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Dilute-acid hydrolysis for fermentation of the Bolivian straw material Paja Brava

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Abstract

Hydrolysis of the straw material Paja Brava, a sturdy grass characteristic for the high plains of Bolivia, was studied in order to find suitable conditions for hydrolysis of the hemicellulose and cellulose parts. Dried Paja Brava material was pre-steamed, impregnated with dilute sulfuric acid (0.5% or 1.0% by wt), and subsequently hydrolyzed in a reactor at temperatures between 170 and 230 °C for a reaction time between 3 and 10 min. The highest yield of xylose (indicating efficient hydrolysis of hemicellulose) were found at a temperature of 190 °C, and a reaction time of 5–10 min, whereas considerably higher temperatures (230 °C) were needed for hydrolysis of cellulose. Fermentability of hemicellulose hydrolyzates was tested using the xylose-fermenting yeast species *Pichia stipitis*, *Candida shehatae* and *Pachysolen tannophilus*. The fermentability of hydrolyzates decreased strongly for hydrolyzates produced at temperatures higher than 200 °C.

Keywords: Two-stage hydrolysis; Dilute acid; Fermentation; Yeasts; Inhibition

1. Introduction

Ethanol, produced from biomass, is one attractive alternative for partial replacement of fossil fuels, in particular for transportation purposes. Ethanol can be produced from biomass with a very low net CO_2 formation, thereby decreasing the emission of greenhouse gases as called for in the Kyoto protocol in 1997 (Oberthur and Ott, 1999). Ethanol has desirable properties as a fuel, both with respect to its combustion, and the possibility to use it in low-blend mixtures together with gasoline (Galbe and Zacchi, 2002). An addition of up to 10% of ethanol to gasoline can be used directly without modifications of car engines. In most countries there is thus already an existing infrastructure enabling a fast market penetration of low-blend ethanol fuels.

Large-scale production of fuel ethanol today is based on sucrose from sugar cane in Brazil, or starch—mainly from corn—in the US (Wheals et al., 1999). These processes for ethanol production can be said to be well established. However, to increase the ethanol production potential, also other sources of biomass are needed, and/or a larger fraction of the already established sources of biomass should be utilized. In both cases, this will involve utilizing the cellulosic and hemicellulosic part of the biomass. There exists a great variety of lignocellulosic feedstocks, which potentially can be used for ethanol production. The potential feedstocks include biomass resources, which are already in industrial or agricultural use. Examples are forest residues or agricultural residues, such as corn stover, wheat straw (Nigam, 2001), sunflower stalks (Sharma et al., 2002) or sugarcane bagasse (Roberto et al., 1991; Cuzens and Miller, 1997). Alternatively, dedicated energy crops, such as fast growing poplar wood, can be used as feedstocks. As a third option, straw materials can be a potential substrate for ethanol production in some areas. In the current study, the possibility of using a particular straw material, the Paja Brava, as a feedstock for ethanol production was investigated.

The Paja Brava is a straw, which is rather typical for the high plains of Bolivia. There is abundant vegetation of Paja Brava (brave straw) in the surroundings of the Poopó lake and Desaguadero river in the Bolivian

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highland. The Paja Brava is a sturdy grass, capable of growing up to more than 1 m height, also at the semi-desert conditions in the high plateau of the Andes. The straw is traditionally used mainly in the construction of the rustic housings of the region, being used both in the construction of walls and roofs. In terms of chemical composition, the Paja Brava predominantly contains the monosaccharides glucose (53 wt% of the sugars) and xylose (38 wt% of the sugars). Minor amounts of arabinose (6 wt%) and galactose (2 wt%) are also present, but very little mannose (<1%). Since, as in other straw materials, the amount of xylose is high, xylose fermentation is important to consider when using this feedstock for ethanol production.

To render the monosaccharides accessible for fermentation, depolymerization of the material is necessary. This can be obtained by either purely chemical hydrolysis (Lee et al., 1999) or by enzymatic hydrolysis (Sun and Cheng, 2002) In the present study conditions for obtaining a high sugar yield using dilute-acid hydrolysis of Paja Brava were examined. In dilute-acid hydrolysis the hemicellulose fraction is depolymerized at lower temperatures than the cellulose fraction. If higher temperatures (or longer residence times) are applied, the formed monosaccharides from the hemicellulose will degrade (Saeman, 1945), which gives rise to furan compounds and carboxylic acids (Taherzadeh et al., 1997; Larsson et al., 1999). For this reason, it is normally suggested that the hydrolysis process be carried out in at least two stages; the first stage at relatively mild conditions during which the hemicellulose fraction is hydrolyzed and a second stage at higher temperatures, during which the cellulose is hydrolyzed. The liquid phase, containing the monosaccharides, is removed between the treatments, thereby avoiding degradation of the monosaccharides formed. Avoiding degradation of monosaccharides is important not only to improve the yield, but also to avoid inhibition problems, since the degradation products are toxic to the fermenting microorganisms (Taherzadeh et al., 1997, 1999, 2000a,b; Larsson et al., 1999). These by-products may be present in low amounts, but still cause substantial inhibition. The fermentability of hydrolyzates therefore needs to be assessed.

In the present study, a bench-scale hydrolysis reactor was used for hydrolyzing Paja Brava. The hydrolysis temperature was varied in the range of 170–230 °C, and the residence time in the range of 3–10 min. Obtained yields of sugar and furan compounds were determined and the fermentability of the hydrolyzates obtained were evaluated using the common Baker's yeast—*Saccharomyces cerevisiae*—as well as the xylose fermenting yeasts *Pichia stipitis*, *Candida shehatae* and *Pachysolen tannophilus*. From analysis of the sugars formed, it was possible to determine conditions at which a maximum yield of sugars from the hemicellulose was obtained and also conditions at which the cellulose fraction was hydrolyzed. Conditions for a two-stage hydrolysis process could thus be found, and a two-stage hydrolysis experiment was performed at those conditions.

2. Methods

2.1. Analysis of Paja Brava composition

Paja Brava was collected from a region close to Lake Poopo on the Bolivian Altiplano. The composition of the material was analyzed for monosaccharides, Klason lignin and ash. The analyzes were performed at the Swedish Pulp and Paper Research Institute, Stockholm, Sweden. Samples of 2 cm length were taken starting 3.5 cm from the beginning of the stem (root excluded). The samples were washed in water before analysis. Monosaccharides were analyzed after acid hydrolysis by GC using a method described by Theander and Westerlund (1986). A BP 225 column (12 m length, 0.32 mm id, and 0.25 μm layer) was used with He as carrier gas. The initial oven temperature of 150 °C was maintained for 1 min, and was thereafter increased at a rate of 14 °C/min up to the final temperature of 220 °C. Eluted compounds were detected by an FID. The Paja Brava was found to have the composition given in Table 1. The ash content was determined to be 7.6% of the dry weight, and the extractives to be 2% of the dry weight.

2.2. Hydrolysis

2.2.1. Pretreatment of straw material

Dry Paja Brava straw having a length of 0.3 m was cut into 5 cm pieces. The material was further processed in a hammer mill, which, however did not affect the particle size noticeably, due to the recalcitrance of the straw material. The dry Paja Brava pieces were presteamed with low-pressure steam of 10^5 Pa for 20 min in a vessel to make sure that the pores of the straw were free from air, which may have an adverse effect on the acid-impregnation step. The pretreated material was stored in a cool-room overnight.

Table 1
Composition of Paja Brava

Compound	Content (wt%)[a]
Glucose	32.2
Xylose	22.7
Arabinose	3.7
Galactose	1.4
Mannose	0.3
Klason lignin	23.1
Acid soluble lignin	0.9

[a] Based on dry extracted sample. Sugars are reported as anhydro sugars.

2.2.2. First stage hydrolysis

The pre-steamed Paja Brava straw was impregnated with 0.5–2% (w/w, based on the water content) H_2SO_4 in plastic buckets and allowed to stand overnight and then steam treated at 170, 180, 190, 200, 210, 215 or 220 °C for 3, 5, 7 or 10 min. Steam treatment was performed in a 2 l reactor with a diameter of 0.1 m. Saturated steam at appropriate temperature was introduced to the reactor from a boiler. After a preset treatment time the material was discharged into a flash vessel, equipped with a cooling jacket, and collected for further processing.

2.2.3. Second stage hydrolysis

In the two-stage experiments, the water content of the filter cake from the first-stage hydrolysis was determined by drying for 2 h at 120 °C. Subsequently, sulfuric acid was added to obtain a sulfuric acid content of 0.5% (w/w, based on the water content). Due to the water content in the filter cake it was not possible to use a solid content higher than 12%. The impregnated filter cake was stored in a cool room overnight before hydrolysis. The second stage hydrolysis was conducted at 230 °C with a reaction time of 10 min in the same way as described above. The hydrolyzate was collected for further analyses.

2.2.4. Calculation of yields

For calculation of total sugar yields, the liquid phase was first separated from the solid phase by filtration using a Buchner funnel, and its volume was determined. The solid fraction was then washed with pure water and the washing water was collected for further analysis. Typically the amount of water used for washing was 5–10 times larger than the amount of liquid separated after hydrolysis. Sugar concentration in both the primary liquid collected and the washing water was determined by HPLC. The amount of sugars initially retained in the unwashed wet filter cake typically accounted for as much as 30–50% of the sugar yield. The weight of the washed filter cake was determined after drying at 120 °C overnight. All yields were normalized with respect to the initial dry biomass.

2.3. Fermentation

2.3.1. Yeast strains

Commercial Baker's yeast *S. cerevisiae* obtained from Jästbolaget AB, Stockholm, Sweden, *Pichia stipitis* CBS 6054, *C. shehatae* CBS 4410, and *Pachysolen tannophilus* CBS 4044 obtained from Centraalbureau voor Schimmelcultures, Delft, The Netherlands were used in the fermentation experiments. The cells were maintained on agar plates made from yeast extract 10 g/l, peptone from soy 20 g/l, and agar 20 g/l with glucose 20 g/l as an additional carbon source. Inocula were prepared aerobically in 1000 ml baffled conical flasks in a rotary shaker at 120 rpm at 30 °C for 24–36 h. The growth medium was a defined medium according to Taherzadeh et al. (1997) with the exception of an addition of xylose 1 g/l. Cells were harvested by centrifugation and washed with 0.9% (w/v) NaCl. Cell dry weight was determined from duplicate 10 ml samples, which were centrifuged, washed with distilled water and dried for 24 h at 105 °C.

2.3.2. Fermentation of hydrolyzates

Semi-aerobic fermentations were carried out in 40 ml flasks (vials) with a total medium volume of 34 ml placed in a shaker bath at 120 rpm at 30 °C. The fermentation medium contained per liter: $(NH_4)_2SO_4$ 0.47 g, KH_2PO_4 12.8 g, Na_2HPO_4 0.51 g, $MgSO_4 \cdot 7H_20$ 0.47 g, yeast extract 0.94 g and hydrolysate 874 ml. The hydrolysates were pH adjusted to 5.5 with 2 M NaOH. Each flask was equipped with a cotton stopper through which a syringe for sample withdrawal was inserted. Fermentations were started by adding inoculum to an initial cell concentration of 2–5 g/l. Sampling was conducted until 140 h, and metabolite samples were immediately centrifuged and stored at −18 °C for later analysis.

2.4. Analyses

Samples for HPLC-analysis were centrifuged and filtered through 0.2 μm filters. The concentrations of glucose, xylose, galactose, mannose and arabinose were determined using a polymer column (Aminex HPX-87P, Bio-Rad, USA) at 85 °C. The concentrations of ethanol, glycerol, furfural and 5-hydroxymethyl furfural (HMF) were determined on an Aminex HPX-87H column (Bio-Rad, USA) at 65 °C. All the compounds were detected with a refractive index (RI) detector. The content of oligosaccharides was determined on an Aminex HPX-42A column (Bio-Rad, USA) at 85 °C.

3. Results

3.1. One-stage hydrolysis

3.1.1. Sugar yields

The conditions for the first stage hydrolysis were chosen so that the temperature was varied at four levels (170, 180, 190, 200 °C) and the residence time at three different levels (3, 5, 10 min). (The shortest residence time was not tested at 170 °C.) Optimal conditions for hydrolysis of the hemicellulose part, which for the present substrate can be said to be equivalent to a maximum yield of xylose, was expected to be within this selected range. The obtained sugar yields are shown in Table 2. The yield of xylose passed through a maximum at about 190 °C for a residence time of 5 and 10 min, whereas the optimum temperature was above 200 °C for a residence time of 3 min.

Table 2
Sugar yields obtained from dilute acid hydrolysis of Paja Brava

Temperature (°C)	Residence time (min)	Yield of xylose (g/g)[a]	Yield of arabinose (g/g)	Yield of glucose (g/g)
170	10	0.130	0.006	0.018
170	5	0.069	0.006	0.012
170	3	0.061	0.006	0.009
180	10	0.142	0.006	0.018
180	5	0.155	0.009	0.028
180	3	0.083	0.006	0.014
190	10	0.209	0.007	0.025
190	5	0.191	0.007	0.023
190	3	0.142	0.005	0.018
200	10	0.175	0.012	0.028
200	5	0.158	0.008	0.023
200	3	0.191	0.009	0.032

The concentration of H_2SO_4 used was 0.5% w/w of water. The initial dry weight content was 25%.
[a] Based on wood dry weight.

The degradation of hemicellulose is a gradual process during which long polymers are gradually degraded to oligosaccharides and finally monosaccharides. The oligosaccharides are rather short-lived (Lee et al., 1999), and very often they are not analyzed. In the present study, the presence of oligosaccharides was analyzed by HPLC. Oligosaccharides were indeed present at the lowest hydrolysis temperatures. However, for a temperature of 200 °C, the concentrations of oligosaccharides were almost non-detectable (Fig. 1). No distinction could be made between oligosaccharides consisting of xylose and those consisting of glucose units. However, given the fact that very little glucose was found, it appeared likely that the oligosaccharides detected consisted of xylose units.

Very little hydrolysis of cellulose was found for the conditions used (cf. Table 2). In order to find suitable conditions also for a second hydrolysis stage, additional experiments were therefore conducted in which the severity conditions were increased by the use of a higher temperature, longer residence time and higher acid concentrations. Surprisingly severe conditions (230 °C) were needed in order to obtain hydrolysis of cellulose (Table 3). The highest glucose yields obtained, 0.118 g/g corresponded to slightly more than 30% of the theoretically maximal glucose yield. The remaining solids yield at that temperature was found to be 0.46 g/g (Fig. 2). For the conditions in the study, the yield of furfural increased with the increasing temperature (cf. Fig. 2). This showed that the increased degradation of xylose more than compensated for the increased degradation of furfural at the studied conditions.

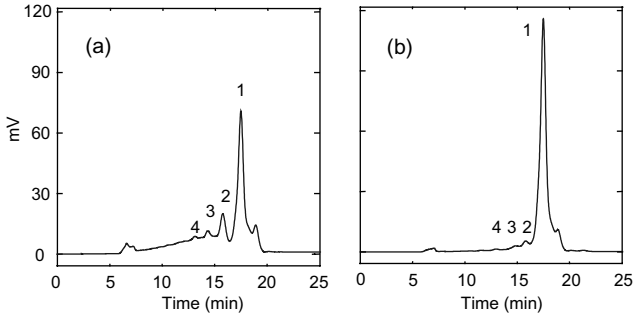

Fig. 1. HPLC chromatograms showing mono- and oligomers in hydrolysates treated at: (a) 170 °C and (b) 200 °C. (1) Monomers; (2) dimers; (3) trimers and (4) tetramers.

3.1.2. Fermentabilities

A representative set of hydrolyzates from the one-stage hydrolysis experiments was tested in fermentation experiments to assess their toxicity. Four different yeast species, three of which were natural xylose fermenting yeasts (*Pichia stipitis*, *C. shehatae* and *Pachysolen tannophilus*), were used. The compositions of the hydrolyzates are given in Table 4. Since xylose fermentation by xylose fermenting yeasts is known to require small amounts of oxygen (Skoog and Hahn-Hägerdal, 1990) the fermentations were conducted in cotton-plugged flasks, allowing some oxygen transfer. Obviously, the maximum attainable ethanol concentration for *S. cerevisiae* was low compared to that of the xylose fermenting yeasts.

The highest ethanol concentrations for all hydrolyzates were obtained with the yeast *Pichia stipitis*, with *C. shehatae* as the second best strain (Fig. 3). *Pachysolen tannophilus* did not perform well under the conditions examined. The highest ethanol concentration obtained was found for the hydrolyzate produced at 180 °C. The time profile of these fermentations for *S. cerevisiae* and *Pichia stipitis* are shown in Fig. 4. The maximum attainable ethanol concentration, $c_{EtOH,max}$, can be calculated as 0.51 times the sum of glucose and xylose concentrations (in units of g/l). Using these values it can be calculated that more than 90% of $c_{EtOH,max}$ was found

Table 3
Sugar yields obtained from dilute acid hydrolysis of Paja Brava

Temperature (°C)	Residence time (min)	Acid concentration (% w/w)[a]	Yield of xylose (g/g)[b]	Yield of arabinose (g/g)[b]	Yield of glucose (g/g)[b]
210	5	0.5	0.130	0.018	0.022
215	5	0.5	0.124	0.019	0.025
220	5	0.5	0.100	0.018	0.027
220	7	0.5	0.101	0.020	0.028
220	10	0.5	0.080	0.015	0.076
220	10	2	0.045	0.008	0.051
230	5	0.5	0.10	0.020	0.033
230	10	0.5	0.094	0.019	0.118
230	10	2	0.029	0.006	0.047

The initial dry weight content was 25%.
[a] Based on total water content.
[b] Based on wood dry weight.

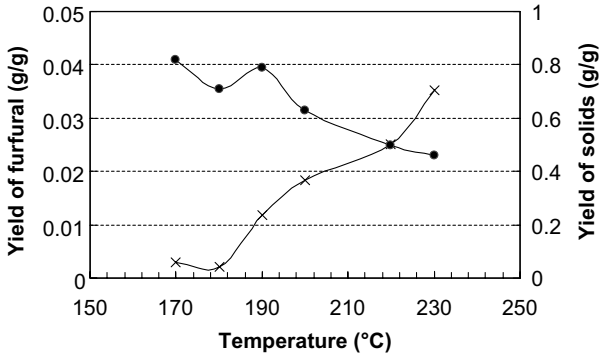

Fig. 2. Yield of furfural (×) and solids (●) obtained for hydrolysis of Paja Brava at different temperatures. 0.5% w/w of H_2SO_4 was used and the residence time was 10 min.

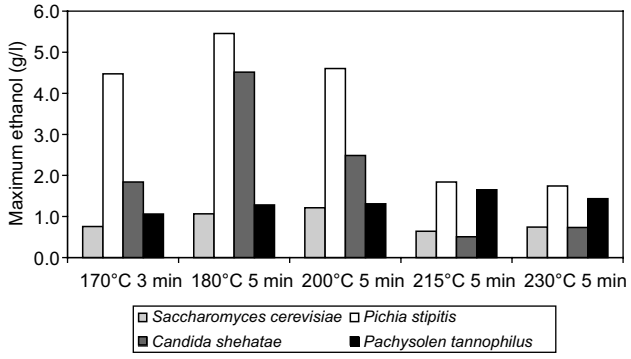

Fig. 3. Maximum ethanol concentration obtained in fermentation of hydrolyzates from Paja Brava. Four different yeast strains were used and five different hydrolyzates obtained from one-stage hydrolysis were tested.

Table 4
Composition of hydrolyzates used in the fermentability test[a]

Conditions of hydrolyzate preparation	Glucose (g/l)	Xylose (g/l)	Arabinose (g/l)	Furfural (g/l)	HMF (g/l)
170 °C, 3 min	1.7	9.3	2.0	<0.1	N.D.
180 °C, 5 min	2.5	19.8	2.4	0.3	<0.1
200 °C, 5 min	2.9	19.8	2.0	1.1	0.25
215 °C, 5 min	3.5	17.8	2.8	2.5	0.42
230 °C, 5 min	3.7	11.4	2.1	2.6	0.52

All hydrolyzates were produced using 0.5% w/w H_2SO_4.
[a] In the fermentation experiments, the hydrolyzates were diluted somewhat due to addition of other medium components. The concentrations in the E-flasks were 87% of the values above.

for the hydrolyzate produced at 170 °C, whereas about 55% of $c_{EtOH,max}$ was found for that produced at 180 °C. The hydrolyzates produced at temperatures 215 and 230 °C were apparently more severely inhibitory to the yeasts, giving measured ethanol concentrations less than 25% of $c_{EtOH,max}$.

3.2. Two-stage hydrolysis

One important objective of the screening was to find suitable conditions for a two-step hydrolysis, in which the hemicellulose was principally hydrolyzed in the first step and the cellulose part in the second step. From the one-stage hydrolysis experiments, conditions for the two-stage hydrolysis process were thus chosen. The temperature chosen for the first stage was 190 °C with a residence time of 5 min, whereas for the second stage, the temperature chosen was 230 °C with a residence time of 10 min. The results of the two stage hydrolysis are shown in Table 5. The xylose yield in the first stage was lower than expected, even when combining the yield in the two stages. The glucose yield calculated from the solids content in the second stage (0.092 g/g) agreed well with the one-stage experiments. However, the yield based on initial solids from the first stage (0.061 g/g) was lower than expected (cf. Table 3). The total glucose yield obtained was less than 25% of the theoretical.

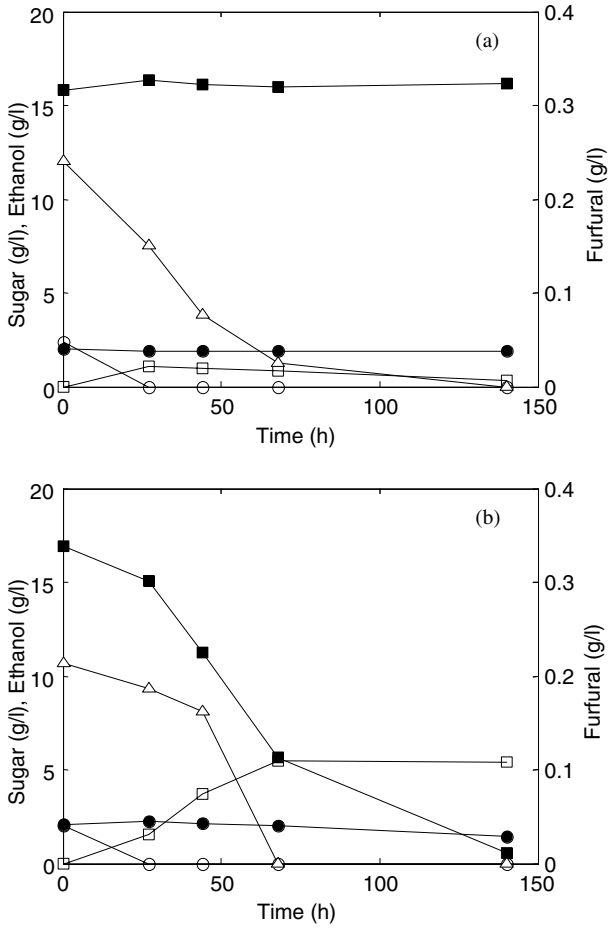

Fig. 4. Fermentation of Paja Brava hydrolyzate produced at 180 °C and 5 min hydrolysis time: (a) *Saccharomyces cerevisiae*; (b) *Pichia stipitis*. (●) arabinose; (○) glucose; (■) xylose and (□) ethanol.

Table 5
Yields obtained in a two-stage hydrolysis[a]

Temperature (°C)	Xylose (g/g)	Arabinose (g/g)	Glucose (g/g)	Furfural (g/g)	HMF (g/g)
First stage: 190°C, 5 min residence time	0.113	0.013	0.011	0.02	N.D.
Second stage: 230 °C, 10 min residence time	0.022	0.004	0.061	0.07	0.007

[a] Yields are calculated based on initial dry weight before the first stage.

4. Discussion

4.1. Hydrolysis

The hydrolysis of Paja Brava, like hydrolysis of other lignocellulosic materials, can be divided into two parts: hemicellulose hydrolysis and cellulose hydrolysis. For an H_2SO_4 concentration of 0.5%, the optimum temperature for hemicellulose hydrolysis was found to be about 190 °C for a residence time of 5 min in the present study. The yield of recovered xylose at this condition was close to 80% of the theoretical value. Bagasse is a straw material, which like Paja Brava has a relatively high xylan content. Lavarack et al. (2002) reported a total pentosan content of 0.268 g/g in bagasse, with a ratio of arabinose to xylose of 0.11. The maximum xylose and arabinose yields from bagasse was thus 0.274 and 0.03 g/g, respectively. In an extensive study concerning dilute acid hydrolysis of the hemicellulose, Lavarack et al. (2002) reported a maximum xylose yield of about 80%, which is very similar to the present study. Previous reports of yields of recovered sugars from hemicellulose hydrolysis of birch and spruce hemicellulose (Taherzadeh et al., 1997) also closely agree with those found in the present study. Furthermore, the optimal conditions found for dilute acid hemicellulose hydrolysis of birch and spruce were comparable to those found in the present study.

In comparison to the hydrolysis of hemicellulose, hydrolysis of cellulose requires rather severe conditions. Only marginal degradation of cellulose in Paja Brava was found below a temperature of 220 °C. The glucose liberated at lower temperatures (<0.03 g/g) most likely originated from hemicellulose, possibly in the form of glucuronic acids attached to the xylan backbone, as has been reported for bagasse (Lavarack et al., 2002). A temperature as high as 230 °C and a residence time of 10 min, was required in the present work to reach a glucose yield of about 30% of the theoretical. These conditions are somewhat more severe than found necessary to obtain hydrolysis of cellulose in e.g. spruce and birch (Taherzadeh et al., 1997). However, the obtained yield of glucose from cellulose are similar.

The relatively low yield of glucose from cellulose in batch-wise performed dilute acid hydrolysis is in fact one of the prime motivations for efforts to conduct the second stage hydrolysis enzymatically. The reason for the lower sugar yields from cellulose than from hemicellulose can be understood from the simple kinetic model proposed already by Saeman (1945). The hydrolysis reactions can be regarded as a series of consecutive reactions according to

$$\text{(Hemi)cellulose} \xrightarrow{k_1} \text{monosaccharides} \xrightarrow{k_2} \text{furans} \xrightarrow{k_3} \text{break-down products}$$

This scheme is obviously a simplification, and a number of modifications of the scheme have been suggested. The polymer may for example be divided into an easily degradable part and a part which is more difficult to degrade, but the essential reactions are captured in the simplified scheme above. As discussed by Lee et al. (1999), the maximum yield possible in a batch reactor (with a certain solids to liquid ratio) will be decided by

the ratio of the rate constants, which in turn will depend on temperature and acid concentration. For cellulose, the activation energy of the first reaction is higher than that of the second reaction, and as high a temperature as possible is therefore desirable to maximize the glucose yield. For practically obtainable temperatures, the ratio between the first and second rate constant for cellulose hydrolysis is in the order of 1, whereas for hemicellulose the value is probably one order of magnitude higher (Lee et al., 1999). Lavarack et al. (2002) report a higher activation energy for the breakdown of xylose than that of hemicellulose degradation, which indicates that a lower temperature (and thus longer hydrolysis times) may be advantageous for optimizing xylose yields. However, experimentally determined maximum yields of xylose reported appear rather similar over a substantial temperature range, indicating that the activation energies probably do not differ much.

The obtained yields from the hemicellulose in the current study, are close to the limits suggested by the kinetic analysis of Lavarack et al. (2002). However, for cellulose, a kinetic analysis would indicate that glucose yields of about 0.5 g/g for temperatures around 230 °C (Lee et al., 1999) should be reachable. This is clearly above the yield obtained in the present work. Most probably, the acid hydrolysis of the cellulose in Paja Brava could therefore be improved. In comparison to most wood materials, Paja Brava contains a relatively high amount of ash, 7%. This is in agreement with what was found in wheat straw (Nigam, 2001). The minerals in the material may work as buffers, thereby decreasing the active acid concentration (Kim and Lee, 1987). Similar problems were in fact seen in hydrolysis of bark-containing willow by Taherzadeh et al. (1997).

4.2. Fermentation

Xylose is the completely dominating monosaccharide in hemicellulose hydrolyzates from Paja Brava, accounting for more than 80% of the monosaccharides (Table 4). A xylose fermenting microorganism is therefore needed for efficient utilization of the sugars, and in the current study the xylose fermenting yeasts *Pichia stipitis*, *C. shehatae* and *Pachysolen tannophilus* were used. The highest ethanol concentrations were found for *Pichia stipitis* (CBS 6054). This yeast has previously been used in fermentation of several dilute acid hydrolyzates of different origin (cf. Table 6), and is most likely the best suited natural xylose fermenting yeast species (Gong et al., 1999).

A critical issue in the conversion of dilute acid hydrolyzates has been the ability to withstand inhibitors (Olsson and Hahn-Hägerdal, 1993), and most often a detoxification step is needed to improve fermentability. However, the introduction of a detoxification step increases the process complexity and may give precipitation problems. Furthermore, the most common method used, i.e. overliming, may result in a certain loss of

Table 6
Fermentation studies of dilute acid hydrolyzates using *Pichia stipitis*

Material	Hydrolysis conditions	Composition of hydrolyzate used	Strain and technical ethanol yield (g ethanol/g sugar in medium)	Reference
Sugarcane bagasse	35 mM H_2SO_4; temperature: 190 °C; residence time: 5 min	Total reducing sugars 140 g/l, furfural 0.2 g/l, HAc 6 g/l	*Pichia stipitis* (CBS 5773) medium detoxified by activated charcoal $Y_{E/S} = 0.35$ g/g	Roberto et al. (1991)
Eucalyptus wood hemicellulose	0.5% w/w H_2SO_4; temperature: 120 °C; residence time: 3 h	Xylose 30 g/l; glucose 1.5 g/l arabinose 2.8 g/l; galactose 3.7 g/l; mannose 1.0 g/l; furfural not detected; HAc 10 g/l[a]	*Pichia stipitis* (Y-7124) $Y_{E/S} = 0.35$ g/g	Ferrari et al. (1992)
Corn cobs	0.83% w/w H_2SO_4; temperature: 160 °C; residence time: 10 min	Xylose 32.6 g/l; glucose 6.2 g/l arabinose 4.8 g/l HAc 3.2 g/l furfural not reported	*Pichia stipitis* (CBS 5773) $Y_{E/S} = 0.18$ g/g without detoxification, $Y_{E/S} = 0.33$ g/g after detoxification; *Pichia stipitis* (CBS 6054) $Y_{E/S} = 0.03$ g/g and 0.31 g/g, respectively	Hahn-Hägerdal et al. (1994)
Wheat straw	1.85% w/vol H_2SO_4; temperature 90 °C; residence time: 18 h	Xylose 45 g/l; glucose 6.4 g/l arabinose 9.0 g/l; furfural 0.27 g/l HAc 6.9 g/l[a]	*Pichia stipitis* (NRRL Y-7124) $Y_{E/S} = 0.03$ g/g, $Y_{E/S} = 0.12$ g/g (with adapted strain), detoxified medium $Y_{E/S} = 0.21$ g/g, $Y_{E/S} = 0.32$ g/g (with adapted strain)	Nigam (2001)
Water hyacinth	1% vol/vol H_2SO_4; residence time: 7 h	Xylose 54 g/l; glucose 3.5 g/l arabinose 4.5 g/l; mannose 3.5 g/l furfural 1.72 g/l; HMF 0.35 g/l[a]	*Pichia stipitis* (NRRL Y-7124) 0.35 g/g (detoxified hydrolyzate)	Nigam (2002)

[a] The hydrolyzate was concentrated under vacuum.

sugars in the detoxification step (Nigam, 2001; Millati et al., 2002; Nilvebrant et al., 2003). In the current work, no detoxification was used. Still relatively high ethanol yields (0.2 g ethanol/g sugar initially present) were obtained at temperatures up to 200 °C. However, at a temperature of 200 °C, the yield was not obtained until after a fermentation time of more than 100 h, indicating a need for detoxification. At even higher temperatures, both the available sugar formed in hydrolysis and the yield of ethanol decreased. As previously observed for wood hydrolyzates (Taherzadeh et al., 1997), furfural was completely converted in the samples that were fermentable (Fig. 4), but only partially converted in the poorly fermentable hydrolyzates. The concentration of furfural in the hydrolyzates produced above 200 °C were generally above 1 g/l (Table 4).

In conclusion, the Paja Brava straw was found to be surprisingly resistant with respect to hydrolysis of cellulose. Furthermore, the maximum glucose yield obtained was a mere 30% of the theoretical. Given the fact that the hydrolyzates produced at temperatures higher than 200 °C were also severely inhibitory to the yeast, an enzymatic process for hydrolysis of the cellulose part would appear to be advantageous. With respect to the hydrolysis of hemicellulose, however, good sugar yields and acceptable fermentability were obtained using dilute-acid hydrolysis.

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