabinose concentration. The sulphate anions in 10 mL acidic filtrate were precipitated by 0.5 g $Ba(OH)_2{\cdot}8H_2O$ and the supernatant was diluted 1 : 1 with 4 m*M* H_2SO_4. Glucose, xylose, arabinose, acetic acid, and ethanol were quantified by HPLC as described above.

Enzymatic Hydrolysis of Solid Fraction

The enzymatic conversion of the solid fraction was evaluated at 2% DM in 50 m*M* sodium acetate pH 4.8 using Cellubrix L added to obtain 30 FPU/g DM. The solid fibers were enzymatically hydrolyzed as milled samples. After 24 h at 50°C, the reaction was stopped by centrifugation at approx 3000*g* for 10 min. The concentration of glucose, xylose, and arabinose was measured by HPLC as described earlier.

Simultaneous Saccharification and Fermentation

Prehydrolysis (liquefaction) and, simultaneous saccharification and fermentation (SSF) was performed in 200-mL fermentation flasks. Eight grams

of the dried solid fiber fraction were mixed with 60 mL of a 0.2 *M* acetate buffer (pH 4.8) or 60 mL of the pH adjusted (pH 4.8) filtrate originated from the same pretreatment (13% DM). Prehydrolysis of the WO solids was performed at 50°C for 24 h at an enzyme loading of 10 FPU/g DM filter cake, using Cellubrix L. After liquefaction, the fermentation flasks were supplemented with a second batch of enzymes (Cellubrix L) at an enzyme loading of 10 FPU/g DM, added 0.2 mL of a sterile filtered urea (24%), and inoculated with 0.2 g yeast after having cooled down to room temperature. The flasks were sealed with a yeast lock filled with glycerol and incubated at 32°C for 6–8 d. The cellulose to ethanol conversion was monitored by CO_2 loss, determined by weighing of the flasks at regular intervals. The final ethanol concentration was also determined by HPLC analysis as described above.

Calculations

Recoveries were calculated according to Eq. 1. Yields were calculated as percent of theoretical (in g/g original cellulose or hemicellulose in raw material) (Eqs. 2 and 3). Total yields were calculated as the total yield of hemicellulose/glucose in the liquid fraction and after enzymatic hydrolysis of the solid fraction (Eq. 4). The theoretical ethanol production based on the pretreatment and hydrolysis yields was calculated according to Eq. 5. The ethanol yield in SSF experiments was calculated as percentage of theoretical based on cellulose content of the fiber fraction and glucose in the filtrates (Eq. 6).

$$\text{Recovery} = \frac{\left(\text{sugar in filtrate (g / 100 g)} + \text{sugar in solid (g / 100 g)}\right)}{\left(\text{sugar in raw material (g / 100 g)}\right)} 100\% \quad (1)$$

$$\text{Yield}_{\text{hemicellulose}} = \frac{\left(\text{mass}_{\text{hemicellulose}} \text{ in filtrate}\right)}{\left(\text{mass}_{\text{hemicellulose}} \text{ in raw material}\right)} 100\% \quad (2)$$

$$\text{Hydrolysis yield}_{\text{glucose}} = \frac{\left(\text{mass}_{\text{glucose}} \text{ after enzymatic hydrolysis} \times 0{,}9\right)}{\left(\text{mass}_{\text{cellulose}} \text{ in raw material}\right)} 100\% \quad (3)$$

$$\text{Total yield}_{\text{sugar}} = \frac{\left(\text{mass}_{\text{sugar}} \text{ in filtrate}\right) + \left(\text{mass}_{\text{sugar}} \text{ after enzymatic hydrolysis}\right)}{\left(\text{mass}_{\text{sugar}} \text{ in raw material}\right)} 100\% \quad (4)$$

$$\text{Theoretical ethanol production} = \text{TSC}^* \times 0.51 + \text{TSH}^* \times 0.5 \quad (5)$$

*TSC = Total sugar from cellulose (after pretreatment and enzymatic hydrolysis) (g/100 g raw material)
*TSH = Total sugar from hemicellulose (after pretreatment and enzymatic hydrolysis) (g/100 g raw material)

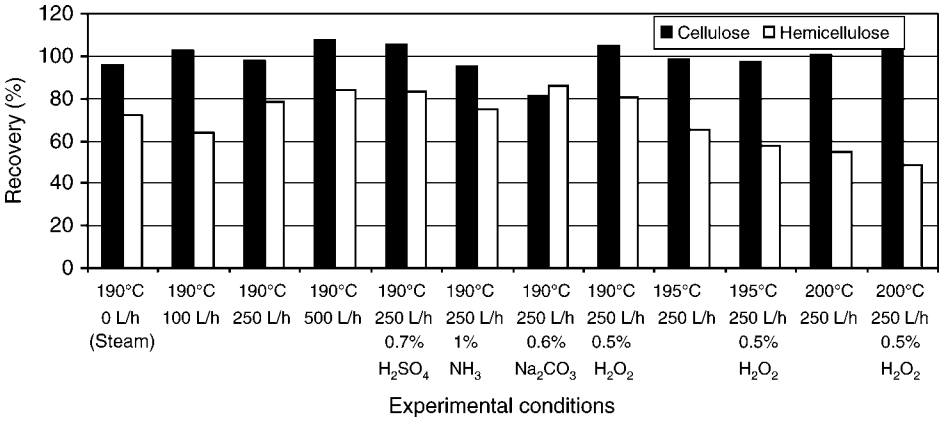


Fig. 4. Recovery of cellulose and hemicellulose in optimization experiments.

$$\text{EtOH yield} = \frac{\text{EtOH}_{\text{Gravimetric/HPLC}}}{\text{glucose in solid} \times 0.51 + \text{glucose in filtrate} \times 0.51} \times 100 \quad (6)$$

Results and Discussion

The different pretreatments were evaluated with respect to recovery of sugars, depolymerization and extraction of hemicellulose (C5) sugars and cellulose convertibility (C6 sugars). The fermentability was evaluated in SSF fermentations with Baker's yeast. SSF of the cellulose-fraction was performed both in a buffer medium and in the hydrolysate to examine the inhibitory effect of the hydrolysates.

Recovery of Sugars

Very good cellulose recoveries (80–100%) were found in all trials (Fig. 4). The highest hemicellulose recovery (86%) was found in the experiment with Na_2CO_3 addition, but also the experiments with H_2SO_4, high flow (500 L/h), and H_2O_2 (wet oxidation) showed hemicellulose recoveries above 80%. In the experiments with low flow a substantial part of the hemicellulose sugars were lost (36%), and increasing the temperature to 195°C and 200°C also caused significant degradation of the hemicellulose sugars (40–50%) (Fig. 4).

Depolymerisation of Hemicellulose and Extraction of Sugars Into the Liquid

Figure 5 shows the yield of hemicellulose and glucose (in the liquid) after the pretreatment process. Especially, the experiment with H_2SO_4-addition stands out regarding extraction of hemicellulose sugars. In this experiment approx 50% of the hemicellulose sugars were extracted into the liquid. In the next best trials with high flow (500 L/h) and H_2O_2 addition around 30% of the hemicellulose sugars were extracted. The lowest

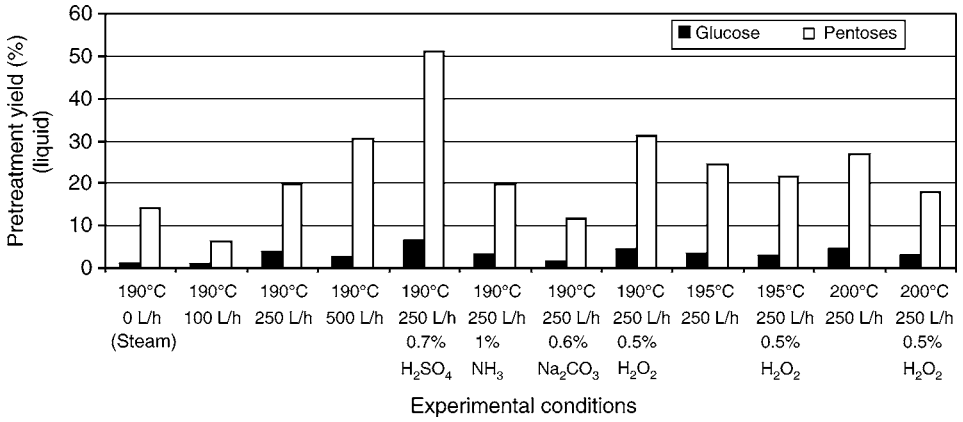


Fig. 5. Yield of glucose and pentoses after pretreatment.

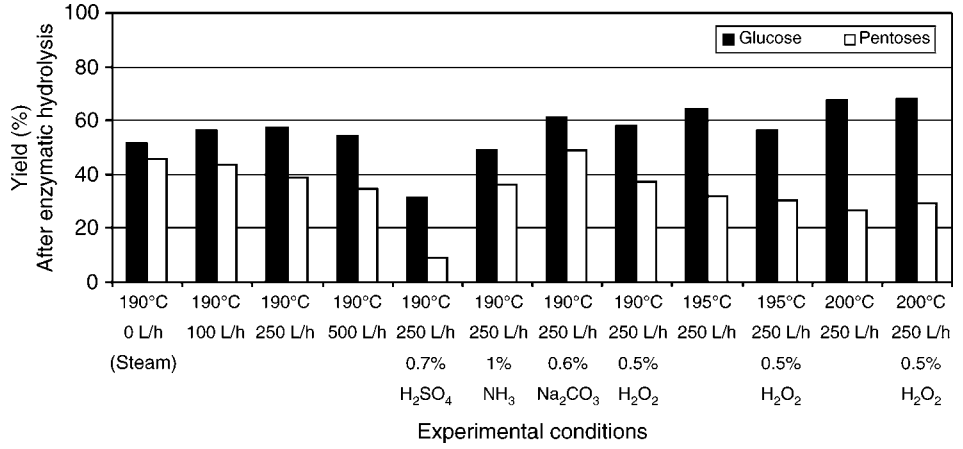


Fig. 6. Yield of glucose and pentoses after enzymatic hydrolysis of the solid fraction.

extraction of hemicellulose sugars was found in the experiment with low flow (100 L/h). By washing the solid (straw) fraction from the experiment with low flow in warm water (80°C), the hemicellulose yield was increased fourfold (data not shown). This showed that washing of the straw in the reactor was insufficient at low water flow and can be improved at higher flow.

Enzymatic Hydrolysis of the Solid (Straw) Fraction

One objective in this process is to use part of/or the entire solid fraction for bioethanol production, so the accessibility of the solid fraction for enzymatic hydrolysis is an important parameter. Figure 6 shows the yields of glucose and hemicellulose sugars after enzymatic hydrolysis of the solid fraction. In the majority of the optimization experiments performed at 190°C the yield of glucose is 50–60%. In the experiment with addition of H_2SO_4 the glucose yield is significantly lower (32%) than in the other experiments, despite the high extraction of hemicellulose sugars found

Table 2
Total Yield of Glucose and Pentoses After Pretreatment and Enzymatic Hydrolysis

Conditions			Yields	
Temperature (°C)	Flow (L/h)	Chemicals (%)	Glucose yield (%)	Pentoses yield (%)
190	0 (steam)	–	52.9	60.5
190	100	–	57.3	49.3
190	250	–	61.4	57.9
190	500	–	56	64.6
190	250	0.7% H_2SO_4	37.8	60
190	250	1% NH_3	52.1	55.5
190	250	0.6% Na_2CO_3	61.8	60.8
190	250	0.5% H_2O_2	62.0	68.3
195	250	–	67.8	56.2
195	250	0.5% H_2O_2	59.2	51.7
200	250	–	71.4	53.9
200	250	0.5% H_2O_2	70.5	47.2

in this experiment. Increasing the reaction temperature improves the convertibility of the cellulose, and the best hydrolysis of the cellulose fraction is found in the experiments performed at 200°C, in which the glucose yield is 67–68% (after 24 h of hydrolysis at 50°C). Also a part of the hemicellulose (left in the straw) was hydrolyzed by the enzymes. This conversion was most significant in the experiment with Na_2CO_3-addition, in which 49% of the hemicellulose was hydrolyzed in the enzymatic process.

Total Sugar Yields

The total yield of glucose was 50–60% in most of the experiments performed at 190°C (Table 2), but was significantly lower in the experiment with H_2SO_4-addition (38%) owing to the poor cellulose convertibility in this experiment. The best glucose yield was obtained in the experiments performed at 200°C. In these experiments the cellulose yield was improved by 10% in comparison with the best result obtained at 190°C.

In the best experiments a total hemicellulose yield of 60–68% was obtained, these are the experiments with H_2O_2, Na_2CO_3, high water flow (500 L/h), and H_2SO_4. It is also in these experiments the best hemicellulose recovery was found (Fig. 4). In the experiment with Na_2CO_3-addition most C5 sugars was obtained after enzymatic hydrolysis of the solid, whereas in the experiment with high water flow almost equal amounts of C5 sugars were obtained by pretreatment (in the liquid) and enzymatic hydrolysis (of the solid). The highest total yield of C5 sugars (68%) was found in the experiment performed at 190°C with H_2O_2 addition (wet oxidation).

Theoretical Ethanol Production

The theoretical ethanol production was calculated based on the total yields of C6 and C5 sugars (Eq. 5, Fig. 7). Because two different batches

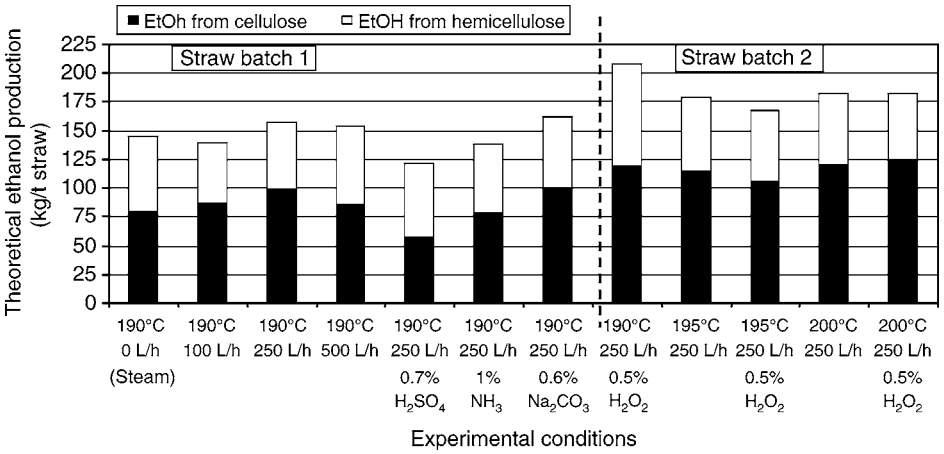


Fig. 7. Theoretical ethanol production based on the yields of C6 and C5 at different pretreatment conditions (Table 2). Two different batches of straw (with different sugar-content) were used in these trials.

of straw (with different sugar contents) were used, the results were not directly comparable. In the best experiment using straw no. 1 (pretreatment at 190°C with Na_2CO_3-addition) the theoretical ethanol production was 162 kg/t straw. Higher theoretical ethanol production was found in the experiments using straw no. 2. owing to the higher sugar content of this batch of straw. The highest theoretical ethanol production of 208 kg/t straw was obtained at 190°C with addition of H_2O_2 (wet-oxidation). For an optimal two-step process as described earlier, using the maximum obtained sugar yields from these trials (68% for hemicellulose and 71% for glucose, Table 2) and straw no. 2, the total theoretical ethanol production has been calculated to 223 kg/t straw.

SSF-Experiments

SSF of the cellulose with Baker's yeast has been performed on material from selected trials to examine the fermentability of the pretreated material (Fig. 8). The SSF were performed both in a buffer medium and in the liquid from the pretreatments (hydrolysates) to test the liquid for possible inhibitors.

In experiments with no addition of H_2O_2 (Fig. 8A), fermentation in the buffer medium takes place after a small lag phase, and very high yields are obtained (close to 100%), showing that under these conditions all of the cellulose can be converted to glucose and fermented to ethanol. Initially, some kind of lag phase is seen, however, 70% the ethanol produced was obtained within the first 50 h, after which the productivity decreased significantly (Fig. 8A). SSF in hydrolysate (instead of buffer medium) showed almost no ethanol production, indicating that the hydrolysates from these experiments contained too many inhibitors for fermentation to take place. A most reasonable explanation is degradation

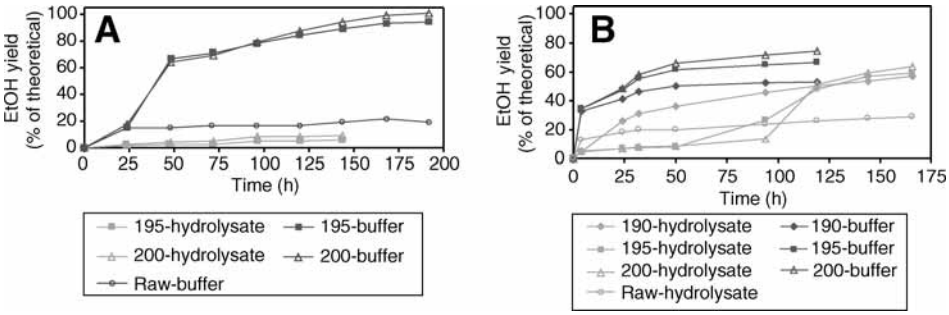


Fig. 8. SSF of solid fraction from pretreatments performed at temperatures ranging from 190–195°C in buffer and hydrolysate media. **(A)** is experiments performed with-out addition of H_2O_2 and **(B)** is experiments performed with addition of H_2O_2 (wet-oxidation).

of C5 sugars to carboxylic acids and furfurals, that are known inhibitors for most microorganisms, but also lignin degradation product have severe inhibition effects *(6)*. Analysis of specific inhibitors was not made in this study.

Using material from experiments with H_2O_2 addition the lag phase in buffer medium was avoided, and the initial productivity of the yeast was faster. Growth starts immediately in hydrolysate treated at 190°C, after approx 50 h in hydrolysate treated at 195°C, and after 94 h in hydrolysate treated at 200°C. The yeast probably uses these long lag phases to detoxify the medium *(6)*, and the more toxic the medium the longer the lag phase. The end yield is highest in fermentation of material pretreated at 200°C, which is owing to the better cellulose convertibility of these materials.

Conclusion and Future Experiments

Optimal depolymerisation and extraction of hemicellulose sugars was obtained at 190°C but improved cellulose conversion of the solid fraction was achieved at 200°C. A water flow of minimum 250 L/h through the reactor was of great importance to obtain optimal extraction of hemicellulose sugars. Addition of Na_2CO_3 and H_2O_2 both improved recoveries of sugars, giving a theoretical ethanol production of 162 kg/t straw with Na_2CO_3 and 208 kg/t straw with H_2O_2, when performed at 190°C. It was demonstrated that H_2O_2 improved the fermentability of the pretreated material and for further experiments a combination of Na_2CO_3 and H_2O_2 should be tested. For an optimal two-step process as suggested earlier, based on the maximum obtained sugar yields from these trials (68% for hemicellulose and 71% for glucose), the expected total ethanol production (theoretical) is 223 kg/t straw.

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