Universidade de Aveiro
2013

Departamento de Química

Sara Patrícia Magalhães da Silva

Uso de Solventes Alternativos no âmbito das Biorefinarias

Alternative Solvents in the Biomass Refinery Concept

Universidade de Aveiro
2013.

Departamento de Química

Sara Patrícia Magalhães da Silva

Uso de Solventes Alternativos no âmbito das Biorefinarias

Alternative Solvents in the Biomass Refinery Concept

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Engenharia Química, realizada sob a orientação científica do Dr. Rafał Marcin Bogel-Łukasik, Investigador Auxiliar da Unidade de Bioenergia do Laboratório Nacional de Energia e Geologia de Lisboa e, do Professor Doutor Armando Jorge Domingues Silvestre, Professor Associado com Agregação do Departamento de Química da Universidade de Aveiro.

Dedico este trabalho aos meus pais, irmão e à minha tia.

o júri

presidente

Professor Doutor Dmitry Victorovitch Evtyugin
Professor Associado com Agregação do Departamento de Química da Universidade de Aveiro

Doutor. Rafał Marcin Bogel-Łukasik
Investigador Auxiliar da Unidade de Bioenergia do Laboratório Nacional de Energia e Geologia

Professor Doutor Armando Jorge Domingues Silvestre
Professor Associado com Agregação do Departamento de Química da Universidade de Aveiro

Professor Doutor Carlos Manuel Santos Silva
Professor Auxiliar do Departamento de Química da Universidade de Aveiro

agradecimentos

Em primeiro lugar gostaria de agradecer ao Doutor Rafał Łukasik pela possibilidade de ter realizado a minha tese de mestrado sob a sua orientação. Os seus conselhos, perseverança e carácter único contribuíram não só para a realização de um melhor trabalho, mas sobretudo para a minha formação como pessoa. Gostaria também de agradecer ao Professor Armando Silvestre pela sua disponibilidade, ajuda e acompanhamento do meu trabalho.

Não posso também esquecer o excelente acompanhamento por parte do André Lopes. A sua dedicação, amizade e personalidade tornaram este percurso muito mais enriquecedor. A ele, o meu muito obrigado!

Um muito obrigado, também, à Dra. Luísa Roseiro pela disponibilidade, ajuda e conhecimentos transmitidos relacionados com a eletroforese capilar.

Gostaria também de agradecer à Rita Morais e à Sofia Graça pela amizade, simpatia e pelo apoio que me proporcionaram ao longo desta experiência no LNEG.

A passagem pela universidade fez-me, não só adquirir conhecimentos, mas também pessoas que sempre estarão presentes na minha vida. Sem nunca esquecer ninguém, gostaria de agradecer ao Nuno Costa, Cristiano Ramos e Luís Pereira por todos os momentos que partilhamos. Não podia deixar de agradecer à Sara Ribeiro e à Andreia Miguéis por todo o apoio e amizade partilhada durante estes anos. Além disso, gostaria de agradecer à Jéssica Machado e Tânia Borges pela excelente amizade e companheirismo.

À Rute Tavares e Andreia Lopes, companheiras de uma vida, pelo seu apoio, amizade e conselhos que tornaram a pessoa que sou hoje.

À minha prima Raquel Melfe por todo o apoio e companhia durante a minha estadia por Lisboa.

Por fim, e por isso não menos importante, gostaria de agradecer aos meus pais, irmão e às minhas tias pelo infinito e incondicional apoio que me deram durante este curso. Isto é para vocês!

palavras-chave Biorrefinaria, biomassa, líquidos iónicos, fluídos supercríticos, pré-tratamento, fracionamento, celulose, hemicelulose, lenhina, produtos de valor acrescentado.

resumo

O presente trabalho foi dedicado ao pré-tratamento de biomassa lenho-celulósica através de dois solventes alternativos – líquidos iónicos (LIs) e fluídos supercríticos (FSCs).

O estudo com líquidos iónicos focou-se em propor uma nova metodologia para o pré-tratamento da palha de trigo com o líquido iónico acetato de 1-etil-3-metilimidazólio ([emim][OAc]), que permitiu o fracionamento da mesma em frações de celulose, hemicelulose e lenhina num simples processo de três etapas. Com este processo de pré-tratamento foram estudadas diferentes temperaturas (80-140°C) e tempos (2-18h) de dissolução da biomassa no [emim][OAc]. A técnica de Espectroscopia de Infravermelho com Transformadas de Fourier (FTIR) permitiu efetuar análises qualitativas e quantitativas de todas as frações obtidas nos pré-tratamentos realizados. Para as frações ricas em celulose foram efetuados ensaios de hidrólise enzimática para avaliar o conteúdo em glucose. Além disso, a dependência da recuperação destas mesmas frações nas diferentes condições experimentais do pré-tratamento foi avaliada através de análises de regressão linear múltipla. O pré-tratamento a 140°C durante 6h deu o melhor resultado, no que toca à recuperação das frações ricas em celulose, onde se obteve uma recuperação de 37.1% (m/m) relativamente à massa inicial de biomassa utilizada. Para as mesmas condições, também foram obtidos resultados com elevada pureza, tanto para a fração de hemicelulose (96% em hidratos de carbono), bem como para a fração em lenhina (97%). Do mesmo modo, foi verificado um elevado teor em glucose (81.1% $m/m_{biomassa}$) presente na fração celulósica. No final de cada pré-tratamento realizado, o LI foi recuperado atingindo sempre valores de recuperação superiores a 86% (m/m). Para avaliar a pureza dos LIs após os pré-tratamentos utilizou-se a técnica de espectroscopia de ressonância magnética nuclear (RMN). Para além disso, os LIs recuperados foram analisados através da técnica eletroforese capilar (EC) para investigar a presença de compostos fenólicos de valor acrescentado. Desta análise, foi possível verificar a presença de compostos de vanilina e seus derivados, bem como outros produtos derivados da lenhina.

O outro estudo centrou-se num processo de auto-hidrólise assistido com CO_2 da palha de trigo, com o objetivo de dissolver seletivamente a fração hemicelulósica. A formação *in situ* de ácido carbónico resultou numa maior dissolução da hemicelulose comparativamente a um processo de auto-hidrólise da palha de trigo em condições análogas (temperatura e razão líquido-sólido (RLS)). Um aumento da quantidade de CO_2 obtido através da diminuição da quantidade de biomassa levou a um aumento de mais de 60% de xilo-oligossacáridos (XOS) dissolvidos. Nomeadamente, a 210°C foi verificada uma recuperação de 15.75$g \cdot L^{-1}$ em XOS comparativamente a uma recuperação de 9.54 $g \cdot L^{-1}$ obtida num processo de auto-hidrólise sem adição de CO_2. Nestas condições, foi também verificado um enriquecimento de 20% (m/m) em glucose no sólido recuperado.

keywords Biorefineries, lignocellulosic biomass, ionic liquid, supercritical fluids pre-treatment, fractionation, cellulose, hemicellulose, lignin, value-added products.

abstract The present work is devoted to the pre-treatment of lignocellulosic biomass by two alternative solvents – ionic liquids (ILs) and supercritical fluids (SCF).

The IL study was focus on proposing a new methodology for the wheat straw pre-treatment with the ionic liquid (IL), 1-ethyl-3-methylimidazolium acetate ([emim][OAc]), that allowed to obtain cellulose, hemicellulose and lignin-rich fractions in a rapid and simple three-step fractionation process. Various temperatures (80-140°C) and processing times (2-18h) of the pre-treatment were studied. The quantitative and qualitative analysis of each lignocellulosic biomass fractions were determined by FTIR measurements. The glucan content in recovered cellulose-rich fractions was investigated by an enzymatic hydrolysis. Cellulose recovery dependency on pre-treatment conditions was ascertained through the regression analysis. The optimal result for the recovery of the cellulose-rich fraction was obtained at 140°C during 6h achieving 37.1 % (w/w) of the initial biomass loading. For the same conditions, optimal results were also produced regarding the amount of the glucan present (81.1 % $w/w_{biomass}$) in cellulose-rich fractions, the carbohydrate enrichment in the hemicellulose fraction (96% wt) and the purity of lignin (97% wt).The recovery of IL was performed after each pre-treatment and the obtained yields were up to 86% (w/w). The recovered ILs were analyzed by ^{13}C- and ^{1}HNMR in order to verify their purities. The presence of value-added phenolic compounds in the recovered ILs was analyzed by capillary electrophoresis. Vanillin and its derivatives, as well as other lignin-based products were identified.

The other study was centered in a CO_2-assisted autohydrolysis treatment of wheat straw, in order to selectively dissolve the hemicellulose fraction. The *in situ* formation of carbonic acid resulted in a higher hemicellulose dissolution in comparison to autohydrolysis of wheat straw with analogous conditions (temperature and LSR). In addition, higher amount of CO_2 obtained by the relative reduction of biomass amount treatment guided to an increase by more than 60% of xylo-oligosaccharides (XOS) recovered. Namely, at 210°C a XOS recovery of 15.75 $g \cdot L^{-1}$ was obtained versus a 9.54 $g \cdot L^{-1}$ recovery with an autohydrolysis pre-treatment without CO_2 addition. Furthermore, an enrichment of 20% (w/w) of the glucan content in the recovered solid fraction was also verified at the same conditions with the CO_2-assisted autohydrolysis pre-treatment.

List of contents

List of Figures

List of Tables

Nomenclature

AcO	Acetyl groups linked to oligosaccharides
AFEX	Ammonia Fiber Explosion
[amim][Cl]	1-allyl-3-methylimidazolium chloride
[bmim][OAc]	1-butyl-3-methylimidazolium acetate
°C	Degree Celsius
$(C_6H_{10}O_5)_n$	Cellulose
CE	Capillary Electrophoresis
CO_2	Carbon dioxide
Cp_m	Molar heat capacity
d	doublet
DP	Degree of Polymerization
e.g.	example
[emim][OAc]	1-ethyl-3-methylimidazolium acetate
EtOH	Ethanol
EU	European Union
FPU	Filter Paper Units
FTIR	Fourier Transform Infra-Red
GLcOS	Gluco-Oligosaccharides
HAPs	Hazardous Air Pollutants
HLVL	High-Value Low-Volume
HMF	Hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
i.d.	Internal diameter
IL(s)	Ionic Liquid(s)
LHW	Liquid Hot Water
Log R_0	Severity factor
LSR	Liquid-to-Solid ratio
LVHV	Low-Value High-Volume
M	Molarity
m_{IL}	Ionic liquid mass

N	Nitrogen
NMR	Nuclear Magnetic Resonance
NREL	National Renewable Energy Laboratory
P	Pressure
P_c	Critical Pressure
PID	Proportional-Integral-Derivative
PR-EOS	Peng-Robinson Equation Of State
q	quartet
R	Gas constant
R^2	Correlation coefficient
RI	Refractive Index
$scCO_2$	Supercritical Carbon Dioxide
SCF(s)	Supercritical Fluid(s)
scH_2O	Supercritical Water
s	singlet
SLR	Solid-to-Liquid ratio
SPE	Solid Phase Extraction
t	time
T	Temperature
T_c	Critical temperature
T_{room}	Room temperature
u	Standard Deviation Error
UV/VIS	Ultraviolet/Visible
VLE	Vapor Liquid Equilibrium
VOCs	Volatile Organic Compounds
XOS	Xylooligosaccharides
β	Regression coefficient
δ	Chemical shift
ρ	Density
Ø	Diameter
ω	Acentric Factor

“Be less curious about people and more curious about ideas”

Marie Skłodowska Curie

1. Introduction

1.1 Green Chemistry

According to Anastas et al.[1] Green Chemistry is "*the design, development, and implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and the environment*". It is an innovative, non-regulatory, economically driven approach toward sustainability. Green Chemistry aims to design and utilize matter and energy in a way that increases performance and value while protecting human health and the environment.[2] This definition is also elucidated by the Twelve Principles of Green Chemistry that are used as a design framework (Figure 1). The principles of Green Chemistry need to become essential for future chemistry, in order to integrate sustainability into science and its innovations. It is also needed the application of these principles at early stages of the product development.

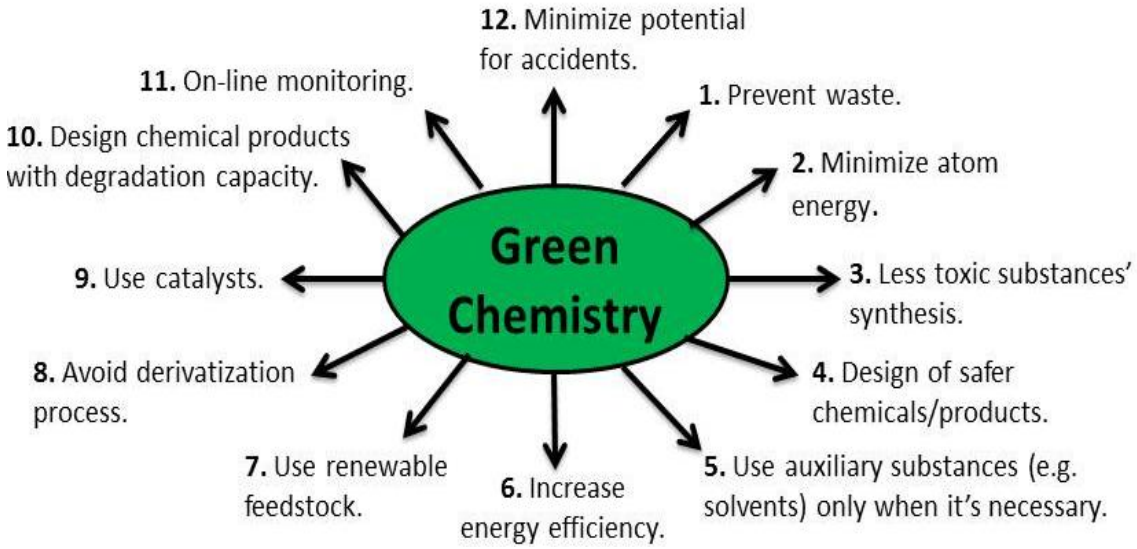

Figure 1 – Twelve Principles of Green Chemistry (adapted from Anastas et al.)[1]

Green Chemistry is an important tool in achieving sustainability. The Brundtland Commission (1987)[3] defined sustainable development as *"development that meets the needs of the present without compromising the ability of future generations to meet their own needs"*. The challenges of global sustainability are complex, in a way that the three dimensions of sustainability, environmental, social and economic must be recognized. These three elements are essential to move sustainability forward.

The application of the Twelve Principles of Green Chemistry demonstrated the possibility of achieving this synergism by working at the most fundamental level.[4] Green Chemistry provides solutions to such global challenges as climate change, sustainable agriculture, energy, toxics in the environment, and the depletion of natural resources.

A major concern regarding sustainability is the release of hazardous substances into the environment. Green Chemistry can have a significant impact in this area. It is important to use alternative solvents to organic solvents in order to decrease the negative environmental impact generated by the last ones, namely the release of volatile organic compounds (VOCs), hazardous air pollutants (HAPs). A green alternative to organic solvents is the use of ionic liquids and supercritical fluids. For instance, considering the supercritical fluids, supercritical carbon dioxide ($scCO_2$) can be used. This compound has an easily accessible critical point and is non-toxic, non-flammable and inexpensive.[5] Regarding to ionic liquids (ILs), these salts are attractive because of their negligible vapor pressure, thermal stability[6] and great solvent power.[7] Their low vapor pressure reduces the risk of exposure which is an advantage related to classical VOCs.[7] This class of solvents has the potential to design next generation of ILs thus is a significant promise for improved environmental benefits.[8] However, the "green" concept of ILs sometimes is questionable as limited data about toxicity and biodegradability are available. Thus, it is important that ILs should be treated with the same caution as other solvents.[7, 9-11] Nevertheless, ILs are still considered as more environmentally friendly compared to the other organic counterparts.[7]

1.2 Biorefinery Concept

The National Renewable Energy Laboratory defined Biorefinery as "*a facility that integrates conversion process and equipments to produce fuels, power, chemicals and materials from biomass."* The main goal of the biorefinery is the generation of a variety of goods from different biomass feedstocks through a combination of technologies.[12-14] In other words it is the production of high-value low-volume (HVLV) and low-value high-volume (LVHV) products. The operations are designed to minimize the waste streams by converting LVHV intermediates into energy, while HVLV products enhance profitability.[12] The biorefinery concept is presented in Figure 2.

The efficient production of transportation biofuels is seen as one of the main promoting factors for the future development of biorefineries.[15] Once, biofuels have the potential to reduce greenhouse-gas emissions and to contribute to energy security by diversifying supply sources. Also, to respond to determined mitigation measures, such as the EU directive[16] which put the objective to achieve a 20% share of energy from the renewable resources. Referring to biofuels in

Portugal, it is necessary to accomplish a level of 10% by 2020. Additionally, a reduction of greenhouse-gas emissions of 20% by 2020 was also established.[17] Although, the implementation of biofuels as main source of energy is still a difficult and challenging process due to the technological limitations.[17] However, to satisfy social and environmental aspects of biofuel production, a complex biorefinery concept must be explored leading to a maximal valorization of non-food and non-feed competitive feedstocks.

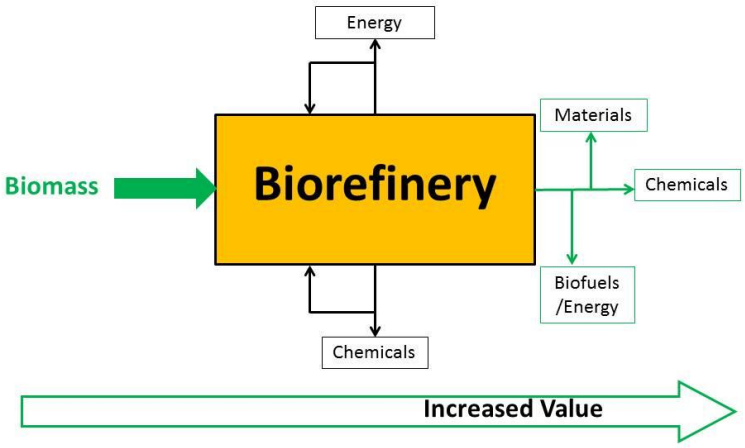

Figure 2 – The Biorefinery Concept (Adapted from Carvalheiro et al.).[18]

1.3 Lignocellulosic Feedstock

Within biorefinery processes the major lignocellulosic feedstock are lignocellulosic biomass, such as softwood (Pine, Spruce), hardwood (Willow, Poplar and *Eucalyptus*), grasses (Miscanthus, Switchgrass). In addition, agricultural residues (wheat straw, sugarcane, bagasse and corn stover), forest residues (sawdust, thinning rests), domestic and municipal solid wastes, and food industry residues are considered as biomass too.[19] Annually are produced approximately 200 billion tones worldwide which make this type of biomass a potential source of a clean, uniform and a low-cost raw material for large-scale and environmentally sustainable biorefineries.[20]

Lignocellulosic biomass from agricultural residues, forestry wastes, waste paper are currently being studied due to their potential use as a starting material for production of bioenergy/biofuels.[20] Furthermore, using this type of biomass can also minimize the competition with the food/feed chain, which represents an advantage comparatively to 1st generation biofuels.[21] Since, the 1st generation of biofuels appear unsustainable regarding to the source of feedstocks, including the impact that it may cause on biodiversity and land use.[22] From lignocellulosic biomass not only biofuels (bioethanol, biobutanol) can be produced, but also other

valued-added products such as reducing sugars, organic acids (acetic acid, propionic acids and butyric acid), compost (organic fertilizer), bio-composites, furfural and its derivatives and other miscellaneous compounds.[23]

Lignocellulosic biomass is composed mainly of cellulose (35-50%), hemicellulose (20-35%) and lignin (5-30%).[24] The composition of lignocellulose materials depends mainly on the species, age and origin of biomass. The contents of macromolecular materials in some residues and waste crops are illustrated in Figure 3.

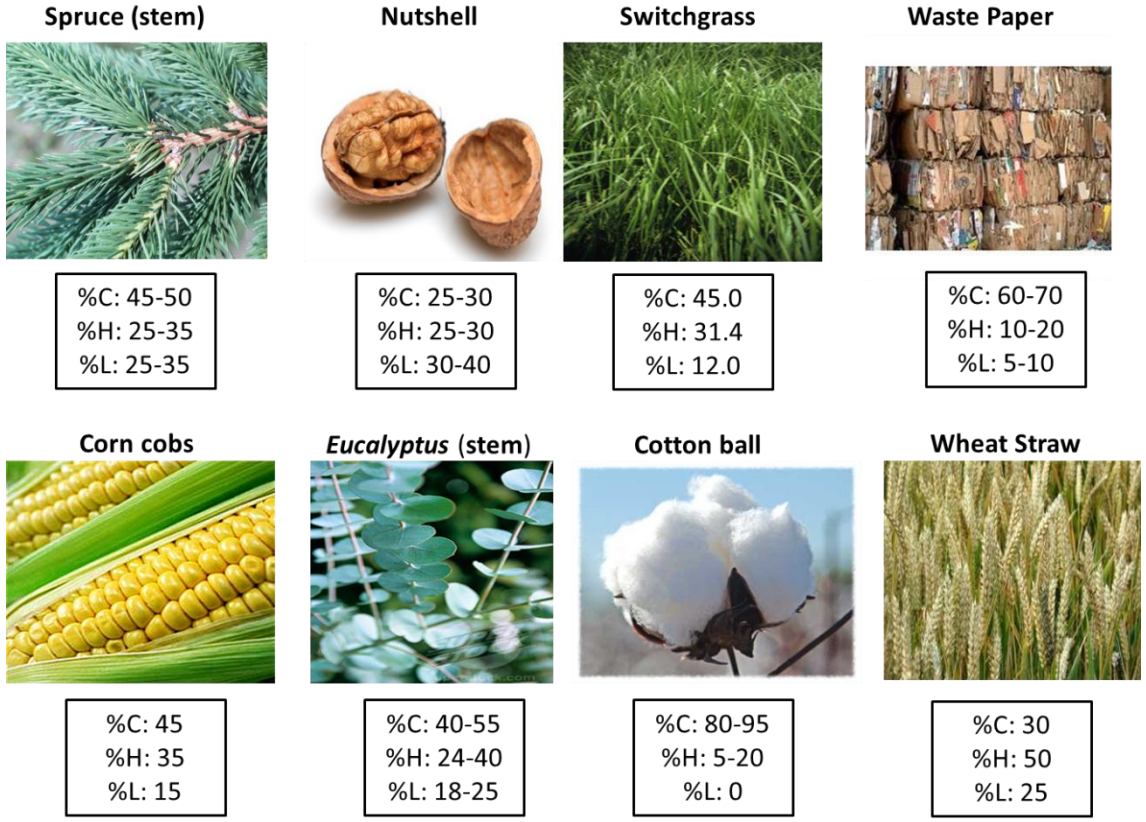

Figure 3 - Contents of cellulose (%C), hemicellulose (%H) and lignin (%L) in common residues and wastes. (Adapted from Sun et al.)[25]

A simplified picture of the lignocellulosic materials' macromolecular structure is that, cellulose forms a skeleton which is surrounded by other substances forming a matrix (hemicelluloses) and encrusting (lignin) materials.[26] Cellulose and hemicellulose represent the carbohydrate fraction, while lignin is a very complex and amorphous phenylpropanoid polymer. Cellulose $(C_6H_{10}O_5)_n$ is a homopolysaccharide composed of $\beta - D -$ glucopyranose units linked together by (1→ 4)-glycosidic bonds generating cellobiose units. Cellulose is located predominantly in the secondary cell wall. Cellulose molecules are completely linear and have a strong tendency to form intra- and intermolecular hydrogen bonds. Bundles of cellulose molecules are thus aggregated together in form of microfibrils where highly ordered (crystalline)

regions are alternatives to less ordered (amorphous) regions. Microfibrils build up fibrils and finally cellulose fibers. The example of cellulose structure is presented in Figure 4.

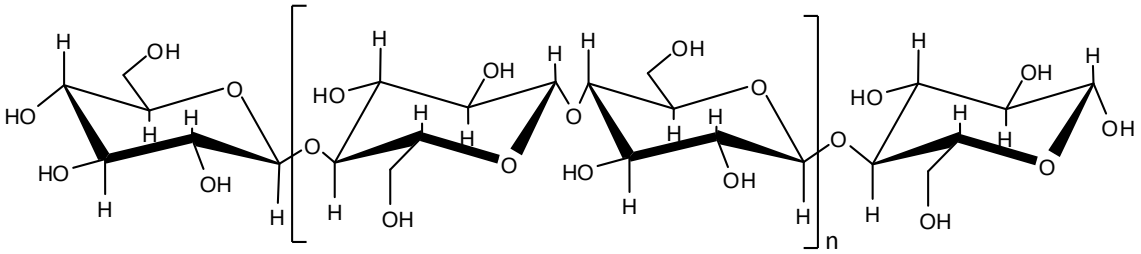

Figure 4 - Cellulose stereochemical formula. The dimeric unit repeated is cellobiose.

The number of glucose units that make up one single chain of cellulose is recognized as degree of polymerization (DP). Molecular weight measurements have shown that wood cellulose consists of about 10000 glucose residues. Based on properties cellulose in solution behaves like most of synthetic polymers, so this means that the molecules have no preferred structure in solution.[26] In terms of solubility cellulose is insoluble in water and other common solvents due to its fibril structure and the presence of intra- and intermolecular hydrogen bonds. At the same time is hygroscopic at normal atmospheric conditions absorbing around 8-14% of water. In order to accomplish various demands for its industrial applications, cellulose is often modified by chemical, physical, enzymatic or, genetic procedures to improve solubility and others properties of the polymer.[27] Cellulose is mostly hydrolyzed to sugar monomers, and then converted into alcohols (ethanol, butanol), hydrogen or methane by fermentation process. Apart from biofuel/bioenergy production, cellulose can be also used for the production of other valuable products, such as hydroxymethylfurfural (HMF) and levulinic acid.[28]

Hemicellulose is a non-crystalline, highly branched, water-insoluble heteropolysaccharide. Similarly to cellulose most of hemicelluloses has a supporting function in the cell walls.[26] The term hemicellulose represents a family of polysaccharides such as arabino-xylans, gluco-mannans, galactans, and others which have different composition and structure depending on their source and the extraction method.[29] The high complexity of hemicellulose, namely type, ratio contents of sugar units and structural conformation, is associated to the different building blocks present (Figure 5).

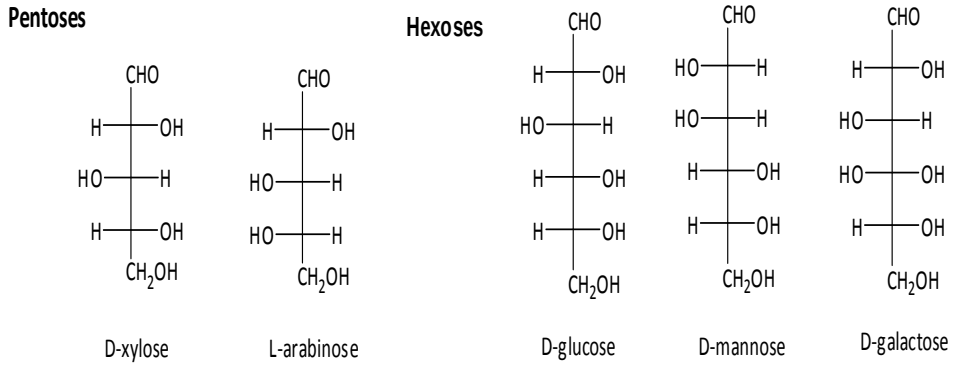

Figure 5 - Chemical structure of sugar building blocks.

The major type of hemicellulose polymers' content is xylans which have different structures depending on the type of branching. As can be seen from Figure 6, the backbone consists of $\beta - D -$ xylopyranose units, linked by (1→ 4)-bonds. Most hemicelluloses have a DP of only 200. Additionally, the highly branched structure of hemicellulose and the presence of acetyl groups linked to the polymer chain lead to lack of crystalline structure of hemicellulose.

Hemicelluloses are relatively easily hydrolyzed by acids to their monomeric compounds consisting of pentoses (D-xylose and L-arabinose), hexoses (D-glucose, D-mannose and D-galactose), and small amounts of desoxyhexoses (L-rhamnose and L-fucose) in addition to glucuronic acids (D-glucuronic acid, 4-O-methyl-D-glucuronic acid and D-galacturonic acid).

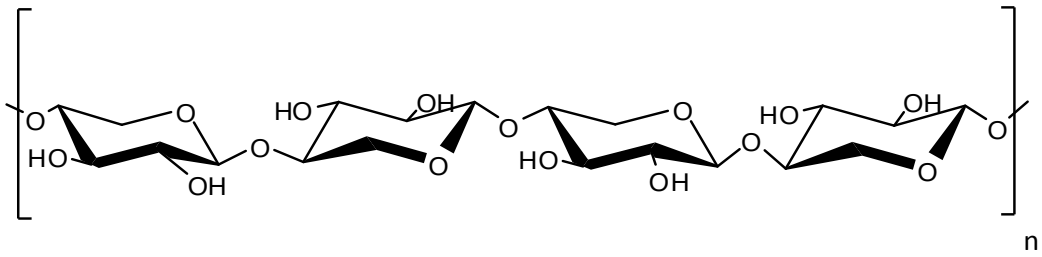

Figure 6 – Chemical structure of xylans' backbone.

Xylans can be isolated using alkali solutions such as KOH or NaOH. However, disadvantage of alkali extractions is almost complete hemicelluloses deacetylation. Then, the polysaccharides can be precipitated from the alkaline extract by acidification (acetic acid). The addition of a neutral organic solvent, for example ethanol, results in a more complete precipitation.[26] Hemicelluloses are used as emulsifiers, stabilizers and binders in food, pharmaceutical (as food fibers, therapeutic agent in lowered immune conditions),[30] cosmetics industries, production of xylitol, xylo-oligosaccharides (used as prebiotic),[31] furfural and other value-added products.[32]

Lignin is an amorphous three-dimensional polymer of phenylpropane units which has an important role in the cell's endurance and development. Thus, it affects the transport of water,

nutrients and metabolites in the plant cell. More specifically these phenylpropane units are three monolignol precursors, coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol (Figure 7) with various degrees of oxygenation/substitution on the aromatic ring. Moreover, these precursors are covalently linked to hemicelluloses.[33]

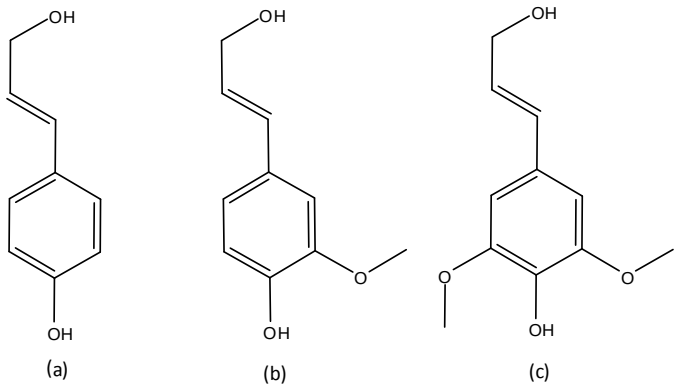

Figure 7 – Chemical structure of the three lignin precursors: (a) p-coumaryl alcohol, (b) coniferyl alcohol, (c) sinapyl alcohol.

The nature of the lignin polymerization reactions results in the formation of a three-dimensional, highly-branched network of essentially infinite molecular weight (Figure 8).[26] In terms of properties, high polydispersity degree characterizes lignin structure, once different branching and bonding in otherwise similar molecules are encountered. Lignin has a very low solubility in most solvents and exhibited a low viscosity. The polymeric properties are also extremely important when evaluating the suitability of lignin by-products for technical applications. Other issue is the isolation of lignin from wood without causing degradation.

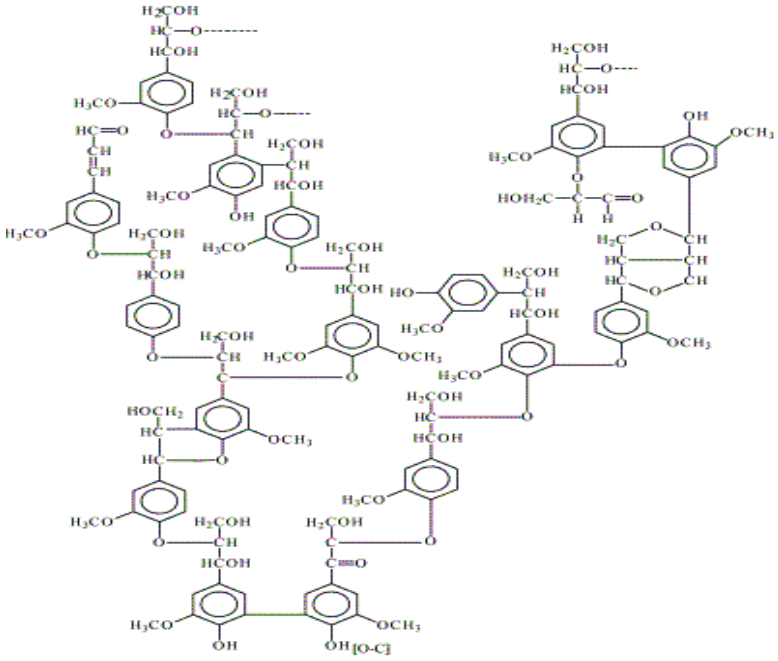

Figure 8 – Macromolecular representation of lignin structure.[34]

Lignin has been used as a low-value heating fuel, binder, dispersant and emulsifier. Recently, there is noticed a growing interest in the characterization of isolated lignin extracted from different crop residues in order to find novel applications. Lignin can be used as source of polyols for various applications such as the synthesis of polyurethane and phenol–formaldehyde resins, for the production of bioproducts such as vanillin, guaiacol and other phenolic compounds.[35] Beyond that it can also be used in automotive brakes, wood panel products, biodispersants, polyurethane foams, epoxy resins for printed circuit boards and surfactants.[36]

1.4 Ionic Liquids

ILs are salts composed of large organic cations and organic or inorganic anions with low lattice energy leading to low melting point usually below 100°C.[37] Although ILs possess immeasurable combinations of anions and cations (more than 10^6), only a small number (≈1000) of these compounds are described and characterized in the literature.[11] Examples of cations and anions studied are illustrated In Figure 8.

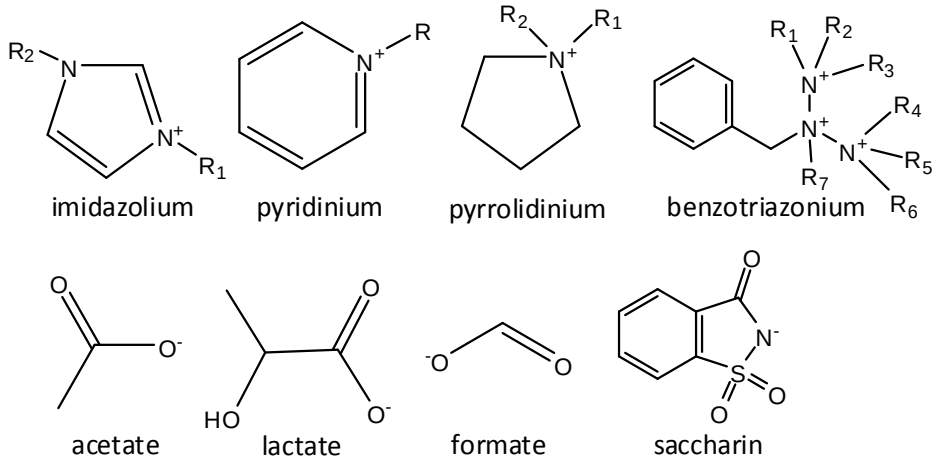

Figure 9 - – Chemical structures of cations and anions commonly used to form ILs. $R_{i=1,\ldots,7}$ = alkyl group(*Adapted from* Zakrzewska et al.).[7]

The ionic nature of ionic liquids results in several physical and chemical advantages over conventional and molecular organic solvents such as negligible flammability and vapor pressure, thermal stability and highly solvating capacity either for polar and nonpolar compounds,[10, 38-41] large electrochemical window and high conductivity.[6] Due to its unique properties ILs are interesting as an alternative to VOCs with the aim to facilitate sustainable chemistry. Since a large number of cationic and anionic structural combinations is possible, desired physicochemical properties of ILs for a particular processes can be easily tuned.[42] Thus hydrophobicity, viscosity or density can be adjusted by changing the alkyl chain of the cation.[43, 44] The viscosity is sometimes one of the obstacles in chemical reactions due to mass transfer limitations, once viscosity values'

for ILs can be generally up to 3 times higher than that of conventional organic solvents.[45] The water content in ILs can be considered as an impurity in a way that it was found that decreases the solubility of carbohydrates.[46] Also, their solvent properties can be varied by adjusting the anion and the alkyl constituents of the cation.

Due to the outstanding ILs' properties they have been applied in numerous areas such as (bio-) catalysis,[47] organic synthesis,[48] separations processes such as liquid-liquid extraction,[49-51] electrochemistry in solar and fuel cells,[52-54] lubricants,[55] and cosmetics.[56]

1.5 Supercritical Fluids

A supercritical fluid (SCF) is a state above the critical temperature, T_c, and critical pressure, P_c, but below the pressure required to condensate into a solid.[57] Under these conditions, some properties of the fluid are placed between those of a gas and those of a liquid. Namely, the density of a supercritical fluid is similar to a liquid and its viscosity is similar to a gas, although its diffusivity is intermediate between the two states.[57] It is also important to point out the great solvating power of the SCF similar to a liquid. SCFs' solvent power is the highest for non-polar or slightly polar compounds.[58] At the critical point, the density of the gas phase becomes equal to that of the liquid phase, thus the phase distinction between vapor and liquid disappears (Figure 10).

The most commons SCFs are presented in Table 1, their critical parameters where can also be seen. Carbon dioxide (CO_2) is frequently chosen as supercritical fluid due to its moderated critical constants, inertness, low cost, availability in pure form and the easy recovery after use.

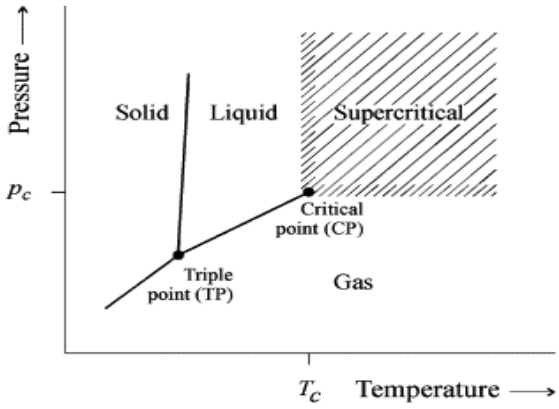

Figure 10 - Definition of supercritical state for a pure component.[59]

In biomass pre-treatment water is often present or added deliberately. It was shown that supercritical water (scH_2O) develops acidic characteristics at high temperatures (at 220°C results a pH of 5.5).[59] However, to achieve critical conditions of water comparing with critical conditions of

CO_2 demands much more energy. Thus combining both compounds can reduce significantly the energy demand.

Table 1 – Critical parameters of the most common compounds. (Adapted from Jessop and Leitner).[57]

Compound	T_c/°C	P_c/bar
Carbon Dioxide (CO_2)	31.1	73.8
Water (H_2O)	374.0	220.6
Propane (C_3H_8)	96.7	42.5

As matter of fact it was shown that water in contact with CO_2 becomes acidic due to formation and dissociation of carbonic acid[60] (Equation 1). By the dissolution of CO_2, the pH of water-CO_2 mixture decreases to approximately 3.0 (depending on pressure and temperature).[60]

$$CO_2 + 2H_2O \leftrightharpoons H_3O^+ + HCO_3^- \quad \textbf{(Equation 1)}$$

Use of SCF as an alternative to conventional solvents (organic acids) offers attractive possibilities, such as the facile removal of CO_2 by the depressurization without the contamination of the reaction mixture, and reduction of operational costs since further neutralization can be omitted. Also, the formation of waste particles and, consequent corrosion of the equipment can be overcome by introducing CO_2 in a autohydrolysis treatment.[59] Therefore the significance of SCF for a sustainable chemistry can be underlined.[61]

Due to their tunable properties SFCs are used in different areas such as food processing (such as decaffeination of green coffee beans or recovery of aromas and flavors from herbs and spices) adsorptive and chromatographic separations,[58] separations or/and extractions,[62-64] pharmaceuticals applications.[65]

1.6 Biomass Pre-treatments

Lignocellulosic biomass is a complex three-dimensional structure constituted by cellulose, hemicellulose and lignin. The concrete use of these lignocellulosic fractions requires a pre-treatment that allows the biomass fractionation into cellulose, hemicellulose and lignin fractions. The principal goal of lignocellulosic pre-treatment is to turn cellulose more easily hydrolysable by

removing hemicellulose and lignin, reducing the surface area, and reducing crystallinity.[66] Hence, the pre-treatment step is the central key in a biorefinery to convert lignocellulosic biomass into fuels and chemicals.[67] A schematic representation of the goal in biomass pre-treatment is illustrated in Figure 11. Therefore, the selection of an efficient pre-treatment method should take in account various features. Namely, (1) result in high recovery of all carbohydrates; (2) to improve sugar yields; (3) to avoid formation of inhibitory by-products to the subsequent hydrolysis and fermentation process; and, (4) require low capital and operational costs.[67-69] Hereafter it is extremely important that all pre-treatment methods must be reassessed at more industrial-like conditions referring to the whole integrated process taking into account the different types of lignocellulosic biomass.[68]

The pre-treatment methods can be categorized according to various criteria.[25, 68-70] Among these pre-treatment criteria they can be divided into conventional and alternative methods. Since this thesis is based on alternative methods, namely ionic liquids and supercritical fluids, just a brief introduction to conventional methods is presented below.

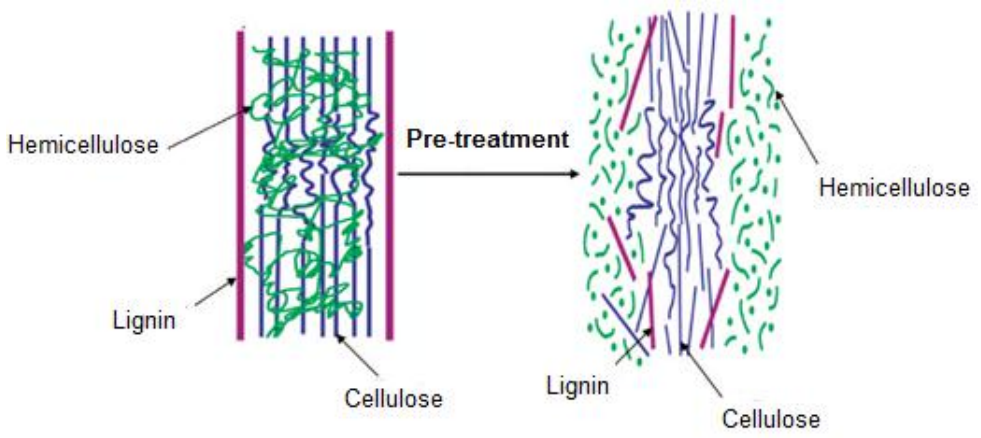

Figure 11 - Schematic of the role of pre-treatment.[71]

1.6.1 Conventional Methods

Conventional pre-treatment methods can be classified as biological, physical, chemical and physical-chemical. Depending upon the pre-treatment method chosen different effects might be caused. These effects are clearly presented in Table 2. It is important to notice that the selection of the feasible pre-treatment method for a specific process configuration with a certain type of co-products is not necessarily optimal for another process configuration.[68]

Table 2 – Types of pre-treatment methods (Adapted from Mohammad et al.),[70] Carolina et al.[72]).

Pre-treatment	Examples of Process	Effect
Biological	*Fungi and actinomycetes*	Delignification
Physical	Milling Irradiation Hydrothermolysis	Increase surface area and pores size; Partial depolymerize of lignin; Disrupts plan cell; Partial hydrolysis of hemicelluloses.
Chemical	Alkaline Hydrolysis (Na^+, K^+, Ca^+ and NH^{4+}) Acid Hydrolysis (H_2SO_4, HCl and HNO_3) *Organosolv* (ethanol, acetone) Wet Oxidation (water/air or O_2, T>120°C) Ozonolysis	Decrease cellulose crystallinity; Partial or complete hydrolysis of hemicellulose; Delignification.
Physical-Chemical	Steam Explosion (autohydrolysis, SO_2 addition) Liquid hot water (LHW) Ammonia fiber explosion (AFEX) Irradiation (Microwave)	Combination of all effects referred to above.

It is also important to point out that none of these pre-treatments is highly selective and efficient. Also, these methods are frequently environmentally detrimental, creating hazardous pollutants and demanding much energy. Moreover, conventional pre-treatments use severe conditions, such as strong base or mineral acids. Thus, new, less toxic, green methodologies are currently studied. Examples of new methodologies are ionic liquids and SFCs which have already demonstrated promising perspectives against conventional methods.

1.6.2 Alternative Methods

1.6.2.1 Pre-treatment with Ionic Liquids

In 2002, Rogers and co-workers[73] have demonstrated that imidazolium-based ILs can efficiently dissolve cellulose. These ILs show a relatively low viscosity and stronger hydrogen bonding basicity,[46] which facilitates the breakdown of inter and intramolecular hydrogen bonds. More specifically Sun et al.[74] report the 1-ethyl-3-methylimidazolium acetate ([emim][OAc]) for dissolution of cellulose due to its desirable properties such as low toxicity, viscosity and corrosiveness, low melting point (< -20°C) and favorable biodegradability. The importance of a strong hydrogen bond basicity[46] that facilitates the breakdown of inter- and intramolecular hydrogen bonds is commonly known. Imidazolium acetate ILs exhibit a promising dissolution behavior for cellulose, although there is a lack of thermal properties of these ILs. The calorimetric analyses of [emim][OAc] were performed by Wender et al.,[75] where a significant mass loss is observed for temperatures close to 200°C. From thermal gravimetric analysis (TGA) measurements it can be concluded that acetate generally decreases the thermal stability, which was reported by Ohno et al.[76]

ILs are not only capable of dissolving cellulose, but can be good solvent for other biomaterials, such as lignin[77] and even wood.[78, 79] When wood dissolution was investigated in ILs, the water content and the particle size of wood chip had large effects on the dissolution efficiency. As mentioned before the water content reduces the solubility of carbohydrates. Sun et al.[74] investigated the effect of wood chip particle size (0.125 - 1.000 mm) of southern yellow pine (softwood) and red oak (hardwood) in [emim][OAc] and 1-butyl-3-methylimidazolium chloride ([bmim][Cl]) at 110°C for 16h. A 98% of dissolution of southern yellow pine for the smaller particles (<0.125 mm) with [emim][OAc] was obtained. As it was expected, smaller particles have larger surface areas, which cause more efficient dissolution. In the same study the effect of initial wood load was also investigated. It was found that an optimum wood load would be approximately 5 parts of wood added to 100 parts of IL that resulted in 98.5% of wood dissolution. In addition, Zavrel et al.[21] performed high-throughput screening systems where six ILs capable to completely dissolve cellulose were identified. Among the studied ILs, [emim][OAc] was the most efficient solvent for cellulose. The same result was reported from Coutinho and co-workers[40] where among eight ILs studied [emim][OAc] was the best candidate to dissolve cellulose. In fact the basicity of the acetate anion makes the dissolution more efficient at

disrupting the inter- and intramolecular hydrogen bonds.[74] However, it should be noted that IL selection for wood dissolution not only depends on water content, wood chip particle size, wood-to-IL ratio, but also on wood type, stirring and dissolution conditions (time and temperature).

1.6.2.1.1 Fractionation of Lignocellulosic Material

One challenge of wood pre-treatment is the recovery of all lignocellulosic materials released from wood. A general procedure is the processing of the biphasic mixture (lignocellulosic material and IL at the certain solid/liquid ratio) at determined temperature and time with rigorous stirring.[19] It is important to note that to improve efficiency of the pre-treatment complete dissolution is required. Usually, after the dissolution step the regeneration of biomass with a precipitating solvent addition is performed. Cellulose can be separated from IL by the addition of anti-solvents, such as water,[78] acetone/water solution (1:1) (v/v),[74] NaOH 0,1M.[80] According to Dadi et al.[81] the regenerated cellulose is completely modified and its crystallinity matrix is changed to an amorphous structure. The natural crystalline form (Cellulose I) is transformed into Cellulose II, which is more stable. Thus, this amorphous structure allows an enhancement of enzymatic hydrolysis that can produce high sugar yields, and consequently produce biofuels from lignocellulosic biomass with IL pre-treatment.[82-85]

Lee at al.[86] has reported a selective method for lignin extraction from maple wood flour in various ILs at 80°C within 24h. [Emim][OAc] provided a very good extractability of the lignin (4.4 g lignin/kg IL from a 50g/Kg of wood flour/IL solution). Despite wood flour presents a very low solubility in [emim][OAc] (<5 g/kg) compared with the high solubility of free cellulose (>100 g/kg). An interesting compromise between high lignin solubility and low wood flour solubility was achieved in [emim][OAc]. Thus, the removal of lignin increases the access of cellulose fraction, and results in a high amount of available cellulose (in this case, over 90%) for enzymatic hydrolysis.

Up to now only few works report the complete fractionation of biomass into the main constituents.[19, 33, 87-89] A pre-treatment of 2% (w/w) sugarcane bagasse with 1-butyl-3-methylimidazolium chloride ([bmim][Cl]) at 110°C for 4h was performed.[33] The complete dissolution was achieved followed by precipitation in acetone/water (9:1) (v/v) and extraction with 3% NaOH solution. The biomass was fractionated into 36.78% cellulose, 26.04% hemicellulose and 10.51% lignin, giving 47.17% (w/w) and 33.85% (w/w) of the original polysaccharides and 54.62% (w/w) of the original lignin. About 26.67% of the original bagasse was lost. This fractionation method resulted in a high purity of cellulose fractions (> 92%). In addition,

[bmim][Cl] was easily recycled after concentration (removal of water) and treatment with acetonitrile. Additional research was focused on 1-allyl-3-methylimidazolium chloride ([amim][Cl]) used as solvent to dissolve and fractionate a solution of 5% (w/v) bamboo powder/IL at 100°C for 12h.[88] After dissolution, bamboo was regenerated with distilled water, followed by consecutively extraction with 0.5M NaOH solution at 80°C for 3h with a solid-to-liquid ratio (SLR) of 1:30 (g/mL). After that, the obtained solid residue was washed with distilled water and, then cellulose-rich residual was extracted with 70% ethanol containing 1.0M NaOH at 80°C for 3h. Hemicellulose was extracted by the adjustment of the solution to a pH 5.5, followed by concentration (removal of water under reduced pressure) and precipitation with 3 volumes of 95% ethanol. To recover lignin-rich fraction, ethanol was removed under reduced pressure and the remaining solution was acidified to pH 1.5-2. A content of 93.88% and 92.02% wt of glucose was obtained for cellulose and hemicellulose-fractions, respectively, along with a recovery of lignin-fraction containing a small amount of carbohydrates (2.19% wt). However, it was noticed that the lignin and hemicellulose fractions were slightly degraded due to [amim][Cl] treatment. Rice hulls, a biomass rarely investigated with IL pre-treatment was reported by Lynam et al.[89] The effect of [amim][Cl], [emim][OAc] and 1-hexyl-3-methylimidazolium chloride ([hmim][Cl]) was investigated in a 10% (w/w) mixture of rice hulls/IL. The effect of temperature (90 or 110°C) and time (4 or 8h) on dissolution were studied. It was found that [emim][OAc] at 110°C within 8h completely removes lignin using 95% ethanol as a precipitating agent. In addition, [emim][OAc] was the only one that gave complete removal of lignin while a solid fraction mainly consisting of cellulose was obtained. However, higher hemicellulose content is removed when the lower temperature (90°C) was used. Another interesting study was reported by Simmons and co-workers[87] where it was investigated a mixture for precipitation of cellulose and lignocellulose biomass from solutions with [emim][OAc]. The main goal of this mixture is to prevent the formation of gel phases during precipitation of cellulose or cellulose-rich biomass fractions from concentrated IL solutions. It was defined as an optimum molar ratio composition of acetone: ethanol: IL solvent mixture to be 4:6:1:1. After the dissolution of 10% (w/w) (corn stover/IL) 93% cellulose, 80% hemicellulose and 58% lignin of the initial composition were recovered. Moreover, a novel method to recover IL after pre-treatment without the addition of acid or other catalysts was also studied. Basically, this method comprises an IL-acetone-ethanol-water phase-splitting process where a recovery of 89% from initial IL was achieved. Considering other studies ,[33, 74, 80] a new fractionation method was developed to obtain high purity samples.[19] A dissolution of 5% (w/w) initial load of wheat straw/[emim][OAc] at 120°C for 6h was performed. Biomass was fractionated into the main components which purities were

measured by FTIR (Fourier Transform Infrared Spectroscopy) analysis. It was verified high carbohydrate content in 86% cellulose and 85% hemicellulose samples while 87% of pure lignin was obtained. The recovery of [emim][OAc] was up to 94.9% from the initial mass.

Determining the optimal dissolution conditions, temperature and time, appears to be essential when working with lignocellulosic biomass. The increase in dissolution temperature increases the diffusivity, both adding thermal energy to the system and by decreasing IL's viscosity. Longer times allows the IL to penetrate farther into the biomass.[89] However, higher temperatures and times do not guarantee the efficiency of the pre-treatment. For instance, the effect of pre-treatment's temperature (50 – 130°C) and time (0.5 – 70h) was reported by Lee at al.[86] A higher content of lignin was extracted and lower content of biomass was recovered with increasing pre-treatment temperatures. The increase in pre-treatment time led to increased lignin extraction while little cellulose and hemicellulose was recovered up to a 70h pre-treatment. Regarding IL's thermostability it was recommended that moderately short dissolution times should be applied for [emim][OAc],[74] and that the degradation temperature of [emim][OAc] can be 200°C.[75] It is also expected that higher temperatures favor hemicellulose hydrolysis with [emim][OAc].

It is important to emphasize that an ideal set of conditions that is feasible to every aspect probably will not exist. However, it is important to consider that the optimum dissolution conditions should be determined in order to avoid depolymerization as well as formation of low molecular products.[90]

Concerning to "Biorefinery Concept" the ILs recycle is a crucial aspect related to economical applicability of the IL pre-treatment process, once this novel solvent is more expensive than conventional organic solvents. The effect of IL recycling was analyzed through the pre-treatment of cotton stalk with [emim][OAc], which maintained its effectiveness upon 3 recycles.[91] The amount of extracted lignin decreased with an increase of number of cycles due to the continuous accumulation of biomass components, mainly lignin.[86] Additionally, it was confirmed that [emim][OAc] could be reused even six times without losses in biomass pre-treatment.[19]

1.6.2.2 Biomass Pre-treatment with sub-/supercritical CO_2 / water

For lignocellulose ethanol bioproduction, hemicelluloses are commonly removed during the initial step of biomass processing in order to destroy the lignocellulosic materials' complex structure. Cellulose and hemicellulose must be hydrolyzed into their respective monomers (sugars) to enhance enzymatic hydrolysis. Once again the pre-treatment step is crucial in this

process, so that selective fractionation from biomass can be achieved. Different pre-treatment methods towards a selective fractionation of hemicellulose from biomass have been developed covering the use of acids, water (liquid or steam), organic solvents and alkaline agents. The two last methods also remove lignin with hemicellulose which in turn can decrease the bioprocess' valorization once lignin-derived compounds usually act as microbial growth inhibitors. Therefore, acid/water/steam pre-treatments are usually applied yielding a selective dissolution of hemicelluloses and a production of hemicellulose-rich liquids that can be partially or totally hydrolyzed into oligomeric and monomeric sugars. Moreover, cellulose-rich solids are recuperated for further bioprocessing.[46, 71]

The autohydrolysis process uses compress hot water (pressure above saturation point) and temperatures usually between 150 – 230°C with reaction times that can vary from seconds up to hours.[46] Hydronium ions generated in situ by water autoionization and acetic acid resulting from acetyl substituents of hemicelluloses act as catalysts in this process. Several studies reported a relatively high hemicellulose recovery (55 – 84%) combined with low levels of inhibitory by-products[92, 93] although, the dissolved hemicellulose, available mainly in oligomeric form, is a drawback to bioethanol production by autohydrolysis process. Steam explosion process consists of heating the material (preferably below 240°C) using high-pressure steam up to few minutes where this thermomechanochemical process led to a desegregation of lignocellulosic matrix. The steam condenses under the high pressure thereby "wetting" the material, that it is then "exploded" when the pressure within the reactor is rapidly released.[46] Similarly to autohydrolysis, steam explosion treatments yielded high solubilized hemicellulose also in oligomeric form. However, slight lignin is present along with dissolution. The addition of acid catalysts, such as H_2SO_4 or SO_2 is known to improve sugar yields.[94]

Hydrothermal technologies, especially sub- and supercritical treatments have been investigated for lignocellulosic materials pre-treatments. In these treatments sub- (near its critical conditions) or supercritical water and supercritical CO_2 are used. Previous studies demonstrated the pre-treatment of cellulose with scH_2O followed by enzymatic hydrolysis for biofuel and chemicals production.[95] Due to the high solvent and catalytic capacity of scH_2O this kind of fluid has been reported to be suitable for processing fermentable hexoses from lignocellulosic materials owing to the efficient lignin separation and cellulose hydrolysis.[96, 97] As referred to in section 1.5, water under sub- or supercritical conditions behaves differently that under normal conditions. It is expected that the acidic characteristics developed by scH_2O enable the disruption of chemical bonds. Thus, hemicellulose can be separated from the lignocellulose and enzymatic

digestibility of cellulose can be increased. However, it was found that the hydrolyzed hemicelluloses monomers can further react to furfural and other toxic by-products.[46] Furthermore, in scH_2O hexoses generated from cellulose decompose rapidly into non-fermentable fragmentation products mainly erythrose and HMF. Figure 12 illustrates the water phase diagram where the typical ranges for different water-based pre-treatments are presented. In subcritical water the decomposition of hexoses is slower than in scH_2O. Thus, several studies were performed in a sub-/supercritical combined process, i.e. lignocelluloses are pretreated and hydrolyzed in scH_2O to remove lignin and to produce oligosaccharides from cellulose, followed by a secondary hydrolysis in subcritical water to convert oligosaccharides into fermentable hexoses. Comparatively to the conventional hydrothermal treatments, the combined sub-/supercritical process demonstrates a much higher reaction rate, requires neither the use of additional catalyst, nor inhibits reaction of intermediates.[98, 99]

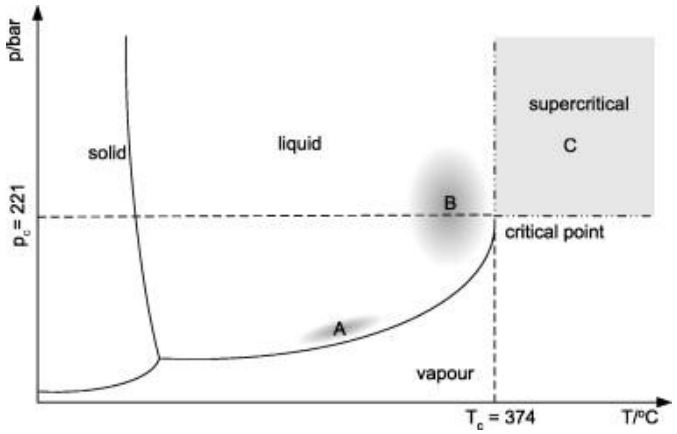

Figure 12 - Water phase diagram: (A) Autohydrolysis; (B) Subcritical conditions; (C) Supercritical conditions.[46]

Supercritical carbon dioxide ($scCO_2$) mostly used as an extraction solvent is studied in lignocellulosic pretreatments. One of the first works reported that the use of $scCO_2$ did not cause significant change in microscopic morphology of wood.[100] Kim et al.[101] investigated the effect of $scCO_2$ on raw lignocellulose with different moistures contents at various pretreatment conditions (temperature, time and pressure). Within this particular experiment it was found that an increase in moisture content to 73% (w/w) at 214 bar and 165°C resulted in a significant increase of final sugar yields from the enzymatic hydrolysis. Therefore, it was concluded that the moisture content has a pronounced effect in pretreatments with $scCO_2$. A study reported by Van Walsum demonstrated an increase of xylan hydrolysis by the addition of CO_2 to a autohydrolysis pre-treatment of beech wood.[102] On the other hand, a study showed that the addition of CO_2 in autohydrolysis process at 100 bar did not lead to a higher degree of biomass dissolution.[103] In this case, the effect of the acidic pH system water/CO_2 was not achieved. The employment of CO_2

usually decreases the temperature of the process leading to a minor xylose degradation and a higher yield of the reaction.[46] Additionally, the use of $scCO_2$ can overcome the drawbacks (corrosion, formation of waste particles) resulted from the conventional pre-treatments with organic acids, once CO_2 can be neutralized by a simple pressure reduction.

The present work is devoted to the pre-treatment of lignocellulosic biomass by two alternative solvents – ionic liquids (ILs) and supercritical fluids (SCFs). In Chapter 2 is presented the wheat straw pre-treatment with 1-ethyl-3-methylimidazolium acetate [emim][OAc]. The aim of this study is to present a new methodology where a complete fractionation of the wheat straw biomass into cellulose, hemicellulose and lignin is accomplished. Moreover, the effect of temperature and processing time on the pre-treatment of the wheat straw with [emim][OAc] was studied. To evaluate the efficiency of a new method on the production of cellulose for further potential applications, the enzymatic hydrolysis of cellulose-rich fractions was performed. The dependency of cellulose-rich fractions from the applied conditions was evaluated by a regression analysis concerning the cellulose recovery (% (w/w)) and the glucan content ($\%w/w_{biomass}$). Additionally, the presence of phenolic compounds in the recovered ILs was investigated through a capillary electrophoresis (CE) methodology. The other part of the work is presented in Chapter 3 which is dedicated to a CO_2 assisted autohydrolysis pre-treatment of wheat straw. The objective of this study was to investigate the effect of CO_2 addition in an autohydrolysis treatment of wheat straw, in order to selectively dissolve the hemicellulose fraction. Moreover, the effect of temperature and non-isothermal reaction time on the composition of both liquid and solid fractions was evaluated by the severity factor ($Log\ R_0$). Additionally, the influence of CO_2 density was also analyzed through the application of Peng-Robinson Equation of State (PR-EOS).

2. IL Pre-treatment

2.1 Materials and Methods

2.1.1 Materials

For the pre-treatment experiments [emim][OAc] IL was used (>95% purity, Iolitec GmbH, Heilbronn, Germany). The water content was measured to be 2800 ppm by a volumetric Karl – Fischer titration. Prior to use, [emim][OAc] was dried under vacuum at room temperature for at least 24 hours. Wheat straw was the feedstock material, supplied by Estação Nacional de Melhoramento de Plantas (Elvas, Portugal). The material was ground with a knife mill (IKA® WERKE, MF 10 basic, Germany) to particles smaller than 0.5 mm, and stowed at room temperature. The wheat straw moisture was found to be 8% (w/w).

For the IL pre-treatment experiments the following reagents were used: 1M and 4M HCl aqueous solutions prepared from fuming 37%(w/w) HCl bought from Merck – Darmstadt, Germany; 1M and 3% (w/w) NaOH aqueous solutions prepared from pure (99%) NaOH pellets supplied from Eka Chemicals/Akzonobel - Bohus, Sweden. To prepare HCl and NaOH aqueous solutions, distilled water (17 $M\Omega cm^{-1}$) produced by the PURELAB Classic of Elga system was used. From 4M HCl aqueous solution, an acidic water solution ($pH \leq 2$) was prepared. Ethanol (purity of 96% (v/v)) and acetonitrile were bought from Carlo Erba Group - Arese, Italy. Paper filter membranes (Ø=47 mm, nº 1, 1.2 µm porosity) obtained from Whatman, GE Healthcare Life Generations - Buckinghamshire, United Kingdom, and nylon filters (Ø=47 mm, 0.45 µm porosity) from Merck Millipore, Country Cork, Ireland, were used. For FTIR analysis, all samples were prepared with KBr (≤ 99% trace metal basis) purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Deuterium oxide with an isotopic purity > 99.8 % from Fluka, Sigma-Aldrich Química, S.L. Sintra, Portugal, was used to prepare IL samples for NMR analyses.

Enzymatic hydrolysis was performed using a 0,1M sodium citrate buffer (pH 4.8) prepared from citric acid monohydrate (99.7% purity) and tris-sodium citrate (>99% purity), both bought from VWR International Ltd. - Leicester, England; a 2% (w/w) sodium azide solution was prepared from sodium azide (99% purity; Merck - Darmstadt, Germany). Commercial enzymes Celluclast® 1.5L (activity 60 $FPU \cdot g^{-1}$, 105.89 $FPU \cdot mL^{-1}$) and β-glucosidase Novozym 188 (activity 64 $NPGU \cdot g^{-1}$, 797.25 $pNPGU \cdot mL^{-1}$), both purchased from Novozymes - Bagsvaerd, Denmark, were used in this work.

Capillary electrophoresis (CE) analysis was performed using an electrolyte solution containing 15mM of sodium tetraborate decahydrate (>99.5% from Sigma-Aldrich, Aldrich Co., St. Louis, MO,

USA) in 10% (v/v) methanol (>99,9% from Carlo Erba Group, Arese, Italy). This solution was adjusted to pH 9.13 using 0.1M NaOH.

Nylon syringe filters (Ø=13mm, 0.22µm porosity), purchased from Red® analytical, Cambridgeshire, UK, were used to filtrate all the samples before running on HPLC and CE.

2.1.2 Pre-treatment of the wheat straw using [emim][OAc]

A new methodology was developed based on our previous work dedicated to this subject.[104] Various pre-treatment times and temperatures were examined using a constant biomass/IL ratio of 5% (w/w). A schematic presentation of the described method is shown in Figure 13.

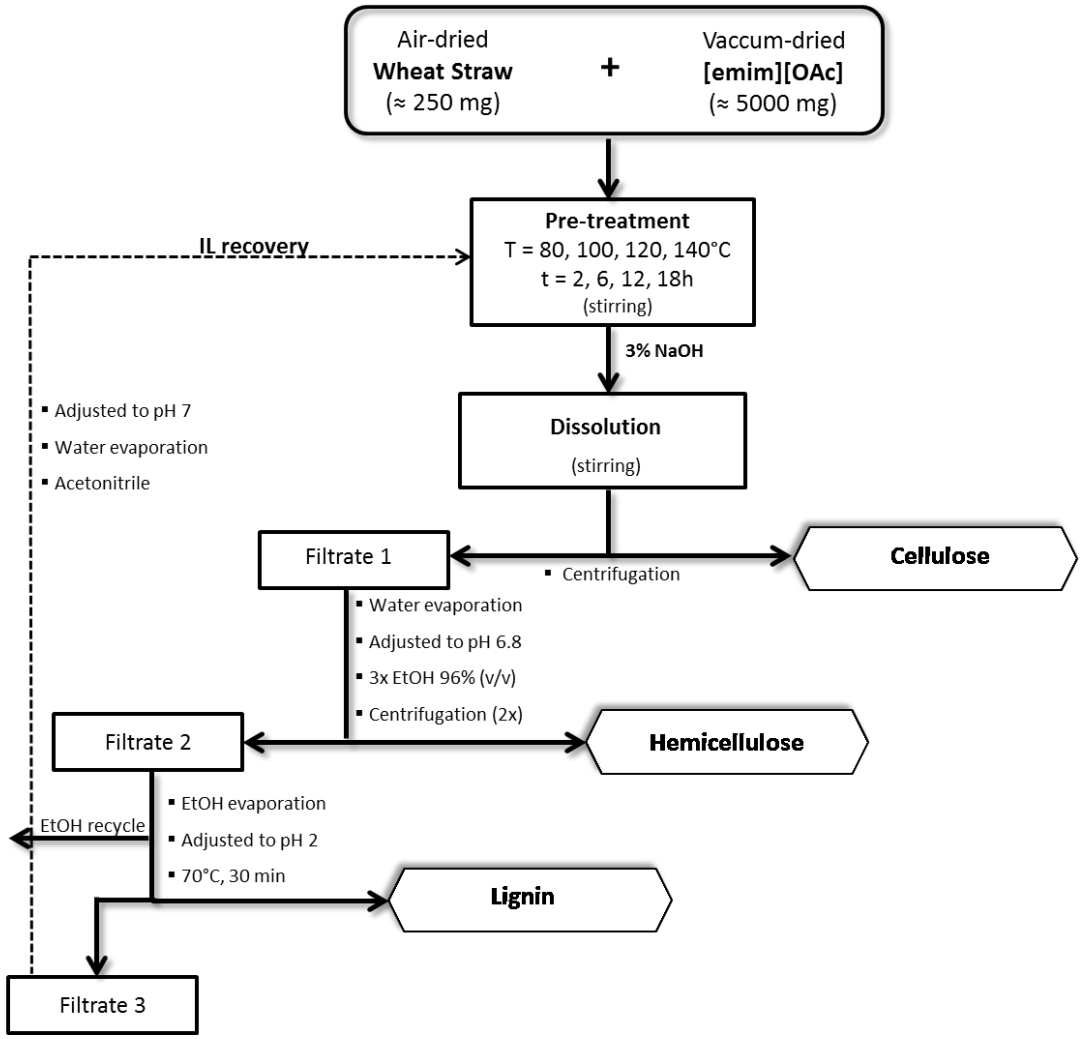

Figure 13 - Schematic presentation of the optimized method.

In a 15mL vial [emim][OAc] was mixed with the wheat straw under a continuous stirring for a defined period of time and temperature. The pre-treatment times and temperatures used in this work are shown in Table 3. After dissolution, a 3% NaOH was added until the vial was filled up.

Next, the mixture was transferred to a 100mL Erlenmeyer flask and a vigorous agitation was applied during 1h. To the formed solution 3% NaOH was added to promote the regeneration of cellulose. The resulting solution was subject to a centrifugation (Digicen 21R centrifuge, Orto Alresa, Madrid, Spain) at 6000rpm, 20°C for 20min. The supernatant was filtrated under vacuum and distilled water was used to wash the cellulose pellet. A new centrifugation step (6000rpm, 20°C for 20min) was performed and both supernatant and cellulose pellet were filtrated. During the vacuum filtration, the cellulose pellet was washed with distilled water until the filtrate demonstrated neutral pH (measured with pH indicator strips from Merck Millipore, Country Cork, Ireland). Subsequently, cellulose was collected and dried in the oven at 60°C for at least 24h.

Table 3 - Pre-treatment temperature and time studied in this work.

T/°C	**80**			**100**			**120**		**140**	
t/h	12	18	6	12	18	2	6	2	6	

Water was evaporated under reduced pressure (Rotavapor R-210/215, Büchi, Flawil, Switzerland) from the filtrate to a final volume of 30mL. Then, the solution was adjusted to pH=6.8 (GLP 21 pH meter, Crison, Barcelona, Spain) with 4M and 1M HCl solutions, and hemicellulose was precipitated with 3 volumes of 96% EtOH with the continuous stirring. Once again, the resulting solution was centrifuged at the conditions referred earlier. After the first centrifugation, the liquid fraction was filtrated while the solid fraction (hemicellulose) remained on the centrifugation flasks. This first liquid fraction was kept to further use. Distilled water was added to the centrifugation flasks in order to wash the hemicellulose precipitate and a last centrifugation step was performed. After that, using the same filter, the hemicellulose precipitate was filtrated and continuously rinsed with distilled water. The washing water was also kept to posterior use, and hemicellulose was dried at 60°C for at least 24h in the oven. Ethanol present in the first liquid fraction was evaporated under reduced pressure and the solution was acidified to pH 2, with a 4M HCl solution, to precipitate the lignin material. The solution was heated up to 70°C for 30min and a subsequent filtration was performed without cooling. The lignin material was washed with 10mL of acidified water ($pH \leq 2$) and dried in the oven at 60°C for at least 24h.

For [emim][OAc] recovery, the washing water from the hemicellulose precipitation step was added to the remaining filtrate (from the lignin filtration) to guarantee that all the IL used is recovered. The solution was then neutralized with the NaOH pellets and water was removed under the reduced pressure resulting in a solid containing NaCl and IL. Subsequently, 130mL of

acetonitrile was added to dissolve IL and the salt was removed by filtration. Acetonitrile was removed under reduced pressure and the recovered IL was dried for at least 24h under vacuum.

All the pre-treatment experiments were at least duplicated and all the weighing was made using an XS205 DualRange analytical balance, Mettler Toledo, Ohio, USA.

2.1.3 Chemical Analysis

2.1.3.1 FTIR characterization of fractionated samples

Perkin Elmer BX Series Spectrum (San Jose, CA, USA) was used to scan all samples. This model features a temperature-stabilized DTGS detector and a KBr beam splitter and is capable of routine mid-IR work. The operating system used to analyze all the spectra was Spectrum software Version 5.3.1, Perkin Elmer, Inc., San Jose, CA, USA). Thirty two scans were taken for each sample with a resolution of $4cm^{-1}$ in an absorbance mode in the range of $4000\text{-}400cm^{-1}$. For each spectrum the air background spectrum was subtracted.

For the composition analysis two calibration curves were built – carbohydrates and lignin – using an acid hydrolyzed wheat straw with known composition as a standard.[105] For the preparation of these curves, bands with a maximum linearity were selected. Specifically, $898cm^{-1}$ band for carbohydrates and the range at $1503\text{-}1537cm^{-1}$ for lignin were used. All samples were scanned at least three times and the average number was used. For each series of analyzes calibration curves were validated.

2.1.3.2 NMR analysis of recovered IL

Bruker ARX-400 spectrometer was used to analyze ^{1}H NMR and ^{13}C NMR spectra of all the recovered ILs using deuterium oxide solution as a solvent.

2.1.3.3 Capillary electrophoresis of recovered IL

Electrophoretic analyses for the presence of phenolic compounds in each recovered IL were carried out using an Agilent Technologies CE system (Waldbronn, Germany) equipped with a diode array detector (UV-DAD). An uncoated fused silica extended light-path capillary from Agilent, i.d.=50µm, total length 62 cm (56cm to the detector) was also used. The Agilent 3D-CE ChemStation data software (Rev B.04.01) was used to perform qualitative analysis of the electropherograms. The temperature in capillary was kept constant at 30°C and it was preconditioned by rinsing sequentially a 1M sodium hydroxide, 0.1M sodium hydroxide and Milli-Q water, for 20 minutes each solution. Between runs, the capillary was washed with 0.1M NaOH followed by buffer solutions. Electropherograms were recorded at 200, 280, 320 and 375 nm, and

phenolic compounds were identified by electrophoretic comparisons (migration times and UV spectra) with authentic phenolic standards. Before performing CE, all the IL samples were subjected to a solid phase extraction (SPE) to concentrate and separate the phenolic compounds from the IL solution through a non-polar surface. The SPE was performed to remove polar non-phenolic compounds such as sugars and salts, thus reducing the absorption of [emim][OAc] in the selected UV wavelengths. Separation columns (C18 ec, 6 mL, 1000mg) from Chromabond®, (Macherey-Nagel GmbH&Co. KG, Düren, Germany) were used. The columns were preconditioned with methanol, and then neutralized with ultrapure water (18.2 $M\Omega cm^{-1}$, PURELAB) using a high vacuum system (VacElut 20 Manifold Tall Glass Basin from Agilent Technologies, Santa Clara, CA, USA). The recovered ILs were loaded in the columns, and ultrapure water was used to the water-soluble constituents and simultaneously to remove the IL. The phenolic compounds were then eluted with absolute methanol and concentrated under vacuum prior to CE analysis.

2.1.4 Enzymatic Hydrolysis

Cellulose-rich samples obtained from all the pre-treatments at different conditions were subjected to enzymatic hydrolysis, in order to determine the glucan content. The adopted procedure was based on the standard NREL protocol.[106] The Optic Ivymen® System (Spain) incubator shaker was used to perform the enzymatic hydrolysis at 150 rpm and 50°C for 72h. After hydrolysis all vials were placed in a hot bath (approximately 95°C) for 5 minutes for enzymes denaturation. The concentration of reducing sugars was then analyzed by an HPLC system (Agilent 1100 series HPLC system, Santa Clara, CA, USA) using a Bio-Rad Aminex HPX-87H column (Hercules, CA, USA). A 5mM sulphuric acid was used as a mobile phase. Before running HPLC, all samples were filtrated. The set conditions of the column were: 50°C, $0.6mL{\cdot}min^{-1}$ flow rate 5µL injection volume and the acquisition time of 15min for standards and 30min for samples. Glucose standards with concentrations of 0.25, 0.50, 1.00, 1.75, 2.50 and $5.00g{\cdot}L^{-1}$ were used to construct the calibration curve. The determination of glucan content was made by multiplying glucose with a conversion factor of 0.90.[107]

2.1.5 Experimental Errors

Standard deviation error (u) was determined for all the obtained results. All weighing was made considering a u(*m*)=0.1mg. For all different dissolution conditions in the wheat straw pre-treatment, the applied temperature demonstrated a u(*T*)=1°C. An arbitrary error of 5% was defined to all the FTIR measurements.

2.2 Results

2.2.1 Wheat straw pre-treatment with [emim][OAc]

This work was devoted to the fractionation of the wheat straw from the pre-treatment with [emim][OAc] using a new fractionation pathway based on previously developed methodology.[104] The results of the wheat straw pre-treatment with [emim][OAc] at settled conditions, as well as the yield of IL recovery, are presented in Table 4. Considering the temperature effect, it can be stated that temperature is more important parameter regarding the recovery of cellulose-rich fractions. At 140°C the amount of cellulose-rich fractions obtained (38.4% and 37.1% (w/w)) were equivalent to the content of the cellulose fraction in the native biomass (38.9% (w/w)). An irrelevant effect in the recovery of hemicellulose-rich fractions was verified, where no clear trend was observed at different temperatures (80-140°C). The obtained recovery of hemicellulose varied between 17.4% and 20.8% (w/w) of the initial biomass. A similar effect was also observed for lignin fractions recovery, except those performed at the higher studied temperature. At 140°C, the amount of lignin-rich fractions decreased from ≈10%(w/w) (average observed for the other studied temperatures) to ≈7%(w/w). Regarding the effect of the pre-treatment time, its increase did not affect significantly the amount of lignocellulosic materials recovered. Only at 80°C, a minor effect of the pre-treatment time was verified, where an additional 6h pre-treatment up to 18h led to a decrease by 7%(w/w) of the cellulose recovery.

Table 4 - Results of the wheat straw pre-treatment with [emim][OAc]: fractionation at various pre-treatment temperatures and times. The yield of IL recovery after each pre-treatment is depicted.

T/°C	t/h	Lignocellulosic materials[a]			IL recovery/ %(w/w)
		Cellulose-rich	Hemicellulose-rich	Lignin-rich	
native		**38.9**[b]	**23.5**[b]	**18.0**[b]	
80	12	57.4	17.4	10.1	91.3
	18	50.4	17.6	8.9	95.5
100	6	44.2	19.7	8.9	86.7
	12	48.0	18.7	8.5	94.9
	18	42.1	17.4	10.2	93.2

120	2	45.4	18.8	10.9	91.4
	6	44.5	19.8	11.6	85.9
140	2	38.4	20.8	6.9	95.8
	6	37.1	18.2	7.6	90.3

[a] %(w/w) of dry weight, [b] Wheat straw macromolecular composition.[105]

Concerning the IL recovery at the different conditions, the attained results were at a similar level ranging from 85.9% (w/w) to 95.8% (w/w). However, a noteworthy effect of temperature can be observed in the color of the recovered ILs after pre-treatment. Figure 14 shows the color of the fresh [emim][OAc] and ILs recovered from the pre-treatment at various temperatures. The color of the recovered [emim][OAc] became deeper with an increase of the pre-treatment temperature comparatively with the color of the fresh IL, probably due to temperature effect on the lignin degradation products.

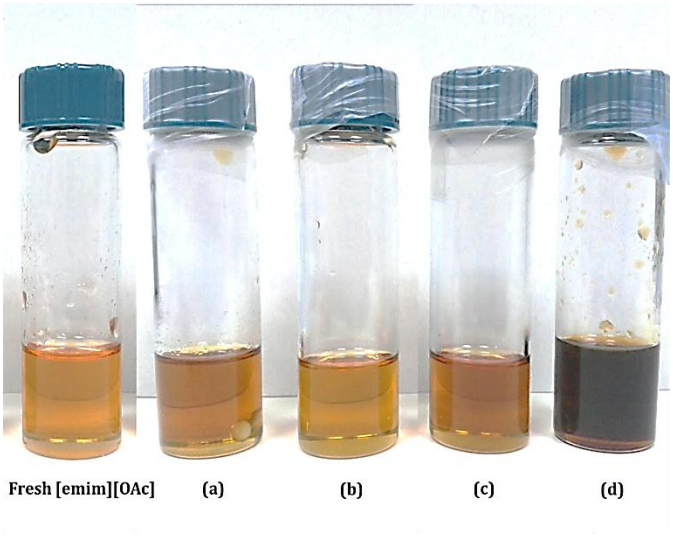

Figure 14 - Color of the fresh [emim][OAc] and recovered ILs after the wheat straw pre-treatment at different dissolution temperatures: (a) 80°C; (b) 100°C; (C) 120°C and (d) 140°C.

2.2.2 FTIR Characterization

2.2.2.1 Qualitative Analysis

Fourier Transform Infrared (FTIR) technique was selected to analyze the chemical characterization of the solid materials recovered from pre-treatments. The main chemical bond vibrations of lignocellulosic materials are identified in the region of 1800-800cm^{-1}. The characteristic absorption bands attributed to carbohydrates – cellulose and hemicellulose – are 1161, 1112-1120 and 897cm^{-1}. Absorption bands at 1376, 1061 and 1025cm^{-1} are also recognized

as cellulose characteristic bands. Characteristic bands of hemicellulose are observed at 1251, 1046 and 990-996cm^{-1}.[108-110] The lignin characteristic absorption bands are displayed at 1718, 1702, 1654, 1508, 1597, 1458, 1420, 1261, 1242, 1224, 1127, 1033 and 840cm^{-1}.[111, 112] An absorption band at 1734cm^{-1}, which is characteristic for untreated lignocellulosic biomass, is associated to ester-linked acetyl, feruloyl and p-coumaroyl groups between hemicellulose and lignin.[113] All the above-mentioned bands are characterized in detail elsewhere.[104]

The FTIR spectra of lignocellulosic materials obtained after the pre-treatment of the wheat straw in [emim][OAc] at different conditions were analyzed.

All spectra of cellulose-rich fractions presented the characteristic vibration bands of cellulose in the region of 1250-850cm^{-1}. The obtained samples using temperatures at 100, 120, 140°C presented well-defined vibrations bands at 897, 1025, 1061, 1161 cm^{-1}. However, the experiments at 80°C demonstrated a slight difference in the shape of cellulose characteristics bands that were not well-defined. Additionally, a vibration band at 1376cm^{-1} assigned to bending of C-H group in cellulose of the native wheat straw was also observed. Furthermore, the band at 1112cm^{-1} is detected in all the cellulose-rich samples, but with low intensity. Pre-treatment at 140°C for a 2h cellulose-rich fraction exhibits a pronounced band at 996cm^{-1} which indicates the presence of arabinosyl side chains (arabinose). Insignificant bands at 1420, 1508, 1654cm^{-1} are observed in all cellulose spectra indicating the presence of lignin. Moreover, the band at 1734cm^{-1} which is related to ester-linked groups between hemicellulose and lignin appears only in spectra of cellulose-rich fractions recovered from both pre-treatments at 80°C. Besides, absorption bands at 2850 and 2920cm^{-1} that are attributed to asymmetric and symmetric C-H stretching of CH, CH_2 and CH_3 are present in all the spectra.

FTIR spectra of hemicellulose-rich fractions presented characteristic absorption bands at 1251, 1046 and 990-996cm^{-1}. The various hemicellulose-rich samples recovered from the pre-treatments at different conditions exhibited a great similarity. Moreover, a vibration band at 2918cm^{-1} is visualized in all the spectra.

The lignin-rich samples' spectra displayed numerous vibration bands owing to the complex structure of lignin. All the recovered lignin materials were successfully fractionated revealing all the lignin characteristic bands. Absorption bands are observed at 1127, 1508, 1597 and 1654cm^{-1} in all the recovered lignin-rich samples. Also, an absorption band at 1033cm^{-1} which is related to the aromatic C-H bond in the plane deformation for guaiacyl units is clearly identified in all the lignin samples. Vibration bands at 2850 and 2920cm^{-1} are present in lignin-rich samples, except in

samples recovered at 120°C and 140°C both for 6h. The absence of carbohydrates is plainly observed in all the lignin-rich samples.

2.2.2.2 Quantitative Analysis

Based on FTIR measurements, a quantitative analysis was performed for each recovered solid fraction in order to evaluate the efficiency of the new method to fractionate lignocellulose. The quantification was made for carbohydrate and lignin contents following the same technique presented elsewhere.[104, 114] The composition determined for the recovered solid fractions at different pre-treatment conditions is presented in Table 5. A general overview indicates that different temperatures and times applied in the pre-treatment of the wheat straw with [emim][OAc] had a significant effect on the purity of the fractionated materials.

Table 5 - Results of the FTIR quantification of the fractionated samples obtained at different pre-treatment temperatures and times.

		Purity/%wt					
T/°C	t/h	**Cellulose**		**Hemicellulose**		**Lignin***	Carbohydrate yield/%
		Carbohydrates	*Lignin*	*Carbohydrates*	*Lignin*		
80	12	64	6	78	6	67	81
	18	79	7	83	7	61	87
100	6	80	9	80	6	63	82
	12	80	7	88	6	78	88
	18	90	8	92	7	65	86
120	2	67	8	80	7	69	73
	6	86	8	96	6	92	92
140	2	84	8	73	7	72	76
	6	91	6	96	8	97	82

*Lignin samples were free from carbohydrates.

The recovered carbohydrate fractions – cellulose and hemicellulose – showed that for each temperature, the prolonged pre-treatment time resulted in higher carbohydrate content. Likewise, higher temperatures led to the higher carbohydrate content. For instance, the pre-treatment at 80°C for 18h gave the carbohydrate content of 79% wt in cellulose-rich samples, while in the pre-treatment at 140°C during 6h a 91% wt carbohydrate content was obtained. Following the same line for hemicellulose-rich samples, pre-treatment at 80°C for 18h gave the carbohydrate content of 83% wt, while at 140°C during 6h a 96% wt carbohydrate content was attained. The lignin content of both cellulose and hemicellulose fractions was relatively low varying from ≈6% to ≈9% wt. On the other hand, the temperature seemed not to have a pronounced effect on the purity of the recovered lignin-rich solid fractions in the pre-treatments performed at 80 and 100°C**.** However, at 120 and 140°C, longer time such as 6h, resulted in high pure solid lignin-rich fractions with 92 and 97% wt of purity, respectively. One of the greatest accomplishes was that all the lignin-rich fractions obtained were free from carbohydrates. Additionally, it can be assumed that dissolution times superior to 12h led to a decrease in the lignin purity.

2.2.3 Enzymatic Hydrolysis

After FTIR analysis of cellulose-rich samples enzymatic hydrolysis was performed in order to investigate the amount of the glucan present in cellulose-rich samples. Glucose yields (% $w/w_{biomass}$) and the amount (mg) of pure cellulose recovered after each pre-treatment, as well as the quantity of other compounds present on cellulose-rich fractions, are displayed in Table 6. The amount of pure cellulose was determined by equation 2.

$$\text{Cellulose}_{\text{mg}} = \text{Recovered Cellulose}_{\text{mg}} \times \%\text{Cellulose}_{\left(\frac{\text{w}}{\text{w}_{\text{biomass}}}\right)} \quad (\textbf{Equation 2})$$

Considering the results presented in Table 6, it can be noticed that with the pre-treatment temperature increase, an enrichment of the glucan content from 59.8 to 81.1% ($w/w_{biomass}$) is observed. The same trend is visualized with the increase of pre-treatment time at one fixed temperature. Furthermore, it can be also observed that higher temperatures (120°C and 140°C) resulted in a more efficient fractionation by the lower amount of other compounds present in cellulose fractions. However, in the experiments using 140°C, a lower amount of pure cellulose was achieved in comparison to pre-treatments at 120°C.

Table 6 - Glucose yields (% $w/w_{biomass}$) and the amounts (mg) of pure cellulose and other compounds present on the recovered cellulose after pre-treatment at different conditions.

Pre-treatment conditions		%Cellulose /($w/w_{biomass}$)	Cellulose /mg	Other compounds* /mg
T/°C	**t/h**			
80	12	59.8	79.0	53.1
	18	60.9	70.7	45.3
100	6	67.9	69.1	32.7
	12	67.5	74.6	35.9
	18	71.9	69.5	27.2
120	2	73.8	77.1	27.2
	6	78.2	79.9	22.4
140	2	74.8	66.1	22.3
	6	81.1	69.2	16.2

*****Other compounds are mainly hemicellulose and lignin.

2.2.4 NMR Analysis

The purity of the recovered ILs after pre-treatments was verified using ^{1}H- and ^{13}C-NMR techniques. The determined chemical shifts of pure [emim][OAc] were as follows: ^{1}HNMR (400 MHz; D_2O) δ(ppm): 1.34 (t, 3H, NCH_2CH_3); 1.77 (s, 3H, CH_3COO); 3.76 (s, 3H, NCH_3); 4.10 (q, 2H, NCH_2CH_3); 7.32 (d, 2H, NCHCHN); 8.58. (s, 1H, CH_3COOH). ^{13}CNMR (D_20) δ(ppm): 14.44 (NCH_2CH_3); 23.26 (CH_3COO); 35.55 (NCH_3); 44.73 (NCH_2CH_3) 121.81 (NCHCHN); 123.37 (NCHCHN); 135.52 (NCHN) and 181.21 (CH_3COO).

2.2.5 Capillary Electrophoresis Analysis

The change in the IL color after the pre-treatment with an increase of the pre-treatment temperature coupled to the loss of almost ≈50% (w/w) of lignin present in the wheat straw led to investigate the presence of phenolic compounds associated to the delignification process during

the pre-treatment with IL. Coupling SPE with CE it was possible to identify the presence of phenolic compounds in the IL solutions. As an example, Figure 15 shows the electropherogram recorded at 320 nm for the phenolic profile of the recovered IL after the pre-treatment at 100°C during 18h. By comparison with electrophoretic data obtained with authentic standards, it was possible to identify vanillin, ferulic acid and coumaric acid with >90% matching. Another unidentified phenolic compound (migration time 7.922min) with a characteristic flavonoid spectrum[115] was also detected. Between the four studied temperatures the phenolic compounds identified were the same. However, it was verified that the most abundant compound differs according to the pre-treatment temperature. Vanillin was a major compound in the recovered ILs from the pre-treatments occurred at 100 and 120°C, but at 140°C the unidentified phenolic compound was the main component present. This was probably due to some degradation caused by the effect of temperature.

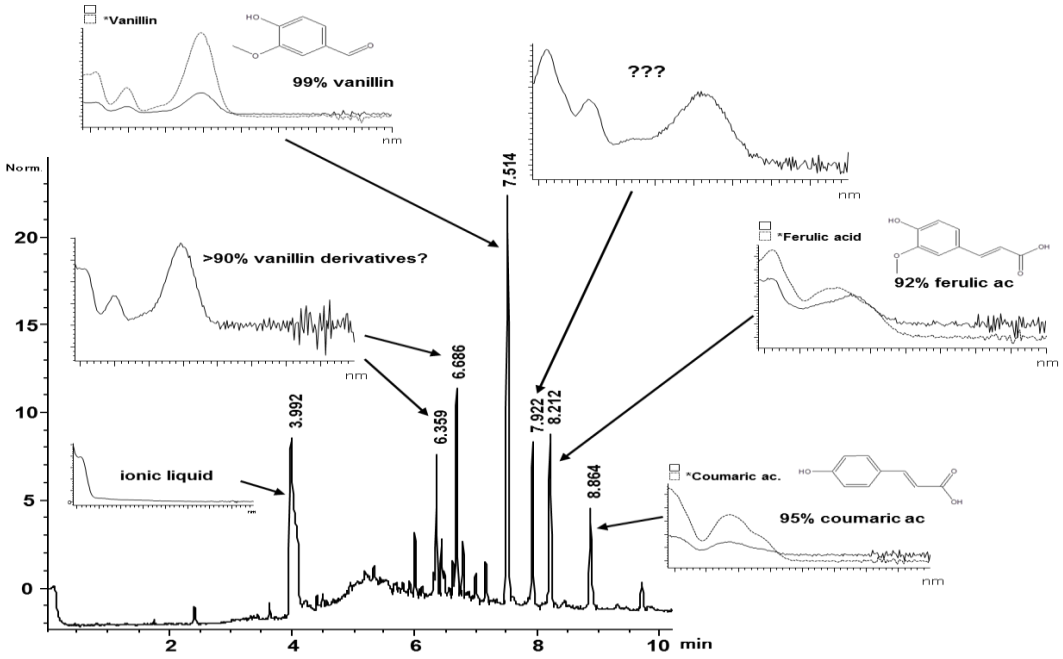

Figure 15- Electropherogram recorded at 320 nm showing the phenolic profile of the recovered IL after pre-treatment at 100°C during 18h.

2.3 Discussion

2.3.1 A three-step fractionation process

A novel three-step fractionation process was developed. Comparatively to previous work, the increase of NaOH concentration from 0.1 M to 3% (≈ 0,75 M) leads to a more effective fractionation of wheat straw into cellulose, hemicellulose and lignin-rich fractions. In this work, the cellulose-rich material is obtained directly by the addition of an anti-solvent. Obtained results

show that the increase of the hydroxyl group concentration leads to higher solubility of lignin and hemicellulosic fractions, therefore, the fractionation process of the carbohydrate-rich material is eliminated. This is contrary to the previous work where the carbohydrate-rich fraction was obtained and subsequently fractionated into cellulose and hemicellulose was needed.[104]

2.3.2 Effect of temperature and time on the pre-treatment of biomass

The effect of the pre-treatment temperature (80-140°C) and time (2-18h) was studied regarding the recovery and purity of the separated lignocellulosic materials. As it is presented in Table 4 and Table 5, at higher temperatures lower amounts of cellulose-rich fractions were recovered, although more pure fractions were achieved. Likewise, longer times at a set temperature gave higher purity cellulose-rich fractions. On the other hand, by changing the conditions on the pre-treatment, approximately the same amount of hemicellulose-rich fractions was obtained. Therefore, the temperature and time have no significant effect on hemicellulose recovery. Notwithstanding, the pre-treatments at 120°C and 140°C during 6h gave high pure hemicellulose-rich fractions attaining a 96% wt carbohydrate content. The same trend was observed in the purity of lignin-rich fractions, where at 120°C and 140°C during 6h the fractions with higher purities (92 and 97% wt, respectively), were obtained. Additionally, close to a 4% (w/w) mass loss of the lignin-rich fraction was noticed with increasing temperature from 120°C to 140°C. This can be justified by the fact that the recovery of lignin can be hampered due to stronger interactions between lignin and [emim][OAc] at higher temperatures, and 140°C is closeness to the glass transition temperature of lignin (165°C).[116]

The temperature affects the viscosity as well as the conductivity of ILs. The [emim][OAc] IL exhibits a relatively low viscosity that facilitates dissolution of cellulose at lower temperatures. The increase in the dissolution temperature increases the diffusivity, both adding thermal energy to the system. However, at higher temperatures thermal degradation of cellulose can occur.[117] Longer times allow the IL to penetrate further into the biomass.

The pre-treatment efficiency of the new method at different pre-treatment conditions was also evaluated by enzymatic hydrolysis of the cellulose-rich fractions. The results of enzymatic hydrolysis (Table 6) followed the same trend exhibited by FTIR results (Table 4). It is clearly visible that at lower temperatures not only glucan is present in cellulose-rich fractions. A larger amount of other compounds, mainly hemicellulose and lignin, are also present. With the temperature increase, enrichment in the glucan content is observed. At the highest temperature studied, an 81.1% ($w/w_{biomass}$) content of glucan was obtained, comparatively to only 59.8% ($w/w_{biomass}$) of the

glucan content obtained from the pre-treatment at 80°C (for 12h process). Concerning the amount of pure cellulose present in each cellulose-rich fraction (Table 6), the higher values obtained for samples recovered at lower temperatures are a consequence of the increase amount of the cellulose-rich material recovered. The selection of the best set of conditions should have in consideration the final use of these fractions. For example, for biofuel production it will probably be more feasible to obtain higher mass of cellulose, even with lower purity, as long as the contamination compounds will not interfere with the rate and performance of enzymatic hydrolysis process. In this way, higher yield of glucose can be accessed for fermentation. On the other hand, high purity cellulose is desirable to produce value added derivative products. Thus, pre-treatment with ILs can be perfectly tuned for biomass processing in biorefinery.

Since the different conditions studied had a direct effect on cellulose recovery, a multiple linear regression with an interaction factor (Equation 3) was made to describe the dependency of cellulose recovery on pre-treatments temperature and time.

$$y = \beta_0 + \beta_1 T + \beta_2 t + \beta_{12} Tt \quad (\textbf{Equation 3})$$

where y is the desired response, T and t correspond to temperature and time and ($\beta_0, \beta_1, \beta_2$ and β_{12}) are the regression coefficients. Matlab® software (version 7.12.0.635) was used to determine the regression coefficients by minimizing the sum of the squares of the deviations of the data from the model (least-squares fit). Regression coefficients were calculated with a 95% level of confidence. The obtained and adjusted models are $\%CR = 79.32 - 0.2753T + 0.3138t - 0.0077Tt$ with R^2 = 0.96 and $\%C = 48.49 + 0.1857T - 2.0157t + 0.0226Tt$ with R^2 = 0.97, where RC – cellulose recovery (% w/w); %C - glucan yield from the enzymatic hydrolysis ($w/w_{biomass}$); T – temperature (°C); t – time (h). From the analysis of the results, it can be assumed a strong dependency between the different pre-treatment conditions and the recovery of cellulose-rich fractions – in terms of the quantity recovered as well as of the enhancement of the enzymatic hydrolysis (Figure 16).

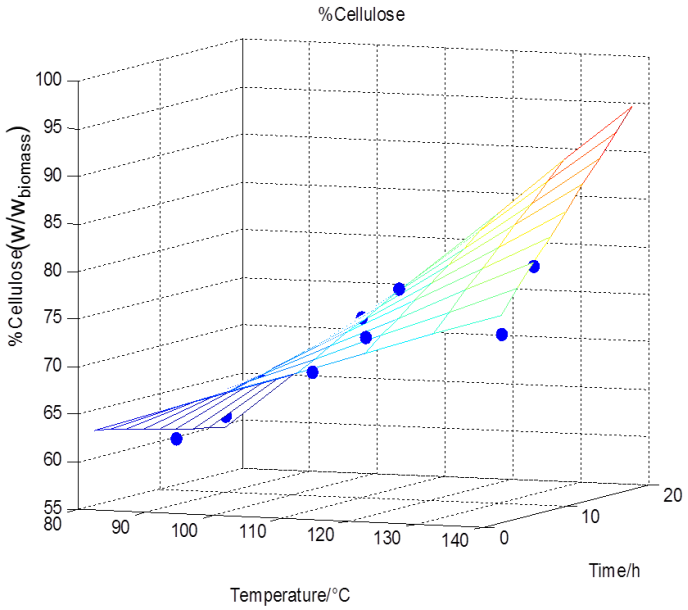

Figure 16- Representation of the adjusted model for %cellulose ($w/w_{biomass}$) obtained from the enzymatic hydrolysis. (●) experimental data.

The optimal result achieved in this work for the recovery of cellulose-rich fractions (37.1% (w/w)) was obtained at 140°C during 6h. Furthermore, the same conditions gave the optimal result in terms of higher amount of the glucan present (81.1% $w/w_{biomass}$) in cellulose-rich fractions. At such conditions a recovery of 18.2% (w/w) was obtained for hemicellulose-rich fractions (purity of 96% wt), and for lignin-rich fractions a recovery of 7.6% (w/w) was attained (purity of 97% wt). Also, at the end of the process it was recovered 90.3% (w/w) of [emim][OAc] from the initial load. Nevertheless, the choice of the optimal pre-treatment conditions has to be optimized according to the biomass and the IL used.

An interesting association can be performed on the relation between the amount of energy required for the pre-treatment process and the purity of the fractionated lignocellulosic materials. An estimation of the energy spent was calculated according to Equation 4. The molar heat capacity (Cp_m) of [emim][OAc] for each dissolution temperature was estimated from the results reported for [bmim][OAc][118] which were adjusted for the temperatures examined in this work taking into account the difference between the literature data for [bmim][OAc][118] and for [emim][OAc].[119] The estimated energy values obtained are illustrated in Figure 17.

$$E = Cp_{m,T} \times m_{IL} \times (T - T_{room}) \times t \quad \textbf{(Equation 4)}$$

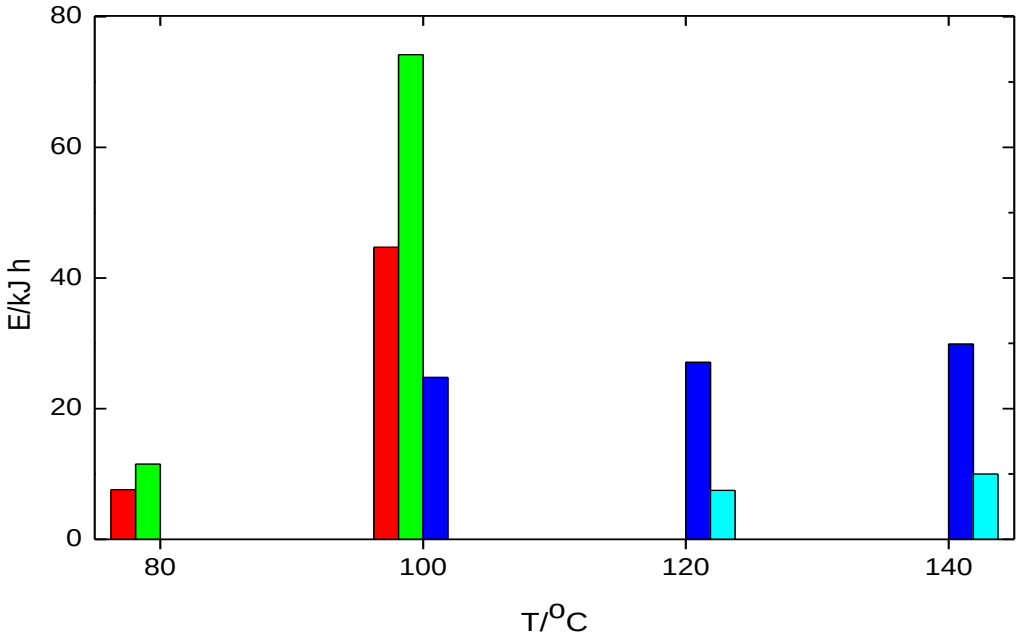

Figure 17- Graphical presentation of the amount of energy spent in the pre-treatments at the different dissolution conditions. The vertical bars present various pre-treatment time in the following manner: light blue – 2 hours, dark blue – 6 hours, red – 12 hours, green – 18 hours.

Considering the cellulose-rich fraction recovered from the pre-treatment at 100°C for 18h (purity of 90% wt) and those recovered from the pre-treatment at 120°C during 6h (purity of 86% wt), a slight difference is observed in the purity of cellulose samples, but in terms of the energy required a significant increase of ≈47 kJ·h is noticed. On the other hand, an increase of 20°C (140°C) in the pre-treatment temperature gave an increase of 5% wt in purity (from 86 to 91% wt), with a small noticeable increase in the energy demand (from 27.1 to 29.9 kJ·h). Concerning the purity of hemicellulose-rich fractions, an identical result was obtained for the experiments at 120 and 140°C during 6h (96% wt). However, a demand of an extra 2.8 kJ·h is required. The same tendency can be observed in the case of the purity of lignin-rich fractions recovered at the same conditions referred earlier. Also, a difference of ≈5% wt between purity of lignin-rich fractions from 120°C and 140°C was attained. Once again, the selection of the best pre-treatment conditions in terms of energetic consumption should be taken into account according to the final use of the processed lignocellulosic materials.

2.3.3 Ionic liquid recovery

The [emim][OAc] recovery obtained in the presented method at different dissolution conditions was mainly superior to 90% (w/w) from the original IL load (Table 4). The presence of impurities within the recovered ILs was analyzed by ^{13}C- and ^{1}H-NMR analysis. Although negligible differences in chemical shifts are visualized between all recovered ILs and the pure [emim][OAc],

the intensity of the chemical shifts related to carbons in position 9 and 10 decreases significantly. According to ^{1}H NMR analysis, the same difference in chemical shift is observed to hydrogen in position 10. The aforementioned chemical shifts are associated with the acetate anion (Figure 18). Therefore, this can suggest the substitution of the acetate anion by hydroxide anion, for instance, due to the use of NaOH as the antisolvent and a pH neutralizer in the IL recovery step.

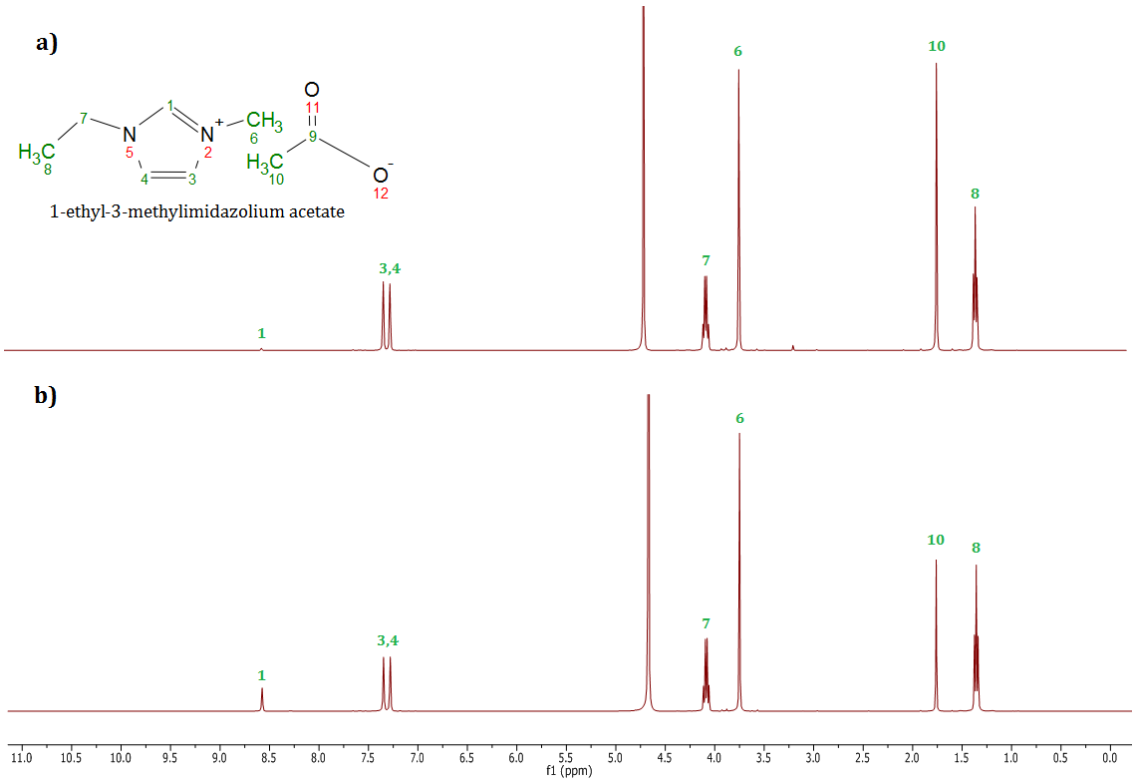

Figure 18- ^{1}H NMR spectra of the a) pure [emim][OAc]; b) recovered [emim][OAc] from pre-treatment at 140°C, 6h.

From Figure 14 it is clearly visible that ILs' color became increasingly darker with the temperature increase. Currently, very limited information concerning the chemical analysis of the recovered ILs is available. Due to the similarity of the color revealed by the recovered ILs with that exhibited by the lignin material, and a loss of ≈36-62% (w/w) of the lignin material from the theoretical lignin,[105] it was found crucial to study the presence of phenolic compounds in the recovered ILs. Additionally, a few papers also refer that the dark color exhibited by the recovered ILs is due to the presence of lignin materials.[86, 120, 121] In fact, Lee et al. [86] reported that the accumulation of lignin in [emim][OAc] is given by the high solubility of lignin in this IL.

In fact, after SPE-CE analysis (Figure 15), the presence of phenolic compounds, namely vanillin and phenolic acids, in the recovered ILs suggests the presence of lignin degradation products. Thus, the change in color intensity with increased temperatures could indicate the existence of other compounds in the recovered IL.

3. CO_2 assisted Pre-treatment

3.1 Materials and Methods

3.1.1 Materials

Wheat straw was supplied by Estação Nacional de Melhoramento de Plantas (Elvas, Portugal). The material was ground with a knife mill (IKA® WERKE, MF 10 basic, Germany) to particles smaller than 1.5 mm, and stowed at room temperature. The wheat straw moisture was found to be 8% (w/w). A CO_2 with purity ≥99.99% bought from Air Liquide, AlphaGaz™ gamma, Paris, France was used. For post-processing filtrations, paper filters (Ø=150 mm, nº 1238) from Filter-Lab, Microchip Technology Inc., Arizona, USA were used. For all experiments the following reagents were used: distilled water (17 $M\Omega cm^{-1}$) produced by the PURELAB Classic Elga system, 72% (w/w) H_2SO_4 aqueous solution was prepared from concentrated H_2SO_4 solution (96% purity) bought from Panreac Química, Barcelona, Spain. In addition, ethanol of 96% purity (v/v) for gas phase capturing was acquired from Carlo Erba Group - Arese, Italy.

3.1.2 CO_2-assisted autohydrolysis of wheat straw

The CO_2-assisted autohydrolysis treatments of wheat straw were performed in a stainless steel 600 mL reactor (series 4560, Parr Instruments Company, Moline, Illinois, USA). The reactor was equipped with two four-blade turbine impellers, and the temperature and pressure were controlled by a Parr PID controller, model 4842. An external fabric mantle was used to heat the reactor, while an internal stainless steel loop was used to cool with cold water the system. In Figure 19 is illustrated a representation of the apparatus used.

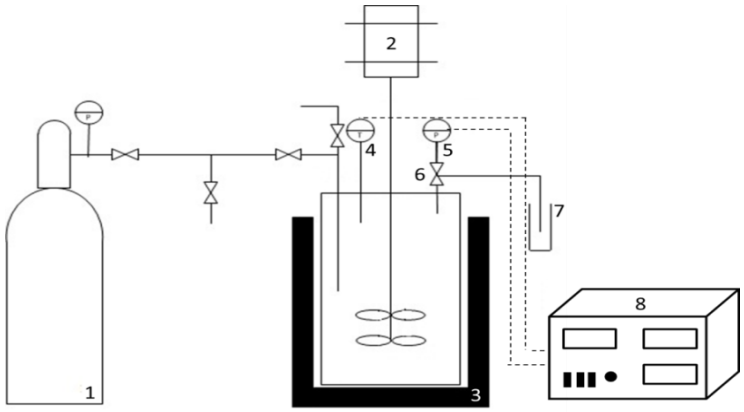

Figure 19 - The scheme of CO_2-assisted autohydrolysis pre-treatment apparatus. 1 – CO_2 cylinder; 2 – magnetic drive; 3 – heating mantle; 4 – thermo par; 5 – pressure transducer; 6 – depressurization valve; 7 – vial filled with ethanol; 8 – pressure and temperature PID controller.

The CO_2-assisted autohydrolyses of wheat straw were carried out at three temperatures, namely 180, 200 and 210°C, selected based on the literature data.[92] An initial pressure of 60 bar at room temperature was maintained constant in all experiments. The reaction mixtures were prepared in the following manner: the liquid to solid ratio was maintained equal in all reactions however various mixture loadings versus volume of the reactor were used: – 250g of H_2O/25g of wheat straw; 150g of H_2O/15g of wheat straw and 75g of H_2O/7.5g of wheat straw. A fixed agitation speed of 70 rpm was used. When the final desired temperature was attained, the reactor was rapidly cooled down to quench the reaction. A slow depressurization (2 bar $minute^{-1}$) of the reaction mixture was executed when the temperature was lower than 20°C to minimise the presence of volatile compounds in the vapour phase. The depressurised gas phase passed through a vial placed in the ice bag filled with known amount of ethanol. This procedure allows for dissolution of volatile compounds for posterior qualitative and quantitative analyses. The liquid (liquor) and solid fractions were recovered through filtration. A GPL 21 pH meter (Crison, Barcelona, Spain) was used to measure the pH of the recovered liquors.

The effect of temperature and non-isothermal operational mode on the composition of both liquid and solid fractions was evaluated in the function of the severity factor (Log R_0)[122] according to the following equation:

$$R_0 = \int_0^t e^{\left(\frac{T(t)-100}{14.75}\right)} dt \quad \textbf{(Equation 5)}$$

, where *t* is time expressed in minutes, T abbreviates temperature (°C) and 14.75 is an empirical parameter related with temperature and activation energy. The qualitative and quantitative analyses of all fractions were performed using the procedures presented below.

3.1.3 Chemical Analyses

3.1.3.1 Characterization of the feedstock material composition

The feedstock material was ground in a knife mill to a particle size <0.5 mm and the moisture was determined by drying at 105°C for at least 16h to obtain constant weight. The biomass was characterized for glucan, xylan, arabinan and acetyl groups after treatment with 72% (w/w) H_2SO_4 according to the standard methods.[123] Syringe filters (0.2 µm) from Whatman, GE Healthcare Life Generations, Buckinghamshire, United Kingdom were used to filtrate all samples before running on HPLC. Monosaccharides (glucose, xylose and arabinose) and acid acetic were analysed by high performance liquid chromatography (HPLC). An Agilent 1100 series HPLC system, Santa Clara, CA, USA equipped with a Bio-Rad Aminex HPX-87H column (Hercules, CA, USA) was used. The set

conditions of the column were: 50°C, 0.4mL·min^{-1} flow rate with 5mM H_2SO_4. A refractive index (RI) detector was employed to examine sugars and acetic acid content. The acid insoluble residue was considered as Klason lignin after correction for the acid insoluble ash (determined by igniting the content at 575 °C for 5 h). Protein quantification was performed by the Kjeldahl method using the Nx6.25 conversion factor.[124]

3.1.3.2 Characterization of the processed solids

The solid fractions were washed with distilled water at room temperature, and oven-dried at 40°C for at least 48h. The processed solids recovered were subjected to the same chemical characterisation of the feedstock except the determination of protein and ash.[123]

3.1.3.3 Liquor and post-hydrolysate characterization

The concentration of reducing sugars (glucose, xylose and arabinose), as well as acetic acid, furfural and hydroxymethylfurfural (HMF) present in the liquor recovered from the CO_2 pre-treatment were analyzed by HPLC. In this case, a flow rate of 0.6 mL·min^{-1} and furfural and HMF analyses occurred with UV/VIS detector at 280nm. The liquor sample was subjected to hydrolysis with 4% (w/w) H_2SO_4 at 121°C for 1h in an autoclave (Uniclave, Portugal) to convert soluble hemicelluloses into their constituent sugar monomers.[125] After post-hydrolysis, oligosaccharides concentrations were expressed as the increase in sugar monomers analyzed through HPLC.

3.1.3.4 Gas phase

The gas phase recovered during slow depressurization was analyzed by HPLC to examine the presence of volatile degradation products, namely furfural, and acetic acid.

3.2 Results

3.2.1 Feedstock composition

Chemical composition of the wheat straw used in this work is presented in Table 7. The wheat straw moisture was found to be at the level of 8% (w/w). A total of 63% (w/w) of wheat straw biomass are polysaccharides among which 38.5% (w/w) is cellulose (estimated as glucan). Wheat straw hemicellulose is constituted by a β-D-(1,4)-linked xylopyranosyl backbone, substituted with arabinofuranose, 4-O-methylglucuronic acid, acetyl groups, xylose and phenolic acids.[92] The total hemicellulose was measured as the sum of xylan, arabinan and acetyl groups content revealing 24.9% (w/w). In relation to the Klason lignin content, the obtained value was corrected for the ash content of acid insoluble residue and it was determined to be at the level of 17.7% (w/w). The obtained data are in a good agreement to those reported by Carvalheiro et al.[92]

Table 7 - Macromolecular composition of wheat straw (% of dried weigh).

Component	This work[a]	Carvalheiro et al.[92]
Cellulose[b]	38.5±0.1	38.9±0.2
Hemicellulose	24.9	23.5
Xylan	19.1±0.6	18.0±0.3
Arabinan	3.0±0.1	3.0±0.2
Acetyl groups	2.7±0.2	2.5±0.1
Klason lignin	17.7±0.1	18.0±0.5
Ash	10.7±0.1	9.70±0.03
Protein	4.7±0.1	4.5±0.5
Others	3.5	5.5

[a] Average of two replicates; [b] Determined as glucan; [C] Determined by difference.

3.2.2 Composition of the liquors

The wheat straw CO_2-assisted autohydrolyses resulted in liquors containing a mixture of sugar oligomers (mainly XOS), monosaccharides (glucose, xylose and arabinose), acetic acid (from acetyl groups present in hemicelluloses) and sugars degradation products, namely HMF and furfural. According to the literature reports, the formation of these chemicals depends on the pre-treatment conditions that are biomass type, temperature and treatment time. The composition of liquors obtained from the CO_2-assisted autohydrolysis at various conditions is depicted in Table 8.

Table 8 – Composition of the liquors ($g \cdot L^{-1}$) from CO_2–assisted autohydrolysis of wheat straw with initial CO_2 pressure equal to 60 bar.

Biomass loading	**250/25**[a]		**150/15**[a]			**75/7.5**[a]	
T (°C)	*210*	*180*	*200*	*210*	*180*	*200*	*210*
Log R_0	3.54	2.58	3.16	3.44	2.60	3.08	3.48
pH	3.85	4.38	4.13	3.93	4.55	4.39	4.03
Composition ($g \cdot L^{-1}$)							
XOS	10.03	5.46	11.38	11.79	10.64	12.91	15.75
GlcOS	4.34	3.52	3.43	3.20	5.24	5.09	4.14
AcO	0.70	1.48	1.22	1.11	1.78	1.28	1.21
Xylose	3.40	2.04	2.44	4.03	0.49	0.51	3.34
Arabinose	0.86	1.17	1.25	2.04	0.42	0.51	2.14
Glucose	1.20	1.13	1.22	1.76	0.38	0.40	2.03
Acetic Acid	2.36	0.60	1.03	3.03	1.14	1.59	2.71
HMF	0.14	0.00	0.08	0.20	0.03	0.04	0.13
Furfural	5.38	0.06	0.52	4.60	0.33	0.72	3.19
Klason Lignin[b]	0.44	0.31	0.48	0.22	0.21	0.31	0.23

[a] g of water/g of wheat straw; [b] dissolved lignin (g); XOS - xylooligosaccharides; GlcOS - gluco-oligosaccharides; AcO - acetyl groups linked to oligosaccharides.

Xylooligosaccharides (XOS) were the main components present in liquors in all reactions. Considering all biomass loading studied, the highest amount of XOS produced was found to be at the most severe condition. On the other hand, at the highest biomass loading (250/25) and at 210°C, the concentration of XOS is 10.03 $g \cdot L^{-1}$ and it is comparable to the concentration (10.64 $g \cdot L^{-1}$) obtained with the lowest biomass loading (75/7.5), but at the less severe conditions (180°C). The remaining oligosaccharides (GlcOS and AcO) exhibited a significant concentration in the liquid fraction, which decrease with the increase of the severity of the conditions. On the other hand, xylose was the main monosaccharide present in all assays, revealing the highest concentration value at the most severe conditions. The same trend can be observed for monomers of glucose, arabinose as well as for acetic acid. The sugar degradation products, HMF and furfural, were detected in low amount in reactions executed except the reactions at the harshest conditions for which an increase is more pronounced as furfural concentration increases by 9 and 4.5 time in case of transition from 200 to 210°C for 150/15 and 75/7.5 biomass loading, respectively. The analysis of the influence of CO_2 amount shows that larger amount of CO_2 obtained by the relative reduction of biomass amount present in the reactor by half guides to an increase of XOS

recovered by 1/3 (at 210°C) and is counterbalanced by a reduction of xylose and furfural concentrations by a 17% and 30%, correspondingly.

3.2.3 Composition of the processed solids

The results of the composition of the processed solids as well as the solid yield are demonstrated in Table 9. The hydrolysis condition affects the solid yield recovery. The elevated temperature and thus *Log* R_0 guides to the decrease of the solid yield and it decreases from approximately 70-78% to around 55% depending on the biomass loading used.

Table 9 - The solid phase composition (g·(100g processed solids)$^{-1}$) and solid yield (g·(100g feedstock)$^{-1}$) obtained after CO_2-assisted autohydrolysis of wheat straw for different biomass loading.

Biomass loading[a]	**250/25**	**150/15**			**75/7.5**		
T (°C)	*210*	*180*	*200*	*210*	*180*	*200*	*210*
Log R_0	3.54	2.58	3.16	3.44	2.60	3.08	3.48
Solid Yield	56.27	77.84	60.54	55.98	70.54	62.89	54.56
Glucan	58.41	45.50	49.89	55.56	54.10	54.36	64.33
Xylan	8.90	15.56	6.72	5.00	9.16	5.89	2.23
Arabinan	0.04	0.53	0.00	0.00	0.06	0.00	0.00
Acetyl groups	1.35	2.63	0.46	0.00	2.67	1.21	0.00
Klason Lignin	28.30	19.99	23.87	28.88	20.99	21.52	26.74

[a] g of water/g of wheat straw.

On the other hand, the amount of xylan in the processed solids decrease as the severity of the conditions increase and a complete removal of arabinan from the processed solids is verified except for the reaction at 180°C in which a noticeable amount of arabinan (0.53 g·(100g feedstock)$^{-1}$) was detected in the solid phase. Additionally, harsher reaction conditions facilitate the complete dissolution of acetyl groups as they are absent in the solid phase. Additionally it can be observed that the applied treatment does influence neither cellulose nor Klason lignin.

3.2.4 Composition of the recovered gas phase

The presence of significant amounts of furfural was detected in the gas phase recovered from the reaction mixture depressurization procedure. Figure 20 depicts the influence of the biomass loading and reaction temperature on the amount of furfural recovered from the gas phase. The increase of furfural formation is observed with the increase of the process temperature.

Furthermore, lower amount of biomass loaded to the reactor counterbalanced by the larger amount of CO_2 presented affects the furfural removal as well.

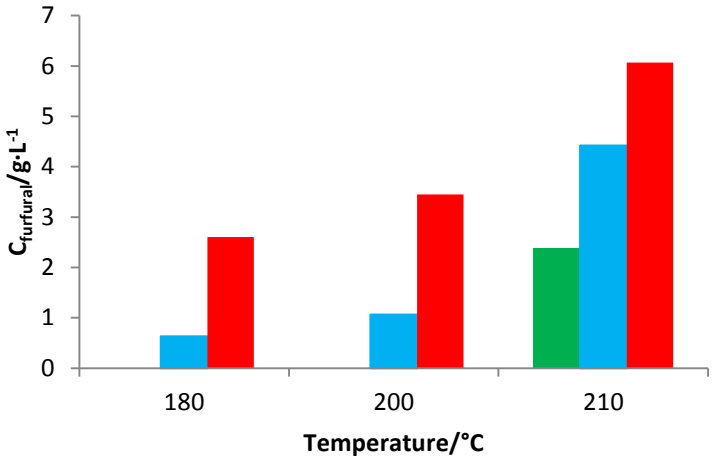

Figure 20 - Furfural concentration in the recovered gas phase from depressurization for the studied temperatures and biomass loadings. Green bar - 250g of water/25g of biomass, blue bar - 150g of water/15g of biomass, red bar - 75g of water/7.5 g of biomass.

3.3 Discussion

3.3.1 Effect of temperature

The wheat straw CO_2-assisted autohydrolyses were carried out at three temperatures (180, 200 and 210°C). The temperature selection has been performed based on the literature data.[92] Two different biomass loadings were used to study the influence of temperature (Table 8). In case of both examined ratios (150/15 and 75/7.5) the release of xylose and XOS increased with the increase of temperature of the process. In fact the increase of reaction severity is responsible for the *in situ* water autohydrolysis that enables the disruption of the recalcitrant structure and, hence, guides to easier hydrolysis of hemicellulose producing XOS-rich liquors.[126] Similarly to xylose and XOS, the increase of arabinose and acetic acid concentrations is observed with the increase of temperature. At 210°C a 3-fold higher acetic acid concentration in the liquor was observed than at 200°C. Furthermore, achieved results permit to conclude that higher temperature of the process lead to the formation of more sugar derived degradation products. Although at 180 and 200°C an insignificant concentrations of furfural and HMF were detected but at 210°C furfural in concentration equals to 4.60 $g \cdot L^{-1}$ was observed. It is caused by the fact that at the examined condition, 85% of initial xylan present in the raw feedstock was dissolved while for example at 180°C much lower xylan dissolution occurred 37%. It is worth to underline that at the same conditions the concentration of HMF remains very low (0.20 $g \cdot L^{-1}$) although the dissolution

of cellulose occurs 19.3%. Therefore the severity of the pre-treatment seems to be insufficient to produce degradation products such as HMF.

The chemical composition of the processed solids (Table 9) shows the enrichment of glucan and lignin contents with the increase of temperature. At the most severe condition 19.3% of glucan present in raw feedstock was dissolved to the liquid fraction. In addition, a high recovery of 91% of lignin from the initial amount of lignin present in wheat straw (Table 7) was also attained and only a 0.22g of lignin was dissolved to the liquor. This strongly indicates that the CO_2 presence does not drive to the significant dissolution of lignin. The amount of xylan present in the processed solid decreased with the increase of temperature, and at 210°C a recovery of 14.6% was obtained. Therefore to achieve a complete removal more severe conditions are required although the results obtained in this work as well as presented in literature[92] show that at higher temperature more degradation products are formed (Table 8). A complete removal of acetyl groups and arabinan from the solid was attained at 210°C.

The pH of the liquor obtained after the CO_2-assisted treatments varies with temperature. The increase of the pre-treatment temperature led to lower pH for the same biomass loading. These results are in good agreement with the previous observation. The higher temperature decreases the solubility of gases therefore less CO_2 is soluble in the liquid phase. Following the aforementioned explanation, after the depressurization, the equilibrium in the system can be achieved easier thus the pH of the liquor should be higher as it is observed.

3.3.2 Effect of CO_2

3.3.2.1 Influence of CO_2 presence

The obtained results show that the presence of *in-situ* formed carbonic acid enhances the hydrolysis of hemicellulose fractions. A previous literature results demonstrate that with pure xylan, carbonic acid significantly increases hydrolysis activity comparing to the CO_2-free autohydrolysis process.[102] Similar conclusion can be taken from the results presented in this work as they illustrate that addition of CO_2 guide to an increase of XOS concentration when compared to CO_2-free autohydrolysis as reported elsewhere.[92] It is especially evident for the same severity factor ($Log\ R_0$ = 3.53) and for the same biomass loading (250g water/25g biomass) as reported in literature[92]. An increase by 65% and 100% of XOS and xylose concentration respectively can be observed. The presence of GlcOS (gluco-oligosaccharides) in the liquor was also found to be higher when CO_2 was added (6-fold higher) to the reaction. This indicates that the presence of CO_2

leads to the dissolution of cellulose, even in less severe conditions than without CO_2[92] but further degradation of hexoses to HMF was negligible as HMF was detected in the minimal concentration (0.14 $g \cdot L^{-1}$). On the other hand, the presence of CO_2 contributes to the formation of further degradation products from hemicellulose fraction e.g. furfural. This is caused by the easier degradation of pentoses to furfural while hexoses are less susceptible for the degradation to HMF.

Furthermore, CO_2 plays an important role in the pH of hydrolysate. It was found that pH varies from 3.85 to 4.55. The decrease of the hydrolysate pH can be explained by the fact that carbonic acid is formed *in-situ* especially that no additional acetic acid comparing to CO_2-free autohydrolysis reaction[92] is produced. Conversely, Walsum et al.[127] revealed that CO_2 addition drive to increase of the final pH of the hydrolysate in comparison to autohydrolysis without CO_2. This inconsistence in results between presented by Walsum et al.[127] and in this work comes from the difference in the reaction conditions. The work of Walsum shows that the CO_2/water ratio is equals to 0.04 while in this work the CO_2/water ratio is at least 3-fold higher. Therefore, relatively higher amount of CO_2 guides to considerable lower pH created in the course of the reaction, thus after the depressurisation, CO_2 dissolved in the liquid phase acts as acidifier of the medium.
Comparison of this result with that obtained by Carvalheiro et al.[92] illustrates that the amount of XOS depends on the CO_2 presence, and the same concentration of XOS can be achieved at less severe conditions. To produce 10 $g \cdot L^{-1}$ of XOS a 5°C higher temperature is needed that also translates to by 11% higher *Log* R_0.

The effect of CO_2 is also observed in the composition of the processed solids. The main differences between treatments with and without CO_2[92] are visible in enrichment of glucan content and Klason lignin in case of CO_2-assisted reactions, as well as a complete removal of arabinan. In addition, lower xylan content is observed in the wheat straw CO_2 pre-treatment. It indicates that CO_2 augment the dissolution of hemicellulose (xylan, arabinan and acetyl groups) and retains cellulose and lignin.

3.3.2.2 Influence of CO_2 concentration

To examine the influence of CO_2 on the obtained results, the CO_2 concentration has been calculated. For this purpose the Peng-Robinson equation of state (PR-EOS) with the initial temperature of 20°C and pressure of 60 bar were used. The CO_2 density was calculated using the following relation:

$$P = \frac{RT\rho}{(1-b\rho)} - \frac{a\rho^2}{1+2b\rho-b^2\rho^2} \quad (\textbf{Equation 6})$$

where $a \equiv a_c\alpha$; $a_c \equiv 0.45723553\frac{R^2{T_c}^2}{P_c}$; $\alpha \equiv \left[1+\kappa(1-\sqrt{T_r})\right]^2$;

$\kappa \equiv 0.37464 + 1.54226\omega - 0.26993\omega^2$; $b \equiv 0.07779607R\frac{T_c}{P_c}$

The constants used are: T_c (CO_2) = 304.2K; P_c (CO_2) = 73.8 bar; ω (acentric factor) = 0.228;[128] R (gas constant) = $8.314\cdot10^{-2}$ L·bar·K^{-1}·mol^{-1}.

Table 10 - The CO_2 density predicted by the Peng-Robinson (PR-EOS), as well as number of CO_2 moles present in the reactor at initial conditions.

Biomass loading [a]	250/25	150/15	75/7.5
ρ(CO_2)/mol·dm^{-3}		5.071	
"Head space" [b]/mL	325.0	435.0	517.5
n(CO_2)/mol	1.65	2.21	2.62

[a] g of H2O/g of wheat straw; [b] "Head space" was determined by the difference between the reactor volume (600 mL) and the volume occupied by the biomass loaded.

The CO_2 amount affects the XOS recovery. The increase of CO_2 concentration makes liquors more reach in hemicellulose products. Figure 21 depicts that at 210°C, an increase of 17% and 57% of XOS concentration is attained with the reduction of water/wheat straw loading from 250/25 to 150/15 and to 75 g of H_2O/7.5 g of wheat straw, respectively.

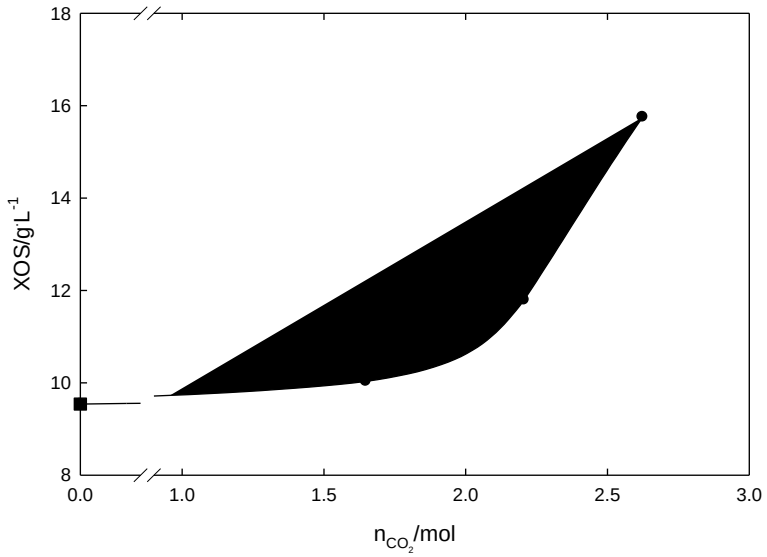

Figure 21- The XOS concentration (g·L^{-1}) as a function of CO_2 number of moles at 210°C. The data for CO_2-free reaction (■) taken from literature.[92]

A biomass loading reduction by half (from 150/15 to 75/7.5) led to an increase of number of moles of CO_2 by more than 20% (Table 10). Therefore it is expected that more CO_2 present in the system should catalyse hydrolysis of hemicellulose to XOS by the *in-situ* generation of carbonic acid thus pH of hydrolysate should be lower. As it is expected the XOS concentration increases with the increase of CO_2 concentration in the system however the pH of the solution became less acidic. This controversial result can be explained by the fact that after the CO_2 decompression, a prolonged time is needed to achieve the equilibrium in the system. In other words, the pH measurement done immediately after the experiment is performed at non-equilibrium conditions. Furthermore, time needed to achieve the equilibrium is strongly depended on the amount of water present in the system due to the diffusion limitation of CO_2 in the liquid phase.

3.3.3 Volatile products

The volatile compounds being formed from the hemicellulose fraction has been found in the gaseous phase. The obtained data depicted in Figure 20 show that the biomass loading and reaction temperature plays an important role in the amount of furfural recovered. The increase of furfural formation is observed with the increase of the process temperature as well as a function of CO_2 present. Temperature effect on furfural formation has already been discussed in this work. Other important aspect influencing the furfural volatility is the presence of acetic acid. To examine the acid-base interaction between furfural and acetic acid, the effect of different contents of acetic acid on the distribution behaviour of furfural and the solvent properties of the weak acid on carbon dioxide has to be taken into account. The literature results show that up to a concentration of 5 wt% acetic acid has modifier properties and enhances furfural extraction.[129]

Other interest aspect is that, although acetic acid is present in the liquor it was not detected in the gas phase entrapped after the reaction. The estimation of the VLE data for system CO_2+water+acetic acid shows that at the reaction conditions, the solubility of acetic acid is negligible.[130] This occurs also due to the fact that in the presence of CO_2 exists equilibria between CO_2, H_2O and acetic acid thus acetic acid is dissolved in water not only in molecular but also ionic form[131] inhibiting its volatility.

4. Conclusions

Lignocellulosic biomass is among the most promising renewable feedstocks for the production of energy and chemicals. Due to the complex and recalcitrance structure of lignocellulosic materials pre-treatment technologies are needed in order to valorize the low value feedstock. This work showed the potential existing in two different alternative solvents, namely ionic liquids and supercritical fluids, which were successfully used in the pre-treatment of wheat straw.

A new methodology of fractionation of the wheat straw into cellulose, hemicellulose and lignin-rich fractions at various temperature and the pre-treatment time using [emim][OAc] in a rapid and simple three-step process was developed. Among studied parameters (temperature (80-140°C) and time (2-18h)), higher temperature and prolonged reaction time favored production of high purity of fractionated lignocellulosic materials, as well as the release of glucose from cellulose-rich fractions. In more detail, the wheat straw pre-treatment at 140°C and at 6h gave the highest purity fractions of lignocellulosic materials (91% wt cellulose, 96% wt hemicellulose and 97% wt lignin) and a glucan content of 81.1% ($w/w_{biomass}$). Moreover, the presence of valued-added phenolic compounds (e.g. vanillin) was detected in the recovered ILs. After IL pre-treatment it was possible to recover cellulose, hemicellulose and lignin-rich solid fractions, along with a recovery of [emim][OAc] mainly superior than 90% (w/w) from the initial IL load.

Second type of alternative solvent used in the biomass fractionation was applied in the autohydrolysis process. The CO_2-assisted autohydrolysis treatment of wheat straw was investigated, in order to selectively dissolve the hemicellulose fraction. In other words, the autohydrolysis with CO_2 allowed to produce a liquid fraction rich in hemicellulose (mainly in oligomeric form) and a solid containing mainly glucan together with lignin. The *in-situ* formation of carbonic acid resulted in a higher hemicellulose dissolution in comparison to CO_2-free autohydrolysis of wheat straw at the analogous conditions (temperature and LSR). Additionally, higher amount of CO_2 obtained by the relative reduction of biomass amount treatment guided to an increase by more than 60% of XOS recovered. Furthermore, an enrichment of 20% (w/w) of the glucan content in the recovered solid fraction was also verified at the same conditions with the CO_2-assisted autohydrolysis pre-treatment. One of the principle advantages of the use of CO_2 over the conventional solvents, namely organic acids, is facile removal of CO_2 by the depressurization without the contamination of the reaction mixture.

Several mathematical, analytical, chemical and biochemical techniques employed in the analysis of the executed processes have been used. Regression analyses, Fourier-transform Infrared Spectroscopy, High Performance Liquid Chromatography, Nuclear Magnetic Resonance, Solid Phase Extraction, Capillary Electrophoresis, enzymatic hydrolysis, post-hydrolysis, quantitative acid hydrolysis were used in this work proving their robustness, versatility, and usefulness in the executed works.

Both presented alternative processes demonstrate several technological and environmental benefits, mainly related to solvent recycle, limited equipment corrosion, reduction of operational costs (no catalysts are needed) and lower water usage. Thus, examined processes facilitate their integration in the broader concepts such as biorefinery and in general are in the agreement with the Green Chemistry approach.

Within the biorefinery framework it can be sustained the production of value-added products from the both presented alternative methods, specifically cellulose-rich fractions which can be subjected to enzymatic hydrolysis for bioethanol production, extraction of phenolic compounds, production of XOS and other bio-based products. Therefore, an integrated biorefinery approach that enables the selective fractionation of wheat straw into its main molecular components and its subsequent individual upgrade is one of the advantages of the examined processes.

The study of different biomass and ILs as well as different processing conditions (temperature, time, biomass/IL ratio) should be investigated in order to verify the applicability and versatility of the optimized method developed. The major costs involved in the overall process could be associated to ionic liquid losses, thus ionic liquid recovery should be one of the principle aims of future works. Additionally, further studies are required for the purification of the recovered ILs and the extraction of phenolic compounds from ILs. Furthermore, to perform a quantitative CE of the phenolic compounds produced during the pre-treatments would be also advantageous to evaluate the economic efficiency of the process.

Regarding the effect of CO_2 in an autohydrolysis process, additional studies are needed in order to determine the optimal conditions at which the consensus between temperature/initial pressure along with hemicellulose dissolution is attained without the extensive degradation product formation. In addition, to construct the equipment that permits for on-line measurements of pH and analysis of phase compositions.

Moreover, the scale up of the optimized pre-treatments should be attained to verify the feasibility for a future application in industry.

5. References

1. Anastas P.T. and Warner J.C., *Green Chemistry: theory and practice*, Oxford University Press:New York, 1998.
2. Manley, J. B., Anastas, P. T. and Cue Jr, B. W., Frontiers in Green Chemistry: meeting the grand challenges for sustainability in R&D and manufacturing. *Journal of Cleaner Production*, 2008. **16**(6). 743-750.
3. Brundtland, G., *Our common future: the world commission on environment and development*, ed. Oxford, Oxford University Press, 1987.
4. Manley J.B., Anastas P.T. and Berkeley, W. a. C. J., Frontiers in Green Chemistry: meeting the grand challenges for sustainability in R&D and manufacturing. *Journal of Cleaner Production* 2008. **16**. 743-750.
5. Kirchhoff, M. M., Promoting sustainability through green chemistry. *Resources, Conservation and Recycling*, 2005. **44**. 237–243.
6. Domanska, U. and Bogel-Lukasik, R., Physicochemical properties and solubility of alkyl-(2-hydroxyethyl)-dimethylammonium bromide. *The Journal of Physical Chemistry B*, 2005. **109**(24). 12124-12132.
7. Zakrzewska, M., Bogel-Łukasik, E. and Bogel-Łukasik, R., Solubility of Carbohydrates in Ionic Liquids. *Energy & Fuels*, 2010. **24**(2). 737-745.
8. Anastas, P. T. and and Kirchhoff, M. M., Origins, Current Status, and Future Challenges of Green Chemistry. *Accounts of chemical research*, 2002. **35**. 686-694.
9. Chowdhury, S., Mohan, R. S. and and Scott, J. L., Reactivity of ionic liquids. *Tetrahedron*, 2007. **63**(11). 2363-2389.
10. Sowmiah, S., Srinivasadesikan, V., Tseng, M. and Chu, Y., On the Chemical Stabilities of Ionic Liquids. *Molecules*, 2009. **14**(9). 3780-3813.
11. Plechkova, N. V. and Seddon, K. R., Applications of ionic liquids in the chemical industry. *Chemical Society Reviews*, 2008. **37**(1). 123-150.
12. Fernando, S., Adhikari, S., Chandrapal, C. and Murali, N., Biorefineries: Current Status, Challenges, and Future Direction. *Energy & Fuels*, 2006. **20**(4). 1727-1737.
13. Kaparaju, P., Serrano, M., Thomsen, A. B., Kongjan, P. and Angelidaki, I. , Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. *Bioresource Technology*, 2009. **100**(9). 2562-2568.

14. Laser, M., Jin, H., Jayawardhana, K. and Lynd, L. R., Coproduction of ethanol and power from switchgrass. *Biofuels, Bioproducts and Biorefining*, 2009. **3**(2). 195-218.

15. Cherubini, F., The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. *Energy Conversion and Management*, 2010. **51**(7). 1412-1421.

16. Council, E. P., Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing, 2009.

17. IEA *CO_2 emissions from fuel combustion - Highlights*. 2011. 1-134.

18. Carvalheiro, F., Duarte, L.C. and Gírio, F.M., Hemicellulose biorefineries: a review on biomass pretreatments. *Scientific & Industrial Research*, 2008. **67**. 849-864.

19. Lopes, A. M. S. Pre-treatment of lignocellulosic biomass with ionic liquids. *Master.* Universidade de Aveiro, 2012. 1-134

20. Fu, D., Mazza, G. and Tamaki, Y., Lignin Extraction from Straw by Ionic Liquids and Enzymatic Hydrolysis of the Cellulosic Residues. *Journal of Agricultural and Food Chemistry*, 2010. **58**(5). 2915-2922.

21. Zavrel, M., Bross, D., Funke, M., Büchs, J. and Spiess, A.C., High-throughput screening for ionic liquids dissolving (ligno-)cellulose. *Bioresource Technology*, 2009. **100**(9). 2580-2587.

22. Naik, S. N., Goud, V. V., Rout, P. K. and Dalai, A. K., Production of first and second generation biofuels: A comprehensive review. *Renewable and Sustainable Energy Reviews*, 2010. **14**(2). 578-597.

23. Godliving, Y. S. M., Recent advances in pretreatment of lignocellulosic wastes and production of value added products. *African Journal of Biotechnology*, 2009. **8**(8). 1398-1415.

24. Van Spronsen, J., Cardoso, M.A.T., Witkamp, G., Jong, W. and Kroon, M.C., Separation and recovery of the constituents from lignocellulosic biomass by using ionic liquids and acetic acid as co-solvents for mild hydrolysis. *Chemical Engineering and Processing: Process Intensification*, 2011. **50**(2). 196-199.

25. Sun, Y. and Cheng, J., Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 2002. **83**(1). 1-11.

26. Sjöström, E., *Wood Chemistry: Fundamentals and Applications*. 2 ed 1993: Academic Press. 293.

27. Richardson, S. and Gorton, L., Characterisation of the substituent distribution in starch and cellulose derivatives. *Analytica Chimica Acta*, 2003. **497**(1–2). 27-65.

28. Zakrzewska, M. E., Bogel-Łukasik, E. and Bogel-Łukasik, R., Ionic liquid-mediated formation of 5-hydroxymethylfurfural-a promising biomass-derived building block. *Chemical Reviews*, 2011. **111**(2). 397.

29. Harmsen, P., Huijgen, W., Bermudez, L. and Bakker, R., *Literature review of physical and chemical pretreatment processes for Lignocellulosic biomass* 2010: Wageningen UR, Food & Biobased Research.

30. Singh Sanjay, K. and Gross Richard, A., *Overview: Introduction to Polysaccharides, Agroproteins, and Poly(amino acids)*, in *Biopolymers from Polysaccharides and Agroproteins*2001, American Chemical Society. 2-40.

31. Moure, A., Gullón, P., Domínguez, H. and Parajó, J. C., Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. *Process Biochemistry*, 2006. **41**(9). 1913-1923.

32. Belgacem, M. N. and Gandini, A., *Monomers, Polymers and Composites from Renewable Resources* 2008: Elsevier Science.

33. Lan, W., Liu, C.-F. and Sun, R.-C., Fractionation of Bagasse into Cellulose, Hemicelluloses, and Lignin with Ionic Liquid Treatment Followed by Alkaline Extraction. *Journal of Agricultural and Food Chemistry*, 2011. **59**(16). 8691-8701.

34. Cathala, B., Saake, B., Faix, O. and Monties, B., Association behaviour of lignins and lignin model compounds studied by multidetector size-exclusion chromatography. *Journal of Chromatography A*, 2003. **1020**(2). 229-239.

35. Buranov, A. U., Ross, K. A. and Mazza, G., Isolation and characterization of lignins extracted from flax shives using pressurized aqueous ethanol. *Bioresource Technology*, 2010. **101**(19). 7446-7455.

36. Mansouri, N.-E. and El Salvadó, J., Structural characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins. *Industrial Crops and Products*, 2006. **24**(1). 8-16.

37. Freire, M. G., Carvalho, P. J., Fernandes, A. M., Marrucho, I. M., Queimada, A. J. and Coutinho, J. A. P., Surface tensions of imidazolium based ionic liquids: Anion, cation, temperature and water effect. *Journal of Colloid and Interface Science*, 2007. **314**(2). 621-630.

38. Freire, M. G., Santos, L. M. N. B. F., Fernandes, A. M., Coutinho, J. A. P. and Marrucho, I. M., An overview of the mutual solubilities of water–imidazolium-based ionic liquids systems. *Fluid Phase Equilibria*, 2007. **261**(1–2). 449-454.

39. Reichardt, C., Polarity of ionic liquids determined empirically by means of solvatochromic pyridinium N-phenolate betaine dyes. *Green Chemistry*, 2005. **7**(5). 339-351.

40. Freire, M. G., Teles, A. R. R., Rocha, M. A. A., Schröder, B., Neves, C. M. S. S., Carvalho, P. J., Evtuguin, D. V., Santos, L. M. N. B. F. and Coutinho, J. A. P., Thermophysical Characterization of Ionic Liquids Able To Dissolve Biomass. *Journal of Chemical & Engineering Data*, 2011. **56**(12). 4813-4822.

41. Rogers, R. D. and Seddon, K. R., Ionic Liquids--Solvents of the Future? *Science*, 2003. **302**(5646). 792-793.

42. Seddon, K. R., Ionic Liquids for Clean Technology. *Journal of Chemical Technology & Biotechnology*, 1997. **68**(4). 351-356.

43. Gardas, R. L. and Coutinho, J. A. P., A group contribution method for viscosity estimation of ionic liquids. *Fluid Phase Equilibria*, 2008. **266**(1–2). 195-201.

44. Fendt, S., Padmanabhan, S., Blanch, H. W. and Prausnitz, J. M., Viscosities of Acetate or Chloride-Based Ionic Liquids and Some of Their Mixtures with Water or Other Common Solvents. *Journal of Chemical & Engineering Data*, 2010. **56**(1). 31-34.

45. Marsh, K. N., Boxall, J. A. and Lichtenthaler, R., Room temperature ionic liquids and their mixtures—a review. *Fluid Phase Equilibria*, 2004. **219**(1). 93-98.

46. Gírio, F. M., Fonseca, C., Carvalheiro, F., Duarte, L. C., Marques, S. and Bogel-Łukasik, R., Hemicelluloses for fuel ethanol: A review. *Bioresource Technology*, 2010. **101**(13). 4775-4800.

47. Zhao, D., Wu, M., Kou, Y. and Min, E., Ionic liquids: applications in catalysis. *Catalysis Today*, 2002. **74**(1–2). 157-189.

48. Zhao, H. and Malhotra, S. V., Applications of Ionic Liquids in Organic Synthesis. *Aldrichimica Acta,*, 2002. **35**(3). 75-83.

49. G. Huddleston, J. and D. Rogers, R., Room temperature ionic liquids as novel media for 'clean' liquid-liquid extraction. *Chemical Communications*, 1998(16). 1765-1766.

50. Cammarata, L., Kazarian, S. G., Salter, P. A. and Welton, T., Molecular states of water in room temperature ionic liquids. *Physical Chemistry Chemical Physics*, 2001. **3**(23). 5192-5200.

51. Passos, H., Ferreira, A. R., Cláudio, A. F. M., Coutinho, J. A. P. and Freire, M. G., Characterization of aqueous biphasic systems composed of ionic liquids and a citrate-based biodegradable salt. *Biochemical Engineering Journal*, 2012. **67**(0). 68-76.

52. Pandey, S., Analytical applications of room-temperature ionic liquids: A review of recent efforts. *Analytica Chimica Acta*, 2006. **556**(1). 38-45.

53. Ohno, H., *Frontmatter*. Electrochemical Aspects of Ionic Liquids 2005: John Wiley & Sons, Inc. 199-204.

54. Valkenburg, M. E. V., Vaughn, R. L., Williams, M. and Wilkes, J. S., Thermochemistry of ionic liquid heat-transfer fluids. *Thermochimica Acta*, 2005. **425**(1–2). 181-188.

55. Zhou, F., Liang, Y. and Liu, W., Ionic liquid lubricants: designed chemistry for engineering applications. *Chemical Society Reviews*, 2009. **38**(9). 2590-2599.

56. Davis, J. J. H. and Fox, P. A., From curiosities to commodities: ionic liquids begin the transition. *Chemical Communications*, 2003(11). 1209-1212.

57. Jessop, P. G. and Leitner, W., *Chemical Synthesis Using Supercritical Fluids* 2008: Wiley.

58. Brunner, G., Supercritical fluids: technology and application to food processing. *Journal of Food Engineering*, 2005. **67**(1–2). 21-33.

59. Schacht, C., Zetzl, C. and Brunner, G., From plant materials to ethanol by means of supercritical fluid technology. *The Journal of Supercritical Fluids*, 2008. **46**(3). 299-321.

60. Toews, K. L., Shroll, R. M., Wai, C. M. and Smart, N. G., pH-Defining Equilibrium between Water and Supercritical CO2. Influence on SFE of Organics and Metal Chelates. *Analytical Chemistry*, 1995. **67**(22). 4040-4043.

61. Hauthal, W. H., Advances with supercritical fluids [review]. *Chemosphere*, 2001. **43**(1). 123-135.

62. Mukhopadhyay, M., Extraction and processing with supercritical fluids. *Journal of Chemical Technology & Biotechnology*, 2009. **84**(1). 6-12.

63. Andrews, A. T., Ahlert, R. C. and Kosson, D. S., Supercritical fluid extraction of aromatic contaminants from a sandy loam soil. *Environmental Progress*, 1990. **9**(4). 204-210.

64. Sarrade, S., Guizard, C. and Rios, G. M., New applications of supercritical fluids and supercritical fluids processes in separation. *Separation and Purification Technology*, 2003. **32**(1–3). 57-63.

65. Reverchon, E., Adami, R., Cardea, S. and Porta, G. D., Supercritical fluids processing of polymers for pharmaceutical and medical applications. *The Journal of Supercritical Fluids*, 2009. **47**(3). 484-492.

66. Wettstein, S. G., Alonso, D. M., Gürbüz, E. I. and Dumesic, J. A., A roadmap for conversion of lignocellulosic biomass to chemicals and fuels. *Current Opinion in Chemical Engineering*, 2012. **1**(3). 218-224.

67. Costa Sousa, L., Chundawat, S. P. S., Balan, V. and Dale, B. E., 'Cradle-to-grave' assessment of existing lignocellulose pretreatment technologies. *Current Opinion in Biotechnology*, 2009. **20**(3). 339-347.

68. Galbe, M. and Zacchi, G., Pretreatment: The key to efficient utilization of lignocellulosic materials. *Biomass and Bioenergy*, 2012. **46**(0). 70-78.

69. Alvira, P., Tomás-Pejó, E., Ballesteros, M. and Negro, M. J., Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology*, 2010. **101**(13). 4851-4861.

70. Taherzadeh, M. and Karimi, K., Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. *International Journal of Molecular Sciences*, 2008. **9**(9). 1621-1651.

71. Kumar, P., Barrett, D. M., Delwiche, M. J. and Stroeve, P., Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Industrial & Engineering Chemistry Research*, 2009. **48**(8). 3713-3729.

72. Conde-Mejía, C., Jiménez-Gutiérrez, A. and El-Halwagi, M., A comparison of pretreatment methods for bioethanol production from lignocellulosic materials. *Process Safety and Environmental Protection*, 2012. **90**(3). 189-202.

73. Swatloski, R. P., Spear, S. K., Holbrey, J. D. and Rogers, R. D., Dissolution of Cellose with Ionic Liquids. *Journal of the American Chemical Society*, 2002. **124**(18). 4974-4975.

74. Sun, N., Rahman, M., Qin, Y., Maxim, M. L., Rodriguez, H. and Rogers, R. D., Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. *Green Chemistry*, 2009. **11**(5). 646-655.

75. Wendler, F., Todi, L.-N. and Meister, F., Thermostability of imidazolium ionic liquids as direct solvents for cellulose. *Thermochimica Acta*, 2012. **528**(0). 76-84.

76. Ohno, H. and Fukaya, Y., Task Specific Ionic Liquids for Cellulose Technology. *Chemistry Letters*, 2009. **38**(1). 2-7.

77. Pu, Y., Jiang, N. and Ragauskas, A. J., Ionic Liquid as a Green Solvent for Lignin. *Journal of Wood Chemistry and Technology*, 2007. **27**(1). 23-33.

78. Kilpeläinen, I., Xie, H., King, A., Granstrom, M., Heikkinen, S. and Argyropoulos, D. S., Dissolution of Wood in Ionic Liquids. *Journal of Agricultural and Food Chemistry*, 2007. **55**(22). 9142-9148.

79. Fort, D. A., Remsing, R. C., Swatloski, R. P., Moyna, P., Moyna, G. and Rogers, R. D., Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-n-butyl-3-methylimidazolium chloride. *Green Chemistry*, 2007. **9**(1). 63-69.

80. Tan, S. S. Y., MacFarlane, D. R., Upfal, J., Edye, L. A., Doherty, W. O. S., Patti, A. F., Pringle, J. M. and Scott, J. L., Extraction of lignin from lignocellulose at atmospheric pressure using alkylbenzenesulfonate ionic liquid. *Green Chemistry*, 2009. **11**(3). 339-345.

81. Dadi, A. P., Varanasi, S. and Schall, C. A., Enhancement of cellulose saccharification kinetics using an ionic liquid pretreatment step. *Biotechnology and Bioengineering*, 2006. **95**(5). 904-910.

82. Perez-Pimienta, J. A., Lopez-Ortega, M. G., Varanasi, P., Stavila, V., Cheng, G., Singh, S. and Simmons, B. A., Comparison of the impact of ionic liquid pretreatment on recalcitrance of agave bagasse and switchgrass. *Bioresource Technology*, 2013. **127**(0). 18-24.

83. Poornejad, N., Karimi, K. and Behzad, T., Improvement of saccharification and ethanol production from rice straw by NMMO and [BMIM][OAc] pretreatments. *Industrial Crops and Products*, 2013. **41**(0). 408-413.

84. Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H. V., Auer, M., Vogel, K. P., Simmons, B. A. and Singh, S., Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification. *Bioresource Technology*, 2010. **101**(13). 4900-4906.

85. Sant'Ana da Silva, A., Lee, S.-H., Endo, T. and P.S. Bon, E., Major improvement in the rate and yield of enzymatic saccharification of sugarcane bagasse via pretreatment with the ionic liquid 1-ethyl-3-methylimidazolium acetate ([Emim] [Ac]). *Bioresource Technology*, 2011. **102**(22). 10505-10509.

86. Lee, S. H., Doherty, T. V., Linhardt, R. J. and Dordick, J. S., Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. *Biotechnology and Bioengineering*, 2009. **102**(5). 1368-1376.

87. Dibble, D. C., Li, C., Sun, L., George, A., Cheng, A., Cetinkol, O. P., Benke, P., Holmes, B. M., Singh, S. and Simmons, B. A., A facile method for the recovery of ionic liquid and lignin from biomass pretreatment. *Green Chemistry*, 2011. **13**(11). 3255-3264.

88. Yang, D., Zhong, L.-X., Yuan, T.-Q., Peng, X.-W. and Sun, R.-C., Studies on the structural characterization of lignin, hemicelluloses and cellulose fractionated by ionic liquid followed by alkaline extraction from bamboo. *Industrial Crops and Products*, 2013. **43**(0). 141-149.

89. Lynam, J. G., Toufiq Reza, M., Vasquez, V. R. and Coronella, C. J., Pretreatment of rice hulls by ionic liquid dissolution. *Bioresource Technology*, 2012. **114**(0). 629-636.

90. Mäki-Arvela, P., Anugwom, I., Virtanen, P., Sjöholm, R. and Mikkola, J. P., Dissolution of lignocellulosic materials and its constituents using ionic liquids—A review. *Industrial Crops and Products*, 2010. **32**(3). 175-201.

91. Haykir, N. I., Bahcegul, E., Bacak, N. and Bakir, U., Pretreatment of cotton stalk with ionic liquids including 2-hydroxy ethyl ammonium formate to enhance biomass digestibility *Industrial Crops and Products*, 2013. **41**. 430-436.

92. Carvalheiro, F., Silva-Fernandes, T., Duarte, L. and Gírio, F., Wheat Straw Autohydrolysis: Process Optimization and Products Characterization. *Applied Biochemistry and Biotechnology*, 2009. **153**(1-3). 84-93.

93. Garrote, G. and Parajó, J. C., Non-isothermal autohydrolysis of Eucalyptus wood. *Wood Science and Technology*, 2002. **36**(2). 111-123.

94. Cara, C., Ruiz, E., Ballesteros, M., Manzanares, P., Negro, M. J. and Castro, E., Production of fuel ethanol from steam-explosion pretreated olive tree pruning. *Fuel*, 2008. **87**(6). 692-700.

95. Nakata, T., Miyafuji, H. and Saka, S., Bioethanol from cellulose with supercritical water treatment followed by enzymatic hydrolysis. *Applied Biochemistry and Biotechnology*, 2006. **130**(1-3). 476-485.

96. Garrote, G., Domínguez, H. and Parajó, J. C., Hydrothermal processing of lignocellulosic materials. *Holz als Roh- und Werkstoff*, 1999. **57**(3). 191-202.

97. Resende, F. L. P., Neff, M. E. and Savage, P. E., Noncatalytic Gasification of Cellulose in Supercritical Water. *Energy & Fuels*, 2007. **21**(6). 3637-3643.

98. Zhao, Y., Lu, W.-J., Wang, H.-T. and Yang, J.-L., Fermentable hexose production from corn stalks and wheat straw with combined supercritical and subcritical hydrothermal technology. *Bioresource Technology*, 2009. **100**(23). 5884-5889.

99. Goto, M., Obuchi, R., Hirose, T., Sakaki, T. and Shibata, M., Hydrothermal conversion of municipal organic waste into resources. *Bioresource Technology*, 2004. **93**(3). 279-284.

100. Ritter, D. and Campbell, A., Supercritical Carbon Dioxide Extraction of Southern Pine and Ponderosa Pine. *Wood and Fiber Science*, 1991. **23**(1). 98-113.

101. Kim, K. H. and Hong, J., Supercritical CO2 pretreatment of lignocellulose enhances enzymatic cellulose hydrolysis. *Bioresource Technology*, 2001. **77**(2). 139-144.

102. Walsum, G. P., *Severity Function Describing the Hydrolysis of Xylan Using Carbonic Acid*, in *Twenty-Second Symposium on Biotechnology for Fuels and Chemicals*, B. Davison, J. McMillan, and M. Finkelstein, Editors. 2001, Humana Press. 317-329.

103. Rogalinski, T., Ingram, T. and Brunner, G., Hydrolysis of lignocellulosic biomass in water under elevated temperatures and pressures. *The Journal of Supercritical Fluids*, 2008. **47**(1). 54-63.

104. da Costa Lopes, A., João, K., Rubik, D., Bogel-Lukasik, E., Duarte, L. C., Andreaus, J. and Bogel-Lukasik, R., Pre-treatment of lignocellulosic biomass using ionic liquids: wheat straw fractionation. *Bioresource Technology*, 2013. 10.1016/j.biortech.2013.05.032.

105. Carvalheiro, F., Silva-Fernandes, T., Duarte, L. C. and Gírio, F. M., Wheat Straw Autohydrolysis: Process Optimization and Products Characterization. *Applied Biochemistry and Biotechnology*, 2009. **153**(1-3). 84-93.

106. Selig, M., Weiss, N. and Ji, Y., *Enzymatic Saccharification of Lignocellulosic Biomass: Laboratory Analytical Procedure (LAP): Issue Date, 3/21/2008* 2008: National Renewable Energy Laboratory.

107. Zhu, L., O'Dwyer, J. P., Chang, V. S., Granda, C. B. and Holtzapple, M. T., Structural features affecting biomass enzymatic digestibility. *Bioresource Technology*, 2008. **99**(9). 3817-3828.

108. Sun, R. C. and Tomkinson, J., Characterization of hemicelluloses obtained by classical and ultrasonically assisted extractions from wheat straw. *Carbohydrate Polymers*, 2002. **50**(3). 263-271.

109. Sain, M. and Panthapulakkal, S., Bioprocess preparation of wheat straw fibers and their characterization. *Industrial Crops and Products*, 2006. **23**(1). 1-8.

110. Liu, R., Yu, H. and Huang, Y., Structure and morphology of cellulose in wheat straw. *Cellulose*, 2005. **12**(1). 25-34.

111. Yang, Q., Wu, S., Lou, R. and LV, G., Structural characterization of lignin from wheat straw. *Wood Science and Technology*, 2011. **45**(3). 419-431.

112. Kubo, S. and Kadla, J. F., Hydrogen bonding in lignin: a Fourier transform infrared model compound study. *Biomacromolecules*, 2005. **6**(5). 2815-2821.

113. Wang, B., Feng, H., Wang, X., Zhou, B. and de Frias, J. A., Deconstructing lignocellulosic biomass with a two-step method, 2012, The Board of Trustees of the University of Illinios: United States.

114. Li, Q., He, Y. C., Xian, M., Jun, G., Xu, X., Yang, J. M. and Li, L. Z., Improving enzymatic hydrolysis of wheat straw using ionic liquid 1-ethyl-3-methyl imidazolium diethyl phosphate pretreatment. *Bioresource Technology*, 2009. **100**(14). 3570-3575.

115. Naseem, B., Shah, S. W. H., Hasan, A. and Sakhawat Shah, S., Interaction of flavonoids, the naturally occurring antioxidants with different media: A UV–visible spectroscopic study. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2010. **75**(4). 1341-1346.

116. da Costa Lopes, A., João, K., Morais, R., Bogel-Lukasik, E. and Bogel-Lukasik, R., Ionic liquids as a tool for lignocellulosic biomass fractionation. *Sustainable Chemical Processes*, 2013. **1**(1). doi:10.1186/2043-7129-1-3.

117. Maki-Arvela, P., Anugwom, I., Virtanen, P., Sjoholm, R. and Mikkola, J., Dissolution of lignocellulosic materials and its constituents using ionic liquids--A review. *Industrial Crops and Products*, 2010. **32**(3). 175-201.

118. Strechan, A. A., Paulechka, Y. U., Blokhin, A. V. and Kabo, G. J., Low-temperature heat capacity of hydrophilic ionic liquids [BMIM][CF3COO] and [BMIM][CH3COO] and a correlation scheme for estimation of heat capacity of ionic liquids. *Journal of Chemical Thermodynamics*, 2008. **40**(4). 632-639.

119. Freire, M. G., Teles, A. R. R., Rocha, M. A. A., Schröder, B., Neves, C. M. S. S., Carvalho, P. J., Evtuguin, D. V., Santos, L. M. and Coutinho, J. A. P., Thermophysical Characterization of Ionic Liquids Able To Dissolve Biomass. *Journal of Chemical and Engineering Data*, 2011. **56**(12). 4813-4822.

120. Wu, H., Mora-Pale, M., Miao, J., Doherty, T. V., Linhardt, R. J. and Dordick, J. S., Facile pretreatment of lignocellulosic biomass at high loadings in room temperature ionic liquids. *Biotechnology and Bioengineering*, 2011. **108**(12). 2865-2875.

121. Shill, K., Padmanabhan, S., Xin, Q., Prausnitz, J. M., Clark, D. S. and Blanch, H. W., Ionic liquid pretreatment of cellulosic biomass: enzymatic hydrolysis and ionic liquid recycle. *Biotechnology and Bioengineering*, 2011. **108**(3). 511-520.

122. Overend, R. P., Chornet, E. and Gascoigne, J. A., Fractionation of Lignocellulosics by Steam-Aqueous Pretreatments [and Discussion]. *Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences*, 1987. **321**(1561). 523-536.

123. Browning, B. L., in *Methods in Wood Chemistry*, K.V. Sarkanen and C.H. Ludwig, Editors. 1967, John Wiley & Sons: New York.

124. AOAC, *Official Methods of Analysis, eleventh ed.* AOAC 1975, Washington, DC.

125. Duarte, L. C., Carvalheiro, F., Lopes, S., Marques, S., Parajo, J. C. and Girio, F. M., Comparison of two posthydrolysis processes of brewery's spent grain autohydrolysis liquor to produce a pentose-containing culture medium. *Applied Biochemistry and Biotechnology*, 2004. **113**. 1041-1058.

126. Girio, F. M., Fonseca, C., Carvalheiro, F., Duarte, L. C., Marques, S. and Bogel-Lukasik, R., Hemicelluloses for fuel ethanol: A review. *Bioresource Technology*, 2010. **101**(13). 4775-4800.

127. van Walsum, G. P. and Shi, H., Carbonic acid enhancement of hydrolysis in aqueous pretreatment of corn stover. *Bioresource Technology*, 2004. **93**(3). 217-226.

128. Elliott, J. R. and Lira, C. T., *Introductory chemical engineering thermodynamics* 1999: Prentice Hall PTR Upper Saddle River, NJ.

129. Gamse, T., Marr, R., Froschl, F. and Siebenhofer, M., Extraction of furfural with carbon dioxide. *Separation Science and Technology*, 1997. **32**(1-4). 355-371.

130. Perakis, C. A., Voutsas, E. C., Magoulas, K. G. and Tassios, D. P., Thermodynamic modeling of the water plus acetic acid + CO2 system: The importance of the number of association sites of water and of the nonassociation contribution for the CPA and SAFT-type models. *Industrial & Engineering Chemistry Research*, 2007. **46**(3). 932-938.

131. Rumpf, B., Xia, J. Z. and Maurer, G., Solubility of carbon dioxide in aqueous solutions containing acetic acid or sodium hydroxide in the temperature range from 313 to 433 K

and at total pressures up to 10 MPa. *Industrial & Engineering Chemistry Research*, 1998. **37**(5). 2012-2019.