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# Hydrothermal treatment followed by enzymatic hydrolysis and hydrothermal carbonization as means to valorise agro- and forest-based biomass residues

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## Abstract

The suitability of several abundant but underutilized agro and forest based biomass residues for hydrothermal treatment followed by enzymatic hydrolysis as well as for hydrothermal carbonization was studied. The selected approaches represent simple biotechnical and thermochemical treatment routes suitable for wet biomass. Based on the results, the hydrothermal pre-treatment followed by enzymatic hydrolysis seemed to be most suitable for processing of carbohydrate rich corn leaves, corn stover, wheat straw and willow. High content of thermally stable components (i.e. lignin) and low content of ash in the biomass were advantageous for hydrothermal carbonization of grape pomace, coffee cake, Scots pine bark and willow.

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**Keywords:** Hydrothermal carbonization; Hydrothermal treatment; Enzyme hydrolysis; Lignocellulose; Biomass

## 1 Introduction

Due to the need to find alternative renewable sources to replace fossil raw materials for the production of fuels and chemicals, new ways to exploit lignocellulosic biomass are being extensively studied. At the same time the amount of lignocellulosics available for new applications is limited. Thus, the challenge of increasing the usage of lignocellulosic biomass as a feedstock for various applications e.g. bioenergy and biochemicals can be answered by the utilization of unexploited raw material sources.

Various types of side-streams are generated in agriculture and forest industry. Most of these streams are poorly utilised or combusted for energy. The challenges in the cost-efficient utilization of these side-streams for added value products are their heterogeneity, seasonal availability and their remote locations (Leboreiro and Hilaly, 2011; Li et al., 2016; Mosier et al., 2005). To overcome these complexities mobile and flexible processing steps suitable for various raw materials are needed. Pre-treatment steps that can fractionate and sort different raw material streams enable utilization of wider raw material basis in different applications. It is, however, critical to recognize that all biomass fractions are not equally suitable for conversion into biofuels, biochemicals or biopower (Li et al., 2016). The quality of biomass will affect the material handling, conversion efficiency and thus product yield and even product quality. Thus, the basis for efficient and sustainable utilization of biomass side-streams is in the deep understanding of the biomass chemistry, structure and the factors affecting the suitability of biomass for various processes.

Numerous biotechnical as well as thermochemical processing routes for biomass have been suggested over the years. E.g. enzymatic hydrolysis of polysaccharides of plant biomass into monomeric sugars followed by

fermentation has been claimed to be one of the most promising approach to produce renewable chemicals, e.g. ethanol, with high yields (Hendriks and Zeeman, 2009; Jørgensen et al., 2007; Sun and Cheng, 2002). For enzymatic hydrolysis of lignocellulosic biomass, a pre-treatment of some sort is a pre-requisite in order to achieve high saccharification yields (Hendriks and Zeeman, 2009). The purpose of the pre-treatment is to make the cell wall polysaccharides more susceptible for enzymes, as in their native state lignocellulosic materials are for the most part resistant to hydrolytic enzymes. The most common pre-treatment method used prior to hydrolysis of lignocellulosic material is steam explosion (Pielhop et al., 2016; Sun and Cheng, 2002). Another commonly used steam pre-treatment is hydrothermal treatment. The chemical changes caused by these methods are nearly similar, and the main difference between them is that steam explosion is usually carried out in high dry matter content, whereas more water is present in hydrothermal treatment (Kallioinen, 2014). Due to these treatments cellulose in biomass becomes more accessible to enzymatic hydrolysis as most of hemicelluloses are solubilized into the liquid phase (Mosier et al., 2005). A hydrothermal treatment is a potent option for pre-treatment of lignocellulose biomass due to the simplicity of the reactor needed and the use of water as solvent.

Processing biomass of diverse origins by hydrothermal carbonization (HTC) has also gained a lot of interest during recent years (Funke and Ziegler, 2010; Hu et al., 2010; Libra et al., 2011; Titirici et al., 2015; Titirici and Antonietti, 2010; Wikberg et al., 2015a). By HTC biomass can be converted to coal like material in an energy-efficient carbonization process at moderate temperatures (~180-260 °C). The various chemical reactions in HTC, e.g. hydrolysis, dehydration, decarboxylation, polymerization and condensation result in a solid carbonaceous material, a liquid phase consisting of small molar mass degradation products and a minor gas phase (Funke and Ziegler, 2010). The solid carbonaceous material, defined as hydrochar in the text, consists of particles of different shapes, size and composition (Sevilla and Fuertes, 2009; Titirici and Antonietti, 2010). Due to the diverse properties of hydrochar, several possible end uses have been proposed in many areas including environmental, catalytic, electronic and biological applications (Titirici et al., 2012).

Hemicellulose and cellulose are among the most studied raw materials in acidic hydrothermal conditions of HTC (Falco et al., 2011; Sevilla and Fuertes, 2009). Under these conditions, water breaks the glycosidic bonds of hemicellulose degrading it into sugar monomers, which further degrade into furfurals and other compounds, including 5-HMF (hydroxymethyl furfural). HMF forms polymeric carbonaceous particles and simultaneously decomposes into low molecular weight acids by time (Falco et al., 2011; Titirici et al., 2008). Cellulose requires more severe conditions to break the glycosidic bonds. The conversion can occur either via hydrolysis to glucose or via direct transformation through intramolecular condensation, dehydration and decarboxylation (Falco et al., 2011; Sevilla and Fuertes, 2009; Wikberg et al., 2016). Lignin is far more stable in higher temperatures than cellulose and hemicellulose and thus it is only slightly altered in HTC (Dinjus et al., 2011; Kang et al., 2012; Lu and Berge, 2014; Wikberg et al., 2015b). The use of crude biomass instead of pure biomass components would make the hydrochar production economically more feasible. Several studies concerning the use of complex lignocellulosic biomasses in HTC have recently been published (Hoekman et al., 2011; Lynam et al., 2015; Mumme et al., 2011; Poerschmann et al., 2014; Reza et al., 2014, 2013a, 2013b).

In this work, the processability of several abundant, but underutilized agro and forest based biomass residues by hydrothermal treatment followed by enzymatic hydrolysis as well as by hydrothermal carbonization was studied. The selected paths, representing simple biotechnical and thermochemical routes are both suitable for wet biomasses, having thus the advantage that no drying is needed prior to processing. The processing routes were compared in terms of suitability for the different biomass fractions.

## 2 Materials and methods

### 2.1 Raw materials

Nine European biomass residues were used in this study, i.e. corn stover (leaves and stalks of corn plants left in the field after harvest), corn leaves (leaves around the corn cob), coffee cake (roasted coffee beans from which the aromas have been extracted with hot water to produce coffee concentrate i.e. instant coffee), grape pomace (solid remains of grapes after mechanical and water extraction in wine making), green house residues (plant waste resulting from the maintenance and renewal of public and private green spaces), Salix willow, Scots pine bark, wheat straw and brewers spent grain (BSG).

The corn stover, corn leaves and greenhouse residues were milled as such (in wet stage) using a garden waste mill (Bosch AXT 23 TC, Robert Bosch GmbH, Stuttgart, Germany) prior to analysis and processing. Willow stalks, Scots pine bark and wheat straw were dried at <70° in a flat-bed drying wagon with forced ventilation prior to shredding using a single-shaft shredder (Lindner Micromat 2000, Lindner-Recyclingtech GmbH, Spittal, Austria) with a 15 mm screen. Other fractions were used as such if not otherwise mentioned later in the text.

### 2.2 Hydrothermal pre-treatment followed by enzymatic hydrolysis

The hydrothermal pre-treatment utilised in this work was a hot pressurized water pre-treatment performed in a laboratory scale fixed bed flow-through reactor. The reactor had a total volume of 6 L, with an electric heater and liquid phase circulation pump. For each pre-treatment trial 550-1000 g (wet basis) material was loaded into reactor and water was added in order to obtain the initial DM:water rate about 1:6 to 1:7 w/w, and the pH was adjusted to 12 with 5 M NaOH. The pH was adjusted to this level to ensure that the final pH after the treatment would remain above pH 4 as unwanted degradation of hemicellulose is known to take place at lower pH. The substantial release of acidic

compounds, especially acetic acid from biomass in the treatment conditions evidently neutralizes the amounts of alkali used. Liquid phase was pumped through the packed material bed at a circulation flow rate of 1 L/min. Before process start the reactor temperature was raised to 85–90 °C and held for 30–45 min for better wetting. Reaction time measurement was started at the moment when the temperature build-up had been developed. The average heating rate was 10–12 °C/min, thus a pre-treatment time of 15 min at 190 °C required a total time of 30 min. After the reaction was stopped, liquid was driven out from the bottom relief valve and cooled down in a double-pipe heat exchanger. The pressure was released, the reactor cover was opened and solid phase removed from reactor. Both liquid and solid fractions were weighed, and pH and dry matter content of extract was measured. Both solid and liquid fractions were stored in freezer (−18 °C) prior to further use.

Enzymatic hydrolysis of both untreated biomass samples and samples processed by the hydrothermal treatment was carried out to evaluate the effect of the hydrothermal treatment. The untreated materials were air-dried and milled with a Wiley mill (2 mm sieve) in order to get homogenous samples. The pre-treated samples were used as such except the grape pomace, pine bark and willow samples, which were dried and milled with a Wiley mill as described above to obtain more homogenous samples. Enzymatic hydrolysis was performed in triplicates at 1% consistency (á 50 mg oven dry biomass), at 45 °C and pH 5.0 (100 mM sodium acetate buffer including 0.02% sodium azide as biocide). The enzymes used in the hydrolysis were Celluclast 1.5 L and Novozym188 (Novozymes, Bagsvaerd, Denmark) and the enzyme dosage was 10 FPU/g of dry matter for Celluclast and 200 nkat of  $\beta$ -glucosidase/g of dry matter.

## 2.3 Hydrothermal carbonization

HTC of the studied biomass side-streams was carried out in a 2 L Hastelloy C276 stirred autoclave reactor (Amar Equipments PVT. Ltd., India) equipped with an electrical heater band, temperature control, digital and analog pressure indicator, internal water cooling coil and PC controlled data logger. Pressurised nitrogen was introduced (~35 bar) to flush and leak test the reactor. Nitrogen was vented before reaction. The reaction sequence consisted of reactor heat up to 260 °C (~60 min), hold up for 360 min, and water cooling to ambient temperature before venting the residual pressure and opening the reactor. The carbonaceous slurry was weighed and filtered in a Bücher funnel using qualitative filter paper MN 616 (Macherey-Nagel). Char was rinsed in a Büchner funnel with ~1 L of deionised water and then dried in oven at 105 °C overnight.

## 2.4 Analyses

### 2.4.1 Composition analysis of raw materials before and after hydrothermal pre-treatment

The chemical compositions of the materials were determined both before and after the hydrothermal pre-treatment. Prior to composition analyses, the air-dried materials were ground using a Fritsch Pulverisette 14 mill. The extractives were determined gravimetrically after extraction with heptane. To determine the carbohydrate and lignin composition, the heptane-extracted materials were hydrolyzed with sulphuric acid and the resulting monosaccharides were determined by HPAEC with pulse amperometric detection (Dionex ICS 3000 equipped with CarboPac PA1 column) according to NREL method (Hausalo, 1995; Sluiter et al., 2008).

The polysaccharide content in the samples was calculated from the corresponding monosaccharides using an anhydro correction of 0.88 for pentoses and 0.9 for hexoses. Acid soluble lignin in the hydrolysate was detected at 215 and 280 nm using equation described by Goldschmid (Goldschmid, 1971).

Klason lignin content i.e. the insoluble residue from the hydrolysis was determined gravimetrically. The Klason lignin content was corrected for protein and ash. Elemental analysis was carried out for both Klason lignin and the whole material using FLASH 2000 series analyser (Thermo Scientific). Protein contents of the whole material and Klason lignin were determined by multiplying the nitrogen content with 6.25. Ash content of Klason lignin was calculated by subtracting the contents of nitrogen, carbon, oxygen, hydrogen and sulphur from 100%. Ash content of the whole material was measured gravimetrically after burning the samples at 550 °C for 23 h.

### 2.4.2 Composition analysis of liquid fractions from hydrothermal treatment

The liquid samples were filtrated prior to analysis in order to remove all solid particles. The solids removed were included in the mass balance calculations.

The alkaline soluble lignin was detected at 280 nm and the obtained value was corrected by subtracting absorbencies of HMF and furfural. The absorbencies for furfural and HMF were calculated based on analysed concentrations in the liquids (Rocha et al., 2012). Furfural and 5-HMF (5-Hydroxymethyl-2-furaldehyde) concentrations were quantified using a Flexar™ HPLC-RI (PerkinElmer, Waltham, MA, USA) system equipped with an Aminex column (HPX-87H, 300 mm × 7.8 mm, Bio-Rad, CA, USA) under isocratic conditions (40 °C) at a flow rate of 0.5 mL/min of 2.5 mM H<sub>2</sub>SO<sub>4</sub> (Patrick and Kracht, 1985). The results were calculated by using corresponding standards: Furfural (Sigma-Aldrich) and 5-HMF (Sigma-Aldrich).

Total carbohydrates in the liquid fractions were determined after a one-step sulphuric acid hydrolysis (4% w/w sulphuric acid at 120 °C for 50 min) by HPAEC (Dionex ICS-3000) with pulse amperometric detection using CarboPac PA1 column according to Hausalo (Hausalo, 1995).

### 2.4.3 Analysis of degree of enzymatic hydrolysis

The release of soluble sugars in the enzymatic hydrolysis was monitored at 0, 24 and 48 h by analysing the reducing sugars using the dinitrosalicylic acid (DNS) method (Bernfeld, 1955) scaled down to a 96-well microtiter plate. Glucose was used as standard. The solubilized sugars measured as glucose equivalents were converted to polymeric carbohydrates by multiplying with a factor of 0.9.

### 2.4.4 Analyses of HTC hydrochars and liquid fractions

Elemental composition (C, H, N and O) of the HTC samples was analysed using FLASH 2000 series analyzer. The ash content was analysed as described above. Total organic carbon (TOC) of the liquid fractions was measured using Shimadzu TOC analyzer (TOC-Vcsh, Shimadzu Corporation) after filtration.

Heating values for biochars and liquid fractions were calculated according to Reed (1979).

## 3 Results and discussion

### 3.1 Raw material characterisation

The chemical compositions (i.e. carbohydrates, lignin, extractives, protein and ash) of the selected nine biomasses representing side streams from forestry and agriculture, i.e. corn stover, corn leaves, coffee cake, grape pomace, brewers spent grain (BSG), green house residues, willow, Scots pine bark and wheat straw were analysed. Klason lignin content was corrected for both ash and protein as most of the samples were known to contain these components.

The chemical composition analyses revealed that the selected samples formed a rather heterogeneous group where each biomass fraction had a unique chemical composition (Table 1). Corn stover, corn leaves, BSG, wheat straw and willow were rich in carbohydrates, whereas coffee cake had especially high extractive content. Greenhouse residues contained a significant amount of ash, which probably came from to the presence of soil and sand in the material. Scots pine bark and grape pomace had high lignin contents. BSG was rich in protein.

**Table 1** Chemical compositions of the studied biomasses before and after hydrothermal pre-treatment and the end pH of the treatment. For the chemical composition, the left-hand column represents the value before the pre-treatment and the right-hand column after the treatment.

	Carbohydrates (%)		Klason lignin (%)		Acid-soluble lignin (%)		Total lignin (%)		Protein (%)		Extractives (%)		Ash (%)		Not analysed (%)		End pH
	Prior	After	Prior	After	Prior	After	Prior	After	Prior	After	Prior	After	Prior	After	Prior	After	
Grape pomace	16.2	16.8	41.8	44.2	6.5	2.2	48.3	46.4	11.9	8.8	7.5	14.4	3.9	9.5	12.1	4.3	7.0
Coffee cake	32.9	30.3	18.2	18.9	0.8	0.8	19.0	19.7	14.1	12.1	28.9	27.6	0.4	5.1	4.7	5.2	7.7
Brewer's spent grain	43.2	43.8	11.1	9.1	5.0	3.0	16.1	12.1	24.4	13.4	7.4	12.3	4.1	10.8	4.7	7.7	9.4
Greenhouse residues	25.2	27.6	24.4	26.1	3.0	2.3	27.4	28.4	8.7	9.7	2.3	2.7	26.0	28.2	10.5	3.4	6.4
Corn stover	48.6	51.4	12.9	9.9	3.9	2.9	16.9	12.8	8.0	5.2	1.5	1.8	6.6	11.7	18.4	17.1	8.5
Corn leaves	64.9	55.7	6.7	9.4	3.4	3.5	10.1	12.8	9.4	7.3	2.0	2.7	2.8	4.4	10.8	17.1	4.8
Pine bark	39.0	35.9	36.1	39.3	1.5	0.8	37.7	40.1	1.6	1.1	4.5	4.9	2.4	6.1	14.9	11.9	6.1
Wheat Straw	57.1	56.3	18.2	14.3	1.7	1.4	19.9	15.7	1.9	1.6	1.7	1.4	8.6	11.5	10.9	13.6	6.7
Willow	48.5	49.1	23.6	28.0	1.9	1.9	27.2	29.9	2.4	2.9	1.4	1.7	1.9	4.4	18.4	12.0	4.3

### 3.2 Hydrothermal treatment followed by enzymatic hydrolysis

In this study, the polysaccharides in the selected biomass fractions were made more accessible for enzymatic hydrolysis by hydrothermal pre-treatment. In the treatment, the biomass structure is opened up for the following enzymatic hydrolysis as hemicelluloses are removed. Steam treatments have also been shown to have a significant effect on distribution and chemical structure of lignin (Marchessault, 1991; Pereira Ramos, 2003).

The yields of dry matter after hydrothermal treatment varied significantly. For instance, the solubilization of BSG in the treatment was as high as 52%, whereas for coffee ground and greenhouse residues it was only 10 and

14%, respectively (Fig. 1, darker grey part of the columns). The solubilization of different components was most likely enhanced by the alkaline pH (pH 12) at the beginning of the pre-treatment, as hemicelluloses, lignin and lipids are known to dissolve in alkaline conditions. The pH dropped (Table 1) in the course of the pre-treatment, most probably due to the release of acids, e.g. via deacetylation, weakening thus the dissolving effects of the alkali. The change in pH during the pre-treatment varied between materials, which was presumably caused by the different chemical compositions, especially acetylation of hemicellulose.

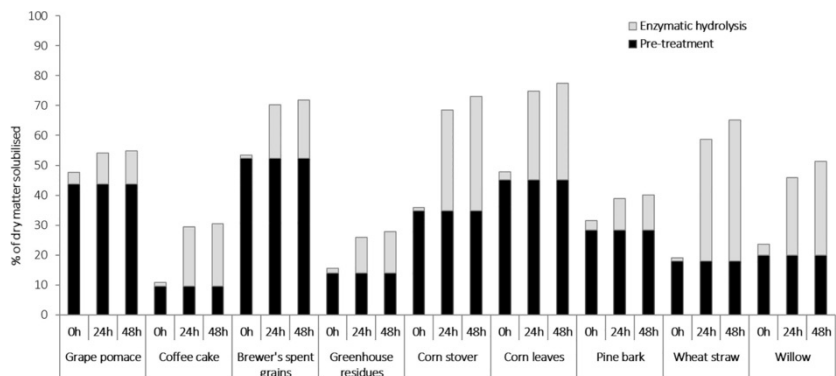


Fig. 1 Total solubilization of biomasses in sequential hydrothermal pre-treatment (darker grey part of the columns) and enzymatic hydrolysis (lighter grey part of the columns).

Interestingly, the overall chemical compositions did not change dramatically due to the hydrothermal treatment indicating that the pre-treatment did not selectively dissolve certain components but affected all of them (Table 1, Fig. 2). Some more notable changes were, however, observed. Even though the relative amount of carbohydrates was notably ~~decreased~~ only for corn leaves, the analysis revealed that the hemicelluloses were solubilised more readily than cellulose during the pre-treatment (results not shown). In corn leaves the carbohydrate content decreased from 65 to 56%. A notable change was even seen in the glucose content (data not shown), which was probably due to the presence of starch in the material, as the corn leaves also contained residual corn kernels. With corn leaves having the highest carbohydrate content and rather high degree of solubilization, i.e. 45%, the mass balance calculations revealed that a significant amount of carbohydrates could be recovered from the liquid fraction after hydrothermal treatment (Fig. 2). The protein content of BSG was decreased from 24 to 13%. Even though the majority of proteins in BSG are water-insoluble storage proteins, the high protein solubility can be explained by the fact that BSG contains also proteins that are soluble in water and alkaline solutions (Celus et al., 2006). Additionally, due to the complex and linked structure of BSG, it is possible that some proteins are released from the structure as other components are dissolved to the water phase (Ohra-Aho et al., 2016). Hydrothermal treatment of grape pomace and pine bark, having the highest lignin contents, resulted in rather high solubilization and thus with liquid fractions having high lignin contents (Fig. 2). The total amount of components detected as lignin in the solid and liquid fractions was somewhat higher after hydrothermal treatment. This can be explained by tannins that are dissolved from the material during hot water extraction and are, as lignin, detected at 280 nm.

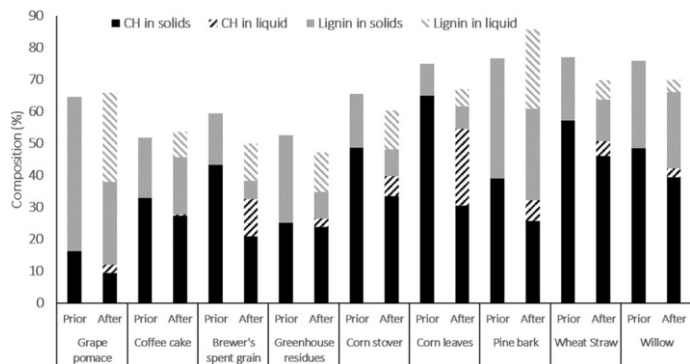
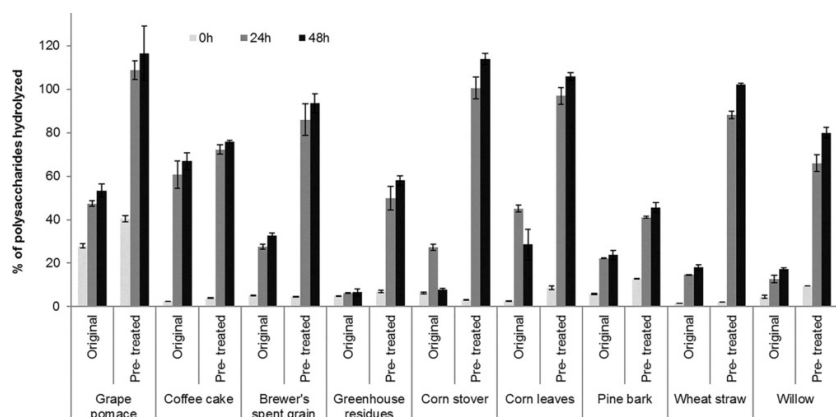


Fig. 2 Carbohydrate and lignin recovery in solid and liquid fractions prior and after hydrothermal pre-treatment.

The effect of the hydrothermal treatment on enzymatic hydrolysability was estimated based on the difference in the degree of hydrolysis before and after the pre-treatment (Fig. 3). For corn leaves, corn stover, wheat straw and grape pomace the pre-treatment was clearly beneficial for the enzymatic hydrolysis and enabled practically complete hydrolysis of carbohydrates. BSG and willow carbohydrates were also efficiently hydrolyzed after the pre-treatment,

whereas the pre-treatment had a smaller effect on hydrolysability of pine bark and coffee cake. The values exceeding 100% (Fig. 3) are most likely caused by inaccuracies in the chemical composition analysis. The analytical acid hydrolysis step was not optimised for each material and thus some sugars may have degraded or remained unhydrolyzed and have thus not be included in the total amount of carbohydrates. Therefore, when the amount of sugars released from the biomass during the enzymatic hydrolysis step was compared with the analysed amount of carbohydrates, hydrolysis yields over 100% could be obtained.



**Fig. 3** Enzymatic hydrolysis of carbohydrates before and after hydrothermal pre-treatment.

When counting both the dry matter solubilized in the in hydrothermal treatment and enzymatic hydrolysis, the total solubilization of the materials ranged from 28 to 78% (based on 48 h hydrolysis) (Fig. 1). In case the aim is to produce fermentable sugars from solid fraction in more concentrated solutions, it can be considered that the higher the enzymatic solubilization is compared to the solubilization in the pre-treatment, the better. If high amounts of carbohydrates are dissolved in the pre-treatment, it may affect the sugar yield and process economy, as the sugars may be difficult to recover economically from the dilute liquid phase containing a mixture of solubilized compounds. Considering these aspects, the hydrothermal pre-treatment with the used conditions seemed most suitable for corn stover, corn leaves, wheat straw and willow. As the hydrolysis of willow was not complete, likely slightly harsher pre-treatment conditions would be needed.

### 3.3 Hydrothermal carbonization

HTC was selected as the other processing route as it is a simple and easily scalable technology basically suitable for any organic raw material. HTC can be used to convert various biomasses into bio-based carbonaceous materials with improved physical and chemical properties to be used in specific applications.

The applicability of the selected biomass fractions to the HTC process was evaluated based on parameters describing the total feasibility. The yield inevitably affects the economic feasibility of the process. High carbon content is advantageous for many applications aiming at replacing existing fossil-based carbon materials. Additionally, high solid carbon recovery level is important not only for the process efficiency but for the environmental perspectives, as waste fractions are minimised. However, the final value of the hydrochars is set by their functionality in the potential applications which was not studied in this paper.

The biomasses were hydrothermally carbonized at 260 °C for 6 h under acidic conditions. Fairly high HTC processing temperature combined with rather long residence time was selected to promote the full conversion of the inhomogeneous biomasses into homogeneous hydrochars. The processing conditions were fixed in order to detect clear differences between the biomasses behaviour in the HTC processing. Further optimization of the process conditions for each biomass relative to hydrochar yield and quality needs to be done separately.

There were large differences in the hydrochar yields between the raw materials, as shown in Table 2. The hydrochar yields varied from ~21 wt% for corn stover to ~55 wt% for coffee cake. The obtained yields were partly lower than the yields reported previously for similar biomasses treated in the same temperature range (Hoekman et al., 2011; Lynam et al., 2015, 2013). The observed difference may be explained by the longer residence time used in this study. In general, differences in the yields are related to the biomass composition and the used reaction temperatures (Hoekman et al., 2011; Lynam et al., 2015). Higher temperatures result in increased formation of components departing from the solid fraction into the liquid- and gas phases thus decreasing the hydrochar yield. Lowering the temperature would increase the yield but would have a negative effect on the hydrochar quality, especially when processing heterogeneous biomasses having large original particle size. Chemical composition of the biomass, solubility of biomass components into water and the reaction mechanisms taking place during the process have also impact on the yield and the final composition of the produced fractions. Thus, the amount of cellulose, hemicelluloses, lignin and extractives in the biomass has a substantial effect on the yields. Corn leaves and corn stover as well as wheat straw had

lowest yields due to high amount of carbohydrates in the biomass. According to previous studies, biomass with higher content of hemicellulose and water-soluble extractives results in lower hydrochar yields while high amount of lignin in the biomass contributes to higher yields (Lu and Berge, 2014; Lynam et al., 2015; Reza et al., 2013b). Hemicelluloses degrade at reaction temperatures of approximately 200 °C while lignin structure is only slightly altered in conventional HTC temperatures. Consequently, high content of lignin contributed to high hydrochar yields for grape pomace (~48%) and pine bark (~50 wt%). The highest hydrochar yield was, however, obtained for coffee cake.

**Table 2** Yield, elemental composition including, ash content including ash recovery and heating values for biochars before and after HTC as well as TOC values for the liquid fractions.

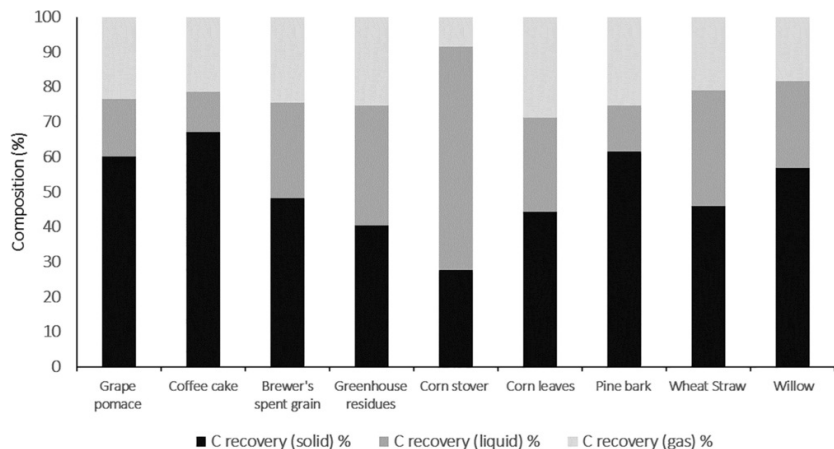
	Solid yield (%)	C (%)		H (%)		O (%)		N (%)		Ash (%)		Ash recovery (%)	Heating value (MJ/kg)		TOC (mg/L)
		Prior	After	Prior	After	Prior	After	Prior	After	Prior	After		Prior	After	
Grape pomace	47.7	54.0	68.0	6.1	5.6	35.5	18.6	1.9	1.9	4.0	3.1	38	22	28	3867
Coffee cake	54.7	60.0	73.4	7.7	7.2	31.9	15.6	2.3	3.1	0.4	0.3	39	27	32	5762
Brewer's spent grain	34.5	50.0	69.3	6.6	6.7	39.1	15.4	3.9	3.8	4.1	3.7	31	21	30	5786
Greenhouse residues	28.4	39.1	55.5	4.7	4.8	30.8	16.1	1.4	1.7	26.0	23.4	26	15	23	4568
Corn stover	21.1	46.5	66.2	5.7	5.2	39.3	15.9	1.3	2.3	6.6	9.4	28	19	27	8418
Corn leaves	28.7	46.8	72.2	6.3	5.3	44.4	15.8	1.5	2.5	2.8	1.6	17	19	30	3816
Scots pine bark	49.9	53.0	70.1	5.9	4.9	41.7	21.8	0.3	0.3	2.4	1.3	25	21	28	5429
Wheat Straw	24.2	45.6	67.3	5.8	5.0	42.9	19.0	0.3	0.7	8.6	6.3	23	18	27	3507
Willow	40.3	49.3	69.5	6.0	5.0	45.3	21.4	0.4	0.6	1.9	0.7	15	19	28	4954

The coffee cake contained plenty of extractives, which based on literature contain lipophilic compounds, such as free fatty acids (Pujol et al., 2013). Most of the fatty acids are reported to retain in HTC increasing thus the hydrochar yield (Lu et al., 2015).

The reactions occurring in HTC result in a loss of carbon, hydrogen and oxygen from the biomass. Table 2 presents the elemental composition and ash content before and after HTC as well as the computational heating value. Carbon is an important element regarding many applications where hydrochars could be used, e.g. as soil amendment or fuel. Hydrochars produced from coffee cake, corn leaves and pine bark had the highest carbon content being over 70%.

Fig. 4 presents the distribution of carbon in the biomass to solid, liquid and gas phases in HTC. The amount of carbon in the solid fraction was measured using elemental analysis while carbon in the liquid fraction was measured using TOC analysis. The remaining carbon was assumed to be in the gas phase. Coffee cake and pine bark together with grape pomace had the highest solid carbon recovery levels i.e. over 60%. The result correlates with the high overall solid yields. Low solid carbon recovery levels for corn stover, corn leaves and wheat straw correlated with low solid yields. The high amount of carbon in the liquid fraction of corn stover can be explained by the high amount of degradation products soluble in water in the used conditions. The products are known to be mainly monomeric sugars (Chen et al., 2007; Reza et al., 2013a).





**Fig. 4** Carbon distribution to solid, liquid and gas fractions in HTC.

Generally, the HTC liquid fraction contains various organic compounds, mainly acetic, lactic and formic acids but also sugars and phenols (Hoekman et al., 2011). The HTC liquid stream containing high amount of dissolved carbon is a potentially problematic waste fraction. Anaerobic treatment (Oliveira et al., 2013; Poerschmann et al., 2014) and wet air oxidation has been suggestions for reducing its organic load (Baskyr et al., 2014; Reza et al., 2016).

The biomass fractions contained different amounts of inorganics. Ash recovery in Table 2 describes the amount of ash retaining in the hydrochar. The ash content was significantly reduced in HTC for all the biomasses. Greenhouse residues had originally the highest amount of ash mainly due to dirt in the sample. Loose sand and soil were probably partly leached out from the sample to the hot liquid fraction during processing. Also, the loss of mass in HTC causes simultaneous loss of the ash (Reza et al., 2013a). The high temperatures used in this study, have been reported to cause higher reduction in the ash recovery due to more porous hydrochar structure enabling leaching of the entrapped or loosely bonded inorganics (Reza et al., 2013a).

The extent of reduction of oxygen and hydrogen in the carbonization process can be described by a Van Krevelen diagram (Fig. 5) where molar ratios of O/C and H/C illustrate the carbonization of each biomass (Ramke et al., 2009). The conversion proceeds gradually from the upper right corner of the diagram, which is a typical biomass region, to lower left to regions resembling lignitic brown coal, brown coal, black coal and finally anthracite, as the carbonization advances. The intensity of the carbonization is described by the length of the vector and it is mainly affected by the dehydration and decarboxylation reactions in HTC. Dehydration during HTC can result from both chemical and physical processes through the elimination of hydroxyl groups or from dewatering, when water is ejected from the biomass due to the increased hydrophobicity of the hydrochar (Reza et al., 2014). As a result, the biomass is carbonized and the O/C and H/C molar ratios are decreased. The hydrochars in this study exhibited O/C ratios of 0.16-0.23 and H/C molar ratios of 0.84-1.18. Interestingly, the length of the input-output vector showed that corn leaves had reacted most intensively reaching the area of lignitic black coal. Hydrochars from coffee cake and BSG had the lowest O/C molar ratios (0.159 and 0.1667) indicating removal of carboxyl groups from the biomass extractives, hemicellulose, and cellulose in the HTC process. At the same time the lowest H/C molar ratios were found for woody biomasses willow and pine bark, indicating elimination of hydroxyl groups in the process. The heating values or hydrochars were calculated based on the elemental composition reaching maximum values  $\geq 30 \text{ MJ kg}^{-1}$  for coffee cake, corn leaves and BSG correlating with high carbon content and low H/C mol ratio. The increase in hydrochar energy density compared to raw biomass were comparable to previously reported results (Hoekman et al., 2011; Mumme et al., 2011).

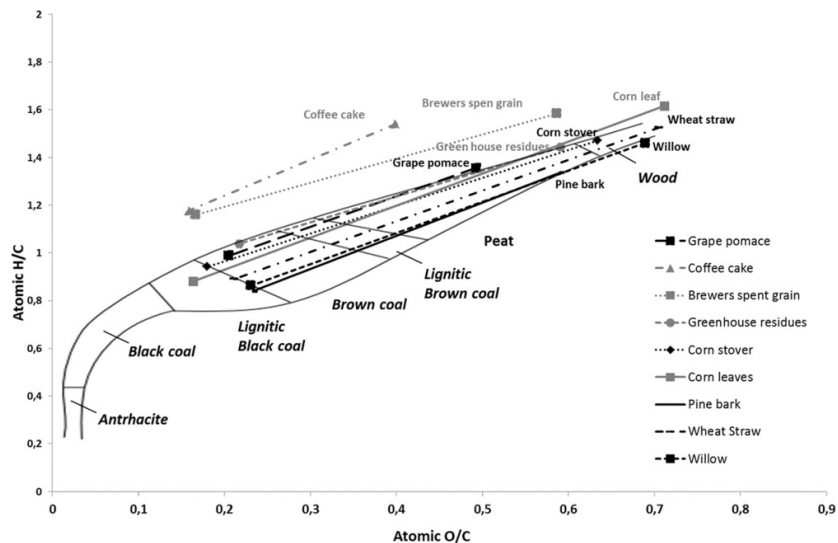


Fig. 5 Van Krevelen diagram describing the extent of carbonization of the studied biomasses (Ramke et al., 2009).

## 4 Conclusions

The obtained results showed that all biomass fractions were not equally suited for [saccharification](#) and hydrochar production. If aiming at fermentable sugars the hydrothermal pre-treatment prior to enzymatic hydrolysis was most suitable to materials having high carbohydrate content (i.e. corn leaves, corn stover, wheat straw and willow). Based on yield, high carbon content and solid carbon recovery level the HTC route was most suitable for biomasses having high content of thermally stable components, such as lignin and low content of ash (i.e. coffee cake, grape pomace, Scots pine bark and willow).

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## Graphical abstract



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## Highlights

- Biomass composition defines its feasibility for alternative process routes.
- High carbohydrate content was favourable for the saccharification route.
- Hydrothermal carbonisation yields were high for biomasses with high lignin contents.