

## CHANGES IN THE CHEMICAL COMPOSITION OF BLACK LOCUST WOOD AFTER HOT-WATER PRETREATMENT BEFORE BIOETHANOL PRODUCTION

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### ABSTRACT

Bioethanol produced from various lignocellulosics, mainly wood, have the potential to be a valuable substitute for gasoline. The initial step in this process is a pretreatment to remove hemicelluloses, break down the lignin structure and make the cellulose accessible to hydrolysis for conversion to fuels. Hot-water pretreatment is one of the most promising technologies due to that no additional chemicals are required. In this paper, we present changes in the chemical composition of *Robinia pseudoacacia* wood as well as the amounts of carbohydrates in the solids after hot-water pretreatment at 180°C during 5, 10, 20, 30 and 50 minutes. Main wood components were determined by wet chemistry methods, whereas saccharides were analysed by high-performance liquid chromatography according to the National Renewable Energy Laboratory (NREL) analytical procedure.

Hot-water pretreatment of black locust wood resulted in increased contents of both cellulose and extractives, and a decrease in the amounts of lignin, holocellulose and hemicelluloses. The amount of holocellulose decreased during pretreatment, especially at the beginning of the process because the content of hemicelluloses diminished rapidly. In the first stage of experiment (5 min), approximately 65% of hemicelluloses were released from black locust wood, and only 20% of hemicelluloses remained in the solid at the end of pretreatment (50 min). We have found that optimum time for black locust wood hot-water pretreatment at 180°C is 20 minutes.

**Key words:** black locust, hydrolysis, cellulose, hemicelluloses.

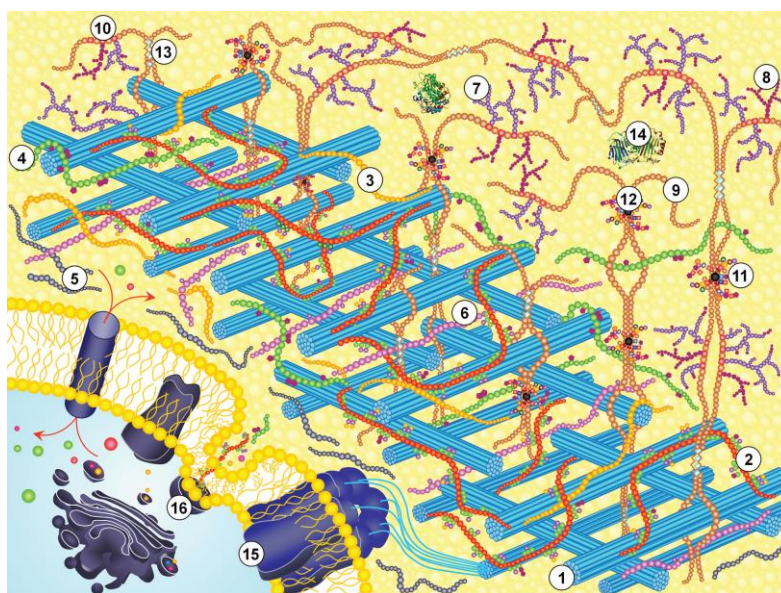
### INTRODUCTION

To meet the increasing demand for bioenergy several raw materials must be considered for the production of alternatives to fossil fuels: one possible option is to consider the production of bioethanol from lignocellulosic materials. Therefore it is important to search for new raw materials and to study the different steps that lead to bioethanol production from those materials (FERREIRA *et al.* 2010, GNANSOUNOU 2010).

Biomass materials can be hydrolysed to monosaccharides that can be fermented to ethanol or other alcohols. To achieve high hydrolysis yields, pretreatment is required to break away lignin that is closely associated with the cellulose and disrupt the structure so enzyme can penetrate the cell wall and depolymerize cellulose and hemicelluloses (XIMENES *et al.* 2013). One of the most promising methods is the pretreatment with Liquid Hot Water (LHW) or thermohydrolysis. Under optimal conditions, this method is

comparable to dilute acid pretreatment but without addition of acids or production of neutralization wastes. In addition, this technology presents elevated recovery rates of pentoses and does not generate inhibitors for enzymatic hydrolysis and fermentation, respectively (LASER *et al.* 2002, SÁNCHEZ, CARDONA 2008). However, some authors reported that aqueous pretreatment can release a range of inhibitors, such as phenolics, furans, aromatic and aliphatic aldehydes and acids, xylooligosaccharides, etc. (KINKLE *et al.* 2004, THOMSEN *et al.* 2009, PARAWIRA, TEKERE 2011).

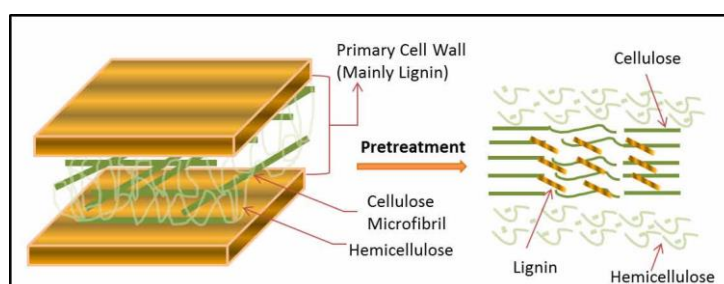
Aqueous pretreatment utilises the chemical and catalytic properties of liquid water at temperatures and pressures significantly above room temperature and atmospheric pressure, respectively. Biomass is primarily composed of three polymers: cellulose, hemicelluloses, and lignin (Fig. 1). As hemicelluloses are hydrolysed under the temperatures and pressures associated with aqueous pretreatment, organic acids (especially acetic acid) are released into the water and make the solution more acidic. Protons in the solution catalyse the hydrolysis of glycosidic bonds within the backbone of the polysaccharides. In general, pretreatment significantly improves the accessibility of the cellulose present in the plant cell-wall matrix to the enzyme-catalysed hydrolysis to fermentable glucose. This is achieved through several physical and chemical changes to the biomass that occur during pretreatment. Aqueous pretreatment affects hemicellulose, lignin and cellulose in different way to improve the enzyme accessibility and enhance the reactivity of the cell-wall polysaccharides (Figs. 2 and 3) (MOSIER *et al.* 2005, MOSIER 2013).



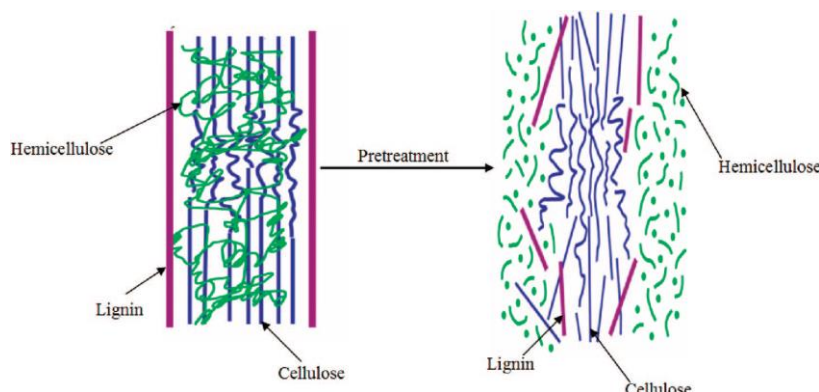
**Fig 1 Model of the polysaccharide framework in a plant cell wall, generalized for poalean and non-poalean walls. 1, Cellulose: cellulose microfibrils; 2–6, hemicelluloses: 2, xyloglucan; 3, mixed-linkage glucan; 4, xylan and related heteroxylans; 5, callose; 6, mannan and related heteromannans; 7–11, Pectins: 7, galactan; 8, arabinan; 9, homogalacturonan; 10, rhamnogalacturonan I; 11, rhamnogalacturonan II; 12, boron bridge; 13, ‘egg-box’ with calcium bridges; 14–16, Non-polysaccharide components: 14, enzymes and structural proteins; 15, cellulose synthase complex; 16, transport vesicles (FRANKOVÁ, FRY 2013).**

The objective of pretreatment is to structurally alter the cell walls of plant biomass, thus rendering the polysaccharides fraction (cellulose and hemicelluloses) more accessible to hydrolytic enzymes (CAO *et al.* 2012). The cleavage of aryl ether linkage in lignin

during hydrothermal pretreatment can result in lignin fragmentation thus disrupting the biomass cell wall matrix and facilitating cellulase accessibility to cellulose. In addition, the acidic pretreatment might also cause cleavage of some labile linkages between lignin and carbohydrates (mainly hemicellulose) therefore facilitating hemicellulose dissolution, which in turn increases pore volume and available surface area in pretreated biomass (PU *et al.* 2013). Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis, (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and (4) be cost-effective (KUMAR *et al.* 2009).



**Fig. 2** Schematic diagram showing the effect of pretreatment on lignocellulosic biomass. The primary cell wall becomes compacted by a dense lignin network structure as the plant grows and ages. (TONG *et al.* 2013).



**Fig. 3** Schematic of the role of pretreatment in the conversion of biomass to fuel (KUMAR *et al.* 2009).

Although numerous information about various biomass resources for bioethanol production across literature are available, they are mainly focused to selected wood species (Eucalyptus, poplar, etc.) and data for *Robinia pseudoacacia* are rare. The aim of our research was therefore to study the influence of hot-water pretreatment to black locust wood and to find the optimum conditions for its further conversion to bioethanol.

## EXPERIMENTAL

Ten grams of 28-years old black locust (*Robinia pseudoacacia* L.) wood sawdust (particle size from 0.5 mm to 1.0 mm) were added to a 300 mL Parr 4561 reactor. 200 mL of deionized water was then added. The reactor was then sealed and heated by stirring at 80 rpm to the temperature of 180°C. Once the reactor reached 180°C, the reaction proceeded 5, 10, 20 30, and 50 minutes, respectively and then was stopped by submerging

the reactor in an ice bath. The contents were then separated into liquid and solid fractions by filtration. Each pretreatment was performed twice and the solids were analyzed separately. Chemical analyses were performed twice for both of solids, so four results were obtained for each time of pretreatment.

Wood sawdust was extracted in the Soxhlet apparatus with a mixture of ethanol and toluene according to the ASTM Standard Test Method for Ethanol – Toluene Solubility of Wood D1107-96. Lignin content was determined according to the ASTM Standard Test Method for Acid-Insoluble Lignin in Wood D1106-96. Holocellulose was determined using the method of Wise *et al.* (1946), and cellulose by the Seifert method (SEIFERT 1956). Hemicelluloses were calculated as a difference between holocellulose and cellulose. Data are presented as the percentages of oven-dry weight (odw) per unextracted wood. Qualitative and quantitative analyses of saccharides were carried out by HPLC according to the NREL procedure (SLUITER *et al.* 2011). The samples were hydrolysed in a two-stage process. In the first stage, 72% (w/w) H<sub>2</sub>SO<sub>4</sub> at a temperature of 30°C was used for 1 h, and in the second stage the formed oligomers were hydrolysed to monosaccharides after dilution to 4% (w/w) H<sub>2</sub>SO<sub>4</sub> at 121°C for 1 h. The analyses were performed with an Agilent 1200 HPLC chromatograph (Agilent Technologies) equipped with an Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA, USA) at a temperature of 80°C and a mobile phase flow rate of 0.6 mL min<sup>-1</sup>.

## RESULTS AND DISCUSSION

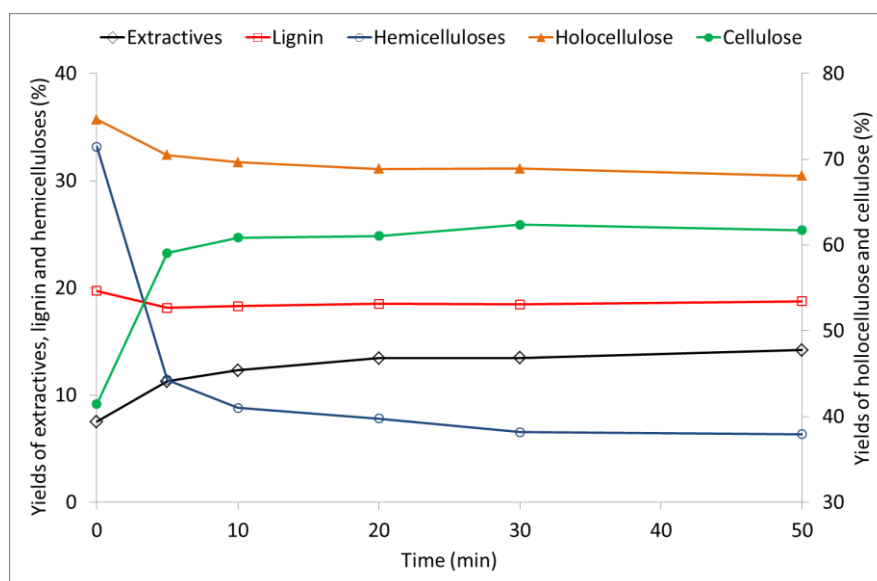
The results of the chemical composition of black locust wood (dry mass) were as follows: extractives, 7.50%; lignin, 19.73%; holocellulose, 74.62%; cellulose, 41.46%, and hemicelluloses, 33.16%. These findings are similar to chemical composition reported by other authors for various aspen, e.g. species cellulose, 43.1; xylan, 18.7; acid insoluble lignin, 20.7; acid soluble lignin, 5.2; extractives, 8.3; acetic acid, 4.8; formic acid, 1.6 and ash, 1.1 for *Acacia dealbata* (FERREIRA *et al.* 2011); cellulose, 42.37%; xylan, 16.18%; uronic acids, 3.96%; arabinan, 0.23%; acetyl groups, 3.90%; acid insoluble residue, 18.90%; protein 1.6%; ash 0.50%; extractives, 5.85% for *Acacia dealbata* (YANEZ *et al.* 2009a); extractives in ethanol benzene, 3.0%; extractives in hot water, 3.7%, extractives in cold water, 2.5%; cellulose, 40.6%; carbohydrates, 63.8%; pentosans, 20.1%; acid insoluble lignin, 22.7%; ash, 2.3% for acacia wood (HOA *et al.* 2008). Differences between presented values were due to various tree species as well as different sites where the examined trees were growing. The dry raw material of our sample consisted of approximately 41.5% cellulose and about 33% of hemicelluloses, which could be used for ethanol production.

Hot-water pretreatment of black locust wood resulted in increased contents of both cellulose and extractives (Fig. 4). The cellulose content increased with the time of pretreatment to reach a maximum value of 62.36% in the experiment carried out at 180°C for 30 min. The experimental data confirmed the high recovery of cellulose in the solid phase and thus the preferential solubilization of hemicelluloses. This result agrees with the findings of YANEZ *et al.* (2009b) aimed at hydrothermal pretreatments on *Acacia dealbata* at the temperature range from 170°C to 240°C. On the other hand, these authors observed the increased content of Klason lignin. We have found a decrease in lignin content at the beginning of the process, then its amount slightly increased with the time of pretreatment (Fig. 4).

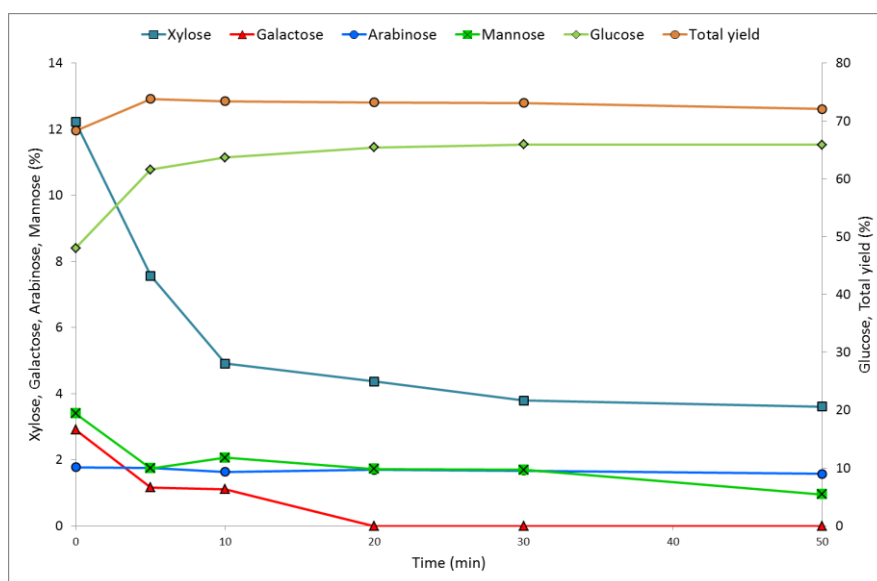
Increased content of extractives can be explained as a result of polysaccharide degradation (ROSA and PEREIRA 1994) and similar trends were observed from thermal and

hydrothermal pretreatment of softwoods and hardwoods (NUOPPONEN *et al.* 2003, NIEMZ *et al.* 2004). According to Esteves *et al.* (2008), most of the original wood extractives disappear upon heat treatment, especially the most volatile compounds, while new extractives appear as degradation products of structural polymers.

The amount of holocellulose decreased during pretreatment, especially at the beginning of the process, because the hemicelluloses content diminished rapidly. In the first stage of experiment (5 min) approximately 65% of hemicelluloses were released from black locust wood; and only 20% of hemicelluloses remained in the solids at the end of pretreatment (50 min).



**Fig. 4** Amounts of extractives, lignin, hemicelluloses, holocellulose and cellulose in black locust wood before and after hot-water pretreatment.

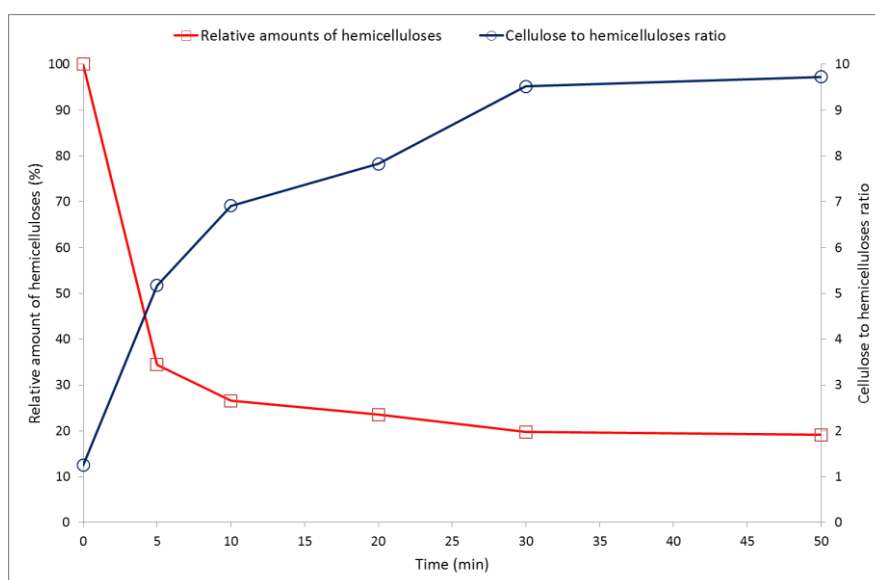


**Fig. 5** Amounts of monosaccharides in black locust wood before and after hot-water pretreatment.

Cellulose is a linear chain homopolymer consisting of (1→4)-β-D-glucopyranosyl units with a varying degree of polymerization (DP) and with a tendency to form intra- and

inter-molecular hydrogen bonds through hydroxyl groups on glucan chains. Hemicellulose is a broad class of mixed heteroglycans of pentoses and hexanoses which are linked together and frequently have branching and substitution groups (CAO *et al.* 2012). These differences in polysaccharide structures considerably influence their solubility in water at elevated temperature. As it was reported by PU *et al.* (2013), xylan, the main hemicellulose in hardwoods, is hydrolysed to xylose or xylo-oligomers during hydrothermal pretreatment, whereas glucomannan is relatively stable in acidic process. In general, the degree of xylan hydrolysis increases as the hydrothermal pretreatment severity increases.

The sound black locust wood had a typical composition of hardwood with 19.73% Klason lignin and 74.62% holocellulose. The carbohydrate fraction was predominantly composed of glucose (48.03%) and xylose (12.22%), while arabinose (1.78%), mannose (3.40%) and galactose (2.91%) were relatively minor components. Our results showed the rapid decrease of xylose content at the beginning of pretreatment, and then its decline was slowed down. The amounts of mannose and galactose also decreased, in content of arabinose were observed only minor changes. In contrast to cellulose, hemicelluloses are more easily hydrolysable and as a result, the amount of glucose increased in black locust wood during hot-water pretreatment (Fig. 5). This result is consistent with findings of other authors (CAO *et al.* 2012, KANG *et al.* 2012).



**Fig. 6 Loss of hemicelluloses and the ratio of cellulose to glucose in black locust wood at hot-water pretreatment.**

The ratio cellulose to hemicelluloses rapidly increased with the time of pretreatment from 1.25 to 9.73 (Fig. 6), and the content of hemicelluloses decreased up to 80% of its original amount in the sound wood. These changes were very fast at the beginning of pretreatment, and then slowed down. This implies that almost all accessible hemicelluloses were removed from black locust wood at the temperature of 180°C during 30 minutes of hot water pretreatment. From our previous results, we know that the maximum yields of carbohydrates in liquid phase were found at the hydrolysis time of 20 minutes (KAČÍK *et al.* 2014). The data presented in this study extend the previous work that was aimed at the assessment of black locust wood hydrolysates after hot-water pretreatment (Kačík *et al.* 2015), and all these results are mutually in a good agreement.

## CONCLUSIONS

In this study, the chemical composition of *Robinia pseudoacacia* wood as well as the amounts of carbohydrates in the solids after hot-water pretreatment at 180°C during 5, 10, 20, 30 and 50 minutes, were investigated.

Hot-water pretreatment of black locust wood resulted in increased contents of both cellulose and extractives, and a decrease in the amounts of lignin, holocellulose and hemicelluloses. The main effect of pretreatment was the removal up to 80% of the original amount of hemicelluloses which resulted in the enrichment of cellulose content up to 150% in comparison with untreated wood. Pretreatment caused the rapid decrease of xylose content at the beginning of the process, and then its decline was slowed down. The amounts of mannose and galactose also decreased, whereas only minor changes were observed for the content of arabinose.

It can be concluded that optimum time for black locust wood hot-water pretreatment at 180°C is 20 minutes.

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