A novel alkaline oxidation pre-treatment for spruce, birch and sugar cane bagasse

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Highlights

- Alkaline oxidation is an efficient pretreatment method for various raw materials.
- Clearly lower enzyme dosages could be used as compared to steam exploded materials.
- An ethanol yield of 80% could be obtained with both bagasse and spruce in 1–3 days.

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Abstract

Alkaline oxidation pretreatment was developed for spruce, birch and sugar cane bagasse. The reaction was carried out in alkaline water solution under 10 bar oxygen pressure and at mild reaction temperature of 120–140°C. Most of the lignin was solubilised by the alkaline oxidation pretreatment and an easily hydrolysable carbohydrate fraction was obtained. After 72 h hydrolysis with a 10 FPU/g enzyme dosage, glucose yields of 80%, 91%, and 97%, for spruce, birch and bagasse, respectively, were achieved. The enzyme dosage could be decreased to 4 FPU/g without a major effect in terms of the hydrolysis performance. Compared to steam explosion alkaline oxidation was found to be significantly better in the conditions tested, especially for the pretreatment of spruce. In hydrolysis and fermentation at 12% d.m. consistency an ethanol yield of 80% could be obtained with both bagasse and spruce in 1–3 days.

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1. Introduction

Targets to decrease greenhouse gas emissions and dependency of fossil fuels, amongst others, have increased the interest for fuel ethanol in the industrialised world. Currently ethanol is mainly produced from the sugars derived from sucrose or starch, which are also important constituents of food and feed. A more sustainable way to produce ethanol would be to use cellulose as the main carbohydrate raw material. The most promising cellulosic raw materials, in terms of industrial scale, include the various side-streams generated when sugar cane, sugar beet, corn, grains (e.g. wheat) and wood are processed, dedicated energy crops as well as industrial and municipal wastes. However, cellulose is typically tightly bound to hemicelluloses and lignin forming a matrix structure that is very difficult to break down. Consequently, producing monomeric sugars from cellulose and hemicellulose at high yields is far more difficult than deriving fermentable sugars from sucrose- or starch-containing crops, e.g. sugar cane and corn.

The ethanol production concept relevant for this study consists typically of three main processing steps: Firstly, the native, polymeric carbohydrates are hydrolysed by acid or enzymatic treatment into their respective monomer sugars. Secondly, the monomer sugars are fermented to ethanol, and thirdly, ethanol is separated and purified (dehydrated). As such, enzymatic hydrolysis is inefficient without first making the lignocellulosic biomass matrix more accessible to enzymes by a so-called 'pretreatment'. Pretreatments can affect biomass in many different ways. Desirable outcomes of pretreatment include liberation of cellulose from the matrix, decrease in crystallinity of cellulose and increase in accessible surface area and pore size of cellulose (Gong et al., 1999; Sun and Cheng, 2002). An effective pretreatment should, at the same time, avoid degradation or loss of carbohydrates, avoid formation of inhibitory by-products for subsequent hydrolysis and fermentation and be cost-effective.

Various pretreatment methods have been recently reviewed by Mosier et al. (2005), Galbe and Zacchi (2007), Yang and Wyman (2008), Hendriks and Zeeman (2009) and Alvira et al. (2010). Over 0960-8524/$ - see front matter ©2013 Elsevier Ltd. All rights reserved.

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1. Introduction

Targets to decrease greenhouse gas emissions and dependency of fossil fuels, amongst others, have increased the interest for fuel ethanol in the industrialised world. Currently ethanol is mainly produced from the sugars derived from sucrose or starch, which are also important constituents of food and feed. A more sustainable way to produce ethanol would be to use cellulose as the main carbohydrate raw material. The most promising cellulosic raw materials, in terms of industrial scale, include the various side-streams generated when sugar cane, sugar beet, corn, grains (e.g. wheat) and wood are processed, dedicated energy crops as well as industrial and municipal wastes. However, cellulose is typically tightly bound to hemicelluloses and lignin forming a matrix structure that is very difficult to break down. Consequently, producing monomeric sugars from cellulose and hemicellulose at high yields is far more difficult than deriving fermentable sugars from sucrose or starch-containing crops, e.g. sugar cane and corn.

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Various pretreatment methods have been recently reviewed by Mosier et al. (2005), Galbe and Zacchi (2007), Yang and Wyman (2008), Hendriks and Zeeman (2009) and Alvira et al. (2010).
the last few decades, several pretreatment methods, especially thermochemical methods, have been shown to be promising for a variety of feedstocks. A comprehensive comparison study of several state-of-the-art pretreatment technologies has been reported by the CAFI consortium (Wyman et al., 2005; Eggeman and Elander, 2005; Kumar et al., 2009; Wyman et al., 2011). The 2005 CAFI study concluded that all five methods studied, i.e. dilute acid, hot water, ammonia fiber explosion (AFEX), ammonia recycle percolation (ARP) and lime, were effective in corn stover pretreatment and produced similar overall sugar yields (Wyman et al., 2005). On the other hand, methods applying alkaline pH provided a possibility to decrease the enzyme dosage.

Alkaline pretreatments have one advantage compared to acidic pretreatments and hot-water pretreatments, i.e., alkalids readily remove lignin and xylan side chains, resulting in a dramatic increase in enzymatic saccharification (Sun and Cheng, 2002; Chang and Holtzapple, 2000; Nlewem and Thrash, 2010). In addition, alkaline pretreatments remove acetyl substitutions on the hemicellulose. The addition of oxygen to the reaction mixture greatly improves the delignification of biomass, especially with highly lignified materials (Chang et al., 2001). Particle size reduction is often applied before alkaline treatment of woody materials like poplar (Sierra et al., 2009; Chang et al., 2001) or wet oxidation of spruce (Palonen et al., 2004).

In this study, a novel wet oxidation pretreatment at high alkal concentration (‘alkaline oxidation’) was developed for pretreatment of spruce, bagasse and birch and the effects of the pretreatment on the chemical composition and enzymatic hydrolysis were evaluated. The pretreated materials were compared in terms of hydrolysis and fermentation efficiency to those produced using either catalytic oxidation or steam explosion pretreatments.

2. Methods

2.1. Raw materials

Norway spruce (Picea abies) chips were obtained from UPM-Kymmene Kaipola plant and birch (Betula sp.) chips from Kuusansniemi pulp mill. Sugar cane bagasse from the harvest of 2007 was obtained from Illovo Sugar, Malawi. Bagasse was manually cut <5 cm length. Norway spruce saw dust (2 mm × 2 mm × 10 mm) was provided by VTT Jyväskylä, Finland and was only used in separately specified experiments.

2.2. Pretreatments

Alkaline oxidation (AlkOx) of spruce chips, bagasse, or birch chips was carried out by the method of Hakola et al. (2010) using 0.25 M Na2CO3 but without the Cu-phenanthroline catalyst at 120 °C for 20 h. 10 bar oxygen pressure was used in all the experiments. For the comparison of AlkOx and catalytic oxidation (CatOx) spruce saw dust was pretreated at 120 °C or 140 °C for 5 or 20 h with or without the Cu-catalyst. To study the effect of different alkaline agents in AlkOx, 0.25 M NaOH, KOH or Ca(OH)2 were applied instead of Na2CO3 to remove possible oligosaccharides as a raw material. At the end of the reaction the solid material was separated by vacuum filtration.

Steam explosion (SE) was carried out at different conditions depending on the raw material. Raw material was immersed into dilute sulphuric acid (0.5% or 2% w/w) to get final acid dosages of 0.4% (w/w) acid per dry biomass) for spruce, 1.4% w/w for birch and 0.5% w/w for bagasse, kept at room temperature for 30 min, and the excess acid was drained. Materials were stored overnight at +4°C before SE treatments. SE was carried out in 400 g (dry) batches in a 10 L vessel. The material was heated by steam to 205 °C with spruce and 200 °C with birch and bagasse. The temperature was kept constant for 15 min for spruce and 5 min for birch and bagasse before opening a valve for sudden pressure drop and explosive release of the material. Optimal pretreated steam exploded spruce (2% SO2, 215 °C for 5 min) was obtained from Lund University and used in the fermentation experiments.

2.3. Enzymes

Cellulact 1.5 L, Novozym 188, Cellic Ctec2, and Cellic Htec enzymes were kindly provided by Novozymes. Filter paper activity was measured by the IUPAC-method (Ghose, 1987) and β-glucosidase activity was measured according to Bailey and Linko (1990). Protein content of the enzymes was analysed by the Bio-Rad analys kit using Lowry et al. (1951) method.

2.4. Enzymatic hydrolysis

The solid fraction obtained by filtration was washed with distilled water and hot tap water (–50°C) to remove any soluble material. The sugar compositions of the filtrates and the washing waters were analysed. The remaining solid fractions were enzymatically hydrolysed in order to evaluate hydrolysability of the samples after different pretreatment procedures.

The hydrolysis of the washed solid fraction was carried out using commercial cellulase Cellulact 1.5 L FG (10 FPU/g dry matter) and β-glucosidase Novozym 188 (100 nkat/g dry matter). The hydrolysis experiments were done in 50 mM sodium acetate buffer (pH 5) in test tubes with magnetic stirring at 1% (w/w) dry matter concentration. The temperature was controlled at 45 °C by water bath. Hydrolyses were done as triplicates.

The effect of enzyme dosage was studied by hydrolysing the washed solid fractions with Cellulact 1.5 L FG using 10 FPU/g d.m., 4 FPU/g and 2 FPU/g. In this experiment, Novozym 188 β-glucosidase dosage was kept as constant 100 nkat/g d.m.

2.5. SSF

The simultaneous saccharification and fermentation (SSF) of washed pretreated materials was tested in oil-lock shake flasks at 12% d.m. consistency in 40 mL working volume. Yeast Nitrogen Base (4 mL of 10x stock solution) was used as a nutrient and pH was adjusted to pH 5 by adding 200 mM sodium citrate buffer. A 6 h prehydrolysis with enzyme dosage 15 mg/g d.m. of commercial enzymes (90:10 mixture of Cellic Ctec2 and Htec) was carried out at 50 °C before inoculation with commercial Red Star yeast (3.5 g/L Le Saffre). Fermentation was carried out at 35 °C for 3–6 days.

2.6. Assays

The sugar compositions of the feedstocks were analysed after total acid hydrolysis (Puls et al., 1985). The resulting monosaccharides were analysed by high performance anion exchange chromatography (HPAEC-PAD) using a CarboPac PA-1 column in a Dionex DX 500 series chromatograph equipped with pulse anemometer detection (Tenkanen and Siilka-aho, 2000). The reducing sugars released during pretreatment and in enzymatic hydrolysis were monitored using the DNS method (Bernfeld, 1955). The composition of the material dissolved in the SE was analysed by HPAEC-PAD after acid hydrolysis to hydrolyse possible oligosaccharides to monosaccharides. For acid hydrolysis, 0.5 mL of 70% sulphuric acid was added to 10 mL of sample and autoclaved for 1 h at 120 °C. After cooling to room temperature, sample was diluted with water to 25 mL and analysed according to Tenkanen and Siila-aho.
ka-aho (2000). The monosaccharides in the enzyme hydrolysates were analysed by HPAEC-PAD.

Ash content was analysed from wet samples in a muffle oven by heating samples stepwise first to 103 °C for 7 h to evaporate water and then to 550 °C for 16 h to ash the samples. After that, the ashed samples were cooled and the residue weighted.

To analyse the content of extractives and lignin, air dried samples were extracted with heptane in a Soxhlet extraction system. The heptane extract was dried and the weight of the residue was measured to get gravimetric extractive content. The lignin content was analysed from the extracted samples by acid hydrolysis with 70% sulphuric acid. Klasson lignin was obtained by analysing the acid insoluble residue after drying and soluble lignin by analysing the UV absorbance with wavelength of 203 nm. Extinction coefficients of 128 L/g/cm and 110 L/g/cm were used for spruce and birch, respectively. Acid-soluble lignin was not analysed from bagasse before or after the pretreatments as its extinction coefficient was unknown.

Fermentation was followed by measuring the mass loss due to formation of CO2 and at the end of fermentation analysing ethanol from the broth by HPLC using Aminex HPX-87H column (Bio Rad) with 2.5 mM H2SO4 as eluent and flow rate 0.3 mL/min. The column was maintained at 55 °C. Peaks were detected using a Waters 410 differential refractometer and a Waters 2487 dual wavelength UV (210 nm) detector.

3. Results and discussion

3.1. Raw material characterisation

Carbohydrate content of raw material is important in the production of chemicals via the sugar route as the maximal potential product concentration is dependent on it. The other compounds, lignin, ash and extractives affect the processability of the raw material and can be recovered as side products of the process. The raw materials (spruce chips and saw dust, birch chips and sugar cane bagasse) were characterized regarding their chemical composition (Table 1).

All the selected raw materials consisted mainly of carbohydrates and lignin. Glucan (mainly cellulose) content of spruce, birch and bagasse was high, about 40%. Bagasse had the highest hemicellulose content (24%). In bagasse and birch hemicellulose consisted mainly of xylan whereas in spruce the major hemicellulose was galactoglucomannan. The obtained results are in accordance with the fact that hardwood xylan contains usually methyl glucuronic acid subunits but in grasses glucuronoxylans are prevailing (Harris and Stone, 2008). The highest lignin content, 28.5%, was analysed from spruce saw dust. In addition to carbohydrates and lignin, bagasse had significant ash content. The sum of the analysed compounds was below 100%, especially with birch. One reason is that the acetyl content and monosaccharides from pectic polysaccharides were not analysed. Galacturonic acid, the main component in pectin, could not be analysed as it was partly degraded during acid hydrolysis used in the carbohydrate analysis. Galacturonic acid content in spruce has been reported to be about 1.5% (Bertaud and Holmbom, 2004). Some degradation of pentoses arabinose and xylose, might also occur during the analytical acid hydrolysis. The obtained hemicellulose and lignin contents of birch were also somewhat lower than the respective contents, 28% and 30%, analysed by Miraehdani et al. (2010).

### Table 1

<table>
<thead>
<tr>
<th>Chemical component</th>
<th>Spruce (chips)</th>
<th>Spruce (saw dust)</th>
<th>Birch</th>
<th>Bagasse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucan</td>
<td>40.9</td>
<td>38.5</td>
<td>40.3</td>
<td>42.0</td>
</tr>
<tr>
<td>Xylan</td>
<td>5.1</td>
<td>5.0</td>
<td>16.9</td>
<td>21.5</td>
</tr>
<tr>
<td>Mannan</td>
<td>10.1</td>
<td>11.7</td>
<td>1.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Galactan</td>
<td>1.9</td>
<td>1.9</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Arabinan</td>
<td>1.0</td>
<td>1.3</td>
<td>0.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Rhamnans</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
<td>-0.1</td>
</tr>
<tr>
<td>Total polysaccharides</td>
<td>59</td>
<td>58</td>
<td>60</td>
<td>66</td>
</tr>
<tr>
<td>Methyl glucuronic acid</td>
<td>0.9</td>
<td>na</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>&lt;0.1</td>
<td>na</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Lignin</td>
<td>27.7</td>
<td>28.5</td>
<td>20.3</td>
<td>22.9b</td>
</tr>
<tr>
<td>Extractives</td>
<td>1.2</td>
<td>2.1</td>
<td>1.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Ash</td>
<td>0.3</td>
<td>na</td>
<td>0.4</td>
<td>5.3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>90</td>
<td>89</td>
<td>84</td>
<td>96</td>
</tr>
</tbody>
</table>

*a* = not analysed.

* Extracted with heptane.

** Only Klasson lignin analysed from bagasse.

During AlkOx and SE the raw materials were partially solubilised. The carbohydrate content of the soluble fractions and the chemical composition of the solid fractions were analysed from the hydrolysates. Fig. 2 presents the glucose and xylose yields after the pretreatments and after the enzymatic hydrolysis. As mannan content in spruce is high, mannose yields are also presented. The hydrolysis yields of the monosaccharides were calculated by dividing the monosaccharides solubilised in enzymatic hydrolysis with the total amount of monosaccharides in the fibre fraction remaining after pretreatment. The overall yields of the monosaccharides were calculated by dividing the sum of the monosaccharides solubilised in the pretreatment and in the enzymatic hydrolysis with the content of monosaccharides in the raw material.

### 3.2. Effects of alkaline oxidation and steam explosion pretreatments

To study the effects of AlkOx and SE on the performance of hydrolytic enzymes the solid fractions were enzymatically hydrolysed. After 72 h enzymatic hydrolysis the monosaccharides were analysed from the hydrolysates. Fig. 2 presents the glucose and xylose yields after the pretreatments and after the enzymatic hydrolysis. As mannan content in spruce is high, mannose yields are also presented. The hydrolysis yields of the monosaccharides were calculated by dividing the monosaccharides solubilised in enzymatic hydrolysis with the total amount of monosaccharides in the fibre fraction remaining after pretreatment. The overall yields of the monosaccharides were calculated by dividing the sum of the monosaccharides solubilised in the pretreatment and in the enzymatic hydrolysis with the content of monosaccharides in the raw material.

The cellulose from AlkOx was easily hydrolysed to monosaccharides in 72 h resulting the overall glucose yields of 84%, 91%, and 97%, for spruce, birch and bagasse respectively. Most of the cellulose in the solid fraction of SE bagasse and birch could also be enzymatically hydrolysed giving the overall glucose yields of 78% and 93%, respectively. The major difference between AlkOx and SE could be seen in the enzymatic hydrolysis of pretreated spruce. After AlkOx the solid fraction was easily hydrolysed, only 52% overall glucose yield was achieved with SE spruce.
ally carried out in partly acidic conditions, which is the main difference to alkaline oxidation. The earlier reported wet oxidation pretreatment of spruce (Palonen et al., 2004) was applied to ground wood and 79% carbohydrate yield of theoretical in 72 h hydrolysis with enzyme dosage 30 FPU/g was obtained. Highest yield was obtained in acidic pretreatment conditions. In our study we showed that 87% of carbohydrates in the AlkOx material could be hydrolysed in 72 h with enzyme dosage of 10 FPU/g without grinding the raw material before oxidation.

Pretreatment of birch has not been widely studied. Mirahmadi et al. (2010) studied the alkaline pretreatment of birch with 7% NaOH at 100 °C for 2 h. Glucose yield in the enzymatic hydrolysis for 96 h was 82% with cellulase dosage of 20 FPU/g. Both AlkOx and SE produced clearly higher enzymatic hydrolysability of birch.

Pretreatment of sugar cane bagasse with SE and wet oxidation have been studied for several years. By far, the highest reported overall glucose yield by SE has been 92% with pretreatment conditions 190 °C, 5 min and with SO2-impregnation and enzymatic hydrolysis for 72 h (Carrasco et al., 2010). The optimized wet oxidation conditions for sugar cane bagasse at 195 °C for 15 min at pH 10 followed by enzymatic hydrolysis of solid fraction for 48 h with 25 FPU/g dosage produced 79% glucose yield in hydrolysis and 74% overall glucose yield (Martin et al., 2006). Lime pretreatment has been reported to reach up to 88% overall glucose yield (Rabelo et al., 2008). In our study, as high as 97% overall glucose yield was obtained with AlkOx and enzymatic hydrolysis of cellulose in fibre fraction was complete. SE of bagasse at 200 °C for 5 min also produced very high enzymatic hydrolysability, and 93% of the cellulose was hydrolysed in 72 h hydrolysis. The overall glucose yield was 78% in pretreatment and hydrolysis.

Hemicelluloses i.e. xylan and galactoglucomannan are susceptible to be solubilised and degraded during pretreatments. A fraction of hemicellulose was solubilised as free monosaccharides and oligomers during both SE and AlkOx of all raw materials (Fig. 2). The xylose yields were higher in AlkOx than in SE pretreated spruce and bagasse though a significant part, 30–50%, was degraded during both pretreatments. In contrast, the xylene from birch was almost totally recovered after the pretreatments.

Overall mannose yields were low for both SE and AlkOx pretreated spruce. Similarly to xylan, galactoglucomannan was solubilised in SE and only a low amount remained in the solid fraction. In AlkOx higher proportion of mannose remained in the solid fraction than in SE but nearly similar amount was lost during both the pretreatments. Mannose in the solid fraction of AlkOx spruce was only partially released by enzymatic hydrolysis. The hydrolysis of galactoglucomannan presumably stopped at oligosaccharides, which the Celluclast and Novozym enzyme preparations were not able to hydrolyse further to monosaccharides. AlkOx was shown to be an effective pretreatment method for all the applied raw materials. When compared to SE, higher overall yields of glucose and xylose could be obtained from spruce and bagasse. With birch there was no difference in the overall yield.
3.3. Comparison of catalytic oxidation and alkaline oxidation

In addition to AlkOx studied here, we have previously introduced a catalytic oxidation pretreatment method based on in situ generated copper-phenanthroline catalyst in alkaline conditions (Hakola et al., 2010). The reaction conditions were similar to AlkOx, the major difference of the treatments being the addition of the catalyst.

As in AlkOx, also in CatOx the share of carbohydrates in the solid fraction was significantly increased and a considerable part of the lignin was solubilised. The carbohydrate content of solid fraction was nearly similar, 93% and 96%, after AlkOx and CatOx, respectively. Similarly, the fibre fraction obtained after CatOx was easily hydrolysed by enzymes into monosaccharides. However, material treated for 20 h with a catalyst had a slightly lower enzymatic hydrolysability (glucose yield in hydrolysis 92%) than the respective treatment without a catalyst (98%). It is possible that the CatOx for 20 h is too harsh and makes the structure of cellulose less favourable to enzymatic hydrolysis or causes the formation of inhibiting compounds.

The use of the catalyst in the oxidation shortened the reaction time and allowed lower reaction temperatures. Catalytic oxidation of spruce saw dust for only 5 h at 120 °C gave 95% glucose yield in enzymatic hydrolysis. AlkOx for 5 h at 140 °C or 20 h at 120 °C resulted in similar carbohydrate yields. Recycling the catalyst would lower the additional costs, but, on the other hand, would require additional equipment and the process has not been developed to a commercial scale.

3.4. Effect of oxygen and different alkaline agents on alkaline oxidation

With AlkOx the role of oxygen was found to be essential. At reference conditions, i.e. heat treatment at 120 °C for 20 h with argon pressure in the conditions resembling soda cooking, only 26% of the initial dry biomass was solubilised during the pretreatment and 9% glucose yield in enzymatic hydrolysis was obtained, whereas with oxygen present almost half of the biomass was solubilised. The oxidation of lignin, and decrease in its molecular weight (Rovio et al., 2012) are probably the main reasons for the increased delignification.

The challenges of adding the catalyst into the process are the increased costs and the mandatory toxicity evaluation. Different alkaline solutions, NaOH, KOH, Ca(OH)₂, and Na₂CO₃, were compared in AlkOx of spruce. Carbohydrate yields after AlkOx with different alkalis and after enzymatic hydrolysis for 48 h were analysed. The different alkalis produced quite similar carbohydrate yields (Fig. 3). Overall glucose yields were high: 96–97% of raw material glucose. Only with Ca(OH)₂ pretreatment cellulose had a clearly lower enzymatic hydrolysability than with other alkalis. Nearly identical xylose yields were obtained with all alkalis whereas in mannose yields more variation was observed. As in earlier experiments with spruce, mannose yields were very low, only 13–24%. The lowest mannose yield was obtained with Na₂CO₃. Oxidation of mannose during the pretreatment was evidently high as the yield loss was 50–60%. Higher mannose content was found from liquid fraction after pretreatment with Ca(OH)₂ and KOH than with the other alkalis. A considerable fraction of mannose as galactoglucomannan in the solid fraction, 19–30%, was not hydrolysed to monosaccharides. Galactoglucomannan probably partly remained in the solid fraction after enzymatic hydrolysis or the hydrolysis was stopped to oligomers. With KOH pretreatment the enzymatic hydrolysis of mannose in the fibre was most incomplete. Concerning the glucose and xylose yields, a great variety of different alkalis could be applied in AlkOx without decreasing the efficiency of pretreatment.

3.5. Effect of enzyme dosage on enzymatic hydrolysability of pretreated raw materials

The use of cellulases in total hydrolysis is relatively expensive and the enzymes or the enzyme production costs are important contributors to the overall ethanol production cost (Zhang et al., 2006; Galbe and Zacchi, 2007). The expenses can be decreased by lowering the enzyme price or by reducing their consumption. One way to minimize the enzyme usage is to enhance the pretreatment so that the same hydrolysis level and rate can be obtained with a lower enzyme dosage. The possibility of decreasing the enzyme dosage was studied with AlkOx and SE materials (Fig. 4).

Enzymatic hydrolysability of bagasse and birch was high with both pretreatments with 10 FPU/g dosage as studied in 1% consistency in small laboratory scale. When enzyme dosage was decreased to 4 and 2 FPU/g, hydrolysis level after 24 h was decreased for AlkOx bagasse. With SE bagasse, the hydrolysis with lower dosages was similar to 10 FPU/g in the first 4 h, but after 4 h hydrolysis the reaction was slowed down and the hydrolysis level of SE bagasse was decreased more steeply compared to AlkOx materials. Probably a part of the SE bagasse was very easily hydrolysable and caused the higher hydrolysis levels. SE might also have

![Fig. 3. Glucose and xylose monosaccharide yields after alkaline oxidation pretreatment with Ca(OH)₂, NaOH, KOH, or Na₂CO₃ and after enzymatic hydrolysis for 48 h with 10 FPU/g Celluclast and 100 nkat/g Novozym 188. Spruce saw dust was used as a raw material.](image-url)
decreased adsorption of enzymes to lignin, removal of lignin has probably increased enzymatic hydrolysability by other ways. Lignin removal has been shown to correlate to enhanced enzymatic hydrolysability by removing the lignin barrier for enzymes, increasing the accessible surface area and porosity of substrate (Nlewem and Thrash, 2010; Chang and Holtzapple, 2000).

Standard deviations of the triplicate hydrolysates were quite high in some cases, probably due to inhomogeneous materials containing e.g. sticks and fines. Hydrolysis level was analysed by reducing sugars assay with DNS reagent (Bernfeld, 1955). The method gave in some cases hydrolysis degrees above 100%; probably due to other reducing or coloured compounds released during the hydrolysis and due to inhomogeneity of solid fraction. Monosaccharide analysis from the 72 h hydrolysates by HPAEC-PAD verified the high hydrolysation results (Table 2).

3.6. Ethanol production from alkaline oxidised materials

Alkali recovery after pretreatment by separation and washing the fibre is essential part of AlkOx process concept. For reliable comparison SE materials were also washed. With washed material the amount of the inhibitors was low and thus no lag phase was observed in the beginning of SSF. Washed AlkOx bagasse and spruce fermented well in 12% consistency using commercial yeast and relatively low dosage of hydrolytic enzymes.

Fig. 5. Fermentation of alkaline oxidised spruce and bagasse and steam exploded spruce and bagasse at 12% d.m. consistency at 35°C. 6 h prehydrolysis was carried out with Cellic Ctec2 + Htec enzyme mixture (enzyme ratio was 90:10; total dosage 15 mg/g) at 50°C, pH 5 before inoculation with a commercial yeast Red Star. Ethanol yields calculated from the measured mass loss during fermentation.
the materials were mainly xylose which is in line with the fact that the applied yeast could not ferment pentose sugars. The steam exploded materials after fermentation contained only very low amounts of mono-, cello- and xylooligosaccharides, which indicates that the slow enzymatic hydrolysis with the used dosage (15 mg/g) of pretreated materials was limiting the fermentation. Enzymatic hydrolysis of steam exploded materials did not proceed during fermentation presumably due to inhibition of enzymes, high dry matter content, and suboptimal temperature for the hydrolysis. On the contrary, hydrolysis of AlkOx materials was not limiting the fermentation to the same extent than hydrolysis of SE material and the fermentation of produced hexoses was efficient.

4. Conclusions

Alkaline oxidation is an effective pretreatment method for different lignocellulosic raw materials. Compared to steam explosion higher glucose and xylose yields were obtained from spruce and bagasse. The enzyme dosage required for efficient hydrolysis could be reduced by at least 60% as compared to steam exploded raw materials. The method was not restricted to the use of Na₂CO₃ but various alkalis could be applied. Ethanol yield of 80% and ethanol concentration of 49 g/L could be obtained with alkaline oxidisation but various alkalis could be applied. Ethanol yield of 80% and ethanol concentration of 49 g/L could be obtained with alkaline oxidised spruce at 12% d.m consistency in 3 days using commercial yeast and relatively low dosage of hydrolytic enzymes.

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