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Investigation of the sugar content in wood hydrolysates with iodometric titration and UPLC-ELSD

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Abstract. Autohydrolysis of birch wood is a mild pretreatment process, which gives a notable yield of sugars – monosaccharides and oligosaccharides – in the aqueous hydrolysate, while a solid lignocellulose fraction can be further processed into other valuable products within a biorefinery concept. In this work two analytical methods – iodometric titration and ultra-high performance liquid chromatography with evaporative light scattering detection (UPLC-ELSD) – have been optimized and compared for the determination of the sugar content in series of birch wood hydrolysates. The results of both methods were consistent and showed that the highest yield of sugars, mostly xylose, was obtained by hydrolysis at 180 °C after 75 min.

Key words: sugar analysis, birch wood hydrolysis, pentoses, hexoses, xylose, oligosaccharides.

INTRODUCTION

A biorefinery producing a combination of value-added products is at the core of a bio-based economy, which employs renewables instead of fossil resources (Star-COLIBRI, 2011). The biorefinery concept involves sequential processes for transforming biomass, such as wood. Hydrolysis is often the first stage in a biorefinery concept, during which wood is pretreated yielding a sugar-rich aqueous fraction (hydrolysate) and a solid lignocellulose fraction, which can be further subjected to other processes, such as pyrolysis (de Wild et al., 2011).

Hot water treatment is a pretreatment method which is obtaining popularity by its green and sustainable approach and moderate temperatures, and the hydrolysate mostly contains the destruction products of hemicelluloses. Hemicelluloses are a group of polysaccharides in wood or other plant-based biomass, and the qualitative and quantitative composition (e.g. the ratio of pentosans and hexosans) of hemicelluloses differs depending on the species of the material, but the composition of the hydrolysates is also influenced by the processing conditions – time, temperature, the presence or absence of a catalyst (Borrega & Sixta, 2015; Nitsos et al., 2016; Chen et al., 2017). Autohydrolysis is a comparatively mild process without the addition of a catalyst (Silva-Fernandes et al., 2015). However, acetic acid produced from hemicelluloses themselves acts as a catalyst. The products of autohydrolysis of wood are mostly C_5

monosaccharides and lower oligosaccharides, which can be used for the production of bioethanol (Luque et al., 2014) or 5-hydroxylmethylfurfural.

The most popular sugar analysis methods are classical chemical methods, such as titration using Fehling's reagent (Porreta et al., 1992), spectrophotometry (Timmel et al., 1956), or liquid chromatography (Tihomirova et al., 2016). In this work two different methods are used – iodometric titration and ultra-high performance liquid chromatography with evaporative light scattering detection (UPLC-ELSD).

Iodometric titration of sugars is based on the oxidation of α-diols by sodium periodate. Unlike most of other titrimetric sugar determination methods, this method does not determine all reducing agents, but only α-diols, so it is more specific (Meile et al., 2014). While the titrimetric method is suitable for determining all sugars present in the samples as a chemical class, liquid chromatography is used to determine individual compounds. HILIC or ion exchange type liquid chromatography columns are used to analyse sugars (Oliver et al., 2013), but the separation of the many saccharides can still be a challenge, especially the separation of isomers (Wang et al., 2012; Nagy & Pohl, 2015). There are also limitations to the choice of detectors for sugar analysis, because due to the lack of chromophores the most widely used UV spectrophotometric detector is not applicable, but refraction index detectors, which are inexpensive and easy to operate, are not compatible with gradient elution and are also not very sensitive. A more recent alternative for sugar detection is the evaporative light scattering detector (Schuster, 2011).

The purpose of this work is to evaluate the applicability of iodometric titration and UPLC-ELSD analysis methods to determine the sugar content in wood or other industrial biomass hydrolysis products. Therefore, we present an overview of the method optimization, as well as results obtained for a series of birch (*Betula pendula*) wood hydrolysates.

MATERIALS AND METHODS

Materials

All chemicals were of analytical grade – purchased from Sigma Aldrich, and used without further purification.

Birch Wood Hydrolysis

300 g oven dry birch wood with particle size 0.2–0.63 mm was hydrothermally treated in a Parr 4554 high pressure reactor with 7.5 L volume. The treatment media was demineralized water at temperatures 150–200 °C. The ratio of water and wood was 15:1. Two hours after the necessary temperature had been reached, the reaction mixture was rapidly cooled, then it was filtered and washed, obtaining a solid lignocellulose residue 61–75 w% of the dry feedstock. The liquid samples for analysis were taken when temperature was reached (~90 min after heating started) after 0, 15, 30, 45, 60, 75, 90, 120 min.

Iodometric Titration

Kinetic curves for sugar oxidation with sodium periodate were obtained as follows. Aqueous solutions of hexoses (D-(+)-glucose, D-(+)-galactose and D-(+)-mannose) and pentoses (L-(+)-arabinose and D-(+)-xylose) were prepared with 4 mg mL^{-1}

concentration. A birch wood hydrolysate was diluted from 8 to 50 mL with deionized water. 0.1 mL 15% H_2SO_4 and 1 mL 0.2 M $NaIO_4$ was added to a series of 2 mL of each of the solutions and they were placed in a thermostat at 40 °C. After certain time (5, 60, 120, 180, 240 and 300 min) 10% ammonium molybdate was added to the oxidated solutions. After 15 min 1 mL acetic acid and 1 mL 10% KI was added and after another 15 min the solutions were titrated with 0.1 M sodium thiosulfate with starch as an indicator. Sample analysis was done by the same method with oxidation at 40 °C for 240 min. All analysis were performed in triplicate.

UPLC-ELSD

The UPLC experiments were performed on Waters ACQUITY UPLC equipment with a Waters ACQUITY UPLC BEH Amide column (1.7 μm, 2.1×100 mm) and a Waters ACQUITY UPLC ELS detector. The optimal ELS detector drift tube temperature was 50 °C, the nebulizing gas was nitrogen with 45 psi pressure, gain was set for 50. The gradient program was set for phase A – 80:20 acetonitrile/water with 0.1% ammonium hydroxide; phase B – 30:70 acetonitrile/water with 0.1% ammonium hydroxide. The results were acquired, integrated and processed using Waters Empower 3 software.

For the optimisation of separation conditions standard solutions of different sugars – 1,6-anhydro-β-D-glucose, D-(+)-xylose, L-(+)-arabinose, D-(+)-mannose, D-(+)-glucose, D-(+)-galactose, D-(+)-sucrose, D-(+)-cellobiose and D-(+)-raffinose were prepared in 50:50 acetonitrile/water. For quantitative analysis standard solutions of xylose were prepared with 0.02-0.24 μg mL^{-1} concentrations. A linear ($R^2 = 0.998$) calibration curve was used with equation $y = (4.35\times10^6)x-1\times10^6$. Hydrolysate samples were diluted from 10 to 25 mL with 50:50 water/acetonitrile. Solutions were filtered with KX Syringe filters (pore size 0.22 μm). The injection volume was 1 μL. All analysis were performed in triplicate.

Determination of by-products

Furfural and 5-HMF were determined by UPLC-PDA, using the same equipment as described before with a Waters ACQUITY UPLC BEH C18 column (1.7 μm, 2.1×50 mm). The gradient program was set for phase A – water with 0.1% formic acid; phase B – acetonitrile. For detection a Waters ACQUITY UPLC PDA detector was used with wave length 275 nm.

Acetic acid was determined by potentiometric titration with 0.1 M KOH (standardised with potassium hydrogen phthalate), using Radiometer Analytical SAS titrator TitraLab980.

RESULTS AND DISCUSSION

Iodometric Titration

Oxidation curves (Fig. 1) of solutions of several wood based sugars showed that the reaction rate was faster for pentoses than hexoses, however, all monosaccharides were completely oxidized at 120 min. Therefore, oxidation of samples for a time period at least 120 min would give accurate results for pentosan or hexosan hydrolysis products. There are differences in the formulas used for calculating the amount of pentoses and hexoses, because the periodate oxidation equations are different. Eqs 1 and 2 show that 1 mol pentoses reacts with 4 mol $NaIO_4$, but 1 mol hexoses – with 5 mol $NaIO_4$. On the

other hand, this stoichiometric difference is not significant, because taking into account the ratio of the stoichiometric coefficients and molecular masses, the final coefficients in the calculations are almost the same: 180 by 5 giving 36.0 for hexoses, and 150 by 4 giving 37.5 for pentoses. So, even if the sample contained a mixture of hexoses and pentoses, the accuracy of the results would still fall within 5%.

$$C_5H_{10}O_5 + 4\ NaIO_4 \rightarrow 4\ HCOOH + H_2CO + 4\ NaIO_3 \quad (1)$$

$$C_6H_{12}O_6 + 5\ NaIO_4 \rightarrow 5\ HCOOH + H_2CO + 5\ NaIO_3 \quad (2)$$

The oxidation curve of the birch wood hydrolysate sample was significantly different, as the reaction did not stop after 120 min. There are two likely reasons for the continuous increase of the reduced sodium periodate. Firstly, oligosaccharides are known to take longer to oxidize completely (Meile et al., 2014), so the presence of di-, tri- and higher saccharides or other sugar derivatives could be responsible for the rising oxidation curve after 120 min. Secondly, phenols produced from lignin or extractives could react with periodate unstoichiometrically (Pennington & Ritter, 1947; Antolovich et al., 2004; Gosselink et al., 2011) and increase the amount of the reduced periodate.

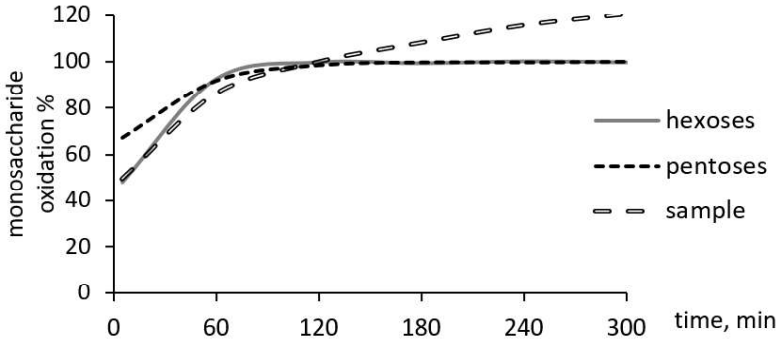

Figure 1. Oxidation curves of hexoses (mixture of glucose, galactose and mannose), pentoses (mixture of arabinose and xylose) and a sample of birch wood hydrolysate.

Even though the oxidation curves showed, that some extra periodate was reduced by the hydrolysis products, the iodometric titration method could be applied to characterize the content of sugars in hydrolysates, if a certain time of oxidation was maintained. The results might have a tendency to be increased by up to 20%, but this method could be a simple way of process monitoring by comparing the relative content of sugars in large series of wood hydrolysis product samples. It must also be noted that many samples can be prepared and oxidized simultaneously, thus decreasing the analysis time per sample. For example, if a series of 10 samples is analysed in triplicate, the oxidation time per sample is only 4 min (120 min by 30 titrations).

UPLC-ELSD

For the tested biomass related sugars the elution order from the amide-functionalized column was according to the molecular size and number of hydroxylgroups, as follows: levoglucosan, xylose, arabinose, mannose, glucose,

galactose, sucrose, cellobiose, raffinose. Fig. 2 shows the effect the column temperature had on the retention time and peak width. As described in literature (McCabe & Hudalla, 2010), because of mutarotation monosaccharides had broad and even split peaks at moderate column temperatures. This proved to be also true for the disaccharide cellobiose, which contained a reducing sugar moiety, unlike the nonreducing disaccharide sucrose and trisaccharide raffinose. Levoglucosan with its anhydrogroup between C1 and C6 also had a narrow peak regardless of the column temperature. With increasing the column temperature from 30 to 70 °C, the peak width for nonreducing sugars decreased by < 10% due to mass transfer processes, but the peak width of the reducing sugars decreased by up to 50%.

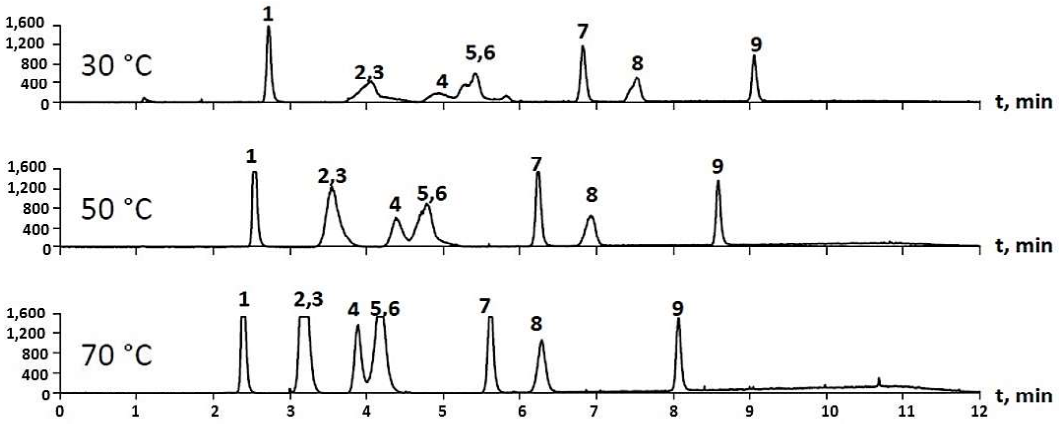

Figure 2. UPLC-ELSD chromatograms of several biomass related sugars with column temperature 30, 50 and 70 °C: 1 – levoglucosan; 2 – xylose; 3 – arabinose; 4 – mannose; 5 – glucose; 6 – galactose; 7 – sucrose; 8 – cellobiose; 9 – raffinose.

Despite our experiments with the composition of the mobile phase (varying proportions of water and acetonitrile with the addition of ammonium hydroxide or triethylamine), the peak pairs of xylose/arabinose and glucose/galactose remained overlapping, making it impossible to determine xylose in the presence of arabinose, just like glucose in the presence of galactose and vice versa. For the purpose of enzymatic processing of the sugars separation of C_5 and C_6 sugars as groups is sufficient, because many microorganisms that process the sugars into alcohol are pentose or hexose specific, instead of being specific for individual sugars (Azhar et al., 2017). However, there are some liquid chromatography columns which can separate xylose and arabinose, for example by ion chromatography (Zhang et al., 2017) or ligand exchange chromatography (Tiihonen et al., 2002). Neither of these methods are supported by UPLC columns, but the biggest disadvantage is that mobile phases containing sodium hydroxide (for ion chromatography), sulphuric acid or non-volatile salts (for ligand exchange chromatography) are not compatible with ELS detection or mass spectrometry. Since ELSD is a semi-universal detector, giving similar signals for analytes with similar volatilities, xylose and arabinose can be determined together as pentoses using the calibration curve of xylose, because in birch wood hydrolysates xylose is typically more abundant than other monosaccharides (Goldmann et al., 2017).

Analysis of Birch Wood Hydrolysates

Both sugar determination methods – titration and UPLC-ELSD – were applied to analyse series of birch wood hydrolysates. Fig. 3 shows a chromatogram of a sample of the hydrolysis products with complete separation of pentoses at 3.1 min and hexoses at 4.0 min. The precision for the methods was described as the relative standard deviation of the parallel measurements, which was < 4% and < 2% in all cases for titration and UPLC-ELSD, respectively. Results of recovery in spiked samples were 91 ± 2% and 92 ± 1%, respectively.

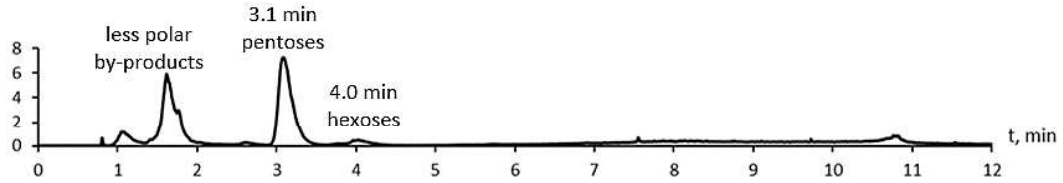

Figure 3. UPLC-ELSD chromatogram of birch wood hydrolysis products: pentoses eluted at 3.1 min, hexoses eluted at 4.0 min and less polar by-products co-eluted from 1 to 2 min.

Hydrolysis of birch wood was performed at six temperatures from 150° to 200 °C for up to 120 min. Fig. 4 illustrates the sugar content in the recovered hydrolysis liquids, depending on the treatment temperature and time. The results obtained by iodometric titration (total sugars) and UPLC-ELSD (pentoses, mostly xylose) were compared, showing a good agreement between the trends determined by the two methods. Both methods confirmed that the yield of sugars grew with the increase of the process temperature up to 180 °C. The highest yield of sugars was obtained at 180 °C after 75 min. Similar hydrolysis conditions have been proposed for optimal wheat straw pretreatment (Sidiras et al., 2011). After 75 min as well as with higher temperatures the yield of sugars significantly decreased.

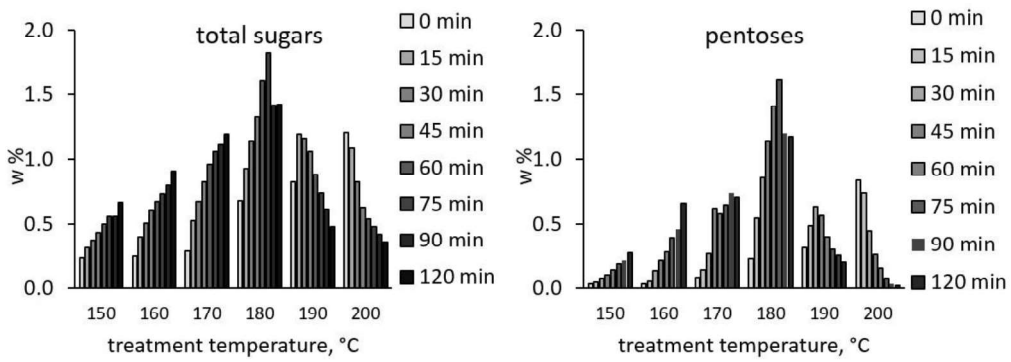

Figure 4. Sugar content in birch wood hydrolysates depending on the hydrolysis temperature: a comparison of the iodimetric titration (total sugars) and UPLC-ELSD (pentoses) results.

Fig. 5 shows the results of sugar analysis in the hydrolysates obtained at 180 °C. As mentioned before, the highest yield of sugars was obtained after 75 min, namely, the sugar concentration in the hydrolysate was 1.8%, which corresponds to 27% yield from the feedstock. UPLC-ELSD showed that the yield of hexoses was considerably lower

than that of pentoses, but the sum of the UPLC-ELSD sugar results (pentoses + hexoses in Fig. 5) were mostly in line with the titration results of total sugars, except for the hydrolysates which were treated for 30 min or less. This divergence of the two methods can be explained by the presence of oligosaccharides in the hydrolysates at the beginning of the treatment. Since the titration results included oligosaccharides, the total sugar concentration was about two times higher than the monosaccharide results obtained by UPLC-ELSD. After 30 min treatment the oligosaccharides were broken down to monomers, so the results obtained by both methods corresponded. The total sugars determined by iodometry had an increased result at 120 min, most likely because of a higher concentration of some lignin origin phenols, which could also react with sodium periodate.

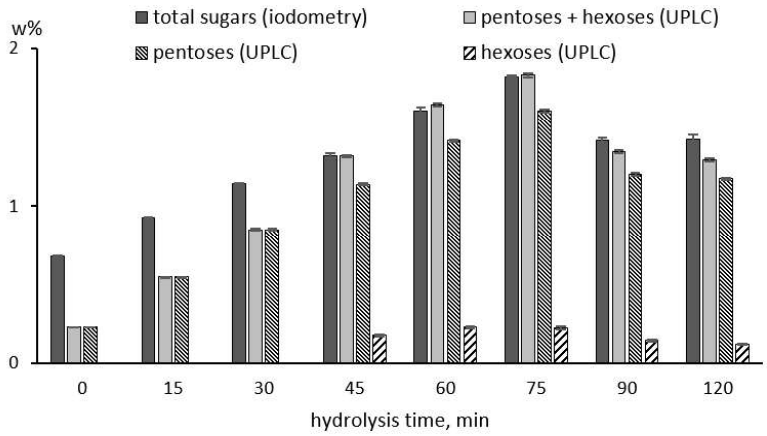

Figure 5. Sugar content in birch wood hydrolysates depending on treatment time at 180 °C, determined by titration as total sugars or UPLC-ELSD as pentoses and hexoses separately.

For a deeper understanding of the hydrolysis process acetic acid and aldehydes – furfural and 5-hydroxylmethylfurfural (5-HMF) were determined. The concentration of acetic acid in the hydrolysates increased almost lineary up to 6% with treatment time, acting as a catalyst and increasing the yield of the other products. The concentration of furfural and 5-HMF, which are known as the dehydration products of pentoses and hexoses (Rasmussen, 2014), increased up to 0.2% and 0.03% in the hydrolysates, respectively. The formation of the aldehydes was responsible for the decrease of the sugar content after 75 min treatment time.

CONCLUSIONS

Consistent quantitative results of sugar determination in birch wood hydrolysates were obtained by two methods – iodometric titration and UPLC-ELSD, showing that the highest yield of sugars (27% of oven dry feedstock) was obtained by hydrolysis at 180 °C for 75 min. Both analytical methods showed acceptable precision with relative standard deviation < 5% and xylose recovery from a spiked sample about 90%, and could be used to monitor the yield of sugars in the wood pretreatment process. The advantages of UPLC-ELSD are the speed of analysis and superior selectivity, which allows not only to quantify pentoses and hexoses separately, but also to avoid possible interferences of

other non-sugar components in the samples. However, the iodometric method is a simple, inexpensive method for determining the total content of sugars, including oligosaccharides. Besides, more than ten samples can be oxidised for iodometry simultaneously to decrease the necessary time of analysis per sample.

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