Bioresource Technology 369 (2023) 128470

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Optimization of softwood pretreatment by microwave-assisted deep eutectic solvents at high solids loading

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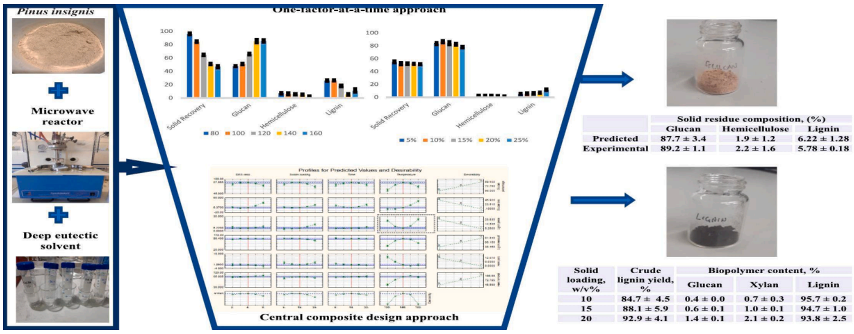
HIGHLIGHTS

- ChCl:FA (1:4) was optimal for milled softwood mixture pretreatment.
- Temperature had the highest effect on the biomass pretreatment.
- At optimum conditions 93.5% glucan retention and 90.1% delignification was achieved.
- At 10–15% solid load, pretreated biomass had similar characteristics.
- High lignin yield (88.1%) and purity (94.7%) was obtained at the optimum condition.

GRAPHICAL ABSTRACT



	Solid residue composition, (%)		
	Glucan	Hemicellulose	Lignin
Predicted	87.7 ± 3.4	1.9 ± 1.2	6.22 ± 1.28
Experimental	89.2 ± 1.1	2.2 ± 1.6	5.78 ± 0.18

Solid loading, w/v%	Crude lignin yield, %	Biopolymer content, %		
		Glucan	Xylan	Lignin
10	84.7 ± 4.5	0.4 ± 0.0	0.7 ± 0.3	95.7 ± 0.2
15	88.1 ± 5.9	0.6 ± 0.1	1.0 ± 0.1	94.7 ± 1.0
20	92.9 ± 4.1	1.4 ± 0.1	2.1 ± 0.2	93.8 ± 2.5

ARTICLE INFO

Keywords:
Lignocellulosic biomass
Deep eutectic solvent
Microwave-assisted extraction
Optimization approach
Lignin removal

ABSTRACT

Microwave-assisted deep eutectic solvent (DES) has received attention as an ultrafast pretreatment method in lignocellulose fractionation. This study investigated the improvement of milled softwood mixture (MSM) fractionation with chlorine chloride-formic acid (ChCl:FA) to obtain residues with high glucan retention and purity while removing majority of the lignin and hemicelluloses. At the optimum pretreatment conditions i.e., ChCl:FA (1:4), 140 °C, 14 min, 800 W and 15 % (w/v), 96.2 % hemicellulose removal, 90.1 % delignification and 93.5 % glucan retention were achieved. About 85 % lignin was recovered with a 95 % purity when solid loading was 10–20 % (w/v). This study showed that microwave assisted ChCl:FA pretreatment was a suitable means to fractionate MSM to achieve high quality glucan and lignin at high solid loading.

1. Introduction

The growing demand for energy has placed more stress on the fossil fuel reserves resulting in faster depletion. The use of fossil fuel has caused an increase in greenhouse gas emission further deteriorating the global warming issues. Lignocellulosic biomass (LCB) is renewable, sustainable and, if correctly managed, contributes little to greenhouse gas emission. Therefore, LCB has been targeted as carbon–neutral source of materials and fuels to reduce the negative effects from fossil resources. The production of energy and platform chemicals from LCB is usually reliant on monomeric sugars such as glucose and xylose (Tan et al., 2020). The glucose yields obtained are inherently related to the digestibility and composition of the cellulosic substrate used under consideration. Therefore, LCB use requires an efficient pretreatment

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https://doi.org/10.1016/j.biortech.2022.128470
Received 29 September 2022; Received in revised form 3 December 2022; Accepted 6 December 2022

process to improve its cellulosic content, porosity and digestibility by enzymes and microorganisms.

The recalcitrance of LCB to chemical and biological conversion is mostly due to the presence of lignin, which acts both as a binder of the LCB components and a shield against both chemical and enzymatic attacks. Over the years, various pretreatment methods have been developed to overcome this challenge. Chemical pretreatments using alkaline, acid and organic solvents have gained much attention with alkaline pretreatment being mostly used (Rezania et al., 2020). Furthermore, the pretreatment process contributed to about 40 % of the total production cost, necessitating the search for a low cost and recyclable pretreatment process and solvent, respectively (Wang and Lee, 2021). Recently, the development of deep eutectic solvents (DESs) have come of interest in LCB pretreatment due to their low cost, low energy requirement, simple preparation, recyclability and environmental friendliness (Xie et al., 2021). An evaluation of the performance effects of the various chemicals for LCB pretreatment and reducing sugar yields showed that DESs performed better than water, acid and organosolv pretreatment while alkaline pretreatment showed similar performance (Sai and Lee, 2019). However, given that alkaline pretreatment required an additional operational unit for neutralization, DESs were reported as the best performing chemicals.

DES is formed by homogeneously mixing a hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). The hydrogen bond formed between the HBA:HBD results in a reduction in freezing point maintaining the produced DES in a liquid state (Elgharbawy et al., 2020). Choline chloride (ChCl) has remained as the most used HBA for most biomass pretreatment processes due to both its effectiveness and cost (Loong et al., 2021). Acid and polyalcohol based HBDs have been reported to be very effective for biomass pretreatment when combined with ChCl (Tan et al., 2020). Furthermore, the ability to recycle the DES and obtain comparable results to the initial pretreatment during the first three cycles has made it an interesting solvent to study (Bajpai, 2021). Kwon et al. (2020) found that at similar reaction conditions, the acidic DES, ChCl:Lactic acid (LA) removed more lignin and hemicellulose than the polyalcohol DES, ChCl:Glycerol when used in pinewood pretreatment. Oh et al. (2020) investigated the action of various DES on pinewood and found out that ChCl:Formic acid (FA) was the most effective acidic-based DES removing 70 % lignin and 86 % hemicellulose while resulting in about 18 % glucan loss. However, DES pretreatments to remove the maximum amount of lignin from the biomass are usually conducted at low solid loading (5–10 %) or at extensive treatment times (6–12 h) (Tan et al., 2020). At such solid loading, the DES pretreatment was about twice more costly than a sulfuric acid pretreatment without recycling (New et al., 2019). Additionally, the pretreatments resulted in more than 10 % loss of glucan, the prime component for the production of glucose.

The introduction of microwave-assisted pretreatment served as a potential means to resolve these concerns while reducing the energy requirement to less than half (Kumar et al., 2019). The industrial feasibility of the pretreatment is reliant on the ability to process the biomass at high solid concentration to reduce equipment size and operation expenses. However, the effects of high solids loading on the solid composition yield has been minimally discussed in literature. Furthermore, the effect of the high solid loading on the characteristics of the recovered solid after pretreatment needs to be accessed to determine its practicability.

The various research works conducted so far have only selected certain microwave-DES assisted pretreatment factors to study. For instance, Isci et al. (2020) studied the effect of irradiation power, reaction time and DES ratio on wheat straw whereas Kohli et al. (2020) studied the effect of temperature and time on *Miscanthus*. A limitation of these studies is that not all the factors affecting the microwave-assisted DES pretreatment were considered in the one factor-at-a-time (OFAT) approach that was used. Furthermore, the effects of each factor were considered independently from the other factors present in the pretreatment resulting in a situation where factor interactions were not accounted for.

In this study, a mixture of milled softwood predominately made up of pinewood was used as a model feedstock. ChCl:FA was considered as the DES of choice since it has been proved to be the most effective binary DES for pinewood (Kumar et al., 2019). The DES molar ratio, solids loading, microwave-irradiation power, temperature and reaction time were the factors considered. A systematic statistical study of the effects of the main factors, including the concentration of solids, was developed using both a one factor-at-a-time (OFAT) analysis and a central composite design (CCD) to determine factor interactions and optimum treatment conditions. The treatment performance was evaluated based on the contents of glucan, hemicellulose and lignin in the pretreated solid. Finally, the effect of high solids loading on the recovered solid was determined by characterizing the composition, functional groups and crystallinity to enhance process industrialization.

2. Methodology

2.1. Materials

A homogeneous batch milled softwood mixture (MSM), predominantly consisting of *Pinus insignis* was purchased from M.I.M.S.A (Lleida, Spain; product ID: MFQ). The pre-milled biomass was received sieved to a maximum size of 100 μm. Formic acid (98 %) was purchased from Fischer Scientific. Choline chloride (ChCl) and all other chemicals used in this study were reagent grade purchased from Merck and Sigma Aldrich.

2.2. Preparation of the deep eutectic solvent

DES was prepared by physically weighing and mixing ChCl and FA at 80 °C for 2 h with continuous stirring at 250 rpm until a homogeneous, colourless mixture was formed (Chen et al., 2019). The DES termed as ChCl:FA were produced at different mole ratios (1:2, 1:3, 1:4, 1:5 and 1:6). Subsequently, the solvents were maintained at 80 °C overnight to achieve complete conversion of unreacted free acids and remove residual moisture. The produced DES were stored in tightly sealed bottles and kept at room temperature in a desiccator to prevent humidity.

2.3. Microwave-assisted DES pretreatment and fractionation of lignocellulosic biomass

The biomass pretreatment was conducted by adapting the method described by Yan et al. (2021). Samples of ca. 1.0 g of extractives-free MSM were mixed with DES at different mass ratios and placed in a Milestone Synthwave Single Reaction Chamber (SRC) with a constant stirring of 200 rpm. The samples were treated at different combinations of temperature, time, irradiation power, and solids load (Table 1). After the pretreatment, the samples were filtered through a vacuum filter and washed with 50 % (v/v) acetone–water. The pretreated solid (SR) was dried at 60 °C for 24 h and then cooled to room temperature and stored for further analysis. Lignin was recovered from the filtrate by evaporating acetone under vacuum in a rotary evaporator, followed by centrifugation at 7000 rpm for 10 min. The recovered lignin (LR) was then washed with distilled water until neutrality and dried at 50 °C for 24 h. Compositional analysis was conducted for SR and LR as described below. Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) were conducted for SR while LR was qualitatively analyzed by FTIR.

$$Solid\,yield\,(\%) = \frac{Mass\,of\,SR(g)}{Mass\,of\,raw\,biomass(g)} \times 100\% \tag{1}$$

$$Glucan\,recovery\,(\%) = \frac{Mass\,of\,glucan\,in\,SR}{Mass\,of\,glucan\,in\,raw\,biomass} \times 100\% \tag{2}$$

Table 1
Process conditions for microwave-assisted deep eutectic solvent for both one-factor at a time and central composite design analysis.

Pretreatment parameter for OFAT					
Pretreatment conditions	I	II	III	IV	V
Varied DES mole ratio (ChCl:FA) at 120 °C, 800 W, 10 min and 10 w/v%	1:2	1:3	1:4	1:5	0:1
Varied temperature (°C) at 1:4, 800 W, 10 min and 10 w/v%	80	100	120	140	160
Varied reaction time (min) at 1:4, 140 °C, 800 W and 10 w/v%	2	6	10	14	18
Varied irradiation power (W) at 1:4, 140 °C, 14 min, 800 W and 10 w/v%	400	600	800	1000	1200
Varied solid loading (w/v%) at 1:4, 140 °C, 14 min and 800 W	5	10	15	20	25
Pretreatment parameters for CCD					
Factors	-α	−1	0	1	+α
DES mole ratio (HBA: HBD)	1:2	1:3	1:4	1:5	1:6
Temperature, C	100	120	140	160	180
Reaction time, min	6	10	14	18	22
Solid loading, w/v%	5	10	15	20	25

$$Lignin\ recovery\ (\%) = \frac{Mass\ of\ lignin\ in\ LP}{Mass\ of\ lignin\ in\ raw\ biomass} \times 100\% \tag{3}$$

$$Residual\ hemicellulose_{SR}\ or\ lignin_{SR}\ (\%) = \frac{Mass\ of\ hemicellulose\ or\ lignin\ in\ SR}{Mass\ of\ hemicellulose\ or\ lignin\ in\ raw\ biomass} \times 100\% \tag{4}$$

$$Hemicellulose\ or\ lignin\ removal\ (\%) = 100 - Residual\ xylan_{SF}\ or\ Residual\ lignin_{SF} \tag{5}$$

$$Glucan\ removal\ (\%) = 100 - Glucanrecovery(\%) \tag{6}$$

2.4. Characterization

2.4.1. Compositional analysis

The chemical composition of the samples was determined according to the National Renewable Energy Laboratory (NREL) analytical procedures. The biomass moisture content was determined gravimetrically by drying at 105 ± 5 °C. The ash content was determined by ashing at 575 ± 25 °C for 5 h (Sluiter et al., 2011). The water and ethanol extractives were determined by performing successive extractions through a Soxhlet apparatus (Sluiter et al., 2008). The carbohydrate sugars and Klason lignin content were determined by performing a two-step hydrolysis (Sluiter et al., 2011). In the first step, ca. 0.3 g of sample was hydrolyzed in 3 mL of 72 % H_2SO_4 at 30 ± 2 °C for 1 h with stirring after every 10 mins. The acid concentration in the sample was diluted to 4 % H_2SO_4 by adding 84 mL distilled water and autoclaved at 121 ± 2 °C for 1 h. The hydrolyzed sample was cooled, and vacuum filtered through a microfiber glass filter. The recovered solid was washed with hot water and dried overnight at 105 ± 5 °C to determine the lignin content of the pretreated biomass, and it was ashed at 575 °C for 5 h to determine the ash content. Aliquots of the filtrate were analyzed with a high-performance liquid chromatography (HPLC).

The hydrolyzed samples were neutralized to pH 7 with $BaCO_3$ and centrifuged to remove the precipitated barium sulfate. A sample of the supernatant liquid was then analyzed on an Agilent 1100 series chromatograph using a refractive index detector. An Aminex HPX-87P column was used at 80 °C with 0.6 mL min^{-1} of Milli Q water as solvent to determine the monosaccharides. The instrument was calibrated with standards prepared from analytical grade glucose, arabinose, xylose, galactose and mannose. An Aminex HPX-87H column was also used to analyze compounds such as acetic acid, formic acid, levulinic acid, furfural and 5-hydroxymethylfurfural (HMF) present in the samples. This column was operated at 50 °C and 0.6 mL min^{-1} of 0.005 M H_2SO_4 as the mobile phase. The HPLC peaks form the samples were identified by comparing with the retention times of the analytical grade compounds used for calibration.

2.4.2. Fourier transform infrared (FTIR)

FTIR of the samples was conducted using a Jasco FT/IR-600 Plus equipped with ATR Specac Golden Gate. Each sample was pressed into a compact film that was analyzed within a spectra range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. The functional groups assignment for the various observed peaks were done in accordance with literature (Ahorsu et al., 2019).

2.4.3. X-ray diffraction (XRD)

The crystallinity of the samples was analyzed by means of a Bruker D2 Phaser X-ray diffractometer equipped with a mono- chromatic CuKα radiation source (λ = 1.54184 Å, 40 kV) and a maximum tube flow of 40 mA. The samples were scanned between the range 5-50° (1°/min) at room temperature at a step of 0.02°. The crystallinity was calculated from the formula (Ceaser and Chimphango, 2021):

$$CrI\ (\%) = \frac{I_{002} - I_{am}}{I_{am}} \times 100\% \tag{7}$$

Where I_{002} is the diffraction peak intensity of the 002 crystal plane at 2θ = 22.5°, and I_{am} is the diffraction peak intensity of the amorphous region at 2θ = 18.0°.

2.4.4. Design of experiments and statistical analysis

A preliminary screening of the various pretreatment factors (DES molar ratio, temperature, reaction time, microwave irradiation power and solid loading) were conducted using a one-factor-at-a-time (OFAT) approach (Table 1). Each pretreatment factor was varied while maintaining the other factors constant (Lim, Gunny, Kasim 2019). The OFAT approach was used to investigate the main influence of each variable.

Process conditions were optimized following a response surface methodology based on a central composite rotatable design (CCD) to determine the number of experimental runs required (Table 1). Optimization was aimed at obtaining a high glucan content in the pretreated biomass while achieving high hemicellulose and lignin removals. The experimental results were fitted into a second-order mathematical polynomial equation that was used to express the models for the various response: where x_i are the values of the i^{th}-factor, k is the total number of factors considered, and b_0, b_i, b_{ii}, and b_{ij} are the coefficients determined by fitting the model to the experimental data.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j}^{k} \sum_{j}^{k} \beta_{ij} x_i x_j \tag{8}$$

To select the best pretreatment conditions to obtain the most optimum multivariate responses the desirability function was used. The desirability function is the most applied method in the optimization of multiple response problems. In general, the desirability function converts each response into desirability value, “d”. A value of 1 implies that the response is at the target value whereas a value of 0 implies that the response is beyond the acceptable limit (Mesa, Martinez, Barrio et al. 2017). Based on this approach, the optimum multivariate conditions were determined, and additional experiments were conducted to validate the prediction of the models at the predicted optimal conditions.

The OFAT experiments were performed in triplicates and the data expressed in terms of mean ± standard deviation. The CCD was statistically analyzed at 95 % confidence interval (p = 0.05) using a one-way analysis of variance (ANOVA). This helped to determine the significance of each factor and their interactions with each other. The fit of the models was evaluated by the coefficient of determination (R^2) and the adjusted R^2. The statistical analysis for the CCD was performed with Statistica 10.0 software. The validation of the optimum conditions was expressed in terms of mean ± standard deviation and compared to the predicted values based on the model and desirability function.

3. Results and discussion

The effectiveness of the microwave-assisted DES pretreatment can be evaluated by the changes to the chemical composition of the biomass and the percentage removal of the individual biopolymers. Therefore, the chemical composition of the untreated biomass was determined before pretreatment. The untreated milled softwood mixture composition was 45.8 % glucan (ca. 50.9 % glucose), 18.1 % hemicellulose (ca. 6.2 % xylose, 7.6 % mannose, 4.0 % galactose and 2.8 % arabinose), 29.3 % lignin (ca. 28.8 % acid insoluble (AIL) and 0.46 % acid soluble lignin (ASL)) comparable to the values (40.43–50.16 % glucose, 6.3–8.7 % xylose, 8.4–13.9 % mannose, 3.7–4.9 % galactose, 2.0–2.5 % arabinose, 27.1–28.7 % ASL and 0.5 % AIL reported in literature for pinewood (Acquah et al., 2018; Kwon et al., 2020; Martínez et al., 1997).

3.1. Microwave-assisted DES pretreatment conditions using the OFAT approach

3.1.1. Effect of DES molar ratio

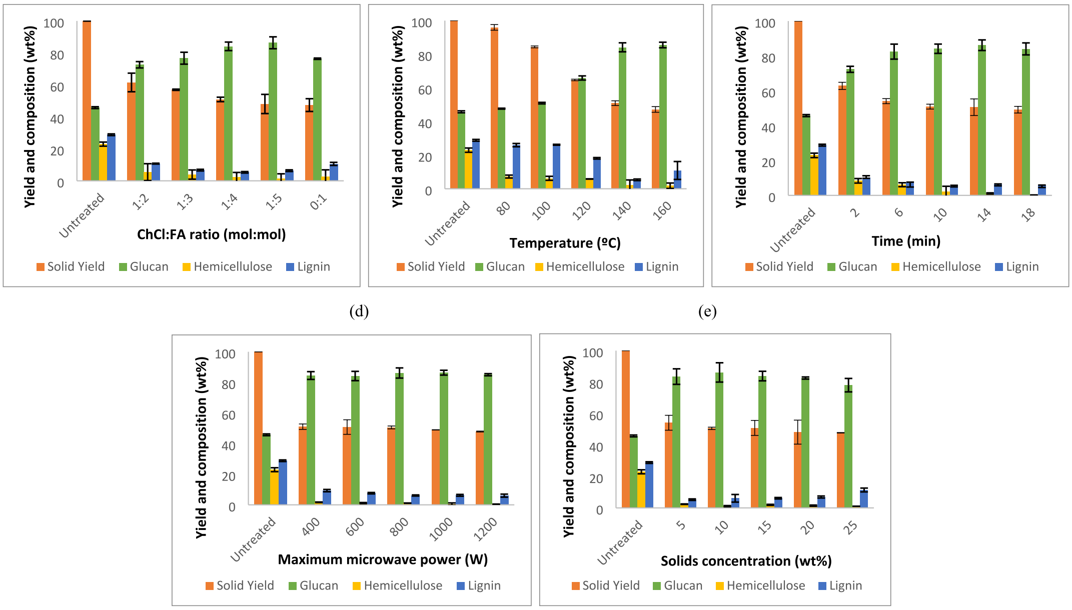
The various molar ratios tested for the ChCl:FA DES were shown in Fig. 1a. At a ChCl:FA molar ratio of 1:1, the DES formed was unstable and could not maintain its homogeneity after standing for 48 h. Therefore, ChCl:FA, 1:1 was excluded from further pretreatment experiments. The effect of the ChCl:FA molar ratio on biomass composition were determined at 140 °C, 10 min, 800 W, and 10 % (w/v) solid loading. The glucan content increased from a molar ratio of 1:2 to 1:4 while remaining almost constant between 1:4 and 1:5 (Fig. 1a). The hemicellulose and lignin content within SR reached their lowest at the 1:4 ratio. A further increase in molar ratio to 1:5 did not considerably change the hemicellulose content whereas the lignin content increased implying the occurrence of lignin recondensation at 1:5. A comparison with the microwave-assisted pretreatment with pure FA indicated that the 1:4 DES results in less glucan loss than with pure FA. Furthermore, similar hemicellulose removal and higher lignin removal were obtained at molar ratio of 1:4 as compared to pure FA. At the microwave-irradiation pretreatment pressure of 10 bars, the boiling point of pure FA was 269 °C indicating that the lower effectiveness of pure FA as a pretreatment solvent could not be attributed to its vaporization at the reaction condition. Therefore, the DES proved to be a better solvent for biomass pretreatment than pure FA. Tan et al. (2019) and Yu et al. (2017) also observed similar trends upon increasing the molar ratio from 1:2 to 1:5. This was correlated to the weaker hydrogen bond formation present when the acid content was increased thereby reducing the available ChCl to react with the FA to produce the DES. Thereby causing the produced DES at and above 1:5 to act more similar to the FA, thus hindering delignification and increasing glucan removal. Consequently, a DES molar ratio of 1:4 was selected to study the effect of reaction temperature.

3.1.2. Effect of temperature

The effects of temperature on the biomass composition and the biopolymer removal were determined with ChCl:FA (1:4) at 10 min, 800 W and 10 % (w/v) solid loading (Fig. 1b). Generally, an increase in temperature reduces the viscosity of the DES, reduces the number of hydrogen-bonds in the solvent and improves its diffusivity into the biomass structure. This leads to higher removal of lignin and hemicellulose while increasing the glucan content of the pretreated biomass (Kohli et al., 2020). As temperature increased from 80 to 140 °C the glucan content of the pretreated biomass increased, and glucan removal was lower than 10 %. At 160 °C the glucan content of the pretreated biomass was statistically equal to that at 140 °C, but the yield of pretreated solid was lower. Hemicellulose content reduced as temperature increased from 100 to 160 °C from 6.2 % to 1.8 %. Similarly, lignin content reduced to 5.3 % at 140 °C. However, at 160 °C an increase in lignin content to 10.9 % was observed which could be due to repolymerization of lignin. Kohli et al. (2020) proposed that the reduction in lignin removal at temperatures above 150 °C was due to lignin repolymerization which was observed with a correlated recondensation into the solid residues thereby resulting in a marginal reduction from 50.9 % to 47.2 % in the solid yield at 140 and 160 °C, respectively. Therefore, 140 °C and ChCl: FA molar ratio of 1:4 was selected for further studies on the effects of reaction time.

3.1.3. Effect of reaction time

Reaction time is another essential parameter that influences product quality and process cost. Fig. 1c illustrates the effect of reaction time on biomass composition at ChCl:FA (1:4), 140 °C, 800 W and 10 w/v% solid loading. The ramp-up time required to reach 140 °C at 800 W was 8 min. Although, it has been reported that a high lignin removal could be achieved during the ramp time (Muley et al., 2019), at the same irradiation power and temperature, the ramp-up time was kept constant, implying a similar effect along this study. The holding time after



(d)

(e)

Fig. 1. Solid biopolymer composition at α = 0.05 for a triplicate of each experiment a) HBA: HBD mole ratio b) Temperature c) Time d) Power e) Solid loading.

reaching the required reaction temperature was considered as the reaction time in this study. Microwave pretreatment from 6 to 18 min marginally changed the pretreated biomass glucan content (82.5–86.2 %). Furthermore, at 18 min the solid yield of 49.2 % implied that about only 10 % glucan was lost. Hemicellulose content sharply declined after exceeding 10 min. Lignin content also reduced from 10 to 14 min before reaching a plateau of ca. 5.6 % where no visible change was observed. The results indicated that an extensive reaction time had a higher effect on the hemicellulose content of the biomass than the glucan and lignin contents. Zhong et al. (2022) similarly observed that the glucan yield did not change when the reaction time was increased from 5 to 30 min whereas lignin content remained almost constant at 15–20 min. Since a longer reaction time would result in more energy consumption but no significant changes to the yield and quality of the pretreated biomass, reaction time was fixed at 14 min for ChCl:FA (1:4) and 140 °C for the subsequent study of the microwave-irradiation power.

3.1.4. *Effect of microwave-irradiation power*

Fig. 1d shows the effects of the microwave-irradiation power on the pretreated residue. An increase in power from 400 to 1200 W did not influence the glucan, hemicellulose and lignin contents of the pretreated biomass residue. Similarly, a marginal difference was observed with the glucan removal. The hemicellulose and lignin removals slightly increased from 400 to 800 W, before remaining almost constant with an increase in irradiation power to 1200 W. This was mainly due to the regulation of the ramp time required to reach the set temperature at the various microwave irradiation power settings, thereby ensuring that the conditions remained almost similar. Therefore, further treatments were conducted keeping the microwave irradiation power at 800 W.

3.1.5. *Effect of solid loading*

The effect of the solid loading was determined by conducting the experiment at ChCl:FA (1:4), 140 °C, 14 min and 800 W changing the solid load from 5 to 25 % (Fig. 1e). The glucan content of the pretreated biomass remained almost constant between 5 and 25 w/v% solid loading, albeit slightly lower at 25 %. However, based on the 47.8 % solids yield at 25 w/v%, a considerable glucan loss of 18.5 % was obtained. Between 10 and 25 w/v%, there was almost no change to the hemicellulose content of SR whereas the lignin content only increased when the solid residue was increased to 25 w/v%. The increase in the lignin content could be attributed to the reduction in glucan content of the SR at 25 w/v%. Due to this, the lignin content (11.4 %) of pretreated residue at 25 w/v% was almost twice the lignin content observed between 5 and 20 w/v% (5.2–6.9 %, respectively). Chen et al. (2018) similarly reported that increasing solid loading to 27 % led to a decrease in lignin removal. This result indicated that the solid loading could potentially be increased to up to 20 w/v% without drastically affecting the composition of the pretreated residue.

3.1.6. *Factors interaction and optimization of microwave-assisted DES pretreatment using central composite design*

A 28 run CCD was conducted with four factors (DES molar ratio, temperature, reaction time and solid loading) and three levels to ascertain the individual effects of the various factors as well as their interactions and the results represented as response surface profiles in Fig. 2. A second order polynomial and a statistical analysis of variance (ANOVA) was conducted for each response variable (Table 2) and pareto charts (supplementary material). For all the responses, the lack of fit was insignificant showing that the responses could be explained by the model.

The R^2 values obtained for the biopolymer residue glucan, hemicellulose and lignin content were 0.91, 0.96 and 0.95, respectively (Table 2) whereas for the percentage biopolymer removal during microwave-assisted DES pretreatment values of 0.94, 0.98 and 0.94 were obtained for glucan, hemicellulose and lignin respectively (supplementary material). A comparison of the R^2 values with the adjusted R^2 values showed that the response variations within the model could be predicted by the mode. The p-values obtained showed the significance of each factor as well as the interactions patterns of the individual factors. The smaller the p-value obtained the more significant the coefficients obtained.

The p-values obtained for the percentage glucan in the biomass

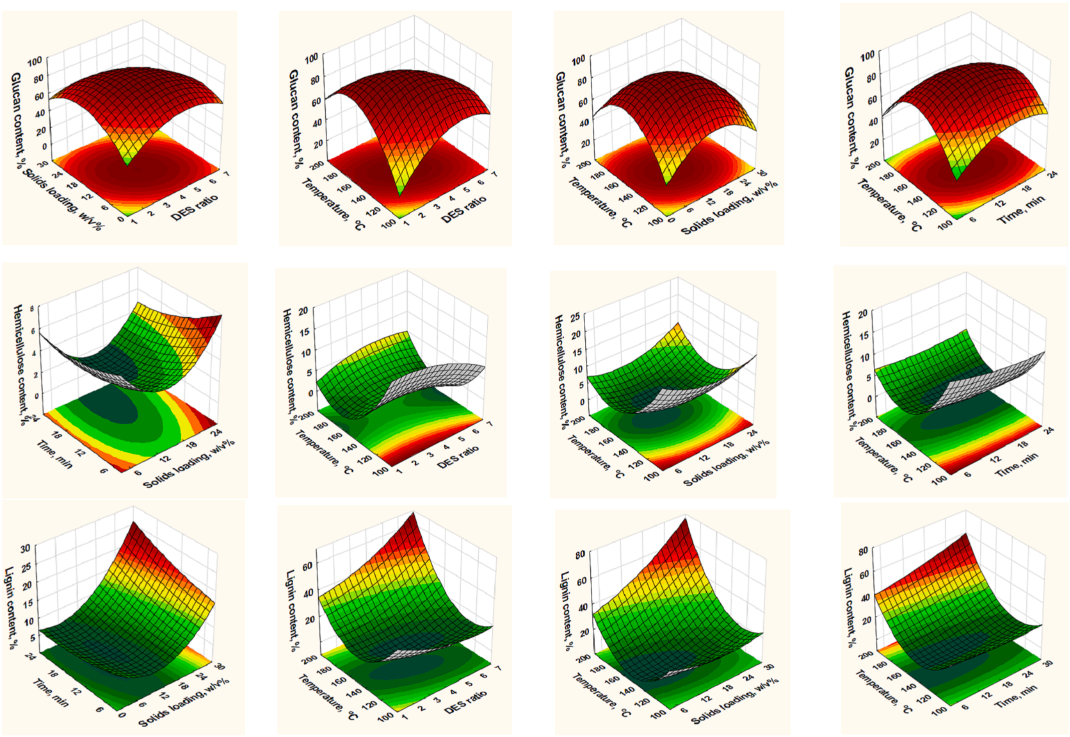


Fig. 2. Response surface profiles of solid residue biopolymer composition after microwave-assisted DES pretreatment.

Table 2
Summarised analysis of variance (ANOVA) for solid residue glucan, hemicellulose and lignin content after microwave-assisted DES pretreatment based on central composite design models.

Factor	Solid residue composition					
	Glucan		Hemicellulose		Lignin	
	F-value	p-value	F-value	p-value	F-value	p-value
A	14.0322	0.0332 *	14.9420	0.0306 *	12.6304	0.0380*
B	42.3065	0.0074 *	3.2030	0.1714	21.0188	0.0195*
C	37.7131	0.0087 *	0.1135	0.7583	213.7805	0.0007*
D	57.9289	0.0047 *	33.2151	0.0104 *	30.9651	0.0115*
A2	0.8329	0.4287	10.8458	0.0460 *	14.7979	0.0310*
B2	40.9879	0.0077 *	0.8794	0.4175	1.5775	0.2980
C2	71.5172	0.0035 *	528.8072	0.0002 *	13.9860	0.0333*
D2	244.6433	0.0006 *	117.8479	0.0017 *	797.9711	0.0001*
AB	12.3913	0.0389 *	0.2111	0.6772	1.2181	0.3503
AC	1.2619	0.3431	1.9210	0.2598	3.6510	0.1520
AD	57.9584	0.0047 *	4.1938	0.1330	89.6997	0.0025*
BC	0.0045	0.9510	0.3784	0.5820	5.0569	0.1101
BD	15.1966	0.0300 *	4.3547	0.1282	113.7284	0.0018*
CD	11.9644	0.0407 *	0.6685	0.4735	17.5318	0.0248 *
Lack of fit	3.9503	0.1426	2.3917	0.2561	6.9627	0.0686
Pure Error		9.7009		1.2370		1.5762
R-squared		0.9190		0.9640		0.9480
Adjusted R-square		0.8340		0.9260		0.8920

* Significant at 95 % confidence level with p value less than 0.05.
A is DES ratio, B is solid loading, C is time and D is temperature.

residue showed that temperature had the most significant effect whereas the linear response (L) for time, the interaction between solid loading and time and the interaction between DES molar ratio and time were found to be insignificant. The biomass residue glucan percentage reached 89.5 % as the various factors increased until ChCl:FA molar ratio 1:4, microwave treatment conditions of 140 °C, 14 min and 15 w/v % solid loading before declining with a further increase in the various factors to 72.0 % (Fig. 2).

The hemicellulose content of the pretreated residue was majorly affected by treatment temperature whereas the solid loading, DES molar ratio and reaction time had little effects. The interaction of the various factors was found to have no effect on the residue hemicellulose content. The residue hemicellulose content sharply declined to 1.3 % as temperatures increased until 140 °C before staying almost constant with further temperature increase in temperature (Fig. 2). This indicated that at 140 °C, the pretreatment was able to remove about 95.0 % hemicellulose from the raw biomass.

The lignin content of the pretreated biomass was mainly affected by reaction temperature and the solid loading whereas the DES ratio and time had very little effect (Table 2). Additionally, the interactions between temperature and solid loading (DES ratio or time) were observed to affect the response. Lignin content reduced sharply to a minimum of 5.3 % as temperature increased to 140 °C before increasing with a further increase in temperature. Yu et al. (2017) similarly found that the reaction temperature was a limiting factor for the lignin removal from Akebia herbal residues when treated with ChCl:FA DES. Between 5 and 20 w/v%, the lignin content remained almost constant as was observed in the OFAT approach whereas an increase was observed with a further increased to 25 w/v%. Furthermore, the CCD showed that increasing time above 18 min would increase the lignin content.

The ANOVA results (Table 2) show that although the OFAT analysis could give a general indication of the pretreatment effects, the interactions observed with some of the factors were not considered. The interactions related to temperature had the most significant effect on both glucan and lignin associated responses. Contrarily, hemicellulose was the least affected by any factor interaction and could therefore be almost predicted by using the OFAT analysis. This implies that to fully understand the effects of the various factors on the responses to obtain the most suitable treatment conditions, a CCD was more beneficial than an OFAT analysis.

3.1.7. Validation of predictive model responses and comparison with past studies

The software Statistica was used to determine the optimum microwave-assisted DES conditions to obtain a pretreated solid with maximum glucan content, lowest lignin and lowest hemicellulose content while achieving minimal glucan removal, maximum lignin removal and maximum hemicellulose removal during the pretreatment process. The predicted optimum conditions to achieve this were 1:4 M ratio, 140 °C, 14 min and 15 % solid loading. The experimental results showed that at these conditions the pretreated biomass residue obtained had a glucan, hemicellulose and lignin content of 89.2 %, 2.2 % and 6.5 %, respectively while the glucan, hemicellulose and lignin removals were 6.8 %, 94.2 % and 90.4 % which were within the range of the predicted values (glucan, hemicellulose and lignin content of 87.7 ± 3.4 %, 1.9 ± 1.2 % and 6.22 ± 1.3 %, respectively while the glucan, hemicellulose and lignin removals were 6.4 ± 2.7 %, 95.0 ± 3.1 % and 89.4 ± 3.1 %). Given the high multi-response desirability value of 0.91 and the correlation between the experimental and predicted values, the regression models obtained were adequate in predicting each response.

The results obtained at the optimum treatment conditions were compared to other studies conducted using either a conventional-DES pretreatment, microwave-assisted or a combination of ultrasonic and microwave-assisted DES pretreatment (supplementary material). The lignin removal obtained in this study was similar to the 90.1–92.0 % obtained by Ji et al. (2020) and Zhong et al. (2022). Interestingly, Ji et al. (2020) was only able to achieve this result by conducting a sequential ultrasonication and microwave treatment on garlic skin using ChCl:Glycerine:Al whereas Zhong et al. (2022) obtained this result using GH: LA at 130 °C for 30 min on castor stalk. On the other hand, Li et al. (2019) was only able to achieve a lignin removal of 42.8 % from pinewood after microwave-assisted ChCl:LA pretreatment. Surprisingly, Xie et al. (2021) obtained 87.0 % lignin removal from Radiata pine pretreated with benzyltrimethylammonium chloride:FA (1:2) at 150 °C for 2 h in an oil bath. This shows that DES with FA as the HBD is effective in enhancing delignification of pinewood.

The higher performance of this DES in this study could also be influenced by the use of an extractive-free biomass. Tajmirriahi et al. (2021) confirmed that extractive removal could act as a weak pretreatment thereby helping to enhance the performance of successive pretreatments. To address this issue, we conducted additional experiments at the optimal conditions with raw unextracted biomass. The results showed a significant decrease in the glucan content (82.2 %) with an increase in the hemicellulose (4.7 %) and lignin (9.9 %) content as compared to the extractive-free biomass used. Nevertheless, the results obtained with the raw unextracted biomass were still higher than the results obtained by Li et al. (2019) on *Pinus bungeana Zucc* (58.4 % gluan, 6.6 % hemicellulose and 29.7 % lignin content in the recovered solid). The pretreatment was observed to provide better results when biomass was first subjected to extraction, which implies that the removal of the extractives could slightly loosen up the biomass structure thus enhancing the effect of the DES pretreatment.

The hemicellulose removal obtained in this study was the highest (94.2 %) which could be attributed to the high optimum temperature in this study. Chen and Wan (2018) similarly observed a high hemicellulose removal (90.1 %) when the microwave treatment was conducted at 152 °C. Yan et al. (2021) performed a sequential ultrasound pretreatment for 30 min followed by a microwave-assisted pretreatment at 100 °C for 20 min to obtain a hemicellulose removal of 91.3 % from corn straw. The glucan, hemicellulose and lignin content of the pretreated solid were similar to the values obtained by Tan et al. (2019) when oil palm fruit bunch was treated with ChCl:FA (1:2) at 10 wt% solid loading at 120 °C for 8 h in an oil bath. The results from our study are among the highest for the various responses observed indicating the need to fully consider all the factors involved in the microwave-assisted DES pretreatment to optimize process conditions.

3.1.8. Effects of microwave-assisted DES on chemical structure and crystallinity of milled softwood mixture

The FTIR spectra of the untreated MSM and pretreated MSM at both 10 % and 15 % solid loading were analyzed (supplementary material). The O=H stretching vibration of glucan was observed at 3332–3336 cm^{-1} in all the samples. The peaks at 2895, 1315, 1160 and 1030 cm^{-1} attributed to the CH_2 stretching vibration, CH_2 wagging, asymmetric stretch and C—O stretch of cellulose, respectively (Ceaser and Chimphango, 2021). The increase in these peaks indicated an increase in cellulose content as observed in the pretreated samples. The amorphous and crystalline bands of cellulose can be observed at 896 and 1428 cm^{-1}, respectively (Hassan and Mutelet, 2022). The intensity of crystalline peak increased after pretreatment for both 10 % and 15 % solid loading whereas the amorphous peak reduced with respect to the untreated biomass.

The presence of lignin in the untreated MSM observed at peak 1508 cm^{-1} representing skeletal vibrations associated with C=C stretching of the aromatic ring of lignin was absent after microwave-assisted DES pretreatment (Slathia et al., 2019). Similarly, the peak at 1263 cm^{-1} attributed to the C—O elongation of the acetyl group was also absent after the pretreatment process owing to the significant hemicellulose removal (Slathia et al., 2019).

The effect of the microwave-assisted DES pretreatment on the cellulose obtained was analyzed and shown in the XRD diffractogram (supplementary material). The intensity of the peaks at 22° and 18° represent the crystalline and amorphous regions of cellulose, respectively for both the raw and pretreated biomass were present. Additionally, the crystalline structure of cellulose I was present in all samples at peak 34.2°. The crystallinity of the untreated biomass was 59.9 % while after the microwave-assisted DES pretreatment the crystallinity increased to ca. 72 %. The results showed that at 10–15 % solid loading, the crystallinity of the pretreated MSM was similar (ca. 72 %). The 20 % increase in crystallinity observed after the microwave-assisted DES pretreatment were due to the disruption of lignin-carbohydrate bonds thereby removing the amorphous lignin and hemicellulose. The FTIR and XRD results were consistent with the compositional analysis showing that the microwave-assisted DES pretreatment exposed the MSM glucan to chemical or enzymatic treatments which could benefit hydrolysis and later bioethanol production processes.

3.1.9. Recovery and characterization of DES lignin samples

At the optimum condition, the LR yield was 84.7–90.9 % at solid loading of 10 to 20 % (w/v). The LR yields obtained were higher than the 80.6 % and 81.5 % obtained by Xie et al. (2021) and Liang et al. (2021), respectively. The value-added use of LR is dependent on its purity. Therefore, the lignin purity was confirmed for the samples. The LR purities at 10–20 % solid loading were relatively high (greater than93.8 %) with less than 4 % carbohydrate sugars present (Table 3). This is in agreement with the study conducted by Chen et al. (2018) where an increase in solid loading had no major effect on the lignin purity. The main polysaccharide present in LR was xylan (0.7–2.1 %) with glucan making up only 0.4–1.4 %. Other carbohydrates such as mannan, galactan and arabinan were not observed. The presence of the carbohydrates in LR is expected since native lignin is known to form complexes with the carbohydrates, especially with hemicellulose improving the mechanical strength of the biomass fiber (Terrett and Dupree, 2019). The microwave-assisted DES treatment affected the ether and ester bonds between lignin-hemicellulose resulting in the breaking of the bonds and a release of lignin with a minimal attached xylan. Xie et al. (2021) also reported 95.2–97.5 % purity for the LR from pinewood pretreated with benzyltrimethyl-ammonium chloride:FA DES. This result is consistent with other literature observations (Hou et al., 2018; Liang et al., 2021; Zhong et al., 2022). The yield and purity of the LR obtained without further purification in comparison with the literature shows that it could potentially be used as a feedstock to produce other value-added products.

The lignin recovered at the optimcum condition was analyzed by FTIR (supplementary material). The peaks representing the C=C and C—C stretching vibration of the aromatic ring and phenolic ring of lignin were observed at 1512 and 1595 cm^{-1}, respectively (Lou and Zhang, 2022). The high purity of LR can be noticed by the prominence of the guaiacyl (G) and syringyl (S) unit peaks. The G units peaks observed at 1267, 1155 and 1035 cm^{-1} indicating the C—O stretching vibration, asymmetric stretching vibration and C—O bond in aromatic G units. The C—O stretching vibration of the S unit peak was also observed at 1363 cm^{-1} (Fernandes et al., 2021; Lou and Zhang, 2022). The abundance of G subunit peaks from the recovered lignin was expected since softwood lignin is composed of about 95 % G subunits. Therefore, the DES-extracted lignin should be explored as a potential feedstock for the production of aromatic derivatives and fine chemicals (Tan et al., 2020).

The main product, glucan obtained from the pretreatment at such high purity could serve as a feedstock to produce nanocellulose, hydroxymethylfurfural, levulinic acid and ethanol (Tan et al., 2020). However, a major challenge to the commercialization of the microwave system is the inability of the irradiation to penetrate larger feedstock slurry volumes resulting in a non-uniform temperature distribution in the slurry (Ocreto et al., 2021). Additionally, the study of the dielectric properties of feedstock is necessary to determine the permittivity of irradiation penetration (Ocreto et al., 2021). Without the feedstock dielectric properties, the reproducibility of the optimum conditions obtained at the lab-scale cannot be accurately reproduced with an industrial microwave reactor. Regardless, of these issues, preliminary technoeconomic studies have shown the potential of DES pretreatment (Tan et al., 2020).

Table 3
Yield and associated polysaccharide content in recovered lignin at different solid loadings.

Solid loading, w/v%	Lignin yield, %	Biopolymer content, %		
		Glucan	Xylan	Lignin
10	84.7± 4.5	0.4± 0.0	0.7± 0.3	95.7 ± 0.2
15	88.1± 5.9	0.6± 0.1	1.0± 0.1	94.7 ± 1.0
20	90.9 ± 4.1	1.4± 0.1	2.1± 0.2	93.8 ± 2.5

4. Conclusion

Microwave-assisted DES pretreatment fractionated MSM effectively. The optimal conditions, i.e. ChCl:FA (1:4), 140 °C, 800 W, 14 min and 15 w/v% solid loading resulted in 89.2 % residue glucan content, 90.1 % delignification and 96.2 % hemicellulose removal. Temperature had the most significant effect on the biopolymers during pretreatment. The composition of the pretreated biomass was similar between 10 and 20 %, however the was a 14.9 % glucan loss. At 10–15 w/v% there was no structural, functional and crystallinity difference between the residues obtained making this beneficial for industrial processes.

CRediT authorship contribution statement

Regan Ceaser: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Silvia Rosa:** Investigation, Data curation, Formal analysis, Validation. **Daniel Montané:** Methodology, Formal analysis, Writing – review & editing, Supervision. **Magda Constantí:** Methodology, Formal analysis, Writing – review & editing, Supervision. **Francesc Medina:** Methodology, Formal analysis, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

Regan Ceaser is grateful to the financial support from the European Union's Horizon 2020 research (ref. 2020MFP-COFUND-16) and innovation programme under the Marie Skłodowska-Curie grant agreement No. 945413. This research was developed within the project PID2021-123665OB-I00 funded by MCIN/AEI/10.13039/501100011033/ FEDER "Una manera de hacer Europa", project TED2021-129343B-I00 funded by the Spanish Ministry of Science and Innovation and the project 2022/12 funded by Diputació de Tarragona and the Universitat Rovira i Virgili under the collaboration framework agreement for the period 2020–2023.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.128470.

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