Two-Stage Fractionation of Hardwoods

Mehmet S. Tunc,[a,b,*] Juben Chheda,[c] Evert van der Heide,[d] Jerry Morris,[c] and Adriaan van Heiningen [a]

A two-stage lignocellulosic biomass fractionation process consisting of a formic- or acetic acid-reinforced wood autohydrolysis step followed by an ethanol-water treatment was applied to a mixture of Southern hardwood chips. The wood products in the hydrolysate were mostly monomeric xylose, other monomeric sugars, polymeric hemicelluloses, acetic acid, and a relatively small amount of lignin. The second step mostly dissolves sulfur-free lignin, while the fibrous residue consists mainly of cellulose fibers, which may be used for liquid fuels or pulp production. The yield composition, and quality of these products were determined as a function of treatment conditions, with the aim to develop an economic and robust biorefinery fractionation technology.

Keywords: Acetic acid; Biomass; Cellulose; Formic acid; Fractionation; Hardwood; Hemicelluloses; Lignin; Lignocellulose; Xylan

*Contact information: a: University of Maine, Department of Chemical and Biological Engineering, Orono, ME 04469 USA; b: American Process Inc., Atlanta, GA 30308 USA; c: Shell Global Solutions (US) Inc., Houston, TX USA; d: Shell Global Solutions International B.V., Amsterdam, the Netherlands; *Corresponding author: sefik.tunc@umit.maine.edu*

INTRODUCTION

The increasing concentration of greenhouse gases in the atmosphere and the high cost of oil have given impetus to the concept of a biorefinery. This technology involves converting biomass into a wide range of products, such as fuels, chemical intermediates, nanocellulose, and biomaterials. Woody biomass has great potential for the production of affordable fuels, in addition to traditional fiber products following the Integrated Forest Products Biorefinery concept (van Heiningen 2006).

Wood, the most abundant renewable raw material on earth, primarily consists of cellulose, hemicelluloses, and lignin, with minor amounts of extractives and ash. Cellulose is a homopolymer of glucose that is distributed in about equal amounts of crystalline and amorphous forms and represents about 40 to 50% of the wood. The amorphous hemicellulose consists of several different hexose and pentose sugars, acetic acid, and uronic acids, while lignin consists mostly of aromatic phenyl-propane units. The percentages of these components vary depending on the wood species. Generally, about 70% of all wood consists of polysaccharides (cellulose and hemicelluloses), with lignin representing most of the remaining 30% (Sjöström 1993).

Because the three main components in wood have very different characteristics, fractionation rather than "pretreatment" enables optimum economic utilization of the separate components (Bozell 2010). Currently, the biggest barrier to utilization of lignocellulosic feedstock for the production of fuels and chemicals is the economic and efficient fractionation of biomass into its constituents. Consequently, biomass fractionation is critical to the success of the biorefinery concept (Myerly *et al.* 1981).

Hemicelluloses are relatively easy to remove from hardwoods (and annual fibers) by autohydrolysis, *i.e.*, hydrolysis in water catalyzed by organic acids (mostly acetic acid) that are released from the wood itself (Brasch and Free 1965; Conner 1984; Conner and Lorenz 1986; Garrote *et al.* 1999; Tunc and van Heiningen 2008a; 2008b; 2009). The hemicelluloses are removed in oligomeric form (up to a DP of about 40 to 70), while the cellulose remaining in the wood is not dissolved (Tunc and van Heiningen 2011). Thus, a logical approach would be to remove the hemicelluloses in the first stage of the fractionation process. For biofuel and chemical production, the extracted hemicelluloses must be hydrolyzed to monosugars and organic acids before further processing. Therefore, it would be advantageous if the hydrolysis could be combined with the hemicellulose extraction stage. Because acetic acid, a major component of hardwood hemicelluloses (Sjöström 1993), must be separated from the extract in downstream processing, a practical approach for the first stage of the fractionation process is to extract the hemicelluloses with water reinforced by recycled acetic acid and formic acid, the latter being a sugar degradation product (Fengel and Wegner 1984).

Water pre-hydrolysis of hardwood chips leads to the formation of lignin-based precipitates that adhere to reactor walls and piping and are very difficult to remove. These precipitates form when the temperature of the extract is lowered (due to the release of pressure) and also when the extracted hemicellulose oligomers are further hydrolyzed over time (Leschinsky *et al.* 2009). However, the precipitates may not form, or may be dissolved, if a 50% (w/w) ethanol-water mixture is used to displace the hemicellulose extract in a subsequent treatment without pressure release, because ethanol is a much better lignin solvent than water.

Autohydrolysis followed by organosolv (mostly 60% w/w ethanol-water) delignification has recently been reported by a number of groups. This two stage approach has been applied to *Eucalyptus globulus* wood meal (Romani *et al.* 2011), a mixture of eastern Canadian hardwood chips (Liu *et al.* 2011), wheat straw (Huijgen *et al.* 2012), and grape stalks (Amendola *et al.* 2012). The temperatures during autohydrolysis are generally about 180 °C or higher, while the organosolv stage is performed at temperatures ranging from 180 to 220 °C. In the present study lower temperatures are used in the prehydrolysis step (120-170 °C) because of the reinforcement with formic or acetic acid, while the organosolv temperature range is also lower at 120-170 °C. Thus, in the present study the organosolv treatment is functioning more as a lignin dissolution and washing step rather than as a delignification step. Also, the ethanol concentration was taken as 50% (w/w), similar to the ALCELL process (Ni and van Heiningen 1996).

Acid-catalyzed prehydrolysis has been studied by many groups and applied to a large number of lignocellulosic feedstocks, including wood and annual plants. The acids include organic acids such as acetic acid (Conner and Lorenz 1986), maleic acid and oxalic acid (Lee and Jeffries 2011; Lim and Lee 2013), and p-toluenesulfonic acid (Amarasekara and Wiredu 2012) and inorganic acids such as hydrochloric acid (Conner *et al.* 1985), phosphoric acid (Avci *et al*. 2013) and sulfuric acid, with the latter being the standard to compare with. Formic acid in very high concentrations (about 80%) has been used for delignification of woody material (Dapia *et al.* 2002; Sixta *et al.* 2004), and several processes have progressed to the pilot scale (Sundquist 1996; Anttila *et al.* 2006; Delmas 2008). However, we only identified one study (Xu *et al.* 2009) where formic acid was used at low concentrations of 4 g/L in prehydrolysis at 195 °C for 15 min. A very comprehensive comparison of the catalytic activities of three acids during prehydrolysis

with focus on the minimization of lignin precipitation was recently reported by Gütsch *et al.* (2012). The study was performed on *Eucalyptus globulus* wood particles (2.5-3.5 mm) at 0.02-0.10 M acetic acid, 0.01-0.10 M sulfuric acid, and 0.01-0.10 M oxalic acid, and the results were compared to that of autohydrolysis. The objective of the present study is to develop an effective but mild severity two-stage hardwood/agricultural lignocellulosic fractionation process consisting of an autohydrolysis step reinforced with formic acid or acetic acid, followed by an ethanol-water treatment to produce three product streams: a hemicellulose extract, a lignin extract, and cellulosic fibers.

EXPERIMENTAL

Materials

The chemical composition of the Southern hardwood mixture (SHM) used as a feedstock for development of the fractionation process of wood is summarized in Table 1.

Table 1. Chemical Composition of Southern Hardwood Mixture

Component	%	Component	%
Arabinan	0.51±0.01	AcG	3.23±0.05
Galactan	1.00±0.01	UAG	4.36±0.10
Glucan	42.80±0.61	Lignin	28.03±0.19
Xylan	15.17±0.03	Extractives	2.00±0.11
Mannan	2.13±0.05	Ash	0.38±0.08
AcG: Acetyl groups UAG: Uronic acid groups			

The SHM contained sweet and black gum (35%), oak (35%), maple (15%), poplar and sycamore (12%), and southern magnolia (3%). The average wood chip dimensions were 31 mm in length, 14 mm in width, and 4 mm in thickness.

Analytical grade acetic acid, formic acid, and ethanol with purity of 99.7%, 95%, and 99.5%, respectively, were purchased from Sigma-Aldrich and used for the experiments.

Methods

Experimental procedures

The fractionation experiments were conducted in a Modified ASE100 apparatus manufactured by Dionex. In addition to the modification described earlier (Tunc and van Heiningen 2008a), three more solvent bottles were connected to the system through a four-way switching valve immediately before the extraction cell for multi-stage extraction, as illustrated in Fig. 1.

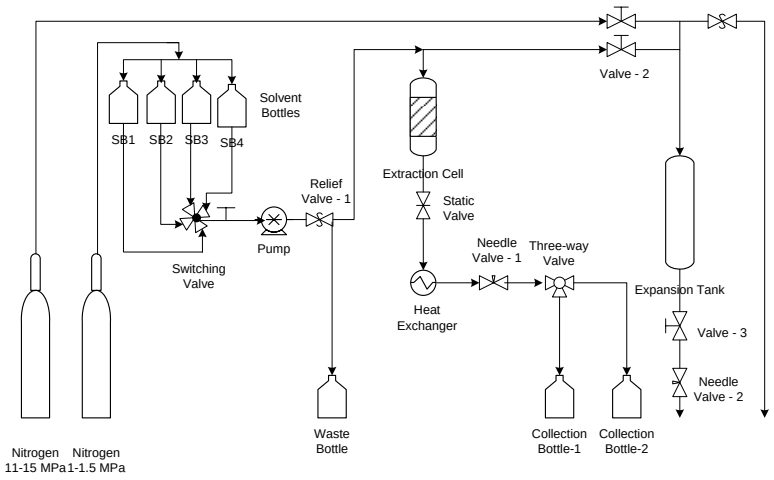

Fig. 1. Modified Dionex ASE100 extractor

Approximately 20 g of oven dried (od) wood chips were placed in a 100-mL extraction cell. Then, the cell was filled completely with aqueous solutions of acetic acid (HAc), formic acid (FA), or ethanol (EtOH). The liquid to wood ratio (L/W) in the extraction cell was about 4.5 (L/kg). The modified ASE100 can be maintained for a predetermined time at a desired extraction temperature. The time at the extraction temperature was corrected for the heat-up time by an algorithm provided by Dionex. At the end of extraction, the liquid in the cell was displaced using 150 mL of the same solvent/liquid that was used in the extraction stage at the extraction temperature. The displacement step was immediately followed by nitrogen gas purging for 5 min to free liquid in the extraction cell.

Analysis of solid phase

The ash content of the original SHM summarized in Table 1 and extracted wood was determined according to TAPPI Standard Method T211 om-85. The acid-insoluble lignin content, Klason lignin, was determined according to a method reported by Effland (1977), while the acid-soluble lignin content was determined using TAPPI Method 250. The uronic acid groups (UAG) were determined using the chromophoric group analysis method developed by Scott (1979). Hydrolysate was produced by a two-step hydrolysis with 72 and 4% sulfuric acid, and the monosugar content was determined by high-performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) (Davis 1998). Acetic acid in the hydrolysate was determined by HPLC using a refractive index detector and a BIO-RAD Aminex HPX-87H column. The mobile phase used was 5 mM H_2SO_4 with a flow rate of 0.6 mL/min and an oven temperature of 60 ºC.

Analysis of liquid phase

Monosugar content of the liquid phase was determined by direct injection of the samples into a Dionex HPAEC-PAD and also after hydrolysis of the sample with 4% H_2SO_4 at 121 °C in an autoclave for 1 h. The yield of oligomeric sugars was calculated from the increase in monosugar content due to the 4% H_2SO_4 hydrolysis. The lignin content of each extract was also determined using UV-Vis at an absorbance of 205 nm according to TAPPI Method 250. The UV absorption of sugar degradation products such

as furfural and hydoxymethylfurfural (HMF) is minimal at 205 nm. Acetic acid, furfural, and HMF in the liquid phase were also determined by HPLC, as described earlier.

RESULTS AND DISCUSSION

Two-Stage Wood Fractionation Process

The two-stage autohydrolysis-ethanol (EtOH) extraction process used is illustrated in Fig. 2. The first stage was autohydrolysis or acid-reinforced (acetic or formic acid) autohydrolysis at certain operation conditions (temperature and time varied). The second stage was extraction with 50% (w/w) EtOH at the selected temperature and time. Finally, the pre-extracted wood chips were subjected to a water-washing stage for 30 min without heat.

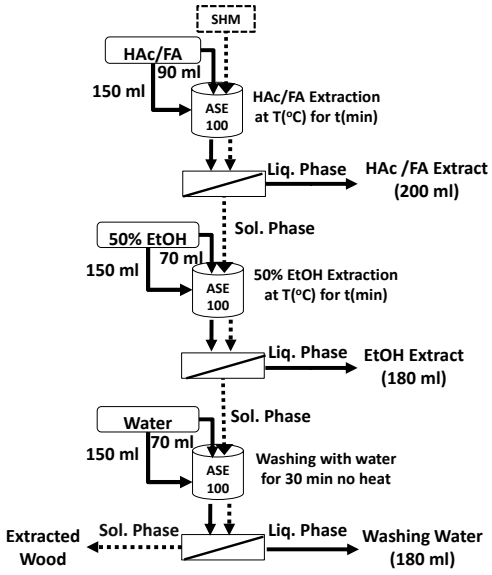

Fig. 2. Two-stage fractionation process of SHM chips with 50% EtOH following acid-reinforced autohydrolysis

Optimum Conditions for EtOH Extraction of Two-Stage Wood Fractionation

The total amount of wood dissolved and delignification obtained with 50% EtOH for 90 min following autohydrolysis at 160 ^{o}C for 90 min is shown in Fig. 3. Although the extraction yield and lignin removal increased with increasing EtOH extraction temperature, the increase was more pronounced at the highest temperature. It is clear from Fig. 3 that when the temperature of the EtOH stage increased from 150 ^{o}C to 160 ^{o}C, the total wood dissolution was more pronounced than that of the lignin removal. For this reason, the increased extraction yield with increasing EtOH temperature (from 150 ^{o}C to 160 ^{o}C) of water-pretreated SHM was probably due to the increased dissolution of carbohydrates in the EtOH extract at higher temperatures. These carbohydrates are presumably in the form of lignin-carbohydrate complexes (LCCs) (Tunc *et al.* 2010, Tunc and van Heiningen 2011), because pure oligomeric saccharides are rather insoluble in ethanol-water. The severity factor (Overend *et al.* 1987) of the present autohydrolysis of 160 °C for 90 min is 3.72. The total amount of wood dissolved at the highest EtOH temperature of 170 °C of 26% is much smaller than a value of about 37% obtained by

Romani *et al.* (2011) in their two stage autohydrolysis-organosolv process on eucalyptus at the same severity factor and organosolv conditions except for 60% (w/w) EtOH. Presumably, the higher EtOH concentration leads to more lignin dissolution (about 15% Klason lignin on original wood *vs.* about 8% in the present study).

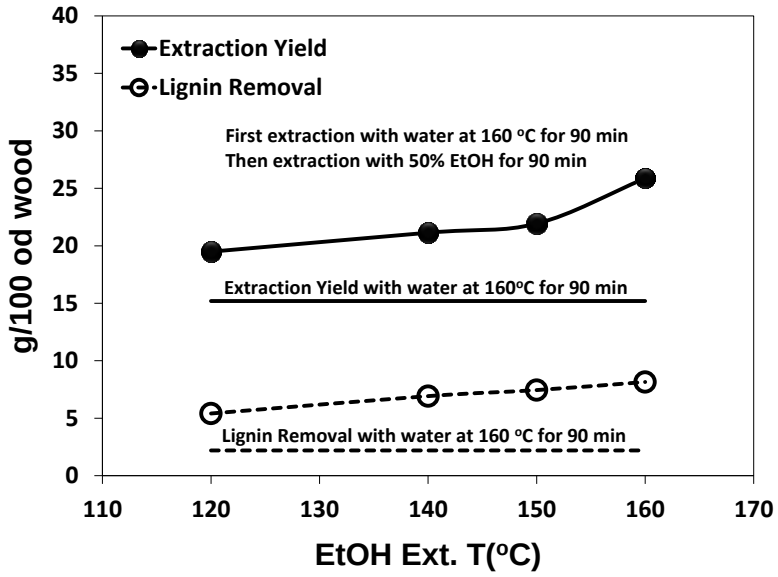

Fig. 3. Total extraction yield and lignin removal during 50% EtOH extraction for 90 min following autohydrolysis of SHM at 160 °C for 90 min *versus* EtOH extraction temperature

Figure 4 shows the amount of wood and lignin removal during multiple stage extraction with 50% EtOH at 160 °C for 60 min following autohydrolysis at 160 °C for 90 min. It is clear from Fig. 4 that delignification was only significant during the 50% ethanol extraction stage and that carbohydrates were mainly removed during the water extraction stage. Figure 4 also shows that the amount of lignin removed significantly increased with an increasing number of ethanol extraction stages, each of which was applied for 60 min. The total amount of wood removed after three ethanol extraction stages was only slightly higher than the amount after two ethanol extraction stages. In addition, during the third ethanol extraction stage, lignin-like condensation products were observed in the extract. Therefore, the use of two EtOH stages for 60 min at 160 °C is considered optimal. However, further investigation showed that a single ethanol extraction stage for a longer time is sufficient for efficient wood fractionation, as will be detailed below.

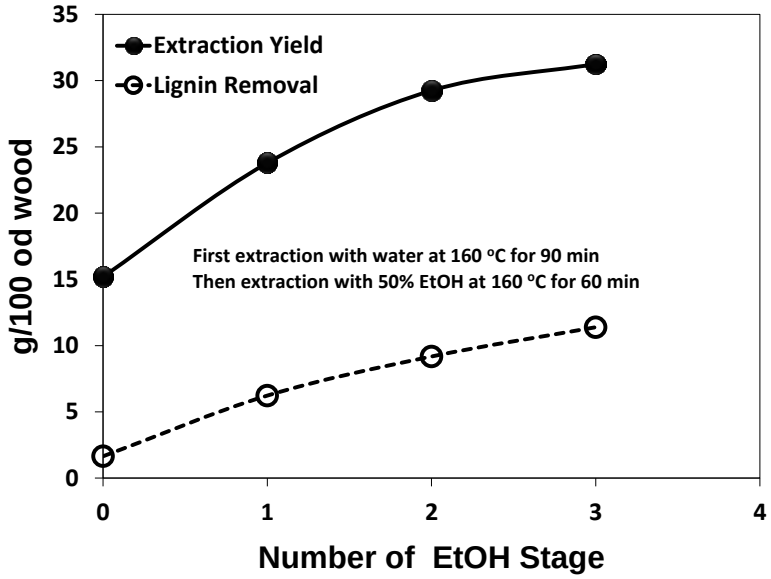

Fig. 4. Total extraction yield during 50% EtOH extraction at 160 °C for 60 min following water extraction at 160 °C for 90 min *versus* number of EtOH stage

To reduce the number of EtOH extraction stages, the kinetics of a single EtOH extraction stage after autohydrolysis was investigated. The total wood dissolution and delignification kinetics with 50% EtOH at 160 °C following autohydrolysis at 160 °C for 90 min are shown in Fig. 5. It is apparent from Fig. 5 that the total extraction yield increases only slightly after a third EtOH extraction at 160 °C for 60 min. Based on the results from Figs. 4 and 5, practical considerations of a single EtOH extraction stage at 160 °C for 90 min was adopted following autohydrolysis of SHM chips (at 160 °C for 90 min) in subsequent experiments.

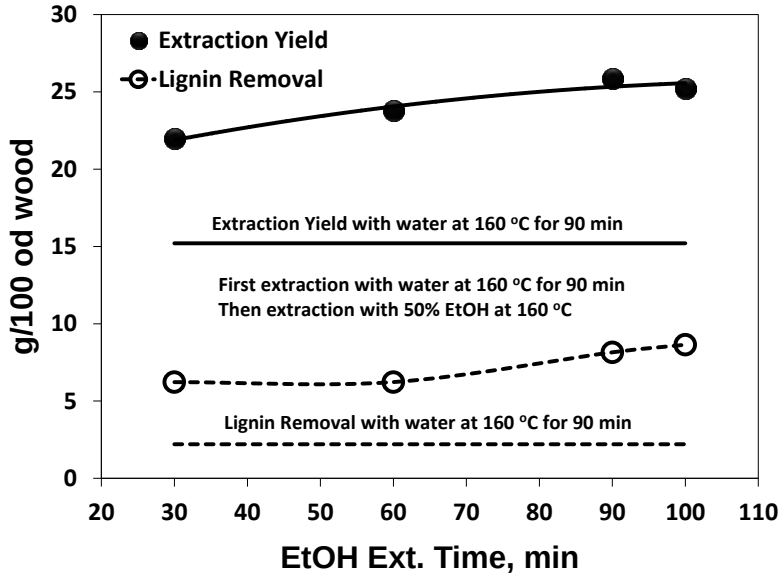

Fig 5. Total extraction yield and lignin removal during 50% EtOH extraction *versus* EtOH extraction time at 160 °C following autohydrolysis of SHM at 160 °C for 90 min

Effect of Acetic Acid Reinforcement of Autohydrolysis on Fractionation

The procedure of two-stage extraction, first with autohydrolysis reinforced with recycled acetic acid (HAc) at 160 °C for 90 min and then with 50% EtOH at 160 °C for 60 min, is illustrated in Fig. 2. Figure 6 shows the total extraction and lignin removal yields of SHM of the two-stage extraction. It is clear that the extraction yield and lignin removal increased with increasing HAc concentration. Gütsch *et al.* (2012) studied the autohydrolysis of *Eucalyptus globulus* with or without acetic acid addition (1.2-9.0 g/L) and reported that over this range of acetic acid reinforcement, the extraction was not significantly different from that of autohydrolysis. Considering that in the present experiments the maximum acetic acid concentration is an order of magnitude larger, the finding of Gütsch is not surprising. However, since it is known that the structure of KL is altered during prehydrolysis, making it easily soluble with organic solvents (Lora and Wayman 1980; Leschinsky 2009), increased lignin removal is expected at the present high acetic acid concentrations. This can be seen when the two-stage extraction is compared to the single-stage extraction with HAc reinforcement in Fig. 6, showing that the increase in total lignin removal yield increases at HAc concentrations higher than 20 g/L during the two-stage fractionation and is mostly due to improved lignin dissolution during the EtOH stage.

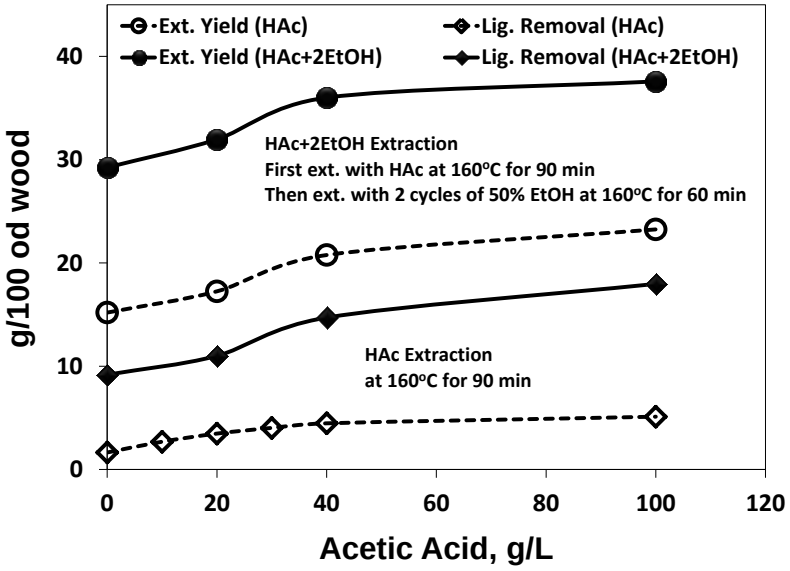

Fig. 6. Total extraction yield during the two-stage extraction *versus* HAc concentration

The retention of wood components after the two-stage fractionation is plotted *versus* HAc concentration in the first stage in Fig. 7. It is clear from Fig. 7 that cellulose (glucan) was retained, while the contents of other wood components, such as lignin, galactan, xylan, mannan, and uronic acid groups, decreased with increasing HAc concentration. However, most of the dissolution of these wood components had already occurred at 0 g/L HAc, *i.e.*, with pure autohydrolysis in the first stage. Therefore, it can be concluded that during two-stage extraction (at 160 °C for 90 min), the acetic acid released from wood during autohydrolysis is mostly responsible for the dissolution of wood components. The pH of the HAc extract ranges from 3.0 to 2.6 at HAc concentrations from 10 to 100 g/L, respectively.

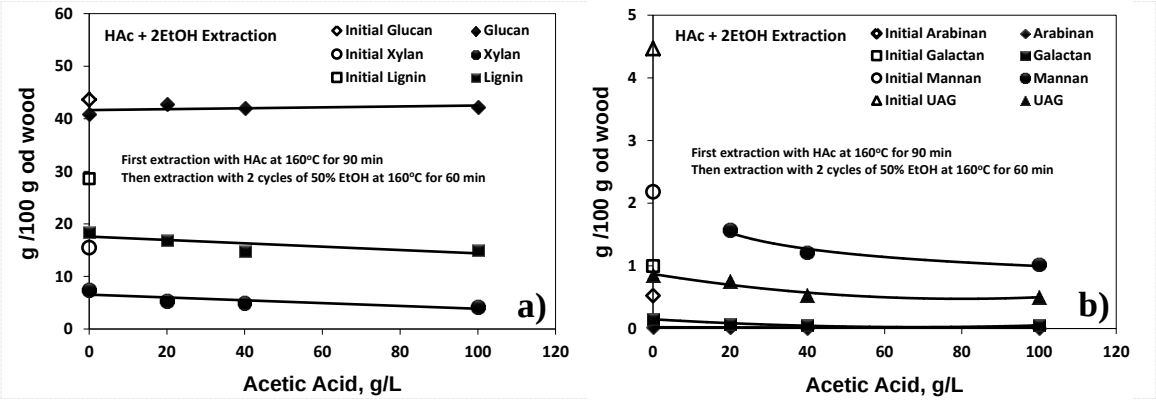

Fig. 7. Retention of wood during the two-stage fractionation versus HAc concentration in the first stage, a) glucan, lignin, and xylan, b) arabinan, galactan, mannan, and uronic acid groups (UAGs)

The sum of the sugar concentrations in the different extracts (HAc-reinforced autohydrolysis extract and two EtOH extracts) are plotted as a function of HAc concentration in Fig. 8. It is apparent from Fig. 8 that the concentration of xylan, the most abundant component in the reaction medium, increased with increasing HAc concentration and reached a maximum around 40 g/L HAc. This strongly suggests that degradation/decomposition of dissolved xylan takes place at HAc concentrations higher than 40 g/L. The concentration of glucan increased with increasing HAc concentration, indicating that some cellulose hydrolysis takes place at the highest HAc concentration. Figure 8 also shows that arabinan and galactan are degraded at HAc concentrations higher than 20 g/L. Thus, it can be concluded that HAc reinforcement of the autohydrolysis step above 20 g/L should be limited.

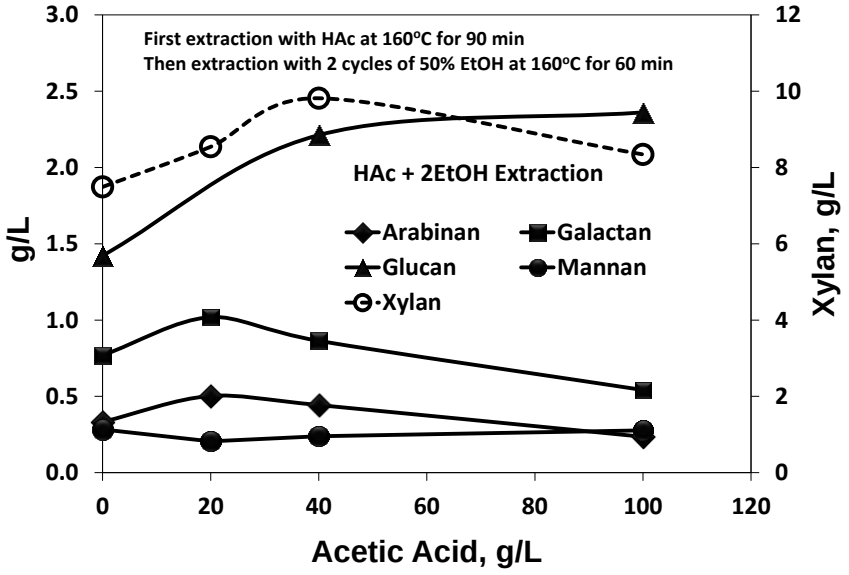

Fig. 8. Total sugar content of the extract during the two-stage fractionation *versus* HAc concentration

Effect of Formic Acid Extraction Temperature

The procedure of the two-stage fractionation of the SHM chips with 50% EtOH at 160 °C for 90 min after 10 g/L FA extraction for 90 min is again illustrated in Fig. 2. The variable in this series of experiments was the temperature of the FA extraction. As usual, the pre-extracted wood chips were subjected to a water-washing stage for 30 min without heat. Figure 9 shows the extraction yield using the two-stage extraction procedure. The extraction yield increased with increasing FA extraction temperature. Approximately 35% of od wood was dissolved during the two-stage SHM chips fractionation with 50% EtOH at 160 °C for 90 min after treatment with 10 g/L FA at 160 °C for 90 min. This compares to about 25% wood removal using autohydrolysis but otherwise the same conditions (see Fig. 5).

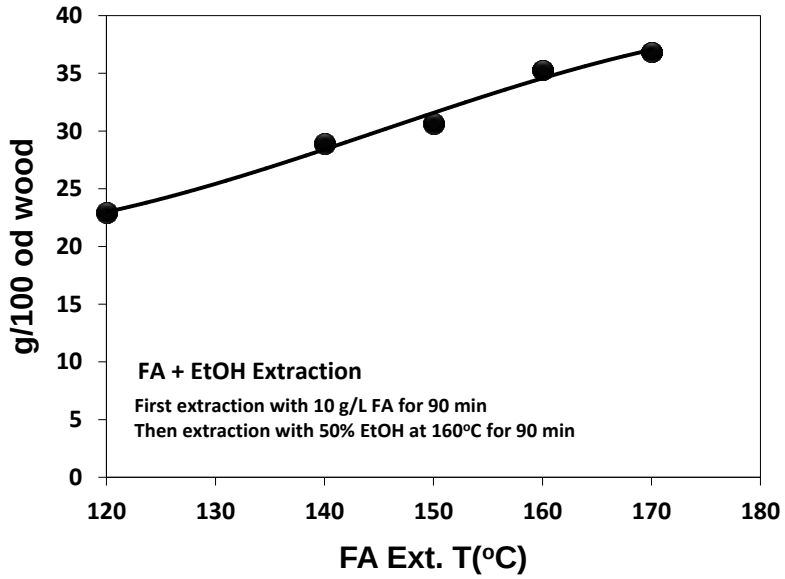

Fig. 9. Total extraction yield during the two-stage fractionation *versus* FA extraction temperature

The retention yields of the wood components after the two-stage extraction are plotted versus FA extraction temperature in Fig. 10. It is clear from Fig. 10 that, except for glucan (cellulose), the content of all other wood components, such as lignin, galactan, xylan, mannan, acetyl groups, and uronic acid groups, decreased with increasing FA temperature. Figure 10b shows that complete dissolution of arabinan has already taken place at the lowest extraction temperature of 120 °C and that the majority of uronic and acetyl groups were dissolved at this low FA extraction temperature. Figure 10b also shows that complete deacetylation and galactan dissolution took place at an FA extraction temperature of 160 °C.

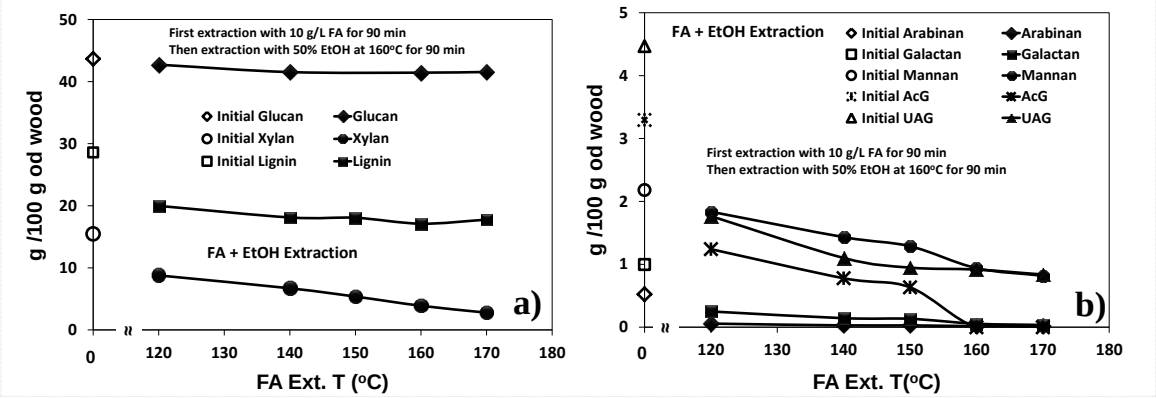

Fig. 10. Retention of wood after the two-stage fractionation *versus* FA extraction temperature, a) glucan, lignin, and xylan, b) arabinan, galactan, mannan, acetyl groups (AcGs), and uronic acid groups (UAGs)

The chemical composition of the EtOH and FA extracts obtained during the two-stage fractionation is shown in Fig. 11 as a function of FA extraction temperature. The pH of the FA extract is around 2.2, similar to that of the fresh 10 g/L formic acid solution. Figure 11a shows that lignin mostly dissolved during the EtOH extraction stage, even though its dissolution increased in both extracts. Figure 11b shows that the most abundant sugar, xylan, reached a maximum at 160 ^{o}C, indicating degradation above this temperature. Sticky (glue-like) lignin condensation products were observed at 170 ^{o}C, which could be problematic at the industrial scale (Leschinsky *et al.* 2008). Therefore, operation temperatures higher than 160 ^{o}C are not suggested. Gütsch *et al.* (2012) found that the formation of insoluble lignin in the extract decreased when the autohydrolysis temperature was decreased. They also found that with addition of strong acids (such as sulfuric and oxalic acid) and allowing the hydrolysis temperature to be decreased while maintaining the same wood dissolution, significantly less insoluble lignin was present in the extract compared to that in an autohydrolysis extract. However, in all cases insoluble lignin is seen in the aqueous extracts. Therefore, the present finding that no precipitates are seen in the FA extract or following EtOH extract is highly significant. The amount of xylan dissolved in the EtOH extract was higher than that dissolved during FA extraction up to an FA temperature of 150 ^{o}C. The likely explanation is that xylan in the EtOH extract was removed with lignin in the form of lignin carbohydrate complexes (LCCs) (Tunc *et al.* 2010, Tunc and van Heiningen 2011). At higher FA extraction temperatures, the chemical bond between xylan and lignin was progressively broken, which led to more xylan in the FA extract and correspondingly less in the EtOH extract. Figure 11 shows that a selective sugar and lignin fractionation can be accomplished during two-stage fractionation with 50% EtOH extraction at 160 ^{o}C for 90 min (for lignin dissolution) following a 10 g/L formic acid extraction at 160 ^{o}C for 90 min (for carbohydrate dissolution).

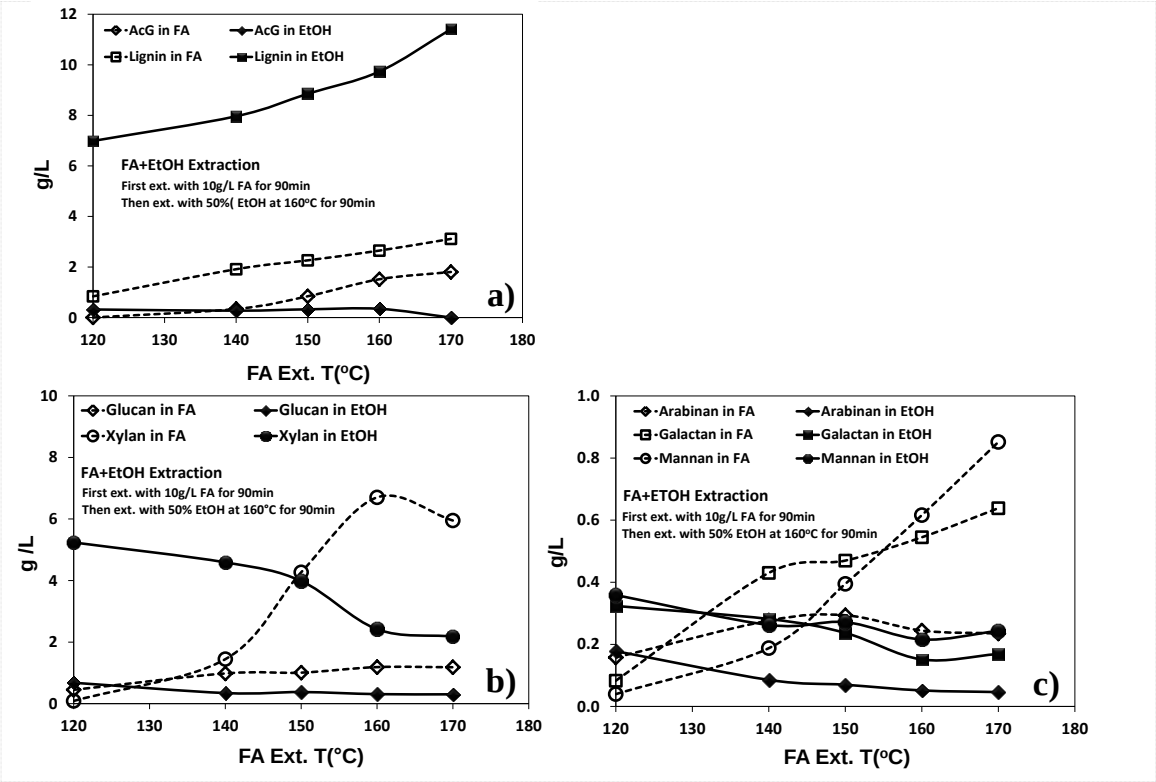

Fig. 11. Composition of the FA and EtOH extracts during the two-stage fractionation *versus* FA extraction temperature, a) acetyl groups (AcGs) and lignin, b) glucan and xylan, c) arabinan, galactan, and mannan

The sum of the monomeric and oligomeric concentrations in the FA plus EtOH extracts for the different sugars is shown in Fig. 12. At lower FA extraction temperatures ($T < 150\ ^{o}C$), all the sugars except arabinan dissolved in oligomeric form. When the FA extraction temperature increased, the amount of monosugars in the extracts increased due to hydrolysis of the oligomeric sugars to monosugars. The main sugars in the reaction medium were present almost equally in oligomeric and monomeric forms at 160 ^{o}C. Thus, formic acid addition at 10 g/L during prehydrolysis leads to more hydrolysis of the sugar oligomers to monomers than that seen in autohydrolysis. However, complete hydrolysis of the oligomers obtained with sulfuric acid or oxalic acid addition as found by Gütsch *et al.* (2012) at a similar wood dissolution was not achieved. This agrees with the observation of Gütsch that the wood dissolution is governed by the severity factor, but the sugar oligomer hydrolysis is governed by the pH of the fresh extract, with the pH of the FA solution of 2.2 lying in between that of 0.1 M oxalic acid (pH 1.3) and 0.15 M acetic acid (pH 2.9). Figure 12b shows that decomposition of dissolved arabinan takes place at FA extraction temperatures higher than 150 ^{o}C.

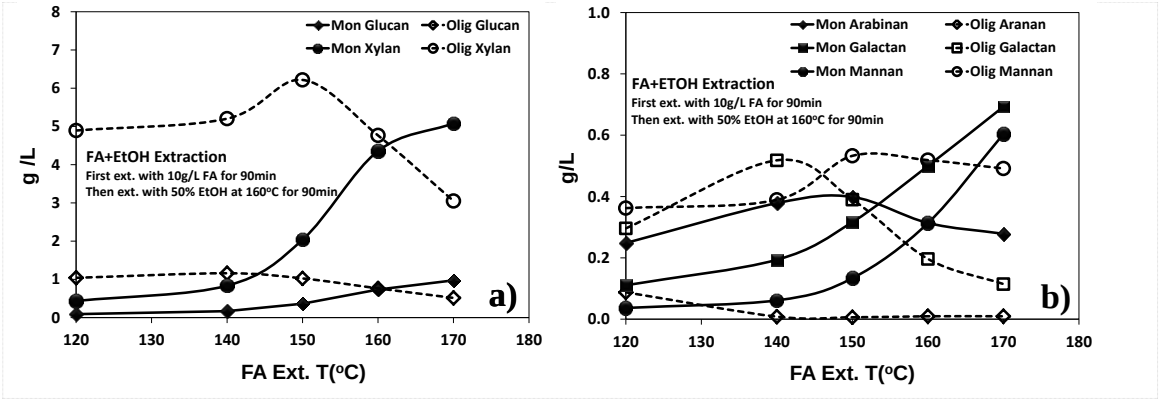

Fig. 12. Mono- and oligosugars in the extract during the two-stage fractionation *versus* FA extraction temperature, a) glucan and xylan, b) arabinan, galactan, and mannan

CONCLUSIONS

1. A two-stage lignocellulosic fractionation process of aqueous FA/HAc extraction (which mostly removes carbohydrates) followed by EtOH-water (50% EtOH) extraction (which mainly removes lignin) was explored.
2. The selectivity of the lignin removal in the second stage was positively affected by an increase in the severity of the acid treatment in the first stage.
3. Based on the study of the effects of operating parameters such as (acetic/formic) acid concentration, time, and temperature of the extraction, a selective sugar and lignin fractionation process was achieved for SHM chips without the formation of sticky lignin condensation products (LCPs). The conditions were as follows: extraction with 10 g/L formic acid (FA) at 160 ^{o}C for 90 min, which led to a 12.4% carbohydrate and 3.0% lignin dissolution (all % on an original wood basis), followed by extraction with 50% EtOH at 160 ^{o}C for 90 min, which gave 9.7% lignin and 3.4% carbohydrate dissolution.
4. Somewhat smaller amounts of wood sugars were extracted with acetic acid, but only at an acetic acid concentration of about 40 g/L, *i.e.*, almost one order of magnitude higher than that of formic acid (5 to 10 g/L). This is mostly because formic acid (pKa of 3.75) has an acidity one order of magnitude higher than that of acetic acid (pKa of 4.76) (Weast and Astle 1981).

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