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Comprehensive Wheat Straw Processing with Deep Eutectic Solvent to Deliver Reducing Sugar

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Abstract

Pretreatment is one of the bottlenecks in the cost and energy-efficient biomass valorization. Deep eutectic solvents are potential candidates for being used to address these challenges. In this work, the deep eutectic solvent composed of choline chloride, and acetic acid was studied for its use in wheat straw fractionation. The pretreated biomass was assessed concerning the lignin and glucan content. Under optimized time and temperature conditions, defined using Doehlert matrix chemometric tool, of 3 h 47 min and 139.6 °C, the processed wheat straw contained as much as 42.5 ± 0.42 wt.% and 38.59 ± 1.26 wt.% of glucan and lignin contents, respectively. The need for biomass washing after the pretreatment with deep eutectic solvents and before the enzymatic hydrolysis step was also evaluated. The obtained enzymatic hydrolysis results, i.e., glucan to glucose yield of 27.13 ± 0.25 vs. 25.73 ± 0.08 for washed or unwashed biomass correspondingly, are equally good substrates. Fractal kinetic analysis of the data showed similar values of *k* and *h* for both glucose and xylose reactions between washed and unwashed biomass. This confirmed that biomass washing is an unnecessary step, which in turn opens room for biomass processing intensification.

Keywords Process intensification · Enzymatic hydrolysis · Pretreatment · Design of experiments · Choline chloride

Introduction

Concerns regarding the social, economic, and environmental aspects of worldwide energy provisions have prompted efforts to identify unique sources and create innovative, eco-friendly approaches for generating fuels and chemicals. Lignocellulosic biomass has garnered considerable interest due to its potential as a bioenergy and biomaterials source and as an abundant renewable resource [1]. To minimize land use and to avoid competition with food resources, waste biomass from numerous economic sectors, e.g., agriculture, offers significant potential. Furthermore, using waste materials helps prevent the release of greenhouse gasses, associated with the cultivation, landfill degradation, biomass burning, and others [1]. Wheat straw is an agriculture residual biomass with low or null economic value, the second most abundant biomass feedstock globally, following rice straw, and the most abundant in Europe. Depending on the cultivation practice, this residual biomass may be left on the soil for fertilization purposes, removed, or even burned on site. However, the latter option faces growing restrictions due to concerns including fire propagation. Hence, these factors make wheat straw an ideal candidate for bioeconomy development [2].

Wheat straw, like any lignocellulosic biomass, is a complex matrix primarily composed of cellulose (32–47%), hemicellulose (19–27%), and lignin (5–24%), which is highly resistant to degradation [3]. The conversion of biomass involves several stages. Among them are biomass pretreatment, which breaks down the lignocellulosic matrix and makes reactive cellulosic intermediates available and enzymatic hydrolysis, in which cellulases and hemicellulases transform cellulosic and hemicellulosic intermediates

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into fermentable sugars turning them a substrate for further valorization via, e.g., fermentation. Biomass pretreatment typically involves physical, chemical, or physicochemical procedures such as milling and grinding and acid, alkali, or oxidative delignification. This stage often serves as an economic bottleneck in developing biorefineries for biomass-to-biofuel conversion due to high energy and operational costs [4]. Therefore, there is a growing need for new pretreatment technologies that maintain high delignification rates while being low-cost, eco-friendly, and energy efficient. Deep eutectic solvents (DES) have recently gained prominence in biomass processing as complementary to ionic liquids (ILs) "green" solvent [5]. Their extraction effectiveness heavily relies on DES composition, biomass type, and reaction conditions. DES are created by combining a hydrogen bond acceptor (HBA) with a hydrogen bond donor (HBD) at a specific ratio, yielding a product with at least 20 °C lower melting point than pure substance, hence, the term deep eutectic solvent. Although HBDs vary significantly and include acids, alcohols, amides, and polysaccharides, choline chloride (ChCl) is one of the most widely used HBA and it is produced in large quantities for, e.g., chicken feeding [6]. A key advantage of using DES is their lower sensitivity to water content, which eliminates the need for energy-intensive drying processes for wet biomass processing, providing economic benefits over other chemical pretreatment methods [7]. Additionally, DES have been studied for their promising potential in the intensification of the biomass pretreatment process [8]. Due to the biocompatibility advantages of some DES, there is a possibility of skipping the biomass washing step between biomass pretreatment and enzymatic hydrolysis that is usually crucial when managing organic solvents as they can inhibit enzymes. A biomass washing step is not only a time-consuming step but also brings additional costs and can lead to biomass loss, making the process less efficient. For example, ChCl and glycerol DES have been successfully used in a one-pot production of cellulosic ethanol with Cellic® CTec 2 enzymes and *Saccharomyces cerevisiae*, achieving yields > 80% after enzymatic hydrolysis [9].

Moreover, recovering lignin from biomass alongside its cellulosic content can bring economic benefits to a biorefinery by enabling the creation of value-added products from this fraction. Lignin residues can be used to produce phenolic resins, adhesives, polyphenols, or fuel additives. Furthermore, lignin/starch blends can be employed in coatings, food packaging, and drug delivery systems. The properties and applications of lignin vary greatly depending on its source, environmental context, and the biomass pretreatment approach used [10].

Recent advances in wheat straw pretreatment include the use of eutectic solvents composed mainly of ChCl and HBDs, such as lactic acid, urea, malonic acid, oxalic acid, malic acid, ethanediol, and formic acid [11, 12]. Such studies have demonstrated the high selectivity properties of DES on biomass dissolution, in particular, to lignin removal, with yields as high as 99% concerning glucose recovery, in the case of microwave-assisted ChCl: lactic acid pretreatment [11].

ChCl: acetic acid has a by almost 22 °C melting point lower than pure ChCl or acetic acid; hence, ChCl: acetic acid can be considered DES as reported in the literature [13]. This DES has been widely studied for the removal of phenolic compounds from several biomasses too [14–17]. However, studies for biomass delignification and conversion to reducing sugars are yet limited, specifically for wheat straw biomass [18]. Using acetic acid in DES processing can be an advantage since it is a cheap and abundant chemical, is widely used for food preservation, and can be sustainably produced from biomass [13].

In this work, a DES composed of ChCl and acetic acid, at a molar ratio of 1:2, was studied for wheat straw processing under defined optimal conditions to scrutinize the influence of the variables on sugars and lignin recovery yields and to optimize the reaction in terms of the pretreated biomass composition. The major novelty of this work is the attempt of process intensification, particularly in terms of avoidance of washing step between the biomass pretreatment and enzymatic hydrolysis, which is time and energy-consuming step found in biomass processing.

Materials and Methods

Raw Materials and Chemicals

The wheat straw used in this work was received from Estação Nacional de Melhoramento de Plantas (Elvas, Portugal). The biomass was kept in a closed glass container at room temperature. The particle size of the biomass used was kept irregular. To analyze the granulometry distribution of the raw biomass sieving was conducted using a Retsch (Haan, Germany) vibratory sieve shaker (EVS1, Endecotts, England) for 30 min, using sieves with pore diameters of 3.55, 2.36, 1.60, 1.00, 0.50, 0.25, and 0.15 mm. After the procedure, the material that remained in each sieve was weighted to calculate the different mass fractions.

The lignocellulosic biomass pretreatment was carried out using a DES comprising ChCl (> 98 wt.% Sigma Aldrich, St. Louis, USA) as the HBA and acetic acid (> 99.8 wt.% Fluka-Honeywell) as the HBD, with a HBD to HBA molar ratio of 1:2, as described elsewhere [19]. The DES (with a pH of 1.7) was prepared by mixing the HBD and HBA, and later, the flask containing both components was placed in a water bath (Memmert W200) heated to 80 °C for 30 min.

Distilled water (15 MΩ/cm) used in all procedures was produced by the PURELAB Classic Elga system. The

biomass processing experiments utilized Baysilone M350 oil as the heating medium, which was acquired from Bayer, Leverkusen, Germany.

After pretreatment, the biomass-DES mixture was washed with ethanol 96% v/v purchased from AGA-Álcool e Géneros Alimentares, S.A. (Prior Velho, Portugal). Filtration membranes (Ø = 150 mm, No. 1235 from Filter-Lab, Filtros Anoia, S.A., Barcelona, Spain) were utilized for the post-processing separation of solids from their supernatant. Sulfuric acid (72 wt.%), used in quantitative acid hydrolysis, was purchased from Carlo Erba (Milan, Italy) reagents.

Glucose, xylose, and arabinose standards of analytical grade were used for HPLC analysis and purchased from Sigma Aldrich (St. Louis, USA). A 0.22 µm nylon membrane (Agilent Captiva, Agilent Technologies, USA) was used for sample filtration before HPLC analysis.

Enzymatic hydrolysis assays were performed using the commercial enzyme Cellic CTec2, kindly provided by Novozymes (Denmark). Sodium azide 99 wt.% was purchased from Merck (Darmstadt, Germany) and used to keep the medium aseptic during enzymatic hydrolysis. Then, 50 mM sodium citrate buffer (pH = 4.8) was used for enzymatic hydrolysis sample preparation and was prepared using citric acid monohydrate (99.7 wt.% purity) and tris-sodium citrate (> 99 wt.% purity), both purchased from VWR International Ltd. (Leicester, England).

Biomass Pretreatment

The biomass pretreatment was carried out using methodology developed by authors on the basis of previous studies with ionic liquids [20] as well as was a simplification of other methods given in the literature, e.g., [21]. The pretreatment reactions were conducted using a 1:10 solid-to-liquid ratio, 5 g of biomass (dry weight), to 50 g of DES, and 20 g of biomass (dry weight) to 200 g of DES. The pretreatment was conducted by mixing the biomass and DES in a Schott flask of twice the volume of the mixture, 100 mL for 5 g of biomass to be pretreated and 500 mL for 20 g of biomass to be pretreated. The flask was then immersed in a pre-heated Baysilone M350 oil bath to the desired temperature set in the design of experiments presented in Fig. 1, using an IKA C-MAG HS 7 heating plate controlled by an IKA ETS-D5 electronic contact thermometer, and for the designated experimental time while constantly stirring.

After the reaction, the cellulose- and lignin-rich fractions of the resulting pretreated biomass were separated by cooling the reaction mixture to room temperature, washing, and centrifuging twice with the first 20 mL, and lastly, 10 mL of ethanol for every gram of pretreated biomass used. Each centrifugation was conducted at 4000 rpm and 15 min long (Sigma 6-16KS centrifuge). The mixture was then vacuum filtered, and the solids were dried in a 45 °C oven for at least 24 h. The liquid fraction was separated from ethanol by distillation in a rotavapor (Buchi R-210 rotavapor and Buchi V-850 vacuum controller) at a 100-mbar pressure and temperature of 45 °C for at least 1 h. Following this, the resulting liquid lignin fraction was added to four times its volume of distilled water and stirred to precipitate for at least 15 min. The lignin-rich fraction was then centrifuged for 15 min at 4000 rpm and washed twice with 33 mL of a 1:9 ethanol-to-water solution for every gram of initially treated biomass, centrifuging twice also for 15 min at 4000 rpm and discarding the liquid fraction. The resulting solid fraction was vacuum filtered onto filtration membranes (Ø = 150 mm, No. 1235 from Filter-Lab, Filtros Anoia, S.A., Barcelona, Spain) and left to dry in a 45 °C oven. Once the cellulose-rich fraction of the biomass was dried, a washing process was conducted with 40 mL of distilled water for every 0.5 g of biomass. This process entailed soaking the biomass in a container with distilled water and stirring for 20 min, after which the solids were separated through vacuum filtration and dried for an additional 24 h at 45 °C. Figure 1 shows a simplified scheme of the pretreatment of wheat straw.

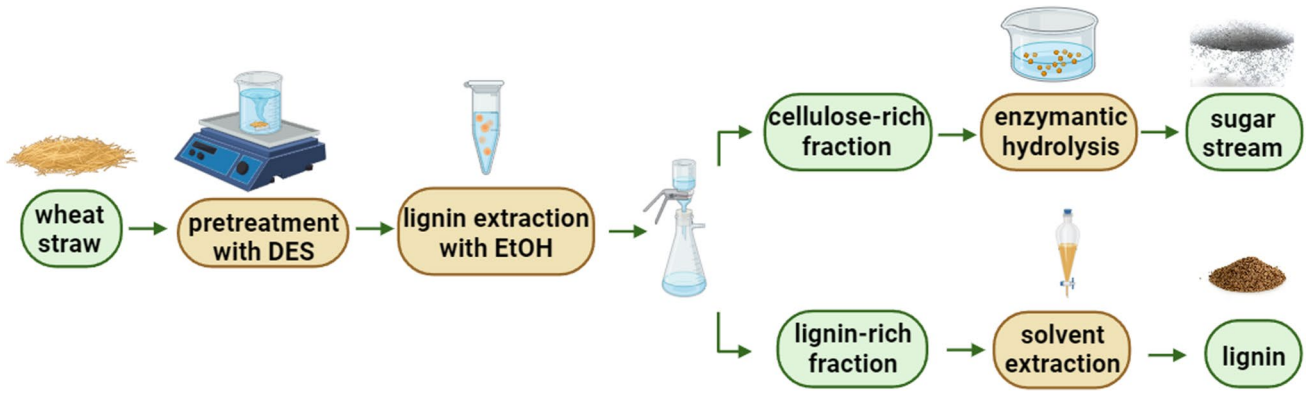

Fig. 1 Biomass conversion approach with DES

Compositional Analysis of Solids

The moisture content of the biomass was determined by drying a sample of 0.5 g at 105 °C for 18 h, as presented in the literature [22]. To determine the ash content, the dried sample was incinerated in a muffle furnace at 550 °C for at least 5 h, following the NREL/TP-510–42622 protocol [23]. The total extractives of the raw wheat straw were determined according to the NREL/TP-510–42619 method [24] and the protein content according to the corresponding ISO method [25].

The composition of the biomass, including the cellulose (glucan), xylan, arabinan, and acetyl group contents, was determined by acid hydrolysis following the NREL/TP-510–42618 protocol [26]. To perform this test, 0.3 g of biomass was hydrolyzed with 3 mL of sulfuric acid 72 wt.% at 30 °C, using a water bath. Subsequently, 84.0 g of distilled water was added to create a 4 wt.% sulfuric acid solution, and the mixture was autoclaved for 1 h at 1 bar and 121 °C. After cooling down to room temperature, the content was vacuum filtered into pore number 3 glass crucibles, which were then dried at 105 °C for 18 h and subject to 550 °C for at least 5 h to assess the acid-insoluble lignin content. Monosaccharide (glucose, xylose, and arabinose) and acetic acid contents in the hydrolysates were determined using an Agilent 1100 series HPLC system (Santa Clara, CA, USA) with a Bio-Rad Aminex HPX-87H column (Hercules, CA, USA) and a refractive index (RI) detector. The column was operated at 50 °C, applying a 0.6 mL/min flow rate using 5 mM H_2SO_4 as the mobile phase. Acid-soluble lignin was quantified in acid hydrolysates using UV spectrophotometry at 320 nm [27].

Enzymatic Hydrolysis

Enzymatic hydrolysis of pretreated biomass was performed with a 15% (w/v) loading rate in a total volume of 10 mL using 50 mM acetate buffer (pH 4.8), distilled water, and a 2% (w/v) sodium azide solution, to prevent any microbial contamination. The enzymatic activity was determined using the procedure described by Ghose [28], and according to the NREL/TP-510–42628 method [29], it was 154.17 FPU/mL.

The experiments were conducted in 50 mL glass, and the conditions used were as follows: 50 °C and 125 rpm achieved using an orbital shaker. Then, 1 mL of samples was taken at 4, 24, 48, and 72 h, next were boiled for 10 min to inactivate the enzymes, centrifuged at 1400 rpm for 5 min to separate the liquors from solid particles, and filtered and analyzed by HPLC. The yields of glucose from glucan and xylose from xylan were calculated using the following equations:

$$\text{Glucan to glucose yield (wt\%)} = \frac{\left(\frac{162}{180}\right) \times [\text{Glucose}] \times V}{\text{glucan content}} \times 100\%$$

$$\text{Xylan to xylose yield (wt\%)} = \frac{\left(\frac{162}{180}\right) \times [\text{Xylose}] \times V}{\text{xylan content}} \times 100\%$$

where V stands for the volume of the enzymatic solution (L), [Glucose] and [Xylose] are glucose and xylose concentrations, respectively, in g/L, and the glucan and xylan contents refer to those present in the pretreated biomass before the enzymatic hydrolysis trials.

Fractal Kinetics Modeling of Enzymatic Hydrolysis

The kinetics of the enzymatic hydrolysis reaction was evaluated using the fractal kinetics analysis, which is based on a pseudo-first-order reaction according to the following equation [30]:

$$P(t) = S_0[1 - e^{-kt^{1-h}}]$$

where $P(t)$ is the product concentration released, S_0 is the initial substrate concentration, k is the rate coefficient (h^{-1}), t is the reaction time (h), and h ($0 \leq h < 1$) is the fractal exponent. The values of h and k were calculated by minimizing the sum of squared errors (SSE) between the simulated values of $P(t)$ and the experimental data, using the Excel solver tool.

Design of Experiments

Pretreatment of biomass with DES was performed under pre-defined reaction conditions regarding temperature and time. The Doehlert matrix chemometric tool was used for this purpose [31]. For the first iteration test, the reaction interval was established in the range of 100 to 150 °C and 1 to 5 h. This resulted in the experimental conditions gathered in Table 1 as X_1* and X_2*. For the final optimization, as described in the results and discussion section, the model was expanded to a range of temperatures from 100 to 160 °C, and a time ranging from 1 to 6 h. This resulted in the experimental conditions presented as X_1 and X_2 also given in Table 1. These experimental domains were chosen based on similar pretreatment reactions using ChCl and acetic acid DES given in the literature [19, 32, 33].

Table 1 Experimental domain concerning time (t) and temperature (T) and their respective codifications using Doehlert matrix position for the design used in the first iteration attempt (X_1* and X_2*) and for the final optimization (X_1 and X_2)

		First iteration		Final optimization	
Absolute values		Codified values			
t/h	T/°C	X_1*	X_2*	X_1	X_2
3.0	125.0	0.00	0.00	−0.20	−0.17
5.0	125.0	1.00	0.00	0.60	−0.17
1.0	125.0	−1.00	0.00	−1.00	−0.17
4.0	146.7	0.50	0.87	0.20	0.56
2.0	103.4	−0.50	−0.87	−0.60	−0.89
4.0	103.4	0.50	−0.87	0.20	−0.89
2.0	146.7	−0.50	0.87	−0.60	0.56
6.0	155.0	-	-	1.00	0.83
6.0	160.0	-	-	1.00	1.00

Statistical Analysis

To statistically analyze the obtained results, multiple regression analysis was performed using the Design-Experiments 7.0 software (Stat-Ease, Inc., Minneapolis, MN, USA). The quadratic response surface model was applied for this analysis, which is defined by the equation $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$. In this equation, Y represents the response, and β_i are the regression coefficients. β_0 represents the response at the center of the experimental domain, while β_1 and β_2 describe the effect of time and temperature, respectively. The interaction parameter, β_{12}, shows the influence of time and temperature on the response, while β_{11} and β_{22} demonstrate how the response surface folds downward (negative values) or upward (positive values) quadratically for both studied variables. The ANOVA was used to determine the statistical significance of the regression coefficients and their effects.

With the same software, an optimization of the reaction conditions was made. This was done by setting up the objectives to minimize the lignin content and to maximize the glucan content in the pretreated biomass. In order to perform this multiresponse optimization, a desirability function was used as follows: $D(x) = (d_1 \times d_2)^{\frac{1}{n}}$ [34]. Where d_i are the desirable values for lignin and glucan content, which vary between 0 and 1, from least to most desirable and n is the number of responses in the measurement, i.e., $n = 2$.

Experimental Uncertainty

The standard uncertainty (u) for each measured weight, $u(m)$, was 0.1 mg. Pretreatment reactions were performed with a temperature uncertainty, $u(T)$, of 0.1 °C. All other procedures that required heat were performed with a $u(T)$ of 1 °C. All reactions, pretreatment, and enzymatic hydrolysis were done in duplicates, and the results presented are average values with their standard deviation values. Reaction times and all other procedures that needed an exact time interval measurement were done with an uncertainty $u(t)$ of 5 s. The calibration technique employed to quantify product concentrations determined the extent to which other experimental errors related to measurements occurred, and a uniform arbitrary error of 10% of the measured value was estimated for all analyses.

Table 2 Wheat straw size distribution

Particle size range (mm)	Wheat straw fraction (wt.%)
> 3.55	0.79
3.55–2.36	8.93
2.36–1.60	31.94
1.60–1.00	36.71
1.00–0.50	14.68
0.50–0.25	4.37
0.25–0.15	1.19
< 0.15	1.39

Results and Discussion

The present work is focused on applying the DES composed of ChCl and acetic acid (1:2) for the pretreatment of wheat straw for lignin separation and subsequent enzymatic hydrolysis of cellulose-rich fraction. Additionally, the biocompatibility of the DES was assessed, evaluating the effect of non-washed in comparison to the washed pretreated biomass. To measure this effect, the efficiency of the enzymatic hydrolysis reactions of both types of pretreated biomasses was used.

Wheat Straw Composition and Size Distribution

Wheat straw used in this study presented a total glucan content of 35.77 ± 0.02 wt.% (as glucose), 22.48 ± 0.001 wt.% of xylan, and 4.49 ± 0.01 wt.% of arabinan. Additionally, acetyl groups accounted for 2.74 ± 0.01 wt.% of the total composition. Total lignin accounted for 23.11 ± 0.09 wt.%, of which 20.92 ± 0.07 wt.% was acid-insoluble lignin and 2.18 ± 0.03 wt.% acid-soluble lignin. Protein constituted 7.31 ± 0.01 wt.% and ash for 9.11 ± 0.05 wt.% of the dried mass. The total moisture content of the biomass was 8.14 ± 0.05 wt.%. The composition of wheat straw used in this study is in general agreement with those presented in literature [12].

The used biomass was subject to particle size distribution measurements, and the results are given in Table 2. As can be seen, more than 2/3 (68.65 wt.%) of the biomass has a granulometry from 1.00 to 2.36 mm.

Optimization of Reaction Conditions

To perform the optimization of the reaction conditions to achieve the objective of minimal lignin and maximal glucan contents in the pretreated biomass, the wheat straw was pretreated with DES. The results of these experiments are presented in Table 3.

A first optimization of reaction conditions was attempted using the results of the pretreatment reactions studied under the experimental domain presented in Table 3 as X_1^* and X_2^*. The result of such optimization was 3.5 h and 149.25 °C. Since these values showed to be in the upper range of the experimental domain, more specifically for temperature, and the optimization graph did not show a clear maximum, as can be seen in Fig. 2, the experimental domain was extended and redesigned to add further the conditions of 6 h and 155 °C and 160 °C. This new experimental design is summarized in Table 1 under the conditions presented as X_1 and X_2, and results are presented in Table 3.

Glucan content values ranged from 29.49 ± 1.09 wt.% for reaction conditions of 1 h and 125 °C to 49.68 ± 0.01 wt.% for conditions of 6 h and 155 °C. In the case of lignin, its content changed from 26.24 ± 0.79 wt.% for the reaction conditions of 3 h and 125 °C to 35.58 ± 0.01 wt.% for 6 h and 155 °C.

This data was subjected to an ANOVA statistical analysis to understand the influence of each variable on the content of cellulose and lignin in the pretreated biomass. The results of such analysis for glucan and lignin content are summarized in Table 4.

The quadratic models in terms of time (t) and temperature (T), in coded factors, which resulted from the ANOVA analysis, are as follows for glucan and lignin content in the pretreated biomass, respectively.

$$\text{Glucan content (wt\%)} = 41.91 + 8.25t + 6.85T - 0.30\text{tT} - 1.60\text{tt} - 4.78\text{TT}$$

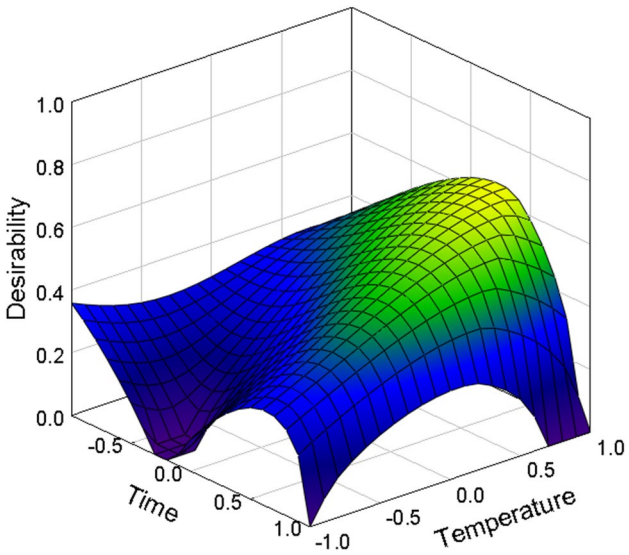

Fig. 2 Desirability function of lignin and glucan contents in the pretreated biomass for different reaction times and temperatures, in coded factors, first iteration attempt

$$\text{Lignin content (wt\%)} = 26.78 + 3.15t + 1.16T - 0.62\text{tT} + 3.95\text{tt} + 1.17\text{TT}$$

Regarding the analysis of the glucan content results, only the linear terms, t and T, of the resulting model showed statistical significance with p values lower than 0.05 (0.004 for time and 0.012 for temperature). Both coefficients have a positive value, which shows that both time and temperature increase will lead to higher glucan content values in the pretreated biomass. The model proved to be significant with a low p value of 0.002. However, the model also showed itself to have a significant lack of fit, with a p value of 0.0002.

Table 3 Results of glucan and lignin content of pretreated biomass used for a first iteration attempt and for the final optimization. X_1* and X_2* represent codifications of experimental domain concerning time (t) and temperature (T) used in the first iteration attempt, and X_1 and X_2 represent the same variables used in the final optimization experiments

First iteration				Final optimization			
X_1*	X_2*	Glucan content (wt.%)	Lignin content (wt.%)	X_1	X_2	Glucan content (wt.%)	Lignin content (wt.%)
0.00	0.00	32.90 ± 0.65	26.24 ± 0.79	−0.20	−0.17	32.90 ± 0.65	26.24 ± 0.79
1.00	0.00	49.51 ± 0.01	30.31 ± 0.01	0.60	−0.17	49.51 ± 0.01	30.31 ± 0.01
−1.00	0.00	29.49 ± 1.09	26.85 ± 0.69	−1.00	−0.17	29.49 ± 1.09	26.85 ± 0.69
0.50	0.87	48.64 ± 1.57	27.98 ± 0.37	0.20	0.56	48.64 ± 1.57	27.98 ± 0.37
−0.50	−0.87	30.47 ± 0.36	26.28 ± 0.74	−0.60	−0.89	30.47 ± 0.36	26.28 ± 0.74
0.50	−0.87	30.44 ± 0.12	27.18 ± 0.20	0.20	−0.89	30.44 ± 0.12	27.18 ± 0.20
−0.50	0.87	39.88 ± 1.62	27.99 ± 0.81	−0.60	0.56	39.88 ± 1.62	27.99 ± 0.81
-	-	-	-	1.00	0.83	49.68 ± 0.01	35.58 ± 0.01
-	-	-	-	1.00	1.00	47.46 ± 0.01	35.30 ± 0.01

Table 4 ANOVA statistical analysis for response surface quadratic model of glucan and lignin content in the pretreated biomass. All model coefficients are presented in coded terms

		Model	Linear		Interactive	Quadratic		Lack of fit	R^2	Adjusted R^2
			t	*T*	tT	tt	TT			
Glucan content	Coefficients		8.25	6.85	−0.30	−1.60	−4.78		0.82	0.73
	F value	8.910	13.46	9.29	0.004	0.13	1.22	33.85		
	p value[a]	0.002	0.004	0.012	0.952	0.721	0.295	0.0002		
Lignin content	Coefficients		3.15	1.16	−0.62	3.95	1.17			
	F value	35.00	55.68	7.57	0.47	23.42	2.07	1.21	0.95	0.92
	p value[a]	< 0.0001	< 0.0001	0.020	0.510	0.0007	0.181	0.375		

[a]Statistically significant when p value < 0.05, not statistically significant when p value > 0.05

As for the lignin content in the pretreated biomass, the statistical modeling results show that both linear terms, *t* and *T*, as well as the quadratic term tt, are statistically significant, with *p* values of < 0.0001, 0.0204, and 0.0007, respectively. The model was significant, with a *p* value of < 0.0001 and not significant lack of fit, with a *p* value of 0.3753, showing that the model is well adjusted to the results, which can also be noted by the values of R^2 and adjusted R^2 of 0.95 and 0.92, respectively. When comparing the model coefficients, it can be noted that reaction time has a greater influence, almost three times higher, on the pretreated biomass lignin content over temperature, with coefficient values of 3.15 and 1.16, respectively.

This data was used to optimize the reaction by minimizing the lignin content and maximizing the glucan content in the pretreated biomass, using a desirability function as described previously. The optimal conditions obtained were a reaction duration of 3 h and 47 min and a temperature of 139.6 °C. These obtained conditions were no longer on the limit of the experimental domain, and the optimization graphic, Fig. 3, showed a much clearer maximum, in contrast with the first iteration. For these conditions, it was expected to obtain a glucan content of 44.52 wt.% and a lignin content of 27.65 wt.%. To confirm the accuracy of the optimization model, wheat straw was pretreated under the obtained optimal conditions. The results showed themselves to be in good agreement with the prediction, having obtained glucan and lignin contents of 41.02 wt.% and of 32.34 wt.%, respectively.

Wheat straw pretreated with a DES composed of ChCl and ethanediol (1:2), with a higher pH of 3.42 than 1.7 for the DES used in the current work, yield biomass contained a slightly lower cellulose and lignin content, namely, 37.64 wt.% and 27.09 wt.%, respectively [35]. In terms of removal of both fractions during the pre-treatment, cellulose was fully intact, and lignin was removed with a yield of 27.26%. However, when wheat straw was pretreated with the DES composed of ChCl and lactic acid (1:2), with a more acidic pH, of 0.25, the glucan content in the processed biomass was higher, i.e., 50.55 wt.% although

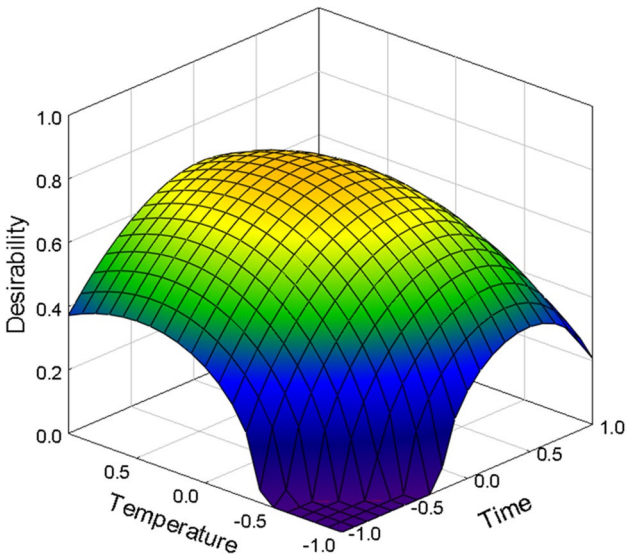

Fig. 3 Desirability function for lignin and glucan contents in pretreated biomass under different coded factors for time and temperatures of reaction, final optimization attempt

with only 80.95 cellulose retention rate. On the other hand, the lignin content was lower and was 17.32 wt.% that is a 69.46% removal rate [35]. These results confirm that cellulose is little affected by the pH of the reaction system; however, a more acidic environment can remove as much as almost 20% of cellulose. Hence, lignin and hemicellulose removal can be easily controlled by changing the DES composition as presented in another study, where the effect of wheat straw composition under different time and temperature pretreatment conditions, using ChCl and lactic acid (1:2), was studied. The authors found that biomass reached higher cellulose content, up to 73.6 wt.%, and lower lignin content, of 5.1 wt.%, for the conditions of 6 h and 150 °C [36]. These results further support the notion that lignin can be efficiently removed by a strongly acidic DES, which exhibit organosolv effect observed for numerous other systems [37, 38].

Enzymatic Hydrolysis

The process intensification was evaluated by testing if there was a need for biomass washing in between the pretreatment and enzymatic hydrolysis steps. For this purpose, a scale-up to 20 g of biomass pretreatment at optimal conditions was done to produce sufficient biomasses for all necessary trials. The pretreated biomass contained as much as 38.21 wt.% and 34.30 wt.% glucan and lignin content, respectively, and these results are in general agreement with those obtained previously with the 5 g biomass pretreatment reaction scale. Enzymatic hydrolysis was performed on the pretreated wheat straw to assess the achieved glucan to glucose and xylan to xylose conversion yields and glucose and xylose concentrations. These trials were performed on both washed and unwashed pretreated biomass to understand if the DES present in the unwashed biomass can affect the hydrolytic potential of enzymes. The enzymatic hydrolysis tests were performed with fixed enzyme loading, i.e., 15.45 FPU/g of glucan. Table 5 presents the glucan to glucose and xylan to xylose conversion yields, and Fig. 4 presents the glucose and xylose concentrations along the reaction time.

Glucose concentration and glucan to glucose conversion yields achieved were similar for washed and unwashed biomass, with the maximum concentrations achieved for glucose of 10.18 g/L and 9.85 g/L, and for conversion yields of 27.13 wt.% and 26.08 wt.%, at 72 h for washed and unwashed biomass, respectively. Wheat straw pretreated with a more acidic DES, of ChCl and lactic acid (1:2) for 6 h at 150 °C, allowed achieving higher glucan to glucose conversion yields of 89.98 wt.% at 72 h of enzymatic reaction time. These results were possible to be obtained due to more organosolv effect of such DES as already discussed above [36].

Regarding xylose, the maximum concentrations achieved were 2.38 g/L and 2.91 g/L, and maximum xylan to xylose conversion yields of 25.73 wt.% and 29.96 wt.%, at 72 h for washed and unwashed biomass, respectively. As can be noted, these values also show to be similar for washed and unwashed biomass.

As described previously, fractal modeling was used to fit the enzymatic hydrolysis data, to determine the kinetic parameters k and h, along with the R^2 value. The obtained values are shown in Table 6, and the models obtained are represented in Fig. 4. High R^2 values show that the fractal kinetic model adequately represents the glucose and xylose saccharification reaction in these conditions. Regarding glucose, the kinetic parameter of k was similar for both enzymatic reactions with washed and unwashed biomasses, with values of 0.096 h^{-1} and 0.093 h^{-1}, respectively. The xylose reaction kinetic parameter of k obtained from both washed and unwashed biomass was 0.094 h^{-1} and 0.142 h^{-1}, correspondingly. Although the value of k was slightly higher for the unwashed biomass reaction, the values are still similar, especially considering that xylose concentrations only varied for 1.3 g/L between the initial and final stages of the enzymatic hydrolysis.

Table 5 Glucan to glucose yield (wt.%) and xylan to xylose yield (wt.%) during enzymatic hydrolysis for both washed and unwashed pretreated wheat straw

	Glucan to glucose yield (wt.%)		Xylan to xylose yield (wt.%)	
Time (h)	Washed	Unwashed	Washed	Unwashed
0	0	0	0	0
4	9.33 ± 0.23	11.79 ± 0.08	11.79 ± 0.08	1.61 ± 0.01
24	19.75 ± 0.65	20.08 ± 0.12	20.08 ± 0.12	2.37 ± 0.06
48	24.43 ± 0.80	23.69 ± 0.20	23.69 ± 0.20	2.72 ± 0.06
72	27.13 ± 0.25	25.73 ± 0.08	25.73 ± 0.08	2.91 ± 0.05

Table 6 Fractal kinetics parameters of enzymatic hydrolysis

	Washed		Unwashed	
	Glucose	Xylose	Glucose	Xylose
k (h^{-1})	0.096	0.094	0.093	0.142
h	0.669	0.714	0.671	0.765
R^2	0.9997	0.9996	0.9998	0.9998

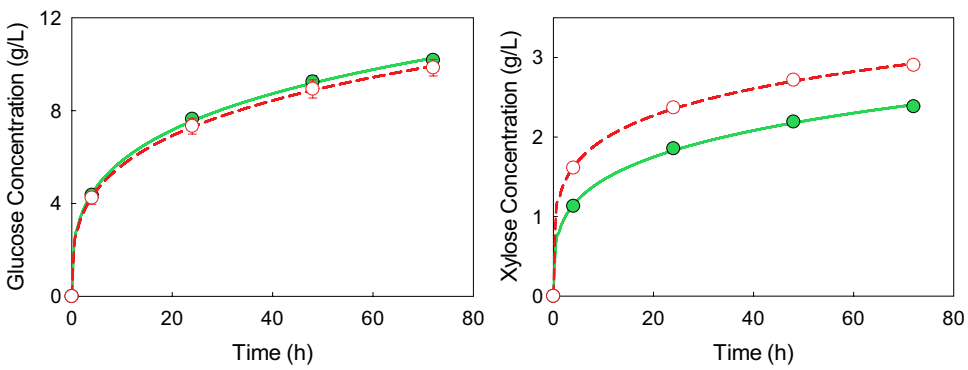

Fig. 4 Glucose concentration (g/L) (left figure) and xylose concentration (g/L) (right figure) obtained during enzymatic hydrolysis of both washed (closed symbols) and unwashed (open symbols) pretreated wheat straw. The solid (washed biomass) and dashed (unwashed biomass) lines represent the fractal kinetic model fitted to enzymatic hydrolysis data

The fractal exponent values of *h* for glucose reaction of washed and unwashed biomasses were similar, of 0.669 and 0.671, respectively. The same can be observed for the xylose reaction for washed and unwashed biomasses, with values of 0.714 and 0.765, respectively. Values of *h* vary between 0 and 1 and can be associated with the accessibility of the substrate, with lower values indicating better accessibility. In this case, the values obtained were somewhat high, and this can be explained by large granulometry of wheat straw, i.e., with 68.65 wt.% of the particle size larger than 1 mm [39–41].

Conclusion

Pretreatment of wheat straw biomass was conducted with the DES composed of choline chloride and acetic acid. The reaction was optimized in terms of time and temperature to minimize lignin and maximize glucose content. No significant difference in the enzymatic hydrolysis of washed and unwashed wheat straw biomass from the pretreatment reaction with choline chloride and acetic acid DES, and therefore, there is room for a process intensification by avoidance of a washing step.

Author Contribution Patrícia J. Piedade: conceptualization, methodology, investigation, software, data curation, and writing—original draft preparation. Veshal Venkat: methodology and investigation. Khaled W. A. Al-Shwafy: methodology and investigation. Mearg A. Aregawi: methodology and investigation. Gabriela Dudek: visualization and writing—review and editing. Mateusz Zygadło: methodology and investigation. Rafal M. Lukasik: conceptualization, methodology, validation, formal analysis, software, supervision, funding acquisition, and writing—reviewing and editing.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

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