

# Two-Step Autohydrolysis Pretreatment: Towards High Selective Full Fractionation of Wheat Straw

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Supporting Information  
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Pretreatment is necessary to increase the enzymatic digestibility of lignocellulosic biomass. Here, degradation reactions of solubilized pentoses to furfural and others are undesired regarding the reduced product yield and increasing downstream processing efforts. In this work, the use of the unit operation configuration was successfully shown to reduce degradation reactions. In the used two-step autohydrolysis pretreatment, the reaction is stopped before degradation takes place. The pentoses are removed by water leaching to make them unavailable for degradation in a second autohydrolysis treatment. The overall sugars yield is increased, and the furfural formation is decreasing while maintaining the high lignin purity using the two-step autohydrolysis pretreatment.

**Keywords:** Autohydrolysis pretreatment, Avoid degradation reactions, Full fractionation, Pentose yield, Two-step autohydrolysis

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## 1 Introduction

The European Commission's Green Deal aiming at zero net emissions of greenhouse gases in the European Union until 2050 [1] will put immense price pressure on materials based on crude-oil in the next 30 years. A promising alternative for chemicals and materials is a biomass-based feedstock [2]. Several techniques have been proposed to fractionate non-edible biomass and convert the resulting molecules into platform chemicals [3]. The carbohydrate biopolymers in lignocellulosic biomass can be hydrolyzed and dissolved in water. The degradation of the dissolved hemicellulose-derived pentoses to furfural is a significant challenge for the technological advancement of lignocellulose biorefineries [4–6].

It is of great interest to fractionate the lignocellulose components (pentoses, hexoses, and lignin) into separate streams in order to convert them into material applications in different routes [7,8]. Processing routes discussed, among others, are pentose to furfural, hexose to HMF (hydroxymethylfurfural), and lignin to functional fillers or lignin oil. Several pretreatment techniques can be considered for biomass fractionation. Dilute acid pretreatment generates high monomeric sugar yields above 90 wt %, but pentoses and hexoses typically occur in the same stream [9,10]. It is assumed that fractionation of the sugars during the pretreatment is economically beneficial. Other disadvantages associated with dilute acid pretreatment are

impure lignin, chemical modification of lignin during the process, additional costs of purchasing and handling of corrosive chemicals, acids neutralization, and salt removal [11,12]. In autohydrolysis pretreatment, where no other solvent or catalyst than water is used, the disadvantages mentioned above do not occur, but low pentose yields, pentose degradation to furfural, or significant thermal energy demands should be taken into account instead [6,13]. In a previous work [4], the batch autohydrolysis of wheat straw resulted in a maximum in hemicellulose concentration in the liquid phase at hemicellulose solubilization of approx. 50 %. In batch autohydrolysis of wheat straw, a maximum yield of 10.5 g xylooligosaccharides per 100 g of feedstock was obtained by Carvalho et al. [14]. Here, approximately 33 % of xylan is still bound to the solids. Both publications report that at maximum hemicellulose solubilization, the hemicellulose content in the liquid phase to have dropped to near zero, indicating severe degradation.

To avoid the pentose degradation, two different autohydrolysis reactor types have been investigated intensively, namely the flow-through pretreatment using a fixed bed

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reactor [15–17] and an inclined continuous screw reactor with countercurrent flow [18, 19]. Both aim to remove the thermally unstable pentoses from the reaction zone in situ. These reactor types suffer either from substantial water consumption at excellent pentose recovery or poor pentose extraction performance at low water usage. Using an inclined reactor with countercurrent flow, Thomsen et al. [18] reported a recovery of hemicellulose into the hydrolysate of approx. 50 % of the hemicellulose present in the feedstock at a water-to-solid mass ratio of 4–5. Increasing water consumption during autohydrolysis pretreatment will lead to increased energy demand and low concentrations in the pentose-rich hydrolysate.

In a previous work, the use of two mild consecutive autohydrolysis steps was proposed for an industrial scale using a screw conveyor reactor (SCR) and saturated steam [4]. The leaching of thermally unstable sugar products in between the hydrothermal processing steps at ambient pressure and low temperature avoids their degradation. Finally, the pretreated biomass is enzymatically hydrolyzed to produce a solid fraction called AS-lignin and a glucose solution. The first pretreatment step in saturated steam or liquid water environment has been studied by many researchers [6, 13, 20–22]. In [4], an approach to operate the first step just until furfural formation takes place was proposed. A process window for this approach, aiming at no furfural production was identified at a severity factor  $\log(R_0)$  less than 4 in a liquid water environment [4]. However, no experimental data for the second mild autohydrolysis pretreatment is available. Therefore, operational conditions in the second reaction step are investigated in this paper aiming at a selective full fractionation of lignocellulose.

## 2 Experimental

### 2.1 Material

Wheat straw pellets of 8 mm diameter from a local farm in Stelle, Germany, with a moisture content of 9.5 wt % were used. The composition was determined according to the NREL procedure “Determination of structural carbohydrates and lignin in biomass” [23]. The xylose and arabinose sugars are reported as hemicellulose. The dry matter composition was lignin 27.7 wt %, cellulose 36.0 wt %, hemicellulose 28.0 wt %, and rest 18.0 wt %.

### 2.2 Hydrothermal Pretreatment

The hydrothermal pretreatment was carried out in a batch screening plant composed of six stainless-steel reactors of 40 mL reactor size each. The setup was equipped with a PTFE inlay, a magnetic stirrer, electrical heating jackets, a thermocouple in the reaction mixture, and a gaseous nitrogen source.

600 mg of dry mass was inserted to the PTFE inlay, and water added to 30 g reaction mixture. The gas-phase pressure was adjusted to 50 bar before starting the heating-up. The time was taken when the reaction mixture reached five degrees below the set point. The reaction was quenched with cold water. The pressure was released as soon as the temperature fell below 50 °C.

The obtained slurry was filtered and centrifuged; the hydrolysate (filtrate) pH was measured. The chemical composition of the hydrolysate was determined using an ion-exchange HPLC and RI detector by the TUHH central laboratory for chemical analysis, method number M03.008. Analytical hydrolysis with 4 wt % of sulfuric acid was carried out to determine the total sugar content in the hydrolysate. The filter cake and centrifugal pellet were washed and dried at 60 °C for 24 h. The dry mass was determined gravimetrically.

The experimental conditions used in the hydrothermal pretreatment are shown in Tab. 1, whereas in Fig. 1 the experimental flow sheet is shown. Three conditions were tested in the first hydrothermal pretreatment experiments, specifically 200 °C and 30 min (Opt1), 170 °C and 60 min (A1), and 200 °C and 10 min (B2). Each of the conditions was carried out and analyzed six-fold. The conditions A1 and B1 are carried out 60 times each to produce enough material to be tested in a second pretreatment step. The two dried solid collections were mixed separately. For the

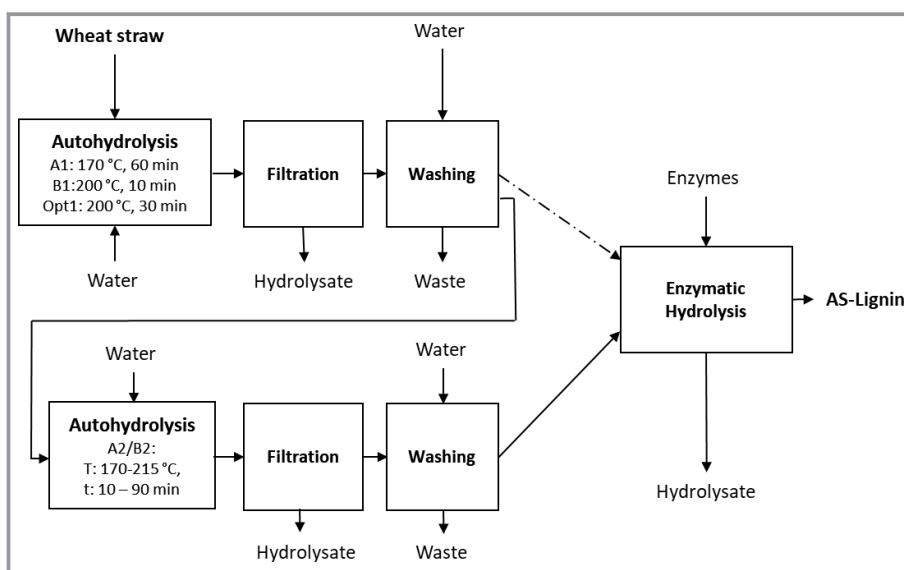


Figure 1. Flow diagram of the experimental procedure for single and two-step autohydrolysis.

**Table 1.** Experimental conditions in the first and second hydrothermal pretreatment.

Name	Temperature [°C]	Time [min]	No. of conditions	Repetitions [-] <sup>a)</sup>
Opt1	200	30	1	6
A1	170	60	1	6
B1	200	10	1	6
A2	170, 185, 200, 215	10, 20, 30, 60	16	2
B2	170, 185, 200, 215	10, 20, 30, 60	16	2

a) Repetitions analyzed.

second step of pretreatment, full factorial design with the temperatures 170 °C, 185 °C, 200 °C, and 215 °C and residence time of 10 min, 20 min, 30 min, and 60 min were conducted in duplicate. The experiments of the second pretreatment step following A1 are called A2. Analogously, B2 are experiments using pretreated solids produced with condition B1.

## 2.3 Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted in 50-mL centrifugal tubes horizontally positioned in a water bath shaker. 600 mg of the pretreated wheat straw and 0.147 mL Novozymes Ctec2 and 30 mL of 0.05 M citrate buffer is used for each trial. The activity dosage was calculated to equal 27 FPU per gram of dry biomass. The reaction conditions are 50 °C, 72 min duration, pH 5, and 2 wt % initial solids loading. Immediately after the experiment, a 500-μL sample of the liquid phase was taken, centrifuged, and the supernatant subjected to the DNS reducing sugars assay, according to [24]. The centrifugal tubes were cooled in ice water, centrifuged, the supernatant removed, and the tube and pellet dried at 105 °C for 4 h. Afterward, the pellet mass was determined gravimetrically. The pretreated biomass from the duplicates of the hydrothermal pretreatment was used as a substrate for the enzymatic hydrolysis. The lignin content of the resulting AS-lignin was analyzed with sulfuric acid hydrolysis and an ash correction based on an NREL method [23].

## 2.4 Calculations

For each reaction step  $j$  the severity factor  $R_{0,j}$  was calculated using the residence time in min, and the reaction temperature in °C (Eq. (1)). The reaction step  $j$  can be 1, 2, EH or combined hydrothermal pretreatment th. For graphical representation, the decadic logarithm of the severity factor is used. The overall severity factor  $R_0$  combines the first and second step conditions (see Eq. (2)).

$$R_{0,j} = \text{texp} \left( \frac{T - 100}{14.75} \right) \quad (1)$$

$$R_0 = R_{0,1} + R_{0,2} \quad (2)$$

The degree of solubilization  $DS_j$  represents the fraction of the initial dry biomass that was solubilized during the treatment step  $j$  (Eq. (3)).  $DS_{th}$  is the degree of solubilization after the hydrothermal treatment and is calculated according to Eq. (4).

$$DS_j = \frac{M_{j,0}^s - M_j^s}{M_{j,0}^s} \quad (3)$$

$$DS_{th} = \frac{M_0^s - M_2^s}{M_0^s} = 1 - (1 - DS_1)(1 - DS_2) \quad (4)$$

The pH was calculated using the measured concentration of acetic acid  $c_{AA}$  and formic acid  $c_{FA}$  in  $\text{mg L}^{-1}$  (Eq. (5)). The formic acid concentration below the detection limit was adapted to the concentration profile above the concentration limit (see Supporting Information).

$$\text{pH} = -\log_{10} \left( \sqrt{\frac{c_{FA}}{46030} 10^{-3.77} + \frac{c_{AA}}{60000} 10^{-4.76}} \right) \quad (5)$$

The recovery of the products  $i$  after step  $j$   $Rec_{i,j}$  is expressed as the component mass in the hydrolysate  $M_{i,j}^l$  to the polymer mass in the untreated biomass from which the component is derived  $M_{i,j=0}^s$ . Here, the volume of the reaction mixture  $V_R$  and the concentration of component  $i$  after step  $j$   $c_{i,j}$  are used (cf. Eq. (6)). The recovery calculation in the second pretreatment step is based on the substrate mass in the untreated component. For this purpose, the untreated, dry biomass  $M_0^s$  was calculated using the initial dry biomass  $M_1^s$  and  $DS_1$  (cf. Eq. (7)). The total recovery of component  $i$  after both hydrothermal pretreatment steps  $Rec_{i,th}$  is the sum of both individual steps (see Eqs. (8) and (9)).

$$Rec_{i,j} = \frac{M_{i,j}^l}{M_{i,0}^s} = \frac{c_{i,j} V_R}{W_{i,0} M_0^s} 10^{-6} \quad (6)$$

$$M_0^s = \frac{M_1^s}{(1 - DS_1)} \quad (7)$$

$$Rec_{i,th} = \frac{M_{i,1}^l + M_{i,2}^l}{M_{i,0}^s} = Rec_{i,1} + Rec_{i,2} \quad (8)$$

$$Rec_{i,th} = Rec_{i,1} + Rec_{i,2} = \frac{c_{i,1} V_{L,1}}{W_{i,0} M_0^s} 10^{-6} + \frac{c_{i,2} V_{L,2}}{W_{i,0} \frac{M_1^s}{(1 - DS_1)}} 10^{-6} \quad (9)$$

The yield of component  $i$   $Y_{i,j}$  is calculated as mass component recovered in step  $j$  to the untreated, dry biomass. The AS-lignin yield is calculated based on the three treatment's degree of solubilization (see Eq. (10)). The yield of the reducing sugars in the enzymatic hydrolysis experiment  $Y_{RS,EH}$  is calculated with the reducing sugar concentration  $c_{RS}$  generated with the DNS test (see Eq. (11)). The yield of the dissolved component  $i$  is calculated based on its total recovery (Eq. (12)). Here,  $Y_{Rest,th}$  represents the organic acid and furfural in both hydrothermal pretreatments.

$$Y_{AS-lignin} = \frac{M_{AS-lignin}}{M_0^s} = \prod_{k=1}^{j=EH} (1 - DS_k) \quad (10)$$

$$Y_{RS} = \frac{M_{RS}^l}{M_0^s} = \frac{C_{RS} V_R (1 - DS_{th})}{M_{EH}^s} \quad (11)$$

$$Y_i = \frac{M_i}{M_0^s} = Rec_{i,th} W_{i,j=0} \quad (12)$$

## 2.5 Uncertainty Analysis

The investigated parameters ( $P_A$ , Eq. (13) or  $P_M$ , Eq. (15)) are based on measured quantities  $a$ ,  $b$ ,  $x$ ,  $y$ . The standard deviations of measured parameters  $\delta a$ ,  $\delta b$ ,  $\delta x$ ,  $\delta y$  affect the uncertainty of the calculated parameter  $\delta P$ . The calculation of the uncertainty for additions was conducted according to Eq. (14). The standard deviation for a product  $\delta P_M/P_M$  was calculated based on the relative uncertainty of its parameters (see Eq. (16)). The standard deviation is calculated from (i) the repetition of the measurement of the same quantity, or when the repetitions are insufficient from (ii) the measurement of the same quantity in a different experiment or from (iii) a regression of correlated quantities.

$$P_A = a + b + \dots + x + y \quad (13)$$

$$\delta P_A = \sqrt{(\delta a)^2 + (\delta b)^2 + \dots + (\delta x)^2 + (\delta y)^2} \quad (14)$$

$$P_M = \frac{ab \dots}{xy \dots} \quad (15)$$

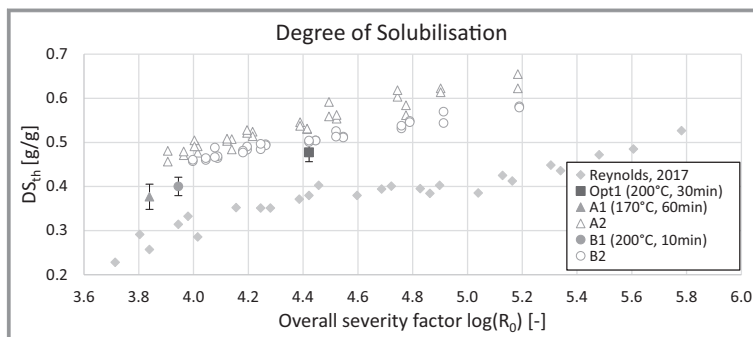
$$\frac{\delta P_M}{P_M} = \sqrt{\left(\frac{\delta a}{a}\right)^2 + \left(\frac{\delta b}{b}\right)^2 + \dots + \left(\frac{\delta x}{x}\right)^2 + \left(\frac{\delta y}{y}\right)^2} \quad (16)$$

## 3 Results and Discussion

### 3.1 Autohydrolysis Pretreatment

The autohydrolysis pretreatment was investigated in one and two consecutive steps to find optimal operation conditions regarding the recovery of pentose, glucose, and lignin, and reduced furfural formations. In the following graphs, the first hydrothermal pretreatment step is marked with full symbols, and the second steps are marked with hollow symbols. The full diamonds are experimental data from Reynolds et al. generated for reactions kinetics investigations [25, 26] using the same setup. These data are used for comparison between the single-step and two-step hydrothermal pretreatment of wheat straw. The experiments for the first step are conducted six-fold. The resulting average and the error bars indicating the standard deviation are displayed. The experiments of the second step are conducted in duplicate, and every single data point is shown, which allows a graphical representation of the experimental and analytical uncertainty.

In Fig. 2 the degree of solubilization  $DS_{th}$  is displayed versus the overall severity factor  $\log(R_0)$ . It can be seen that all data sets follow the same increasing trend. The second step  $DS$  shows higher values than the first step at the same overall severity factor. It is assumed that the water-soluble components, like non-structural carbohydrates, ash, proteins, are removed from the solids rapidly. The increase in  $DS$  with the treatment severity is due to the solubilization of structural carbohydrates, mainly hemicellulose. At higher severities, the solubilized pentoses can degrade to furfural and repolymerize [27]. Thus, the  $DS$  slope would be lowered. The absence of a  $DS$  increase at high severities can indicate that the solubilization and the repolymerization are of equal rates. This phenomenon can be observed with the single-step pretreatment in the range of  $4.4 < \log(R_0) < 5.1$  but not with the two-step pretreatment. It is concluded that repolymerization is reduced for A2 and reduced for B2 at a higher severity factor. The data from Reynolds et al. show

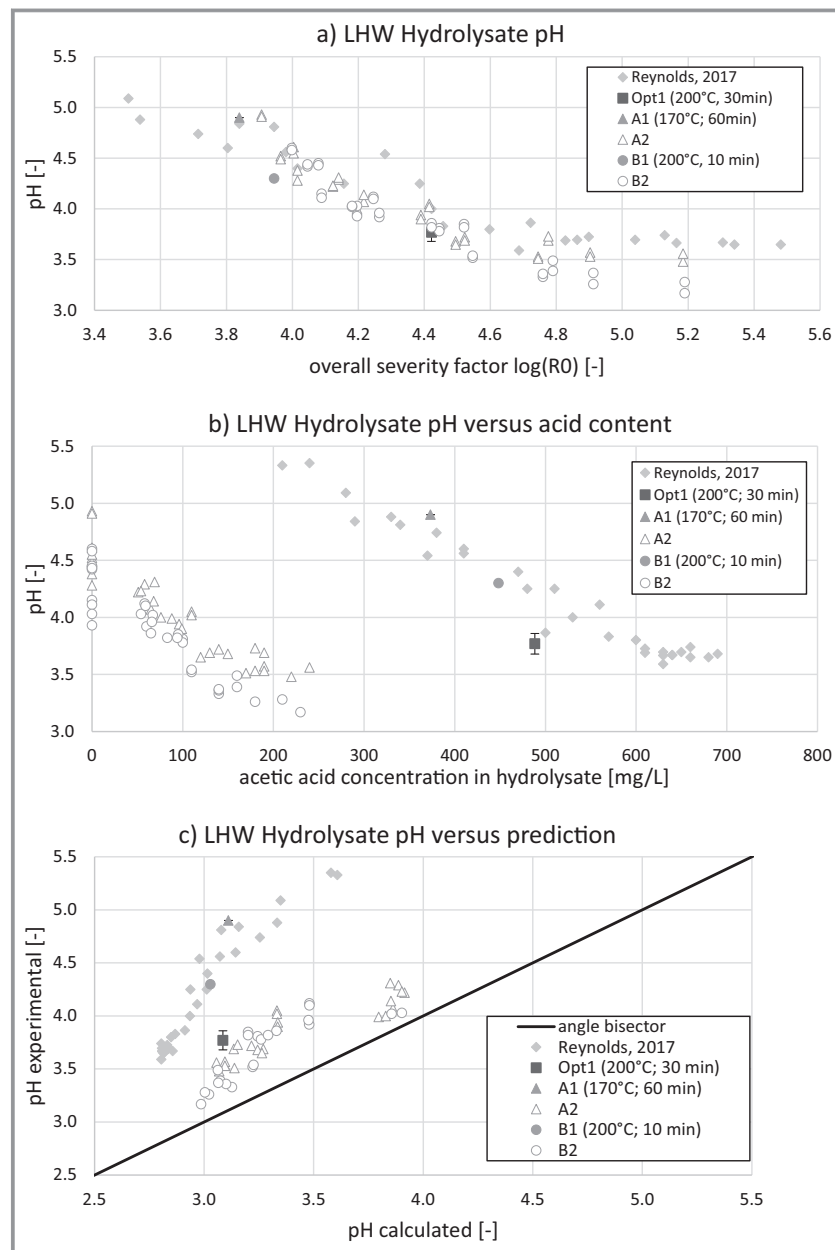


**Figure 2.** Degree of solubilization of the first (full symbols) and the second step (hollow symbols) of autohydrolysis pretreatment versus the overall severity factor. Opt1 (full square), A1 (full triangle), B1 (full circle), A2 (hollow triangle) and B2 (hollow circle). The error bars indicate the standard deviation for the first step hydrothermal pretreatment experiments (six repetitions). For the second step, all experimental results (duplicates) are shown.

lower *DS* values in the first step at the same severity factor. This result is unexpected.

In Fig. 3a pH values of the hydrolysates are displayed versus the overall severity factor. All data points follow the same decreasing trend. The single-step pH curve tends to show higher values and ends on a stagnating value of pH 3.65. In contrast, the lowest two-step pretreatment pH values are 3.50 and 3.25 for B2 and A2, respectively. In

Fig. 3b pH values are displayed versus the acetic acid concentration of the hydrolysate. The detection limit is 50 mg L<sup>-1</sup>; the values below are set to zero. Here, two different curves can be distinguished for the first and the second pretreatment step, respectively. B2 shows a tendency to result in lower pH values than A2. For the first step pretreatment, a three-times higher acid concentration results in the same pH values compared to the second step pretreatment.



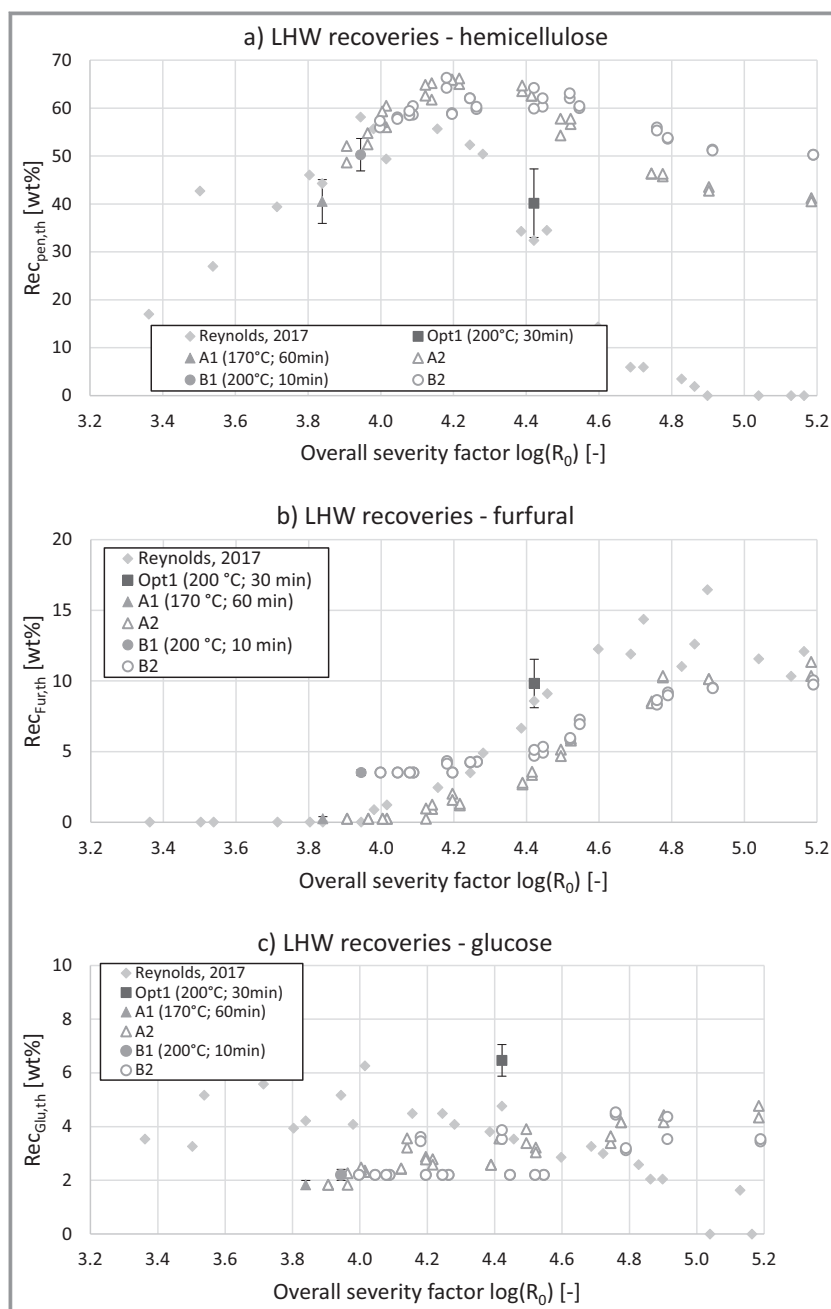
**Figure 3.** Hydrolysate pH value versus a) the overall severity factor, b) the acetic acid concentration, c) the calculated pH value. The first (full symbols) and second step (hollow symbols) of the hydrothermal pretreatment are displayed. Opt1 (full square), A1 (full triangle), B1 (full circle), A2 (hollow triangle) and B2 (hollow circle). The error bars indicate the standard deviation for the first step hydrothermal pretreatment experiments (six repetitions). For the second step, all experimental results (duplicates) are shown.

In Fig. 3c experimental pH values versus the calculated pH are displayed. Formic acid and acetic acid concentrations are used to calculate the pH using Eq. (5). All experimental data points show a higher pH than the calculated one, indicating the presence of bases. Again, the first step and the second step data can be found in different regions. The first step data show a more substantial deviation to the angle bisector compared to second step data.

These observations can be explained with a biomass-inherent neutralizing. The neutralization of acids via cation-exchange with mineral salts in the biomass was reported several times in literature [28–30]. It is assumed that minerals are dissolved, acetic, and formic acid are partially neutralized, and these components are removed in the filtration and washing step after the first pretreatment. With a reduced neutralizing capacity in the second step, the released acetic acid can induce lower pH values. Thus, the hemicellulose solubilization is accelerated. The increased *DS* in the second step supports this interpretation since these *DS* values can otherwise only be achieved at higher severities. The higher severity in B1 presumably removes more minerals in the first step than A1. This reasoning explains the lower pH value of B2 experiments compared to A2 and the lower pH value in the two-step autohydrolysis compared to the single-step autohydrolysis.

In Fig. 4 recoveries of pentoses, furfural, and glucose are plotted versus the overall severity factor. In Fig. 4a the pentose recovery in the first pretreatment step data from Reynolds et al. shows a maximum of 50–60 wt % at  $\log(R_0) = 4.0$ –4.2. At severities larger than  $\log(R_0) = 5.0$  the pentose yield tends towards zero. The conditions A1 and B1 can be found on the rising part of the curve, while Opt1 can be found on the decreasing side. The





**Figure 4.** a) Pentose recovery, b) furfural recovery, and c) glucose recovery versus the overall severity factor. The first (full symbols) and second step (hollow symbols) of the hydrothermal pretreatment are displayed. Opt1 (full square), A1 (full triangle), B1 (full circle), A2 (hollow triangle) and B2 (hollow circle). The error bars indicate the standard deviation for the first step hydrothermal pretreatment experiments (six repetitions). For the second step, all experimental results (duplicates) are shown.

latter indicates that a significant amount of the pentose is lost to side reactions, which decreases the yield and selectivity. Both A2 and B2 possess a maximum of  $(65 \pm 8)$  wt % at  $\log(R_0) = 4.2$ . The Opt1 condition was chosen in previous work as a trade-off between the criteria of a high hemicellulose removal from the solids, >95 wt %, and a high pentose

recovery, 33–47 wt %. For high-quality lignin, a high hemicellulose removal is required. The two-step hydrothermal pretreatment avoids a trade-off between pentose removal and recovery but allows fulfilling both.

In Fig. 4b furfural recoveries are displayed versus the overall severity factor. The first step pretreatment conditions follow the same curve, which increases from zero at  $\log(R_0) = 3.9$ –4.1 and does not exceed  $R_{Fur,th} = 20$  wt %. At a severity of  $4.6 > \log(R_0)$ , there is no significant accumulation in furfural, which indicates further furfural degradation reactions. Furfural resinification or repolymerization with other dissolved components are possible degradation reactions. As discussed above, this may explain the constant DS at  $4.6 < \log(R_0) < 5.2$ . The condition B1 possesses a  $R_{Fur,1} = (3.5 \pm 0.8)$  wt %. Due to the additive nature of  $R_{Fur,th}$ , the second pretreatment cannot reduce the overall recovery. It must be stated that the condition B1 was chosen to show no furfural yield. This was not achieved, highlighting the variance in experimental data in lignocellulose pretreatment. It is concluded that the process window in the first step is revised to  $\log(R_0) < 3.9$ . At the maximal pentose recovery for A2, the furfural recovery is below 1 wt %. In Opt1, the furfural recovery is significantly higher  $(9.8 \pm 1.6)$  wt %.

In Fig. 4c glucose recovery is displayed. All data points, first and second treatment steps, are well below  $R_{Glu,th} = 8$  wt %. It is not possible to affect the glucose recovery in the hydrolysate under the tested reaction conditions. Thus, it does not need to be considered in the selection of process conditions. The hemicellulose contains a small amount of glucose. Thus, it is suspected that the glucose originated from the hemicellulose and not the cellulose.

Regarding the highest pentose recovery and lowest furfural recovery, the optimal conditions for the second pretreatment step, now called OptA2 and OptB2, are observed at an overall severity factor of  $\log(R_0) = 4.1$ –4.3. Tab. 2 shows the first step and optimal second step conditions tested in this work. The recoveries of the pentoses, furfural, and glucose are displayed.

**Table 2.** First step pretreatment experiment. Experimental conditions and recoveries.

	$T$ [°C] <sup>a)</sup>	$t$ [min] <sup>a)</sup>	$\log(R_0)$ [-]	$DS$ [wt %]	$R_{HC}$ [wt %]	$R_{Glu}$ [wt %]	$R_{Fur}$ [wt %]	$R_{Rest}$ [wt %]
A	170	60	3.84	38 ± 3	40.5 ± 4.2	1.8 ± 0.1	0.2 ± 0.5	9.1 ± 0.8
B	200	10	3.94	40 ± 2	50.3 ± 3.1	2.2 ± 0.2	3.5 ± 0.8	12.2 ± 1.9
Opt1	200	30	4.42	48 ± 2	40.1 ± 6.5	6.5 ± 0.5	9.8 ± 1.6	13.0 ± 3.0
OptA2	170; 200	60; 10	4.20	52 ± 5	65.9 ± 7.1	2.8 ± 0.3	1.8 ± 0.2	10.0 ± 1.1
OptB2	200; 185	10; 20	4.18	48 ± 5	65.3 ± 7.0	3.5 ± 0.4	4.2 ± 0.5	12.8 ± 1.4

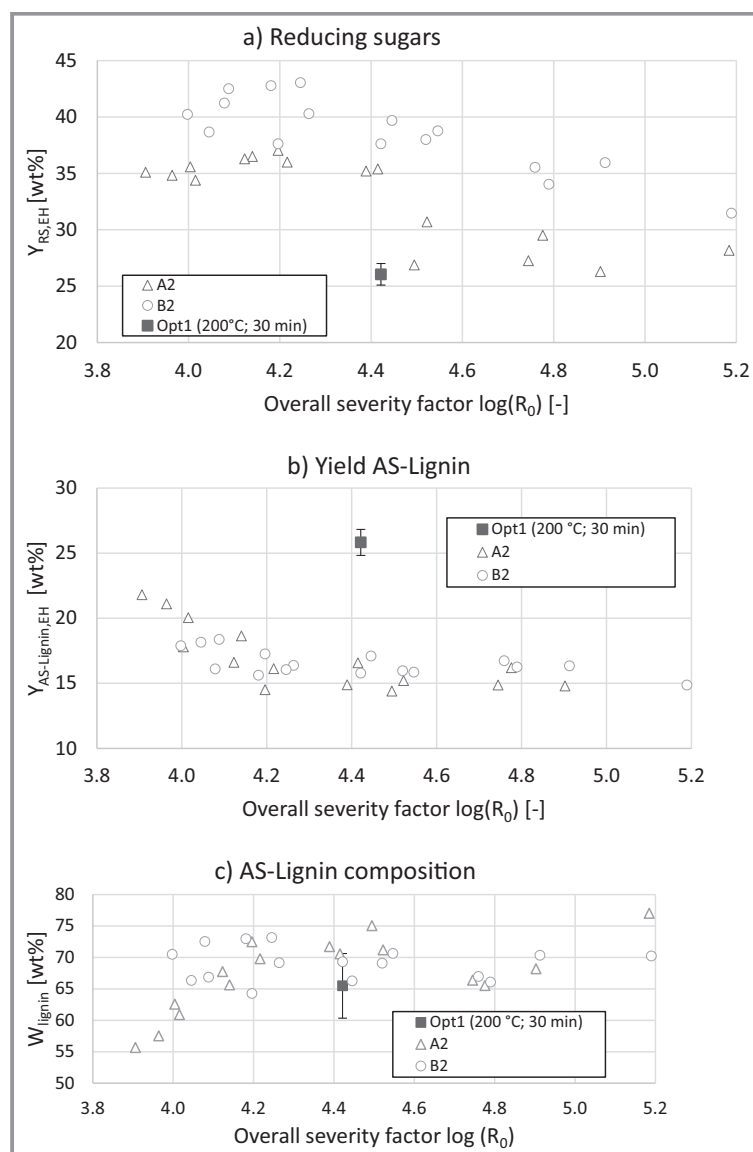
a) Temperatures and residence time in chronological order.

### 3.2 Enzymatic Hydrolysis

The enzymatic hydrolysis was conducted to investigate the effect of the pretreatment on the enzymatic digestibility. The reducing sugar content in the EH hydrolysate represents glucose as well as xylose and arabinose that were not released during the autohydrolysis pretreatment. In Fig. 5 the enzymatic hydrolysis results are displayed versus the overall severity factor applied in the hydrothermal pretreatment. Fig. 5a shows the reducing sugar yield, which is not affected by the pretreatment conditions in the relevant range  $4.0 < \log(R_0) < 4.4$ . The highest yield was observed for B2 conditions, which is  $(47 \pm 5)$  wt %. A2 conditions result in approx.  $(40 \pm 4)$  wt %. The yield of the Opt1 condition being lower is  $(29 \pm 1)$  wt %. The ratio of glucose and pentoses in the hydrolysate remains unknown. Since the pentose recovery in the hydrothermal pretreatment remained below 90 wt %, it is suspected that some pentoses are released during the enzymatic hydrolysis, as was observed by Ertas et al. [22].

Fig. 5b shows the yield of the remaining solid called AS-lignin. A2 and B2 conditions show the same yield of 16–20 wt % for an overall severity factor greater than 4.1. The AS-lignin yield for Opt1 is  $(26.1 \pm 0.9)$  wt %. The differences in yield between the single-step and two-step hydrothermal pretreatment can be explained using the lower recovery of reducing sugars and the assumed repolymerization involving furfural, which are suspected to reduce the enzymatic digestibility [31].

In Fig. 5c the lignin content in AS-lignin is plotted versus the overall severity factor. A2 shows an increasing lignin content until  $\log(R_0) = 4.2$ ; it stagnates until  $\log(R_0) = 4.5$ , then it goes through a minimum at  $\log(R_0) = 4.7$ –4.8. A2 shows the highest lignin content of  $(72 \pm 4)$  wt % at the severity of  $\log(R_0) = 4.2$ –4.5. Even though there is a tendency for the two-step AS-lignin



**Figure 5.** a) Reducing sugar yield, b) AS-lignin yield, and c) AS-lignin composition versus the overall severity factor. The first (full symbols) and second step (hollow symbols) of the hydrothermal pretreatment are displayed. Opt1 (full square), A2 (hollow triangle) and B2 (hollow circle). The error bars indicate the standard deviation for the first step hydrothermal pretreatment experiments (three repetitions). For the second step, all experimental results are shown.

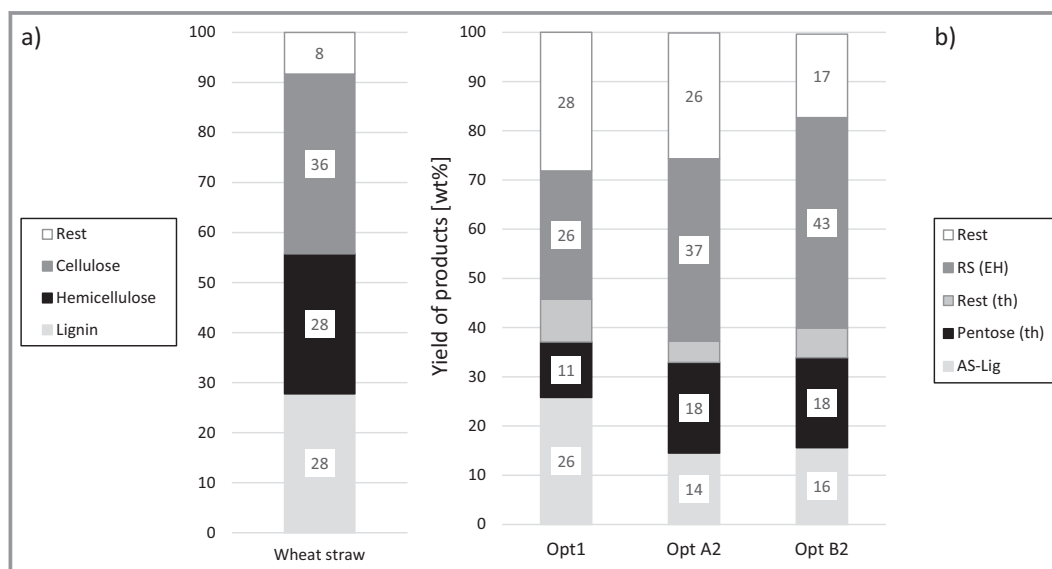
purity to be higher than a single-stage autohydrolysis of ( $65 \pm 5$ ) wt %, there is no significant increase regarding the uncertainty of the measurement.

### 3.3 Fractionation Performance

The pretreatment in second-generation biorefinery aims at a full fractionation of the biomass components. For its evaluation, the product yields are used. Here the reference mass is the dry, untreated biomass (see Eqs. (10)–(12)). In Fig. 6a, the composition of the used wheat straw pellets is shown. Here the fraction Rest is the deviation to a closed mass balance and represents minerals, proteins, waxes, and the analytical error.

Fig. 6b and Tab. 3 display the product yields obtained using the optimized conditions for the single-step pretreatment (Opt1) and the two-step pretreatment (OptA2 and OptB2). For the autohydrolysis pretreatment, four yields are calculated and are denoted with (th). The first  $Y_{\text{pen,th}}$  is the yield of pentoses, namely xylose, and arabinose. The second  $Y_{\text{fur,th}}$  is the furfural yield. The third is  $Y_{\text{Acids,th}}$  and represents the acetic acid and formic acid. The fourth  $Y_{\text{Glu,th}}$  is the glucose yield in the hydrothermal hydrolysate. The

fraction  $Y_{\text{Rest}}$  is the deviation to a closed mass balance and represents undetected dissolved components, like aromatic components and other degradation products. In Fig. 6b the yields  $Y_{\text{fur,th}}$ ,  $Y_{\text{Acids,th}}$ , and  $Y_{\text{Glu,th}}$  are presented combined as Rest(th). The pentose yield is the same using the conditions OptA2 and OptB2. For the other performance parameters, not only the overall severity factor is decisive, but also the severity factor of the first step. OptB2 results in higher furfural yield, reducing sugars yield, and higher mass balance closure compared to OptA2. However, the two-step autohydrolysis (OptA2 or OptB2) is superior in the fractionation performance compared to the single-step autohydrolysis Opt1. The pentose yield is increased by 65 %, from 11.2 wt % (Opt1) to 18.5 wt % (OptA2). The furfural yield is sharply decreased; for OptA2, it is below 1 wt %. The reducing sugar yield is increased from 26.1 wt % (Opt1) to 37.0 wt % (OptA2) and 42.8 wt % (OptB2), which is an increase of 42 % and 64 %, respectively. The overall sugars yield is increased from 39.6 wt % (Opt1) to 56.5 wt % (OptA2) and 62.3 wt % (OptB2), which is an increase of 43 % and 57 %, respectively. Considering the impurity in AS-lignin and the AS-lignin yield, increased lignin solubilization is concluded in the two-step approach.



**Figure 6.** a) Composition of wheat straw used in this work; b) fractionation yields for optimized conditions for a single-step (Opt1) and two-step (OptA2 and OptB2) pretreatment. (th) yield in hydrothermal hydrolysate; (EH) yield in enzymatic hydrolysate; Pentose: xylose and arabinose, Rest (th): glucose, furfural, acetic acid, and formic acid; RS: reducing sugars after the enzymatic hydrolysis.

**Table 3.** The yield of components (wt %) for the optimized condition in single-step and two-step pretreatment.

	$Y_{\text{AS-Lig}}$	$Y_{\text{pen,th}}$	$Y_{\text{glu,th}}$	$Y_{\text{fur,th}}$	$Y_{\text{rest,TH}}$	$Y_{\text{RS,EH}}$	$Y_{\text{rest}}$
Opt1	$25.8 \pm 0.9$	$11.2 \pm 1.8$	$2.3 \pm 0.2$	$2.8 \pm 0.4$	$3.6 \pm 0.9$	$26.1 \pm 1$	$28.2 \pm 3.1$
OptA2	$14.5 \pm 3.6$	$18.5 \pm 1.8$	$1.0 \pm 0.1$	$0.5 \pm 0.1$	$2.8 \pm 0.3$	$37.0 \pm 3.2$	$25.6 \pm 2.8$
OptB2	$15.6 \pm 3.9$	$18.3 \pm 1.8$	$1.3 \pm 0.1$	$1.2 \pm 0.1$	$3.6 \pm 0.4$	$42.8 \pm 3.7$	$16.9 \pm 1.9$



### 3.4 Discussion

The two-step hydrothermal pretreatment aims at a full fractionation of annual lignocellulose with high product yield and high selectivity. The ideal process performance is to produce three streams that each contain only one of the three major substrate components pentoses, glucose, or lignin. During the single-step autohydrolysis pretreatment, the dissolved pentose monomers undergo degradation reactions, before a sufficient digestibility of the solids is achieved. In this work, it was shown that a two-step autohydrolysis pretreatment is a viable approach for the aim of a full fractionation. A yield of 18.3 g pentoses per g of untreated wheat straw was achieved, corresponding to 65.3 % pentose recovery. These results show a significant improvement compared to reported performance in single-step autohydrolysis by Thomsen et al. [18] and Carvalheiro et al. [14].

The two-step autohydrolysis improves the sugar yields by using a second hydrothermal pretreatment step. The pentose yield in the hydrothermal hydrolysate is still significantly lower than 100 wt %. Since the neutralizing capacity of the biomass is mostly removed after the first mild pretreatment, the addition of acetic acid to increase the recovery should be considered. Since acetic acid is produced as a side product, it might be interesting to recycle it as a catalyst. Additionally, a third hydrothermal pretreatment step can be investigated. In this work, the enzymatic hydrolysis was only used to evaluate the effect of hydrothermal pretreatment on the enzymatic digestibility. Since neither the pentose yield nor the lignin purity is maximal, it is suspected that the enzymatic hydrolysis inherits potential for optimization. Ertas et al. [22] showed that mechanical refining after the hydrothermal pretreatment increased the monomeric sugar yield after enzymatic hydrolysis.

The disadvantage of the two-step process is increased investment and processing costs. The primary energy demand is attributed to heating water to reaction temperatures [7]. However, due to the reduced dry biomass in the second pretreatment step, the overall energy demand will be less than two-fold. The two-step autohydrolysis pretreatment might be considered in a biorefinery, including other modules like feedstock storing and preparation, enzymatic hydrolysis, glucose fermentation, chemical conversion of pentoses, and others. The refinery-wide specific product costs might be reduced using the two-step autohydrolysis pretreatment due to its substantially improved yields and small overall increased energy demand.

This work is regarded as a proof of principle for the two-step autohydrolysis. For the advancement of this technology, reactor setups should be tested that are closer to production conditions. A two-step continuous screw conveyor reactor (SCR) operating at high solids loading and using saturated steam as proposed in [4] is regarded as an energy-efficient reactor type for industrial operation [6]. The process investigation using high solid loading and a saturated

steam environment is required to generate data suitable for industrial reactor design. Under these conditions, the sugar concentrations in the hydrolysate are suspected to be higher and more relevant for an industrial application.

### 4 Conclusion

In this work, the two-step autohydrolysis of lignocellulosic biomass is suggested as a measure to increase the total sugar yield by 57 %, selectivity, and purity of its three main constituents, namely lignin, pentoses, and glucose. The two-step autohydrolysis approach is also useful to reduce the furfural yield to below 1 wt % substantially. Thus, its effects as an inhibitor in specific fermentation processes may be neglectable. This technology is more demanding in investment and processing costs but potentially benefits the downstream processing of all streams by producing purer fractions.

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### Symbols used

$c_{FA}$	[mg L <sup>-1</sup> ]	concentration in hydrolysate of formic acid
$c_{AA}$	[mg L <sup>-1</sup> ]	concentration in hydrolysate of acetic acid
$c_{i,j}$	[mg L <sup>-1</sup> ]	concentration in hydrolysate of component $i$ in treatment $j$
$DS_j$	[g g <sup>-1</sup> ]	degree of solubilization of treatment $j$
$M_0^s$	[g]	initial solids mass before treatment
$M_{1,0}^s$	[g]	initial solids mass before treatment
$M_{j,0}^s$	[g]	initial solids mass before treatment $j$
$M_j^s$	[g]	solids mass after pretreatment $j$
$M_{i,j}^s$	[g]	mass of component $i$ after treatment $j$ in solid phase
$M_{i,j}^l$	[g]	mass of component $i$ after treatment $j$ in liquid phase
$R_{0,j}$	[g]	severity factor of treatment $j$
$R_0$	[g]	overall severity factor ( $j = th$ )
$Rec_{i,j}$	[wt %]	recovery of component $i$ in after reaction step $j$
$V_R$	[mL]	volume of the reaction mixture
$W_{i,j}$	[g g <sup>-1</sup> ]	solid mass fraction of component $i$ before treatment $j$
$Y_i$	[g g <sup>-1</sup> ]	yield of component $i$

## Sub- and Superscripts

i	components (pen, fur, glu, AS-lignin, rest)
j	treatment (1, 2, th, EH)
s	solid
l	liquid

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