Organosolv pretreatment of Sitka Spruce wood: conversion of hemicelluloses to ethyl glycosides

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Abstract

A range of organosolv pretreatments, using ethanol: water mixtures with dilute sulphuric acid, were applied to Sitka spruce sawdust with the aim of generating useful co-products as well as improving saccharification yield. The most efficient of the pretreatment conditions, resulting in subsequent saccharification yields of up to 86%, converted a large part of the hemicellulose sugars to their ethyl glycosides as identified by GC/MS. These conditions also reduced conversion of pentoses to furfural, the ethyl glycosides being more stable to dehydration than the parent pentoses. Through comparison with the behaviour of model compounds under the same reaction conditions it was shown that the anomeric composition of the products was consistent with a predominant transglycosylation reaction mechanism, rather than hydrolysis followed by glycosylation. The ethyl glycosides have potential as intermediates in the sustainable production of high-value chemicals.

Keywords: Ethylglycosides, softwood, Organosolv pretreatment, transglycosylation

1. Introduction

Second-generation liquid biofuels, produced from lignocellulosic biomass, are widely seen as a way to satisfy future demand for transportation energy without inflating food prices (Field et al., 2008). Since lignin is not fermentable, the use of lignocellulosic materials leads to lignified residues that can be burnt or utilised as co-products. Lignin must be disconnected from cellulose during a pretreatment step to allow the cellulose to be saccharified for fermentation (Bommarius et al., 2008; Zhu et al., 2008). The lignocellulosic biomass is usually subjected to one- or two-stage pretreatments intended to fractionate the different polymers (hemicelluloses, cellulose and lignin) and increase the glucose yield (Pan et al., 2005; Panagiotopoulos et al., 2013). Softwoods, grown sustainably and disconnected from food markets, are suitable raw materials for biofuel production (Shuai et al., 2010). Their lignin content, typically 30% of the dry matter, must be reduced to permit saccharification. A delignification step using organosolv pretreatment improved saccharification yields from softwoods (Arato et al., 2005; Mabee et al., 2006). The saccharification products of non-cellulosic polysaccharides such as xylans (pentosans) and glucomannans can also be fermented by some organisms, but xylose and, more particularly, its degradation product furfural are unutilised or toxic to many of the most widely used micro-organisms (Pienkos and Zhang, 2009). Pentosans and xylose can therefore have negative value rather than being useful co-products. Efficient production of a range of economically viable co-products is the basis of the biorefinery concept (Ragauskas et al., 2006), and realising this concept is a major 21st-century challenge. New routes to valuable co-products would therefore be desirable but the possibilities depend on the raw material (Pan et al., 2005), and particularly on the nature of the lignin and non-cellulosic polysaccharides.

The research described here demonstrates the feasibility of Organosolv pretreatment applied to Sitka spruce, the most widely grown softwood species in the UK, and identifies a group of co-products derived from hemicelluloses and offering potentially high added value.

2. Materials and Methods

2.1. Materials

Sitka spruce sawdust was supplied from a commercial sawmill handling timber from Southern Scotland and Northern England. The sawdust was freezer-milled to pass a 1 mm sieve and the 300-1000 μm size fraction was selected for this study. The dry matter content of the sawdust was 91.2%. Its composition was 39.2 ± 1.8% Glucan; 9.8 ± 0.4% Mannan; 4.3 ± 0.3% Arabinoxylan; 1.9 ± 0.1% Galactan and 29.8 ± 0.1% Klason lignin. The sawdust sample was stored in the freezer.

2.2. Organosolv pretreatment

A range of organosolv pre-treatments were examined in order to select conditions leading to effective lignin extraction and subsequent saccharification. Sitka spruce sawdust (5.5 g) was heated with acidified aqueous ethanol in a 75 ml stainless-steel pressure vessel (Swagelok) with a liquid: solid ratio of 10:1. A total of 23 pretreatment conditions were tested, of which 6 were examined in detail: these are described in Table 1. The pretreatment temperature, ranging from 150 ˚C to 180 ˚C, was reached with a ramp of 25 ˚C/min and maintained from 25 to 85 min. After cooling, the liquid was filtered through glass filter paper (no.4). The solid residue was washed with 50 ml of ethanol/water and oven dried at 60 ˚C overnight. The filtrate was transferred to a 250 ml vol. flask. A 10 ml aliquot was withdrawn

77   and mixed with 1 ml of myo-inositol (20 mg/l) as internal standard for monosaccharide

78   analysis.

79   Table 1. Selected conditions for Organosolv pretreatment of Sitka spruce sawdust.

	1	2	3	4	5	6
Ethanol (%v)	50	50	60	70	60	70
Temperature (°C)	180	180	180	180	180	180
Time (min)	80	60	60	60	60	60
Sulphuric acid (%w)[a]	1.25	1	1	1	0.75	0.75

80   [a]Based on the dry mass of wood starting material

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82   **2.3. Enzymatic hydrolysis**

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84   The solid residue from each ethanol organosolv pretreatment was saccharified in

85   citrate buffer (50 mM, pH 4.5) at 2% load with β-glucosidase supplemented cellulase at 20

86   FPU/g of dry matter (FPU/CBU = 2/1). To each 200 mg of sample of organosolv residue 92

87   μl of Celluclast 1.5 (Sigma-Aldrich, 44FPU/ml) and 7.2 μl of Novozym 188 (Sigma-Aldrich,

88   282 CBU/ml) were added. Reaction mixtures were incubated at 45 °C for 68 h, on a shaker at

89   60 rpm. The saccharification residues were filtered on sintered crucibles, washed with

90   deionised water and dried at 60 °C overnight.

91   **2.4. Analytical methods**

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93   Moisture content was determined after oven drying at 105 °C for 16h. Polysaccharide

94   composition was determined after two-step hydrolysis (Rémond et al., 2010). Acid insoluble

95   lignin was determined by a two-step acid hydrolysis (Monties, 1984).

Monosaccharides and ethylglycosides were prepared for GC injection (Blakeney et al., 1983) and quantified using a HP 5890 gas chromatograph fitted with a Supelco SLB-5ms capillary column (30m x 0.32mm, 1mm thickness). A volume of 1 μl was injected in splitless mode. The oven temperature was increased from 180 ˚C to 280 ˚C at 2.5 ˚C/min and held at 280 ˚C for 30 min. Flow pressure was set to 12 psi. The injector temperature was set at 250 ˚C and the flame ionisation detector temperature was set at 280 ˚C. Quantification was performed using pure monosaccharide and ethylglycoside standards with myo-inositol as internal standard.

Ethylglycosides were identified using a Finnigan Trace GC Ultra/MS fitted with an Agilent HP-5 capillary column (30m x 0.32mm, 0.25mm thickness). The injection volume was set at 1 μl in 1/10 split mode. The oven temperature was increased from 100 ˚C to 280˚C at 3˚C/min and held at 280 ˚C for 20 min. Helium flow rate was 1ml/min. Injector temperature was set at 250 ˚C.

Furfural and hydroxymethylfurfural (HMF) were quantified using a Merck Hitachi HPLC fitted with a Waters ODS-2 C18 column and a diode array detector (280 nm). A 10 μl aliquot of the clarified solution was directly injected and eluted at 1 ml/min with acetonitrile/water containing 0.1% of tetrafluoro acetate. The solvent programming followed a linear gradient from 5% acetonitrile to 18.5% acetonitrile in 20 min and to 95% acetonitrile in 5 min. Quantification was obtained from furfural and HMF standard solutions.
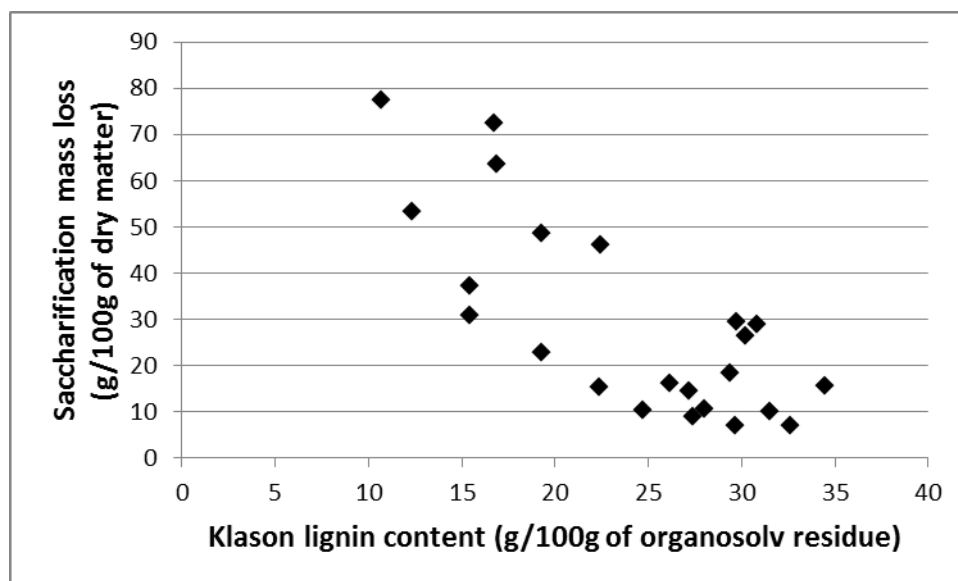
3. Results and Discussion

3.1. Enzymatic digestibility of pretreated Sitka spruce sawdust

Fig. 1. Saccharification mass loss of solid residues from organosolv pretreatments, as a function of their Klason lignin content.

The organsolv residue was then subjected to a saccharification step. Fig. 1 shows the saccharification mass loss as a function of lignin content. Decreasing the lignin content of the organosolv residue from 35% to 22% did not significantly affect subsequent saccharification but for pretreatments giving lignin content lower than 22%, saccharification mass loss increased steeply. It is well-established that delignification of wood facilitates the enzymatic hydrolysis of cellulose by reducing the 'hindering' effect of lignin (Agbor et al., 2011; Heiss-Blanquet et al., 2011; Kim, 2012; Pan et al., 2005). Nevertheless, low lignin content does not always lead to efficient saccharification. Other parameters such as hemicellulose content and the crystallinity and degree of polymerisation of cellulose also influence the degradability (Zheng et al., 2009).

The highest saccharification yield was obtained from the following organosolv conditions : 1% sulphuric acid in 60% ethanol (mass:vol:vol); 60 min at 180 ˚C. Six sets of experimental conditions (cf. Table 1, Pretreatments 1-6), all leading to a saccharification mass loss higher than 40% of the dry matter, were selected for more detailed assessment.

The polysaccharide content of the organsolv residue, estimated from the Klason lignin content and neglecting soluble lignin, ranged from 10 to 22% of the dry matter. The most efficient organosolv treatment, Pretreatment 4, permitted a mass loss of 87% of the polysaccharide content after 68 h saccharification. Increasing the ethanol content from 50% in Pretreatment 2 to 60% in Pretreatment 4 resulted in a 27% increase in saccharification mass loss but no further increase was observed with higher ethanol percentages. When the sulphuric acid concentration was reduced from 1% to 0.75% in Pretreatments 3-6, the saccharification mass loss decreased by 24%.

3.2. Composition of the soluble fraction

In most previous work the fraction remaining in solution after organosolv pretreatment has been considered to be of low value and too complex to be of interest. Here this fraction was extensively characterised and shown to contain products of potentially high added value, thus converting a waste stream into a resource.

During Pretreatments 1-6 the mass loss ranged from 46% to 54% of the starting material (Fig. 2A). The lignin content of the soluble fraction, estimated by difference from the Klason lignin remaining in the insoluble residue, ranged from 18% to 24% of the starting material. The soluble fraction also contained 20% to 24% of monosaccharides (quantified as their anhydrides) and products derived from these including furfurals and ethylglycosides (Fig. 2B). In consequence the unknown content of the soluble fraction did not exceed 6% of the dry matter. It can be assumed that most of the hemicelluloses were converted into monosaccharides, ethylglycosides and furfurals.
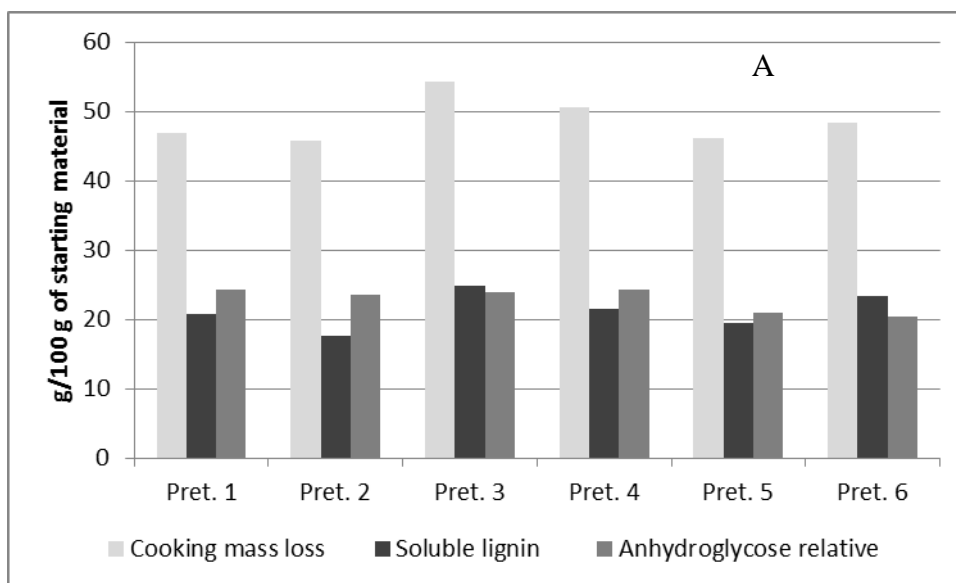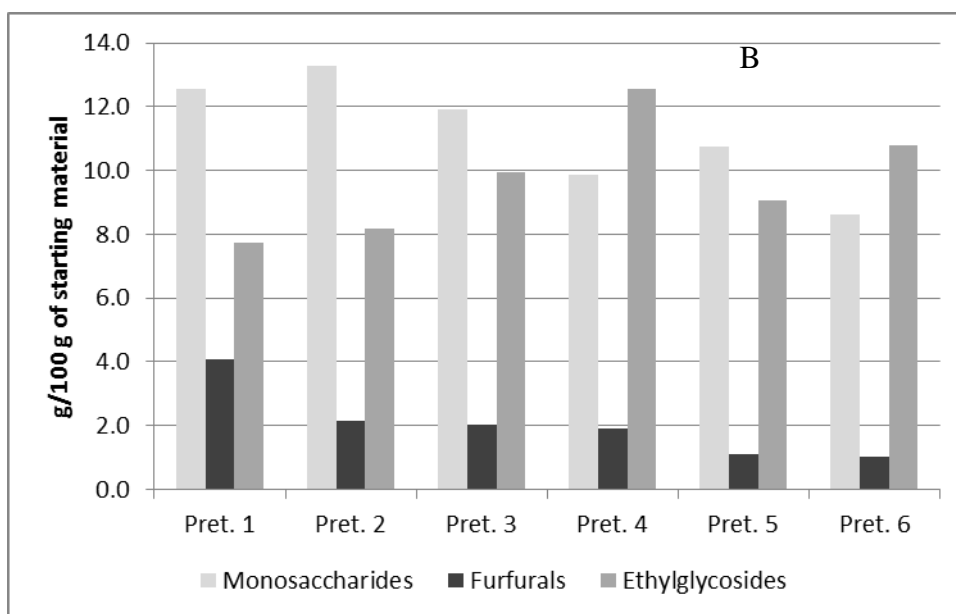
Fig. 2. Overall composition (A) and products derived from sugars (B) in the soluble fraction from orgonosolv pretreatment under six sets of selected conditions.

Within the soluble fraction, monosaccharides accounted for only 9% to 13% of the dry matter, decreasing in abundance with increasing ethanol content at fixed acid concentration (Pretreatments 2-4). This can be explained by the decrease in the apparent acidity of the liquid phase (Kim and Pan, 2010). Including hydroxymethylfurfural (HMF), furfurals ranged from 4% to 1.0% of the dry starting material, decreasing when lower acid concentrations and reaction times were employed and with increasing ethanol concentration

171 (Pretreatments 2-4). As illustrated in Figure 2B, the yield of ethyl glycosides is comparable

172 with the yield of free monosaccharides. Quantification of the products showed that up to

173 12.5% of the anhydroglycose (% dry matter) had been converted into ethylglycosides. The

174 increasing production of ethylglycosides with increasing ethanol content (Pretreatments 2-4)

175 also explained the reduced conversion to furfurals. The production of furfural or HMF from

176 pentoses or hexoses, respectively, requires monosaccharides to be in the ring-opened form.

177 Glycoside formation therefore precludes subsequent degradation to furfurals.

178 As illustrated in table S.1, the best pretreatment conditions allowed the conversion of

179 100 g of dry Sitka spruce sawdust to 38.6 g of D-glucose, 12.5 g of ethylglycosides and 13.7

180 g of isolated lignin. High purity lignin was isolated from the organosolv liquor by simple

181 precipitation in three volumes of water at pH 2 (HCl).

182 **3.3.   Ethylglycoside formation pathway**

183 As illustrated in Figure 2B, ethylglycoside and monosaccharide concentrations were

184 negatively correlated. The molar ratio monosaccharides: ethylglycosides was proportional to

185 the molar ratio water: ethanol. Reaction time and sulphuric acid concentration had no effect

186 on the monosaccharides: ethylglycosides ratio. It is interesting to note that equimolar

187 production of monosaccharides and ethylglycosides was reached at a water: ethanol ratio of

188 2.5:1. The high content of ethylglycosides suggests that transglycosylation was the favoured

189 pathway for polysaccharide breakdown. A further reason may have been the lower stability of

190 the monosaccharides to subsequent degradation, consistent with the lower content of furfurals

191 at higher ethanol: water ratios.

192 To identify the pathway of ethylglycoside production, organosolv conditions were

193 applied to a mixture of free pentoses (L-arabinose and D-xylose) and the products were

194 compared with the soluble fraction from pretreatment of Sitka spruce sawdust. GC/MS

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195 analysis allowed the furanoside and pyranoside ring forms to be distinguished, and

196 substantial differences were found in the proportions of the two ring forms in the mixtures of

197 ethylpentosides (Fig. S1). When organosolv conditions were applied to the free pentose

198 controls, mutarotation of the pentoses led to a near-equilibrium anomeric mixture of

199 ethylpentopyranosides (I) and ethylpentofuranoside (II). In contrast the soluble fraction from

200 pretreatment of Sitka spruce sawdust gave two dominant GC/MS peaks corresponding to the

201 ethylxylopyranoside (I) and the ethylarabinofuranoside (II), suggesting that the

202 ethylpentosides were generated mainly via transglycosylation of the hemicelluloses and to a

203 lesser extent by glycosylation of the free pentoses after hydrolysis.

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206   **4. Conclusions**

207   Organosolv pretreatments were successfully applied to Sitka spruce wood for the

208 production of glucose and co-products. Observations from the

209 ethylglycoside/monosaccharide molar ratio and the ethylglycoside ring structures suggested

210 that transglycosylation was the main pathway. Ethylglycosides are predicted to be a

211 potentially valuable co-product when organosolv pretreatments are applied to softwoods such

212 as Sitka spruce. They are preferred to the monosaccharides for the production of long chain

213 alkylglycosides via simple further transglycosylation (Bouxin et al., 2010). This approach is

214 consistent with the biorefinery concept where efficient production of a range of economically

215 viable co-products is required.

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