



Pretreatments for the Product of Ethanol from Genetically modified Hardwoods with High Cellulose and Low Lignin

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Planned Activities: Continue characterizing the feed stock and also plan to start evaluating the effect of lignin type on pretreatment efficiency and enzymatic hydrolysis (Task 3). We will evaluate both lignin and hemicellulose content.

Actual Accomplishments:

Comparison of the saccharification efficiency of various hardwood species and transgenics with varying lignin content and S/G ratio

Acid and Alkaline (AK) pretreatment

This continuing work will confirm us the influence of the characteristics of hardwoods on the enzymatic hydrolysis. A set of experiments on the behavior of hardwood species (Maple, Sweet Gum, Aspen and Red Alder) pretreated with mild sulfuric acid and alkaline pretreatment were conducted enzymatic hydrolysis. Three species were evaluated earlier. This report evaluates Red Alder. The sulfuric acid pretreatment was carried for 30 minutes at 185C, the concentration of acid is 0.1% (m/m). After this pretreatment, there is about 1.5% lignin removed (Table 1). The alkaline pretreatment were carried out at 160C, 1 hour with active alkaline charge of 12 and 6%. There was a big improvement of lignin remove from 15% for GL6 to 30% for GL12 (Table 1). However, there was no significant difference (sugar yield gain) in the sugar recovery after EH of sawdust pretreated with 12 and 6% AA (Fig.1). If compared to acid pretreatment, more sugar is recovered with alkaline pretreatment . This result further confirmed the tendency between lower lignin content in the original sawdust and higher sugar recovery in the saccharification, and is in good correlation with the results obtained for transgenics. In Figure 2, total sugar recovery of all species in enzymatic hydrolysis (20 FPU/g) with different pretreatments are shown..

Table 1. Composition of residue after pretreatment (express as % of original sawdust) (Red Alder)

Pretreatment method	Yield after pretreatment	Glucan	Xylan	Total sugars	* % Sugar Retained	Lignin	* % Lignin Removed
Acid	71.8	37.7	2.1	41.2	69.5	25.6	1.5
GL6	78.2	43.0	10.5	54.0	91.1	22.1	15.0
GL12	68.0	39.8	9.0	49.3	83.1	18.3	29.6

Note: * % Sugar Retained based on ori-Sugar *% Lignin Removed based on ori-Lignin

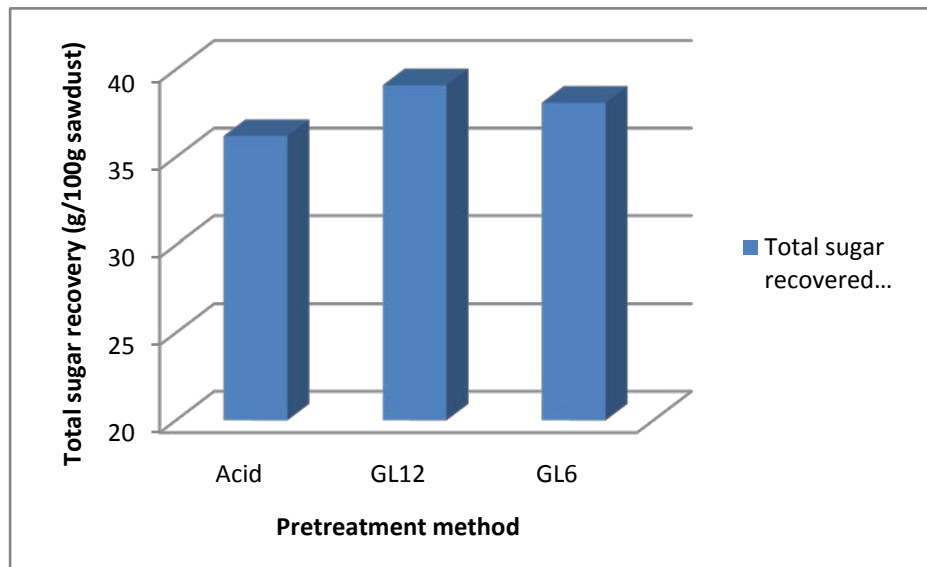


Figure 1. Comparison of total sugar recovery of Red Alder with different pretreatment at enzyme charge of 20 FPU/g. For Acid pretreatment, the amount of sugars include the sum of monomers in AH and EH steps.

comparison of total sugar recovered from different pretreatments at 20 FPU/g

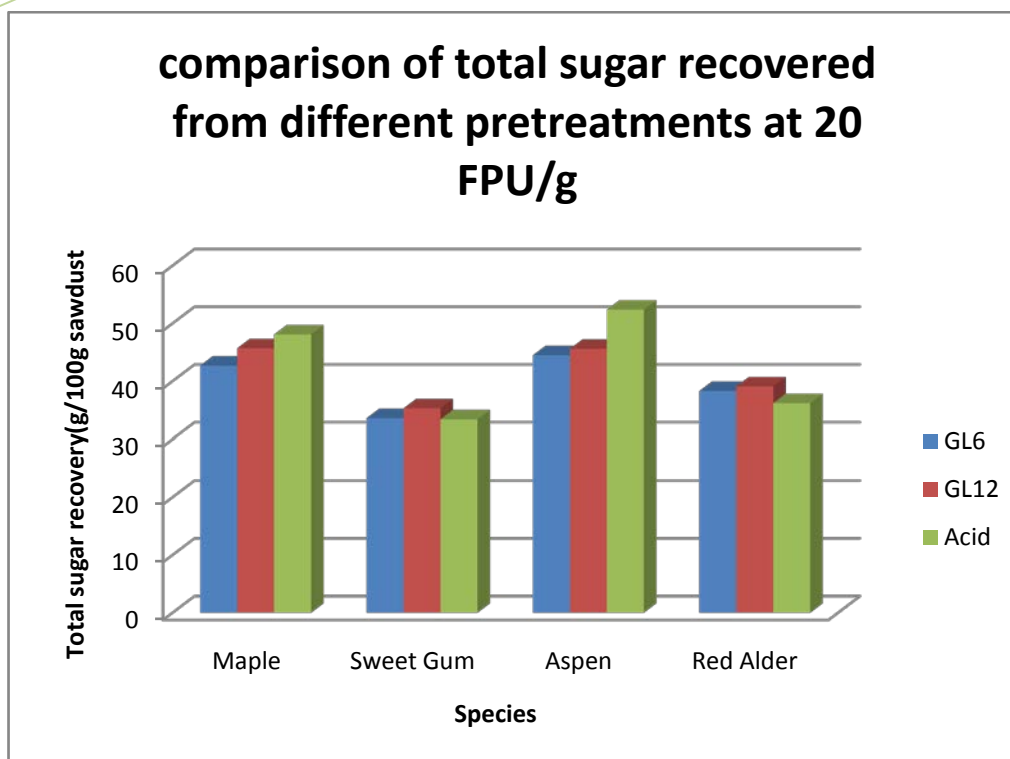


Figure2. Comparison of total sugar recovered of all species from different pretreatments at 20 FPU/g

Low Lignin Transgenics

Changes in lignin and LCC structures in the set of *P. tricarpa* transgenics with low lignin content during wood saccharification process consisting of mild acid pre-treatment (pH=3, 180°C, 20 min.) followed by enzymatic hydrolysis were studied. The set consisted of wild type and transgenic control TG31 (no manipulations done) woods (lignin content ca 22-23%) and two transgenics (TG 4 and 12) with reduced lignin contents, ca 12 and 15%, correspondingly. The protocols for isolation of lignin enriched with LCC moieties and the NMR analysis have been reported earlier. Lignin/LCC preparations were isolated from wood and from the residues after acid pre-treatment (AH) and after enzymatic hydrolysis after pre-treatment (AEH). Lignin/LCC preparations were also isolated from residues after direct enzymatic hydrolysis (EH) of wood (without pre-treatment). Lignin/LCC preparations were comprehensively characterized by a combination of quantitative HSQC and ¹³C NMR. The amount of the major lignin and LCC units after different treatment and the original MWL (orig.) are reported in Table 2.

The results obtained showed that changes in lignin structure during acid pretreatment and enzymatic hydrolysis were very limited. The amounts of major lignin units, such as

β -O-4, β - β , β -5 structures, OMe groups, S/G ratio were not significantly changed. Most of ester moieties (acetyl groups and *p*-hydroxybenzoic acid esters) were cleaved during the acid pretreatment. However, more than half of the original *p*-hydroxybenzoic acid esters were present in the lignin as the corresponding acid indicated that these moieties are incorporated into lignin structure via C-C linkages. The major structural changes were observed in LCC structure, both in the transgenics and control samples. Lignin-xylan linkages of benzyl ether type and γ -ester LCC were degraded during the acid pre-treatment. In contrast, lignin-glucan benzyl ether linkages were more stable in AH in the control samples. Phenyl glycoside LCC linkages were drastically degraded both in AH and EH. Significant degradation of LCC along with *p*-hydroxybenzoic acid esters in the transgenics can explain their higher reactivity in the saccharification processes as compared to that of control trees.