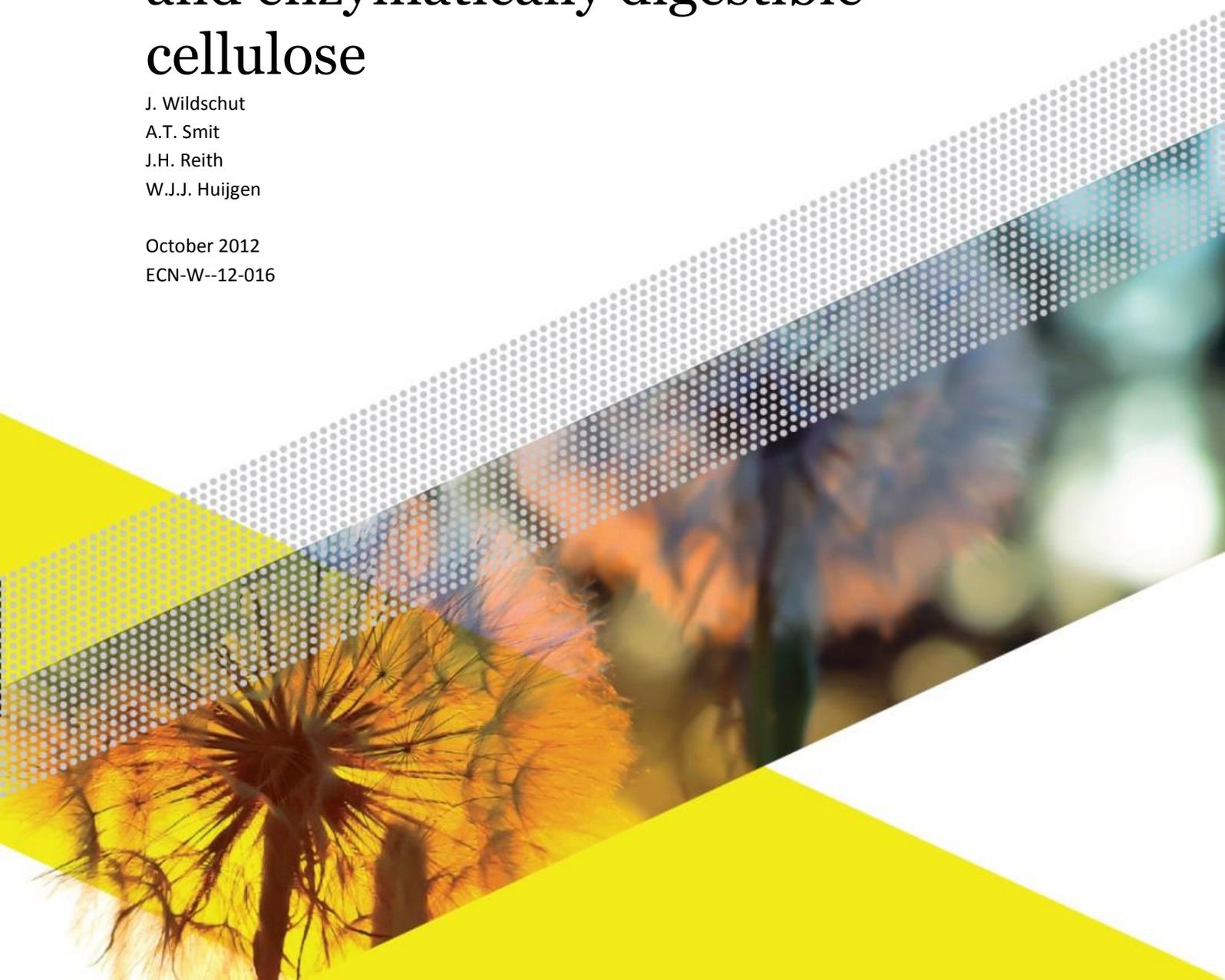


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Ethanol-based organosolv fractionation of wheat straw for the production of lignin and enzymatically digestible cellulose

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HIGHLIGHTS

- ▶ Wheat straw biorefining by ethanol organosolv into lignin and digestible cellulose.
- ▶ Major optimization parameters: temperature, ethanol concentration, and acid dose.
- ▶ Biomass particle size and reaction time less influential process parameters.
- ▶ Optimum enzymatic glucose yield of 86% after autocatalytic organosolv.
- ▶ High yields of lignin, furfural, and sugars by acid-catalysed organosolv.

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ABSTRACT

Wheat straw fractionation by ethanol organosolv was studied as pretreatment for enzymatic cellulose hydrolysis. A parametric study focusing on temperature, reaction time, acid catalyst dose, solvent concentration, and particle size was performed to determine their influence on delignification, xylan hydrolysis, and enzymatic cellulose digestibility. Major process parameters were found to be temperature, ethanol concentration, and acid dose. Optimisation of the process towards enzymatic digestibility resulted in a maximum glucose yield of 86% without the use of a catalyst (lignin yield 84%, organosolv at 210 °C, 50% w/w aqueous EtOH). Using 30 mM H₂SO₄ as catalyst resulted in similar glucose and lignin yields at a lower temperature (190 °C, 60% w/w aqueous EtOH). Lowering the pretreatment temperature by using an acid catalyst substantially improved the yield of the hemicellulose derivatives xylose and furfural. A systematic approach in pretreatment optimisation is vital for development of efficient lignocellulosic biorefineries.

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1. Introduction

Lignocellulosic biomass is a versatile renewable source of chemicals and fuels, available in large amounts and at relatively low costs (Demirbas, 2007; Huber et al., 2006). Current industrial application for energy purposes is often limited to heat and power generation. However, both the sugar and lignin fractions of lignocellulosic biomass have great potential as a resource for fuels and chemicals production with higher added value (Huber et al., 2006; Kumar et al., 2009; Zakzeski et al., 2010). This can be achieved via the biorefinery approach.

Biorefining is the sustainable processing of biomass into a spectrum of marketable products (food and feed, materials, and chemicals) and energy (fuels, power, and heat) (definition International Energy Agency, Bioenergy Task 42). Appropriate fractionation of

the complex lignocellulose material into its constituents is a key operation in a biorefinery. Fig. 1 depicts a possible lignocellulosic biorefinery scheme. In this scheme, lignocellulosic biomass is first fractionated into its major constituents, which are subsequently processed into the desired end-products. The resulting fractions cellulose, hemicellulose and lignin have a variety of potential applications. Cellulose pulp could, for example, be used for paper production or as a carbohydrate source for chemicals or biofuels (e.g., bioethanol or biobutanol by fermentation). Hemicellulose-derived sugars can be used as a substrate for fermentation or anaerobic digestion. Alternatively, sugars derived from the hemicellulose fraction can be (thermo)chemically converted into e.g. furfural (Marcotullio and De Jong, 2011). Furfural, which has many applications e.g. as a solvent, is commercially produced today via thermohydrolysis of biomass residues using mineral acids as catalysts (Zeitsch, 2000). Finally, the lignin fraction is a renewable source of aromatics for a range of chemicals and performance products such as phenolics and ingredients for glues and resins (De Wild et al., 2012; Gosselink, 2011; Zakzeski et al., 2010).

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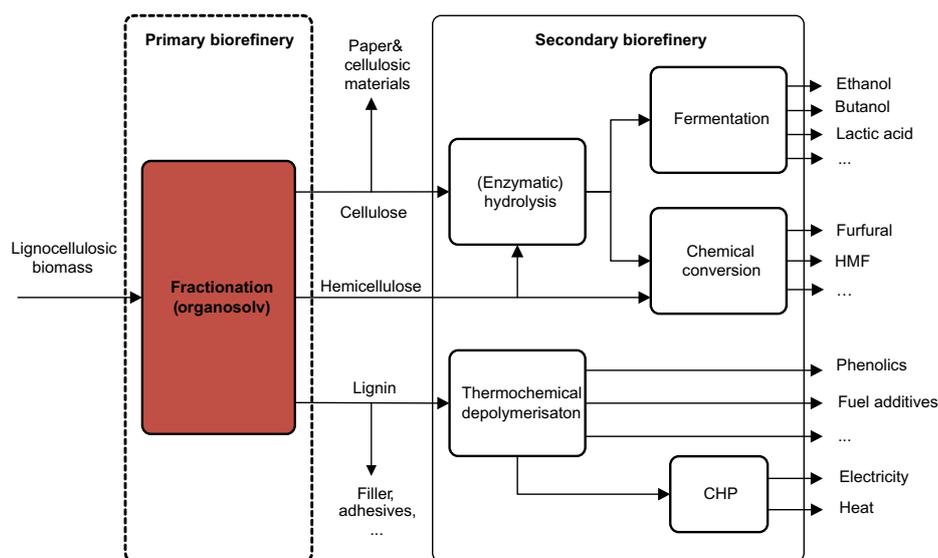


Fig. 1. Organosolv-based lignocellulosic biorefinery (adapted from (De Wild et al., 2012)).

Conversion of cellulose into glucose by enzymatic hydrolysis requires pre-treatment of the lignocellulosic biomass to make the cellulose fraction more accessible to cellulase enzymes. For this purpose several pretreatment techniques are being developed and various overviews can be found in the literature (Alvira et al., 2010; Demirbas, 2007; Hendriks and Zeeman, 2008; Kumar et al., 2009). The current study focuses on organosolv. A key feature of the organosolv process is that it extracts lignin from lignocellulosic biomass prior to enzymatic cellulose hydrolysis in contrast to other common pretreatment technologies such as steam explosion and mild acid pretreatment. Thus, in addition to enzymatically digestible cellulose, lignin with minimum carbohydrate and mineral impurities is produced which facilitates lignin applications with a higher value than heat and power generation e.g. as an ingredient in resins or as feedstock for aromatic chemicals (Zhao et al., 2009; Huijgen et al., 2010, 2012).

Organosolv fractionation consists of cooking lignocellulosic biomass in an (aqueous) organic solvent at elevated temperatures (Zhao et al., 2009). During the process, the hemicellulose is hydrolysed, lignin is dissolved in the liquor and purified cellulose is produced as a pulp. The dissolved lignin can be separated by precipitation by water addition or solvent evaporation. The resulting products are (1) a pulp mainly consisting of cellulose, (2) solid lignin and (3) an aqueous stream containing hemicellulose sugars and derivatives such as furfural (Zhao et al., 2009). Organosolv delignification can be performed with various solvents (e.g. ethanol, acetone, acetic acid) and both autocatalytic (i.e., without any catalyst added) and catalytic. The most well-known autocatalytic organosolv process is the Alcell (now Lignol) process that is based upon autocatalytic ethanol delignification of wood (Pye and Lora, 1991).

Ethanol-based organosolv has been widely studied for pulping as well as for pretreatment purposes. Ethanol is a renewable solvent and its volatility facilitates the extensive recycling required (Zhao et al., 2009). Ethanol-based organosolv pretreatment has been studied for all major types of lignocellulosic biomass including hardwoods (e.g., willow (Macfarlane, 2009), poplar (Kim and Pan, 2010)), softwoods (e.g., lodgepole pine (Pan et al., 2007; Del Rio et al., 2012)), and herbaceous crops (e.g., miscanthus (Brosse et al., 2009)). Wheat straw has been successfully pulped and pre-treated using aqueous ethanol in previous studies (Huijgen et al., 2011; Jimenez et al., 1999; Sidoras and Koukios, 2004; Sun et al., 1998; Xu et al., 2007, 2011).

At present, there is growing interest in organosolv fractionation for biorefineries as well as enhanced interest in lignin as a potential industrial source of renewable aromatics. In the current study, wheat straw is fractionated by using autocatalytic and acid-catalysed ethanol organosolv. The aim is to identify the most influential process parameters and to optimize the process towards enzymatic cellulose hydrolysis to glucose. First, a parametric study is performed focusing on temperature, reaction time, catalyst dose, solvent concentration, and particle size in order to gain insight in the effects on delignification, lignin yield, xylan hydrolysis, and enzymatic digestibility. Subsequently, the parametric effects on yield and quality of fractions are summarised in a 'process product relation scheme'. Finally, the scheme is used to optimize the organosolv fractionation process towards enzymatic cellulose digestibility.

2. Methods

2.1. Biomass

Wheat straw was received ambient-dry from Champagne-Ardennes region, France (harvested summer 2009). The straw was milled with a cutting mill to a particle size smaller than 10 mm (Retsch SM 2000) and stored in closed containers until use. The moisture content of the raw material was determined for each organosolv experiment using a halogen moisture analyser (Mettler Toledo HR83, Columbus, OH) and averaged 7.5 wt.%. The summative composition of the wheat straw was (% w/w dry basis): glucan, 35.4; xylan, 19.8; arabinan, 2.1; galactan, 1.4; lignin, 17.6; ash, 3.5; and extractives, 10.2 (methodology, see Section 2.5). The elemental composition was (% w/w dry basis): C, 47.3; H, 6.4; N, 0.7; and O, 44.6. The acid neutralization capacity of the wheat straw was 0.31 and 0.55 mol H⁺/kg dw for pH 4 and 2, respectively ($t = 48$ h, determined according to CEN TS 14 429 (European Committee for Standardization, 2005)). The enzymatic digestibility of the raw material (<10 mm) without organosolv pretreatment was 21% of its theoretical maximum (20 FPU/g dry biomass, see Section 2.6).

2.2. Experimental program

Table 1 shows an overview of the organosolv experiments performed. In the first part of experiments, the influence of individual

parameters on ethanol-based organosolv fractionation of wheat straw was studied. The effect of these parameters was measured in terms of delignification, xylan hydrolysis, lignin yield, and enzymatic digestibility of the cellulose fraction to glucose. In the second part, an optimisation study was performed taking into account combined parametric effects.

2.2.1. Individual parameters

The following parameters were varied: temperature (160–210 °C), ethanol concentration (50–80% w/w aqueous ethanol), reaction time (60–120 min), catalyst dose (0–30 mM H₂SO₄), and particle size (milled to <0.5–<10 mm). The parameter selection was based upon literature and earlier research (Huijgen et al., 2010, 2011, 2012; Jimenez et al., 1999; Zhao et al., 2009). The maximum particle size was determined by practical experimental considerations. The catalyst doses applied (15 and 30 mM) were chosen based on their expected effect on the pH of the organosolv liquor. In earlier work, it was found that the final pH of the organosolv liquor should not be lower than ~3.5 to avoid cellulose hydrolysis (Huijgen et al., 2011). Based on the acid neutralization capacity of the wheat straw, the catalyst doses applied would result in a pH of around 4 and 2 assuming full dissociation of H₂SO₄. However, since in the same study H₂SO₄ was found not to fully dissociate in aqueous ethanol (Huijgen et al., 2011), the resulting pH was expected to be in the desired range. Afterwards, it turned out that the resulting pH using 30 mM H₂SO₄ was 3.3 (experiment 9, Table 2).

2.2.2. Optimisation

In the second part of experiments, an optimisation study was performed using a low and high value of the four main process parameters (Table 1). Thus, also combined effects of process parameters were taken into account. The goal of the optimisation

study was to determine maximum enzymatic digestibility of wheat straw cellulose after ethanol-based organosolv pretreatment.

In the following paragraphs, the methods applied for the organosolv experiments, analysis of the solid and liquid fractions, and enzymatic hydrolysis are described in short. For more information, we refer to our earlier work (Huijgen et al., 2010, 2012).

2.3. Organosolv pretreatment

Lab-scale experiments were performed in an autoclave reactor (0.5 L Kiloclave, Büchi Glas Uster AG, Switzerland) stirred with an anchor at 100 rpm. For experiments 12 and 13 (Table 1), wheat straw was further milled with the cutting mill, as described in Section 2.1. A suspension of biomass–water–ethanol (10 mL solvent per 1 g biomass) was heated to the reaction temperature, kept at its set-point during the specified reaction time, and subsequently cooled down below 40 °C. The pH of the resulting slurry was determined. After filtration, the solid residue was extensively washed, dried at 50 °C in a vacuum oven, and weighed to determine the solid recovery (pulp yield). Washing was performed at room temperature with aqueous ethanol in similar amount and with the same ethanol concentration as the solvent applied for the organosolv process itself. The filtrate (the “organosolv liquor”) and the washing solution were combined and samples were taken for analysis. Finally, the dissolved lignin was separated from the combined liquor by precipitation upon dilution with water (4 °C, 3:1 w/w dilution ratio H₂O: organosolv liquor). A typical temperature profile of the autoclave reactor is given in Huijgen et al. (2010).

2.4. Liquor composition analyses

Organosolv liquors of selected experiments were analysed for monomeric reducing sugars, oligomeric sugars, and sugar

Table 1
Organosolv pretreatment of wheat straw at varying temperature, reaction time, catalyst dose, ethanol concentration, and particle size.

Series	#	Process conditions				
		T (°C)	t (min)	[H ₂ SO ₄] (mM)	EtOH (%)	d (mm)
<i>Parameter screening</i>						
Centre point	1	190	60	0	60	<10
Temperature	2	160	60	0	60	<10
	3	170	60	0	60	<10
	4	200	60	0	60	<10
	5	210	60	0	60	<10
Reaction time	6	190	90	0	60	<10
	7	190	120	0	60	<10
[H ₂ SO ₄]	8	190	60	15	60	<10
	9	190	60	30	60	<10
Ethanol concentration	10	190	60	0	50	<10
	11	190	60	0	80	<10
Particle size	12	190	60	0	60	<0.5
	13	190	60	0	60	<4
<i>Optimisation study (incl exp 1, 5, 6, 8, 10)</i>						
	10	190	60	0	50	<10
	1	190	60	0	60	<10
	14	190	60	15	50	<10
	8	190	60	15	60	<10
	15	190	90	0	50	<10
	6	190	90	0	60	<10
	16	190	90	15	50	<10
	17	190	90	15	60	<10
	18	210	60	0	50	<10
	5	210	60	0	60	<10
	19	210	60	15	50	<10
	20	210	60	15	60	<10
	21	210	90	0	50	<10
	22	210	90	0	60	<10
	23	210	90	15	50	<10
	24	210	90	15	60	<10

Table 2
pH organosolv liquor, pulp and lignin yields, composition of pulp, fractionation degrees, and enzymatic digestibility.

#	pH organosolv liquor (–)	Yield solids		Composition pulp				Fractionation degree		Enz digest ^a (%)
		Pulp (%)	Lignin (%)	Glc ^b (%)	Xyl (%)	Lignin (%)	Others ^c (%)	Delig (%)	Xyl remov (%)	
Wheat straw				35.4	19.8	17.6	7.3			20.7
1	5.2	67.7	8.2	50.8	20.7	16.2	3.9	37.7	29.2	37.2
2	5.9	86.5	0.0	39.3	21.8	19.4	7.2	4.7	5.0	30.5
3	5.6	81.2	4.6	41.7	22.4	18.6	5.5	14.4	8.4	31.7
4	5.1	63.2	10.4	54.3	21.0	14.2	4.0	49.1	33.2	44.4
5	4.9	53.5	12.9	60.8	15.1	13.1	4.1	60.1	59.3	56.1
6	5.2	65.6	9.6	52.0	21.6	14.4	3.8	46.5	28.5	39.5
7	5.1	64.0	10.6	54.4	21.8	14.1	4.0	48.8	29.7	42.0
8	4.6	62.6	10.6	56.6	18.5	13.3	6.0	52.8	41.6	47.6
9	3.3	43.0	15.1	75.0	2.2	9.9	7.1	75.8	95.3	89.4
10	4.9	64.3	9.8	54.6 ^d	19.6	16.8	4.5	38.8	36.3	44.1
11	5.7	79.3	5.1	44.4	23.3	17.6	7.1	20.8	6.7	33.3
12	5.3	71.2	8.1	49.0	23.5	15.9	4.5	36.0	15.6	40.0
13	5.2	71.9	7.5	48.0	22.5	16.2	4.3	33.9	18.6	38.1
14	4.2	55.4	13.1	63.0	12.5	13.4	5.8	58.0	65.0	71.9
15	4.9	59.7	10.8	58.3	18.2	14.2	3.3	51.9	45.1	48.2
16	4.3	52.6	13.0	65.7	10.8	12.9	5.0	61.5	71.5	75.7
17	4.6	58.4	13.0	60.1	16.8	11.7	5.5	61.3	50.5	67.5
18	4.5	50.7	13.8	67.0	9.3	15.2	3.8	56.3	76.2	75.1
19	4.2	48.8	14.0	64.9	5.0	15.6	6.2	56.8	87.6	83.6
20	4.5	50.8	15.2	68.4	10.4	11.0	6.6	68.5	73.4	78.8
21	4.6	48.6	14.7	69.1	7.6	14.9	3.4	59.1	81.4	85.9
22	4.9	51.6	14.5	64.9	13.4	12.1	3.9	64.7	65.3	64.1
23	4.3	47.8	15.4	67.6	4.0	18.1	5.2	51.0	90.4	78.1
24	4.5	48.1	17.2	69.1	8.2	11.4	5.6	68.9	80.2	76.5

^a Enzymatic digestibility based on raw material (% of theoretical maximum).

^b Abbreviations used: glc, glucan; xyl, xylan; delig, delignification; xyl remov, xylan removal.

^c Others predominantly consist of ash in addition to arabinan, mannan, galactan and rhamnan. Detection limits arabinan, mannan, galactan and rhamnan are 0.1, 0.5, 0.2, 0.1%, respectively.

^d Compositional analysis performed in single.

derivatives. Determination of sugars was performed using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) (ICS3000, Dionex, Sunnyvale, CA). Reducing sugars were analysed on a CarboPac PA1 column using a method described in earlier work (Huijgen et al., 2012). Analysis of reducing sugars was performed both directly to determine monomeric sugars and after post-hydrolysis to determine oligomeric sugars by difference (post-hydrolysis conditions: 1 M sulphuric acid, 100 °C, 120 min). The sugar derivatives furfural, hydroxymethylfurfural (HMF), and levulinic acid were determined using high-performance liquid chromatography (HPLC-RI/UV, with BIO RAD Aminex HPX-87H column, 65 °C, 5 mM H₂SO₄).

2.5. Composition raw and pretreated materials

The summative composition of the raw material and pretreated samples was analysed using analytical procedures described previously (Huijgen et al., 2010, 2012). The ash content of samples was measured by combustion at 550 °C according to protocol NREL/TP-510-42622 (NREL, 2009). The summative composition (i.e., content of extractives, lignin, hemicelluloses, and cellulose) of the solids was determined in duplicate by using a modified hydrolysis protocol based on TAPPI method 249 (TAPPI, 2009). First, in the case of raw materials, extractives were removed with two successive Soxhlet extractions using water and ethanol (NREL/TP-510-42619 (NREL, 2009)). The amount of water extractives reported was corrected for soluble ash removed. Secondly, the sample was milled with a cutting mill and hydrolysed in two steps: (1) 12 M (72% w/w) H₂SO₄ (30 °C, 1 h) and (2) 1.2 M H₂SO₄ (100 °C, 3 h). The amount of solid residue was determined gravimetrically and its ash content was measured according to NREL/TP-510-42618 (NREL, 2009). The acid-insoluble lignin (AIL) content was based on the amount of ash-free residue. Finally, the hydrolysate was

analysed for acid-soluble lignin (ASL, UV-VIS absorption at 205 nm) as well as monomeric reducing sugars (with HPAEC-PAD, procedure described elsewhere (Huijgen et al., 2012)). Pulsed amperometric detection is known to be sensitive to drift in the detector's response. Deviations due to drift within a series of analyses were estimated to be <3%.

2.6. Enzymatic hydrolysis

The biomass feedstock and pretreated materials were subjected to enzymatic hydrolysis using the commercial enzyme mixture Accellerase 1500 obtained from DuPont Industrial Biosciences (Leiden, NL). Cellulase enzyme loading for enzymatic hydrolysis was 20 Filter Paper Units (FPU)/g dw biomass. Enzymatic hydrolysis was performed in 0.05 M sodium citrate buffer (pH 4.8) at 50 °C for 72 h and at 3% (w/v) pretreated material concentration according to NREL/TP-510-42629 (NREL, 2009). Samples were taken every 24 h for colorimetric determination of the glucose concentration (Huijgen et al., 2010, 2012). Enzymatic hydrolysis experiments were performed in duplicate and average results are reported.

2.7. Definitions

Throughout this manuscript, the ethanol concentration of the aqueous ethanol solvent is given in % w/w. Pulp yield and composition of wheat straw and pulps are expressed in % w/w dry biomass. Delignification degree and lignin recovery after precipitation are given as % w/w of the amount of lignin present in the raw wheat straw. The yields of xylan and glucan derivatives, such as sugars and furans, are expressed in % of their theoretical maximum based on the composition of the raw wheat straw. Enzymatic digestibility is determined as % of the theoretical maximum glucose yield based on the glucan content of the raw material.

3. Results and discussion

3.1. Influence process parameters

In the first part of this study, the influence of individual process parameters on organosolv fractionation of wheat straw was studied. Table 2 and Fig. 2 show the experimental results in terms of pulp and lignin yields, composition of the pulp, fractionation degrees, and enzymatic digestibility.

3.1.1. Pretreatment severity

Pretreatment severity is determined by the process parameters temperature, reaction time, and pH (acid catalyst dose) (Chum et al., 1990; Overend and Chornet, 1987). In the subsequent sections, the effects of these three individual parameters are discussed.

3.1.1.1. Temperature. The influence of temperature on organosolv fractionation of wheat straw has been tested in the range of 160–210 °C. An increase of the temperature decreases the pulp yield due to increased delignification and xylan hydrolysis. These results are expected as pretreatment severity increases with a rise in temperature. The compositions of the pulps given in Table 2 reveal that the hemicellulose fraction is the most reactive major biomass constituent. Due to the increased hemicellulose hydrolysis more acetic acid is formed from the acetyl side groups of the hemicellulose. Thus, the pH of the organosolv liquor drops resulting in an increased autocatalytic effect on the process (Table 2).

A higher reaction temperature increases delignification and, thereby, results in an increase of the amount of lignin precipitated (Table 2). Finally, the enzymatic digestibility of the wheat straw pulp increases with organosolv temperature (from 31% at 160 °C to 56% at 210 °C). The enzymatic glucose yield from non-pretreated

wheat straw is 21% (Table 2). In the literature, the various mechanisms by which (organosolv) pretreatment leads to improved enzymatic digestibility of cellulose have been discussed extensively (Alvira et al., 2010; Esteghlalian et al., 2001; Kumar et al., 2009). In case of organosolv, a substantially contributing factor is the removal of lignin leading to cellulose being more accessible for enzymes and to less irreversible absorption of enzymes to lignin. In addition, removal of xylan plays an important role (Huijgen et al., 2011).

3.1.1.2. Reaction time. The reaction time has been varied in the range of 60–120 min (experiments 1, 6 and 7). Extension of the reaction time leads to a decrease of the pulp yield, and at the same time an increase of the delignification degree, the lignin yield, and the enzymatic digestibility (Table 2). However, the reaction time has a negligible effect on the xylan hydrolysis at the process conditions applied. The autocatalytic effect on organosolv fractionation is hardly influenced by the reaction time given the similar pH values found for the organosolv liquors (5.1–5.2). Overall, the reaction time seems not to be a major process parameter for the fractionation at the process conditions tested. Reaction time, however, can be an important parameter with respect to the products formed, such as the yield of xylose from hemicellulose.

3.1.1.3. Catalyst concentration. Since hydrolysis is an acid-catalysed process, addition of an acid catalyst can improve the organosolv fractionation process and thereby the subsequent enzymatic cellulose hydrolysis. In this study sulphuric acid is used, which is commonly used in organosolv studies (Zhao et al., 2009). The concentration of H₂SO₄ has been varied in the range of 0–30 mM in experiments 1, 8 and 9 (Table 1) and, as a consequence, the pH of the organosolv liquor drops from 5.2 to 3.3 (Table 2).

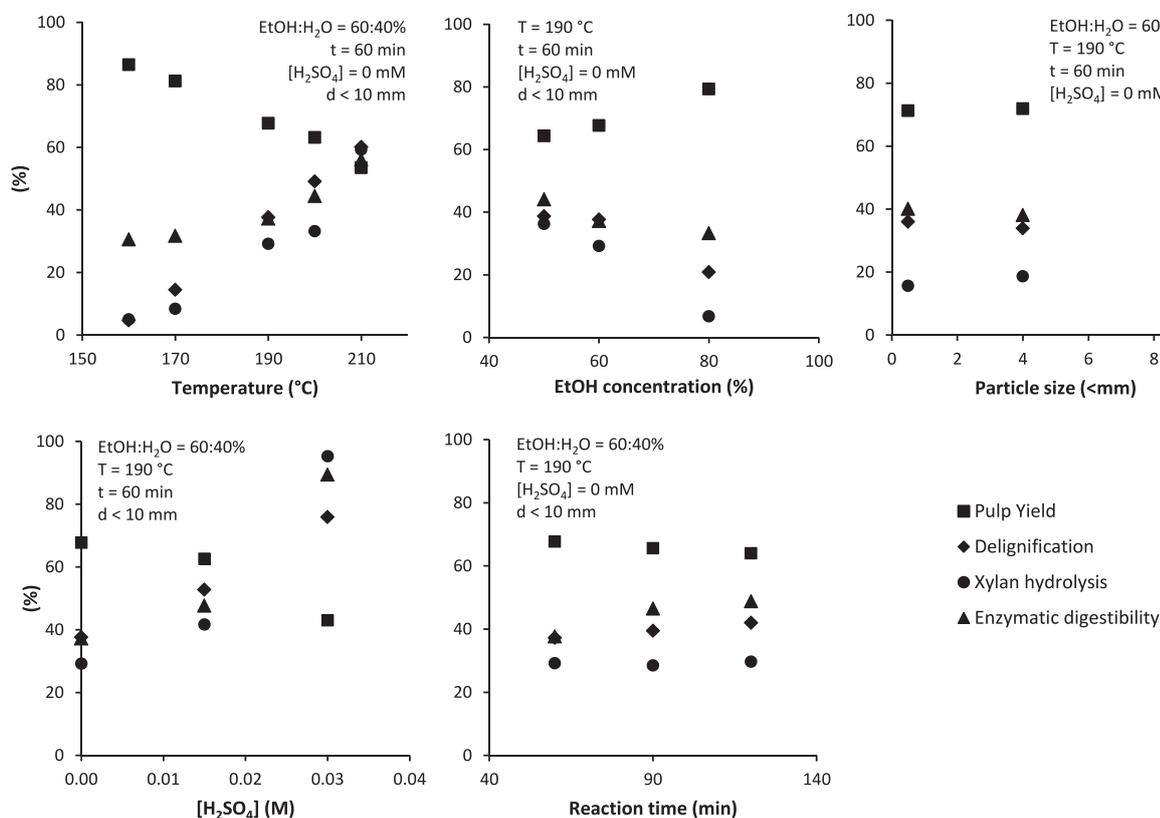


Fig. 2. Organosolv fractionation of wheat straw as a function of temperature, ethanol concentration, particle size, sulphuric acid dose, and reaction time.

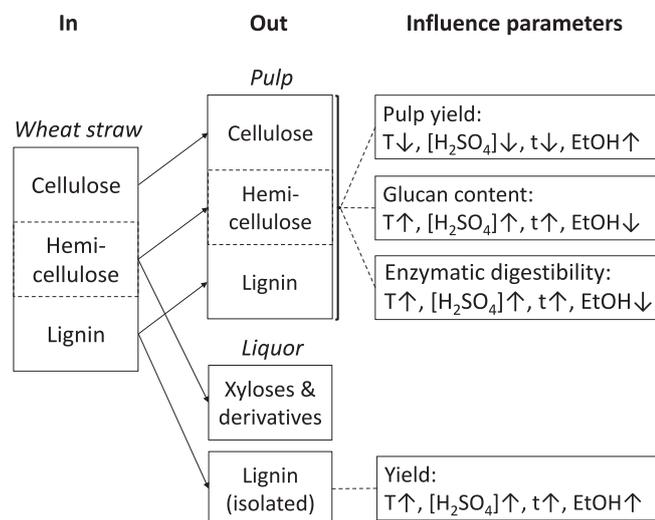
Table 3
Mass balances of four experiments.

#	Cellulose (% of theoretical maximum)				Sum
	Glucan in pulp (%)	Glucose (mon, %)	Glucose (olig, %)	HMF (%)	
1	97.0	0.0	0.6	0.3	97.9
4	96.8	0.0	1.2	0.2	97.9
9	91.0	4.0	0.0	2.0	97.0
21	94.8	0.0	0.6	0.7	95.4
#	Hemicellulose (% of theoretical maximum)				Sum
	Xylan in pulp (%)	Xylose (mon, %)	Xylose (olig, %)	Furfural (%)	
1	70.8	0.2	3.5	1.9	76.5
4	66.8	0.3	7.5	1.1	75.7
9	4.7	29.5	0.0	34.4	68.6
21	18.6	0.2	1.8	4.1	24.7
#	Lignin (% of lignin in straw)				Sum
	Lignin in pulp (%)	Lignin precipitated (%)			
1	62.3	46.3			108.6
4	50.9	58.9			109.8
9	24.2	85.4			109.5
21	40.9	83.1			124.0

Table 4
Effect of parameters on organosolv fractionation efficiency (within ranges of process conditions tested in first part of study).

Parameter	Range	Pulp yield	Delignification	Xylan hydrolysis	Enzymatic digestibility
Temperature ↑	160–210 °C	↓+	↑+	↑+	↑+
EtOH conc. ↑	50–80%	↑+	↓+	↓+	↓+
[H ₂ SO ₄] ↑	0–30 mM	↓+	↑+	↑+	↑+
Time ↑	60–120 min	↓-	↑-	↑-	↑-
Particle size ↑	<0.5–<10 mm	~	~	↓?	~

↑/↓, Trend upwards/downwards; +/–, strong/weak effect; ~, no significant effect found; ?, questionable effect requiring further study.

**Fig. 3.** Schematic summary of trends in yield and quality of fractions (within ranges of process conditions tested in first part of study).

Addition of H₂SO₄ has a large impact on the xylan hydrolysis (29.2–95.3%), delignification (37.7–75.8%), and enzymatic digestibility (37.2–89.4%) (Fig. 2). As a consequence of the increased xylan hydrolysis and delignification, the pulp yield becomes lower and the pulp becomes more enriched in glucan (Table 2). It is observed that, at the highest H₂SO₄ dose, the hemicellulose fraction is almost completely removed as well as a part of the cellulose fraction (cellulose recovery: 91.0%, Table 3). As a result of the

increased removal of lignin, the amount of lignin precipitated also increases from 8.2% to 15.1% (based on weight of raw wheat straw). Finally, it is noted that addition of 15 mM sulphuric acid has a significant, but relatively small effect on the process, while increasing this amount by a factor 2 results in considerably larger effects. The acid neutralisation capacity of the wheat straw neutralises a large part of the first 15 mM H₂SO₄ added, limiting the pH decrease from 5.2 to 4.6 (Table 2, pH 3.3 at 30 mM H₂SO₄).

3.1.2. Ethanol concentration

In earlier studies, the organic solvent concentration in the organic solvent–water mixture used has been identified as a key parameter in organosolv fractionation (Huijgen et al., 2010; Pan et al., 2007). In this study, the effect of the ethanol concentration in the solvent mixture is tested in the range 50–80% (experiments 10, 1 and 11).

At the conditions tested, a higher ethanol concentration increases the pulp yield due to decreased delignification and xylan hydrolysis (Fig. 2). The glucan content of the pulp decreases from 54.6% (50% ethanol) to 44.4% (80% aqueous ethanol) (Table 2). In addition, also the enzymatic digestibility decreases to some extent (44.1% at 50% ethanol and 33.3% at 80% ethanol).

The effect of the ethanol concentration is most pronounced on the xylan hydrolysis which drops from 36.3% to 6.7%. The pH of the organosolv liquor decreases from 5.7 to 4.9 when the ethanol concentration is decreased from 80% (exp 11) to 50% (exp 10) (Table 2). Thus, the higher concentration of water enhances acid-catalysed hydrolysis. Similar results have been reported in our earlier work on acetone-based organosolv pretreatment of wheat straw (Huijgen et al., 2010).

The drop in delignification upon an increase of the ethanol concentration from 60% to 80% might be surprising on first hand, since lignin is known to be soluble in polar organic solvents like ethanol (Macfarlane, 2009; Zhao et al., 2009) and the optimum ethanol concentration for lignin dissolution is around 80% (Huijgen et al., 2010). However, hemicellulose and lignin are structurally linked (Mark Lawther et al., 1996; McDonough, 1993) and in order to dissolve lignin these linkages should be broken first. Moreover, hydrolysis of ether linkages within the lignin polymer will improve lignin solubility due to decreased molecular weight. Overall, the optimum ethanol concentration for delignification is a trade-off between lignin solubility and organosolv liquor pH needed for hydrolysis of ether linkages. Similar results were reported earlier

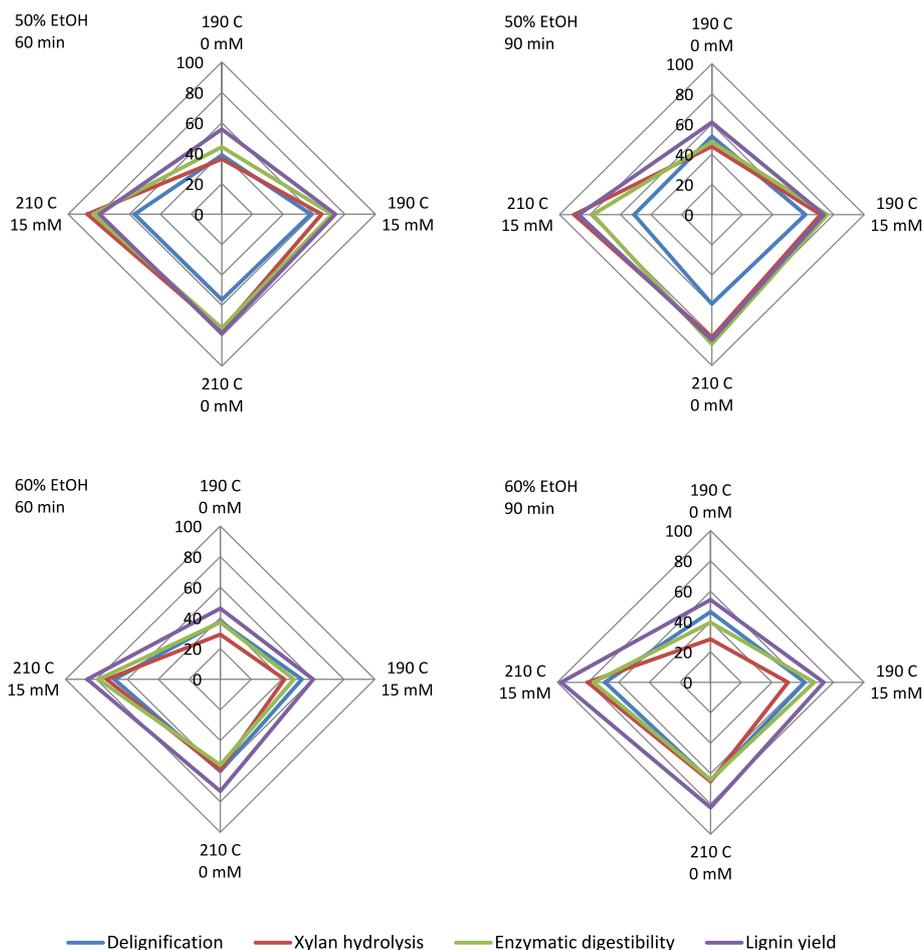


Fig. 4. Optimisation study: fractionation efficiency and enzymatic digestibility as function of process parameters.

for acetone-based organosolv fractionation of wheat straw (Huijgen et al., 2010).

Overall, the optimum ethanol concentration for organosolv is a compromise between the optimum for xylan hydrolysis (0%) and for delignification (typically, ~60%). For this study, 50–60% aqueous ethanol seems most appropriate for further process optimisation.

3.1.3. Biomass particle size

All experiments discussed above were performed using wheat straw <10 mm. In order to verify that the results presented are not dependent on the particle size, reference experiment 1 (190 °C, 60 min, no H₂SO₄, and 60% aqueous ethanol) was repeated using wheat straw <4 mm and <0.5 mm (experiments 12 and 13). No clear effect of the particle size on the pulp yield (67.7–71.9%), lignin yield (7.5–8.2%), pulp composition or the enzymatic digestibility was observed (Table 2, Fig. 2). Recently, Del Rio et al. also reported that fibre size of organosolv-pretreated softwood did not substantially influence its enzymatic digestibility (Del Rio et al., 2012). Delignification varied between 33.9% and 37.7% and enzymatic digestibility between 37.2% and 40.0%. Results suggest that xylan hydrolysis increases with particle size (15.6–29.2%), which seems counterintuitive. Further research is required to elucidate this issue.

Overall, size reduction of the wheat straw feedstock from <10 to <0.5 mm seems not to be an effective way to increase the fractionation and enzymatic digestibility of wheat straw after organosolv

pretreatment. Therefore, all other experiments in this study were performed on wheat straw <10 mm.

3.1.4. Summary influence parameters

The effects of the parameters tested on pulp yield, delignification, xylan hydrolysis, and enzymatic digestibility are summarized in Table 4 and are graphically depicted in Fig. 3. These schemes relate parameter settings to yield and quality of fractions produced. In the remainder of the study, they were used as a basis for process optimisation towards enzymatic cellulose digestibility.

3.2. Optimisation enzymatic digestibility

Based on the influence of the individual process parameters discussed in Section 3.1, parameters were selected for optimisation of the enzymatic cellulose digestibility after ethanol-based organosolv fractionation of wheat straw. The following parameters were applied: temperature 190 and 210 °C, ethanol concentration: 50% and 60%, reaction time: 60 and 90 min, and H₂SO₄ dose: 0 and 15 mM. An optimisation study was performed consisting of 16 (2⁴) experiments (Table 1). These experiments consist of the experiments discussed in Section 3.1 and additional experiments to cover the selected parameter range.

The resulting fractionation degrees and enzymatic digestibility are given in Table 2 and Fig. 4. Delignification, lignin yield, xylan hydrolysis, and enzymatic digestibility vary between: 37.7% and 68.9%, 46.3% and 97.6%, 28.5% and 90.4%, and 37.2% and 85.9%, respectively. The maximum enzymatic digestibility of 85.9% is ob-

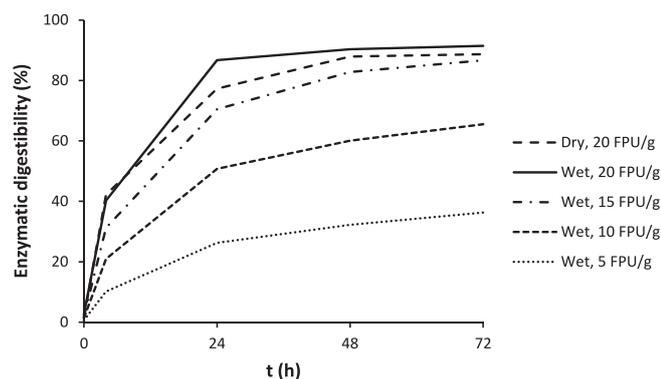


Fig. 5. Enzymatic digestibility of wheat straw after organosolv pretreatment at 190 °C, 60 min, 60% aqueous ethanol, 30 mM H₂SO₄ (exp 9).

tained at 210 °C, 50% aqueous EtOH, 90 min without addition of catalyst (exp 21). At these conditions, delignification (59.1%), lignin yield (83.1%), and xylan hydrolysis (81.4%) are high, but not maximal. Maximum delignification and lignin yield occur at the highest severity (210 °C, 90 min, and 15 mM H₂SO₄) using 60% aqueous EtOH (exp 24). Maximum xylan hydrolysis occurs also at the highest severity, but using 50% aqueous EtOH (exp 23) in line with the discussion in Section 3.1.2.

The optimisation study performed also enables examining combined effect of parameters. Generally, the influence of the parameters is similar as discussed in Section 3.1 based on varying individual parameters. However, in some cases the effect of a process parameter changes due to the level of the other parameters. Particularly, the effect of the ethanol concentration on delignification and lignin yield is very dependent on the level of the other parameters due to its reverse effect on the two steps involved (cleavage of lignin bonds and lignin dissolution, Section 3.1.2). In all cases, an increased ethanol content reduces the enzymatic digestibility due to the reduced xylan hydrolysis. At the most severe conditions using 50% aqueous ethanol (exp 23), the delignification is relatively low. At these conditions, reducing the pretreatment severity by either reducing the temperature (exp 16), reaction time (exp 19) or H₂SO₄ dose (exp 21) results in an increase of the delignification degree in contrast to the general trend. A higher pretreatment severity increases the fractionation degree until a certain maximum after which residual ('pseudo')lignin increases due to lignin condensation and humin formation (see Section 3.3).

3.3. Mass balance organosolv experiments

For four selected organosolv experiments (1, 4, 9, and 21), the mass balances of the major constituents are shown in Table 3. These experiments include both reference conditions (exp 1) as well as pretreatment conditions resulting in the highest enzymatic digestibility (exp 9 and 21).

The mass balances for cellulose are 95.4–97.9% closed. The lowest numbers occur when a higher temperature is applied or sulphuric acid is added. At these conditions, hydrolysis of (amorphous) cellulose occurs resulting in slightly less residual glucan in the pulp. Experiment 9 (30 mM H₂SO₄) yields the least residual glucan in the pulp (91.0%), the most monomeric glucose (4.0%) as well as the most hydroxyl methyl furfural (HMF, dehydration product of glucose) (2.0%). Overall, the cellulose fraction remains to a large extent intact during the organosolv process at the reaction conditions applied.

In contrast to the cellulose fraction, the hemicellulose mass balance is far from closed with mass balances ranging from 24.7% to

76.5%. This difference is due to the higher reactivity of the hemicellulose fraction (Alvira et al., 2010; Huijgen et al., 2012; Zhao et al., 2009). At the reaction conditions applied, hemicellulose is substantially hydrolysed (29.3–95.3%). Reaction products include oligomeric xylose, monomeric xylose, and the dehydration product furfural. Sugars and especially furfural can react further to e.g. humins and lignin-condensation products, which have not been taken into account in the hemicellulose mass balance. The lowest mass balance closures are again observed for the experiments at the most severe conditions (exp 9 and 21). Probably, an extended reaction time leads to an increase in secondary reaction products of the hemicellulose fraction (exp 21). Experiment 9 shows a pronounced effect of the addition of acid resulting in nearly complete xylan hydrolysis (95.3%) and in furfural and monomeric xylose yields of 34.4% and 29.5%, respectively.

The mass balance closure of lignin is over 100% for all experiments (108.6–124.0%). The increase of the lignin mass seems partly due to ethoxylation reactions of e.g. hydroxyl groups present in the lignin. In addition, formation of 'pseudo-lignin' most likely plays a role (i.e., lignin-like components that cannot be distinguished from native lignin by the analytical method applied). 'Pseudo-lignin' can be formed by condensation reactions of the lignin with degradation products from the carbohydrate fraction (e.g., furfural), extractives, and proteins (Huijgen et al., 2010, 2012; Zhao et al., 2009). The contribution of N-containing protein derivatives might be substantial. The wheat straw contains up to 4.3% w/w protein based on its total nitrogen content, corresponding to a potential lignin mass increase to 125%. These condensation reactions particularly occur at extended reaction time and higher temperature. Experiment 21 has the highest sum of identified lignin components and the lowest sum of identified hemicellulose derivatives, which indicates that reaction products of the hemicellulose fraction such as furfural have (as well) reacted with lignin.

Our results show that condensation products resulting from non-lignin biomass fractions are introduced in the lignin product during the organosolv process. The formation of these condensation products can be influenced via the process conditions. Overall, there is a tradeoff between the yield as well as the quality of all three major products (cellulose, hemicellulose-derivatives and lignin) and process optimization should take into account the desired quality of the fractions produced.

3.4. Process improvement

3.4.1. Autocatalytic versus catalytic organosolv

Optimisation of the process as described in Section 3.2 leads to a maximum enzymatic digestibility of 85.9% for non-catalytic organosolv process (210 °C, 50% EtOH, 90 min). The use of a low sulphuric acid dose of 15 mM did not result in an overall higher enzymatic glucose yield. However, experiment 9 in the first part of this study in which 30 mM H₂SO₄ was used as catalyst yielded a slightly higher enzymatic digestibility of 89.4% (190 °C, 60% EtOH, 60 min, 30 mM H₂SO₄). Although organosolv pretreatment using >15 mM sulphuric acid has not been optimised in this study, acid-catalyzed ethanol organosolv pretreatment of wheat straw can yield enzymatically digestible cellulose and lignin in addition to substantially higher yields of hemicellulose derivatives (Table 3 and Section 3.1). An additional optimisation study is required to study whether the enzymatic digestibility following catalytic organosolv pretreatment could be increased further.

3.4.2. Effect of intermediate drying pulp on enzymatic hydrolysis

Intermediate drying of the wheat straw pulp before enzymatic hydrolysis, as performed for practical reasons in this study, has been reported to affect the enzymatic digestibility due to pore collapses resulting in reduced access for enzymes (Huijgen et al.,

2010; NREL, 2009; Esteghlalian et al., 2001). To assess this effect, the experiment yielding the highest enzymatic digestibility (i.e., experiment 9 at 190 °C using 30 mM) was repeated and the pulp was enzymatically hydrolysed without intermediate drying using different enzyme dose (Fig. 5). Although intermediate drying reduces enzymatic hydrolysis between 4 and 48 h, the final enzymatic digestibility after 72 h is similar for dried and non-dried pulps (89% vs 91%). A similar observation was made in a previous study on acetone-based organosolv pretreatment of wheat straw (Huijgen et al., 2010). Direct enzymatic hydrolysis without intermediate drying seems to lower the enzymatic hydrolysis time or enzyme dose required, but not to change the final enzymatic digestibility (at optimised process conditions). Fig. 5 shows that, in this particular case, the enzyme dose can be reduced from 20 to 15 FPU/g while maintaining the enzymatic digestibility after 72 h similar.

4. Conclusions

The most influential process parameters for ethanol-based organosolv fractionation of wheat straw were found to be temperature, acid catalyst dose and ethanol concentration. Reaction time and particle size had limited influence. A maximum enzymatic digestibility of 86% with a lignin yield of 84% was reached without the use of a catalyst (organosolv at 210 °C, 50% w/w aqueous EtOH). Similar results were obtained at 190 °C using 30 mM H₂SO₄ as catalyst and 60% w/w aqueous EtOH. Lowering the pretreatment temperature by using an acid catalyst substantially improved the yield of the hemicellulose derivatives xylose and furfural.

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