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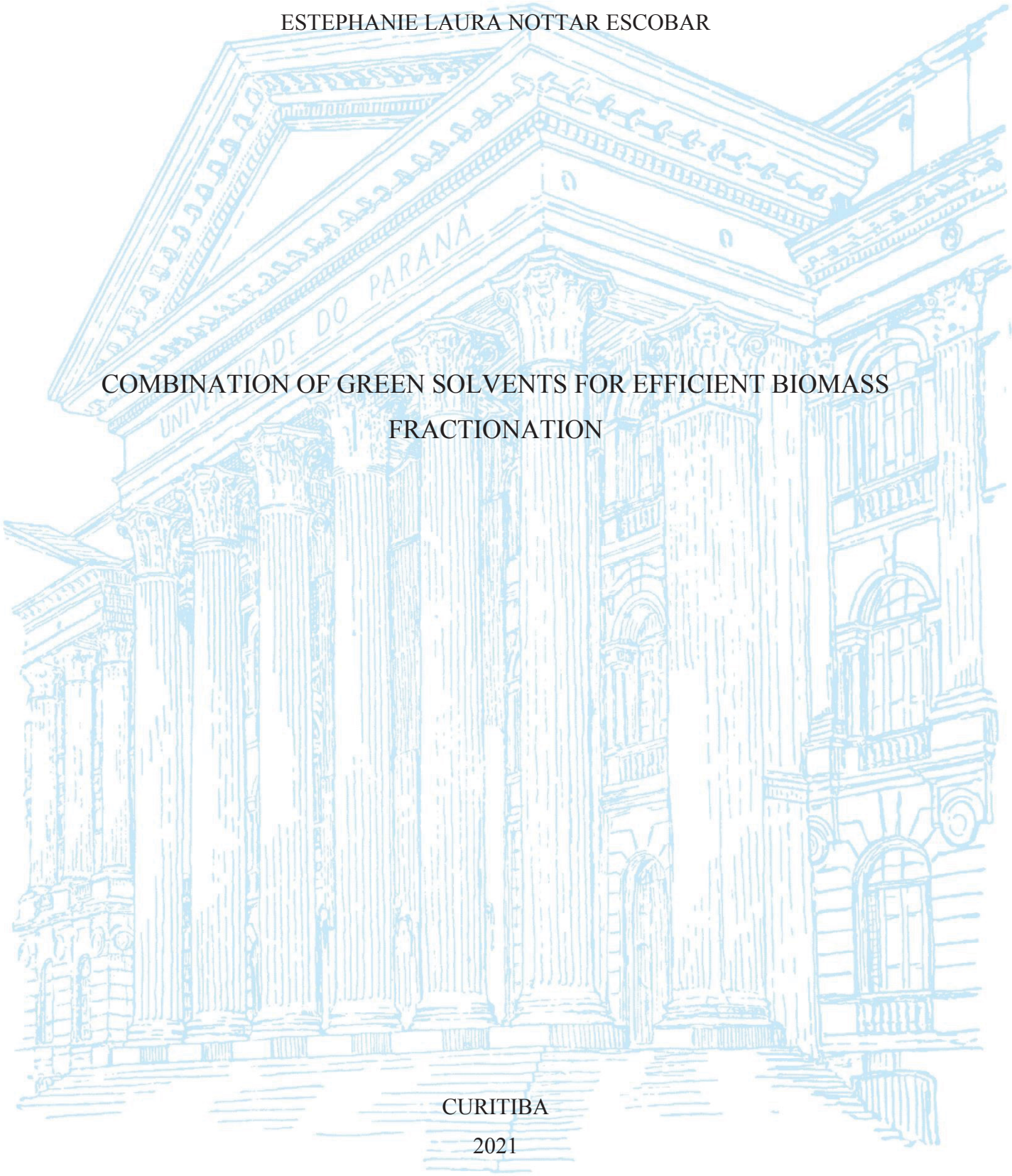
ESTEPHANIE LAURA NOTTAR ESCOBAR



COMBINATION OF GREEN SOLVENTS FOR EFFICIENT BIOMASS FRACTIONATION

CURITIBA

2021

ESTEPHANIE LAURA NOTTAR ESCOBAR

COMBINATION OF GREEN SOLVENTS FOR EFFICIENT BIOMASS FRACTIONATION

Dissertação apresentada ao curso de Pós-Graduação em Engenharia Química, Setor de Tecnologia, como requisito parcial para obtenção de título de Mestre em Engenharia Química.

Orientador: Professor Marcos Lúcio Corazza

Co-orientador: Professor Luiz Pereira Ramos

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ENGENHARIA QUÍMICA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **ESTEPHANIE LAURA NOTTAR ESCOBAR** intitulada: **Combination of green solvents for efficient biomass fractionation**, sob orientação do Prof. Dr. LUIZ PEREIRA RAMOS, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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ATA DE SESSÃO PÚBLICA DE DEFESA DE MESTRADO PARA A OBTENÇÃO DO GRAU DE MESTRE EM ENGENHARIA QUÍMICA

No dia sete de maio de dois mil e vinte e um às 09:30 horas, na sala Microsoft Teams, Plataforma online, em função da portaria 754/2020 da Reitoria - UFPR, foram instaladas as atividades pertinentes ao rito de defesa de dissertação da mestranda **ESTEPHANIE LAURA NOTTAR ESCOBAR**, intitulada**: Combination of green solvents for efficient biomass fractionation**, sob orientação do Prof. Dr. LUIZ PEREIRA RAMOS. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação em ENGENHARIA QUÍMICA da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: LUIZ PEREIRA RAMOS (UNIVERSIDADE FEDERAL DO PARANÁ), FERNANDO AUGUSTO PEDERSEN VOLL (UNIVERSIDADE FEDERAL DO PARANÁ), RAFAL MARCIN LUKASIK (LABORATÓRIO NACIONAL DE ENERGIA E GEOLOGIA). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de mestre está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, LUIZ PEREIRA RAMOS, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

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RESUMO

Biorrefinarias são instalações que objetivam o uso integrado de processos e equipamentos para a conversão de biomassa a produtos de maior valor agregado, como combustíveis, biomateriais, produtos químicos e energia. Resíduos agroindustriais como o bagaço de cana-de-açúcar, que é composto majoritariamente por celulose, hemiceluloses e lignina, correspondem ao tipo de matéria-prima mais abundante no Brasil para essa atividade industrial. Para tirar o maior proveito desse material, estratégias de fracionamento vêm sendo estudadas com o objetivo de destinar cada um de seus componentes principais a aplicações mais nobres do que a queima para geração de energia. Portanto, esse trabalho aborda uma combinação inédita de solventes aplicados ao fracionamento do bagaço de cana-de-açúcar: um solvente eutético profundo (DES - do inglês *deep eutectic solvent*) composto por cloreto de colina e ácido oxálico, etanol (puro ou hidratado) e dióxido de carbono (CO_2). Inicialmente foi verificada a capacidade de remoção de lignina em um sistema contendo DES+CO_2, por meio de um planejamento de experimentos em dois níveis e três variáveis: temperatura, tempo e quantidade de CO_2. Os resultados se mostraram promissores, com deslignificação superior a 40% em apenas 40 minutos de tratamento a 90 °C. Além disso, a remoção de lignina foi acompanhada pela manutenção quase total das glucanas na fração sólida. Posteriormente, uma estratégia *organosolv* a 170 °C foi proposta para maximizar a deslignificação: DES+CO_2+EtOH, em um planejamento de experimentos em dois níveis e três variáveis avaliando-se o efeito da carga de sólidos, da razão mássica entre biomassa e DES e da quantidade de CO_2. Os resultados dessa etapa apontaram para uma remoção de lignina superior a 75% e manutenção virtualmente total de glucanas. O estudo cinético desses efeitos foi realizado em três temperaturas entre 150 e 190 °C, confirmando os melhores resultados quanto à deslignificação a 170 °C. Além disso, neste conjunto de experimentos a retenção de xilanas ficou próxima a 20%, como a adição de água favorece a hidrólise deste carboidrato entende-se que o sistema é beneficiado por sua adição. Portanto, a substituição de etanol anidro por etanol hidratado (20% de água) foi estudada em cinéticas em três temperaturas de 130 a 170 °C. Nessa etapa foi constatada uma remoção de lignina superior a 90%, acompanhada de uma retenção de glucanas superior a 80%. Após o tratamento o resíduo de lignina foi precipitado pela remoção de etanol, seguida da adição de água e resfriamento. Os resíduos precipitados foram caracterizados quanto aos seus teores de carboidratos, resultando em valores inferiores a 3 wt%. Espectros de FT-IR demonstraram a presença de bandas típicas de amostras de lignina advindas de gramíneas. Paralelamente, a distribuição em massas moleculares aparentes foi avaliada por cromatografia de alta performance por exclusão de tamanho. As ligninas obtidas nas melhores condições de rendimento apresentaram massas moleculares médias aparentes baixas, na faixa de 2000 a 3000 $g.mol^{-1}$, com uma dispersidade em torno de 5. Finalmente, como os experimentos realizados forneceram um extenso banco de amostras sólidas pré-tratadas, que apresentaram uma ampla gama de variação dos componentes majoritários, foi aplicado um dispositivo portátil MicroNIR para obtenção dos espectros de cada amostra, que após tratamento, calibração e validação de dados se mostrou uma alternativa viável para a quantificação destes componentes em substituição aos métodos analíticos convencionais, mesmo em amostras sujeitas a diferentes tratamentos termoquímicos. De maneira geral, esse trabalho atestou a viabilidade técnica da utilização de solventes verdes para o fracionamento eficiente do bagaço de cana de açúcar em três frações distintas passíveis de exploração futura e conversão a produtos de alto valor agregado.

Palavras-chave: Fracionamento de biomassa. Organosolv. Bagaço de cana-de-açúcar. Solvente eutético profundo (DES). MicroNIR.

ABSTRACT

Biorefineries are facilities that aim at the integrated use of processes and equipment for converting biomass to products with higher added value, such as fuels, biomaterials, chemicals, and energy. Agro-industrial residues such as sugarcane bagasse, which is composed mainly of cellulose, hemicelluloses, and lignin, correspond to the most abundant type of raw material in Brazil for this industrial activity. To make the most of this material, fractionation strategies have been studied with the objective of allocating each of its main components to more noble applications than burning for power generation. Therefore, this work addresses an unprecedented combination of solvents applied to the fractionation of sugarcane bagasse: a deep eutectic solvent (DES) composed of choline chloride and oxalic acid, ethanol (anhydrous or hydrated), and carbon dioxide (CO_2). Initially, the capability to remove lignin of a system containing DES+CO_2 was verified, through a design of experiments in two levels and three variables: temperature, time, and amount of CO_2. The results were promising, with delignification greater than 40% in just 40 minutes of treatment at 90 °C. In addition, the removal of lignin was accompanied by the almost total maintenance of glucans in the solid fraction. Subsequently, an organosolv strategy at 170 °C was proposed to maximize delignification: DES+CO_2+EtOH in a two-level and three-variable design of experiments evaluating the effects of solids loading, DES to biomass mass ratio, and the amount of CO_2. The results of this stage pointed to a lignin removal greater than 75% and virtually total maintenance of glucans. The kinetic study of these effects was carried out at three temperatures between 150 and 190 °C, confirming the best results regarding delignification at 170 °C. In addition, in this set of experiments the retention of xylans was close to 20%, as the addition of water favors the hydrolysis of this carbohydrate, it is understood that the system benefits from its addition. Therefore, the replacement of anhydrous ethanol with hydrated ethanol (20% water) was studied in kinetics at three temperatures from 130 to 170 °C. In this stage, a lignin removal greater than 90% was observed, accompanied by glucans retention greater than 80%. After treatment, the lignin residue was precipitated by ethanol removal, followed by water addition and refrigeration. The precipitated residues were characterized in terms of their carbohydrate content, resulting in levels below 3 wt%. FT-IR spectra demonstrated the presence of bands typical of lignin samples from herbaceous plants. In parallel, the distribution in apparent molecular masses was evaluated by high-performance size exclusion chromatography. The lignins obtained in the best yield conditions presented low apparent average molecular masses, in the range of 2000 to 3000 $g.mol^{-1}$, with a dispersity around 5. Finally, as the experiments carried out, they provided an extensive bank of pretreated solid samples that presented a wide range of the major components, a portable MicroNIR device was applied to obtain the spectra of the samples, which after treatment, calibration, and data validation proved to be a viable alternative for the quantification of these components instead of the conventional analytical methods, even in samples subjected to different thermochemical treatments. In general, this work attested to the technical feasibility of using green solvents for the efficient fractionation of sugarcane bagasse into three distinct fractions that could be exploited in the future and converted to products with high added value.

Keywords: Biomass fractionation. Organosolv. Sugarcane bagasse. Deep eutectic solvent (DES). MicroNIR.

FIGURES

TABLES

CONTENTS

1 INTRODUCTION

Biorefineries are facilities where equipment and processes are integrated for biomass conversion to biobased chemicals, materials, power, and fuels (DEMIRBAS, 2010). Thereby, one major objective of biorefineries is to add value to vegetable matter through technically and economically feasible processes, increasing the availability of food while producing biofuels and biobased chemicals.

An important role of biorefineries is the valorization of agro-industrial lignocellulosic residues, such as sugarcane straw and bagasse. The main components of lignocellulose are three biopolymers: cellulose, hemicellulose, and lignin. However, as a natural protection, lignocellulose has high recalcitrance, in other words, high resistance to external actions. Therefore, pretreatment steps, or fractionation of the matrix, are necessary to increase the availability of structural polymers for chemical or biological conversion (HIMMEL et al., 2007). The conversion of lignin can originate phenolic compounds used as resins and adhesives (DUVAL; LAWOKO, 2014). At the same time, monomers and oligomers obtained from cellulose and hemicellulose hydrolysis can be converted into organic acids (such as lactic or levulinic), solvents, lubricants, and biofuels such as ethanol.

Among novel techniques for biomass pretreatment and fractionation, one can highlight the use of tailor-made green solvents that cause the partial dissolution of the matrix, as ionic liquids (ILs) and deep eutectic solvents (DESs). However, while ILs raise questions related to their poor biodegradability, high price, and potential of corrosion, DESs have been pointed out as alternatives due to their high biodegradability, low cost, and easy synthesis (QIN et al., 2020).

Lignocellulose valorization using DES has been proposed and technically demonstrated in many studies over the past few years (TAN; CHUA; NGOH, 2020), showing that acidic DESs promote efficient delignification (FRANCISCO; VAN DEN BRUINHORST; KROON, 2012; KANDANELLI et al., 2018; LYNAM; KUMAR; WONG, 2017). Unfortunately, solvents as IL and DES may present high viscosity, impairing transport properties. Therefore, aiming to reduce the resistance to mass transfer limitation – enhancing diffusivity and decreasing viscosity – it is interesting to combine more than one type of solvent, such as short-chain alcohols and supercritical CO_2, as already reported in the literature with a positive effect in pretreatment (KANDANELLI et al., 2018; SILVEIRA et al., 2015a).

Supercritical fluids are any substance or mixtures above their critical point. At this condition, there is no phase transition and its properties (i.e., diffusivity, viscosity, and density) are between those of gases and liquids, and its application may be capable of improving the transport and thermal properties of the system. The more common supercritical fluid employed for biomass valorization is carbon dioxide due to its moderate critical temperature and pressure (31.1 °C and 74 bar), low cost, low toxicity, and wide availability (DEMIRBAŞ, 2001).

Although supercritical CO_2 ($scCO_2$) can be used in biomass pretreatment, this compound itself may have little effect over lignocellulose, majorly due to its low chemical reactivity over the structural biopolymers. Therefore, an alternative to boost $scCO_2$ fractionation of biomass is adding a modifier, generally organic liquids. In this scenario, a common application that has been proposed is the gas-expanded liquid (GXL) technique, where the $scCO_2$ is the compressed solvent used to expand an organic liquid solvent conferring specific thermodynamic and transport properties. This "engineered" solvent system is capable of permeating the cell wall micropores improving its disruption and enhancing the efficiency of the pretreatment process (JESSOP; SUBRAMANIAM, 2007). Additionally, DESs and short-chain alcohols can absorb large amounts of carbon dioxide, and coupling these solvents may reduce mass transfer resistance in the biorefining systems (DÉCULTOT et al., 2019; TRIVEDI et al., 2016; ZHANG et al., 2019).

Given the above and understanding the relevance of researching alternative ways of producing clean and renewable energy and products, this work aims to propose a novel fractionation method that combines the traditional organosolv pretreatment with green solvents, evaluating different fractionation strategies in systems involving deep eutectic solvent, ethanol (anhydrous and hydrated) and CO_2, at sub and supercritical conditions. The main objectives of this study are defined as presented below.

1.1 OBJECTIVES

The main goal of this work is to evaluate the technical feasibility of a green process to fractionate sugarcane bagasse combining deep eutectic solvent, carbon dioxide, and ethanol (anhydrous and hydrated). The process efficiency is evaluated in

terms of two important overall fractions: the glucan-rich solids and the precipitated lignin. Also, a third fraction (liquid) will carry soluble sugars and fractionation co-products. Specifically, this study is outlined by setting and assessing the following topics:

a) To evaluate the suitability of the deep eutectic solvent composed of choline chloride and oxalic acid in sugarcane bagasse delignification;

b) To test different fractionation approaches, involving DES, CO_2, and ethanol with different water contents;

c) To determine the effects of operational conditions on delignification efficiency, as solids loading, temperature, amount of CO_2 and DES;

d) To assess the delignification kinetics and carbohydrate profiles at different conditions;

e) To determine the carbohydrates and lignin content in pretreated sugarcane bagasse;

f) To evaluate the extracted lignin concerning purity and molecular mass distribution.

g) To compare the characterization in terms of lignin and carbohydrates of untreated and pretreated substrates using a portable MicroNIR device with the traditional quantification methods.

2 LITERATURE REVIEW

This section presents a theoretical context about the material used in the work, sugarcane bagasse, and its main constituents, as well as about the fractionation and pretreatment techniques important to understanding the relevance and results of this study.

2.1 SUGARCANE BAGASSE

Modern sugarcane is a hybrid of different wild species of the genus *Saccharum: S. oficcinarum, S. spontaneum, S. barberi, S. sinense, S. edule,* and *S. robustum* (GRIVET et al., 2004; HAMERSKI, 2009). Despite being originated from the Asian southwest, this culture is widely adapted to the Brazilian tropical climate, making this country the world leader in sugarcane harvesting.

According to the Brazilian Ministry of Agriculture, Livestock, and Supply, in the harvest of 2020/2021 (MAPA, 2021), Brazil produced about 660 million tons of sugarcane. This is equivalent to more than 30% of the world's production of this crop and this percentage has remained approximately constant over the last decade (USDA, 2019). In addition, according to Embrapa Technology Information Agency, the fibrous waste generated from sugar and ethanol industries corresponds to approximately 28 wt%, on a dry basis, of the processed sugarcane (AGEITEC).

2.2 LIGNOCELLULOSIC MATRIX STRUCTURE

Woody and agro-industrial biomasses are mainly composed of cellulose, hemicelluloses, and lignin; besides these components, it presents a fraction of ash and minor components that vary widely according to the type of biomass. Among these minor components, it is possible to find extractives - such as terpenes, resins, and phenols - oils, fats, waxes, pectins, and proteins (MCMILLAN, 1994).

2.2.1 Cellulose

Cellulose $(C_6H_{10}O_5)_n$ is a polysaccharide insoluble in water and most organic solvents (BRUNNER, 2005). It is the most abundant biopolymer on the planet and an excellent raw material for the production of biofuels (NILL; KARUNA; JEOH, 2018) through hydrolysis and fermentation. This homopolymer is composed of anhydro-D-glucopyranose units linked by β-1,4-glycosidic bonds, as shown in Figure 1. Conformational analysis of cellulose indicates the disaccharide cellobiose (4-O-β-D-glucopyranosyl-β-D-glucopyranose) as its repeating conformational unit, which in turn is composed of glucose monomers (RAMOS, 2003).

Cellulose exists in amorphous and crystalline regions. The second has a higher level of molecular organization, having inter and intra-layered hydrogen bonds that confer high stability and great resistance to enzymatic hydrolysis (RAMOS, 2003). Although the degree of polymerization (DP), which represents the number of glucose units in the polymer, is an important structural factor to evaluate the accessibility to enzymatic activity, the matrix crystallinity appears to have an even more pronounced effect over hydrolysis (PURI, 1984; ZHENG; PAN; ZHANG, 2009). Therefore, in addition to the biomass fractionation, an impacting pretreatment is expected to be able to contribute to the reduction of cellulose crystallinity, even though the matrix crystallinity index may be enhanced due to the removal of hemicelluloses and lignin.

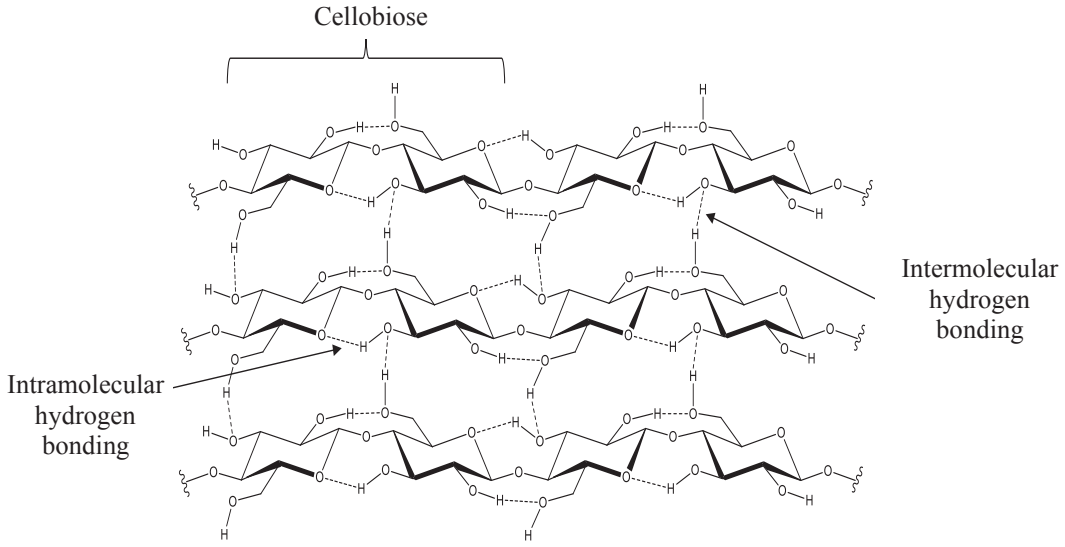


Figure 1: Cellulose representation.

2.2.2 Hemicellulose

Hemicelluloses are structural branched heteropolysaccharides that are covalently bound to lignin and are composed of different polysaccharide units containing pentoses (xylose and arabinose) and hexoses (glucose, mannose, galactose), as well as uronic acids (RAMOS, 2003). Hemicelluloses aid the connection between cellulose and lignin, acting as an interface between them (LIN; DENCE, 1992; NISHIMURA et al., 2018).

In comparison with cellulose, hemicelluloses present themselves in smaller aggregates and their side chains are easily hydrolyzable. Xylans are acetylated heteropolysaccharides with a homopolymeric backbone formed by D-xylopyranose units bonded by β-(1-4) glycosidic linkages. Xylans may also present arabinose, glucuronic, acetic, and ferulic acids, depending on the source of the material (SAHA, 2003). This polysaccharide represents the most abundant hemicellulosic group in herbaceous materials such as sugarcane bagasse and is presented mostly in the form of arabinoxylans. Figure 2 is a general schematic representation of a sugarcane bagasse heteroxylan.

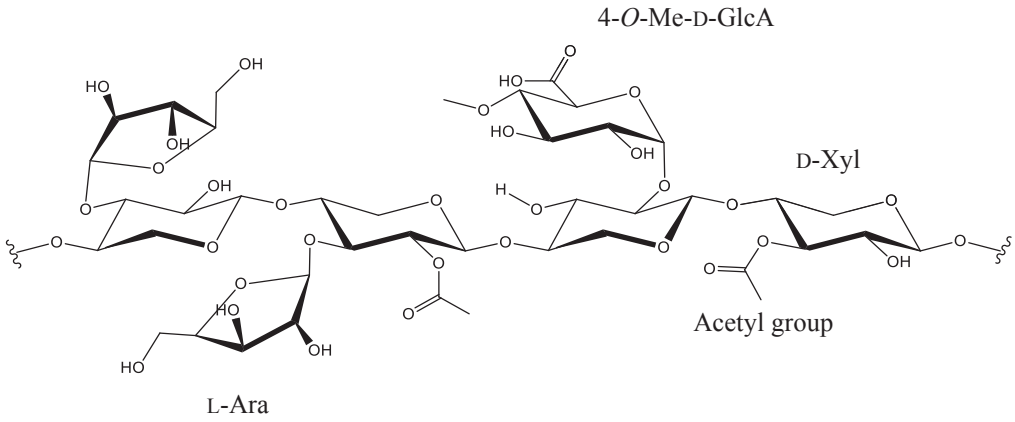


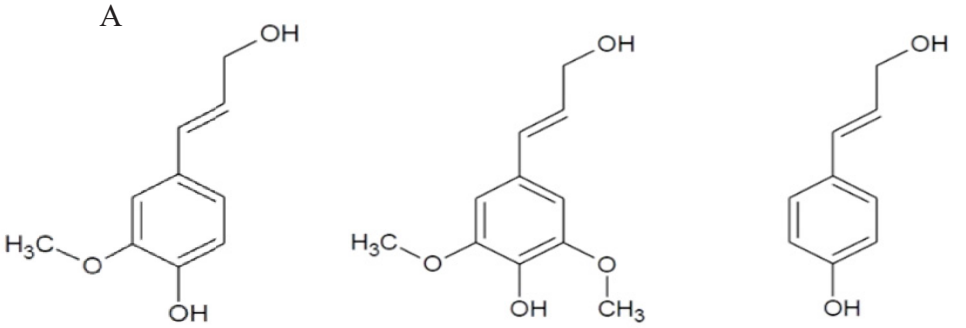
Figure 2: Sugarcane bagasse hemicellulose representation. Reference: Ramos et al., (2020).

2.2.3 Lignin

Lignin is a biopolymer composed of aromatic monolignols derived from cinnamic acids, *p*-coumarylic, coniferylic, and sinapylic, as shown in Figure 3. Lignin confers rigidity and impermeability to cell walls and, together with hemicelluloses, makes up the non-cellulosic portion of lignocellulose (LIN; DENCE, 1992).

The reactivity of lignin relies mainly on the hydroxyl group - in phenolic or aliphatic forms -, and smaller amounts of carboxyl and carbonyl groups. Therefore, lignin can be a source of several monomers, building blocks, and aromatic compounds, essentially phenolic (DUVAL; LAWOKO, 2014).

A



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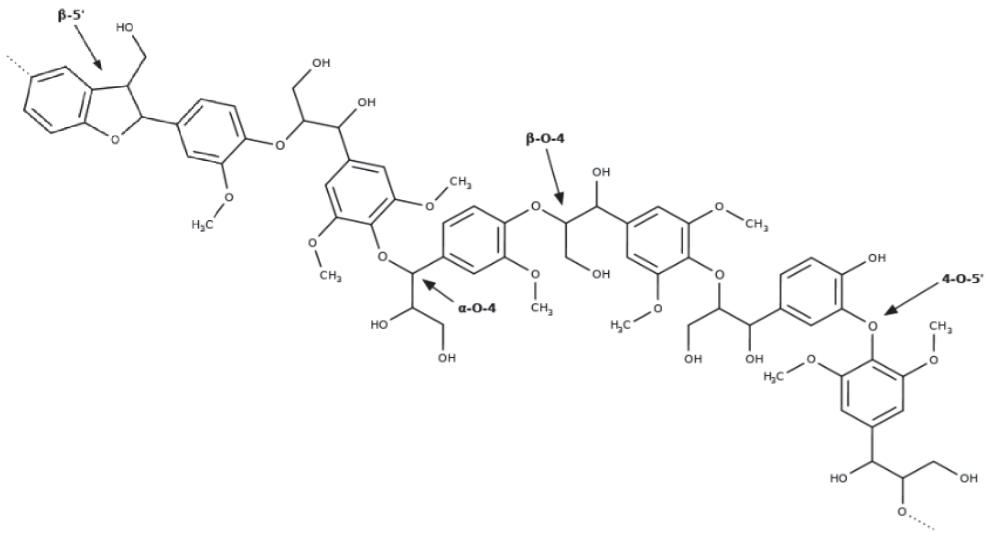

Figure 3: (A) aromatic monolignols that compose lignin structures, and (B) representation of a GS lignin structure. Reference: Ramos et al., (2020)

From coniferyl alcohol derives the structural unit known as guayacil (G), which occurs abundantly in softwood lignin, while hardwood lignins present G and S units (syringyl units derived from sinapyl alcohol). Herbaceous biomass, such as sugarcane, in turn, also contains guayacil-syringil lignins but these present some quantities of the *p*-coumaryl derivative, therefore, they are known as HGS lignins (LIN; DENCE, 1992).

Some types of pretreatment – mainly with lignin dissolution – can benefit enzymatic hydrolysis through different pathways, since enzymes can adsorb onto lignin fragments by hydrophobic interactions (CHANG; HOLTZAPPLE, 2000; LI et al., 2018b). Also, lignin removal is beneficial to fermentative processes, such as second-generation ethanol production, because, besides being a physical barrier to cellulase access to cellulose and compete for enzyme adsorption, the presence of lignin may give rise to phenolic compounds that are toxic to the fermentative microorganisms (PALMQVIST; HAHN-HÄGERDAL, 2000).

2.3 LIGNOCELLULOSIC BIOMASS PRETREATMENT AND FRACTIONATION

Biomass fractionation is a process, or set of processes, that aim the separation of lignocellulose into lignin and carbohydrate-rich fractions. Furthermore, pretreatment techniques involved in fractionation must improve the accessibility of the lignocellulosic matrix to cellulolytic systems. Due to high recalcitrance, untreated herbaceous biomass usually presents enzymatic hydrolysis yields below 20% (LYND et al., 2002). By contrast, a pretreated matrix generally exhibits increased porosity (surface area increase) and lignin removal or redistribution, promoting enzymatic accessibility to cellulose fibers. There are many methods to pretreat a substrate, involving physical, chemical, physicochemical, and chemical methods, among others, each one of them acting over specific components of the matrix (ARSHADI et al., 2016). This section will discuss some methods commonly used in the pretreatment of lignocellulosic biomass.

2.3.1 Traditional pretreatment techniques: acid, steam explosion, hydrothermal, and alkali

Dilute acid pretreatments use acids such as H_2SO_4, HCl and H_3PO_4, to hydrolyze plant polysaccharides, leading to a fraction rich in hemicellulose components, also known as C5 fraction, and to glucan-rich fibers containing variable amounts of lignin. Depending on the severity of the process, carbohydrates may be converted in co-products originated from their dehydration, such as furan derivatives and organic acids (JÖNSSON; MARTÍN, 2016). In general, higher temperatures and longer reaction times characterize more severe processes, but the appropriate combination of these variables with the selection of proper particle size and the addition of a catalyst results in the enhancement in substrate accessibility while avoiding the formation of diluted co-products (SILVEIRA et al., 2015b; TAHERZADEH; KARIMI, 2007). Although the majority of commercial-scale pretreatment units are based on dilute acid technology, this technique still bears some inconvenience as the need for neutralization of the medium after pretreatment, with an over-liming process that hinders catalyst reuse and generates low-value by-products (gypsum).

Steam explosion pretreatment relies on the exposure of the material to high-pressure steam followed by instantaneous decompression, causing a sudden expansion of the fiber network. This explosion increases the exposed surface area and enhances the material's porosity. Also, the main effect of this technique is the deacetylation of hemicelluloses, forming acetic that hydrolyzes labile polysaccharides (auto-catalytic hydrolysis or auto-hydrolysis), releasing hemicellulose sugars in the form of oligo and monosaccharides (MEDINA et al., 2016; PIELHOP et al., 2016; RAMOS, 2003; THEURETZBACHER et al., 2015). In general, since a great extent of hemicelluloses are removed from the solid fibers, the lignin content of the substrate is enhanced by pretreatment. Additionally, the material can be impregnated with acids prior to the steam explosion, improving hemicellulose removal up to the totality. Many biomass steam explosion processes and conditions have been reported in the literature for herbaceous, as sugarcane bagasse and wheat straw, woody materials, and other agricultural residues (CHIARELLO et al., 2020; JACQUET et al., 2015; MEDINA et al., 2016).

Alkaline pretreatments, on the other hand, take place in less severe conditions, which leads to lower carbohydrate losses. The effect of alkaline pretreatment is mostly due to the removal of lignin. The use of aqueous NaOH is one of the most effective alkaline-based techniques, and this technique can enhance enzymatic hydrolysis yields up to 80% of the theoretical maximum (XU et al., 2011). Other alkaline strategies include calcium and ammonium hydroxides, and hydrogen peroxide, and these have been shown promising in terms of enhancing substrate accessibility, with delignification results around 30 to 60% (AITA; SALVI; WALKER, 2011; CHENG et al., 2010).

One important pretreatment technique is based on the use of water to fractionate biomass, hydrothermolysis, which features the advantage of processing high moisture content biomass. It is known that water presents unique solvent properties that can be modulated according to temperature and pressure (BRUNNER, 2005), so this technique consists of a green process that dispenses the use of exogenous catalysts and relies only on the interaction of matrix and water at different ranges of temperatures. The liquid hot water treatment, at temperatures below 200 °C, causes hemicellulose hydrolysis mostly to oligomers, with partial lignin removal or relocation (LI et al., 2017). As the temperature increases from 200 °C, the biomass is liquefied into a slurry of monomers (TEKIN; KARAGÖZ; BEKTAŞ, 2014). Further increasing the temperature above water critical point changes water properties drastically, the viscosity decreases, and the diffusion coefficient increases, enhancing reaction rates. As already demonstrated at both laboratory and pilot-plant scales, ultra-fast hydrolysis in supercritical water leads to the almost total dissolution of carbohydrates, with little release of diluted co-products in very low reaction times (less than 1 s) (CANTERO et al., 2015; CANTERO; BERMEJO; COCERO, 2013; MARTÍNEZ et al., 2019; MARTÍNEZ; CANTERO; COCERO, 2018). However, the disadvantages of such a process lie in its high energy requirement, and mostly in its high capital costs. This is due to the use of low solids loadings, specialized reactors that support drastic conditions (high temperatures and pressures), and the need for protection from corrosion problems associated with the high acidity and high oxygen content dissolved in the medium (PETERSON et al., 2008; SILVEIRA et al., 2015b).

Fockink, Sánchez, and Ramos (2018) studied the pretreatment of sugarcane bagasse using steam explosion at autohydrolysis conditions and with dilute H_2SO_4 or H_3PO_4 as acid catalysts. A combined severity factor, an empirical calculation that

accounts for pH, temperature, and residence time, was calculated for each condition. These authors reported a direct relationship between the combined severity factor and the pretreated substrate properties – chemical composition, rheological behavior, and enzymatic hydrolysis yields. Besides the effect of the combined severity factor, Pielhop et al. (2016) investigated the effect of the explosion *per se* in comparison to steam pretreatment. The explosive decompression had an important effect on digestibility, resulting in a 90% of enhancement in enzymatic hydrolysis yield.

2.3.2 Supercritical carbon dioxide

Supercritical carbon dioxide ($scCO_2$) is one of the most used compressed fluids for biomass processing due to its moderate critical conditions (31.1 °C and 74 bar), non-flammability, low-toxicity, and wide availability. Because of its zero-dipole moment, carbon dioxide is a non-polar molecule that presents its maximum solvation power for non-polar or weakly polar compounds, which is inversely proportional to the molar mass of the solute (BRUNNER, 2005). However, CO_2 presents a significant quadrupole moment, and, related to its microscopic solvent behavior, this molecule may participate in hydrogen bond interactions (RAVEENDRAN; IKUSHIMA; WALLEN, 2005).

The utilization of $scCO_2$ in the extraction of valuable compounds from biomass – as lipids and extracts with pharmaceutical, nutraceutical, antioxidant proprieties, among others – have been extensively studied in the past few years, with excellent results that show it is possible to complement, and even substitute, the use of traditional extraction methods that employ organic solvents (ARAÚJO et al., 2019; CORREA et al., 2016; DA SILVA NONATO et al., 2020; JUCHEN et al., 2019; VEIGA et al., 2021). Besides, the use of $scCO_2$ as a solvent in catalytic systems has also shown promising results in terms of kinetics and equilibrium, favoring multiple reactions, as esterification, hydrogenation, hydroformylation, carbonatation, among others (LEITNER, 1999; MELFI et al., 2020; MORE; YADAV, 2018).

Additionally, $scCO_2$ has been extensively explored in biomass pretreatment (ESCOBAR et al., 2020; MORAIS; DA COSTA LOPES; BOGEL-ŁUKASIK, 2015). However, by itself, $scCO_2$ may have little effect over lignocellulose, majorly due to little chemical affinity with the structural biopolymers. Therefore, an alternative to

boost $scCO_2$ fractionation of biomass is coupling with modifiers, generally pressurized organic liquids such as alcohols. Deep eutectic solvents, as well as alcohols, have been reported as capable of absorbing significant amounts of carbon dioxide (DÉCULTOT et al., 2019). In such cases, the $scCO_2$ can expand the solvent, forming a gas-expanded liquid (GXL) (JESSOP; SUBRAMANIAM, 2007), which is capable of permeating the cell wall micropores, improving its disruption. In fact, the use of supercritical fluids in biomass utilization dates from the ’80s, pointing to positive results on wood impregnation due to enhanced mobility of solvent and solutes (LI; KIRAN, 1988; SMITH et al., 1993). Also, $scCO_2$ facilitates mass transfer by promoting homogeneity and lowering solvent viscosity.

Another action of carbon dioxide may be related to the hydrolysis of hemicelluloses when acting in conjunction with the moisture present in the biomass generating carbonic acid, which could enhance the substrate accessibility to enzymatic hydrolysis as have been extensively reported already in the literature (FOCKINK; SÁNCHEZ; RAMOS, 2018; KIM; HONG, 2001; NARAYANASWAMY et al., 2011; SERNA; ALZATE; ALZATE, 2016).

Considering the versatile nature of CO_2, its multiple applications as solvent and reaction media, and its effects aiding carbohydrate hydrolysis and biomass permeation, it is clear that it can contribute to fractionation processes, promoting homogeneity of the system, and hopefully, enhancing its kinetics.

2.3.3 Organosolv fractionation

The main idea of fractionation processes is to pretreat the lignocellulosic material while making the most profitable use of its components, that is, converting each fraction to value-added products. For example, lignins feature high thermal stability, antioxidant and antimicrobial properties, and they can be used in the production of higher-value polymeric materials, like polyurethanes, polyesters, epoxide, and phenolic resins (GILLET et al., 2017; UPTON; KASKO, 2016). Also, its use in cement mixtures showed positive effects over plasticity and compressive strength (AKOND; LYNAM, 2020). Also, the conversion of lignin to bio-oil and bio-char seems to be a relevant matter. The hydrolyzed carbohydrates, as the hemicellulosic fraction, can be converted to fuels, fuel additives, and organic acids (JI

et al., 2012). Besides, the cellulosic residue can be hydrolyzed to produce fuels and chemicals, but also converted to much more valuable materials such as cellulosic fibers, dissolving pulp, food additives, nanoscale composites, and many cellulose-based pharmaceutical compounds (MARQUES-MARINHO; VIANNA-SOARES, 2013; SHARMA et al., 2019).

One promising technology that envisions a biorefining concept and is capable of utilizing the matrix in its entirety is the organosolv fractionation. This type of process utilizes organic solvents in the presence or absence of exogenous catalysts to promote matrix delignification and increase the surface area of the pretreated material (THORESEN et al., 2020). The use of short-chain alcohols in organosolv pretreatment supports delignification, being able to extract lignin with little chemical alterations. Generally, organosolv lignin presents a low average molecular mass, good antioxidant capacity, and low carbohydrate content; therefore, it can be used in many applications such as dispersants, adsorbents, adhesives, and also in high-value carbon fibers (PAN et al., 2006; ZHANG, 2008).

According to Sannigrahi and Ragauskas (2013), during organosolv fractionation, four main processes occur: 1 – some hemicellulose-lignin linkages are hydrolyzed, along with internal lignin bonds, resulting in their solubilization; 2 – in hemicelluloses, glycosidic bonds are cleaved while glucans are preserved in a large extent, depending on pretreatment severity; 3 – once more, depending on the process conditions and the presence of an acid catalyst, carbohydrates may be partially dehydrated to furfural and 5-HMF (5-hydroxy-2-methyl furfural), with even further degradation to acids such as formic and levulinic; 4 – finally, some condensation reactions may occur. It seems natural to assume that while the first two reactions are desired, the latter is rather unfortunate and must be avoided.

Organosolv efficiency, in terms of carbohydrate utilization and delignification yields, may be enhanced by adding $scCO_2$ in a typical CO_2-expanded liquid (CXL) scheme (LÜ et al., 2013; LV et al., 2013; PASQUINI et al., 2005a, 2005b). At this condition, organic solvents can dissolve large amounts of CO_2, expanding greatly and altering physical properties such as diffusivity, viscosity, and surface tension: the first is increased while the other two are dramatically decreased in the presence of CO_2 (CORAZZA et al., 2019; JESSOP; SUBRAMANIAM, 2007).

The delignification of lignin model compounds during organosolv pulping was evaluated by Schrems et al. (2012) in the absence and presence of $scCO_2$. The

addition of $scCO_2$ seemed to lower the activation energy for delignification by about 40%. Hence, the effects of $scCO_2$, besides reducing mass transfer resistance and enhancing substrate accessibility to hydrolysis, changed chemical pathways by altering solvent polarity and the kinetics of organosolv pulping.

Silveira et al. (2015) evaluated the effect of low concentrations of 1-butyl-3-methylimidazolium acetate ([Bmin][OAc]) in the $scCO_2$/EtOH pretreatment of sugarcane bagasse (SCB). Pretreatment was carried out using 20 mL ethanol and 1–2 mL of the ionic liquid (IL) in a 50 mL agitated Parr reactor containing 2 g of biomass. The variables evaluated were temperature (110–180 °C), pressure (195–250 bar), and IL-to-SCB ratios (0:1–1:1, $ml.g^{-1}$). At the best pretreatment conditions, the delignification extent was 42% and the glucose yield using low enzyme loadings of Cellic CTec2 (Novozymes) reached 70.7 wt.% of the theoretical maximum in relatively short incubation times. High selectivity for delignification was not a requirement to develop high susceptibilities to enzymatic hydrolysis, with emphasis on the small amount of IL applied. Generally, the amount of IL employed for biomass fractionation is at least 10 times higher than the IL loading used in this study (TAN et al., 2009; VERDÍA et al., 2014).

Other delignification methods, often used on an industrial scale – as kraft and lignosulfonate –, deal with chemical removal of lignin that can be extracted with a great variety of chemical properties. Kraft process is the most used method in pulp and paper industries, corresponding to over 80% of the total lignin production in the world (TEJADO et al., 2007). However, one important disadvantage of this process is the sulfur content on the extracted lignin, since this process employs soda and Na_2S to cleave aryl-ether bonds and depolymerize the lignin. Therefore, the general destination of the resulting material is energy production and chemical recovery. Sulfite lignin, on the other hand, is water-soluble and widely employed in industries as dispersants, emulsifiers, and in the production of resins (FATEHI; NI, 2011; TEJADO et al., 2007). Due to its surface-active and binding properties, most of the lignin originated from sulfite pulping is directed to concrete additives.

Considering all the technical lignin production worldwide, less than one-tenth is directed to higher-value products. The development of technologies that enables a higher valorization of lignin compounds is pressing from a biorefinery point of view, and lignins with little modifications, in a pure and unchanged manner, are great resources for upgrading to valuable industrial applications. Hence, improvements in

organosolv processes have the objective of enabling the obtention of good quality feedstocks for upgrading in an economically viable way, which is directly dependent upon high yields, with integrated processes that recover carbohydrates and that allow the reuse of solvents and catalysts.

2.3.4 Deep eutectic solvents in lignocellulosic biomass fractionation

Deep eutectic solvents are a group of liquids at ambient conditions, formed by mixing two solid components that present higher melting points than the eutectic point of the binary mixture (MARCUS, 2019). The formation of a DES relies on the mixture of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), maintaining the chemical identities of its components, while no covalent compound between reactants is formed. These solvents are often compared with ionic liquids (IL) for their main physicochemical properties and applications. Additionally, most DES exhibit advantages in applications, since they are biodegradable, present low toxicity, are much easier to synthesize and can be made up with unexpensive reactants (ABBOTT et al., 2004; ALOK et al., 2018; QIN et al., 2020).

The interest in the fractionation of lignocellulosic biomasses using DES has led to many studies over the past years. Hence, the maximum solubilization of lignocellulosic model compounds in different DES have been studied, indicating a high selectivity for lignin depending on solvent composition, with neglectable cellulose solubility in DESs based in choline chloride with carboxylic acids (FRANCISCO; VAN DEN BRUINHORST; KROON, 2012; MAJOVÁ et al., 2017; MALAEKE et al., 2018). As an example, the solid-liquid transition curve for the binary mixture of choline chloride with oxalic acid (OA) is showed in Figure 4. It is possible to identify a deep eutectic point at 50 mol% of OA, where the freezing temperature is close to 34 °C.

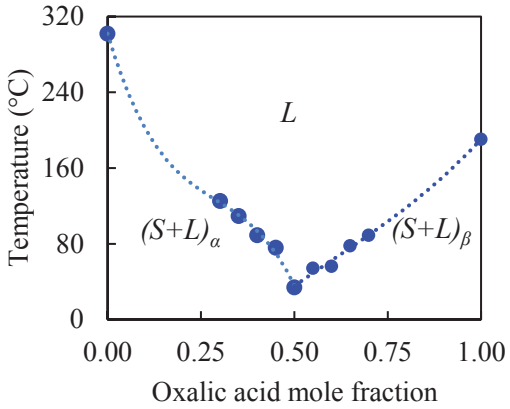

Figure 4: Solid-liquid T-x diagram of mixtures of choline chloride and oxalic acid, according to oxalic acid mole fraction. The blue-filled circles are freeze points obtained by Abbott et al. (2004), and the dotted line is a guide to improve visualization. L region refers to a liquid phase and *S*+*L* regions refer to mixtures of solid and liquid phases.

Figure 5, in turn, depicts the hydrogen bond formation between choline chloride and oxalic acid, the HBA and HBD used in this work.

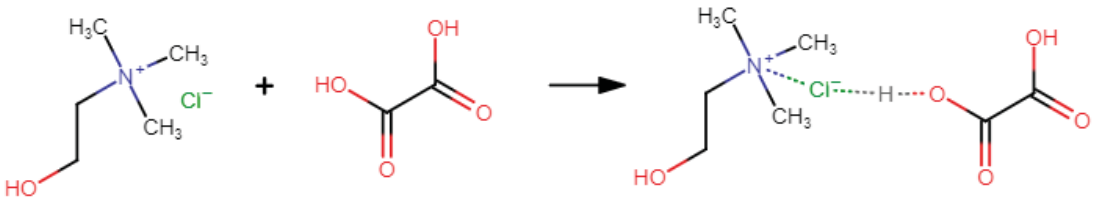

Figure 5: Schematic representation of the hydrogen bond formation between choline chloride and oxalic acid.

The delignification of wheat straw in different choline chloride-based DES was investigated by Jablonský et al. (2015) at low temperatures – 60 to 80 °C – for a long reaction time of 24 h. The DESs tested were mixtures of choline chloride (ChCl) with urea, malonic acid, lactic acid, malic acid, and oxalic acid (OA). Unfortunately, at the studied conditions, little selectivity was found for lignin extraction. It is still worth mentioning that a high fractionation extent could be observed, with the highest decrease in lignin content obtained by ChCl:OA – 57.9 % – but with solubilization of almost 30 % of carbohydrates in the DES used.

Lou et al. (2019) studied the production of lignin nanoparticles from ChCl:LA extraction of wheat straw. A mechanistic understanding of lignin extraction by DES was discussed, in which the extraction rate was governed by the mass transfer resistance. Besides, the effect of water content on lignin removal and purity was evaluated, and an increase in wheat straw water content reduced the purity of extracted lignin. However, Yiin et al. (2016) reported that increasing the water content of low-temperature transition mixtures (malic acid-sucrose-water and malic acid-monosodium glutamate-water) increased their lignin solubility capacity. Still, it is worth mentioning that increasing the water content of these solvents may destroy their integrity by disrupting the hydrogen bonds between DES components while favoring their hydrogen bonding with water (HAMMOND; BOWRON; EDLER, 2017; PASSOS et al., 2016).

A conceptual design of a biomass delignification process using ChCl:LA was assessed by Smink, Kersten, and Schuur (2020), envisioning a process that could replace kraft pulping of *Eucalyptus globulus*. This ambitious work was supported by some experiments evaluating the reuse of the DES after liquid-liquid extraction stages, and optimized solvent usage. After process analysis, calculating mass and energy balances, the authors concluded that the proposed method could result in a heat duty almost 30% lower than traditional kraft pulping.

Considering that the high solubilization of lignin in some DESs is untimely contrasted by slow delignification kinetics, it is natural to look for alternatives to diminish mass transfer limitations. The viscosity of DES is usually high, and thus, to overcome this issue, other solvents can be opportunely added, forming ternary solvent mixtures. Kandanelli et al. (2018) studied the delignification of various biomasses using ChCl:OA in combination with different alcohols, resulting in around 50% of delignification extents.

In conclusion, the present study envisions the valorization of sugarcane bagasse by efficiently fractionating this lignocellulosic material into its main constituents: cellulose, hemicelluloses, and lignin. Therefore, the proposed process unites the advantages of organosolv processes, which generally results in high delignification yields, with the high selectivity for lignin of acidic DES, and with carbon dioxide, which is known to help solvent diffusion through the plant cell wall macromolecular structure, enhancing reaction kinetics and favoring hemicellulose hydrolysis.

2.3.5 Co-products

Several biobased value-added products are currently being studied, especially those that can be used as green solvents, such as furfural, furfuryl alcohol, levulinic acid, ethyl levulinate, and butyl levulinate, among others (LOMBA et al., 2011). In general, many of these co-products are fermentation inhibitors, so their formation must be avoided in second-generation ethanol production. However, acknowledging that they are highly versatile chemicals, it is important to evaluate their production throughout fractionation processes.

The furan derivatives, such as furfural (2-furaldehyde) and 5-HMF (5-hydroxy-2-methyl furfural), originate from dehydration of pentoses and hexoses, respectively (JÖNSSON; MARTÍN, 2016). Besides, some acids such as levulinic, acetic, and formic, originated from the breakdown of furan derivatives (BOZELL, 2010; PALMQVIST; HAHN-HÄGERDAL, 2000), are highlighted as fermentation inhibitors. It is known that fractionation at higher acid concentrations enhances the production of such co-products (RAJAN; CARRIER, 2014). Also, high process temperatures, around 200 °C and up, promote similar effects on pretreatment streams (IRYANI et al., 2013; JAAFARI et al., 2019).

In general, despite their high market value, the production of these co-products in diluted media (mainly in water) is undesirable, because the subsequent steps of separation and purification are very costly. However, the production of these compounds in media free of liquid solvents, meaning ease in downstream processes, should contribute positively to the economic viability of these types of processes, becoming desired in a biorefinery context.

3 MATERIAL AND METHODS

This section presents the material and methods employed in this work, focusing on the procedures and conditions applied at each step, experimental and data treatment, and analysis.

3.1 BIOMASS PREPARATION

The sugarcane bagasse used in this work was supplied by Raízen (Piracicaba – Brazil) and kept in a freezer (-12 °C) until drying. The material was dried in an air circulating oven at 40 °C until moisture content was below 10 wt%. After that, the dry biomass was knife-milled (1 mm sieve), and the particles that passed the 0.42 mm sieve were rejected to prevent the blockage of the depressurization valve. Sequentially, an extraction with ethanol 95 vol% was carried out in a Soxhlet apparatus for 12 h (completing at least 3 cycles every 2 h), which removed most of the low molecular mass components, in order to prevent analytical interferences. This sample, sugarcane bagasse free of ethanol extractives, named SCB_{ee}, was dried in a convection-oven at 40 °C until a moisture content below 10 wt% and stored at 10 °C until use.

3.2 DEEP EUTECTIC SOLVENTS PREPARATION

The DES used in this work was prepared according to Abbott et al. (2004). HBD (di-hydrated oxalic acid, 99.63%, Neon, São Paulo – Brazil) and HBA (choline chloride, Purex, 98%, Inlab Confiança, São Paulo – Brazil) were vacuum dried (600 mmHg) at 60 °C, mixed in a flask with magnetic stirring and heated at 100 °C until a homogeneous colorless liquid appeared. The DES produced was stored in a sealed amber flask until use.

3.3 EXPERIMENTAL APPARATUS AND PROCEDURES

The experimental apparatus used for the sugarcane bagasse pretreatment consisted of a batch reactor with 100 mL volume (Parr Instruments, model 4598), equipped with electrical heating jacketed and with a temperature controller, and mechanically stirred. The reactor was coupled to a syringe pump (Teledyne Isco pump 260D) for the CO_2 loadings, and a thermostatic bath to control the syringe pump chamber temperature. Additionally, an HPLC pump (S1122, Sykam, Eresing – Germany) was coupled to the reactor to inject ethanol during the depressurization of certain experiments, which will be further elucidated. Figure 6 depicts a schematic diagram of the experimental setup used in this study.

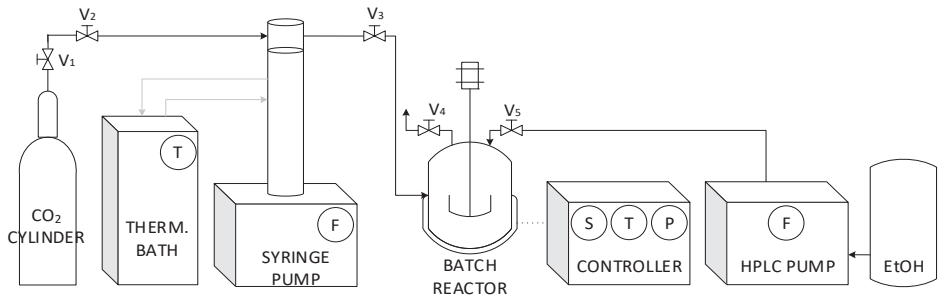

Figure 6: Schematic representation of the experimental apparatus used for SCB_{ee} fractionation.

The pretreatments consisted of different types of experiments, involving three combinations of solvents: DES and CO_2; DES, CO_2 and anhydrous ethanol; and DES, CO_2 and hydrated ethanol, at different water contents.

In a typical experiment using only DES and CO_2, 4 g of SCB_{ee} were loaded into the reactor with 40 g of DES. The reactor was closed and the designated amount of CO_2 in grams (purity in liquid phase > 99 wt%, Air Liquide, São Paulo - Brazil), at 15 °C and 150 bar, was injected (density: 0.92977 $g.mL^{-1}$ – NIST Chemistry WebBook, database number 69). After that, the system was heated up to the desired temperature, while the temperature and pressure profiles were recorded during the dynamic heating process. At the completion of the experiment, the system was cooled down with a water bath until 40 °C. It was observed that the depressurization was impaired by foam formation because the DES solubilized large amounts of CO_2. To work around this inconvenience, the HPLC pump was used to inject ethanol at the end

of each run. An important disclaimer about the use of ethanol on delignified bagasse is that its addition would be inevitable in the separation of the solubilized fraction from the fibers in any case. So, the ethanol addition destabilized the foam and aided in stripping out the CO_2 from the reactant medium. After that, the system was depressurized at an approximately constant rate (around 3 bar/min) until atmospheric conditions.

For the experiments in the presence of ethanol, 6 g of SCB_{ee} were loaded into the reactor vessel with the amounts of DES and ethanol (99.8%, Êxodo Científica, São Paulo - Brazil) designated to each run. The reactor was closed and the CO_2 (at the same conditions mentioned previously) was injected using the syringe pump. After that, the system was heated up to the desired temperature, while the temperature and pressure profiles were recorded during the dynamic heating process. At the end of each run, the system was cooled down with a water bath until 40 °C and depressurized at an approximately constant rate (around 3 bar/min) until atmospheric conditions.

After each experiment, the pretreated fibers were subjected to sequential washing to remove the DES adhered to the fibers. The washing procedure consisted of two steps: 1) filtration and rinsing with 200 mL of ethanol (95 vol%) to avoid lignin reprecipitation over the pretreated fibers; 2) filtration with 250 mL of rinsing water. After washing, the solid samples were oven-dried at 105 °C until constant weight (weight variation < 1.0 mg), weighted, and stored in sealed flasks.

The liquid phase obtained from the ethanol rinsing carries lignin and soluble sugars, which are dissolved in the EtOH+DES mixture. Therefore, to separate the lignin, it was necessary to remove the ethanol in a rotary evaporator, add 250 mL of water to the flask and refrigerate at 4 °C overnight. After that, the lignin precipitated, and a translucid liquid phase appeared, corresponding to the sugars dissolved in the DES+H_2O mixture. The solid was filtered in tared crucibles, dried at 60 °C, weighted, and stored in sealed tubes. To the liquid, water was added to complete 500 mL, and the samples were stored in the freezer at -12 °C until further analysis.

3.3.1 Description of experiments

This work is mainly based on three sets of experiments, comprising the fractionation with DES+CO_2, DES+CO_2+EtOH, and DES+CO_2+EtOH+H_2O.

3.3.1.1. First set of experiments: the first set of experiments aimed to preliminarily evaluate the ChCl:OA capacity of fractionating the SCB_{ee} in a system composed of DES and CO_2 as a co-solvent in order to increase the mass transport rates into the solid matrix. In this 2^3 design of experiments, the fractionation was performed at an SCB_{ee} to DES mass ratio of 1:10 and temperature ranged from 80 to 100 °C, the CO_2 amount ranged from 15 to 45 g, and reaction time from 20 to 60 min. The central point condition was performed in three independent replicates.

3.3.1.2. Second set of experiments: as the main objective of this work was to maximize the lignin extraction from sugarcane bagasse, a fractionation strategy involving an organosolv scheme was planned involving DES, anhydrous ethanol, and CO_2. First, a set of three experiments were performed at fixed solids loading, temperature, time, composition, amounts of solvents, and varying the stirring speeds (150, 300, and 500 rpm) to select a suitable agitation. In the sequence, a 2^3 design of experiments, with a triplicate at the central point, accounted for the effects of DES:SCB_{ee} mass ratio (between 0.5 to 1.5 g/g), the solids loading (within 10 to 15% considering SCB_{ee} to ethanol %mass/volume ratios), and the amount of CO_2 (within 10 and 30 g), during 60 min of pretreatment. Additionally, kinetic studies were carried out based on the second design of experiments and performed at the condition that led to the highest delignification and highest carbohydrate content in the pretreated bagasse, evaluating the kinetics at different temperatures (150, 170, and 190 °C). At each temperature, the reaction times (τ) were 0, 30, 60, and 90 min, where $\tau = 0$ min corresponds to the heating time (meaning that when the desired temperature was reached, the system was immediately cooled down).

3.3.1.4. Third set of experiments: finally, the anhydrous ethanol (99.5 vol%) was replaced by mixtures of water and ethanol (40/60 and 20/80 vol/vol), while other experimental conditions were similar to the highest delignification extent condition from the second set of experiments, and the selected temperatures were 130, 150 and 170 °C, with reaction times (τ) of 0 to 60 min.

Additional experimental measurements that aimed to evaluate the system in the absence of one or another component were also performed, and all the experiments are labeled and described in Table 1.

Table 1: Labeling and description of all experimental conditions performed in this work.

Set	$m_{SCB_{ox}}$[a] (g)	m_{DES}[a] (g)	V_{EtOH}[b] (mL)	m_{CO_2}[a] (g)	time (min)	Temp. (°C)	Stirring speed (rpm)
1st	4	40	0	from 15 to 45	from 20 to 60	from 80 to 100	300
	6	9	48 (anhydrous)	30	30	170	150
	6	9	48 (anhydrous)	30	30	170	300
	6	9	48 (anhydrous)	30	30	170	500
	6	from 3 to 9	from 40 to 60 (anhydrous)	from 10 to 30	60	170	500
2nd	6	9	40 (anhydrous)	10	0 to 120	150	500
	6	9	40 (anhydrous)	10	0 to 90	170	500
	6	9	40 (anhydrous)	10	0 to 90	190	500
	6	9	40 (hydrated, 20 vol%)	10	0 to 60	130	500
3rd	6	9	40 (hydrated, 20 vol%)	10	0 to 60	150	500
	6	9	40 (hydrated, 20 vol%)	10	0 to 60	170	500

	6	0	48 (anhydrous)	20	60	170	500
	6	2.84[c]	48 (anhydrous)	20	60	170	500
	6	9	40 (anhydrous)	0	60	170	500
Additional exp.	6	4.27[c]	40 (hydrated, 20 vol%)	10	60	170	500
	6	9	40 (hydrated, 40 vol%)	10	60	170	500
	6	0	40 (hydrated, 40 vol%)	10	60	170	500
	6	0	40 (hydrated, 20 vol%)	10	60	170	500

[a] $m_{SCB_{\infty}}$, m_{DES}, and m_{CO_2} refers to the mass of each component added to the reactor.
[b] V_{EtOH} refers to the volume of ethanol (anhydrous or hydrated) added to the reactor.
[c] in these runs, the deep eutectic solvent was replaced by its HBD.

3.3.2 Delignification extent, carbohydrate recoveries, and mass balances

The solid mass recovery (Y) was calculated as the mass of bagasse maintained as fibers, as presented by Equation 1:

$$Y(\%) = \frac{M_T}{M_{UT}} \cdot 100 \quad (1)$$

where M_T and M_{UT} stand for the weight on a dry basis of the treated and untreated bagasse, respectively.

The delignification extent (Y_{DL}) was calculated considering the acid-insoluble lignin contents in the SCB_{ee} and in the pretreated pulp, according to Equation 2:

$$Y_{DL}(\%) = \left[1 - \frac{Y \cdot AIL_T}{AIL_{UT}}\right] \cdot 100 \quad (2)$$

where AIL_T and AIL_{UT} correspond to the acid-insoluble lignin content on the treated and untreated substrates, respectively, according to Section 3.4.4.1. Besides, the precipitated lignin mass recovery (Y_{LP}) was calculated according to Equation 3:

$$Y_{LP}(\%) = \frac{M_{LP}}{M_{UT} \cdot (AIL_{UT} + ASL_{UT})} \cdot 100 \quad (3)$$

where M_{LP} is the mass of precipitated material, on a dry weight basis.

The carbohydrate retention in the fibers, $Y_{S,G}$ for the glucans and $Y_{S,AX}$ for the arabinoxylans, were calculated as presented in Equations 4 and 5:

$$Y_{S,G}(\%) = \frac{Y \cdot G_T}{G_{UT}} \cdot 100 \quad (4)$$

where G_T and G_{UT} stand for the glucan content of the treated and untreated samples, respectively.

$$Y_{S,AX}(\%) = \frac{Y \cdot AX_T}{AX_{UT}} \cdot 100 \quad (5)$$

where X_T and Ar_T are the carbohydrate contents in terms of xylose and arabinose in the treated samples, respectively. Similarly, for the untreated material, X_{UT} and Ar_{UT} refer to xylose and arabinose contents.

Also, the total recoveries – or mass balances – of each component (MB_L for total lignin, MB_G for glucans, and MB_{AXA} for arabinoxylans+acetyl groups) were calculated considering liquid and solids fractions following Equations 6 to 8:

$$MB_L(\%) = \left[\frac{Y \cdot TL_T}{TL_{UT}} + \frac{L_P}{M_{UT} \cdot TL_{UT}} \right] \cdot 100 \quad (6)$$

where L_P is the dry weight of the precipitated lignin, TL_T and TL_{UT} are the total lignin content of the treated and untreated substrates, respectively

$$MB_G(\%) = \left[Y_{S,G} + \frac{V_{LQ} \cdot (G_{LQ} + HMF_{LQ})}{M_{UN} \cdot G_{UN}} \right] \cdot 100 \quad (7)$$

where V_{LQ} refers to the total volume of liquid fraction, after lignin precipitation (500 mL), G_{LQ} and HMF_{LQ} refer to the concentration of glucose and 5-HMF on the liquid fraction, respectively.

$$MB_{AXA}(\%) = \left[\frac{Y \cdot (AX_T + Ac_T)}{AX_{UT} + Ac_{UT}} + \frac{V_{LQ} \cdot (AX_{LQ} + Ac_{LQ} + F_{LQ})}{M_{UT} \cdot (AX_{UT} + Ac_{UT})} \right] \cdot 100 \quad (8)$$

where Ac_T and Ac_{UT} are the acetyl groups content in the treated and untreated samples, respectively. And X_{LQ}, Ar_{LQ}, Ac_{LQ}, and F_{LQ} refer to the xylose, arabinose, acetyl groups, and furfural concentrations in the liquid fraction, respectively.

3.4 SUBSTRATE CHARACTERIZATION

The untreated and pretreated fibers were characterized in terms of carbohydrates and lignin, as described in Section 3.4.4. Besides, the native samples were subjected to ashes and extractives analysis (Sections 3.4.2, and 3.4.3), and moisture content determination before each day of experiments (described in Section 3.4.1).

The extracted lignin, after precipitation, was analyzed for carbohydrate content (Section 3.4.4). Additionally, the molecular weight distributions were evaluated by high-performance size exclusion chromatography, and the samples were subjected to FT-IR analysis, as described in Section 3.4.6.

The liquid fraction samples were analyzed for soluble carbohydrates and co-products, as described in Section 3.4.5.

3.4.1 Determination of moisture content

For moisture content determination, based on NREL protocol 510-42621 (SLUITER et al., 2008d), porcelain capsules were oven-dried at 105 °C for 4 h, cooled in a desiccator until room temperature, and weighted. Between 1 and 2 g of sample was weighted in the capsule and oven-dried at 105 °C until constant weight (weight variation < 1.0 mg).

3.4.2 Determination of ashes content

For ashes determination, based on NREL protocol 510-42622 (SLUITER et al., 2008a), porcelain crucibles were placed on the muffle furnace at 575 °C for 4 h, cooled in a desiccator until room temperature, and weighted. Within 1 and 2 g of sample were weighted in the crucible and placed at the muffle furnace, using a ramping program as described by Sluiter et al. (2008a) and kept at 575 °C for 4 h. After that, the samples were cooled in a desiccator and weighed. The procedure was repeated until constant weight (weight variation < 1.0 mg).

3.4.3 Determination of extractives in water

The determination of extractives in water was carried out based on NREL protocol 510-42619 (SLUITER et al., 2008b), using a Soxhlet apparatus with distilled water, in triplicate. For this procedure, approximately 4 g of sample were weighted in paper filter cartridges and place in the Soxhlet chamber, the extraction was carried out for approximately 16 h, corresponding to 10 cycles. The extracts were evaporated in a rotary evaporator, oven-dried at 60 °C until constant weight (weight variation < 1.0 mg), and weighed.

3.4.4 Determination of lignin and structural carbohydrates

The determination of structural carbohydrates and total lignin was based on NREL protocol 510-42618 (SLUITER et al., 2012). Therefore, 300 mg of the dry samples were weighed in glass tubes, 3 mL of 72% sulfuric acid were added and the mixture was placed in a water bath at 30 °C and stirred for 60 min. After that, the content was transferred to autoclave glass bottles, 84 mL of deionized water was added to dilute the acid to 4%. These bottles were placed in an autoclave at 121 °C for 60 min. Thereafter, the mixtures were filtered in medium porosity crucibles, the liquid was analyzed for carbohydrate and acid-soluble lignin content, while the solids were dried to determine the acid-insoluble lignin content.

3.4.4.1 Lignin content

The acid-insoluble residue was oven-dried at 105 °C. Due to the small quantity of insoluble residue samples, the ashes content was admitted to be neglectable. Thus, the acid-insoluble lignin (*AIL*) was calculated according to Equation 9:

$$AIL(\%) = \frac{M_L}{M_S} \cdot 100 \qquad (9)$$

where M_L is the dry weight of the acid-insoluble residue and M_S is the samples' dry weight.

The acid-soluble lignin (ASL) was determined with a UV Spectrometer at 240 nm, using the ε (absorptivity at this wavelength) indicated by NREL protocol 510-42618 (SLUITER et al., 2012) for sugarcane bagasse (25 $L.g^{-1}.cm^{-1}$), using Equation 10:

$$ASL(\%) = \frac{UV_{abs} \cdot V_F \cdot D}{\varepsilon \cdot M_S \cdot L} \cdot 100 \qquad (10)$$

where UV_{abs} is the absorbance read at the mentioned wavelength, V_F is the total volume of the hydrolyzed liquid sample (250 mL), D is the dilution made to maintain the readings between 0.4 and 0.7, and L is the path length of the quartz cuvette (1 cm). Finally, the samples' total lignin content (*TL*) is the sum of *AIL* and *ASL*.

3.4.4.2 Carbohydrate content

The carbohydrate content was analyzed by HPLC (Shimadzu model LC20AD), provided with an autosampler (model SIL10A), a mobile phase degasser (model DGU 14A), column heater (model CTO 10A), and detectors on models RID10A for refractive index and SPD-M10Avp for ultraviolet spectrophotometry. The column used was Rezex RHM (Phenomenex 300 x 7.8 mm) at 65 °C, preceded by a Phenomenex pre-column (8.0 x 3.2 mm) and eluted with 5 $mmol.L^{-1}$ H_2SO_4 mobile phase at a flow rate of 0.6 $mL.min^{-1}$. Quantifications were made by external standardization, based on calibration curves constructed for each monitored component (cellobiose, glucose, xylose, arabinose, and acetic acid), considering their respective hydrolysis factors, which converts each component into its anhydrous derivative: 0.95 for cellobiose, 0.9 for glucose, 0.88 for arabinose and xylose, and 0.72 for acetic acid.

3.4.5 Determination of carbohydrates and co-products in liquid fractions

The aqueous liquid streams, after lignin precipitation, were analyzed based on NREL protocol 510-42623 (SLUITER et al., 2008c). Since all the samples were stored in the freezer after each reaction, the first step for this section was to unfreeze the samples in a refrigerator overnight, after that the samples were homogenized and centrifuged. The supernatant had its pH measured by a pHmeter (MicroNal B474). Following, 2.5 mL of this liquid was taken into a test tube and added by their correspondent amount of sulfuric acid (72%), according to Sluiter et al. (2008c). After homogenizing the liquid, an aliquot was transferred to a vial and placed in the oven for 105 ± 5 °C for 90 min.

The carbohydrate and co-products were analyzed according to section 3.4.4.2, with the addition of the furanic derivatives (furfural and 5-HMF) to the standardization curves. The quantification took into account their dehydration factors considering they were originated from their respective C5 or C6 carbohydrates: 1.37 for furfural, and 1.29 for 5-HMF.

3.4.6 Lignin analysis and molecular weight distribution

The solids precipitated after fractionation underwent three characterization techniques: a carbohydrate and lignin content analysis in the same form described in Section 3.4.4, an HPSEC (high-performance size exclusion chromatography) analysis for apparent molecular weight distribution, and an FT-IR (Fourier Transformed Infra-Red) spectroscopy.

The HPSEC analysis was performed in oven-dry lignin samples. After oven-drying, the precipitated samples were solubilized in tetrahydrofuran (THF 99.8% – Êxodo Científica, São Paulo - Brazil) at the concentration of 3 $mg.mL^{-1}$ and filtered in syringe filters (PTFE 0.45 μm) before injection. The equipment used for the analysis was a Waters 1515 HPLC (Milford, USA), with a model 2487 UV detector operating at 254 nm. The gel column used was a Styragel® HR 4E THF column (7.8 x 300 mm) (Waters, Milford, USA). Injections of 20 μL of the sample were manually made, and THF at 40 °C and 1 $mL.min^{-1}$ was used as the mobile phase. Finally, it was employed a universal calibration using a fifth-order curve using polystyrene standards from $2.8 \cdot 10^6$ to 350 Da ($\alpha = 0.74$; $K = 0.0001179$ - THF at 40 °C).

Finally, the oven-dry lignin samples were subjected to FT-IR spectroscopy. To do so, pellets were prepared by mixing the samples with KBr, in an approximated concentration of 1%. The equipment employed for this analysis was a Bruker Vertex 80 FTIR. All spectra were collected in the transmittance mode with 32 scans, and a resolution of 2 cm^{-1}, in the wavenumber range of 4000-400 cm^{-1}.

3.5 COMPOSITION ANALYSIS USING MICRONIR

The samples' spectra were taken in three independent replicates by near-infrared spectroscopy (NIR) using a portable device (MicroNIR 1700, Viavi, Milpitas, USA) and correlated with the main components' distribution obtained according to section 3.4.

Data processing is a primordial step when using a NIR instrument because NIR absorption bands are not directly associated with specific molecular structures, so the analysis relies on mathematical and statistical tools. In this work, a multivariate calibration – partial least squares (PLS) – was employed. The data processing included

four steps: 1) calculating mean spectra, 2) spectral preprocessing using Savitzky-Golay first-derivative, 3) Kennard and Stone algorithm application for sample division using 80% for calibration and 20% for validation, and 4) PLS application.

3.6 STATISTICAL ANALYSIS AND UNCERTAINTIES

Statistical analyses were performed using the software Statistica 7.0® (Analytical Software, Tallahassee, FL, USA). Experimental data on delignification extents, with the normalized independent variables, were analyzed for variance (ANOVA) with a confidence level of 95%.

The experimental uncertainties were obtained from the replicates at the central points and were considered uniform for each response from the same type of experiments, which means that there is one set of experimental uncertainties for each response (delignification, and carbohydrates retention) for the DES + CO_2 experiments and another set for the organosolv strategies. Also, some experimental conditions were performed in duplicate, and the standard deviation from the average values was reported as experimental uncertainties.

The compositional measurements were performed in three independent replicates. Therefore, the reported analytical uncertainty was the standard deviation from such replicates. However, for some experiments, the mass quantity to perform analysis was limited, and the analytical uncertainty was based on type B error according to the “Simple Guide for Evaluating and Expressing the Uncertainty of NIST Measurement Results” (TAYLOR; KUYATT, 1994). This type of error is based on the analyst's experience with the referred technique, and in this case, it was considered the worst-case observed – the higher standard deviation – for each following component: glucan content, arabinoxylans content, and acetyl groups content.

4 RESULTS AND DISCUSSION

This chapter is dedicated to the presentation and discussion of the main findings from this study. Initially, the raw material characterization is presented. Following, the results related to the two designs of experiments are presented, addressing the effects of each variable on the delignification extent. Then, kinetic studies at varying temperatures are presented, focusing on the delignification and carbohydrate retention on the fibers, and their composition. At this point, the kinetics of carbohydrate extraction is also evaluated. After that, the extracted lignin is characterized as to its carbohydrate and lignin contents, apparent molecular mass distribution, and FT-IR spectra. At last, the composition of pretreated materials was evaluated using MicroNIR, and compared with results of the traditional methods of characterization. Figure 7 presents a block diagram of the process and indicates the analysis performed at each sample.

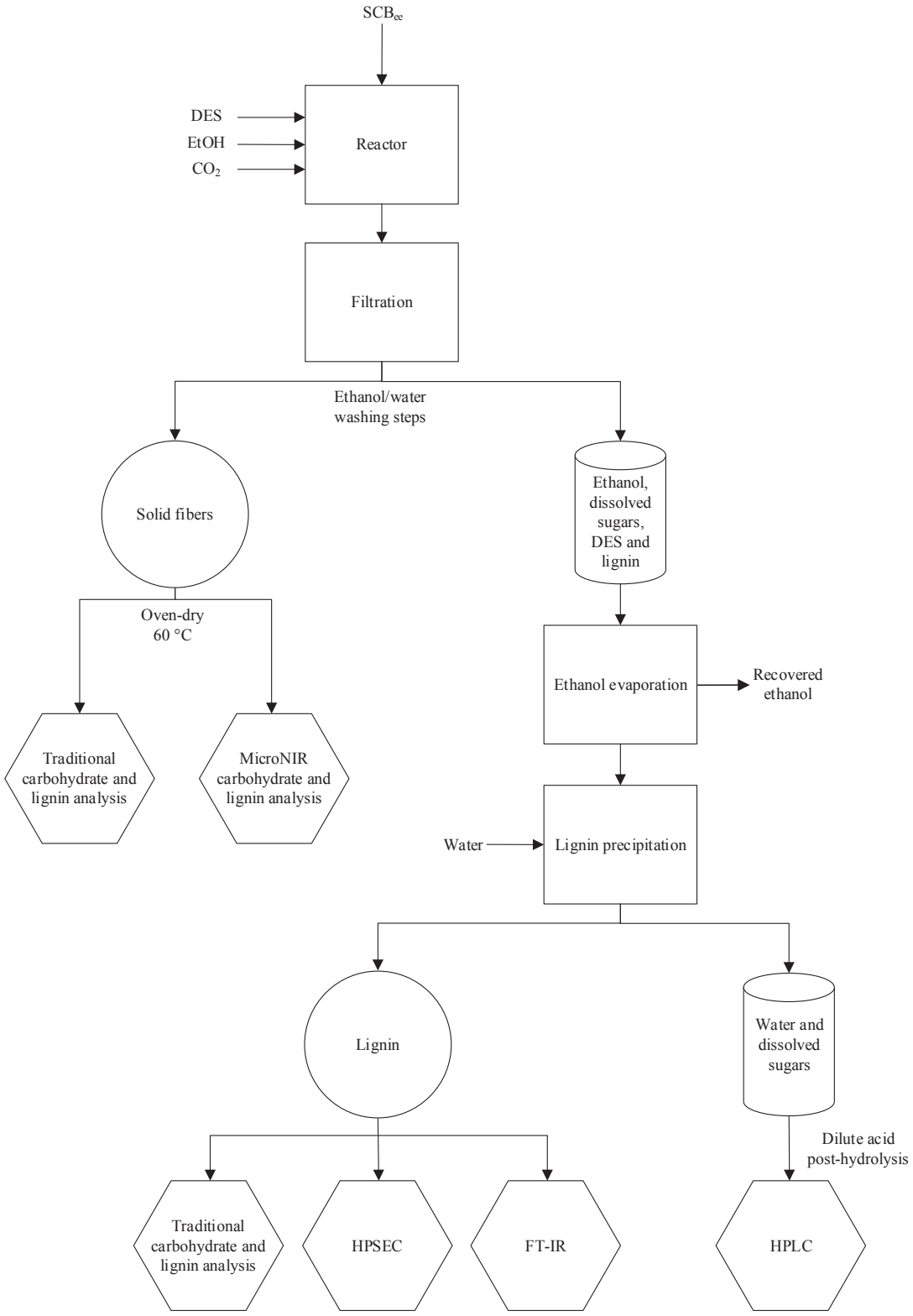

Figure 7: Block diagram of experiments and analysis. The hexagonal symbols indicate analytical procedures.

4.1 BIOMASS CHARACTERIZATION

Before experiments, the native sugarcane bagasse was knife-milled to pass a 1 mm screen (particle size distribution shown in Appendix I) and sieved to remove the fine particles (<0.42mm, which corresponded to 41% of the native biomass). The material with a higher diameter (average diameter of 0.60 mm) was extracted with ethanol 95 vol% to prevent interferences caused by low molecular mass extractives, resulting in 2.3 ± 0.3 wt% of mass loss, and it was used for the experiments. In the sequence, characterization followed the procedures presented in Section 3.4. Table 2 presents the characterization of the ethanol extracted sugarcane bagasse, named SCB_{ee}. Also, all pretreated solids were analyzed for carbohydrate and lignin contents, and the results are compiled in Tables A1 to A5, from Appendix III.

Table 2: Characterization of sugarcane bagasse (dry basis) after extraction with ethanol (95 vol%) and comparison with results from the literature.

Component		**Content (wt%)**		
		Present work	**Silveira et al. (2015a)[a]**	**Fockink et al. (2018)[b]**
Extractives in water		2.5 ± 0.7	-	5.3 ± 0.1
Ashes		1.9 ± 0.3	6.5 ± 0.4	8.3 ± 0.2
Anhydroglucose		43.1 ± 1.3	44.8 ± 0.8	31.8 ± 0.5
Anhydroxylose		20.0 ± 1.0	15.3 ± 0.2	12.2 ± 0.3
Anhydroarabinose		1.0 ± 0.1	1.6 ± 0.2	0.8 ± 0.1
Acetyl groups		3.2 ± 0.2	2.9 ± 0.3	3.2 ± 0.2
Lignin	Acid insoluble	20.2 ± 0.5	29.3 ± 1.1	24.3 ± 0.4
	Acid soluble	5.4 ± 0.6	0.03 ± 0.01	5.2 ± 0.9
Total		*97.3*	*100.8*	*88.9*

[a] those authors used sugarcane bagasse extracted by ethanol 95 vol% in their experiments and measured an anhydrogalactose content of 0.4 ± 0.2 wt%.
[b] those authors found unidentified hexoses (measured as HMF) and unidentified pentoses (measured as furfural) contents of 0.4 ± 0.0 and 2.7 ± 0.7 wt%, respectively.

4.2 FRACTIONATION OF SCB_{ee} USING DES+CO_2

The first set of runs comprises a design of experiments that preliminarily evaluated the capacity of ChCl:OA+CO_2 to fractionate SCB_{ee} into two main fractions: pretreated fibers and a liquid containing the extracted materials. The addition of carbon dioxide is known to improve a system's performance regarding both heat and mass transfers, especially for highly viscous systems. Therefore, CO_2 was added to improve the homogeneity of the reactional system by reducing viscosity. The variables assessed by the design of experiments were the amount of CO_2, time, and temperature. Table 3 displays a description of the conditions applied to this set of experiments and their corresponding delignification extents.

Table 3: Description of the conditions from the 1st design of experiments, and the corresponding delignification extents.

Experiment	m_{DES}[a] (g)	m_{CO_2}[a] (g)	$m_{SCB_{ee}}$[a] (g)	time (min)	Temp. (°C)	Delignification (%)
DC01	40	45	4	60	100	18.1
DC02	40	45	4	20	100	24.5
DC03	40	15	4	20	100	39.4
DC04	40	15	4	60	100	19.2
DC05	40	45	4	60	80	36.3
DC06	40	15	4	60	80	33.1
DC07	40	45	4	20	80	32.8
DC08	40	15	4	20	80	33.9
DC09-1, DC09-2, DC09-3	40	30	4	40	90	41.0 ± 0.4

[a] $m_{SCB_{ee}}$, m_{DES}, and m_{CO_2} refers to the mass of each component added to the reactor.

Concerning the carbohydrate-rich solids, an important measure for their subsequent utilization is the glucan content. Characterization of pretreated solids showed that all experimental conditions led to substrates with enhanced glucan content (Figure 8). The highest glucan content was around 65 wt%, obtained at the highest

temperature tested (DC04 – 100 °C, with 15 g of CO_2 and 60 min). By contrast, the lowest glucan content of 54 wt% was obtained at a milder temperature condition (80 °C, 45 g of CO_2, and 20 min). At the central point condition (DC09 – 90 °C, with 30 g of CO_2, and 40 min) the glucan content on the fibers was still high (64 ± 2 wt%), which corresponded to a value 50% higher than that of the untreated material.

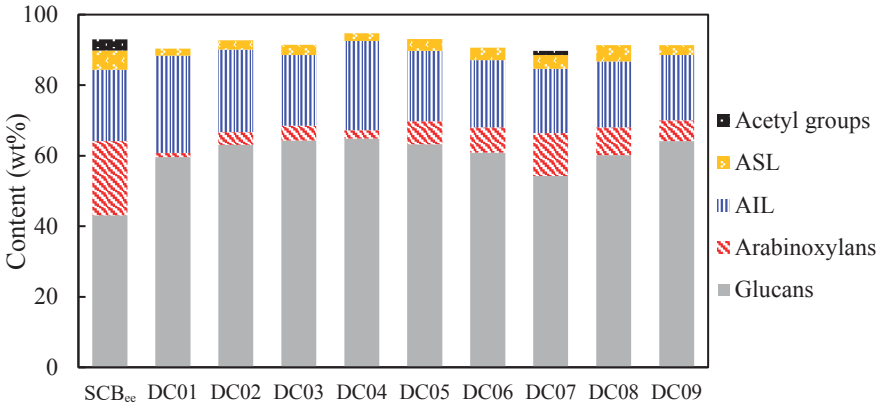

Figure 8: DoE 1 - Solid composition of pretreated substrates. The first design of experiments: fractionation of SCB_{ee} using DES+CO_2 at different temperatures, fractionation times, and amounts of CO_2.

Besides the composition of the pretreated solids, the recovery of carbohydrates is another important measure for demonstrating fractionation efficiency. All conditions tested retrieved high glucan recoveries (Figure 9), and the milder conditions returned total glucan recovery. Even the most severe condition (DC01 – 100°C, 60 min, and 45 g of CO_2) returned a glucan recovery of over 80%. Contrasted to glucans, the arabinoxylans removal seemed to be expressive for all conditions and this was attributed to their higher susceptibility to acid hydrolysis. At the most severe condition (DC01), almost 95% of arabinoxylans were removed, and the highest retention of around 43% was observed at the lowest temperature and time (DC07 – 80 °C, 20 min, and 45 g of CO_2).

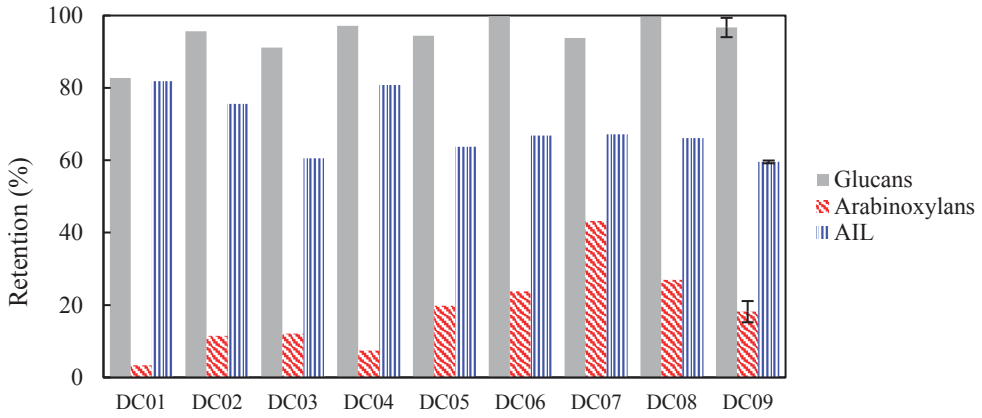

Figure 9: DoE 1 - Solid retention of main constituents of pretreated substrates from the first design of experiments: fractionation of SCB_{ee} using DES+CO_2 at different temperatures, fractionation times, and amounts of CO_2. The error bars refer to the experimental uncertainty.

The liquid fraction of the central condition (DC01 – 90 °C, 40 min, and 30 g of CO_2) underwent chromatographic analysis to evaluate the recovery of arabinoxylans, resulting in around 15% of the initial arabinoxylan content being dissolved in the liquid mostly in the form of mono and oligosaccharides. Also, almost 10% of the initial arabinoxylan content was converted to furfural, however, its exact quantification is not directly available because this component was partially lost during the depressurization or dragged with ethanol during solvent evaporation. The production of furfural from biomass using deep eutectic solvents based on choline chloride with dicarboxylic acids have been studied previously, and furfural yields superior to 25% were obtained when employing oxalic acid as HBD at mild conditions (LEE et al., 2019). Considering that the other common co-products were not found in the samples, and only 43% of the initial arabinoxylans were accounted for either in solid fibers or solubilized in the liquid phase, it is possible to infer that the formation of the volatile by-product may have been way more expressive.

It has been stated in the literature that DES composed of choline chloride with organic acids tends to present good selectivity towards lignin extraction while maintaining glucans undissolved. ChCl:OA presented a solubility of 3.62 wt% for lignin, with a negligible effect on cellulose (FRANCISCO; VAN DEN BRUINHORST; KROON, 2012). In contrast, Hou et al. (2017) reported an even higher lignin dissolution capability by ChCl:OA of 9.4 wt%, but with a 2.9 wt% glucan dissolution; anyhow, the lignin solubility seems to be expressively higher than

cellulose solubility. In terms of selectivity, the results obtained by using DES+CO_2 in the present work corroborates the high affinity of ChCl:OA for lignin.

Around 40% of the lignin present in sugarcane bagasse could be removed at the best pretreatment conditions (DC03 – 100 °C, 15 g of CO_2, and 20 min; and at the central point DC09 – 90 °C, 30 g of CO_2 and 40 min). These results are superior to those reported by Kandanelli et al. (2018) for herbaceous biomass treatment with ChCl:OA at 80 °C for 1 h (delignification around 23-31%). Also, the results found in the present work are comparable with a recent study involving microwave-assisted DES fractionation of Moso bamboo (LING et al., 2021). Those authors obtained up to 48% of delignification in 10 min, but at a much higher temperature (160 °C). Microwave processes are known for reducing reactional times by promoting quick energy transfer and helping the penetration of solvent in the plan cell wall.

However, most of the results on DES-assisted biomass delignification reported so far involve much longer reactional times. Li et al. (2018a) studied the fractionation of rice straw by hydrated lactic acid and choline chloride DES, which led to delignification extents of 48% and 65% at 90 and 120 °C, respectively. The fractionation times applied in their work were 6 h and 3 h, respectively, and the applied biomass-to-solvent ratio was 1:20. It is also worth mentioning that the resulting precipitated solids carried a significant amount of carbohydrates (over 12% of glucans, with less than 70 wt% of total lignin content).

Alvarez-Vasco et al. (2016) reported poplar wood fractionation at 90 °C using DES based on choline chloride with acetic, lactic, and levulinic acids. Delignification extents of 25 %, 18 %, and 21 % were obtained, respectively, after 6 h of pretreatment. Increasing the temperature to 120 °C, or 145 °C for 3 h of fractionation, produced delignification extents over 70%. The precipitation of the extracted lignin followed by multiple water washing steps led to high purity lignins (95 wt% of AIL+ASL, with negligible carbohydrate contents). Another comparison involving DES based on organic acids – levulinic, acetic, formic, and glycolic acids – for the fractionation of Akebia herbal residue at 120 °C resulted in lignin removals of 20, 34, 41, and 58%, respectively, after 8 h of pretreatment (YU et al., 2018).

Comparing the results obtained in the present study with the performance of other acidic DES, one can see that higher delignification levels were achieved in lower pretreatment times. This effect may be related to the important role of CO_2 in increasing the overall mass transfer rates. Besides, the presence of CO_2 in the system

enabled an appropriate stirring, helping solvent permeation through the plant cell walls, soaking the biomass more efficiently, and promoting the homogeneity of a system that would be highly viscous without the addition of CO_2. An attempt at fractionation in the absence of CO_2 was made, in a condition similar to the central one. However, this experiment was disregarded because despite stirring, the fibers were not homogenized, leaving most of the material in the upper part of the reactor without having come in contact with the DES.

After precipitation with water as described in Section 3.3.2, the lignin extracted at the central condition underwent compositional analysis for carbohydrates and total lignin content. The high total lignin content of 95 wt% was observed (82 ± 2 wt% of AIL and 12 ± 1 wt% ASL), with no carbohydrates detected. This high purity of the extracted lignin indicates that this solid could be a good candidate for further conversion to biobased fuels, chemicals, and materials.

The three replicates carried out at the central point of the experimental design (Table 3) had good reproducibility, with an average delignification extent of 41.0 % for a relative experimental uncertainty of 0.4 %. Using the software Statistica 7.0®, the AIL removal data underwent analysis of variance – ANOVA – and a statistical model was adjusted using pair interaction and curvature check, returning a regression coefficient of 0.98, which indicates a good fit. Figure 10 presents the Pareto chart for this ANOVA, and Figure 11 exhibits the observed *vs.* predicted values for the delignification extent. The ANOVA table for this step is presented in Appendix IV, Table B1.

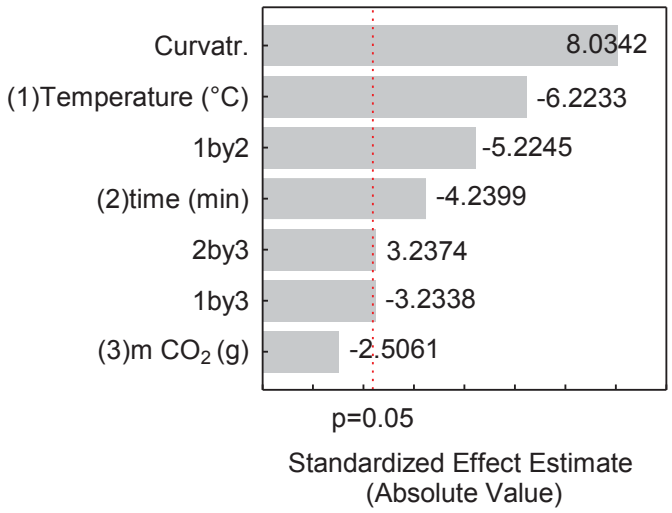

Figure 10: DoE 1 - Pareto chart related to delignification extents. Fractionation of SCB_{ee} using DES+CO_2 at different temperatures, fractionation times, and amounts of CO_2. The statistical model took into account two-way interactions and curvature check, retrieving an R^2 of 0.98.

The variables that retrieved *p-values* lower than 0.05 were considered statistically relevant within a 95% confidence interval. Hence, there was a less than 5% probability for the null hypothesis – where the null hypothesis is that the values found are just random. Based on Figure 10, temperature and time were impacting variables for the reaction response, as well as the binary interaction among all variables. Besides altering the solvent physicochemical properties – like density, diffusivity, viscosity, dielectric constant, among others –, it is expected that temperature favors some degradation reactions. Since competitive depolymerization and condensation reactions are acting over the response, it is natural that temperature impacts the system, while longer times approximate such reactions to their equilibrium. Although the amount of CO_2 did not result in a statistically significant effect, its interaction with other variables did. As expected, CO_2 is acting on promoting homogeneity on the system, and may also be aiding biomass soaking and penetration, but within the limits tested, CO_2 amounts are probably beyond the DES limits for CO_2 absorption. Finally, the adjusted statistical model counted on a significant effect of the curvature, indicating the strong non-linearity of the system.

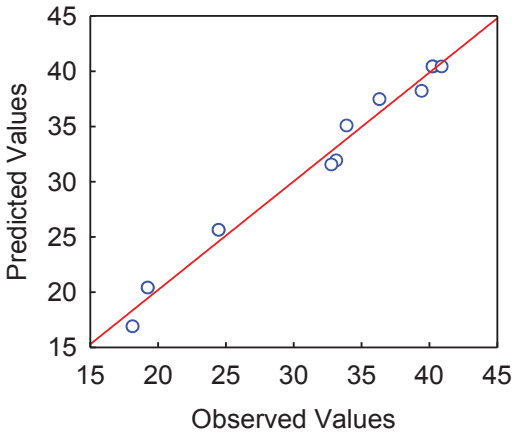

Figure 11: DoE 1 - Statistical model: observed *vs.* predicted values related to delignification extents. Fractionation of SCB_{ee} using DES+CO_2 at different temperatures, fractionation times, and amounts of CO_2. The statistical model took into account two-way interactions and curvature check, retrieving an R^2 of 0.98.

The good correlation between observed and predicted values attest, once again, to the good quality of the statistical model adjusted inferring two-way interactions and curvature check, within the range of variables employed.

In general, the results obtained using the CO_2+DES attest to the high selectivity of the fractionation process for high-purity lignin extraction and maintenance of sugarcane bagasse glucans. In addition, the high production of furfural, in the absence of traditional solvents, could be a further step towards the economic feasibility of a holistic process that integrates lignin and carbohydrate upgrading.

4.3 FRACTIONATION OF SCB_{ee} USING DES+CO_2+ETOH

As stated previously, organosolv fractionation processes lead to high delignification extents, when the appropriate solvents and conditions are employed. Therefore, recognizing that the selected DES is suitable for lignin extraction, and also that this solvent promotes low glucans loss, a novel process was proposed: the conjunction of DES fractionation with an organosolv strategy, using anhydrous ethanol and CO_2. This section preliminary evaluates the effect of stirring speed on the overall responses to aid the selection of a suitable stirring speed. Also, a design of experiments was performed to evaluate the effects of DES:SCB_{ee} mass ratios, solids loadings (SCB_{ee} to ethanol, % wt/vol), and addition of different amounts of CO_2 over

the delignification extent, and guide the selection of process conditions for kinetics studies at different temperatures.

4.3.1 Selection of a suitable stirring speed

First, to define a stirring speed appropriate for the subsequent experiments, preliminary tests were conducted. These experiments were performed at 12.5% total solids considering the SCB_{ee} to ethanol mass/volume ratio, using a DES:SCB_{ee} mass ratio of 1.5, and 30 g of CO_2.

The results from such experiments are presented in Figure 12 and Figure 13. Apparently, the delignification extent was favored by the increase in stirring speed from 150 to 500 rpm. After 30 min of fractionation using 150 rpm, AIL removal was close to 62%. Increasing the stirring speed to 300 rpm enhanced AIL removal to 69% and the best result of 73% was obtained using 500 rpm.

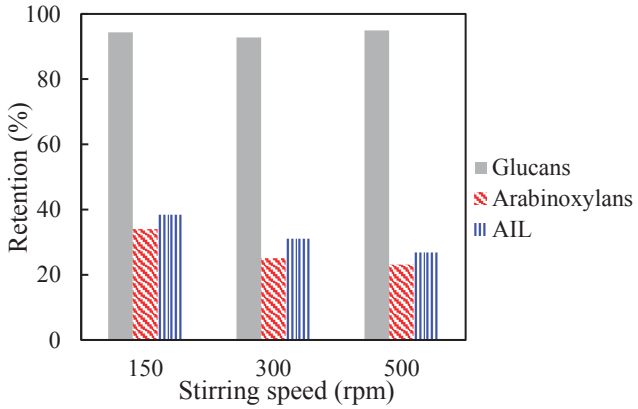

Figure 12: Solid retention of carbohydrates and lignin – fractionation using DES+EtOH+CO_2 at different stirring speeds. Other variables were kept constant – Temperature: 170 °C; time: 30 min; mass of CO_2: 30 g; V_{EtOH}: 48 mL; and DES:SCB_{ee} ratio of 1.5.

All experiments described above resulted in similar lignin and carbohydrate contents, but a trend similar to the delignification was found (Figure 13): increasing the stirring speed slightly enhanced the carbohydrate content while decreasing the lignin content. However, these results lie within the experimental uncertainties for this system (as further discussed). Therefore, it seems that the external mass transfer resistance may not be a limiting factor considering the mixing degree applied in this study, but the agitation promotes an incipient effect in the overall process.

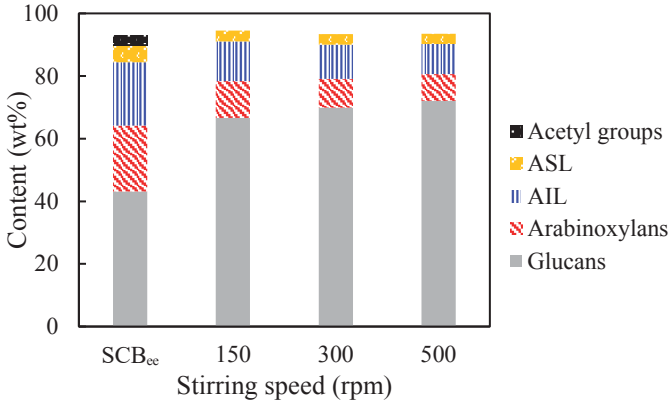

Figure 13: Solid composition of carbohydrates and lignin – fractionation using DES+EtOH+CO_2 at different stirring speeds. Other variables were kept constant – Temperature: 170 °C; time: 30 min; CO_2: 30 g, solids loading of 12.5%, and DES:SCB_{ee} ratio of 1.5.

Although the comparison of different stirring speeds resulted in a quite similar carbohydrate profile and lignin removal, the tendency for better responses from the higher stirring speed (500 rpm) was taken into account and this condition was selected to be used in the subsequent experiments.

4.3.2 Second design of experiments – DES to SCB_{ee} ratio, solids loading and CO_2 amount

As stated previously in section 3.3.1, the experiments performed for the 2nd design of experiments were responsible for evaluating the effects of solids loading (SCB_{ee}-to-ethanol mass/volume ratios) from 10 to 15%, CO_2 addition from 10 to 30 g, and DES:SCB_{ee} mass ratios from 1 to 1.5 on the delignification extent and carbohydrate profiles of the pretreated substrates. The experimental conditions and the delignification results are found in Table 4, while the retention of main components in the solid fibers is presented in Figure 14.

Table 4: Description of the experiments performed in the 2nd design of experiments. Fixed variables – the amount of SCB_{ee}: 6 g, time: 60 min, and temperature: 170 °C.

Experiment	m_{DES}[a] (g)	m_{CO_2}[a] (g)	V_{EtOH}[b] (mL)	Delignification (%)
ED01	3	10	40	42.4
ED02	3	10	60	44.6
ED03-1, and ED03-2	9	10	40	76.1 ± 3.0
ED04	9	10	60	62.9
ED05	9	30	40	59.2
ED06	9	30	60	44.5
ED07	3	30	40	54.3
ED08	3	30	60	47.6
ED09 -1, ED09-2, and ED09-3	6	20	48	64.8 ± 1.4

[a] $m_{SCB_{ee}}$, m_{DES}, and m_{CO_2} refers to the mass of each component added to the reactor.
[b] V_{EtOH} refers to the volume of anhydrous ethanol added to the reactor.

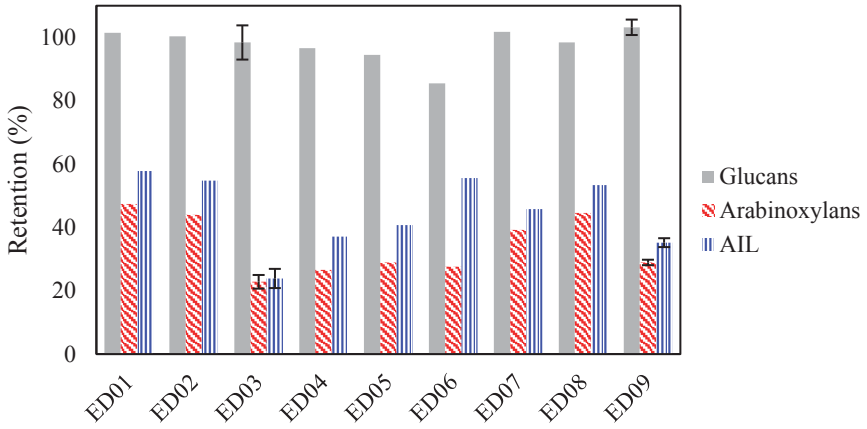

Figure 14: DoE 2 - Solid retention of main components of pretreated substrates. Fractionation using DES+EtOH+CO_2 varying solids loadings, DES:SCB_{ee} ratio, and mass of CO_2. Other variables were kept constant – stirring speed: 500 rpm, Temperature: 170 °C, and time: 60 min. The error bars at ED03 and ED09 refer to the experimental uncertainties.

Regarding glucan retention on the solid fibers, most conditions led to a virtually complete recovery, while ED06 (solids loading of 15%, DES:SCB_{ee} ratio of

1.5, and 30g of CO_2) presented an atypical behavior with almost 15% of glucans removed from the fibers; this point corresponds to the maximum amounts of DES, CO_2, and ethanol. Also, this condition presented an unexpected pressure behavior, which raised beyond 200 bar, although the temperature did not increase accordingly. These observations can be seen in the temperature and pressure profiles in Figure A5 from Appendix II. The arabinoxylans, on the other hand, showed important removal at all conditions tested. The highest removal was around 80% and occurred at ED03 (solids loading of 15%, DES:SCB_{ee} mass ratio of 1.5 and 10 g of CO_2).

Regarding delignification, it is notable once again that ED03 (solids loading of 15%, DES:SCB_{ee} mass ratio of 1.5, and 10 g of CO_2) led to the highest AIL removal. This condition comprises the maximum of DES, maximum of solids loading, with the minimum of CO_2 addition. The comparison of ED03 with ED05 (solids loading of 15%, DES:SCB_{ee} mass ratio of 1.5, and 30 g of CO_2) is inevitable since the latter condition features the maximum of CO_2 while maintaining all other variables constant. Increasing CO_2 addition decreased AIL removal from 76.1 ± 3.0% to 59.2%. This phenomenon may be related to the phase partition inside the reactor. Higher CO_2 addition may have led to the removal of ethanol from the reactant liquid phase, hindering further delignification levels.

At the central condition (ED-09, solids loading of 12.5%, DES:SCB_{ee} mass ratio of 1 and 20 g of CO_2), the delignification extent was close to 65%. This result surpasses those reported by Silveira et al. (2015) for the fractionation of SCB_{ee} using ethanol+scCO_2+IL. In their work, a delignification extent close to 41% was obtained after fractionation at 180 °C with an IL:SCB_{ee} ratio of 1 and 10% solids for 2 h. The best results were obtained in a condition similar to the central point tested in the present work, but with much higher pressure. The central condition of the present study reached values around 70 bar, while Silveira et al. (2015) reported pressures up to 250 bar.

The composition of pretreated solids may be an indicator of the enzymatic hydrolysis potential of the fractionated substrates. Even though there is not a direct relation involving glucan content and enzymatic hydrolysis performance, it is expected that one important factor over the process might be the carbohydrate content. Therefore, observing Figure 15, one notices that all the experimental conditions led to increased glucan and decreased lignin contents. In this regard, the highlight goes to the high glucan content of 75.0 ± 0.8 wt% in condition ED03 (solids loading of 15%,

DES:SCB_{ee} mass ratio of 1.5, and 10 g of CO_2), and the central point (ED-09, solids loading of 12.5%, DES:SCB_{ee} mass ratio of 1, and 20 g of CO_2) with a glucan content 72.7 ± 0.5 wt%. Also, condition ED03 presented the lowest AIL content of 8.5 ± 0.7 wt%. Once more, by comparing the carbohydrate profile obtained in the present work with those of Silveira et al. (2015), one sees that the ChCl:OA DES was highly selective for lignin removal. In their work, those authors attained a maximum glucan content of 59.5 wt%. Thus, considering that ED03 generated the highest glucan content and the highest delignification extent, this condition was selected as a base for further experiments.

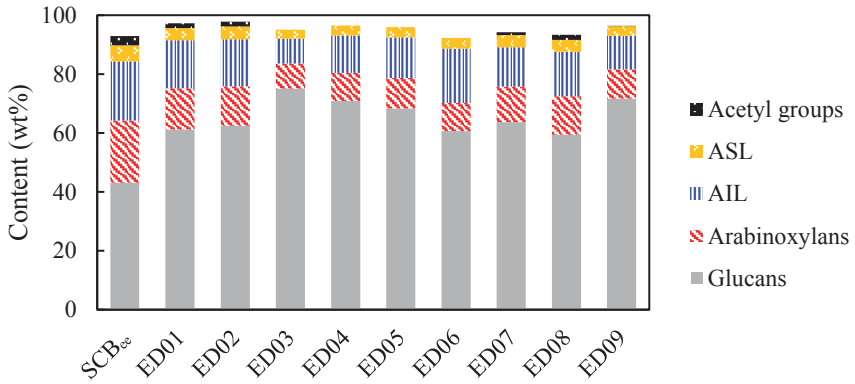

Figure 15: DoE 2 - Solid composition of pretreated substrates. Fractionation using DES+EtOH+CO_2. At varying solids loadings, DES:SCB_{ee} ratio and mass of CO_2. Other variables were kept constant: stirring speed, 500 rpm; Temperature: 170 °C; time: 60 min.

The central point condition and the condition that led to the highest delignification extent (ED09 and ED03, respectively) had their liquid phase subjected to chromatographic analysis to evaluate the retention of carbohydrates, and also their condition as oligomers or monomers. At the post-hydrolyzed liquid sample from the central point (ED-09, solids loading of 12.5%, DES:SCB_{ee} mass ratio of 1, and 20 g of CO_2), arabinose and xylose made up for a bit over 40% of their initial mass of arabinoxylans in the SCB_{ee}. Considering the retention of these components in the pretreated solid (30%), it means that approximately 70% of their initial content was accounted for as carbohydrates. Without the post-hydrolysis, on the other hand, only 1.3% of the initial mass of arabinoxylans was detected in the form of xylose or arabinose, indicating that the extracted sugars were mostly oligomeric.

Likewise, the samples resulting from a condition that led to the highest delignification extent (ED03 solids loading of 15%, DES:SCB_{ee} ratio of 1.5 and 10g of CO_2) presented 36% of their initial arabinoxylans content as soluble carbohydrates. Therefore, considering the xylan retention in the solid fibers (around 23%), the total recovery of arabinoxylans in the form of soluble carbohydrates was close to 60%. Once again, the majority of the soluble sugars were encountered as oligomers, since without the post-hydrolysis step, only 4.7% of the initial arabinoxylans content was found as xylose or arabinose.

Small quantities of furfural were detected in the samples, from 1.6 to 3% of the initial mass of arabinoxylans. However, its exact quantification was not possible because some may have been lost as volatiles during depressurization and ethanol removal in the rotary evaporator.

Considering the three replicates conducted at the central condition of the second experimental design, the results had once again a good experimental reproducibility, with an average delignification extent of 64.8% for an experimental uncertainty of only 1.4%. Additionally, a replicate of the condition that led to the highest lignin removal (ED03 solids loading of 15%, DES:SCB_{ee} ratio of 1.5 and 10g of CO_2) was carried out, and the average delignification of 76.1 ± 3.0%.

Using the software Statistica 7.0®, the delignification data underwent analysis of variance and a statistical model was adjusted using pair interaction and curvature check, returning a regression coefficient of 0.99, which indicates an excellent fit. Figure 16 presents the Pareto chart for this ANOVA, while Figure 17 exhibits the observed *vs.* predicted values for the delignification extent. The ANOVA table for this step is presented in Appendix IV, Table B2.

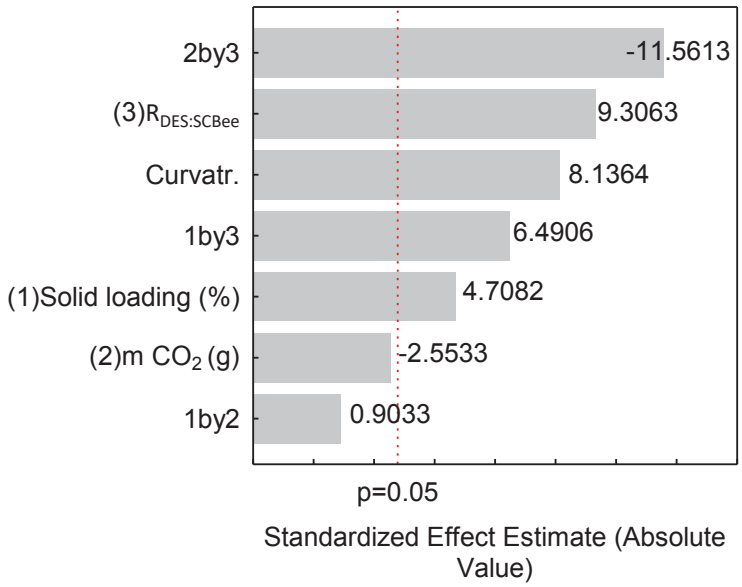

Figure 16: DoE 2 - Pareto chart related to delignification extents. Fractionation of SCB_{ee} using DES+CO_2+EtOH at varying solids loadings, DES:SCB_{ee} ratio, and mass of CO_2. The statistical model took into account two-way interactions and curvature check, retrieving an R^2 of 0.99.

Both DES:SCB_{ee} mass ratio and solids loading were impacting variables for the delignification extent, as well as the binary interaction among all variables. DES capability of solubilizing lignin is a major driving force for delignification, and to lower solids loading means to dilute the system by ethanol addition. Thus, it was expected that those variables would be relevant to the overall process, both favoring higher delignification degrees. On the other hand, by itself, the amount of CO_2 did not result in a statistically significant effect, but the use of CO_2 was primordial given the experimental conditions used in this study. Apart from aiding the solvent penetration on plant cell walls, the main role of CO_2 was on promoting the system's homogeneity at high solids loadings that would be otherwise unviable in the experimental apparatus. Finally, the adjusted statistical model counted on a significant effect of the curvature, indicating the strong system's non-linearity.

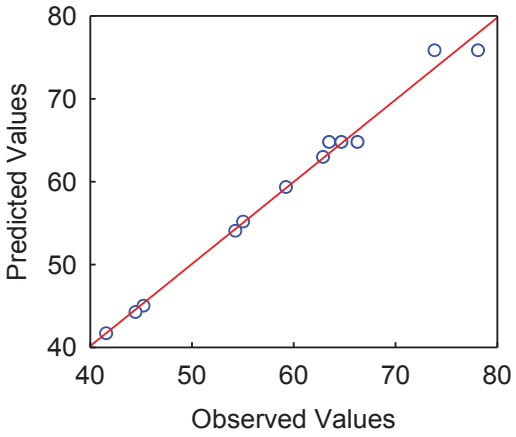

Figure 17: DoE 2 - Statistical model: observed *vs*. predicted values related to delignification extents. Fractionation of SCB_{ee} using DES+CO_2+EtOH at varying solids loadings, DES:SCB_{ee} ratio, and mass of CO_2. The statistical model took into account two-way interactions and curvature check, retrieving an R^2 of 0.99.

Evaluating the observed *vs.* predicted values attest, once again, the good quality of the statistical model based on two-way interactions and curvature check, within the range of variables employed in this study.

Additionally, a control run in the absence of DES was carried out to evaluate the delignification of SCB_{ee} by ethanol and CO_2 in a condition similar to the central point of the experimental design (EX01 - 12.5% of solids loading, and 20 g of CO_2). The resulting pretreated substrate was slightly different from the untreated material, with a delignification extent of merely 12.5%. In contrast, using a similar experimental apparatus, Silveira et al. (2015a) attained an AIL removal of 22 to 30% when operating at 180 °C with pressures of 195 and 250 bar. However, fine particles (<0.42 mm) were also considered in their work, which enhances substrate surface area and contributes to delignification. In the present work, as explained in Section 3.1, to preserve the experimental apparatus, SCB_{ee} was sieved after milling to remove particles below 0.42 mm. Pasquini et al. (2005a) found similar delignification extents (less than 15% for *Pinus taeda* wood chips, and 20% for depithed sugarcane bagasse) after 60 min of fractionation using ethanol at temperatures between 170 and 190 °C and pressures from 160 to 190 bar. Although ethanol can be considered an appropriate solvent for lignin extraction, the lack of a nucleophilic agent hinders further interaction between the solvent system and the biopolymer.

An additional experiment was performed substituting DES for oxalic acid (EX02 – 12.5% of solids loading, and 20 g of CO_2) using the same concentration that

was present theoretically in the DES composition of experiment ED-09 (the central point of the experimental design). This experiment resulted in an AIL removal of only 20.4%. However, the absence of water addition hindered the acid strength, impairing hemicellulose hydrolysis and lignin depolymerization.

Finally, to evaluate the pretreatment in the absence of CO_2, a control run was performed in a condition similar to the one that led to the highest delignification extent (EX03 - 15% of solids loading, and 9 g of DES), resulting in a delignification extent of 69.6% %, which is close to the original result from ED03 (76.1%). However, after the completion of the experiment, it was evident that the bagasse was only partially pretreated, since a significant portion of the material remained at the top of the reactor, with no evidence of having come into contact with the reagents. Therefore, for the experimental arrangement in question, CO_2 was imperative to promote the homogeneity of the system, allowing runs at high solids loading.

4.3.3 Kinetic studies at different temperatures – 150, 170, and 190 °C

Based on the best condition for delignification extent (ED03, solids loading of 15%, DES:SCB_{ee} mass ratio of 1.5, and 10 g of CO_2), a series of experiments were performed, aiming to maximize the response, assess its kinetics, and follow the trends in carbohydrates removal using the conditions described in Table 5.

Table 5: Description of the kinetics involving DES+EtOH+CO_2. Fixed variables – the amount of DES: 9 g, amount of CO_2: 10 g, volume of anhydrous ethanol: 40 mL, and amount of SCB_{ee}: 6 g.

Experiment	t (min)	T (°C)
K170_00	0	170
K170_30	30	170
K170_90-1, and K170 _90-2	90	170
K150_00	0	150
K150_30	30	150
K150_60	60	150
K150_90	90	150
K150_120	120	150
K190_00	0	190
K190_30	30	190
K190_60	60	190
K190_90, and K190_90-2	90	190

One important disclaimer must be made about this kinetic study regarding the heating ramp and the reaction time. As well as what happened in all experiments reported so far, the heating ramp was kept as regular as possible for reactions carried out at the same target temperature. Therefore, the total reaction time is composed by the heating time, starting at 50 °C until the temperature set point is reached, plus the nominal reaction time (τ). Figure 18 shows the delignification kinetics at 170 °C, together with the temperature profile of reactions carried out for different τ values. At other reaction conditions, the temperature profile was suppressed to facilitate the data visualization and analysis, but all temperature and pressure profiles are compiled in Appendix II.

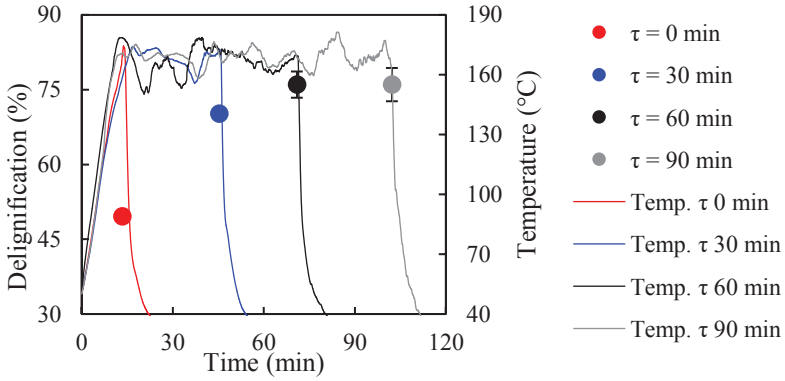

Figure 18: Temperature profile and kinetics of ail removal, fractionation of SCB_{ee} using DES+ETOH+CO_2. Continuous lines refer to the temperature profiles circles refer to delignification extent at the nominal reaction times. The error bars refer to the experimental uncertainties.

In addition, experiments at 150 and 190 °C were performed to evaluate the system response at lower and higher temperatures. The delignification kinetics at all three temperatures are presented in Figure 19, wherein the horizontal axis the time reported is the sum of the heating time and the nominal reaction time (τ).

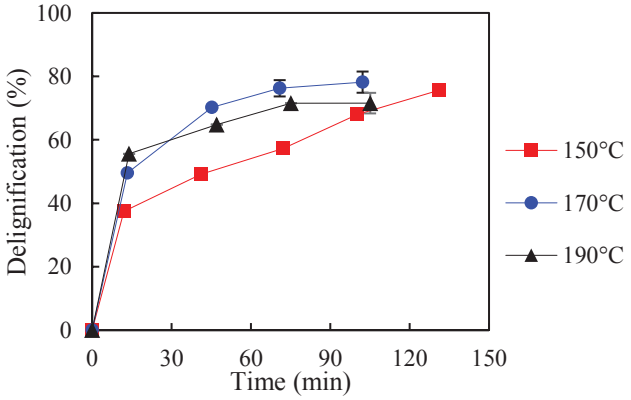

Figure 19: AIL removal from pretreated fibers – kinetics of the fractionation using DES+EtOH+CO_2 at 150, 170, and 190 °C. Other variables were kept constant – stirring speed: 500 rpm; solids loading: 15%, and amount of CO_2: 10 g. The error bars refer to the experimental uncertainties. The corresponding temperature and pressure profiles are presented in Appendix II, Figures A6 to A8.

Initially, the experiments performed at 170 °C indicated that most of the lignin could be separated in 60 min, reaching the highest delignification extent close to 76%. Since the reaction for lignin depolymerization competes with reactions of fragment condensation, it appears to exist an equilibrium after 60 min, and no longer lignin removal was observed. At 150°C, however, it took 120 min to reach a

comparable delignification extent. Still, at 190 °C, the plateau of AIL removal was slightly lower than that at 170 °C. This lower delignification profile may be related to condensation reactions that are possible to occur at higher temperatures, where carbohydrates may be degraded forming furfural and 5-HMF, while these by-products may further degrade into humins – a pseudo-lignin material, that can be analytically read as lignin (SHINDE et al., 2018).

The carbohydrate retention is presented in Figure 20. The arabinoxylan removal followed a similar tendency as delignification, displaying a little enhancement with both time and temperature.

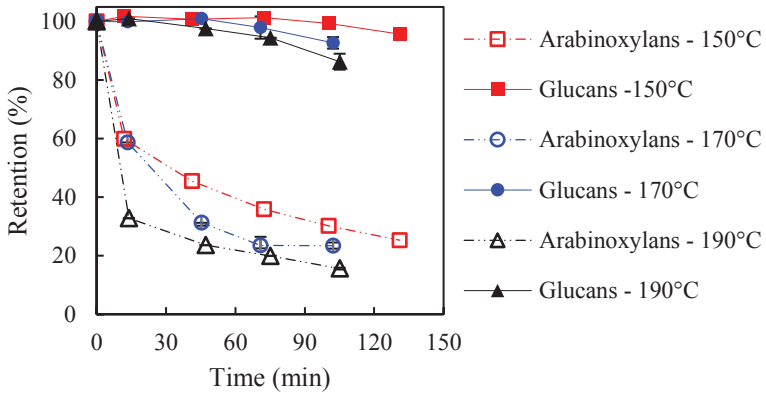

Figure 20: Solid retention of carbohydrates – kinetics of the fractionation using DES+EtOH+CO_2 at 150, 170, and 190 °C. Other variables were kept constant: stirring speed, 500 rpm; solids loading, 15%; amount of CO_2, 10 g. The error bars refer to the experimental uncertainties. The corresponding temperature and pressure profiles are presented in Appendix II, Figures A6 to A8.

By contrast, glucans were well preserved throughout fractionation at all temperatures. The only glucan losses appear to occur at the longest fractionation times, but the glucan retention was still high, around 93% and 86% for 170 and 190 °C, respectively, which corroborates the high selectivity of this fractionation strategy.

There was a notable enhancement in glucan content with time, and this was associated with the drastic decrease in both arabinoxylan and lignin contents (Figure 21). The highest glucan content of around 75 wt% was achieved after pretreating sugarcane bagasse at 170 °C for 60 and 90 min. After τ = 30 min, the arabinoxylan content ranged from close to 14 wt% at 150°C to 9 wt% at 190°C, and the AIL content was reduced to close to 10 wt% for all temperatures.

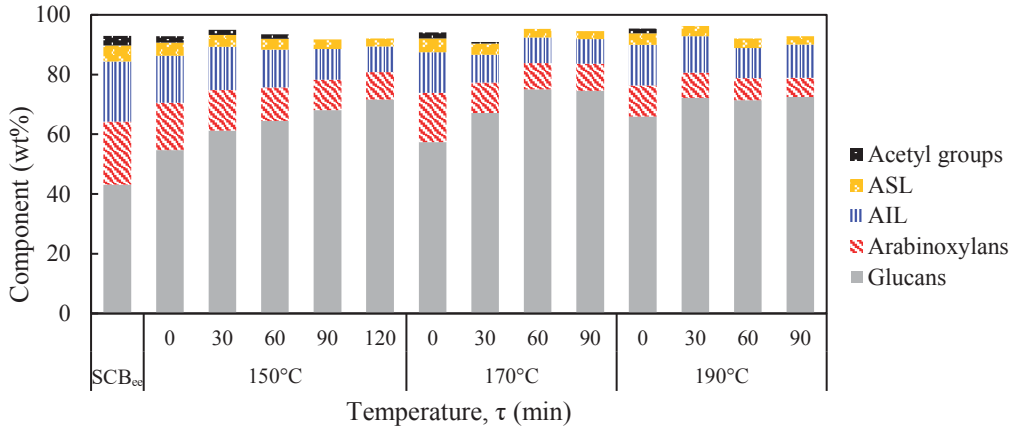

Figure 21: Solid composition of pretreated substrates - kinetics of the fractionation using DES+EtOH+CO_2. Other variables were kept constant – stirring speed: 500 rpm; solids loading: 15%, and amount of CO_2: 10 g.

To better understand the carbohydrate profile in the liquid fraction, as their dissolution or conversion into co-products, carbohydrate analyses were performed using post-hydrolyzed liquid fractions (Figure 22) as described in Section 3.4.5.

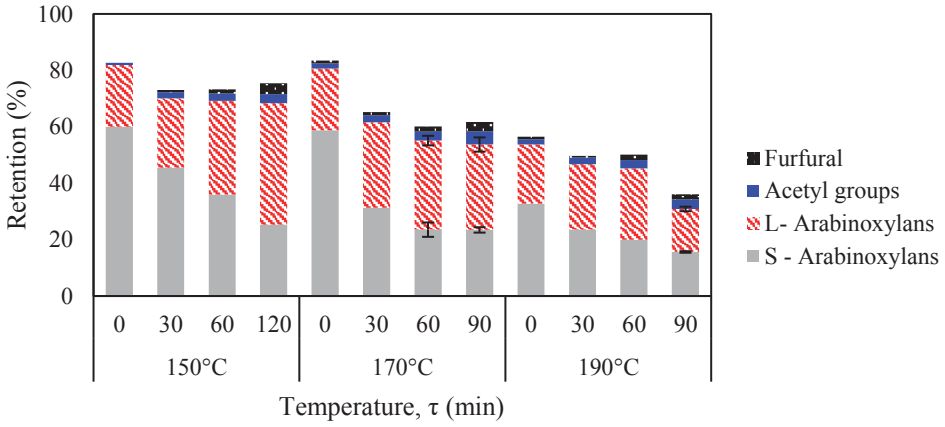

Figure 22: Arabinoxylans+acetyl groups' mass balance considering solid and liquid fractions - kinetics of the fractionation using DES+EtOH+CO_2 at 150, 170, and 190 °C. Other variables were kept constant – stirring speed: 500 rpm; solids loading: 15%, and amount of CO_2: 10 g. The error bars refer to the experimental uncertainties.

The immediate observation is that increased pretreatment temperatures promoted further degradation of arabinoxylan components. At the condition that led to the highest delignification extent, coinciding with the highest glucan content (170 °C, τ = 60 min), the arabinoxylans present in the liquid corresponded to 32% of their initial content. It is important to mention that arabinoxylan removal occurred in the form of oligomers since the analysis of samples prior to post-hydrolysis showed

arabinose plus xylose concentrations varying from 3 to 6% of the original arabinoxylan content. Furfural was produced from dehydration of hemicellulose sugars. However, as explained before, the quantification of furan compounds was underestimated because some were lost during the depressurization or dragged along with ethanol during solvent evaporation.

The mass balances of the main components after fractionation at the three different temperatures, considering furanic compounds arisen from carbohydrates, are displayed in Figure 23A to 23C. Both glucans and total lignin were well accounted for in the pretreatment solids or as the precipitated lignin for all three temperature conditions. However, there was a glucan loss of over 10% at 190 °C with hemicellulose losses that reached over 70% when the pretreatment was carried out for the longest reaction time. This may have contributed to the loss of glucans because sugarcane hemicelluloses contain variable amounts of glucose in their chemical composition, but hydrolysis of amorphous cellulose is also expected to occur at such pretreatment conditions.

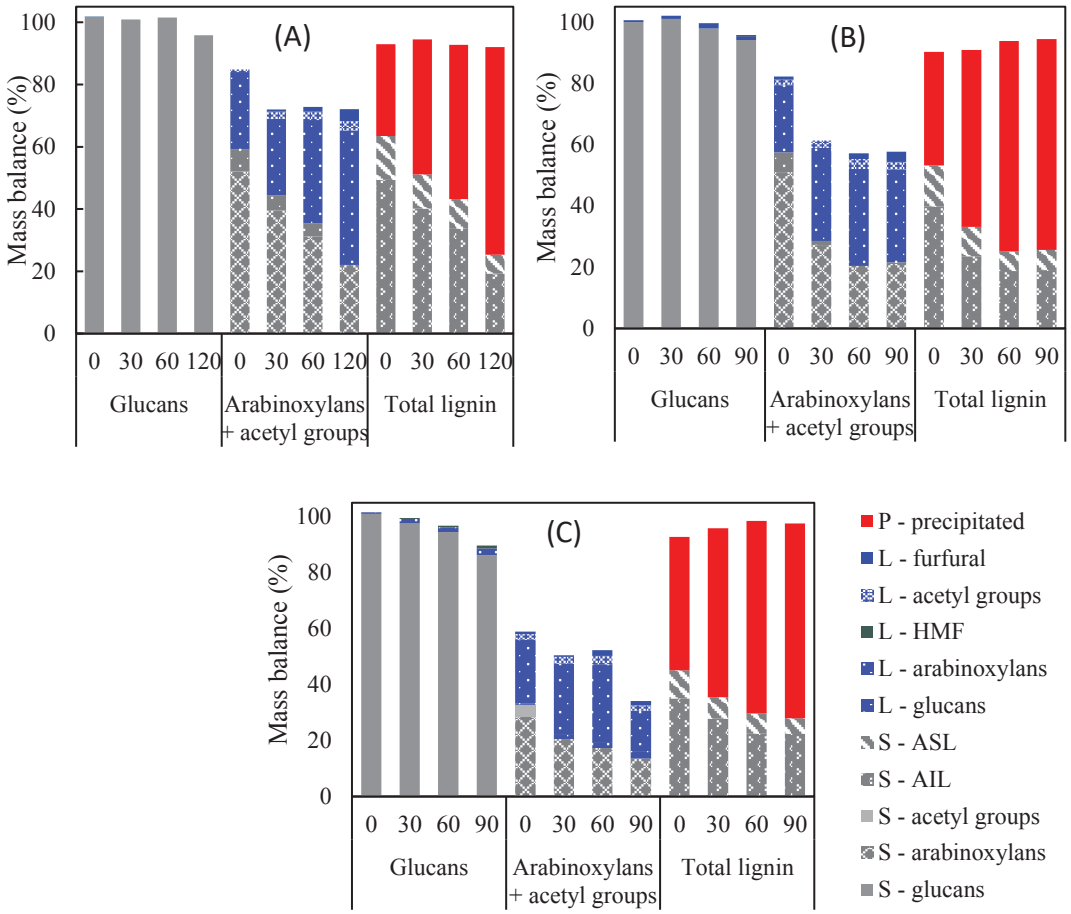

Figure 23: Mass balance by component for glucans, arabinoxylans+acetyl groups and total lignin. Fractionation using DES+EtOH+CO_2 at different temperatures. (A) 150 °C, (B) 170 °C, and (C) 190 °C. The different colors represent the fraction of the process: red (P) is in the precipitated solid, blue (L) is in the liquid fraction and gray (S) is in the solid fibers.

Fractionation of sugarcane bagasse using a DES-assisted organosolv strategy showed excellent results in terms of both delignification and glucans retention, with over 75% of AIL removal and negligible glucan loss at the best conditions. However, the arabinoxylan content in the pretreated fibers remained as high as 20 wt%, which indicates that the addition of water could have a positive effect in enhancing hemicellulose recovery as mono and oligosaccharides.

4.4 FRACTIONATION OF SCB_{ee} USING DES+ETOH+H_2O+CO_2

To enhance the nucleophilicity of the solvent system, it is usual to perform organosolv processes in the presence of water. Therefore, a screening on solvent composition was carried out comparing the delignification extent of SCB_{ee} when anhydrous ethanol was substituted by hydrated ethanol, and later kinetic studies were performed at different temperatures.

4.4.1 Hydrated ethanol: water content screening

The screening on solvent composition was performed with ethanol containing 40 and 20 vol% of water, in the conditions displayed in Table 6.

Table 6: Description of the screening experiments involving DES+ETOH+H_2O+CO_2. Fixed variables – the amount of CO_2: 10 g, amount of SCB_{ee}: 6 g, time: 60 min, and temperature: 170 °C.

Experiment	m_{DES} (g)	V_{EtOH} (mL)
W170_60	9	40 (hydrated, 20 vol%)
EX04	4.27[a]	40 (hydrated, 20 vol%)
EX05	9	40 (hydrated, 40 vol%)
EX06	0	40 (hydrated, 40 vol%)
EX07	0	40 (hydrated, 20 vol%)

[a] in this run, the deep eutectic solvent was replaced by its HBD.

The results on delignification were higher than those obtained in the absence of water. The enhanced performance of this approach may be linked to the effect of water in hemicellulose hydrolysis. Adding 20 and 40 vol% of water led to an AIL removal of 91.2% (W170_60) and 86.0% (EX05), respectively. It is also worth mentioning that glucan retention in the fibers was around 70% with 40 vol% of water, and almost 80% when 20 vol% of water was used (Figure 24).

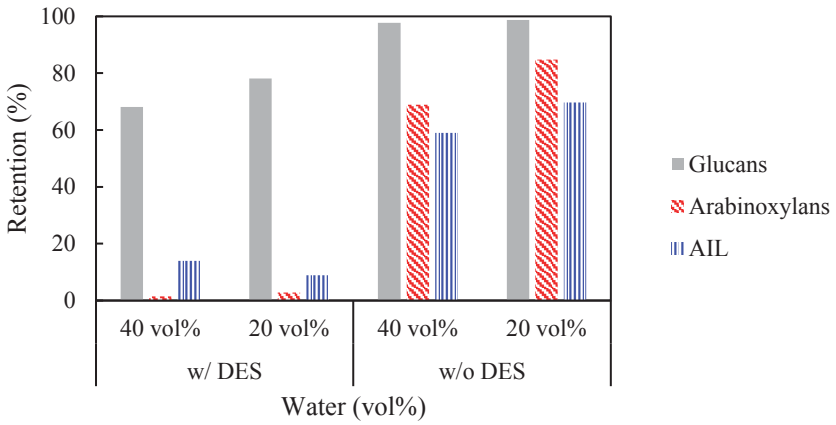

Figure 24: Solid retention of carbohydrates and lignin - kinetics of the fractionation using DES+EtOH+H_2O+CO_2 at 170 °C, varying the water/ethanol composition. Other variables were kept constant: stirring speed, 500 rpm; solids loading, 15%, and amount of CO_2, 10 g.

Interestingly, the highest delignification occurred when using 20 vol% of water in ethanol. One possible reason for worsening the performance with the increase in water content could be the decrease in lignin solubility. However, since this system already counted on a polar solvent, the deep eutectic solvent, which is capable of solubilizing important amounts of lignin, it is more likely that the phenomenon can be related to the condition in which the DES acts in the system. It has been described that increasing the water title in mixtures with deep eutectic solvents tends to disrupt the DES hydrogen bonding while favoring the direct interaction of water with DES components, which occurs in water titles as from 50% (HAMMOND; BOWRON; EDLER, 2017). For example, the water-to-DES ratio in the condition with 40 vol% of water is 0.64, while using 20 vol% of water holds a ratio of 0.47. Hence, at 20 vol% water is probably not affecting DES structure so heavily as when it is at 40 vol%. That is a possible explanation for the results found, but since this field is still not well elucidated yet, any affirmation would require further investigation involving the compounds applied in this work. Concerning the present study, since the system at the lowest water content appear to act more selectively for lignin extraction, this condition was taken as a base for subsequent kinetic study at different temperatures.

The 91.2% delignification extent, obtained by the present work at 170 °C, seems to be close to a general plateau for organosolv processes. Kalogiannis et al. (2018) fractionated fine particles of beechwood sawdust (150-500 μm) using an acid-catalyzed process with hydrated ethanol (60 vol%) at 175 °C for 60 min. These authors

attained over 90% of delignification using 5.6 wt% phosphoric acid, while experiments using 2.6 wt% oxalic acid led to almost 75% of lignin removal.

The delignification of corn stover by water-ethanol mixtures with $scCO_2$ was studied by Lv et al. (2013) in a design of experiments where temperature varied from 160 to 200 °C, pressure ranged from 130 to 170 bar, and reaction times from 40 to 80 min. Delignification extents from 64 to 90% were obtained, depending on the experimental conditions. Kandanelli et al. (2018) treated rice husk, rice straw, and wheat straw with DES and short-chain alcohols at 120 °C. After pre-optimizing the experimental conditions, the best delignification results (around 50%) were obtained using ChCl:OA with n-butanol, using a DES-to-butanol ratio of 2:1. However, the chemical composition of pretreated fibers was not investigated in these previous studies. Suriyachai et al. (2018) treated sugarcane bagasse by organosolv using a mixture of solvents (water, ethanol, ethyl acetate, and formic acid at 25 vol%) with a maximum lignin removal of 85.4% at 170 °C. This delignification was accompanied by glucan retention of 72%, which is a bit lower than the results of the present study using sugarcane bagasse.

Pasquini et al. (2005b) reported a delignification extent up to 93.5% of *Pinus taeda* wood chips using $scCO_2$ with ethanol/water mixtures at 170 °C and 190 bar, with a mass recovery of 35 wt% after 60 min. Their work was performed at much higher pressures, but the CO_2 amount added to the system was not clear. There was a positive relationship of temperature and pressure with the extent of delignification, being the temperature more influential. In another study, Pasquini et al. (2005a) used $scCO_2$ and n-butanol/water mixtures to delignify sugarcane bagasse, with results as high as 94.5% using 60% butanol at 190 °C and 70 bar during 105 min. However, the delignification was also due to the fiber post-treatment with dilute sodium hydroxide. While the present work did not count in any alkaline step for lignin removal, those authors performed an alkaline washing and rinsing, where for each 10 g of dry bagasse (prior fractionation), 500 mL of a NaOH solution (1%) was added to the fibers in a shredder and filtered; after that, another 500 mL of the same solution rinsed the fibers, resulting in high NaOH expense (ratio between pretreated fibers and NaOH over 1:1). Also, the pulp yield at the highest delignification condition indicates a rather non-selective process since less than 9% of the initial mass was obtained in the solid form after fractionation, and the lignin content of the fibers was over 15 wt%.

Compared to the general knowledge in this field, the organosolv ChCl:OA pretreatment developed in this work promoted an extensive delignification of sugarcane bagasse while preserving most of the carbohydrate fractions. This suggests that the proposed strategy is advantageous for integrated processes that aim to the valorization of sugarcane bagasse matrix components.

Control experiments were performed in the absence of DES (170 °C, 60 min, and 10 g of CO_2), and resulted in lower delignification extents. The AIL removal of SCB_{ee} treated with 20 vol% of water was 30.4% (EX07). By contrast, increasing the water content to 40% enhanced delignification to 40.9% (EX06).

Another control experiment (EX04 - 170 °C, 60 min, and 10 g of CO_2) was carried out with oxalic acid alone at the same concentration it would be present in ChCl:OA. The resulting delignification extent and AIL content were quite close to those obtained with DES as solvent. After exposure to oxalic acid, the AIL content of pretreated fibers was 6.7 wt% for a delignification of 85.7%, against an AIL content of 4.3 wt% and 91.2% lignin removal when using DES. The main advantage of DES usage lies, however, in the fact that this solvent can be recycled in the process, or it can be present during saccharification and fermentation processes (ALOK et al., 2018; ROMANÍ et al., 2020). However, the recycling of DES would demand specific studies on methods for its separation from dissolved lignin and hemicellulose sugars, using liquid-liquid extraction or other separation techniques (SMINK; KERSTEN; SCHUUR, 2020b, 2020c).

Figure 25 presents the composition of pretreated fibers after fractionation in the presence and absence of DES or oxalic acid (OA), at varying water/ethanol concentrations.

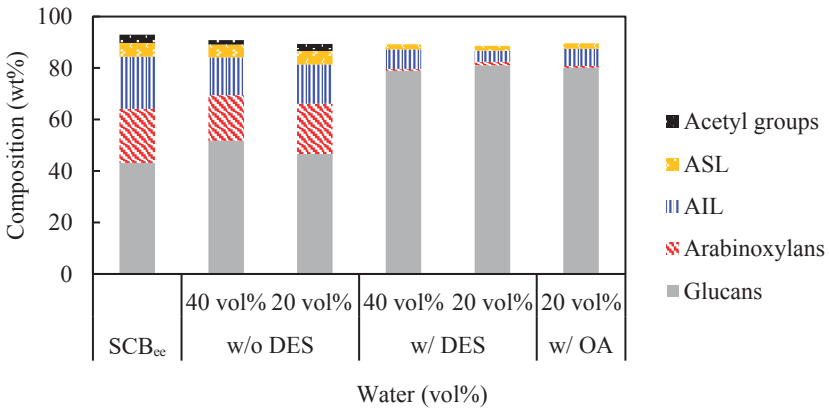

Figure 25: Solid composition of pretreated substrates – using EtOH+H_2O+CO_2 with and without DES, at 170 °C, varying the water vol%; w/o DES refers to the control experiments in the absence of catalyst, w/DES refers to the experiments using DES, and w/ OA refers to the control run where DES was replaced by oxalic acid. Other variables were kept constant: stirring speed, 500 rpm; solids loading, 15%; and amount of CO_2: 10 g.

Analyzing the carbohydrate profiles of Figure 25 leads to interesting conclusions. First is that in the presence of an acid catalyst, almost the totality of the arabinoxylans was removed from the pretreated solids, leading to a highly enhanced glucan content surpassing 80 wt%, which occurs because of the rather selective solubilization of arabinoxylans and lignin. Also, that in the absence of an acid catalyst, the process is rather unselective, provoking little alterations in fiber composition.

Considering that at 170 °C the delignification extent was about the maximum values reported in the literature, a kinetic study of the process in the presence of DES, hydrated ethanol (containing 20 vol% of water), and CO_2 was performed. Also, a temperature reduction to 150 °C and later to 130 °C was intended to verify if it would be possible to maintain the same delignification extent at lower process severities.

4.4.2 Kinetic studies at different temperatures – 130, 150, and 170 °C

The conditions of the kinetic evaluation of the process using hydrated ethanol (with 20 vol% of water) at different temperatures are listed in Table 7.

Table 7: Description of the kinetics involving DES+EtOH+H_2O+CO_2. Fixed variables – the amount of DES: 9 g, amount of CO_2: 10 g, amount of SCB_{ee}: 6 g, and V_{EtOH} (hydrated: 20 vol%): 40 mL.

Experiment	t (min)	T (°C)
W170_00	0	170
W170_15	15	170
W170_30	30	170
W170_60	60	170
W150_00	0	150
W150_15	15	150
W150_30	30	150
W150_60	60	150
W130_00	0	130
W130_15	15	130
W130_30	30	130
W130_60	60	130

The delignification kinetics of the processes can be observed in Figure 26. Although a relation between temperature and delignification extent does exist, these two variables are not directly proportional. Comparing treatments at 150 and 170 °C, one sees that the initial lignin removal at $\tau = 0$ min is higher at the higher temperature. By contrast, after $\tau = 15$ min, the results approached each other: 88.4% at 170 °C and 84.1% at 150 °C. Also, at $\tau = 60$ min, the results are similar within the experimental uncertainty of 3.0%, reaching 91.2% at 170 °C and 86.7% at 150 °C. Nevertheless, further reducing the temperature to 130 °C reduces the delignification extent to close to 70%, attaining the plateau from $\tau = 45$ min, suggesting that the system did not have enough energy to further remove lignin from the fibers.

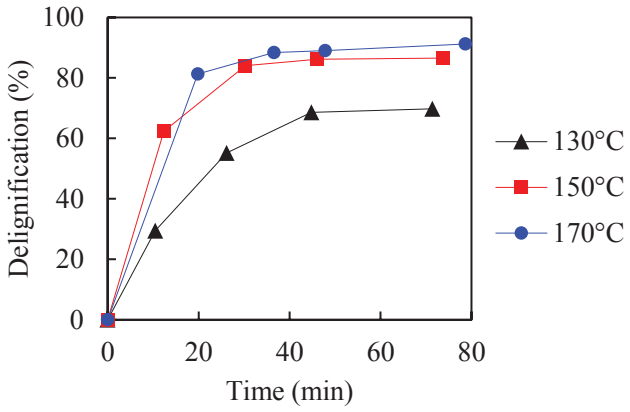

Figure 26: Acid insoluble lignin (AIL) removal from pretreated fibers - kinetics at different temperatures, fractionation using DES+EtOH+H_2O+CO_2. Other variables were kept constant – stirring speed: 500 rpm; solids loading: 15%, and amount of CO_2, 10 g. The corresponding temperature and pressure profiles are presented in Appendix II, Figures A9 to A11.

The carbohydrate profile (Figure 27) suggests that longer reactional times may not be beneficial to these systems, because it leads to glucans removal with little effect over delignification. Comparing results of the kinetic study in the presence of water with those obtained when using anhydrous ethanol (Figure 20), it is clear that the glucan consumption is higher when hydrated ethanol is employed, which is expected due to the enhanced hydrolysis of carbohydrates. At 170 °C, the glucan retention at $\tau = 60$ min decayed from 93% with anhydrous ethanol, to around 78% with hydrated ethanol (20 vol%). Still using hydrated ethanol, at the same τ, decreasing the temperature to 150 and 130 °C, resulted in higher glucan retentions, 86 and 88%, respectively.

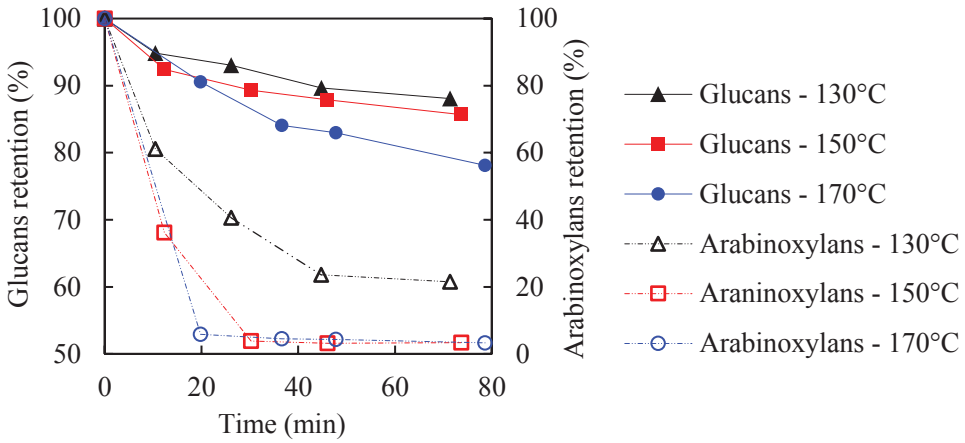

Figure 27: Carbohydrate retention kinetics at different temperatures, fractionation using DES+EtOH+H_2O+CO_2. Other variables were kept constant – stirring speed: 500 rpm; solids loading: 15%, and amount of CO_2, 10 g. The corresponding temperature and pressure profiles are presented in Appendix II, Figures A9 to A11.

Figure 27 also indicates that the arabinoxylan content of pretreated fibers is expressively affected by temperature. At 130 °C, the arabinoxylan removal is slower, and even after τ = 60 min over 20% of this carbohydrate component remains in the solid fibers, while an increase in temperature to 150 and 170 °C leads to their virtually complete removal after τ = 15 min. In fact, the performance at 130 °C is quite similar to the general retention of arabinoxylans after fractionation in the absence of water at higher temperatures (Figure 20), which led to arabinoxylan retention around 15 to 30%, after τ = 60 min, for all temperatures tested. Besides, the delignification profile presents a resemblance between experiments at 130 °C with hydrated ethanol and the results obtained with anhydrous ethanol, reaching plateaus between 70 and 80%. There is evidence of covalent cross-linking, or α-ether linkages, between lignin and hemicelluloses in plant cell walls (NISHIMURA et al., 2018). Hence, to promote delignification the solvent system must be able to solubilize lignin fragments, but also to present nucleophilic species that can cleave the covalent bonds between hemicelluloses and lignin.

In the DES+EtOH+H_2O+CO_2 organosolv systems, CO_2 aided solvent permeation through the plant cell wall, the acidic ChCl:OA DES promoted lignin fragmentation, and ethanol solubilized small lignin fragments, while water enhanced both kinetics and yields of arabinoxylans removal, which – in turn – improved delignification efficiency. On the other hand, in the absence of DES, the addition of water played a decisive role in lignin removal because no other nucleophile was

available in the system, bringing the delignification extent from merely 13% in the experiment with CO_2 and anhydrous ethanol to 41% when ethanol contained 40 vol% of water. In general, the addition of water benefited carbohydrate removal, leading to complete hemicellulose removal at the higher temperatures, but with lower impact over glucans.

The profile of hemicellulose sugars in the liquid fraction is given in Figure 28. First, for reactions carried out at 150 °C and 30 min, over 60% of the initial arabinoxylan content was dissolved in the form of mono and oligosaccharides. Enhancing the temperature to 170 °C, the recovery of hemicellulose sugars decreased to less than 40%. This indicates that although the temperature had a positive effect on delignification, its impact on the recovery of hemicellulose sugars was much higher. As expected, a rise in temperature and reaction time promoted the dehydration of hemicellulose sugars. Furfural was encountered in levels as high as 15% (for 170 °C, 60 min), but since volatiles cannot be accurately accounted for, a high uncertainty lies in the exact quantity of co-products being produced.

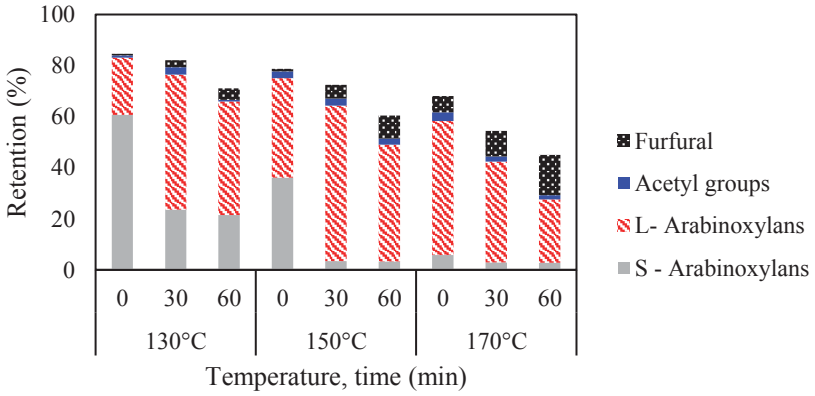

Figure 28: Arabinoxylans+acetyl mass balance considering the components in the pretreated fibers and solubilized in the liquid fraction. Kinetics of the fractionation using DES+EtOH+H_2O+CO_2 at different temperatures. Other variables were kept constant – stirring speed: 500 rpm; solids loading: 15%, and amount of CO_2, 10 g.

The mass balances of the main components in pretreated solids, precipitated lignin, and liquid streams are depicted in Figure 29.

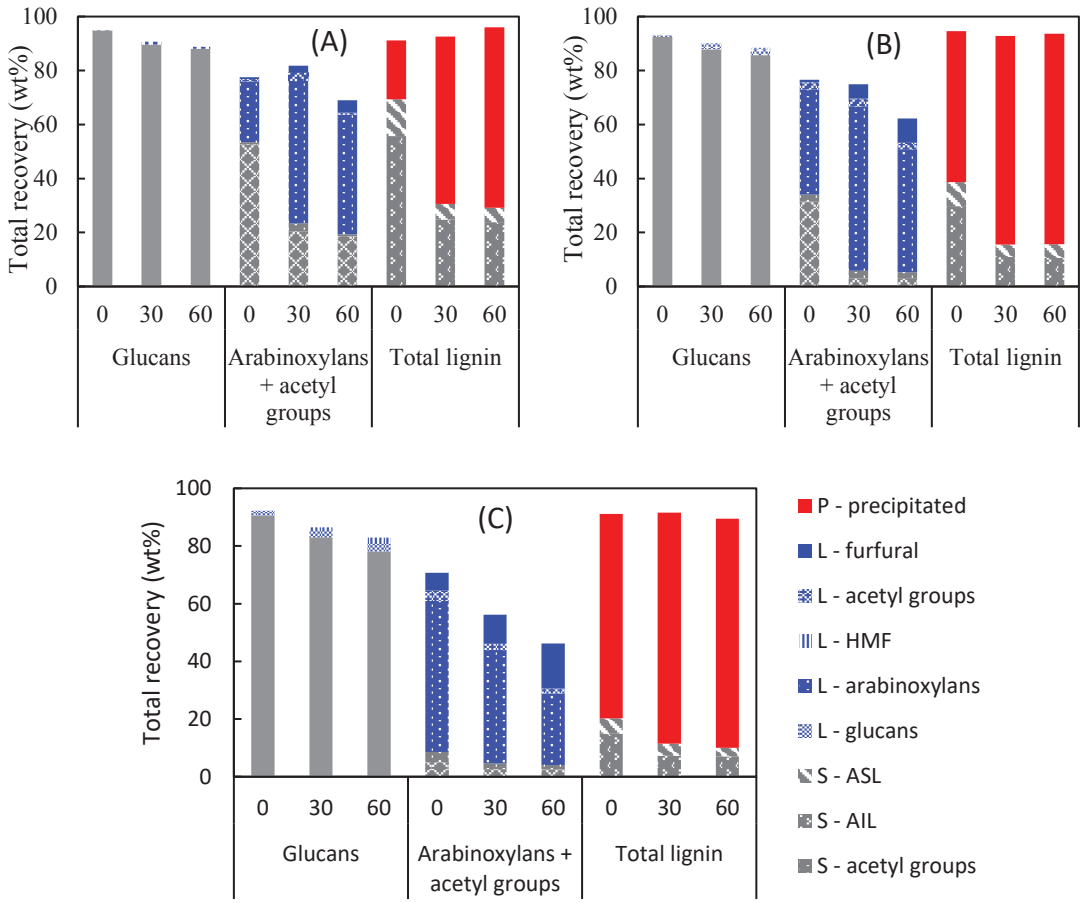

Figure 29: Mass balance by component for glucans, arabinoxylans+acetyl groups, and total lignin. Fractionation using DES+EtOH+H_2O+CO_2 at different temperatures. (A) 130 °C, (B) 150 °C, and (C) 170 °C. The different colors represent the fraction of the process: red (P) is in the precipitated solid, blue (L) is in the liquid fraction, and gray (S) is in the solid fibers.

For all temperatures tested in this study, the vast majority of glucans lay in the solid fibers, with less than 20% of glucan loss. At the lowest temperature, plenty of the arabinoxylans remained in the solid fraction, but from 150 °C onwards, most of this component migrated to the liquid fraction. Also, the lignin distribution was very satisfactory in the two process streams, mainly at the higher temperatures, with almost 80% of the initial total lignin extracted and precipitated from τ = 30 min onwards.

The glucan content of pretreated fibers (Figure 30) increased with time and temperature, reaching a maximum of around 80 wt% after τ =15 min at 170 °C. Also,

an acid-insoluble lignin (AIL) content as low as 4 wt% was observed in these pretreated solids.

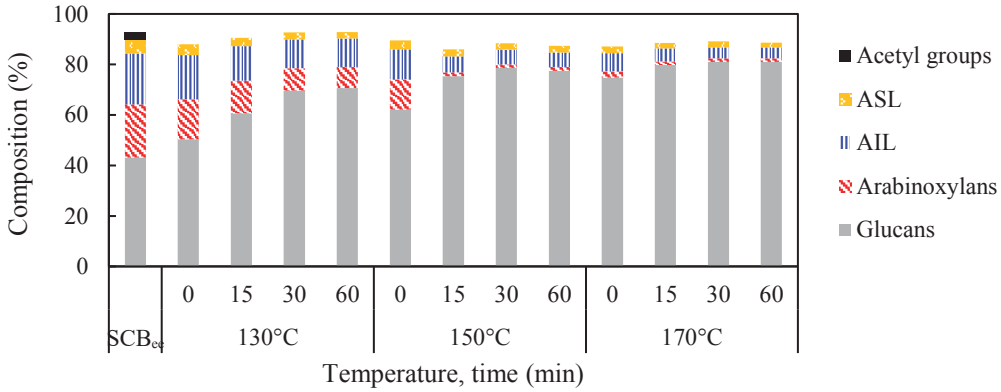

Figure 30: Solid composition of pretreated substrates - kinetics of the fractionation using DES+EtOH+H_2O+CO_2 at 130, 150, and 170 °C. Other variables were kept constant: stirring speed, 500 rpm; solids loading, 15%; amount of CO_2, 10 g.

Considering the results obtained in both delignification extent and carbohydrate retention, it is clear that the proposed pretreatment strategy was successful in fractionating the three main components of sugarcane bagasse, promoting excellent lignin removal and low carbohydrate losses. In addition, it is interesting to know that, when hydrated ethanol was employed, reaction times as low as 15 to 30 min were sufficient to produce high levels of delignification, favoring glucan retention in the fibers and allowing the recovery of hemicellulose sugars in the liquid fraction. Thus, under the best fractionation conditions, the resulting solids were rich in glucans and low in lignin content.

4.5 PRECIPITATED LIGNIN ANALYSIS

After the high lignin yields obtained in this work, it was primordial to evaluate the quality of the extracted material. Hence, the evaluation of the precipitated lignin according to Section 3.4.4 assessed the lignin and carbohydrate content and is presented in Table 8.

Table 8: Composition analyses of precipitated lignins obtained at different experimental conditions.

Experiment	Arabinoxylans (%)	AIL (%)	ASL (%)	Total (%)	Precipitated lignin mass recovery (%)
ED03-1	1.7	85.9	6.1	93.7	62
ED03-2	1.3	87.8	6.0	95.1	73
ED09-1	2.7	84.8	5.2	92.7	45
K150_30	0.0	89.8	5.3	95.0	43
K170_30	0.9	87.7	5.3	93.8	58
K190_30	1.0	89.8	6.0	96.9	60
W130_30	1.5	86.0	7.0	94.6	62
W150_30	0.8	91.1	4.3	96.2	77
W170_30	0.0	95.1	4.5	99.6	80

Based on the experience of the analyzer, the analytical errors were considered as the highest standard deviations obtained from the three independent replicates observed in composition analysis as follows: glucans 4 wt%, arabinoxylans 3 wt%, AIL 2 wt%, and ASL 0.7 wt%.

Although no further lignin purification was performed, all processes yielded precipitated materials with low carbohydrate content and total lignins over 90 wt%. Also, the precipitated lignin mass recovery (Y_{LP}) followed the same tendency as the delignification extent, reaching up to 80% of lignin recovery, with no detectable carbohydrate content in the condition where hydrated ethanol was employed at the highest temperature. These results indicate that the simplest precipitation method based on water addition was already enough to provide high purity lignin.

The evaluation of lignin molecular mass distribution is an important feature to define the utilization of lignin for any practical application. For example, lignins with high molecular mass may be applied to improve the mechanical properties of composites by increasing their cohesive strengths by molecular entanglement, while lignins with low molecular mass may be more suitable to produce adhesives and resins (TOLBERT et al., 2014). In this work, high-performance size exclusion chromatography (HPSEC) was used to determine the average apparent molecular mass of lignins, being M_W the weighted average and M_N the number average, and the ratio between these values reveals the sample dispersity (Đ). The procedure used in this task is found in Section 3.4.6, while the experimental results are summarized in Table 9.

Table 9: Apparent molecular weight averages (M_W), apparent molecular number averages (M_N), and dispersity (Đ) of lignins extracted at different conditions.

Experiment	M_N	M_W	Đ
K150_30	684	2323	3.39
K170_30	574	2900	5.05
K190_30	399	2011	5.04
W130_30	1057	4892	4.62
W150_30	469	3597	7.67
W170_30	492	2656	5.39
W170_15	506	2038	4.02

Although the HPSEC analysis provided some valuable M_W and M_N information, the numerical results obtained in this study may not be completely accurate. First, the term "apparent molecular mass" was used because HPSEC calibration was performed with a series of monodisperse polystyrene standards, whose rheological properties are not aligned with those of technical lignins. Also, some discrepancies may have occurred due to variations in sample injection, which was done manually, such as shifting the curves to either higher or lower apparent molecular masses. In addition, calculations performed to obtain molecular mass distributions – as normalizing the curve –, may generate inconsistencies in the curve fitting, exacerbating some values. Nonetheless, the numerical results are still relevant to understand trends in both molecular mass distributions, which are depicted in Figure 31 and Figure 32. In general, the precipitated lignins presented typical M_W values for organosolv lignin, between 500-5000 $g.mol^{-1}$ (KAI et al., 2016).

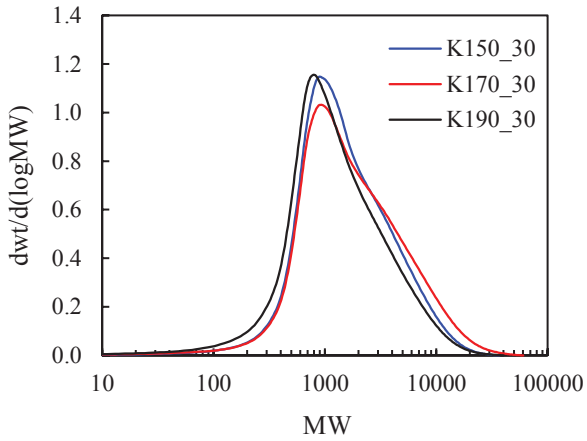

Figure 31: Apparent molecular mass distribution of precipitated lignins extracted using DES+EtOH+CO_2 at different temperatures, during 30 min: the blue line refers to the process at 150 °C, the red line refers to the process at 170 °C, and the black line refers to the process at 190 °C.

Without water addition – K150_30, K170_30, and K190_30 in Figure 31 –, little difference among samples could be noticed, which may happen due to the main role of DES in solubilizing lignin. In fact, depolymerization of lignin and lignin model compounds using acidic DES has been already reported elsewhere (WANG et al., 2020). Besides, there is evidence that milled wood lignins (the closest structure to lignin in its native form) may be composed of linear oligomers, rather than by a polymeric network of high molecular mass, contrary to what is indicated by the analysis of lignins from traditional pulping processes such as kraft, which are known to promote condensation of lignin substructures (CRESTINI et al., 2011). Considering the M_W and M_N values of Table 8, and taken as an illustrative example the molecular mass of the guaiacyl alcohol unit (178 $g.mol^{-1}$), sugarcane bagasse DES+ETOH+CO_2 lignins were extracted in an oligomeric way, with probably around 10 to 20 monolignol units.

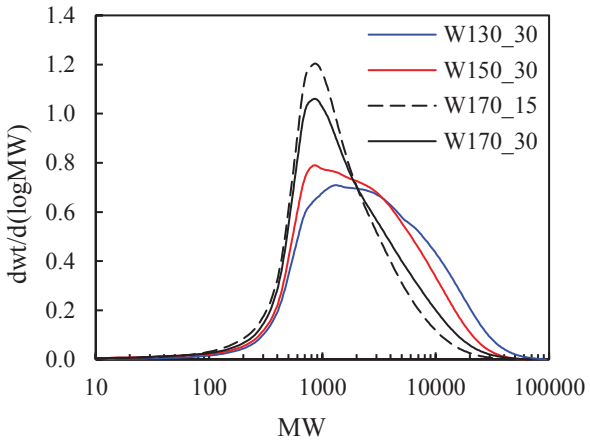

Figure 32: Apparent molecular mass distribution of precipitated lignins extracted using DES+EtOH+H_2O+CO_2. The continuous lines concern the processes during 30 min at different temperatures: the blue line refers to the process at 130 °C, the red line refers to the process at 150 °C, and the black line refers to the process at 170 °C. The black dashed line refers to the process at 170°C during 15 min.

The process in the presence of water (Figure 32), on the other hand, seemed to have followed a different pathway. In this case, there is the hydrolysis of aryl-ether bonds in lignin clusters, increasing extraction efficiency, but depending on the pretreatment temperature, the extracted lignin was more or less depolymerized, affecting sample dispersity. At the lower temperature, this distribution is notably flattened because lignin was extracted as more populous clusters having a large variety of average M_W. As the temperature increased, the number of clusters decreased forming a taller and more uniform distribution at a higher M_W range. In fact, at 170 °C, the M_W profile was quite similar to that originated from experiments carried out in the absence of water, which generated highly depolymerized lignin moieties. Interestingly, halving the reaction time did not affect the M_W distribution, as can be observed in the curves at 170 °C, τ of 15 and 30 min, demonstrating that changes in lignin M_W happen in a kind of equilibrium between depolymerization and condensation reactions.

The FT-IR spectra of lignin samples are given in Figure 33 and Figure 34 and the vertical dotted lines represent the bands assigned according to Table C1 in Appendix V. These spectra show the typical bands found in lignin samples, particularly in the region between 1800 to 800 $cm^{-1,}$ which can be understood as a "fingerprint" of the material. The stretching vibration related to unconjugated C=O axial deformations is noticed around 1707 cm^{-1}, and the conjugated C=O associated

with skeletal vibrations is present around 1603 cm^{-1}. The wavenumber of 1514 cm^{-1} is attributed to aromatic skeletal vibrations. The band typical for H units (present in herbaceous lignin), due to the C-O in *p*-hydroxyphenyl substructures, is located at 1166 cm^{-1}. At 1034 cm^{-1} it was found a band assigned to C–O deformation in aliphatic ethers, and secondary alcohols. Finally, at 834 cm^{-1}, the sign referent to C-H out-of-plane in positions 2 and 6 of S, and in all positions of H units was found.

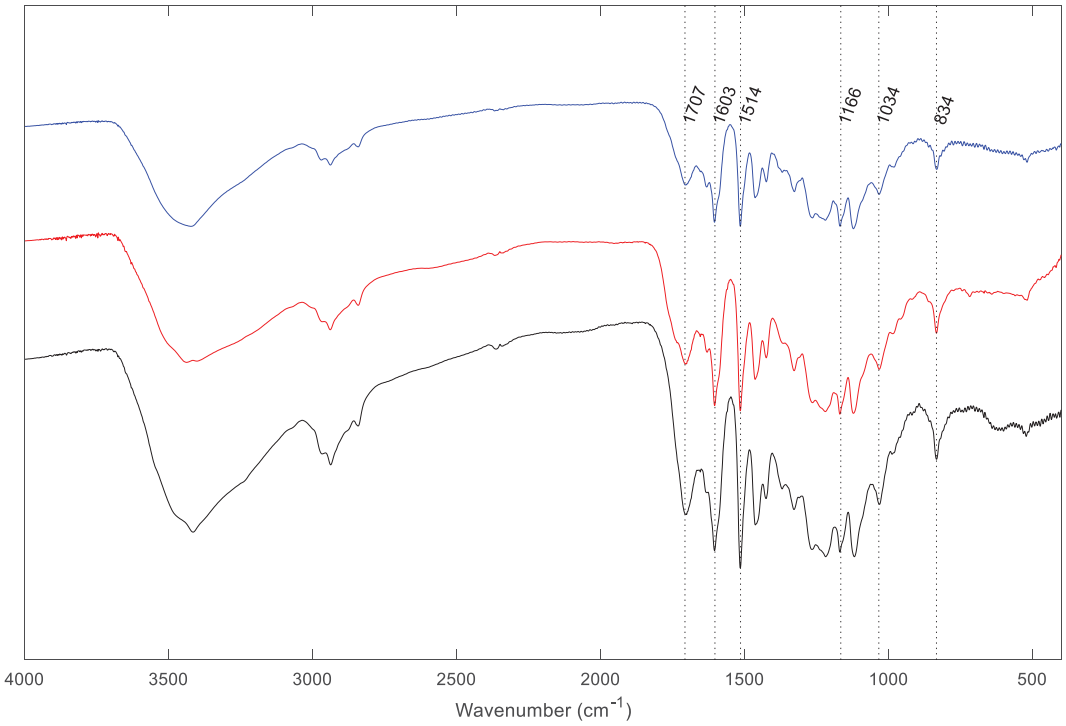

Figure 33: Fourier Transformed Infra-Red spectra of precipitated lignin samples at different temperatures: 150 °C (blue), 170 °C (red), and 190 °C (black). Fractionation using DES+EtOH+CO_2.

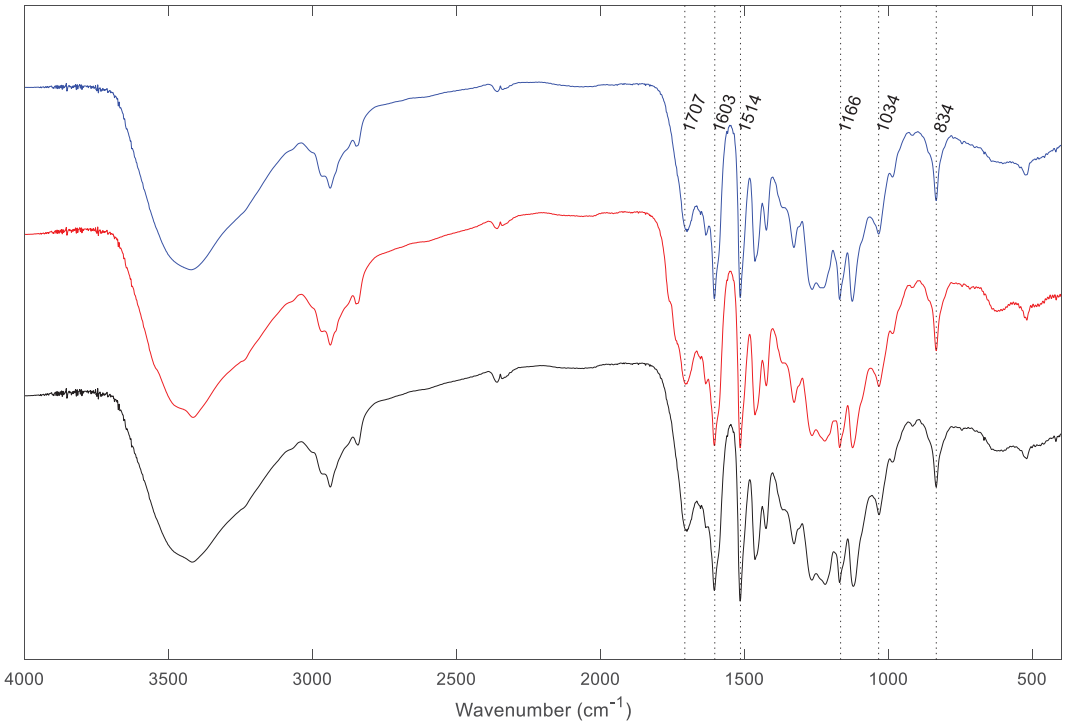

Figure 34: Fourier Transformed Infra-Red spectra of precipitated lignin samples at different temperatures: 130 °C (blue), 150 °C (red), and 170 °C (black). Fractionation using DES+EtOH+H_2O+CO_2.

FT-IR analysis revealed very little differences among different pretreatment types (e.g., in the presence or absence of water). Also, this analysis showed no evident effect caused by an increase in pretreatment temperature. In general, the typical bands for HGS lignin appeared to be well preserved regardless of pretreatment severity.

In short, the characterization of precipitated lignins revealed high purities and a well-depolymerized oligomeric structure. Also, probably due to the reactional pathway, the effect of temperature seems to be less impacting when utilizing anhydrous ethanol, while depolymerization was favored in the presence of hydrated ethanol at high temperatures.

4.6 MICRONIR COMPOSITION ANALYSIS

In the past few years, near-infrared spectroscopy has been used for a number of applications, including in the chemical characterization of biomass components

(PRIETO et al., 2017; SANDERSON et al., 1996; SANTOS et al., 2005). This technique is based on the absorption of radiation in wavelengths between 750 and 2500 nm, including bands resulted from overlapping, combinations, and subtones, which in turn can be correlated to the samples' identity. Since the absorption in the NIR region is less intense than at other spectral regions, it is necessary to apply longer optical path lengths, enabling in-site measurements. Therefore, this technique can be successfully employed in laboratories and industries as an alternative for the compositional analysis of biomasses.

The extractive free sugarcane bagasse and the pretreated samples were scanned in a MicroNIR apparatus, as described in Section 3.5. The data were subjected to Savitzky–Golay first-derivative, and after that to PLS calibration, being 80% of the samples used for calibration to match the average results from the three independent replicates of lignin and carbohydrate analysis, according to Section 3.4.4, and 20% for the prediction test. Additionally, the best adjustment was obtained when taking into account 8 latent variables for cross-validation. The following figures present the actual *versus* the predicted values for total lignin content, arabinoxylans, and glucans, on a dry basis (wt%). Besides, the dotted lines represent the maximum analytical uncertainties as described in Section 3.6.

This method is a non-destructive, easy, and fast way to determine biomass chemical composition, even at a high range of contents, for total lignin contents from 6 to 30 wt% (Figure 35). All the training and test points were located within the analytical standard deviation. Additionally, the regression coefficients for training and test points, regarding the total lignin content, were 0.9735 and 0.9352, respectively.

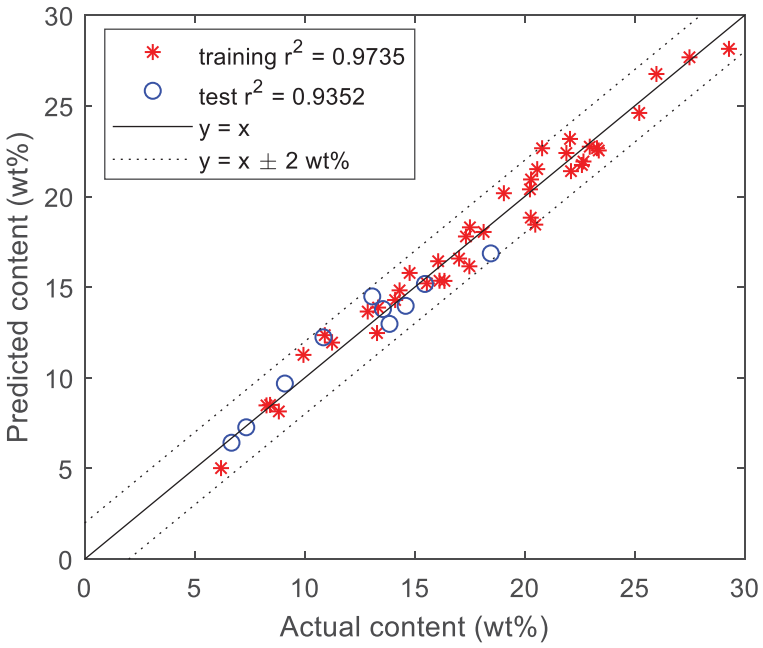

Figure 35: NIR compositional measures: actual *vs*. predicted total lignin content in untreated and pretreated samples.

Along with the lignin results, the capacity of predicting the arabinoxylans content is high, being all the training and test points within the analytical uncertainty (Figure 36). The regression coefficient obtained for both training and test points were satisfactorily high, 0.9940 and 0.9006, respectively.

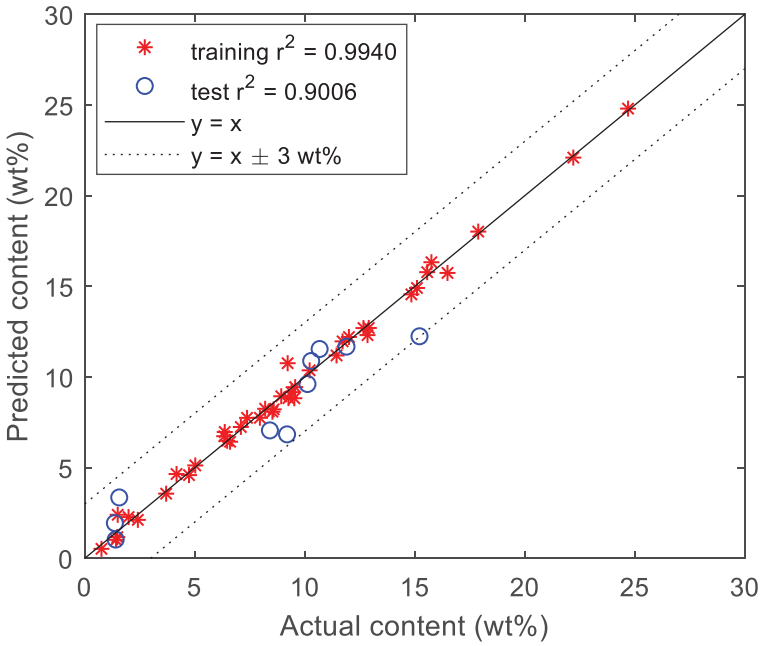

Figure 36: NIR compositional measures: actual *vs*. predicted arabinoxylans content in untreated and pretreated samples.

Finally, the glucans content varied from 43 to 84 wt%, and the adjust generated a regression coefficient of 0.9888 and 0.9387 for training and test points, respectively. Once more, all the predicted values were contained within the analytical standard deviation, as presented in Figure 37.

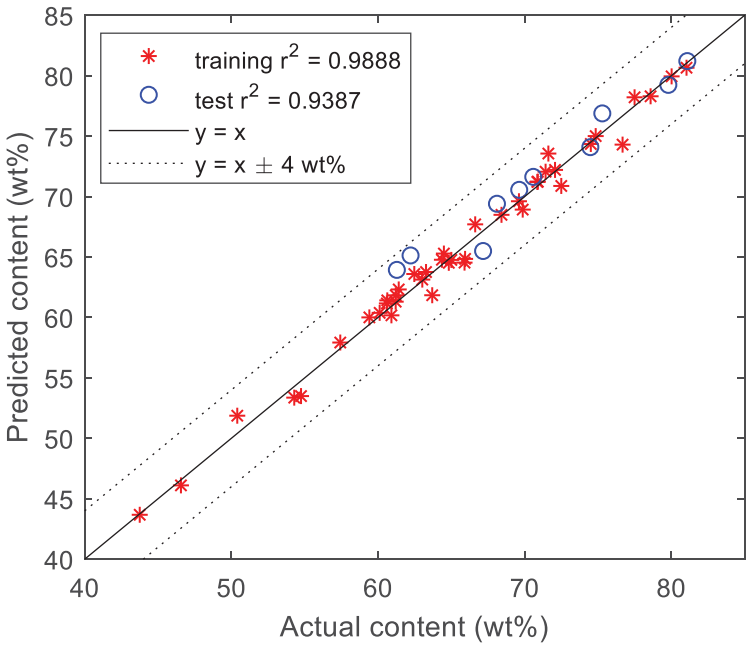

Figure 37: NIR compositional measures: actual *vs*. predicted glucan content in untreated and pretreated samples.

The interpretation of the results obtained by portable MicroNIR analysis leads to the conclusion that this method is capable of predicting biomass composition in a wide range of contents. Also, it is robust enough to deal with substrates subjected to different processes, even those that display very different characteristics in terms of both composition and physical appearance. It attests to the vast possibilities of industrial and practical, applications of portable MicroNIR in providing highly reliable and almost instantaneous compositional analysis of lignocellulosic materials.

5 CONCLUSIONS

This study evaluated the suitability of choline chloride and oxalic acid deep eutectic solvent on sugarcane bagasse fractionation, noting the high selectivity for lignin extraction. In this novel strategy, deep eutectic solvent, ethanol (anhydrous and hydrated), and carbon dioxide were employed to fractionate biomass in three fractions: a high purity precipitated lignin (with total lignin content from 90 to 99 wt%, and carbohydrates content inferior to 3 wt%), a glucan-rich pulp with little glucan losses, and a liquid stream with dissolved hemicellulose carbohydrates and co-products.

Considering the results with anhydrous ethanol, the process at 170 °C yielded over 75% of delignification, reaching the equilibrium around 60 min. Decreasing temperature to 150 °C showed to represent a delay in the process, and even after 120 min, the lignin removal was still increasing. Additionally, further increasing the temperature to 190 °C decreased lignin extraction yields and raised the levels of carbohydrate conversion to co-products. At the best results, the pretreated fibers presented glucan contents as high as 75 wt%, with virtually complete glucan retention. Lignin precipitation by simple solvent evaporation followed by water addition was efficient to generate high purity lignin. HPSEC analysis revealed that lignin had an average low apparent molecular mass and an approximately uniform distribution (at 170 °C, $M_{W,}$ and M_N of 2900 $g.mol^{-1}$ and 574 $g.mol^{-1}$, respectively) for all temperatures tested.

Substituting the anhydrous by hydrated ethanol further enhanced the extraction yields. AIL removal was positively impacted from 130 °C to 150 °C, but further enhancement provoked little effect over lignin yields. Following the kinetics of lignin removal and carbohydrate retention showed that 15 min at 170 °C is enough to remove almost 90% of the AIL, and produce a pulp with 80 wt% of glucans. Examining the lignin extracted at 170 °C, HPSEC analysis showed results similar to the anhydrous process when it comes to molecular mass distribution, but in this case, the process was more dependent on temperature, since lower temperatures produced a lower level of depolymerization.

The role of carbon dioxide over lignin and carbohydrate retention was examined. Although the addition of CO_2 was important for the processes, no direct relation between the amount of CO_2 added to the system and its performance was found. However, the presence of CO_2 enabled the fractionation at high total solids.

And at some extent, the action of CO_2 is related to the mass and heat transfer resistances, improving the system homogeneity.

The quantification of components using a portable MicroNIR device generated results within the limits of standard deviation obtained by independent replicates of the conventional quantification method. Therefore, this method proved to be a viable alternative for the quantification of the main components of sugarcane bagasse, replacing the time-consuming conventional methods, even in samples subjected to different thermochemical treatments.

6 FUTURE PERSPECTIVES

For future studies, it would be interesting to evaluate the enzymatic hydrolysis of the different pretreated materials, since one of the main possibilities for further conversion of cellulosic fibers would be in fermentative processes. However, this type of process is unique for a given combination of pretreatment and feedstock and requires thorough study and optimization of process variables, such as solid loadings, enzyme concentration, media detoxification due to the presence of reaction co-products, and hemicellulose sugars, among others.

In addition, future studies should evaluate the use of the lignin obtained by this work in real-life applications, as its addition in composites and concrete, its conversion to resins, phenols, or even its conversion to biofuels, as bio-oil and syngas.

The production of volatile co-products with high market value, such as furfural, was observed in the present work, however, their total quantification has not been carried out. Therefore, it is reasonable that future studies invest in this process aiming at the recovery of these volatiles, since this fractionation strategy was able to produce these co-products free of solvents such as water, which should positively impact downstream steps, favoring the economics of the process.

Also, to further comprehend the action of DES in the different fractionation schemes, and for processes optimization purposes, it would be necessary to study its interactions with other solvents: as with ethanol and CO_2, the phase partition inside the reactor, and the behavior of a eutectic mixture in the presence of water.

The reuse of catalysts is imperative to the feasibility of the process. Therefore, future research should include the development of suitable DES recovery processes,

approaching different techniques and materials to separate the hemicellulose sugars from the DES, without affecting the successful separation of lignin demonstrated in the present work.

Finally, this work illustrated the technical feasibility of a DES-assisted organosolv fractionation strategy. However, to understand the real application possibilities of such a process, an economic evaluation must be performed, assessing different combinations of matrix conditions, process variables, separation, purification, and further conversion processes.

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APPENDICES

APPENDIX I – Particle size distribution

1- Particle size distribution of milled sugarcane bagasse

Mesh Tyler	Opening (mm)	Mass fraction
16	1.00	0.012
20	0.85	0.066
24	0.71	0.142
28	0.60	0.179
35	0.42	0.189
48[a]	0.30	0.218
<48[a]	<0.30	0.193
Total		1

[a] particles that pass the 35 Mesh sieve were not used in the experiments.

APPENDIX II – Temperature and pressure profiles

1- First design of experiments – ChCl:OA DES+CO_2

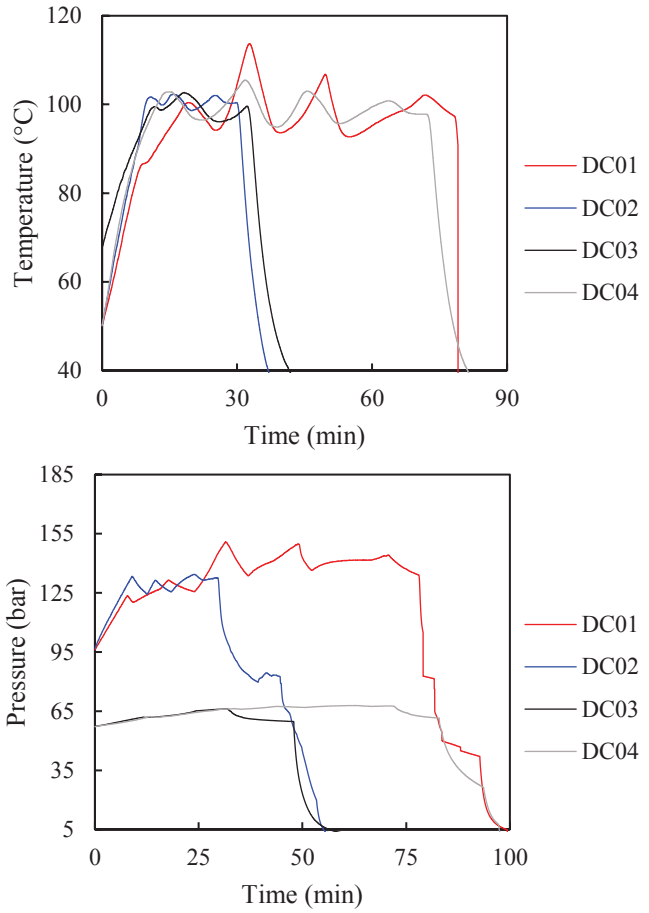

Figure A1: Temperatures and pressures profiles from the first design of experiments - ChCl:OA DES+CO_2. Experiments DC01 to DC04.

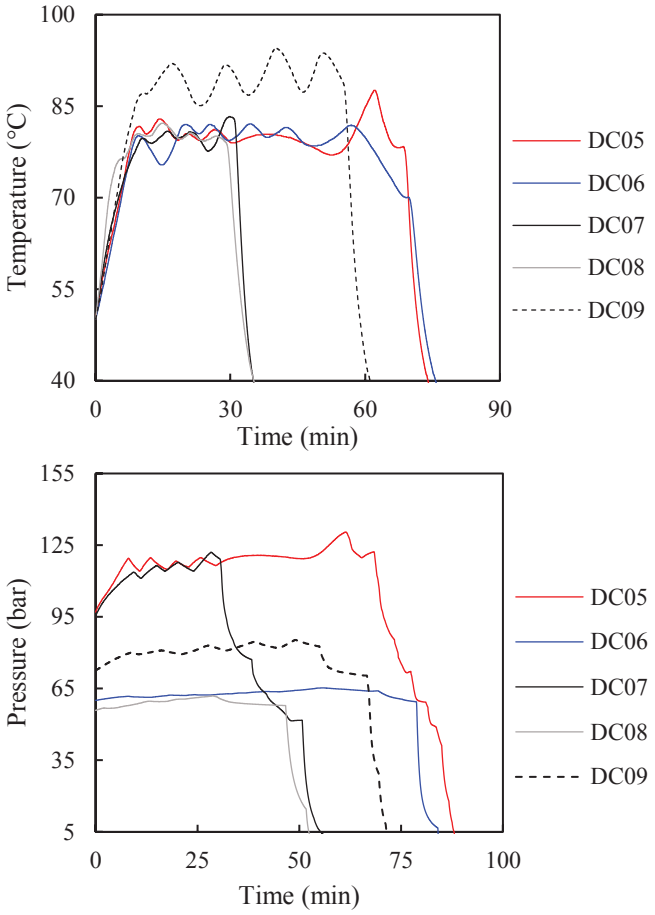

Figure A2: Temperatures and pressures profiles from the first design of experiments - ChCl:OA DES+CO_2. Experiments DC05 to DC09.

1- Experiments involving ChCl:OA DES+EtOH+CO_2

a. Stirring speed evaluation

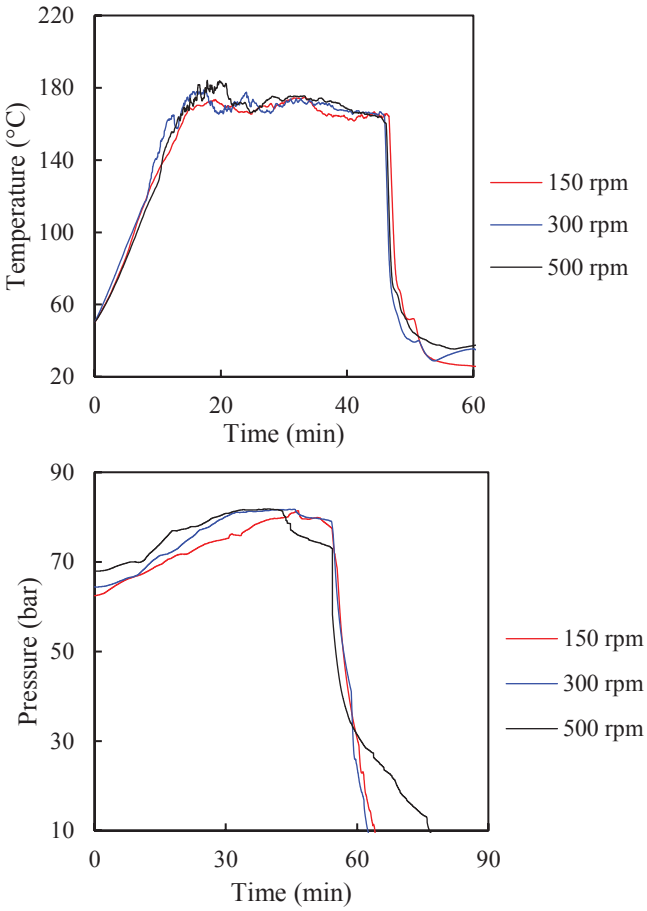

Figure A3: Temperatures and pressures profiles from the stirring speed evaluation experiments – systems involving ChCl:OA DES+EtOH+CO_2.

b. Second design of experiments – ChCl:OA DES+EtOH+CO_2

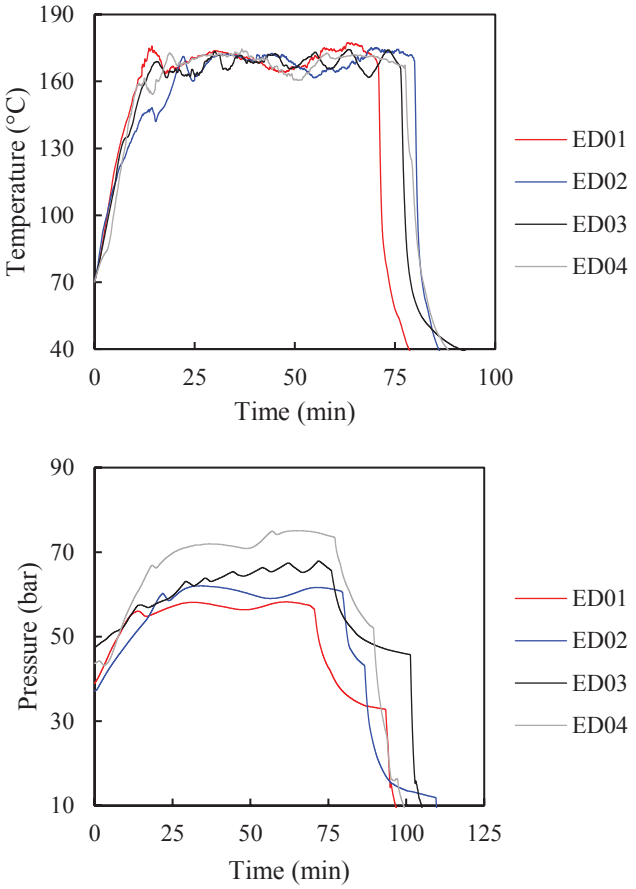

Figure A4: Temperatures and pressures profiles from the second design of experiments - ChCl:OA DES + EtOH+CO_2. Experiments ED01 to ED04.

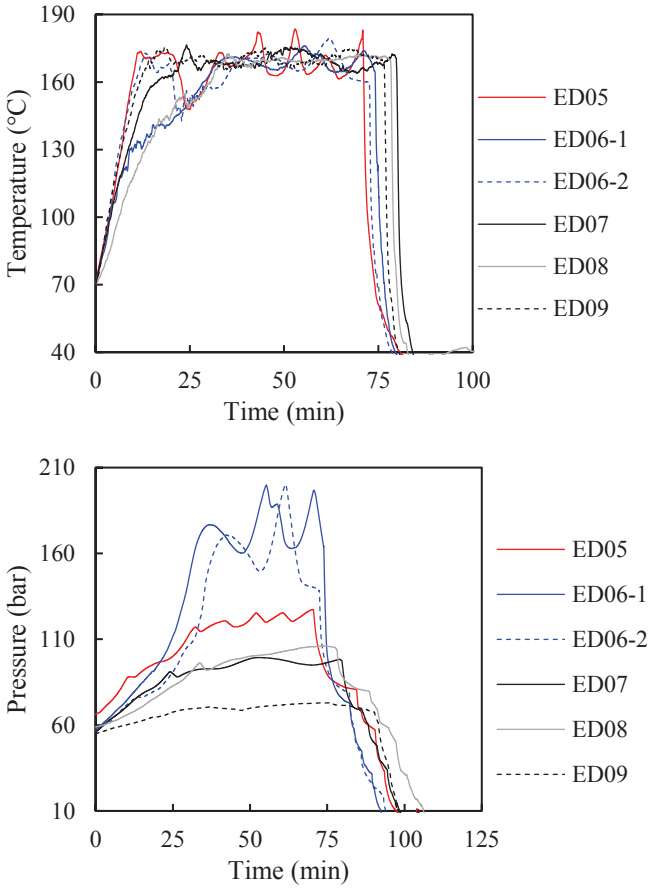

Figure A5: Temperatures and pressures profiles from the second design of experiments - ChCl:OA DES+EtOH+CO_2. Experiments ED05 to ED09.

c. Kinetic at 150°C

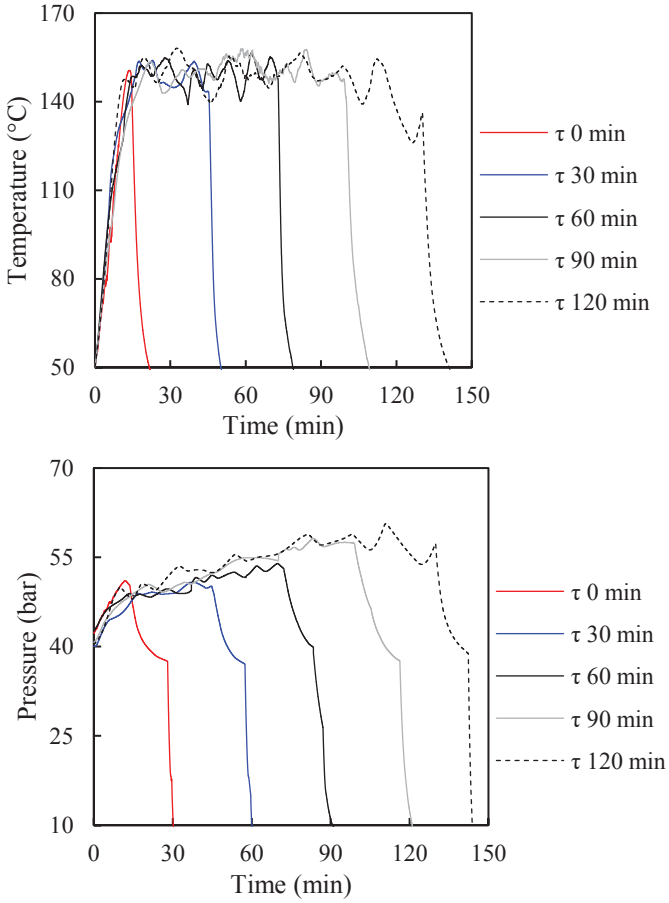

Figure A6: Temperatures and pressures profiles from kinetic points, at 150°C, of the experiments involving ChCl:OA DES+EtOH+CO_2. τ refers to the nominal reaction time, in minutes.

d. Kinetic at 170°C

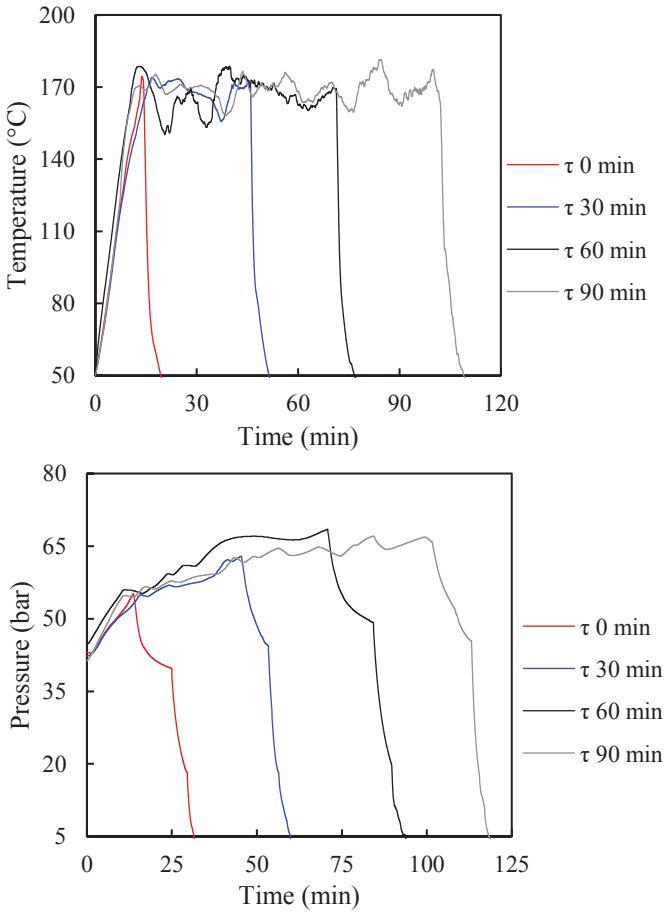

Figure A7: Temperatures and pressures profiles from kinetic points, at 170°C, of the experiments involving ChCl:OA DES+EtOH+CO_2. τ refers to the nominal reaction time, in minutes.

e. Kinetic at 190°C

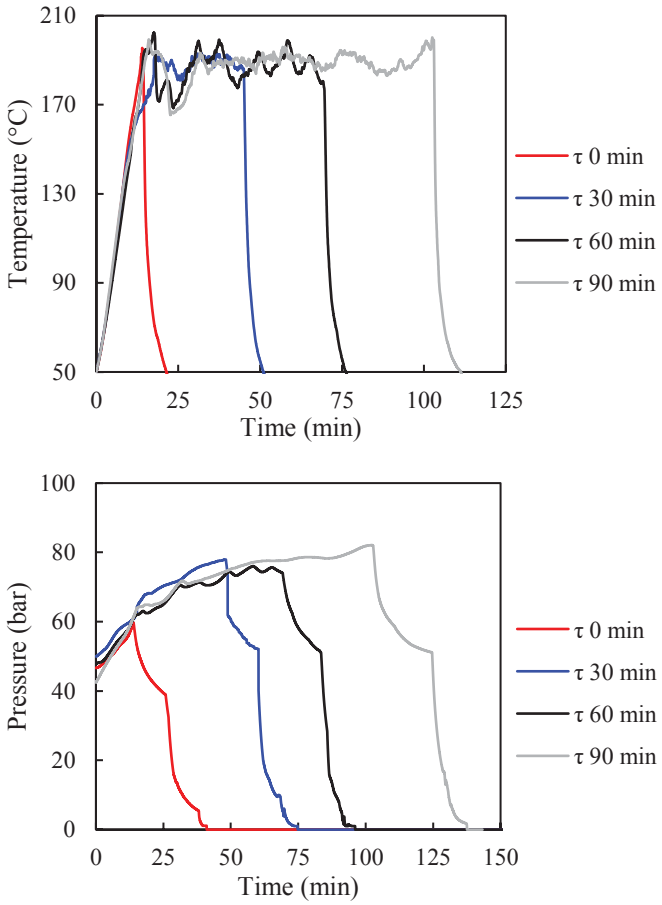

Figure A8: Temperatures and pressures profiles from kinetic points, at 190°C, of the experiments involving ChCl:OA DES+EtOH+CO_2. τ refers to the nominal reaction time, in minutes.

2- Experiments involving ChCl:OA DES+EtOH+H_2O +CO_2

a. Kinetic at 130°C

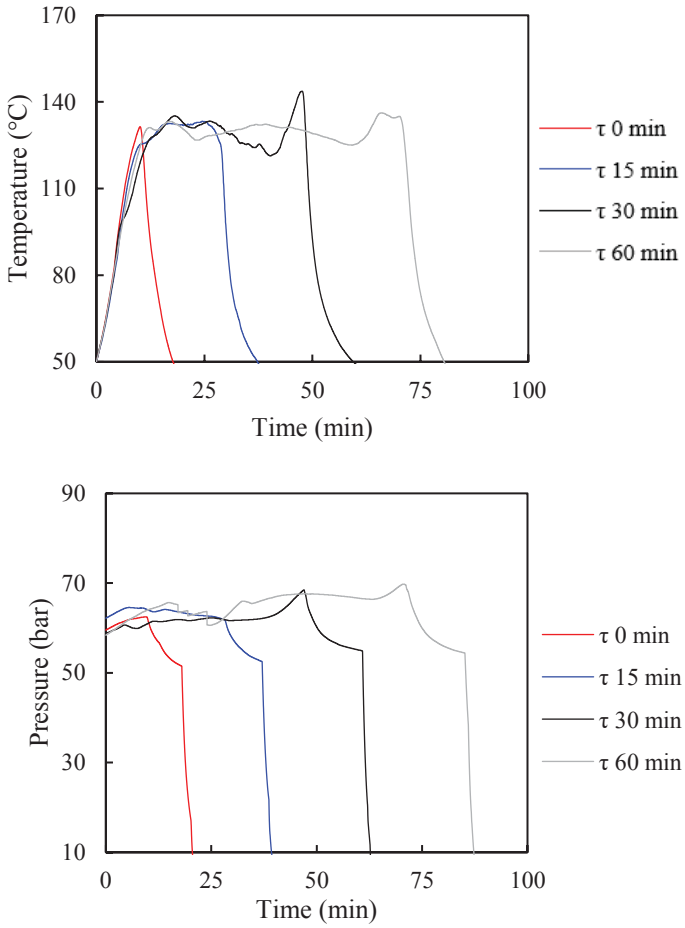

Figure A9: Temperatures and pressures profiles from kinetic points, at 130°C, of the experiments involving ChCl:OA DES+EtOH+H_2O+CO_2. τ refers to the nominal reaction time, in minutes.

b. Kinetic at 150°C

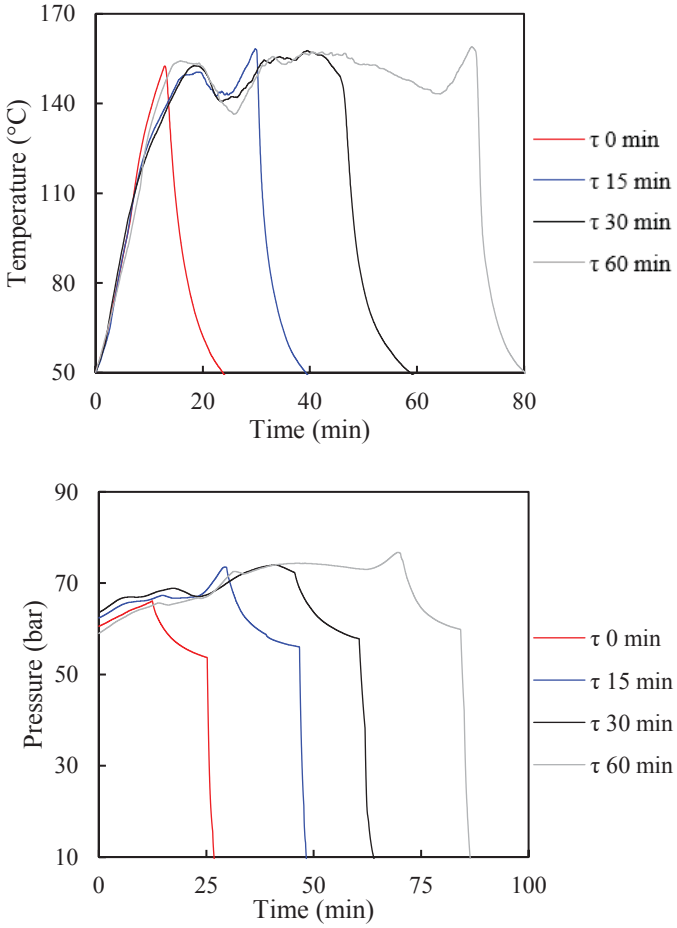

Figure A10: Temperatures and pressures profiles from kinetic points, at 150°C, of the experiments involving ChCl:OA DES+EtOH+H_2O+CO_2. τ refers to the nominal reaction time, in minutes.

c. Kinetic at 170°C

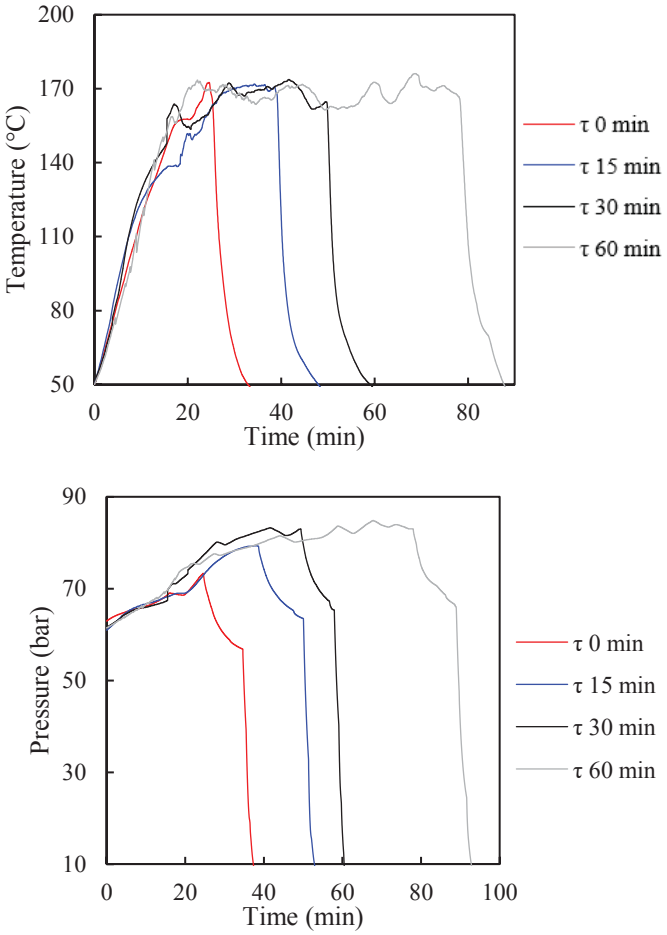

Figure A11: Temperatures and pressures profiles from kinetic points, at 170°C, of the experiments involving ChCl:OA DES+EtOH+H_2O+CO_2. τ refers to the nominal reaction time, in minutes.

APPENDIX III – Compositional analysis tables

1- First set of experiments – ChCl:OA DES+CO_2

Table A1: Analysis of composition from the lignocellulosic samples from the first set of experiments, results are based on three independent replicates (average value ± analytical standard deviation).

Label	Glucans (%)	Arabinoxylans (%)	Acetyl groups (%)	AIL (%)	ASL (%)	Total (%)
DC01	59.6 ± 3.1	2.0 ± 1.1	*bdl*	27.6 ± 0.6	1.9 ± 0.3	90.3
DC02	63.0 ± 1.1	3.7 ± 0.2	*bdl*	23.3 ± 0.3	2.7 ± 0.2	92.7
DC03	64.4 ± 2.0	4.2 ± 0.1	*bdl*	20.0 ± 0.8	2.9 ± 0.4	91.5
DC04	64.8 ± 1.1	2.4 ± 0.1	*bdl*	25.3 ± 0.5	2.2 ± 0.1	94.7
DC05	63.3 ± 0.7	6.5 ± 0.1	*bdl*	20.0 ± 0.1	3.4 ± 0.2	93.1
DC06	60.9 ± 1.2	7.1 ± 0.2	*bdl*	19.1 ± 0.8	3.5 ± 0.2	90.7
DC07	54.2 ± 0.5	12.2 ± 0.7	1.1 ± 0.6	18.2 ± 1.3	3.9 ± 0.3	89.7
DC08	60.1 ± 1.6	8.0 ± 0.3	*bdl*	18.7 ± 1.1	4.6 ± 0.2	91.4
DC09-1	65.0 ± 0.1	4.7 ± 0.3	*bdl*	18.5 ± 1.7	2.5 ± 0.2	90.5
DC09-2	61.4 ± 0.3	6.3 ± 0.3	*bdl*	17.6 ± 0.4	2.9 ± 0.3	88.3
DC09-3	65.9 ± 0.8	6.6 ± 0.5	*bdl*	19.6 ± 0.4	3.1 ± 0.2	95.3

bdl: below detection limit.

2- Second set of experiments – ChCl:OA DES+EtOH+CO_2

Table A2: Analysis of composition from the lignocellulosic samples from the second set of experiments, results are based on three independent replicates (average value ± analytical standard deviation).

Label	Glucans (%)	Arabinoxylans (%)	Acetyl groups (%)	AIL (%)	ASL (%)	Total (%)
SS150	66.6 ± 2.4	11.7 ± 0.2	*bdl*	12.7 ± 0.1	3.4 ± 0.4	94.5
SS300	69.8 ± 2.6	9.2 ± 0.4	*bdl*	11.0 ± 1.2	3.4 ± 0.4	93.4
SS500	72.1 ± 3.8	8.6 ± 1.2	*bdl*	9.5 ± 0.5	3.3 ± 0.3	93.5
ED01	61.2 ± 0.7	14.0 ± 0.1	1.6 ± 0.2	16.3 ± 0.4	4.1 ± 0.1	97.2
ED02	62.5 ± 1.5	13.4 ± 0.4	1.7 ± 0.1	16.0 ± 1.2	4.3 ± 0.3	97.8
ED03-1	76.7 ± 0.7	9.4 ± 0.1	*bdl*	10.0 ± 0.9	3.3 ± 0.1	99.4
ED03-2	74.5 ± 0.5	8.2 ± 0.1	*bdl*	8.0 ± 0.7	2.9 ± 0.1	93.6
ED04	70.9 ± 0.7	9.5 ± 0.3	*bdl*	12.8 ± 0.9	3.3 ± 0.1	96.4
ED05	68.4 ± 3.5	10.2 ± 0.5	*bdl*	13.8 ± 0.8	3.5 ± 0.1	96.0
ED06	60.6 ± 0.9	9.6 ± 0.2	*bdl*	18.5 ± 1.9	3.6 ± 0.4	92.3
ED07	63.7 ± 1.7	12.0 ± 0.4	1.0 ± 0.9	13.4 ± 2.3	4.1 ± 0.2	94.2
ED08	59.4 ± 0.6	13.1 ± 0.1	1.7 ± 0.1	15.1 ± 0.4	4.0 ± 0.4	93.3
ED09-1	71.4 ± 3.8	10.0 ± 1.2	0.4 ± 0.8	11.7 ± 0.4	3.2 ± 0.7	96.7
ED09-2	72.7 ± 1.8	9.5 ± 0.5	*bdl*	11.5 ± 0.2	3.4 ± 0.2	97.0
ED09-3	71.2 ± 3.9	10.0 ± 0.3	*bdl*	11.2 ± 0.3	3.2 ± 0.3	95.5

bdl: below detection limit.

3- Kinetics – ChCl:OA DES+EtOH+CO_2

Table A3: Analysis of composition from the lignocellulosic samples from the kinetic studies using anhydrous ethanol, results are based on three independent replicates (average value ± analytical standard deviation).

Label	Glucans (%)	Arabinoxylans (%)	Acetyl groups (%)	AIL (%)	ASL (%)	Total (%)
K170_00	57.4 ± 0.8	16.5 ± 0.1	2.1 ± 0.1	13.6 ± 2.2	4.5 ± 0.2	94.1
K170_30	67.2 ± 1.3	10.2 ± 0.1	0.5 ± 0.9	9.3 ± 1.2	3.8 ± 0.1	90.9
K170_90-1	73.0 ± 0.1	9.3 ± 1.1	0.5 ± 0.8	9.5 ± 0.5	3.5 ± 0.1	95.7
K170_90-2	74.5 ± 0.6	9.2 ± 0.3	*bdl*	8.2 ± 0.5	2.6 ± 0.1	94.5
K150_00	54.8 ± 1.2	15.8 ± 0.3	2.1 ± 0.1	15.8 ± 1.1	4.5 ± 0.3	92.9
K150_30	61.3 ± 1.1	13.5 ± 0.3	1.7 ± 0.2	14.5 ± 0.1	4.0 ± 0.1	95.0
K150_60	64.5 ± 2.9	11.2 ± 0.8	1.5 ± 0.5	12.7 ± 0.8	3.6 ± 0.3	93.5
K150_90	68.1 ± 2.3	10.1 ± 0.4	*bdl*	10.3 ± 1.5	3.3 ± 0.2	91.8
K150_120	71.6 ± 0.3	9.3 ± 0.2	*bdl*	8.6 ± 1.0	2.7 ± 0.2	92.1
K190_00	65.9 ± 1.7	10.5 ± 0.1	1.6 ± 0.1	13.6 ± 0.3	3.9 ± 0.1	95.4
K190_30	72.2 ± 2.2	8.5 ± 0.2	*bdl*	12.2 ± 0.9	3.4 ± 0.2	96.2
K190_60	71.4 ± 0.9	7.4 ± 0.3	*bdl*	10.1 ± 0.5	3.2 ± 0.2	92.1
K190_90-1	72.5 ± 1.5	6.4 ± 0.2	*bdl*	12.2 ± 1.2	2.6 ± 0.5	93.6
K190_90-2	72.5 ± 0.4	6.5 ± 0.1	*bdl*	10.2 ± 0.1	2.9 ± 0.2	92.1

bdl: below detection limit.

4- Kinetics – ChCl:OA DES+EtOH+H_2O+CO_2

Table A4: Analysis of composition from the lignocellulosic samples from the kinetic studies using hydrated ethanol, results are based on three independent replicates (average value ± analytical standard deviation).

Label	Glucans (%)	Arabinoxylans (%)	Acetyl groups (%)	AIL (%)	ASL (%)	Total (%)
W170_00	74.8 ± 0.7	2.4 ± 0.4	*bdl*	7.3 ± 0.2	2.6 ± 0.4	87.1
W170_15	79.8 ± 2.1	1.4 ± 0.3	*bdl*	5.1 ± 0.8	2.2 ± 0.6	88.5
W170_30	81.1 ± 0.7	1.4 ± 0.8	*bdl*	4.2 ± 0.4	2.5 ± 0.2	89.2
W170_60	81.0 ± 0.5	*bdl*	*bdl*	4.3 ± 0.3	1.9 ± 0.2	87.2
W150_00	62.2 ± 0.4	11.9 ± 0.2	*bdl*	11.9 ± 0.7	3.6 ± 0.6	89.6
W150_15	75.3 ± 0.2	1.6 ± 0.3	*bdl*	6.3 ± 0.8	2.8 ± 0.4	86.0
W150_30	78.6 ± 0.8	1.5 ± 0.1	*bdl*	5.8 ± 1.5	2.5 ± 0.7	88.3
W150_60	77.5 ± 0.7	1.5 ± 0.2	*bdl*	5.7 ± 1.0	2.7 ± 0.3	87.4
W130_00	50.4 ± 0.4	15.8 ± 0.2	*bdl*	17.6 ± 0.1	4.3 ± 0.5	88.0
W130_15	60.6 ± 0.7	12.9 ± 0.2	*bdl*	13.7 ± 0.1	3.3 ± 0.2	90.5
W130_30	69.6 ± 1.5	8.9 ± 0.3	*bdl*	11.4 ± 0.3	2.7 ± 0.4	92.6
W130_60	70.6 ± 0.3	8.4 ± 0.2	*bdl*	11.4 ± 0.7	2.5 ± 0.3	92.8

bdl: below detection limit.

5- Additional runs

Table A5: Analysis of composition of lignocellulosic samples (additional points), results are based on three independent replicates (average value ± analytical standard deviation).

Label	Glucans (%)	Arabinoxylans (%)	Acetyl groups (%)	AIL (%)	ASL (%)	Total (%)
EX_01	44.5 ± 0.5	22.6 ± 0.6	3.0 ± 0.1	18.6 ± 0.7	5.1 ± 0.1	93.8
EX_02	50.1 ± 2.9	18.9 ± 0.7	2.5 ± 0.3	19 ± 1	4.9 ± 0.3	93.4
EX_03	69.6 ± 2.3	10.9 ± 1.9	0.7 ± 0.3	10.2 ± 2.3	3.3 ± 0.6	94.7
EX_04	80.0 ± 0.2	0.8 ± 0.2	*bdl*	6.7 ± 0.3	2.2 ± 0.2	89.6
EX_05	78.9 ± 1.5	0.8 ± 0.1	*bdl*	7.6 ± 1.2	2.0 ± 0.2	89.2
EX_06	51.7 ± 0.9	17.8 ± 0.3	1.8 ± 0.1	14.6 ± 2.4	5.0 ± 0.6	90.8
EX_07	46.6 ± 1.7	19.5 ± 1.6	2.7 ± 0.1	15.4 ± 2.5	5.2 ± 0.5	89.3

bdl: below detection limit.

APPENDIX IV – ANOVA tables

Table B1: ANOVA table related to delignification extents obtained at the first design of experiments.
Fractionation of SCB_{ee} using DES+CO_2 at different temperatures, fractionation times, and amounts of CO_2. The statistical model took into account two-way interactions and curvature check, retrieving an R^2 of 0.98.

	SS	df	MS	F	p
Curvature	253.6044	1	253.6044	64.54893	0.004027
(1) Temperature (°C)	152.1640	1	152.1640	38.72971	0.008365
(2) time (min)	70.6266	1	70.6266	17.97631	0.024023
(3) m_{CO_2} (g)	24.6753	1	24.6753	6.28051	0.087237
1 by 2	107.2380	1	107.2380	27.29487	0.013641
1 by 3	41.0871	1	41.0871	10.45774	0.048075
2 by 3	41.1778	1	41.1778	10.48083	0.047945
Error	11.7866	3	3.9289		
Total SS	702.3599	10			

Table B2: ANOVA table related to delignification extents obtained at the second design of experiments.
Fractionation of SCB_{ee} using DES+CO_2+EtOH at solid loadings, DES:SCB_{ee} ratio, and amounts of CO_2. The statistical model took into account two-way interactions and curvature check, retrieving an R^2 of 0.99.

	SS	df	MS	F	p
Curvature	221.061	1	221.0608	66.2007	0.001241
(1) Solid loading (%)	74.021	1	74.0208	22.1669	0.009252
(2) m CO_2 (g)	21.769	1	21.7695	6.5193	0.063085
(3) $R_{DES:SCB_{ee}}$	289.205	1	289.2052	86.6077	0.000742
1 by 2	2.725	1	2.7249	0.8160	0.417427
1 by 3	140.674	1	140.6739	42.1273	0.002906
2 by 3	446.341	1	446.3405	133.6648	0.000320
Error	13.357	4	3.3393		
Total SS	1447.951	11			

APPENDIX V – Mid-infrared region band assignment

Table C1: Band assignment in the mid-infrared region for typical lignin structures and comparison with non-derivatized HGS type mill-wood-lignin (bamboo MWL).
Reference: adapted from Lin and Dence (1992).

Wavenumber (cm^{-1})			**Band Origin**
Range of Maxima	**This work**	**Bamboo**	
3412-3460	3415	3428	O-H stretch
3000-2842	3040	3002	C-H stretch in methyl and methylene groups
1738-1709	1707	1709	C=O stretch in unconjugated ketones, carbonyls and in ester groups (frequently carbohydrate origin); conjugated aldehydes and carboxylic acids absorb around below 1700 cm^{-1}
1655-1675	1658	-	C=O stretch in conjugated p-subst. aryl ketones; strong electronegative substituents lower the wavenumber
1593-1605	1603	1601	Aromatic skeletal vibration plus C=O stretch: S>G; $G_{condensed}$>$G_{etherified}$
1505-1515	1514	1511	Aromatic skeletal vibrations; G>S
1460-1470	1460	1462	C-H deformations; asym. In $-CH_3$ and $-CH_2-$
1422-1430	1425	1423	Aromatic skeletal vibrations combined with C-H in plane deformation
1365-1370	1368	-	Aliphatic C-H stretch in CH_3, not in OMe; phen. OH
1325-1330	1330	1329	S ring plus G ring condensed (i.e., G ring substituted in position 5)
1266-1270	1270	1267	G ring plus C=O stretch
1221-1230	1225	1229	C-C plus C-O plus C=O stretch; $G_{condensed}$> $G_{etherified}$
1166	1166	1166	Typical for HGS lignins; C=O in ester groups (conjugated)
1128-1125	1123	1127	Aromatic C-H in-plane deformation (typical for S units); plus secondary alcohols plus C=O stretch
1030-1035	1034	1032	Aromatic C-H in-plane deformation, G>S; plus C-O deformation in primary alcohols; plus C=O stretch (unconjugated)
966-990	982	-	-HC=CH- out-of-plane deformation (trans.)
834-835	834	834	C-H out-of-plane in position 2 and 6 of S, and in all positions of H units