

## Enzymatic hydrolysis of steam exploded and alkali pretreated birchwood chips

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### Abstract

*Alkaline pretreatment with NaOH and Steam Explosion (SE) was studied to improve sugar yield from birch wood. Alkali pretreatments were carried out at different temperatures (50°C, 80°C, 100°C and 121°C) in 0,5% and 2% NaOH solutions. The pretreated materials (SE and alkali) were subsequently enzymatically hydrolysed and reducing sugars yield was estimated. Best results were obtained after pretreatment at 121°C for 1 hour with 2% NaOH solution (16 g/l reducing sugars; 37% of theoretical yield), the lowest results were observed for the mildest conditions (50°C, 0,5% NaOH).*

### 1. Introduction:

Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized. Our global economic system is dependent on the use of fossil energy sources for the production of fuels and chemicals. However, fossil fuels are limited resources and global production of conventional oil may already have passed its peak level [1]. Therefore, there is a great interest in exploring alternative energy sources. Unlike fossil fuels, ethanol is a renewable energy source produced through fermentation of sugars [2]. For ethanol production, main feedstock among sugar crops is sugarcane as cane juice or molasses. Among starchy biomass, most of bioethanol is produced from corn or wheat, but also from cassava, rye, barley or sorghum. These raw materials, also employed for animal or even human feeding, seem not to be sufficient for supplying increasing demand [3].

Ethanol production from lignocellulosic biomass is an attractive option for the sustainable production of fuels [4]. Lignocellulosic biomass, including forestry residues, agricultural residues, yard wastes, wood products, animal and human wastes, etc., is a renewable resource that stores energy from sunlight in its chemical bonds [5]. In addition, these resources are renewable, and therefore the carbon released into the atmosphere as carbon dioxide when the liquid fuels are burned is recycled into new plants through photosynthetic fixation [6, 7]. Approximately 90% of the dry weight of most plant materials is stored in the form of cellulose, hemicellulose, lignin, and pectin [8]. The presence of lignin in lignocelluloses leads to a protective barrier that prevents plant cell destruction by fungi and bacteria for conversion into fuel. For the conversion of biomass to fuel, the cellulose and hemicellulose must be broken down into their corresponding monomers (sugars), so that microorganisms can utilize them.

Various pretreatment techniques change the physical and chemical structure of the lignocellulosic biomass and improve hydrolysis rates [7]. The overall purpose of pretreatment is to break down the shield formed by lignin and hemicellulose, disrupt the

crystalline structure and reduce the degree of polymerization of cellulose, so that the acids or enzymes can easily access and hydrolyze the cellulose [9, 10]. Different pretreatment technologies for pretreatment of lignocellulosic biomass include biological, chemical, physical and physico-chemical processes [11].

Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials [12]. In this method, chipped biomass is treated with high-pressure saturated steam and then the pressure is swiftly reduced, which makes the materials undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 160–260°C (corresponding pressure 0.69–4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis [2].

Alkaline pretreatment is basically a delignification process, in which a significant amount of hemicellulose is solubilized as well. The action mechanism is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components, for example, lignin and other hemicellulose. Alkaline pretreatment also removes acetyl and various uronic acid substitutions on hemicellulose that reduce the accessibility of hemicellulose and cellulose to enzymes [14]. Alkaline pretreatment is one approach that has several potential advantages compared to the pretreatment processes including low operation cost, reduced degradation of holocellulose, and subsequent formation of inhibitors for downstream processing [10]. The main mechanisms of alkaline pretreatment are the degradation of ester bonds and cleavage of glycosidic linkages in the lignocellulosic cell wall matrix, which lead to the alteration of the structure of lignin, the reduction of the lignin–hemicellulose complex, cellulose swelling, and the partial decrystallization of cellulose [2, 15, 16].

In the present study, the effect of two different pretreatment methods on enzymatic saccharification of birch were evaluated.

## **2. Material and methods**

### **2.1. Biomass analysis**

Birch is a widespread and readily available biomass throughout Northern Europe and the potential use of this biomass for biofuel production is of major interest. The delivered material was dried at room temperature, chopped into small fragments approximately 3–5 cm long and 0.5–1 cm wide, and stored at room temperature. The chemical composition of raw material was analysed for cellulose, hemicellulose, lignin, ash and dry matter content.

Cellulose, hemicellulose and lignin content were determined according to the method described by Modrzejewski et al. [17]. The percentage dry matter (DM) and the ash content were determined by drying and burning the samples at 105 and 550°C overnight, respectively. The reducing sugar content of the supernatant from the enzymatic hydrolysis was determined by the 3,5-dinitrosalicylic acid reagent (DNS) method using glucose as the standard [18]. The hydrolysis yield of the substrate was defined as follows [19]:

$$\text{Hydrolysis yield (\%)} = \frac{\text{Reducing sugar produced (g)} \cdot 0.9}{\text{Polysaccharide (cellulose+hemicellulose, g)}} \cdot 100\% \quad (2.1)$$

## 2.2. Pretreatment methods

### Alkali pretreatment

Wood chips were pretreated with 0,5% and 2,0% w/v sodium hydroxide solutions at different temperatures of 50°C, 80°C and 100°C for 2 h and autoclaved at 121°C for 1 h. After pretreatment, the biomass was washed with deionized water until the filtrate was neutral. Water insoluble residues were stored at 4 °C until used for enzymatic hydrolysis.

### Steam explosion pretreatment

Steam explosion pretreatment, was carried out in a batch pilot unit equipped with a 5L reaction vessel. Wood chips were introduced into the reaction vessel and exposed to saturated steam at 1,5MPa for 15 minutes. After the saturated steam exposure, a ball valve at the bottom of the reactor was opened suddenly to bring the reactor rapidly to atmospheric pressure. The pretreated fractions were stored at 4°C before the hydrolysis.

## 2.3. Enzymatic saccharification

The untreated and pretreated wood were enzymatically hydrolyzed with 5% w/v dry matter in 50mM sodium citrate buffer at pH 4,8. Penicillin (50 U/mL) and streptomycin (50 µg/mL) were added to prevent bacterial growth during the reaction period. Cellulase (Cellulase from *Trichoderma longibrachiatum*; Sigma Aldrich) and β-glucosidase (Novozyme 188, Novozymes) were added at 20 filter paper unit (FPU)/g cellulose and 15 cellobiose unit (CBU)/g glucose, respectively. The enzyme activities were determined prior to hydrolysis according to NREL [20]. The hydrolyses were carried out at 50°C for 72h, and their progress were followed by analyses of the sugar released by DNS method.

## 2.4. Statistical analysis

All samples were prepared and analyzed in triplicates. The results were statistically tested by analysis of variance and the means were compared by Student-t test at a significance level of P=0.05, by using of Origin 7.5 software.

## 3. Results and discussion

### 3.1. Chemical composition of raw material

Birch wood is classified as hardwood, it grows rapidly, which is advantageous for the bioethanol production, as well as a relatively high content of cellulose and its small demands on the growth conditions. The cellulose, hemicellulose, lignin, extractives, ash and dry matter contents of the raw material used in this investigation are shown in Table 1.

**Table 1.**

Chemical composition of raw material (Results expressed as mean values ± SD(n=3))

	Content	References [21–28]
Dry matter [%]	92,69 ± 0,55	91,4±3,0
Cellulose [% d.m.]	51,39 ± 0,39	43,9±2,7
Hemicellulose [% d.m.]	25,5 ± 0,11	28,9±3,7
Lignin [% d.m.]	18,28 ± 0,41	20,2±0,8
Extractives [% d.m.]	2,6 ± 0,08	3,8±1,3
Ash [% d.m.]	0,20 ± 0,02	1,7±0,5

Total amount of polysaccharides which are a potential source of fermentable sugars (cellulose and hemicellulose) exceeded 75%.

### **3.2. Effect of pretreatment method on enzymatic hydrolysis**

Pretreatment of birch with Steam Explosion (SE) method, as well as 0,5% and 2% NaOH at different temperatures from 50 up to 121°C were performed, and the effects of pretreatments on subsequent enzymatic hydrolysis of lignocelluloses were investigated.

Steam explosion (SE) is known as one of the most efficient pretreatment methods for lignocellulosic biomass [29]. Steam explosion was selected as the processing technology because it requires little or no chemical input and thus is environmentally benign comparing to other technologies. This process involves high temperature steam treatment followed by mechanical disruption of the biomass fibers by a rapid pressure drop (explosion). SE pretreatment works well on hardwood [30, 31]. For *Salix* it has been shown that the pretreatment not only improves enzymatic saccharification but also biogas production through anaerobic digestion [32]. Grous et al. reported that 90% efficiency of enzymatic hydrolysis was achieved in 24 h for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips [33].

Steam pretreatment with addition of a catalyst is the technology that has been claimed to be closest to commercialization [7]. The pretreatment has been tested extensively for a large number of different lignocellulosic feedstocks. The technology has been scaled-up and operated at the pilot-plant scale at the Iogen demonstration plant in Canada [13]. The advantages of steam-explosion pretreatment include the low energy requirement compared to mechanical comminution and no recycling or environmental costs [7].

Alkaline pretreatment of lignocellulosics digests the lignin matrix and makes cellulose and hemicellulose available for enzymatic degradation [34]. Alkali treatment of lignocellulose disrupts the cell wall by dissolving hemicelluloses, lignin, and silica, by hydrolyzing uronic and acetic esters, and by swelling cellulose. Crystallinity of cellulose is decreased due to swelling. Kumar and Wyman reported that NaOH increases hardwood digestibility from 14% to 55% by reducing lignin content from 24-55% to 20% [35].

During the enzymatic hydrolysis, cellulose is degraded by cellulases to reducing sugars that can be fermented by yeasts or bacteria to ethanol. Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process:

- endoglucanase (EG, endo-1,4-D-glucanohydrolase, or EC 3.2.1.4.) which attacks regions of low crystallinity in the cellulose fiber, creating free chain-ends;
- exoglucanase or cellobiohydrolase (CBH, 1,4-β-D-glucan cellobiohydrolase, or EC 3.2.1.91.) which degrades the molecule further by removing cellobiose units from the free chain-ends;
- β-glucosidase (EC 3.2.1.21) which hydrolyzes cellobiose to produce glucose [36].

The enzymatic hydrolysis of pretreated birch wood was performed at 50°C for 72 h using 20 FPU cellulose and 15 CBU per gram of cellulose and glucose respectively. Samples were collected at 24 h intervals in order to determine the dynamics of the formation of reducing sugars. Results are summarized in Fig. 1.



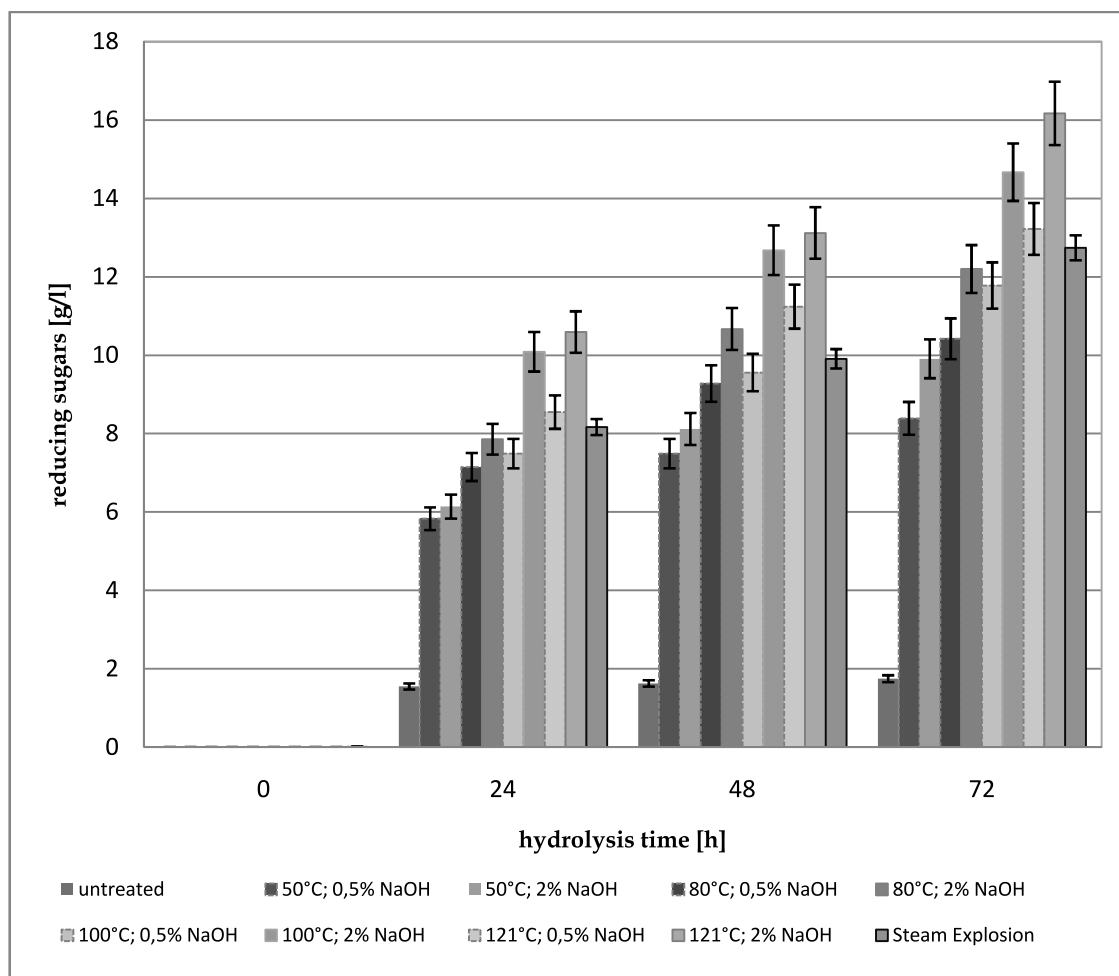


Fig. 1. Enzymatic hydrolysis. Amount of reducing sugars released during enzymatic hydrolysis of the pretreated samples after 0, 24, 48 and 72 h.

Hydrolysis of untreated birch chips resulted in formation  $1,45 \pm 0,009$  g/l reducing sugars, pretreatment allowed to increase the amount of sugars 5 to 10 times.

Fig 1. Shows that 77–89% of reducing sugars are formed during first 48 hours of enzymatic saccharification, only in case of untreated material, hydrolysis was completed in 24 hours.

Among the different pretreatment conditions, the maximum sugar yield ( $16,17 \pm 0,42$  g/l) was obtained when biomass was hydrolyzed with 2% (v/v) NaOH at 121°C for 2 hours. The lowest amount of released sugars was observed when birch wood was pretreated at the mildest conditions (50°C; 0,5% NaOH). No significant effect in sugar release was observed when NaOH concentration was increased (from 0,5% to 2%) at 50°C, while considering temperatures 100°C and 121°C, increase in amount of produced reducing sugars exceeded 20%.

Using equation (2.1), based on total amount of polysaccharides (cellulose and hemicellulose) in raw material, saccharification yield was calculated. Results are summarized in Table 2. The highest hydrolysis yield–37% was observed for birch chips pretreated with 2% NaOH at 121°C.

**Table 2.**

Efficiency of enzymatic saccharification of birch chips pretreated with SE and alkali (Results expressed as mean values  $\pm$  SD(n=3))

No.	Treatment method	Hydrolysis yield [%]
1	Untreated	3,39 $\pm$ 0,03
2	50°C; 0,5% NaOH	19,64 $\pm$ 0,29
3	50°C; 2% NaOH	23,19 $\pm$ 0,39
4	80°C; 0,5% NaOH	24,39 $\pm$ 0,61
5	80°C; 2% NaOH	28,56 $\pm$ 0,71
6	100°C; 0,5% NaOH	27,58 $\pm$ 0,55
7	100°C; 2% NaOH	34,34 $\pm$ 1,32
8	121°C; 0,5% NaOH	30,95 $\pm$ 0,93
9	121°C; 2% NaOH	37,85 $\pm$ 0,98
10	Steam explosion	29,82 $\pm$ 0,82

#### 4. Conclusions

Bioethanol from lignocellulosics is a globally accepted alternative fuel. In this article the influence of SE and alkali pretreatment on sugar release from birch chips was investigated. The efficiency was less than 40% in all cases after pretreatment, while hydrolysis of untreated biomass resulted only in 3% of theoretical yield. Best results were obtained after treatment with NaOH at high temperatures (100°C and 121°C), however steam explosion has also resulted in considerable amount of reducing sugars.

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