RELEASE OF SACCHARIDES DURING HOT-WATER PRETREATMENT OF WILLOW WOOD (*SALIX ALBA* L.)

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Liquid hot-water pretreatment is a hydrothermal process that does not require rapid decompression and the addition of any catalyst or chemicals. It allows the removal of hemicelluloses from lignocellulosic materials, which makes the cellulose more accessible to hydrolysis.
In this study, willow wood (*Salix alba* L.) was subjected to hot-water pretreatment at varying pretreatment times and the temperature of 160 °C. Then, the native willow wood and the hydrolysates were analysed to determine their content of saccharides according to the National Renewable Energy Laboratory analytical procedure. It was determined that the native willow wood contained glucose (49.72%), xylose (12.84%), galactose (2.90%), arabinose (2.00%) and mannose (1.87%), which were also found in the hydrolysates. The optimal amount of saccharides in the hydrolysates was found for a pretreatment time of 60 min (at 160 °C).

Keywords: hot-water pretreatment, willow wood, HPLC, saccharides, bioethanol

INTRODUCTION

Increasing concerns about diminishing fossil fuel resources and energy security have spurred the development of renewable and sustainable energy options.[1] Ethanol is nowadays an important product in the fuel market. Its market grew from less than a billion litres in 1975 to more than 39 billion litres in 2006, and today it is about 100 billion litres. Actually, ethanol has a potential market as big as that of oil and it can potentially entirely replace gasoline on the fuel market.

Lignocellulosic materials can be obtained at low cost from a variety of resources, *e.g.* forest residues, municipal solid waste, waste paper and crop residue resources.[2] They are renewable and abundantly available sources of raw materials, which could be exploited for the production of fuel ethanol. Meanwhile, today, lignocellulosic materials, remain largely unused and are commonly directly burned, which presents another environmental hazard.[3,4] Energy recovery of biomass should be performed at the very end of the life cycle of biomass.[5]

Lignocellulosic biomass has a complex and rigid cell wall structure, which consists of three principal biopolymers, namely cellulose, hemicelluloses and lignin.[1] The proportion of cellulose, hemicelluloses and lignin in a biomass feedstock is a very important criterion to determine its suitability as an economically viable feedstock and also to decide on the optimum pathway for its conversion.[6] The amounts of the carbohydrate polymers and lignin depend on the biomass source. Thus, for example, hardwoods, such as white birch, aspen, red maple, *Eucalyptus*, *Populus* and oak contain 39-54% of cellulose, 14-37% of hemicelluloses and 17-30% of lignin.[7]

One of the most important steps in the processing of lignocellulosic materials is their pretreatment (Fig. 1).[8-13] Saccharides polymerised in the form of cellulose and hemicelluloses can be liberated by pretreatment and subsequently fermented to ethanol by microorganisms.[14,15] The carbohydrate polymers in the lignocellulosic materials need to be converted to simple saccharides before fermentation, through a process called hydrolysis. There are several possible methods to hydrolyse lignocelluloses. The most commonly applied methods can be classified into several groups: liquid hot water pretreatment, chemical pretreatment and enzymatic pretreatment. Cellulose and hemicelluloses can be converted to ethanol, while lignin remains as a by-product.[16]

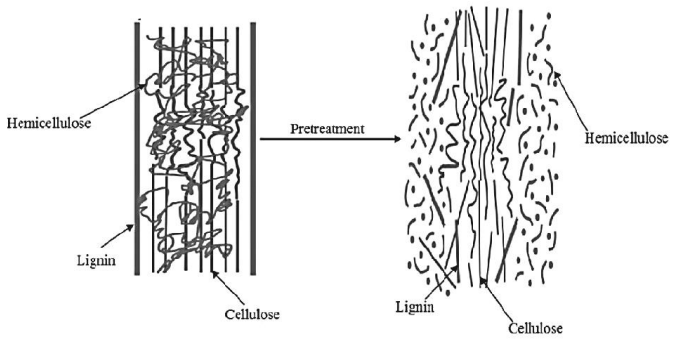

Figure 1: Schematic representation of the role of pretreatment in the conversion of biomass to fuel[19]

Hydrolysis involves cleaving the polymers of cellulose and hemicelluloses into their monomers. Hot-water hydrolysis involves exposure of lignocellulosic materials to hot water for a certain period of time at a specific temperature and results in saccharide monomers from cellulose and hemicelluloses polymers. Complete hydrolysis of cellulose results in glucose, whereas the hemicelluloses give rise to several pentoses and hexoses. While softwood hemicelluloses are mainly composed of mannose, the dominant saccharide in hemicelluloses derived from hardwood and crop residues is usually xylose.[17,18] The main application of xylose is its bioconversion to xylitol, a functional sweetener with important technological properties.[20] In addition, the hydrolysate obtained from willow wood is suitable for the production of feeds, bioethanol or other important products.

The degradation of xylan yields eight main products: water, methanol, formic, acetic and propionic acids, hydroxy-1-propanone, hydroxy-1-butanone and 2-furfuraldehyde.[21] Under high temperature and pressure conditions, xylose is further degraded to furfural. Similarly, 5-hydroxymethyl furfural (HMF) is formed from hexose degradation.[22] These degradation products are known enzymatic hydrolysis inhibitors.[23]

In this study, willow wood (*Salix alba* L.) was subjected to hot-water pretreatment at varying pretreatment times and the temperature of 160 °C. The release of monomeric saccharides and polysaccharides in the hydrolysates was measured by high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Determination of extractives, lignin and saccharides in native wood

Willow wood (*Salix alba* L.) was obtained from 15 year old willow trees grown in the Zvolen region in the Slovak Republic. The sawdust of native wood (untreated) was extracted with a mixture of ethanol and toluene using a Soxhlet apparatus, according to ASTM D1107-96.[24] ASTM Standard Test Method for Acid-insoluble Lignin in Wood[25] was used to determine the lignin content. Data were presented as percentages of oven-dry weight of un-extracted wood.

The wood sample was hydrolysed by sulfuric acid in accordance with the NREL procedure[26] and the obtained product was analysed by a high-performance liquid chromatography (HPLC) system, equipped with an Aminex HPX-87P column, at 80 °C, using ultra-pure water as eluent at a flow rate of 0.5 mL.min^{-1}. Glucose, mannose, arabinose, xylose and galactose were detected with a refractive index detector. Quantitative determination was made by comparison with cellobiose as an internal standard. For all the analyses, four repeated measurements were performed per sample. Data were presented as percentages of oven-dry weight of un-extracted wood.

Hot-water pretreatment

Samples from debarked trunk willow wood were chipped to dimensions of 2 × 2 × 10 mm. The wood chips (2 g) were introduced into stainless autoclaves with an internal volume of 12 mL. Then, a volume of 8 mL of deionized water was added to the chips. Water prehydrolysis was performed in the thermostat at the temperature of 160 °C and pretreatment times of 30, 60, 120 and 240 minutes. The prehydrolysis was stopped by submerging the autoclave into an ice bath at the temperature of 20 °C. The solid fraction was separated from the liquid one by filtration.

In this study, the hot-water pretreatment conditions corresponded to a severity factor (log R_0) ranging from 3.24 to 4.15. The severity factor is defined as follows:[27]

$$\log R_0 = \log\left[\mathrm{t}.exp\left(\frac{\mathrm{T}-100}{14.75}\right)\right] \quad (1)$$

where *t* is the reaction time in minutes (including the pretreatment time); *T* is the hydrolysis temperature in °C, and 100 °C is the reference temperature.

Determination of saccharides in hydrolysates

After the pretreatment, the hydrolysates were neutralised to pH 7 by NaOH. Monosaccharides in the hydrolysates were determined by HPLC, according to the NREL procedure[26] under the same conditions as those applied for the wood samples.

After hydrolysis of the glycoside bonds in the liquor by 4% (w/w) H_2SO_4 at 100 °C for 4 hours, the content of total saccharides was determined by an Agilent 1200 HPLC chromatograph equipped with an Aminex HPX-87P column at a temperature of 80 °C and a mobile phase flow rate of 0.5 $mL.min^{-1}$.

Hot-water pretreatment was performed twice and chemical analyses were performed twice for both hydrolysates. In this way, four series of results were obtained for each time of pretreatment.

Statistical analysis

For all the parameters, multiple comparisons were first subjected to an analysis of variance (ANOVA), and the significant differences between the average values of the pretreated samples were determined using Duncan's multiple range test with a *p*-value of 0.05.

RESULTS AND DISCUSSION

The mean amounts of the main wood components in willow wood were as follows: 5.27% (SD = 0.18) extractives, 37.90% (SD = 0.12) cellulose,[9] 24.44% (SD = 0.22) lignin, 69.34% (SD=0.14) saccharides. Other authors have reported mean amounts of willow wood components of: 2.0% extractives, 54.0% cellulose, 26.3% lignin.[28] Close values for willow wood composition were found by Bodirlau *et al.*: 2.68% hot-water extractives, 50.59% cellulose, 23.45% lignin, 0.91% ash.[29] Also, Stolarski *et al.* reported 41.7% cellulose, 26.7% hemicelluloses, and 24.5% lignin.[30] It is worth noting that the composition of wood, *i.e.* the contents of cellulose, hemicelluloses, lignin and extractives, depends on various factors, such as age, growth conditions, the season of timber harvesting *etc*.

The native willow wood in this study (Fig. 2) was predominantly composed of glucose (49.72 ± 0.15%) and xylose (12.84 ± 0.16%). The galactose (2.90 ± 0.12%), arabinose (2.00 ± 0.09%) and mannose (1.87 ± 0.17%) were relatively minor components. The main constituents of willow wood are cellulose, hemicelluloses and lignin. Cellulose consists of glucose molecules, and hemicelluloses are composed of glucose, xylose, galactose, arabinose and mannose molecules. Hemicelluloses are more easily hydrolysable than cellulose. The hydrothermal treatment is used to achieve the maximum monosaccharide yields and to degrade cellulose, which can be consequently transformed into bioethanol using enzymes.

In the hydrolysates obtained from willow wood, the pH value decreased, while the severity factor increased with the rising time of pretreatment (Table 1). The optimal conditions for maximum saccharide yields were found in the severity parameter range of 3.0-4.5.[31] The acetyl groups bound to hemicelluloses can be cleaved under hot-water pretreatment conditions.[32,33]

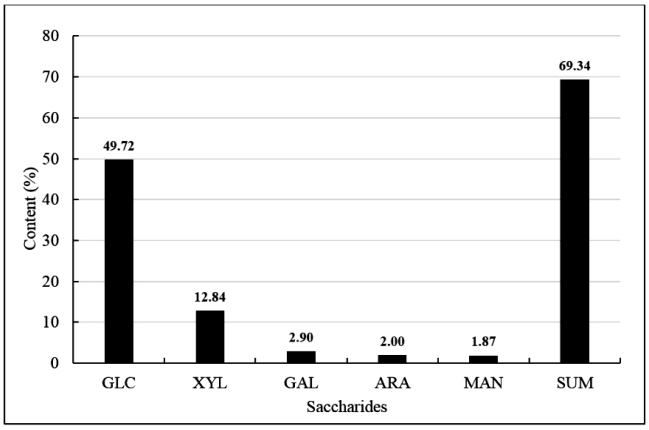

Figure 2: Contents of saccharides in willow wood (%)

Table 1
pH value of hydrolysates and severity factor

Time of pretreatment (min)	30	60	120	240
pH ± SD	3.31 ± 0.01	3.22 ± 0.02	3.13 ± 0.07	3.07 ± 0.01
Severity factor (log R_0)	3.24	3.54	3.85	4.15

Table 2
Duncan's multiple range test p-values for amounts of total xylose in hydrolysates

Temperature (°C)	30	60	120	240
30		0.000	0.000	0.001
60	0.000		0.002	0.000
120	0.000	0.002		0.000
240	0.001	0.000	0.000	

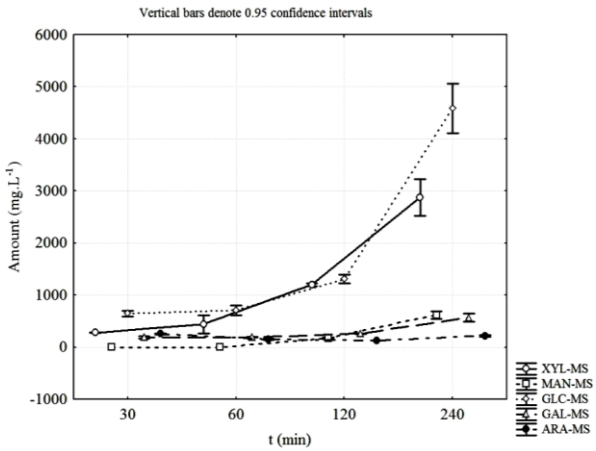

Figure 3: Amount of monosaccharides in willow wood hydrolysates (abbreviations: XYL, D-xylose; MAN, D-mannose; GLC, D-glucose; GAL, D-galactose; ARA, L-arabinose; MS, monosaccharide)

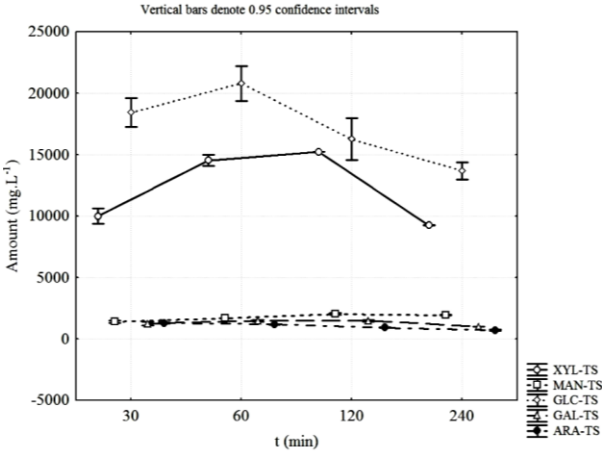

Figure 4: Amount of total saccharides in willow wood hydrolysates (abbreviations: XYL, D-xylose; MAN, D-mannose; GLC, D-glucose; GAL, D-galactose; ARA, L-arabinose; TS, total saccharides)

Previous studies[34,35] found that acidic conditions promote the release of wood components, especially the release of carbohydrates. During the pretreatment, acids are released into the hydrolysates, which causes a decrease of the pH value and an increase of the severity factor, as the pretreatment period is enhanced (Table 1). As has been shown in previous studies,[35,36] these acidic conditions cause the release of wood components, especially saccharides into the hydrolysate. On the other hand, more severe conditions could indeed reduce the concentration of the saccharides dissolved.[37]

The increase of the hydrolysate acidity caused further degradation of willow wood and the glycosidic bonds in the polysaccharides were cleaved. The hemicelluloses were hydrolysed to soluble saccharides. Subsequently, the resulting liquors (hydrolysates) contained a mixture of monosaccharides, specifically, glucose, xylose, galactose, arabinose and mannose, which are typical of hardwood (Fig. 3). After the pretreatment duration of 240 min, glucose, created from hemicelluloses and the amorphous fraction of cellulose, was the main monomeric saccharide product in the hydrolysate. The amount of xylose was smaller than the amount of glucose. Xylose was formed by depolymerisation of xylans. In addition, regarding the amounts of the five saccharides in the hydrolysate, their maximum release yields decreased in the following order: glucose > xylose > mannose > galactose > arabinose.

After total hydrolysis, the basic structural units were identified and their relative amounts (mono- and polysaccharides) in the hydrolysates were calculated. Monosaccharides, such as xylose, glucose, mannose, arabinose and galactose, were determined in the hydrolysates (Fig. 4). Glucose and xylose were the major components found for the investigated pretreatment time range. The amount of glucose decreased after an initial increase, with a maximum being reached for a pretreatment period of 60 min.

The maximum amount of xylose was determined for the pretreatment times of 60 and 120 minutes. According to Duncan's multiple test p-values (Table 2), the amount of xylose varied significantly. The galactose, arabinose and mannose were relatively minor components. Thus, a pretreatment time of 60 min and the temperature of 160 °C were found to be the optimal pretreatment conditions for obtaining high yields of mono- and polysaccharides from willow wood (Fig. 6).

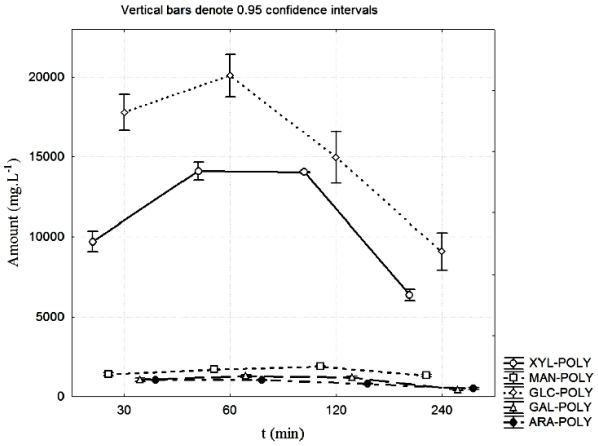

Figure 5: Amount of polysaccharides in willow wood hydrolysates (abbreviations: XYL, D-xylose; MAN, D-mannose; GLC, D-glucose; GAL, D-galactose; ARA, L-arabinose; POLY, polysaccharides)

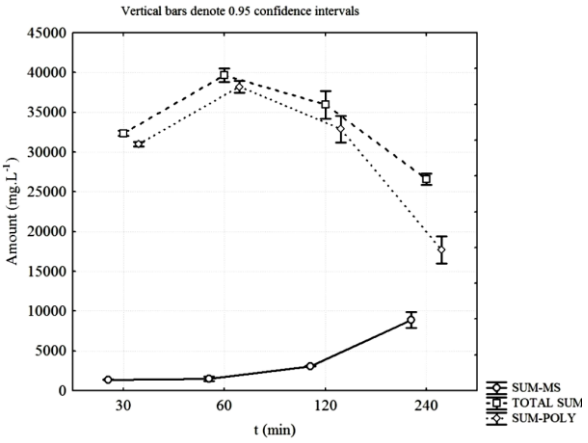

Figure 6: Total monosaccharides, total polysaccharides in willow wood hydrolysates and their sum (abbreviations: SUM-MS, total monosaccharides; SUM-POLY, total polysaccharides; TOTAL SUM, total monosaccharides and polysaccharides)

The variation of the amount of polysaccharides in the hydrolysates as a function of the pretreatment conditions of willow wood chips is shown in Figure 5. The highest glucose yield was obtained at the temperature of 160 °C for 60 min (20,099±838 $mg.L^{-1}$). As may be noted in Figure 5, glucose and xylose were released as polysaccharides into the hydrolysates within the first 60 minutes of the treatment. After that time, the glucosic and xylosic polysaccharides were broken down to monosaccharide units (Fig. 3).

Saccharides in polymeric form were predominant in the hydrolysates obtained after a pretreatment of 60 minutes, while after longer periods of time, the monomeric forms prevailed (Fig. 6). Monosaccharides may be further degraded to volatile compounds (acetic acid, 2-furaldehyde). These compounds and water-soluble lignin degradation products inhibit microbial fermentation to desirable products.

The experimental results indicate (Fig. 6) that an optimal amount of saccharides can be achieved after a pretreatment time of 60 min (at 160 °C) in willow wood hydrolysates.

CONCLUSION

Biomass pretreatment remains a key bottleneck in the bioprocessing of lignocellulosic materials for the production of biofuels and other bioproducts. Although some pretreatment methods show apparent advantages, it is unlikely that one method will become the method of choice for all types of biomass, at least, not for all feedstocks. Willow wood is a suitable raw material for bioethanol production. In this study, we hydrolysed willow wood using hot water of 160 °C during 30, 60, 120 and 240 minutes. The saccharides content of willow wood, as well as that in the hydrolysates after the hot-water pretreatment, were investigated. The native willow wood contained glucose (49.72%), xylose (12.84%), galactose (2.90%), arabinose (2.00%) and mannose (1.87%). Hydrothermal pretreatment of willow wood at 160 °C for 60 min was found to be the most effective to achieve maximum total amounts of saccharides.

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