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Selection and optimization of a suitable pretreatment method for miscanthus and poplar raw material

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Abstract

Miscanthus and poplar are very promising second-generation feedstocks due to the high growth rates and low nutrient demand. The aim of the study was to develop a systematic approach for choosing suitable pretreatment methods evaluated with the modified severity factor ($\log R_0''$). Optimal pretreatment results in a high delignification grade, low cellulose solubilization and increased accessibility for enzymatic hydrolysis while revealing minimal $\log R_0''$ values. In order to do so, several reaction approaches were compared. Acid-catalyzed organosolv processing carried out for miscanthus and poplar revealed the highest delignification grade leading to a relatively high glucose yield after enzymatic saccharification. In both cases, a design of experiments approach was used to study the influence of relevant parameters. Modeling the data resulted in the identification of optimum pretreatment conditions for miscanthus with concentrations of 0.16% H_2SO_4 and 50% EtOH at 185°C for a retention time of 60 min. Experimental validation of these conditions revealed an even higher delignification degree (88%) and glucose yield (85%) than predicted. 0.19% H_2SO_4 and 50% EtOH were determined as optimum concentrations, 182°C and 48 min identified as optimum pretreatment conditions for poplar; the delignification degree was 84% and the resulting glucose yield 70%.

KEYWORDS
design of experiments, miscanthus, modified severity factor, organosolv, poplar, pretreatment

1 | INTRODUCTION

Earlier approaches using renewable resources to gain energy or to produce basic chemicals utilized first-generation feedstocks as sugars from corn, potatoes, or sugar cane. Since these resources compete with food production and so, they are restricted by the amount of fertile soil and require high energy input to grow the crops, the main focus of the research was on the utilization of second-generation feedstocks (Keim, 2010; Lange, 2007). Common raw materials are agricultural residues, municipal waste, or herbaceous crops. Based on this grading, miscanthus and poplar can also be classified as second-generation feedstocks. Miscanthus is a plant that has high growth rates as well as high yields. Low nutrient and water demand makes it an ideal plant for growing on poor soils (Babovic, Drazic, & Dordevic, 2012). As an alternative and rapid-growing wood biomass source, poplar is grown on short-rotation plantations for acquiring energy and/or carbohydrates. As miscanthus, poplar can be cultivated with low agricultural impact and provides high yields and

high coal equivalents (Stolarski, Krzyianiak, Szczukowski, Tworkowski, & Bieniek, 2014).

In contrast to first-generation feedstocks, pretreatment has to be performed prior to enzymatic hydrolysis of carbohydrates based on the imperishable and robust lignocellulosic structure (Galbe & Zacchi, 2007; Sousa, Chundawat, Balan, & Dale, 2009). Pretreatment again can be carried out with biological, chemical, and physical–chemical processes—or optionally in combination or after mechanical pretreatment methods (Chen et al., 2017). Since lignin acts as natural barrier to prevent cellulose and hemicellulose degradation, pretreatment should result in a higher accessibility of the carbohydrates and a higher degree of delignification for efficient enzymatic hydrolysis. This was approved by Adams, Winters, Hodgson, and Gallagher (2018) demonstrating that high carbohydrate:lignin ratios of different miscanthus species increase the glucose release by the means of enzymes. The same applies to poplar wood: Researchers verified that the surface lignin concentration greatly affects the digestibility of the cellulose (Liu, Chen, Hou, Wang, & Liu, 2018). Furthermore, it is approved that the amount and composition of the lignin in the miscanthus has a significant influence on the pretreatment effectiveness (Kärcher, Iqbal, Lewandowski, & Senn, 2016). Several investigations were already carried out concerning the pretreatment of miscanthus (Kärcher, Iqbal, Lewandowski, & Senn, 2015; Pappas, Koukoura, Tananaki, & Goulas, 2014; Yoo, Yang, & Kim, 2016) and poplar (Liu, Liu, Hou, Chen, & Xu, 2015; Yan et al., 2014). However, there is no comprehensive study that investigates the efficiency of several pretreatment methods and whether there is a correlation between the degree of delignification and glucose yield after saccharification.

The introduction of the modified severity factor log R_0'' offered the opportunity to implement pH, temperature, and residence time in one reaction ordinate (Overend & Chornet, 1987; Pedersen & Meyer, 2010). This enables the comparison of different chemical and physical–chemical methods with strongly deviating reaction parameters. However, the reaction parameters combined are the main influencing factors for pretreatment success. The tool of choice to gain knowledge about where there is a correlation between the parameters and degree of delignification or glucose yield after

saccharification is response surface methodology (RSM) based on design of experiment. According to our knowledge, only two studies were conducted with poplar in order to identify the optimum steam explosion conditions via the design of experiments for the subsequent cellulose hydrolysis (Schütt, Puls, & Sake, 2011; Schütt et al., 2013). One study concerns the identification of optimum conditions for the organosolv pretreatment of miscanthus (Goh, Tan, Lee, & Brosse, 2011). Therefore, our approach is to first select the best pretreatment method for miscanthus and poplar wood, followed by the optimization via design of experiments and succeeding evaluation.

Within the presented work, a comparison of different methods for the pretreatment of miscanthus and poplar is shown. The authors demonstrate that exceeding a certain delignification degree is crucial for effective hydrolysis of the resulting carbohydrates. The modified severity factor was then used to identify the most promising pretreatment method and make a point concerning the prospective adaption of relevant parameters. A subsequent DoE approach was used to further identify the quantitative relations between the influencing parameters and glucose yield as well as the degree of delignification for the pretreatment of the two feedstocks.

2 | MATERIALS AND METHODS

2.1 | Resources

Miscanthus (*miscanthus × giganteus*) and poplar wood (*Populus × canescens*) were applied. All resources were chopped with the cutting mill SM 100 (Retsch, Germany) and equipped with a sieve limiting the size of the chopped biomass to a pore size of 2 mm.

2.2 | Pretreatment procedures

Investigations to identify an optimum pretreatment method were performed with the (Versoclave, Büchi AG, Uster, Switzerland) 1 L autoclave. All pretreatment trials regarding miscanthus and poplar wood are performed according to Table 1.

TABLE 1 Overview about the parameters applied for the pretreatment of miscanthus and poplar and the resulting log R_0'' values

	Designation	Solvent	pH	log R_0''
Alkaline	NaOH	2% NaOH	11	8.13
Organosolv	EtOH-OS	50% Ethanol	5	6.13
Organosolv (acidic)	H^+ EtOH-OS	0.2% H_2SO_4, 50% EtOH	4	7.13
Organosolv (alkaline)	OH^- EtOH-OS	0.5% NaOH, 50% EtOH	9	6.13
Aquasolv	H_2O	H_2O	5.6	5.53

Note. The log R_0 amounts to 4.13 for all pretreatment conditions. The values are stated in percent by dry weight.

For each implementation of the trials in the 1 L reactor, 25 g_{DW} biomass was weighed and suspended in the respective solvent. The total mass applied in all trials was 312.5 g. Prior to pretreatment, the reactor temperature was adjusted to 30°C, rendered inert for 1 min with N_2. Subsequently, a pressure of 6 bar was applied. The pretreatment was carried out at 180°C for a retention time of 60 min. After pretreatment was completed, a rough solid–liquid separation was performed with a glass filter crucible. Washing two times with approx. 300 ml of pure solvent applied for pretreatment was followed by washing steps with 500 ml deionized water. The fiber was dried under the fume cupboard. To assess the efficacy of the pretreatment, delignification and glucose yield after enzymatic hydrolysis were compared.

2.3 | Hydrolysis of the resulting fibers

To determine the hydrolysibility degree of the pretreated biomass in function of the delignification degree, an enzymatic hydrolysis was carried out. Therefore, 0.25 g_{DW} fiber was treated with Celluclast® 1.5 L (10 FPU/g_{DW}) and Novozym 188 (10 U/g_{DW}) with a total volume of 10 ml 50 mM pH 5 sodium citrate buffer in 15 ml tubes. The dried fibers were chopped again to a size of <1 mm before hydrolysis. The suspension was incubated for 24 hr and 50°C in a shaking incubator (160 rpm, Amplitude 1.9 cm). After 24 hr incubation, a sample volume of 1 ml was taken and then heated at 95°C for 10 min to inactivate the enzymes. After the heating process, the suspension was centrifuged for 10 min at 10,000 g. The supernatant was subjected to another 1.5 ml tube with a lid and stored at 4°C until analysis process started.

2.4 | Analytics

Analyte concentration was determined with an HPLC equipped with the detector RI 8120 (Bischoff, Leonberg, Germany). The column RPM-Monosaccharide Pb^{2+} was selected for separation of the mixture and heated to 85°C during analysis process. A sample volume of 20 μl was injected into the mobile phase (5 mM sulfuric acid) with a volumetric flow of 0.6 ml/min. Samples were filtrated with a cellulose acetate syringe filter of 0.2 μm pore size before analysis process. The analyte concentrations were determined based on calibration curves collected with pure compounds. The glucose yield (%) can be calculated according to Lu, Li, Zhao, and Qu (2012) as follows:

$$\text{Glucose yield}\,(\%) = I\frac{m_{\text{Glucose}}(g)}{m_{\text{Cellulose}}(g)} \times \frac{M_{\text{Glucose - H}_2\text{O}}\left(\frac{g}{\text{mol}}\right)}{M_{\text{Glucose}}\left(\frac{g}{\text{mol}}\right)} \times 100 \tag{1}$$

With m_{Glucose} (g) as the mass of glucose resulting from enzymatic hydrolysis, $m_{\text{Cellulose}}$ (g) as the total amount of cellulose in the fiber, and M_{Glucose} (g/mol) as molecular weight of glucose (180.2). $M_{\text{Glucose - H}_2\text{O}}$(g/mol) is the approximate molecular weight of the monosaccharide in a cellulose molecule, taking into account the loss of water by the formation (162.2). To simplify the illustration of the results, the values measured for each pretreatment method were normalized with the glucose yields obtained by the best performing pretreatment method—the maximum glucose yield (%).

$$N_{\text{Glucose}} = \frac{\text{Glucose yield}\,(\%)}{\text{Maximum glucose yield}\,(\%)} \tag{2}$$

Based on the glucose yields, the degrees of delignification measured for each pretreatment procedure are normalized by correlating these values with the maximum delignification obtained by a pretreatment method.

$$N_{\text{Delig}} = \frac{\text{Delignification}\,(\%)}{\text{Maximum delignification}\,(\%)} \tag{3}$$

The determination of the biomass composition was performed according to NREL/TP 510–42618 (Sluiter et al., 2008). Total lignin in order to measure the degree of delignification was determined as the sum of the acid-soluble and the acid-insoluble lignin. Xylose and glucose concentrations were summarized to give the holocellulose content. The resulting total composition (in % DM) of both biomass is as follows: Poplar has a lignin content of 30.5% and a holocellulose content of 69.5%; miscanthus lignin content was at 24.5% and holocellulose content at 69.9% with a residue of 5.6%.

2.5 | Modified severity factor

To assess the different pretreatment procedures regarding the experimental data, the temperature T (°C) and the residence time (min) were summarized to a new reaction ordinate—the severity factor $\log R_0$ (Overend & Chornet, 1987). The factor was adapted to the Arrhenius approach introducing relative reaction rates resulting in the general form (Chum, Black, Johnson, Sarkanen, & Robert, 1999):

$$\log R_0 = t \exp\left(\frac{T - T_R}{\omega}\right) \tag{4}$$

The term ω can be determined by means of experiments and corresponds to a reduced level of activation energy applying a first-order reaction. For a first-order reaction,

ω can be fixed to approximately 14.5. T_R is the reference temperature (100°C).

Log R_0 is not suitable for acidic and alkaline pretreatments since the impact of pH was not considered for efficiency. Due to this, the log R_0 was adapted to both, the application of acids and alkali (Pedersen & Meyer, 2010) to give the modified severity factor log R_0'':

$$\log R_0'' = \log R_0 + |pH - 7| \tag{5}$$

2.6 | Optimization of the selected pretreatment methods

The studies of the influence factors within selected pretreatment method were performed with the help of software (Software Design Expert V 8.0; Stat-Ease) with an orthogonal, quadratic experimental design to achieve a response surface with variable-target size dependence. The main process parameters temperature (160–200°C), time (30–90 min), and H_2SO_4 concentration (0.05%–0.2%) were applied to achieve optimum factor values within a DoE approach. By correlation of the influencing factors and the target sizes, optimum conditions for delignification and glucose yield after enzymatic hydrolysis were identified. The correlation can be described by a quadratic model predicting the target size $\bar{y}$ generic provided for two influencing factors (x_1, x_2) including regression coefficients (b_0, …, b_{22}):

$$\bar{y} = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2 \tag{6}$$

The sum of the quadratic deviations $Q_{Residual}$ between the model values $\bar{y}$ and the experimentally determined values y_i is shown by the next equation:

$$Q_{Residual} = \sum_{i=1}^{n} (y_i - \bar{y})^2 \tag{7}$$

For determination of the regression coefficients, the least squares method was applied resulting in a minimization of $Q_{Residual}$.
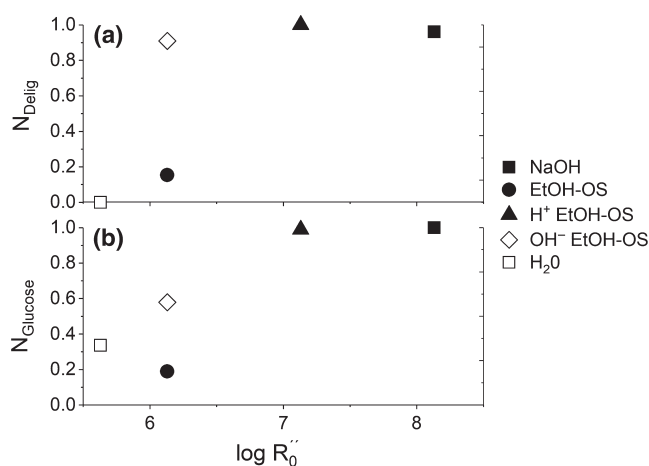
3 | RESULTS

The development and characterization of suitable pretreatment methods for miscanthus and poplar wood is presented in the following sections. Hence, first a systematic comparison of different pretreatment techniques was performed. After selecting the preferred pretreatment method, optimum reaction conditions were identified with DoE and RSM.

Pretreatment procedures should be optimized regarding the solubilization of lignin and the concomitant conservation of a cellulose-rich fiber. Log R_0'' was selected as a basic input factor for the comparison of the different pretreatment methods. A suitable pretreatment method can therefore be defined resulting in a high delignification grade, low cellulose solubilization, and increased availability for enzymatic hydrolysis while calculating minimal log R_0'' values.
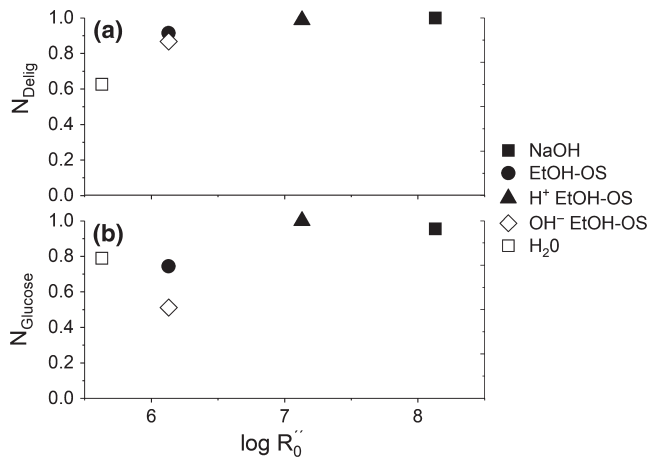
3.1 | Investigation of different pretreatment processes applied to miscanthus and poplar wood

To select a suitable pretreatment method for miscanthus, different methods were compared on the basis of log R_0''. Figure 1 shows the delignification and the glucose yield in function of the modified severity factor.

The highest delignification was achieved using acid-catalyzed organosolv process followed by alkaline ($N_{Delig} = 0.96$) and base-catalyzed organosolv pretreatment ($N_{Delig} = 0.91$). The noncatalyzed organosolv process and the hot water treatment show delignification grades with values ≤15% compared to the catalyzed processes. Consequently, the related glucose yield after enzymatic hydrolysis of the pretreated fiber was lower in comparison with the acid-catalyzed organosolv and alkaline treatment. In our study, the alkaline process and the acidic organosolv pretreatment ($N_{Glucose} = 0.99$) resulted in the highest glucose yields, whereas the acid-catalyzed organosolv pretreatment has a reasonable low log R_0'' value of 7.13. Together with the possibility to recycle the solvent, the acid-catalyzed organosolv process was chosen as a suitable method for the pretreatment of miscanthus.

FIGURE 1 (a) Delignification of miscanthus in function of different pretreatment conditions expressed as modified severity factor; (b) glucose yield from an enzymatic hydrolysis of the solid resulting from different pretreatment conditions expressed as modified severity factor

FIGURE 2 (a) Delignification of poplar in function of different pretreatment conditions expressed as modified severity factor; (b) glucose yield from an enzymatic hydrolysis of the solid resulting from different pretreatment conditions expressed as modified severity factor

Promising results for the delignification of poplar wood lignocellulose were achieved with an alkaline (NaOH) treatment and an acidic organosolv process (Figure 2).

The acidic organosolv process provides advantages due to the low severity factor representing softer reaction conditions. Even the uncatalyzed organosolv process for poplar wood resulted in high delignification degree ($N_{Delig} = 0.92$) at likewise low severity (6.13) compared with the pretreatment processes for miscanthus. The results of delignification are also reflected in the glucose yield obtained after enzymatic hydrolysis of the fiber. The hot water treatment also leads to a saccharification efficiency ($N_{Glucose} = 0.79$) which is significantly higher compared to the hot water treatment of miscanthus ($N_{Glucose} = 0.34$). However, the acid-catalyzed organosolv process, presented in this study, was the most suitable method for the pretreatment of poplar wood. Thus, this method was further investigated and optimized by statistical methods (DoE).

TABLE 2 Pretreatment of miscanthus. Model summary statistics for response factor degree of delignification

Source	SD	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	10.46	0.7520	0.6947	0.4901	2,923	
2FI	7.47	0.9028	0.8444	0.3044	3,988	Suggested
Quadratic	7.81	0.9255	0.8296	0.0966	5,179	
Cubic	4.15	0.9910	0.9518	−1.2851	13,101	Aliased

TABLE 3 Pretreatment of miscanthus. Model summary statistics for response factor glucose yield

Source	SD	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	14.22	0.7529	0.6959	0.6231	4,011	
2FI	15.38	0.7778	0.6444	0.2168	8,336	
Quadratic	10.33	0.9299	0.8397	0.4091	6,290	Suggested
Cubic	7.81	0.9828	0.9084	−5.9396	73,865	Aliased

TABLE 4 Pretreatment of poplar wood. Model summary statistics for response factor degree of delignification

Source	SD	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	10.13	0.6535	0.5736	0.3607	2,460	
2FI	9.19	0.7806	0.6490	0.1104	3,422	
Quadratic	7.18	0.9062	0.7856	0.2280	2,970	Suggested
Cubic	3.04	0.9928	0.9616	0.5509	1,728	Aliased

TABLE 5 Pretreatment of poplar wood. Model summary statistics for response factor glucose yield

Source	SD	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	9.99	0.5195	0.4086	0.1373	2,331	
2FI	9.55	0.6622	0.4596	−0.5801	4,269	
Quadratic	6.85	0.8784	0.7220	0.3742	1,690	Suggested
Cubic	3.45	0.9868	0.9295	0.7491	678	Aliased
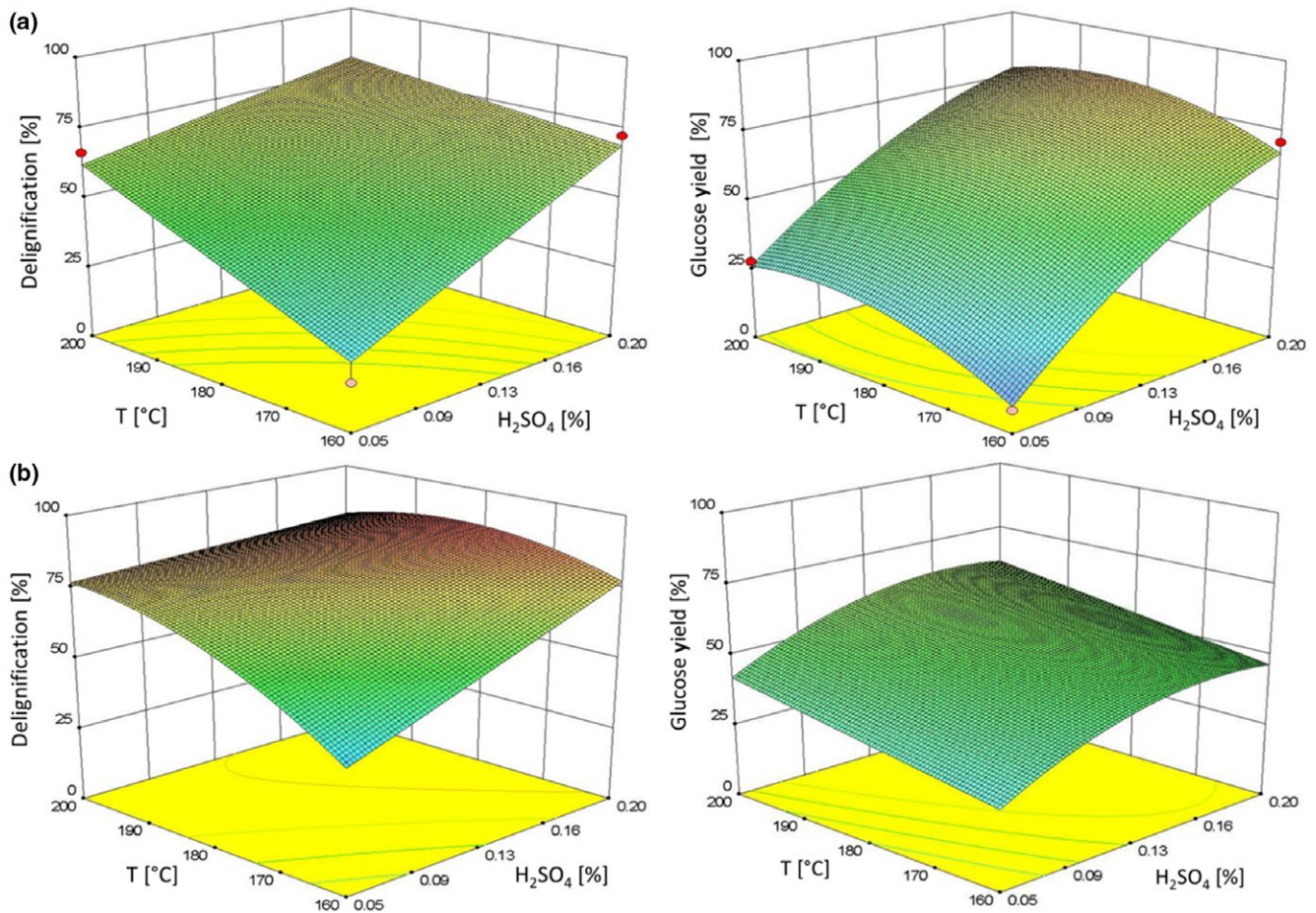
FIGURE 3 (a) Delignification of (left) and glucose yield (right) from miscanthus in function of temperature and acid concentration; (b) delignification of (left) and glucose yield (right) from poplar in function of temperature and acid concentration

3.2 | Investigation of the optimal conditions for pretreatment of miscanthus and poplar wood

For the investigation of the optimum of the pretreatment method H_2SO_4 concentration, temperature T and residence time t were varied. A summary of the statistical analysis was carried out with the software Design Expert V 8.0 and is listed in Tables 2–5.

The different model equations were evaluated regarding R^2, adjusted R^2, predicted R^2, and PRESS (predicted residual sum of squares). The most suitable model for the delignification of miscanthus was a two-factorial model (2FI), whereas a quadratic model for glucose yield was chosen. Regarding poplar wood, the most suitable model for delignification and glucose yield was a quadratic model. The graphs of the corresponding response surface plots are shown in Figure 3.

In order to find the optimum conditions for the pretreatment method in terms of H_2SO_4 concentration, temperature T and residence time t were varied. The delignification (left) and glucose yield (right) in function of H_2SO_4 concentration and temperature are shown in Figure 3a for miscanthus

at a residence time of 60 min, since residence time did not represent a significant factor at the given parameter ranges. Delignification of the miscanthus fiber slopes shows a trend that was linear and upward with increasing temperature and sulfuric acid concentration. The main influencing factors that have an impact on the glucose yield were temperature and H_2SO_4 concentration which was identified by statistical analysis. By increasing the sulfuric acid concentration to 0.2%, a glucose yield of 80% was achieved at a temperature of 180°C and residence time of 60 min. The process was therefore optimized by using a software simultaneously taking into account the delignification and glucose yield after enzymatic hydrolysis. The aim was to carry out the process with minimal temperature, minimal residence time, and minimal sulfuric acid concentration while increasing the target sizes at the same time. Optimum operating conditions were identified by the model at 0.16% H_2SO_4, 185°C, 60 min residence time, and 50% EtOH. At that point, measured delignification was $88 \pm 3\%$ and glucose yield at $85 \pm 4\%$.

Results on poplar are shown in Figure 3b at a residence time of 48 min. The glucose yield showed a linear dependence on temperature. The H_2SO_4 concentration had a

quadratic effect on the glucose yield with a maximum value of 70% at 0.16%–0.2% H_2SO_4. The residence time showed no significance regarding the range investigated. According to miscanthus, software-assisted optimization was performed concerning the delignification and glucose yield. The subsequent optimum operating parameters were identified with a H_2SO_4 concentration of 0.19% solubilized in a 50% EtOH at 182°C for 48 min to achieve $70 \pm 3\%$ glucose yield after 24 hr of hydrolysis and a delignification of $84 \pm 1\%$.

4 | DISCUSSION

4.1 | Identification of a suitable miscanthus pretreatment and optimization thereof

The assessment of the data from our experiments shows that a high degree of delignification (N_{Delig}) could only be realized by acid- and base-catalyzed organosolv processes and alkaline treatment. The reason for this is the high proportion of stable intramolecular β-O-4 ether linkages (46%–93%; El Hage et al., 2009; Villaverde, Li, Ek, Ligero, & Vega, 2009) that commonly require homogenous catalysis, for example, at alkaline or acid conditions for cleavage. This was already reported by Serrano et al. who ascertained that the application of EtOH resulted in a delignification of 30%–40% in contrary to 70%–75% in the presence of catalysts (Serrano, Egues, Gonzalez Alriols, Llano-Ponte, & Labidi, 2010). There are indications from model substance studies that the uncatalyzed cleavage of β-aryl-ether linkages is delayed with regard to the breakage of α-aryl-ether bonds (Kishimoto & Sano, 2003). This is due to the higher activation energy of the β-aryl-ether linkages (150 vs. 80–118 kJ/mol). However, a decrease in the activation energy was already approved for α-aryl-ether bonds in the presence of acidic catalysts (Li, Sun, Xu, & Sun, 2012) and can be also proposed for β-aryl-ether linkages. The acid-catalyzed breakage of β-aryl-ether linkages is elucidated for model compounds and is also likely to occur via a heterolytic, unzipping mechanism in lignin depolymerization (Sturgeon, Kim, & Lawrence, 2014). The alkaline cleavage of aryl ether has been extensively studied in the past with regard to lignin model compounds. The breakage of α-aryl-ether bonds occurs readily at ambient conditions via an elimination mechanism. The cleavage of the β-aryl-ether bond follows a displacement mechanism requiring the deprotonation of an hydroxyl group at a neighboring C atom (Meister, 1995). In our study, the alkaline treatment also resulted in very high glucose yields. However, alkaline treatment had the highest log R_0'' value with 8.13 revealing more harsh conditions than the acidic organosolv pretreatment with the help of ethanol resulting in the highest delignification degree (78%). Furthermore, this method also showed a very high glucose yield with relatively low log R_0'' values of 7.13 at the same time. Additionally, it can be expected that the degree of lignin purity is higher than one that was obtained via base catalysis (Schutyser et al., 2017). Together with the possibility to recycle the solvent, the acid-catalyzed organosolv process was chosen as a suitable method for the pretreatment of miscanthus and selected for optimization: The main influencing factors and the significant variable-target size interactions were identified with the aid of a software: temperature and H_2SO_4 concentration. Accordingly, pretreatment conditions had the highest glucose yield after hydrolysis valued at 0.16% H_2SO_4 and 50% EtOH at 185°C for 60 min. However, at this temperature, xylose and glucose degradation were also observed. These findings are in the same operational range that was recently summarized by Zhao, Li, Wu, and Liu (2017) for an alcohol-based organosolv process.

To confirm the parameter sizes, two additional experiments were performed with these operating points. The model-predicted value for the glucose yield was 74% and the value $85 \pm 4\%$ which was found within the scope of an experiment. With 60%–88%, this value was in the confidence interval of the model. Furthermore, the model predicted a delignification of 72% under these parameter settings whereby data from the experiments revealed a higher delignification degree of $88 \pm 3\%$. Unfortunately, this value is outside the confidence interval. Hence, in future, further data points have to be included into the model to increase its significance. However, the test with higher delignification resulted in a higher glucose yield of $85 \pm 4\%$. Cha et al. (2016) achieved a delignification of only 64% with an alkaline twin screw process using 2.4% NaOH at ~100°C. In this case, they observed a glucose yield of about 56% after 24 hr. A dilute acid pretreatment of miscanthus was also extensively optimized by DoE (Yoo et al., 2016). However, the achieved glucose yields were only <50% after 24 hr approving the demand for the concomitant application of acid and an organic solvent for the efficient pretreatment of miscanthus. The so-called OrganoCat process utilizes a biphasic system of oxalic acid in water and 2-methyltetrahydrofuran (Damm et al., 2017) in addition to ethanol as a solvent for an organosolv process. This method was carried out using miscanthus as feedstock, as well. However, a delignification level of <70% was achieved which led to low glucose yields (<50%) after 24 hr confirming the efficacy of the given process.

4.2 | Identification of a suitable poplar pretreatment and optimization thereof

Best performing methods for the delignification of poplar lignocellulose were achieved for both, the acidic organosolv process and alkaline treatment. In addition to that, the other pretreatment processes with lower modified severity factors had moderate to good results (N_{Delig} and $N_{Glucose} > 0.5$), too. This is based on the higher proportion

of α-aryl ether linkages in poplar, which can simply be cleaved in contrast to the β-aryl-ether bonds (Kishimoto & Sano, 2003). Furthermore, investigations confirmed that the β-aryl-ether bonds present in hardwood revealed increased hydrolysis rates. Another reason is that the poplar also contains syringyl units, which provide higher reactivity (Sannigrahi, Ragauskas, & Tuskan, 2010). At the same time, the condensation of lignin fragments, which usually takes place in acid-catalyzed organosolv counteracting the lignin cleavage (Schutyser et al., 2017), is minimized. Hence, by the supplementation of mineral acid catalysts, the cleavage can be further increased in an organosolv process, since acid-soluble lignin is extracted from the fiber (Chum, Johnson, & Black, 1990, 1988). Because of the miscanthus, the acid-catalyzed organosolv process was thus identified as most suitable method for the pretreatment of poplar wood.

The main influencing factors (temperature and the H_2SO_4 concentration) and significant variable-target size interactions were identified with the assistance of a software. Higher temperatures and H_2SO_4 concentrations enhance the delignification and lead to the augmented cleavage of ether and ester bonds, as mentioned above. A maximal delignification of 80% and a concentration of 0.13% H_2SO_4 were measured for temperatures above 180°C. Also, xylose and glucose degradation were observed at this temperature. Analogous to the delignification, temperature and H_2SO_4 concentration are the significant parameters affecting glucose yield from the enzymatic hydrolysis. The glucose yield showed a quadratic dependence from the H_2SO_4 concentration with a maximum value of 70% at 200°C and 0.16%–0.2% H_2SO_4. The residence time is not a significant parameter in the range considered (data not shown). However, an optimal dwell time can still be determined and the optimum operating parameters were as follows: 0.19% H_2SO_4, 182°C, 48 min, and 50% EtOH. At optimum conditions, we achieved 70 ± 3% glucose yield after 24 hr hydrolysis and a delignification of 84 ± 1%.

A combination of hot pressurized water treatment followed by a treatment with peracetic acid was applied to increase accessibility of poplar wood (Lee et al., 2017). In this case, 70% of the lignin was removed and the glucose yield exceeded the value obtained within our investigations of more than 15% but after 72 hr (no data for 24 hr). Pan et al. (2006) reported values of 92% glucose yield when applying acidic organosolv pretreatment with a higher acid concentration of 1.25% H_2SO_4. However, the amount of endo-cellulase activity applied was twice as high and the β-glucosidase activity was four times higher. By optimizing our enzymatic hydrolysis conditions, the glucose yield exceeded 90% at ≤5% fiber content (data not shown).

As a conclusion, it can be said that the pretreatment methods with higher modified severity factors are more effective than those with lower ones. The acidic organosolv process is suitable for both, the pretreatment of miscanthus and poplar wood. The delignification degree after optimization exceeded 85% in both processes, whereby the glucose yield is higher than 70%. The data show that a gain of fermentable sugars is possible from both resources in the form of high yields. However, the authors are convinced that a further increase in the glucose yield is feasible by implementing a prospective optimization of the hydrolysis process itself. Furthermore, the lignin isolation and the assessment of its quality are to be carried out in the context of investigations in the future.

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