Ana Vanessa Antunes Carvalho

Licenciada em Ciências da Engenharia Química e Bioquímica

Ionic liquids as catalysts of lignocellulosic biomass processing

Dissertação para obtenção do Grau de Mestre em
Engenharia Química e Bioquímica

Orientador: Rafal Bogel-Lukasik

Presidente: Professora Doutora Ana Maria Martelo Ramos
Arguente: Professora Doutora Susana Filipe Barreiros
Vogal: Doutor Rafal Bogel-Lukasik

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

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Abstract

The present work is devoted to study the pre-treatment of lignocellulosic biomass, especially wheat straw, by the application of the acidic ionic liquid (IL) such as 1-butyl-3-methylimidazolium hydrogen sulphate. The ability of this IL to hydrolysis and conversion of biomass was scrutinised. The pre-treatment with hydrogen sulphate-based IL allowed to obtain a liquor rich in hemicellulosic sugars, furans and organic acids, and a solid fraction mainly constituted by cellulose and lignin. Quantitative and qualitative analyses of the produced liquors were made by capillary electrophoresis and high-performance liquid chromatography. Pre-treatment conditions were set to produce xylose or furfural. Specific range of temperatures from 70 to 175 °C and residence times from 20.0 to 163.3 min were studied by fixing parameters such as biomass/IL ratio (10 % (w/w)) and water content (1.25 % (w/w)) in the pre-treatment process. Statistical modelling was applied to maximise the xylose and furfural concentrations. For the purpose of reaction condition comparison the severity factor for studied ionic liquid was proposed and applied in this work. Optimum conditions for xylose production were identified to be at 125 °C and 82.1 min, at which 16.7 % (w/w) xylose yield was attained. Furfural was preferably formed at higher pre-treatment temperatures and longer reaction time (161 °C and 104.5 min) reaching 30.7 % (w/w) maximum yield.

The influence of water content on the optimum xylose formation was also studied. Pre-treatments with 5 and 10 % (w/w) water content were performed and an increase of 100 % and 140 % of xylose yield was observed, respectively, while the conversion into furfural maintained unchanged.

Keywords: acidic ionic liquids, lignocellulosic biomass, pre-treatment, hydrolysis, hemicellulose, biorefinery

Resumo

O seguinte trabalho é dedicado ao estudo de pré-tratamento de biomassa lignocelulósica, em especial palha de trigo, através da aplicação do liquido iónico (LI) ácido 1-butil-3-metilemidazolio sulfato de hidrogénio. A capacidade deste LI para hidrolisar e converter biomassa foi examinada. O pré-tratamento com LI á base de sulfato de hidrogénio permitiu obter um licor rico em açúcares a partir da hemicellulose, furanos e ácidos orgânicos, também como uma fracção sólida constituída maioritariamente por celulose e lignina. Análise quantitativa e qualitativa do licor produzido foi feita através de electroforese capilar e cromatografia líquida de alta-performance. Condições de pré-tratamento foram selecionadas para produção de xilose ou de furfural. Gamas específicas de temperaturas a partir de 70 até 175 °C e tempos de residência de 20.0 a 163.3 min foram estudados, fixando parâmetros como a razão biomassa/LI (10 % (w/w)) e o conteúdo de água (1.25 % (w/w)) no processo de pré-tratamento. Modelação estatística foi aplicada por forma a maximizar a concentração de xilose e furfural. Com o objectivo de comparar as condições de reacção, o factor de severidade para o LI estudado foi proposto e aplicado neste trabalho. Condições óptimas para a produção de xilose foram identificadas a 125 °C e 82.1 min, onde 16.7 % (w/w) de rendimento em xilose foi obtido. Furfural foi formado preferencialmente a temperaturas mais elevadas e tempos de reacção mais longos (161 °C e 104.5 min, respectivamente) alcançando um rendimento máximo de 30.7 % (w/w).

A influência do conteúdo de água nas condições óptimas para formação de xilose foi analisado. Pré-tratamentos com 5 e 10 % (w/w) de humidade foram executados e um aumento de 100 % e 140 % no rendimento em xilose foi observado, respectivamente, enquanto a conversão em furfural manteve-se constante.

Palavras-chave: líquidos iónicos ácidos, biomassa lignocelulósica, pré-tratamento, hidrólise, hemicellulose, biorefinaria

List of Publications

The work presented in this thesis was presented in various forms as listed below:

Peer-reviewed papers

1. V. Carvalho, A. M. da Costa Lopes, R. Bogel-Lukasik, Direct xylose and furfural production from biomass using acidic ionic liquids, Green Chemistry, 2014, submitted.

Oral communications

1. F.Relvas, M. Brenner, A. da Costa Lopes, V. Carvalho, A. R. C. Morais, S. P. Magalhães Silva, A. Mata, L. B. Roseiro, R. Bogel-Lukasik, Biorefinery concept with green solvents towards the phenolic valorization, 8th World Congress on Polyphenols Applications, VI.2014, Lisbon, Portugal.
2. V. Carvalho, F.Relvas, A. da Costa Lopes, A. R. C. Morais, S. P. Magalhães da Silva, A. Mata, L. B. Roseiro, R. Bogel-Lukasik, Green chemistry and biorefineries – common future?, Lignocellulosic Crops as feedstock for Future Biorefineries, Summer Course of FIBRA Project,VII.2014, Caparica, Portugal.
3. M. da Costa Lopes, V. Carvalho, S. P. Magalhães da Silva, L. B. Roseiro, R. Bogel-Lukasik, Ionic liquids as new solvents for residues, COST Action TD 1203 Workshop on Valorisation of Vegetable Waste, VIII.2014, Novi Sad, Serbia.

Poster presentation

1. A. M. da Costa Lopes, M. Brenner, V. Carvalho, S. P. Magalhães da Silva, L. B. Roseiro, R. Bogel-Lukasik, Extraction of phenolic compounds from wheat straw using ionic liquids, 8th World Congress on Polyphenols Applications, VI.2014, Lisbon, Portugal.

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1. Introduction

In the course of the depleting petrochemical feedstock, increasing concerns regarding the climate changes and market globalisation, one of the most important drivers for nowadays development is the sustainability. Thus, towards this trend the concept of green chemistry was created following twelve principles as mentioned in Table 1.1.[1]

Table 1.1 Anastas and Warner's twelve principles of Green Chemistry [1]

1. It is better to prevent waste than to treat or clean up after it has been formed.	**7.** A raw material of feedstock should be renewable rather than depleting wherever technically and economically practicable.
2. Synthetic methods should be designed to maximise the incorporation of all materials used in the process into the final product.	**8.** Unnecessary derivatisation (blocking group, protection/deprotection, and temporary modification of physical/chemical processes) should be avoided whenever possible.
3. Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.	**9.** Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
4. Chemical products should be designed to preserve efficacy of function while reducing toxicity.	**10.** Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products.
5. The use of auxiliary substances (e.g. solvents, separation agents etc.) should be made unnecessary wherever possible and innocuous when used.	**11.** Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
6. Energy requirements should be recognised for their environmental and economic impacts and should be minimised. Synthetic methods should be conducted at ambient temperature and pressure.	**12.** Substances and the form of a substance used in a chemical process should be chosen so as to minimize the potential for chemical accidents, including releases, explosions and fires.

The development and implementation of principles proposed by Anastas and Warner show the ways to achieve sustainability at the industrial level.[2] One of the means to achieve this is the use of sustainable feedstock such as biomass. The use of biomass as the raw material for the processes is accomplished by the biorefinery.[3] Thus, the integration of biorefinery concept into green chemistry principles is nowadays a need to accomplish either principles of biorefinery and green chemistry.

1.1. Biorefinery

The increasing demand for energy, fuels and chemicals is forecasted for the near future that will possibly place additional pressure on the use of fossil resources and the environment. Accordingly, finding new technologies and development of novel processes to bring this growth in line with the social demand for sustainability are major challenges for current and future generations. A biorefinery might be one of the ways to achieve this goal. A biorefinery is a facility, which concept is analogous to today's petroleum refinery consisting on multi-step platform for the production of fuels and chemicals, but starting from renewable sources (e.g. biomass) as raw material instead crude oil (Figure 1.1). Therefore, biorefinery refers to refining biomass into various separated products that undergo further biological, (bio)chemical, physical and/or thermo-chemical processing and downstream separation. Within biorefinery processes, both (high) added-value products (chemicals and/or materials) and secondary energy carriers (transportation fuels, gaseous energy carriers, power, heat) are co-produced.[3] Market competitive products could be produced, especially when the biorefinery processes may possibly be integrated into existing industrial ((petro)chemical, food/feed, power production) infrastructures.

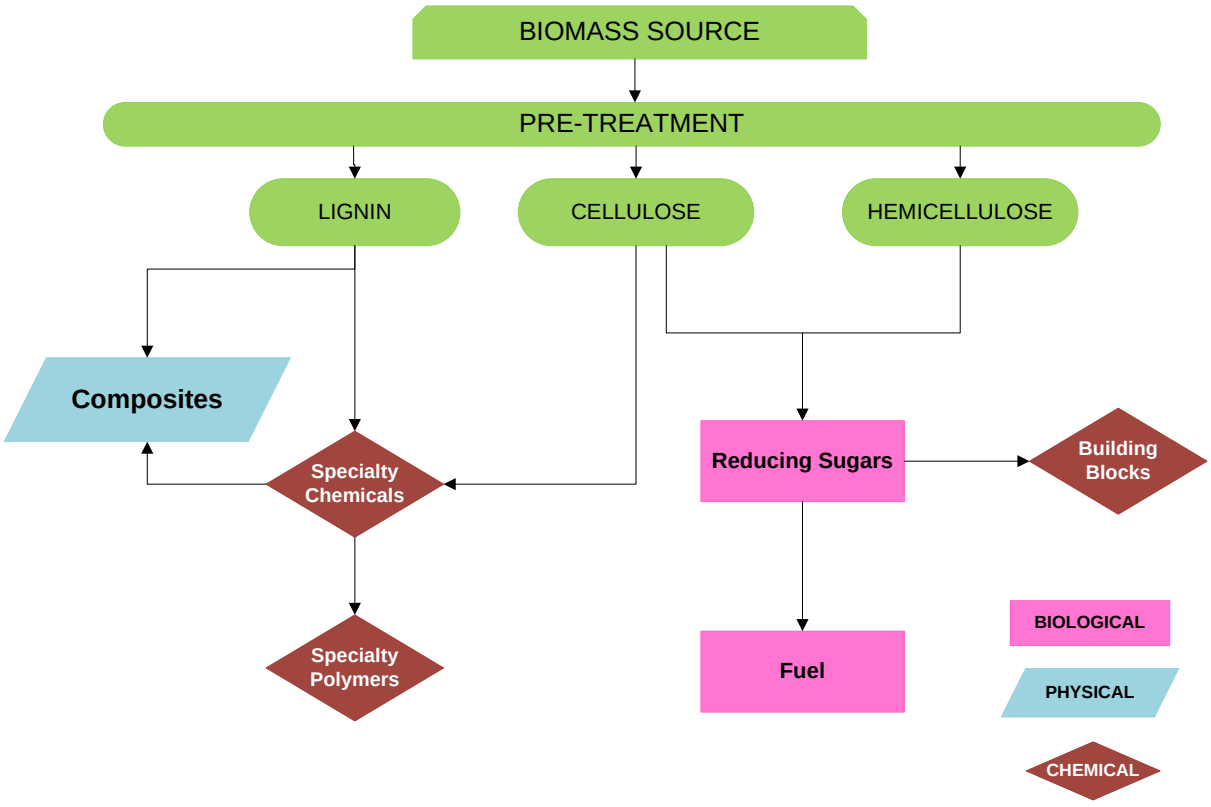


Figure 1.1 Conceptual biomass biorefinery (adapted from [4]).

1.2. Biomass

Biomass is a readily available and low-cost feedstock and is one among a few resources that can facilitate the large-scale and sustainable production of the substantial volumes of energy and materials.[5] Figure 1.2 illustrates biomass resources available in the world and their respective share.

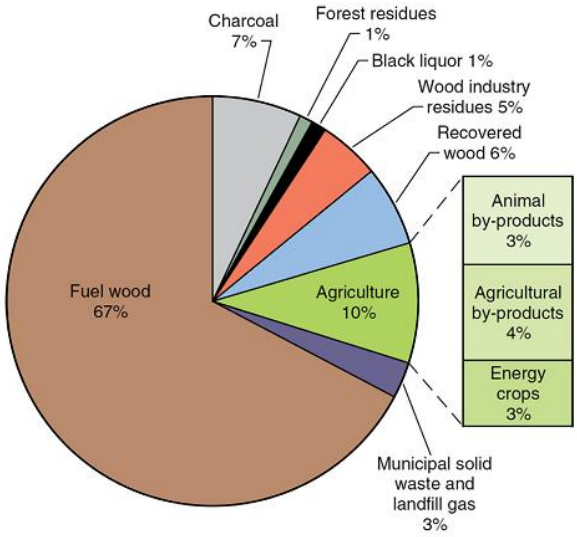


Figure 1.2 Share of biomass sources in the world.[6]

Biomass refers to biological material derived from living organisms and it can be divided into primary, secondary, and tertiary resources.[7] Primary biomass resources are produced directly by photosynthesis and are taken straight from the land. They include perennial short-rotation woody crops and herbaceous crops, the seeds of oil crops, and residues resulting from the harvesting of agricultural crops and forest trees (e.g., wheat straw, corn stover, and the tops, limbs, and bark from trees). Secondary biomass resources result from the processing of primary biomass resources either physically (e.g., the production of sawdust in mills), chemically (e.g., black liquor from pulping processes), or biologically (e.g., manure production by animals).[7] Tertiary biomass resources are post-consumer residue streams including animal fats and greases, used vegetable oils, packaging wastes, and construction/demolition debris.[7] Lignocellulosic biomass as one of the most available and studied feedstocks in biorefineries can be considered as primary and secondary biomass resource categories.

1.2.1. Lignocellulosic biomass

The plant cell wall of lignocellulosic biomass contains three major biopolymers: cellulose, lignin, and hemicellulose (Figure 1.3) which are described below in more details.

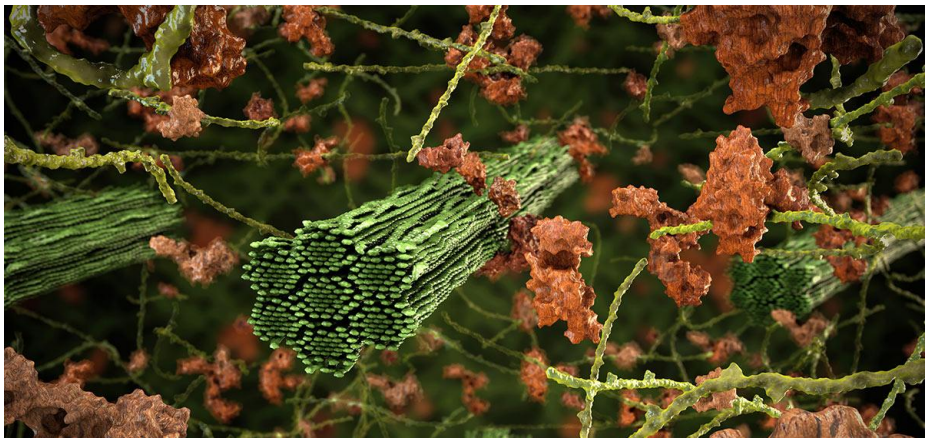


Figure 1.3 Graphical representation of lignocellulosic biomass based on supercomputer models. Cellulose (dark green), lignin (brown) and hemicellulose (light green) Rreproduced from [8]

Cellulose

The cellulose structure is made of glucose units linearly linked by (1→4)-β-D-glycosidic bonds forming a polymer presented in Figure 1.4. An extensive and ordered network of hydrogen bonds and van der Waals forces result in a cellulose structure, with varied size and crystallinity depending on many factors by mostly on the type of biomass. The degree of polymerization (DP) is measured by the number of molecular glucose units in the polymer. Decrystallization and hydrolytic depolymerization to glucose are mechanistically two separate steps prior to rendering crystalline cellulose into usable monosaccharides, either for biocatalytic fermentations or for thermocatalytic processing. In most of studied aqueous systems, hydrolysis of bulk cellulose typically involves hydrolytic fragmentation to reduce the DP followed by hydrolysis to glucose.[9, 10]

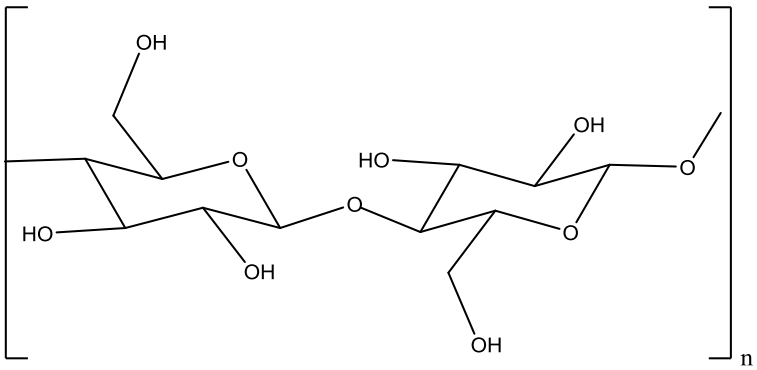


Figure 1.4 Structure of cellulose (adapted from [9, 10])

Cellulose is Earth's most widespread natural organic polymer and therefore the most important bio-renewable resource. It is produced by biosynthesis on land (wood, grasses) and in the sea (algae) as component of plant cells in quantities of approximately 75 x 10^9 tons per year. However, out of these 75 x 10^9 tons that Nature renews every year, only far less than 1% (0,2 x 10^9) tons are used as

feedstock for further processing – mainly for the pulp and paper industry. As a raw material for chemical processes, cellulose is used in very small amounts (5 x 10^6 tons per year, i.e. 0.007%).[11] Cellulose has numerous applications focused on biocompatibility and chirality for the immobilisation of proteins, antibodies, and heparin, for the separation of enantiomeric molecules, as well as the formation of cellulose composites with synthetic polymers and biopolymers.[12] The numerous applications of cellulose are based on its distinct fibre morphology.[12]

Hemicellulose

Hemicellulose is constituted by branched-chain polysaccharides containing pentose and hexose monosaccharide building units, such as xylose (C5), arabinose (C5), galactose (C6) and mannose (C6), as well as acetyl groups, glucuronic and galacturonic acids. Hemicellulose has a random and amorphous structure (two examples are demonstrated in Figure 1.5). Hemicellulose is the easiest component of lignocellulosic biomass to be degraded in acid or in alkaline processes or even in hot water.[13]

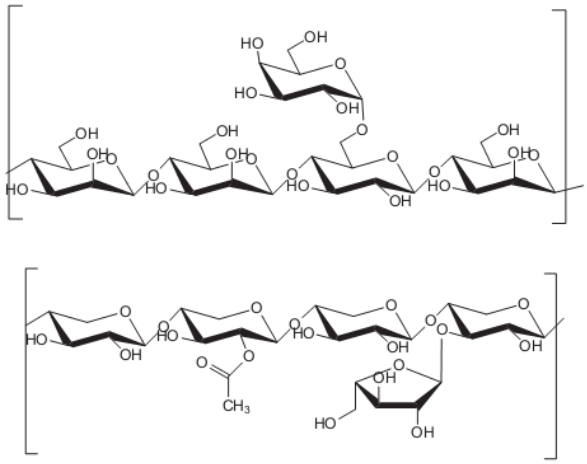


Figure 1.5 Examples of hemicellulose structure [9]

Hemicellulose has diverse applications in both polymeric and hydrolysed forms (Figure 1.6). The polymeric form can be used in industrial applications such as thermoplastic xylan derivatives, biopolymers and hydrogels.[13, 14] When hemicellulose is hydrolysed, the mix of sugars has a value as either an animal feed additive or a feedstock for production of biofuels (ethanol, butanol), chemical intermediates (furfural) or sugars (xylose).[13, 15]

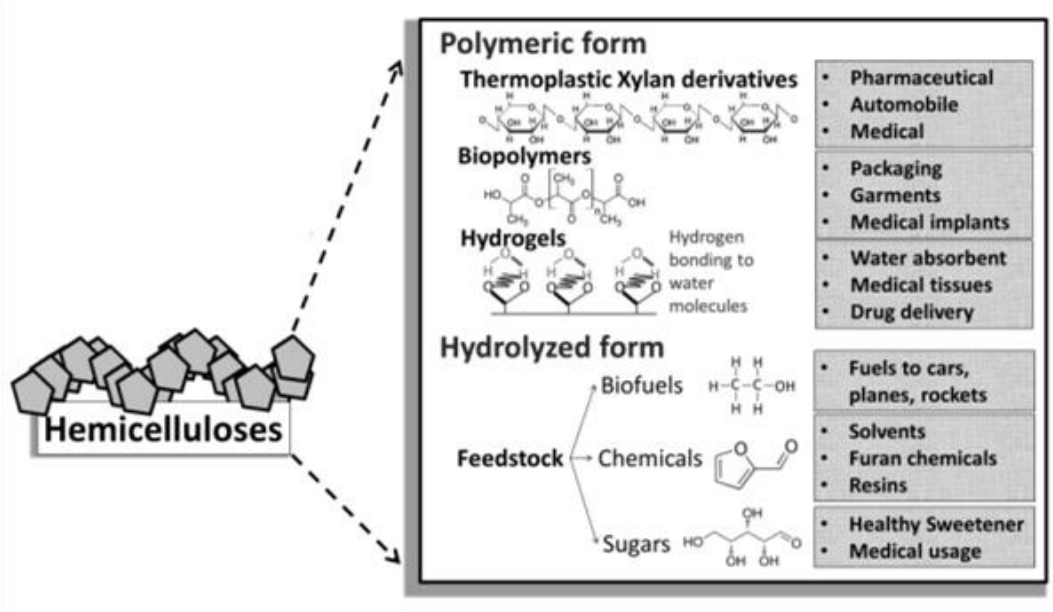


Figure 1.6 Applications of hemicellulose derivatives (adapted from [16])

Lignin

Lignin is polymer with an amorphous three-dimensional structure and is the most abundant aromatic biopolymer in nature. It consists of phenylpropanoid units originated by three aromatic alcohol precursors (monolignols): p-coumaryl, coniferyl and sinapyl alcohol.[16, 17] Lignin (Figure 1.7) is highly branched aromatic polymer with complex structure.[10, 17] High-molecular-weight lignin is insoluble in sulphuric acid (H_2SO_4), whereas low-molecular-weight lignin is considered as acid soluble.

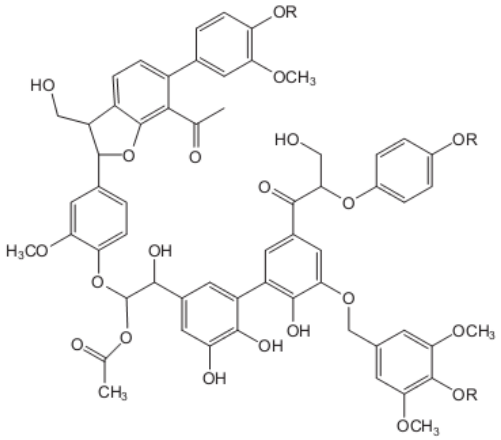


Figure 1.7 Example of lignin structure [9]

Lignin's aromatic nature makes it a potential renewable source of chemicals. Some of the applications of lignin are depicted in Table 1.2.[18, 19]

Table 1.2 Examples of lignin derivatives applications (adapted from [18, 19])

Multi-polarity products		**Materials**	**Agriculture**
Dispersion	**Others**	phenolic resins	soil rehabilitation
Ceramics	complexing agents	polyurethanes	slow release fertilisers
oil well drilling	Flocculating	epoxies	artificial humus
clay brick & tiles	heavy metal binders	particle boards	Fertiliser
Cements	ion exchanging	resin boards	manure treatment
concrete gypsum board	water softening	rubber reinforcing	composting aid
Dyestuffs	destabilization of oil emulsions	bloc copolymers	soil stabilisation
Electrolytes	corrosion protection	polyesters	Insecticides
paper sizing	metal cleaners	composites	Granulation
Emulsion	**Chemicals**	polyolefins	**Miscellaneous**
Wax	Cresols	biodegradables	energy production
Asphalt	Catechols	carbon sieves	diesel fuel
Bitumen	Resorsinols	activated carbons	foam stabilizers
Vitamins	Quinones	carbon fibres	Binders
micronutrients	Vanilin		tanning agent
	Guaiacols		Absorbents

Lignocellulosic biomass has diverse composition however examples of lignocellulosic raw materials and their compositions are given in Table 1.3.

Table 1.3 Composition of examples of lignocellulosic raw materials (wt% on dry biomass) (adapted from [20])

	Cellulose	**Hemicellulose**	**Lignin**
Hardwood steams	40-55	20-40	18-25
Softwood steams	45-50	25-35	25-35
Rice straw	35-45	18-25	10-25
Wheat straw	**38-45**	**20-32**	**7-10**
Tabacco chops	22-30	15-20	15-25
Arundo donax	30-38	18-22	8-20
Miscanthus	35-40	16-20	20-25
Newspapers	40-55	25-40	15-30

1.2.2. Wheat Straw

One of the examples of lignocellulosic biomass is wheat straw. Wheat straw has a typical composition of an agricultural-based lignocellulosic residue and contains on average 38 – 45% cellulose, 20 – 32%

hemicellulose, 7 – 20 % lignin (Table 1.4), as well as a number of minor fractions such as proteins and ash.

Wheat straw is worldwide abundant agricultural residues and the current applications of this biomass feedstock are depicted in Table 1.4.

Table 1.4 Main conventional uses of wheat straw in the EU [21]

Within agricultural sector:	Soil improver Animal fodder supplement Animal bedding Mushroom production (growth substrate) Frost prevention in horticulture Strawberries (preventing damage to the fruit) Compost industry	**Other sector:**	Thatching Traditional building materials, fiber boards, insulation material Energy (heat, power, fuels)

Essentially, wheat straw is used as animal's feed and bedding as well as energy source. Nevertheless, there are two particular limitations of using wheat straw regarding the applications for bioenergy purposes. The high carbon-to-nitrogen content of wheat straw leads to a very low biodegradability in comparison to other agricultural residues. This is of particular interest when straw is used for anaerobic digestion to produce biogas. It means that in many cases, straw needs to be blended with other agricultural residues, in order to speed up the degradation of organic constituents presented in straw. Another limitation, in particular for thermal processes such as combustion and gasification, is the high ash content (typically 6 – 12wt%) as well as the high inorganic composition of wheat straw which promotes slagging, fouling and corrosion in reactor systems. Herein, these disadvantages arise the opportunity to use green and sustainable technologies that could be more adequate for wheat straw processing in the frame of the biorefinery concept.

1.2.3. Biomass pre-treatment

The major aim of biomass pre-treatment is to process biomass to make it more subjectable for for further processing.[4, 13] The pre-treatment makes biomass fractions more subjectable biological and/or chemical treatments aiming further valorisation towards particular products or pivot chemicals.[22]
However, to achieve this goal various challenges must be addressed. Furthermore, depending on the expected results, the most adequate pre-treatment method can be selected. In addition, the choice of pre-treatment should consider the overall compatibility of feedstocks, enzymes and organisms to be applied, overall economic assessment and environmental impact.[23] Up to now, several approaches

have been used for developing low cost pre-treatments to generate sugar-rich liquors from cellulose and hemicellulose.[23, 24]

Table 1.5 Methods for biomass pre-treatment [23-25]

Pre-treatment	Name	Means	Effect
Physical	Mechanical comminution	Ball milling, compression milling, colloidal milling	Decrease of the particle size, crystallinity and DP of cellulose
	Pyrolysis	High temperatures (>300ºC)	Decomposition of cellulose to produce gaseous products and residual char
Physico-chemical	Explosion (steam, CO_2, ammonia, etc)	High-pressure fluid	Decrease of cellulose crystallinity and DP by increase of the surface area; lignin and hemicellulose removal
	Ammonia recycle percolation method (ARP)	Temperatures 150-170ºC, ammonia recycle at low fluid velocity (1 cm/min)	Increase of accessible surface area; removal of lignin and hemicellulose
Chemical	Acid	Mineral acids	Decrease of crystallinity and DP of cellulose; partial or complete degradation of hemicellulose
	Alkaline	Sodium hydroxide, sodium carbonate, ammonia, ammonium sulphate, lime, etc.	Cleavage of hydrolysable linkages in lignin and glycosidic bonds of polysaccharides; decrease of crystallinity and DP of cellulose; disruption of the lignin structure
	Autohydrolysis	Liquid hot water	Hemicellulose removal and lignin transformation; increase of potential of cellulose hydrolysis
	Oxidative delignification	H_2O_2 and peroxidase enzyme	Dissolution of lignin and hemicellulose and glucose production from cellulose saccharification
	Organosolv process	Organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4)	Breaking down of linkages between lignin and hemicellulose; hydrolysis of hemicellulose and lignin
	High pressure fluids	High-temperature and high-pressure exposition to fluids e.g. CO_2	Decrease of cellulose crystallinity and DP by increase of the surface area; lignin and hemicellulose removal
	Ionic Liquids	Simple salts ILs or binary ILs	Dissolution of biomass; Integrated dissolution and hydrolysis of biomass fractionation
Biological	Microorganisms	Bacteria, fungi	Removal of lignin and reduction of cellulose DP

An effective pre-treatment should meet the following requirements:[23]

1) Overcome lignocellulosic biomass recalcitrance, by deconstructing the three-dimensional structure of lignocellulose and breaking down the semi-crystalline cellulose and hemicellulose;

2) Afford high yields to sugars or chemicals and/or give highly digestible pre-treated solid;

3) Avoid carbohydrates degradation and in particular preserve the utility of pentose (hemicellulose) fraction;

4) Avoid the formation of inhibitory toxic by-products;

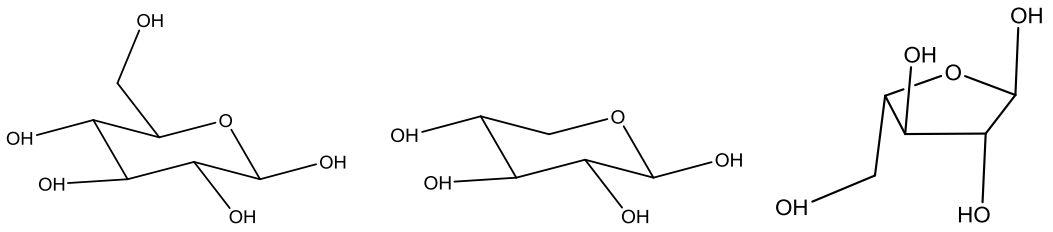
5) Allow lignin recovery and exploitation to give valuable co–products;

6) Be cost–effective, involving reasonable size of reactors, low amount of wastes and low energy demands.

The pre-treatment methods can be classified in a various ways. One of them is a character of forces leading to the biomass processing. Thus physical (pyrolysis and mechanical disruption/comminution), physicochemical (steam explosion, fluid explosion, etc.), chemical (acid hydrolysis, alkaline hydrolysis, organic solvent pre-treatment, ionic liquids, etc.) and biological (degradation by microorganism) methods can be classified as shown in Table 1.5.[23, 24]

1.2.4. Valorisation of lignocellulosic biomass

Biomass polysaccharide fraction is mainly composed by glucan, xylans and arabinan, which after the hydrolysis give mixture of oligo- and monosaccharides namely glucose, xylose, arabinose and other sugars in minor concentration (Figure 1.8).[21] Xylose and arabinose can be obtained from hemicellulose and glucose comes from the cellulose hydrolysis. Thus, sugars obtained could be fermented to produce biofuels, such as ethanol and/or used in a wide range of applications, such as food and feed, pharmaceuticals and other purposes



Glucose Xylose Arabinose

Figure 1.8 Monosaccharides from biomass

The formation of several pivot compounds can be attained directly from the biomass pre-treatment. High temperatures and longer pre-treatments enable the dehydration of reducing sugars into furans, which subsequently can be converted into organic acids, e.g. formic and levulinic.[9]

Depending of the reaction conditions, 5-(hydroxymethyl)-2-furaldehyde (HMF) and/or levulinic acid and formic acid can be formed from glucose (previously obtained after cellulose hydrolysis). Xylose rand arabinose, derived from hemicellulose, follow different reaction pathways resulting in the formation of furan-2-carbaldehyde (furfural) and/or various C-1 and C-4 organic compounds (Figure 1.9). The aromatic fractions of lignin can be converted e.g. by oxidation or hydrolysis to various types of polyphenolics depending on type of structural monomeric units of lignin.

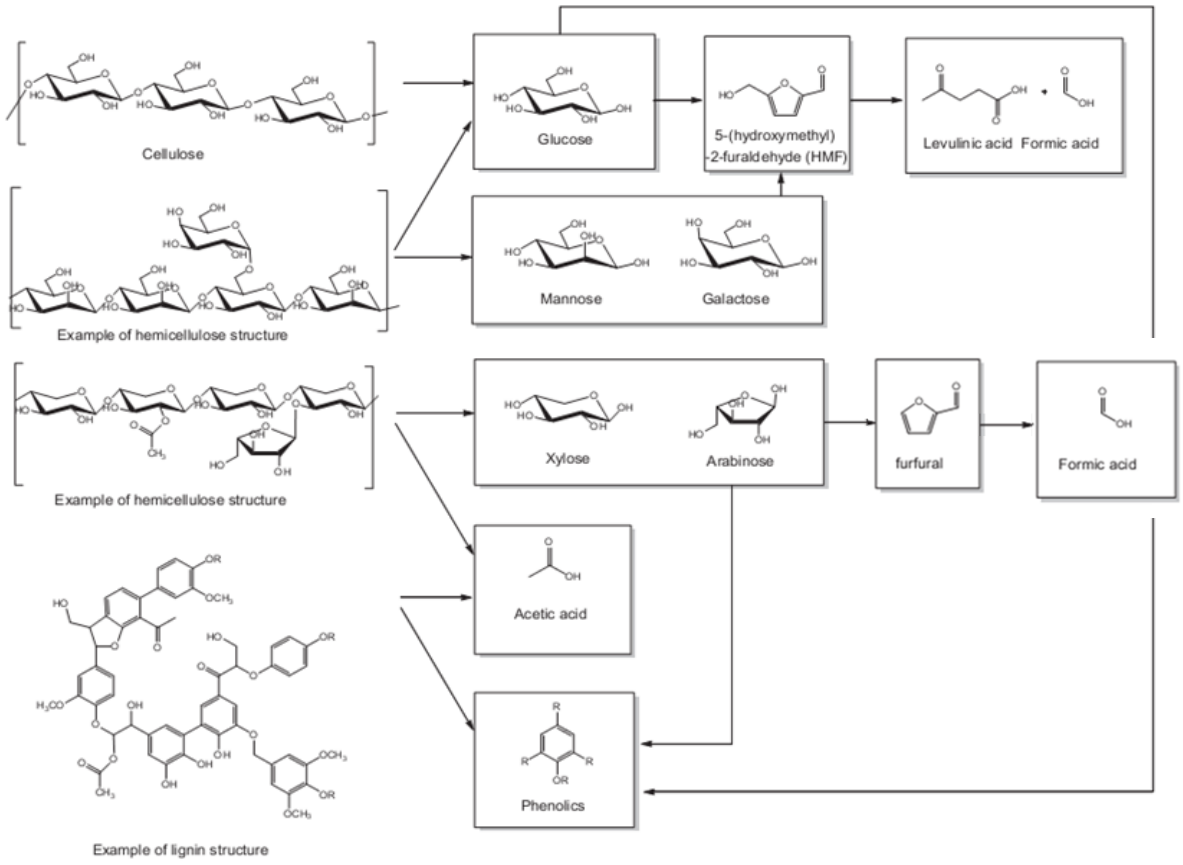


Figure 1.9 Products and summary reaction routes for the degradation of biomass poly- and monosaccharides and lignin during pre-treatment [9]

5-hydroxymethylfurfural (HMF) is a furan based raw material which is considered as one of the top building blocks obtained from biomass.[22] It comprises an aromatic alcohol, aldehyde and furan ring system. HMF is already used as raw material in the production of resins, fine chemicals, pharmaceuticals, polymers (polyester), solvents and liquid transportation fuels (2,4-dimethylfuran, a biofuel that has 40% higher energy density than ethanol).[26] Furfural, similarly to HMF is a heterocyclic aldehyde that has been identified as one of the pivot chemicals to be produced from the lignocellulosic feedstock biorefinery.[22] Furfural is an intermediate commodity chemical used a wide range synthesis of more specialised chemical products, starting mainly with furfuryl alcohol (65 percent of all furfural produced is converted to furfuryl alcohol[27]), which also has many derivatives.

Furfural is used mainly in the production of resin, which is then used as a binding agent in foundry technologies (Figure 1.10). The second main use is as a selective solvent in petroleum production of lubricants. There are many other uses (adhesive, flavouring and as a precursor for many specialty chemicals). Furfural is highly regarded for its thermosetting properties, physical strength and corrosion resistance.

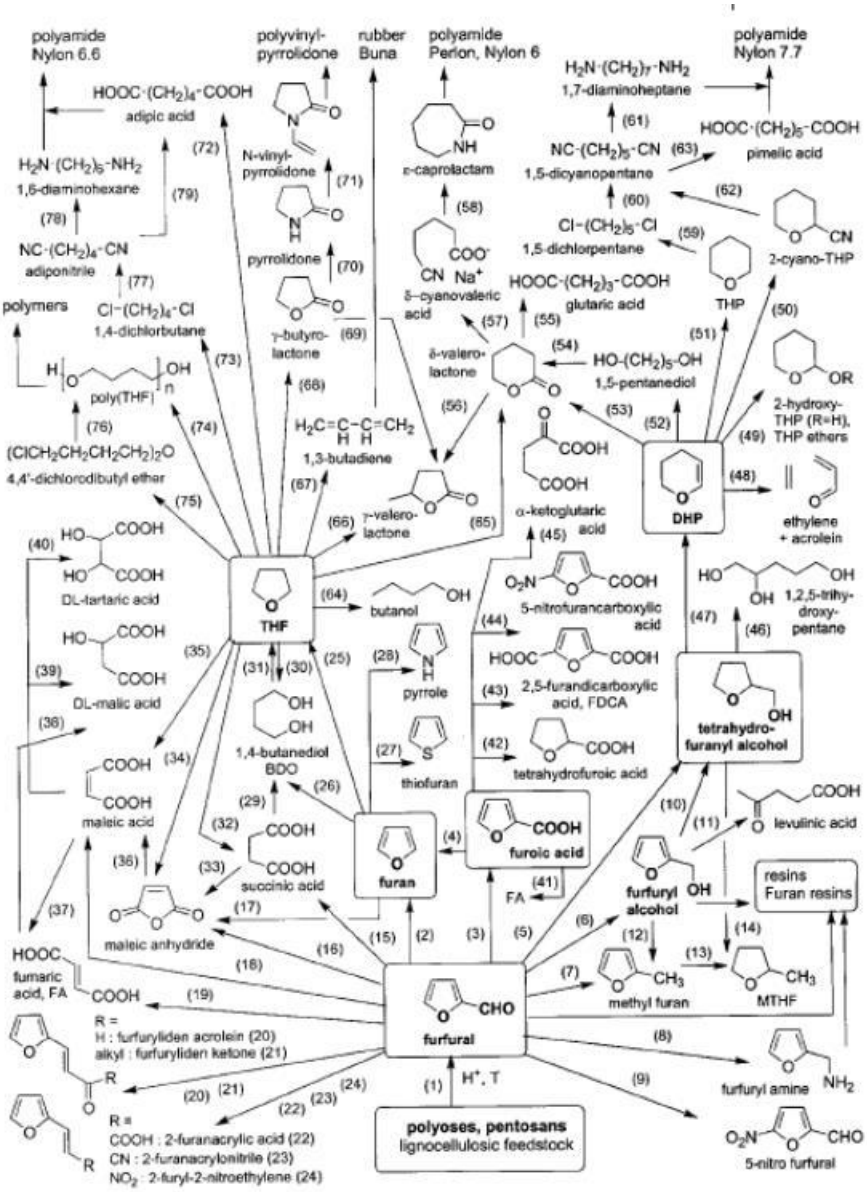


Figure 1.10 Furfural and other chemicals from biomass. For full caption please consult *Biorefineries – Industrial Processes and Products. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.*[28]

Organic acids are degradation products of lignocellulosic materials. Acetic acid is formed by hydrolysis of acetyl groups linked to hemicellulose structure while formic and levulinic acids are degradation products of HMF. Formic acid can also be produced by dehydration of furfural (Figure

1.9).[9] Acetic and formic acids are inhibitors of cellulolytic enzyme activity.[29] Levulinic acid and its derivatives have found a use in highly diverse areas, such as polymers, biological active materials, batteries, personal care products, etc.[30]

1.3. Ionic Liquids

1.3.1. Ionic liquids – novel solvents

Ionic liquids (IL) are salts with melting point below 100ºC composed solely of cation and anions.[31, 32] ILs have broad liquid ranges, negligible vapour pressures and high polarity. Due to the unique properties and numerous combination of cations and anions, IL have been receiving considerable attention (Figure 1.11) and found the use in different areas including industrial application.[33] Among all applications and due to the negligible vapour pressure ILs are mostly used as solvents. Because of this, they allow for easy separation of products providing more favourable chemical environment to carry out clean reactions with minimum waste generation. This drives to name ionic liquids - green solvents however questions related to ILs' toxicity and degradability must be still addressed.[34-36]

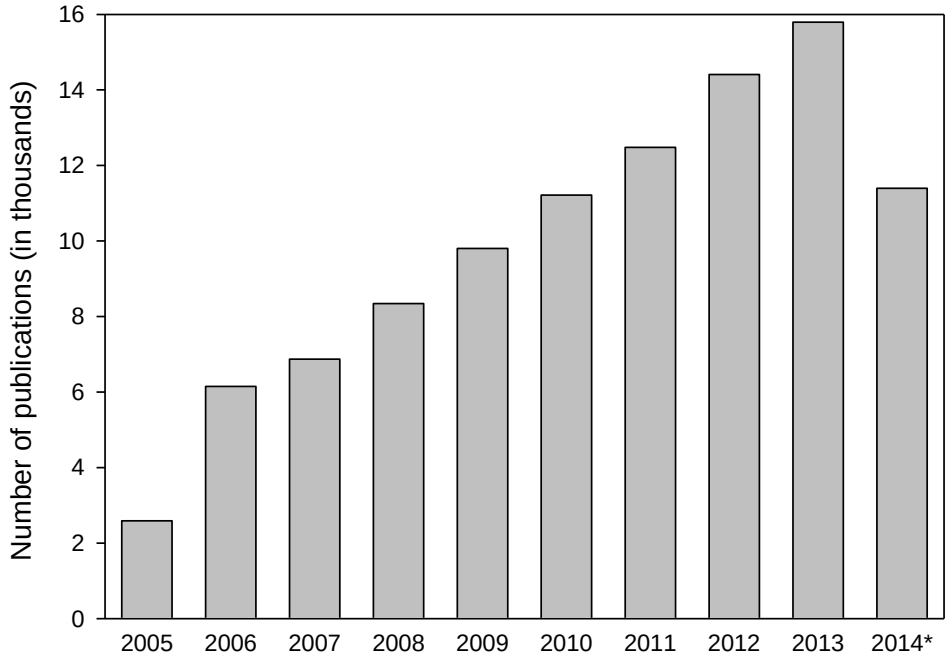


Figure 1.11 Number of literature reports published in last 10 years found in ISI Web of Science search by topic "ionic liquid*"(accessed on 19th September 2014). *up to date of access.

One of the commonly considered drawbacks of ILs' application is their price. However, recent achievements in this field show that IL can be as cheap as organic solvents especially in the bulky quantities.[37]

1.3.2. Dissolution of biomass in ILs

As it was referred before, ILs are great solvents and were broadly used in the biomass dissolution examination. The solubility of either entire biomass or its fractions was extensively studied already.[38] The impact of the IL anion on the dissolution of cellulose was analysed.[39, 40] NMR studies about the dissolution of glucose and cellobiose in ILs showed that the anion strongly coordinates to the carbohydrates' hydroxyl groups. Furthermore, it was shown that IL anion is able to penetrate into the inner structure of cellulose and to form hydrogen bonds with the hydroxyl groups of cellulose, thus disrupting the existing hydrogen bonding network present in crystalline cellulose. The ability to dissolve cellulose was found to correlate proportionally with anion concentration. Nevertheless, the IL cation also contributes to the dissolution.[38] In order to dissolve cellulose, the IL should satisfy the following conditions:

1) Anion must be a good hydrogen bond acceptor;

2) Cation should be a moderate hydrogen bond donor because it has moderate proton capacity bonding with oxygen atoms of the hydroxyls of cellulose;

3) Size of the cation should be as small as possible because of the increasing viscosity affecting negatively the dissolution.

The increase of alkyl chain of the dialkyl imidazolium cation guides to decrease of biomass dissolution.[40] Additionally, the size of the cation interferes with the ability to form hydrogen bonds with cellulose.[38, 41] Therefore, a smaller size of cation favours dissolution of cellulose be promoting the interaction with the oxygen atom of the hydroxyl group in the cellulose structure.[42]

Dissolution of lignocellulosic biomass with ILs is referred as an innovative process where the physicochemical properties of the original biomass are altered in a way not observed before by other solvents.[43, 44] Lignocellulosic biomass interaction with ILs is more complex due to the presence of lignin and extractives, as well as the recalcitrant structure inherent to these materials.[45] The efficiency of lignocellulosic biomass in ILs is associated to the hydrogen bond basicity of the IL anion. Anions with strong hydrogen bond basicity can effectively weaken the hydrogen-bounding network present in the biomass polymers as it was observed for pure biomass fractions.[46]

1.3.3. Biomass pre-treatment with ILs

Pre-treatment with ILs offers advantages superior to conventional methods allowing to: (i) alter physicochemical properties of the biomass macromolecular components, such as reducing the lignin content and cellulose crystallinity; (ii) extract a specific macromolecular component, such as isolation of lignin and cellulose and (iii) perform different fractionation approaches after biomass dissolution in

ILs.[45] Biomass pre-treatment using ILs consists of processing biomass for a determined time and temperature, with or without agitation and no need to apply pressure. This normally results in total or partial dissolution of biomass and alteration of its original properties. The pre-treated biomass is recovered by regeneration, where addition of a protonated precipitating solvent called also anti-solvent is desired.[47] The anti-solvent competes with the dissolved fraction of biomass for the hydrogen bonds formed and creates stronger bonds with ionic liquid that those between IL and biomass fraction. Thus, it results in the precipitation of the desired biomass fraction, and as a result the IL maintains in the liquid phase. The resulted regenerated biomass contains polysaccharides as main content. The regenerated material can be later selectively separated to obtain cellulose and hemicellulose fractions, while IL is recovered from liquid stream with prior to lignin precipitation.[48] During this process a decrease of cellulose crystallinity is observed[48, 49] that guides to enhancement of cellulose saccharification yield.[48, 50, 51]

Pre-treatment conditions can differ depending on: (i) type of IL used; (ii) type, moisture, size and loading of lignocellulosic biomass; (iii) residence time and temperature of pre-treatment; (iv) anti-solvent used and (v) water content. Optimisation of the pre-treatment process is always needed because alteration of the aforementioned condition leads to different pre-treatment results.

The biomass particle size used in the pre-treatment process directly impacts on the contact and diffusion of IL into the lignocellulosic material.[52] Smaller particle size promotes the efficiency of the pre-treatment. However, lower yields of pre-treatment of biomass are verified due to a more extensive depolymerisation to low molecular weight soluble compounds.[53, 54] Concentration of biomass is also an important parameter affecting the pre-treatment efficiency. Better IL performance for dissolution of biomass is favoured in case of less concentrated solution however this is unfavourable due to the lower economic efficiency of the process. On the other hand, extensively high concentrations of solid can hinder agitation of the mixture leading to heat and mass transfer limitations, and as a result low efficiency of the IL action.[45] As such, the ratio of biomass/IL should be optimised and determined depending on the particular biomass and IL used.

Time and temperature of the pre-treatment processes are interlinked. Higher temperature accelerates swelling and dissolution of biomass and normally shorter reaction time is needed, while lower temperatures often require longer times to achieve similar efficiency.[46, 49, 55] Another important aspect of temperature selection is that ILs' properties depend on temperature.[56-58] For example, high temperature lowers viscosity and favours IL effectiveness of the biomass pre-treatment.[59, 60] The disadvantage of higher temperatures is that biomass degradation may occur and as a result the yield of recovered biomass decreases.[61] In other words, high temperatures imply a richer carbohydrate fraction but with a substantial loss in regenerated biomass. The opposite applies to lower temperatures and higher pre-treatment times.[61, 62]

Water content in a pre-treatment with IL can also affect the IL efficiency.[43, 47, 63] Water, being an anti-solvent as mentioned previously,[47] competes with biomass to form hydrogen bonds with IL. Thus the ability of the IL to dissolve biomass is reduced and only partial dissolution of lignocellulose may occur.[43, 63] All of the above mentioned factors vary depending on the IL and biomass used and must be established individually for each studied IL-biomass system.

1.3.4. Acidic ionic liquids as tools to process biomass

Rather than only to dissolve and to pre-treat biomass, some ILs are able to hydrolyse produced fractions and by this convert them to polysaccharides. Acidic ILs can do this kind of integrated processing behaving as both solvent and catalyst.[64-67] Functionalization or alteration of cation or anion is a way to produce acidic ILs. Nowadays, most of the acidic ILs presented in the literature are based on imidazole and pyridine.[26] Acidic ILs functionalised with SO_3H group greatly increase the reaction rate of the cellulose hydrolysis and demonstrate higher catalytic activity for the cleavage of glycosidic bonds.[26] $[HSO_4]^-$ based ILs are another example of acidic ILs able to catalyse biomass. They are extensively used because of their acidic character and their low cost in comparison to other ILs.[37] One of the commonly examined hydrogen sulphate ILs is 1-butyl-3-methylimidazolium hydrogen sulphate ([bmim][HSO_4]), which structure is depicted in Figure 1.12. This IL was reported to provide a selective hydrolysis of hemicellulose observed as a reduced hemicellulose content in the recovered biomass.[48]

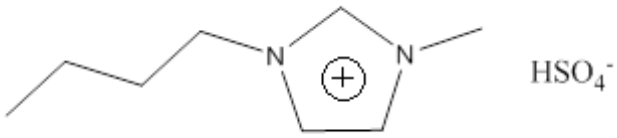


Figure 1.12 Structure of 1-butyl-3-methylimidazolium hydrogen sulphate

2. Objectives

The integration of dissolution, pre-treatment, hydrolysis and conversion of lignocellulosic biomass into particular product is one of the key issues to accomplish the economic efficiency and sustainability of lignocellulosic biorefineries.

The main purpose of this work was to analyse the potential of the acidic IL, [bmim][HSO_4], to examined this approach in case of one of the most common European lignocellulosic residue such as wheat straw. As it was referred, [bmim][HSO_4] demonstrated the potential to fractionate wheat straw and ability to hydrolyse and to convert hemicellulose into furfural. In this work, the mentioned IL hydrolysis and conversion ability was examined in details. For this purpose a wide range of temperature and residence time was studied. The conditions of hydrolysis and conversion capacity to produce either xylose or furfural were selected based on the Doehlert experimental design.

3. Materials and Methods

3.1. Materials

Wheat straw supplied by Estação Nacional de Melhoramento de Plantas (Elvas, Portugal) was used as feedstock. The milling of the raw material was done using a knife mill IKA® WERKE, MF 10 basic, Germany, to get <0.5 mm particles. Humidity of raw biomass used was determined to be 8.3 % (w/w). The used biomass composition is depicted in Table 3.1.

Table 3.1 Composition of wheat straw used as feedstock (% by dry weight)[68]

Component	Composition
Cellulose[a]	38.5 ± 0.1
Hemicellulose	24.9
Xylan	19.1 ± 0.6
Arabinan	3.0 ± 0.1
Acetyl Groups	2.7 ± 0.2
Klason Lignin	17.7 ± 0.1
Ash	10.7 ± 0.1
Protein	4.7 ± 0.1
Others	3.5

[a] Determined as glucan

For the pre-treatment experiments, the [bmim][HSO_4] IL was used (99% purity, Iolitec GmbH, Heilbronn, Germany). The water content in the examined IL was measured by a volumetric Karl – Fischer titration and was 5385 ppm. The [bmim][HSO_4] IL was used as received without further purification.

For the pre-treatment experiments, 4M HCl aqueous solution was prepared from fuming 37 % (w/w) HCl bought from Merck (Darmstadt, Germany) and ultra-pure water (18.2 MΩ/cm) produced by Purelab Classic Elga. Nylon filters (Ø=47 mm, 0.45 µm porosity) from Merck Millipore (Billerica, MA, USA) were also used. Acidified water was made by adjusting the pH of distilled water to value of 2.0 with a solution of 4 M of HCl. Basylone M-350 oil purchased from Bayer (Leverkursen, Germany) was used as the heating medium for pre-treatments experiments.

Capillary electrophoresis (CE) analysis was performed using an electrolyte solution containing 130 mM of sodium hydroxide (NaOH) from EKA (Funchal, Madeira, Portugal) and 36 mM disodium hydrogen phosphate dihydrated ($Na_2HPO_4 \cdot 2H_2O$) from Riedel-de Haën (Sigma-Aldrich Laborchemikalien GmbH, Germany). For the standard sugar samples preparations, D(+)-sucrose, D(+)-xylose, D(+)-cellobiose, D(+)-glucose and D(+)-arabinose were used and were acquired from

Merck (Darmstadt, Germany). Furfural and HMF obtained from Sigma-Aldrich (St. Louis, USA) were also used as standard samples. The standard solutions were prepared using ultra-pure water and contained HMF (0.5-0.03mM), furfural (3-0.05mM) and sugars sucrose, cellobiose, cellulose, arabinose and xylose (4-0.2mM). Solutions of 1.0 mM acetic acid from Panreac (Barcelona, Spain) and 0.1 and 1.0 mM NaOH were applied during the flush phase in CE.

For high-performance liquid chromatography (HPLC) a solution of 5mM sulphuric acid (H_2SO_4) by Panreac Química, (Barcelona, Spain) was used as the mobile phase. Standard samples of acetic acid, fomic acid and levulinic acid from Panreac (Barcelona, Spain) were used in acids analysis using HPLC.

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

For the solid analysis, H_2SO_4 96% (w/w) by Panreac Química, (Barcelona, Spain), Nylon syringe filters (Ø=13mm, 0.22µm porosity) and filtering crucibles with fritted disc, Gooch with porosity grade 4 from SciLabware (Stone, Staffordshire) were used.

3.2. Methodology

3.2.1. Pre-treatment of wheat straw with [bmim][HSO_4]

The pre-treatments performed in this work were carried out according to the procedure represented in Figure 3.1.

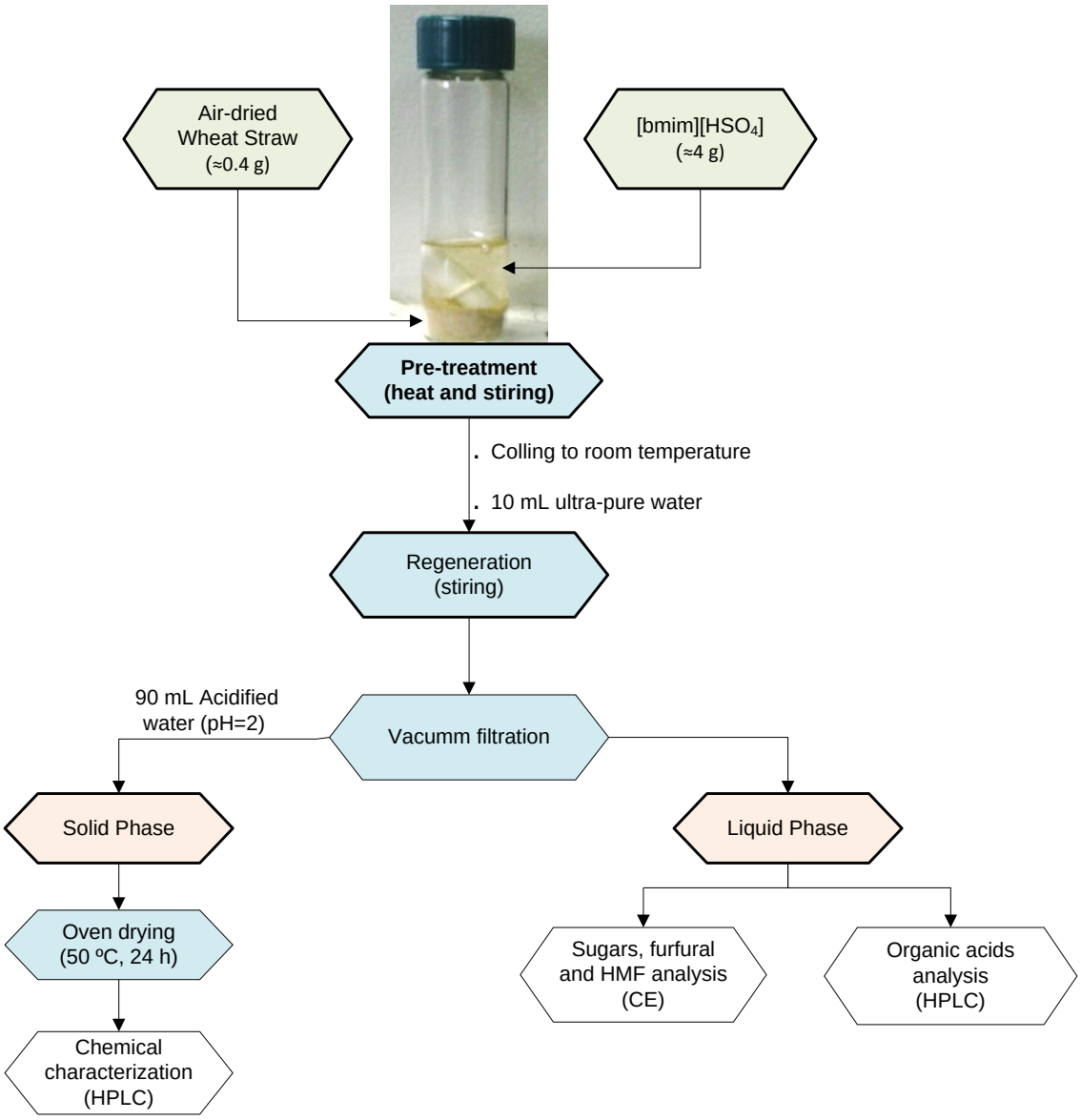


Figure 3.1 Schematic representation of the pre-treatment method

A 4 g of [bmim][HSO_4] was placed into a 15 mL vial and mixed with wheat straw in 10% (w/w) biomass/IL ratio. The vial was submitted to continuous stirring for a defined period of time and temperature in a silicone bath. After pre-treatment, 10 mL of ultra-pure water was added to the flask under continuous agitation. The mixture was filtrated under vacuum and 90 mL of acidified water was used to wash the recovered biomass. The filtrate (liquor) was collected and stored in freezer. The solid phase was dried in oven at 50 °C for 24 h. Afterwards, the recovered biomass was left for a minimum of 1 h at room temperature, and then the recovered mass was measured.

The liquor obtained from each pre-treatment trial was submitted to CE and HPLC analysis while the recovered biomass (solid phase) was submitted to chemical characterisation.

3.2.2. Solid analysis

Solid moisture determination

A nickel plate was placed in oven at 100ºC for at least 5h to remove humidity. After that between 0.1 and 0.06 g pre-treated sample was placed in each plate, heated up in oven for at least 18h and then the dried sample was weighted.

Chemical characterization of the recovered solids

The solid phase resulting from the pre-treatment was washed with ultra-pure water and oven-dried at 50 ºC for at least 48 h. Next, the solids were exposed to room conditions for a minimum of 12 h. The solids obtained from each pre-treatment were subjected to quantitative acid hydrolysis to determine the sugar content (both cellulose and hemicellulose). The protocol of National Renewable Energy Laboratory (NREL)[69] was applied to analyse each recovered solid: (i) 1 mL of H_2SO_4 (72 %) was added to 0.1000 ± 0.0010 g of grounded recovered biomass and the mixture was placed in 30 °C water bath for 1 h; (ii) the treated solution was diluted with 29.65 g of distilled water to obtain acid concentration of approximately 4 %; (iii) the solution sample was conditioned at 121 °C for 1 h using autoclave; (iv) the solution sample was filtered with filtering crucibles grade 4; (v) the resulting liquid was analysed by HPLC to determine the sugar content as mentioned in the following section; (vi) the resulting residue material was dried at 100 °C for 16 h, and lignin content was measured; (vii) ash content was determined by placing the filtering crucibles in a muffle furnace at 550 °C for 5 h.

3.2.3. Liquor analysis

Liquors produced during pre-treatments were subject to two different analyses, namely capillary electrophoresis and high-performance liquid chromatography. Sugars and furans (furfural and HMF) were analysed with CE and the determination of organic acids was made by HPLC analysis.

3.2.4. Chemical analyses

Capillary electrophoresis (CE)

The method applied for sugar and furan determination was based on methodology described in literature.[70] Standard samples with known concentration of sugars, furfural and HMF were produced for the construction of calibration curves. Furthermore, [bmim][HSO_4] was also included as internal standard of stock solutions, always with 200 mM concentration as obtained from the pre-treatment

solution. At this IL concentration the linearity of calibration curves was observed.[71] A solution containing 130 mM NaOH and 36 mM $Na_2HPO_4 \cdot 2H_2O$ was prepared as the electrolyte solution.

The analyses were carried out using Agilent Technologies CE instrument (Waldbronn, Germany), equipped with a diode array detector. The detection was recorded at a wavelength of 270 nm and 200 nm, both with 10 nm bandwidth. Agilent 3D-CE ChemStation data software (Rev B.04.01) was used to perform qualitative and quantitative analysis. An uncoated fused-silica extended light path CE capillary with 50 μm i.d. and 56/64.5 cm total length was used. Between runs the capillary was pre-conditioned by rising sequentially acetic acid 1mM (3 min), sodium hydroxide 1M (3 min), water (3 min) and the electrolyte solution (5 min) and thermostated to +17ºC. The samples were filtered with nylon syringe filters (Ø=13mm, 0.22μm porosity) and injected with a pressure of 35 mbar for 10 s. The separation voltage was fixed at +18 kV for 27 minutes run.

High-performance liquid chromatography (HPLC)

The organic acid content (acetic, formic and levulinic acids) from the collected liquor samples were analysed by an Agilent 1100 Series HPLC equipped with Aminex HPX-87H (Bio-Rad, EUA) column and a refractive index detector (RID). All samples were filtrated using a 0.22 μm syringe filter. The quantification was made by calibration curves using organic acid solutions with known concentrations. Sulphuric acid with 5 mM concentration was flow 0.6 mL/min (sample volume 5 μL) for liquor analysis and 0.4 mL/min (sample volume 5 μL) for solid samples was used as mobile phase. Column temperature was 50ºC and detector temperature of 45ºC was employed.

3.2.5. Optimization of xylose and furfural production

A methodology based on Doehlert[72] experimental design was performed for two different optimisation responses, namely xylose and furfural production from wheat straw hemicellulose. The experimental distribution was considered for two factors: the independent variables "temperature" (X_1) and "residence time" (X_2).Two different experimental distributions were made: the first for xylose production, where 70 °C < X_1 < 160 °C and 20.0 min < X_2 < 120.0 min were considered; the second for furfural production by using 115 °C < X_1 < 175 °C and 63.3 min < X_2 < 163.3 min. Table 3.2 presents the conditions of pre-treatment and respective coded factors, which were used for calculation purposes of the two examined optimisations.

Table 3.2 Pre-treatment temperatures and times studied in this work, and respective coded levels for statistical modeling

T (°C)	*t* (min)	Coded levels (xylose)		Coded levels (furfural)	
		X_1	X_2	X_1	X_2
85	113.3	-0.67	+0.87	-	-
100	70.0	-0.33	0.00	-	-
115	26.7	0.00	-0.87	-1.00	-
	113.3	0.00	+0.87	-1.00	0.00
130	70.0	+0.33	0.00	-0.50	-
	156.6	+0.33	-	-0.50	+0.87
145	26.7	+0.67	-0.87	0.00	-
	113.3	+0.67	+0.87	0.00	0.00
160	70.0	-	-	+0.50	-0.87
	156.6		-	+0.50	+0.87
175	63.3	-	-	+1.00	-1.00
	163.3		-	+1.00	+1.00
	113.3		-	+1.00	0.00

The responses studied were xylan hydrolysis to xylose (Y_1) and xylan conversion to furfural (Y_2). The model used to express the responses was a second order polynomial model and is represented by the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad (1)$$

where X represents the conditions used (independent variables), Y is the response obtained from experimentations and β is the parameters of the polynomial model. The β parameters utilised to estimate the responses have precise meanings: β_0 represents the analysed response in the ` of the experimental domain; the magnitude of β_1 and β_2 indicates the importance of the respective factors (temperature and time, respectively) on the responses; the interaction parameter, β_{12}, indicates how the effect of one factor depends on the level of the other factor. The values of β_{11} and β_{12} determine how the response surface folds downward (negative values) or upward (positive values) quadratically, depending on the magnitude of the absolute value. The relationship between the dependent variables and the response variables was demonstrated by the response surfaces and contour plots obtained using *SigmaPlot*®, Systat Software Inc.

The adequacy of the models to fit the sets of data was performed using the statistical *F*-test for the effectiveness of the factors, which detects whether the source of variance included in the residuals is due to the inadequacy of the models to reproduce experimental data. The adequacy of the model was predicted through the regression analysis (R^2) and the ANOVA analysis ($p < 0.05$), using Microsoft Office Excel 2007 software.

3.2.6. 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 error of $u(T)$ = 1 °C. An arbitrary error of 10% of measured value was defined to all the CE measurements and HPLC analyses.

4. Results

Wheat straw with chemical composition given in Table 3.1 was subject to IL processing with acidic [bmim][HSO_4] IL in temperature range from 70 to 175 °C and residence times from 20.0 to 163.3 min at fixed biomass/IL ratio (10 % (w/w)) and water content (1.25 % (w/w)).

4.1. Production of xylose

Pre-treatments with [bmim][HSO_4] were made focusing on the hydrolysis of hemicellulose fraction from wheat straw to produce xylose as the main product. Based on the literature report[49] the experimental conditions were settled using Doehlert experimental design as shown in Figure 4.1 (orange region) The conditions, 85 °C/26.7 min and 70 °C/70.0 min, were not experimentally validated because less severe reaction condition (85 °C/113.3 min) showed no signs of hydrolysis and conversion of xylan to xylose. For the highest pre-treatment temperature conditions (130 °C/70.0 min), the highest hydrolysis of xylan to xylose was found among all examined conditions. Therefore, following the pattern of Doehlert experimental design, pre-treatments with higher temperatures were studied, namely at 145 °C/26.7 min and 145 °C/113.3 min (blue region) without 160 °C/70.0 min which was not considered for the optimization of xylose production due to the expected extensive hydrolysis.

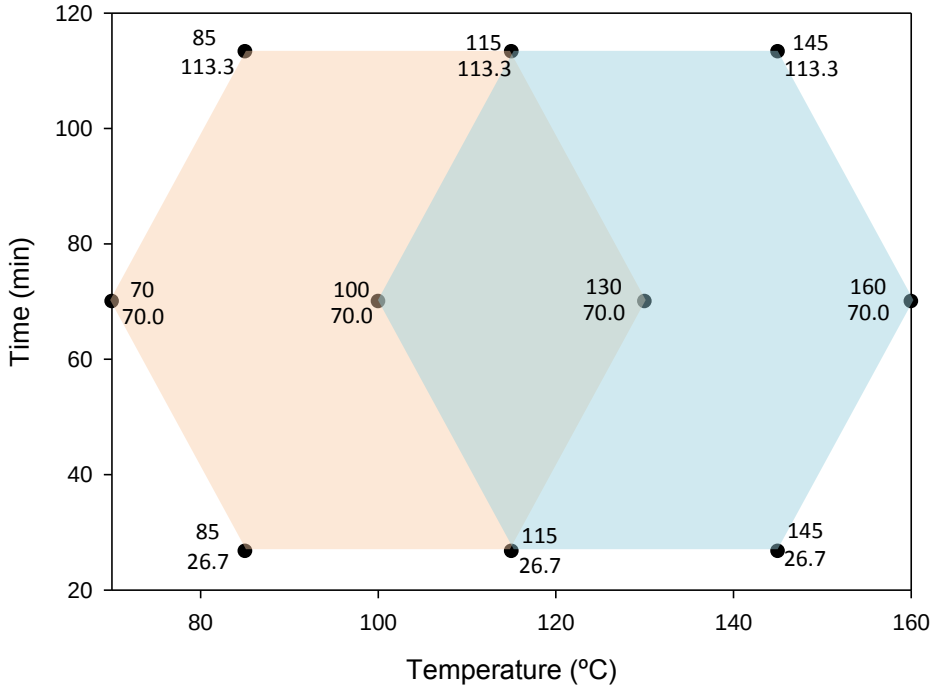


Figure 4.1 Doehlert experimental design for xylose production for 70 °C<X_1<160 °C and 20.0 min<X_2<120.0 min; the points represented by the orange and blue hexagon were the conditions studied.

The results obtained for all performed experiments are given in Table 4.1.

Table 4.1 Liquid phase composition obtained after wheat straw pre-treatment with [bmim][HSO_4] at various temperatures and residence times

T (°C)	*t* (min)	Xylose		Arabinose		Furfural		Glucose		HMF		Acetic acid	
		mg	% (w/w)[a]	mg	% (w/w)[a]	mg	% (w/w)[b]	mg	% (w/w)[a]	mg	% (w/w)[b]	mg	% (w/w)[c]
85	113.3	0.0	0.0	2.5	20.3	0.1	0.1	1.4	1.0	0.9	0.6	0.9	9.5
100	70.0	1.4	2.1	2.5	20.0	0.2	0.2	1.2	0.8	1.1	0.8	1.2	12.4
115	26.7	3.3	4.6	1.9	15.2	0.3	0.3	1.2	0.9	1.2	0.8	1.8	17.8
115	113.3	10.7	15.3	1.4	11.3	2.5	3.1	1.5	1.0	1.3	0.9	3.8	38.3
130	70.0	13.2	18.8	1.3	10.3	11.6	14.3	1.2	0.8	1.0	0.7	5.7	57.9
145	26.7	8.8	12.5	0.6	4.5	8.8	10.9	1.0	0.7	1.2	0.8	5.6	56.6
145	113.3	3.1	4.4	0.5	4.1	21.1	26.1	1.6	1.1	1.3	1.0	8.2	83.3

[a]Xylose, arabinose, glucose and acetic acid production as percentage of initial concentration of xylan, arabinan, glucan and acetyl groups present in untreated biomass, respectively; [b]Furfural percentage was calculated based on sum of initial concentration of xylan and arabinan and HMF conversion was calculated on glucan present in untreated biomass. Formic and levulinic acids were not found in the samples.

The obtained results show that xylose yield in liquor increases with temperature and residence time up to 130 °C and 70.0 min up to 18.8 % (w/w) while for higher temperature (145 °C) the xylose yield decreases to less than ¼ of the maximal one. This decrease is counterbalanced by increase of furfural yield to 26 % (w/w). In case of arabinose, a constant decrease of arabinose yield was found to decrease with the increase of reaction time and temperature. Analysing the glucose yield it can be stated that concentration of glucose remains unchanged in the range of experimental error for all experiments. Similar conclusion can be drawn for glucose degradation product such as HMF, which concentration remains constant in the range of studied parameters. Similarly to furfural, the yield of acetyl groups' hydrolysis to acetic acid increases leading to 83.3 % (w/w) of initial acetyl groups' hydrolysis. Two most common organic acids such as formic and levulinic were not found in the examined samples.

The results of solid produced during the wheat straw processing with [bmim][HSO_4] are summarised in Table 4.2.

Table 4.2 Results of solid phase composition in mass percentage of processed biomass produced in wheat straw pre-treatment with [bmim][HSO_4] at various temperatures and residence times; Y represents the yield of the regenerated biomass

T (°C)	*t* (min)	*Composition* (% w/w)						Y%
		Xylan	Arabinan	Acetyl groups	Glucan	Lignin	Ash	
untreated biomass		19.1	3.0	2.7	38.5	17.7	10.7	
85	113.3	24.3	2.6	2.4	40.7	18.6	2.8	89.4
100	70.0	23.3	2.1	2.1	43.1	20.8	3.5	82.8
115	26.7	21.4	2.0	2.3	44.9	20.0	3.6	78.2
115	113.3	14.5	0.9	2.3	50.3	18.7	4.3	66.1
130	70.0	10.5	1.1	1.1	58.2	20.6	5.8	58.8
145	26.7	9.6	0.8	1.0	54.6	22.1	5.5	58.7
145	113.3	5.6	1.4	1.0	50.9	32.6	5.4	58.1

Characterisation of obtained solids was necessary to determine the amount of biopolymers which did not undergo hydrolysis by [bmim][HSO_4]. The solid yield varies from 89.4% to 58.8% for 85ºC/113.3 min and 145ºC/113.3 min respectively. The obtained data shows that xylan is still present in the recovered solids and its contents decreases from 24.3 % (w/w) of produced solid to 5.6% (w/w) for the lowest and the highest examined conditions. Similar behaviour is observed for arabinan and acetyl groups found in the processed solids. The loss of xylan, arabinan and acetyl group contents in the produced solids is counterbalanced by significant increase of glucan and lignin content. For the highest temperature examined the glucan content is above 50 % (w/w) and lignin reaches as much as 32.6 % (w/w) of obtained solid sample.

The partition of cellulose and hemicellulose fraction between liquid and solid phases is depicted in Figure 4.2. Analysis of this figure reveals that hemicellulose recovery for the lowest temperature is quantitative while for more severe conditions the recovery of hemicellulose fraction decreases significantly to 49.4 % (w/w) and most of the hemicellulose can be found in liquor as hydrolysis and degradation products. In case of cellulose recovery, it also can be observed an analogous decrease of cellulose recovery however decline is much less significant than for hemicellulose. It is also important to mention that for the mildest conditions studied the cellulose was recovered with 88.6 % (w/w) of initial cellulose content in the biomass.

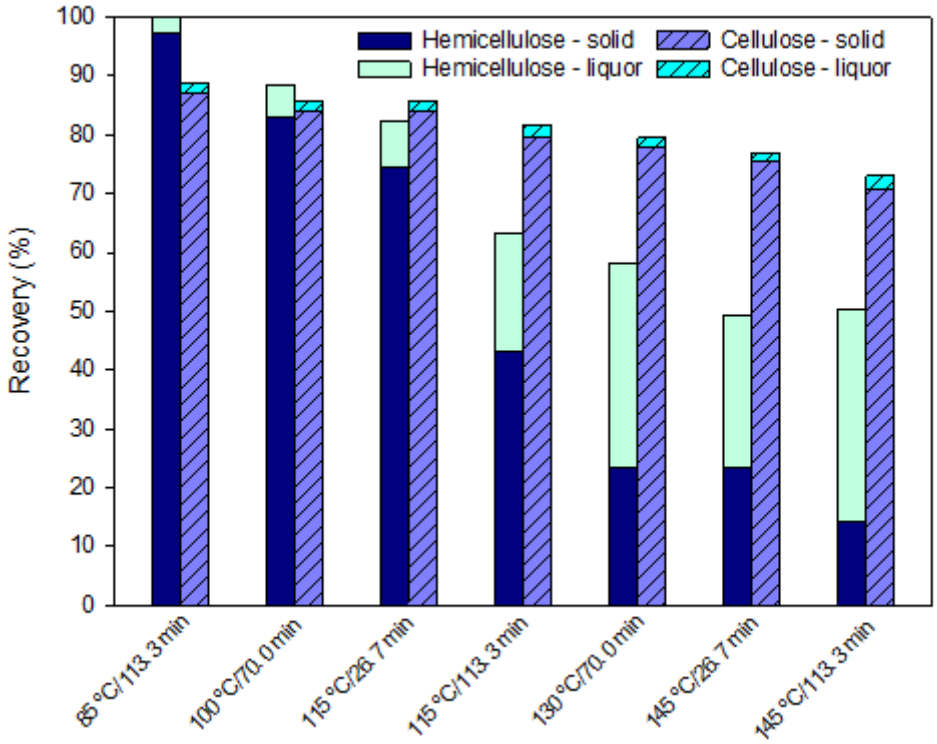


Figure 4.2 Total recovery of hemicellulose and cellulose in pre-treatment of wheat straw with [bmim][HSO_4] for xylose production

4.2. Production of furfural

The pre-treatments previously made demonstrated that xylose is rapidly converted into furfural mostly at higher temperatures. Therefore, the extent of wheat straw pre-treatment and hydrolysis with the aim of xylose conversion to furfural was also studied at more severe conditions. The conditions chosen by Doehlert experimental design to study and to optimise the production of furfural with [bmim][HSO_4] are represented by the green hexagon in Figure 4.3. Furthermore, two additional set of conditions namely 175 °C/163.3 min and 175 °C/63.3 min was also taken into account for the optimisation of furfural production.

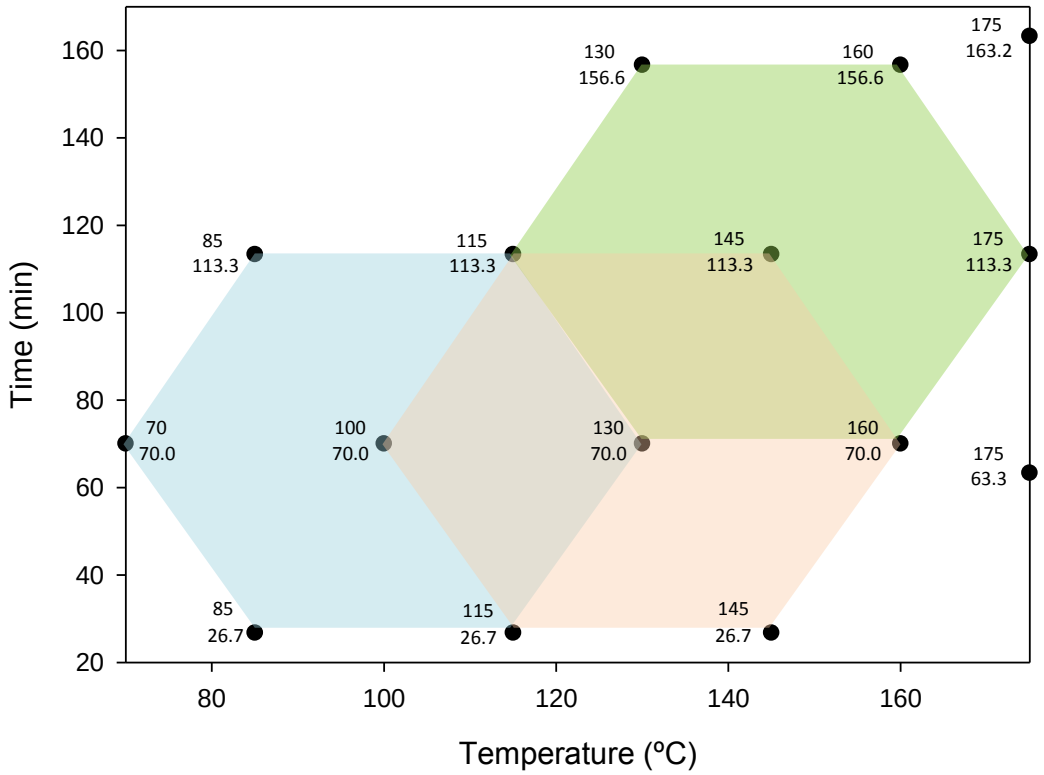


Figure 4.3 Doehlert experimental design for furfural production for 115 °C<X_1<175 °C and 63.3 min<X_2<163.3 min; the points represented by the green hexagon.

Table 4.3 presents the data obtained from the liquor analysis for the new conditions for furfural production. The monosaccharides' present in the liquor disappears faster for more sever conditions. Xylose and arabinose was observed only for a few initial reactions (130 °C/156.6 min, 160 °C/70.0 min and 175 °C/63.3 min), and arabinose was only detected in the liquor obtained from the process at 130 °C/156.6 min but even so in very low concentration. This xylose and arabinose disappearance was counterbalanced by furfural yield which increases and for pre-treatment at 160 °C/156.6 min reached maximum. However, for higher temperature and longer pre-treatment time, its yield decreased significantly. It is worth mentioning that the increase of reaction time between the conditions 175 °C/113.3 min and 175 °C/163.3 min leads to a significant decrease in furfural content compensated by the significant rise of formic acid production. Another product obtained from hemicellulose is acetic acid. The acetic acid formation is very high and is quantitative in all reactions showing that more severe conditions than 160 °C/70 min. are enough severe to solubilise completely acetyl groups present in the hemicellulose. Glucose, HMF and levulinic acid shows similar trend to this observed for xylose and its degradation products. Although the yields are much lower than in case of hemicellulosic-origin products it can be easily discovered that the glucose yield lowers with the reaction severity increase, while HMF presence increases and peaks for 175 °C/113.3 min and its decrease is compensated by the increase of levulinic formation up to 2.1% (w/w) of initial glucan content.

Table 4.3 The liquor composition analysis at various temperatures and residence times for furfural formation

T	*t*	Xylose		Arabinose		Furfural		Glucose		HMF		Acetic acid		Formic acid		Levulinic acid	
(°C)	(min)	mg	% (w/w)[a]	mg	% (w/w)[a]	mg	% (w/w)[b]	mg	% (w/w)[a]	mg	% (w/w)[b]	mg	% (w/w)[c]	mg	% (w/w)[d]	mg	% (w/w)[e]
130	156.6	11.2	16.0	0.9	7.8	19.0	23.4	1.6	1.1	1.5	1.1	6.9	69.5	0.0	0.0	0.0	0.0
160	70.0	0.9	1.3	0.0	0.0	24.9	30.7	1.4	1.0	2.2	1.6	8.9	89.7	0.0	0.0	0.0	0.0
160	156.6	0.0	0.0	0.0	0.0	29.3	36.2	1.3	0.9	3.7	2.6	10.5	106.4	1.7	0.8	2.4	1.7
175	63.3	0.4	0.6	0.0	0.0	24.8	30.6	1.2	0.9	2.6	1.8	9.5	95.9	2.2	1.0	2.1	1.5
175	113.3	0.0	0.0	0.0	0.0	27.9	34.4	1.2	0.9	4.3	3.0	9.4	95.1	0.6	0.3	2.9	2.0
175	163.3	0.0	0.0	0.0	0.0	12.7	15.6	0.0	0.0	2.3	1.6	10.8	108.8	4.6	2.1	3.0	2.1

[a]Xylose, arabinose, glucose and acetic acid production as percentage of initial concentration of xylan, arabinan, glucan and acetyl groups present in untreated biomass, respectively; [b]Furfural percentage was calculated based on sum of initial concentration of xylan and arabinan and HMF conversion was calculated on glucan present in untreated biomass; [c]Formic acid content is presented as percentage of sum of initial concentrations of xylan, arabinan and glucan; [d]Levulinic acid as percentage of initial concentration of glucan.

The yield of solid obtained from pre-treatments was in the range of 59.7 to 68.6 % (w/w) as presented in Table 4.4. Arabinan and acetyl groups were not found in the solid residue. Also xylan was found only in the less severe condition's reaction. The major fractions of produced solid are glucan and lignin. Along the reaction condition severity increase the glucan content decreases by 1/3 to 38.6 % (w/w). At the same time an increase of lignin content is doubled and at the most sever conditions lignin constitute more than 50% (w/w) of the produced solids.

Table 4.4 Results of solid phase analysis obtained from wheat straw pre-treatment with [bmim][HSO_4] at various temperatures and residence times considered for furfural formation;Y represents the yield of the regenerated biomass

T (°C)	*t* (min)	*Composition* (% w/w)						Y (%)
		Xylan	Arabinan	Glucan	Acetyl	Lignin	Ash	
untreated biomass		19.1	3.0	38.5	2.7	17.7	10.7	
130	156.6	6.8	0.0	56.7	0.0	26.0	6.2	59.7
160	70.0	0.0	0.0	52.4	0.0	36.7	6.2	61.5
160	156.6	0.0	0.0	48.6	0.0	46.0	4.9	63.3
175	63.3	0.0	0.0	45.5	0.0	43.7	7.4	63.2
175	113.3	0.0	0.0	45.5	0.0	47.8	5.7	65.5
175	163.3	0.0	0.0	38.6	0.0	52.6	6.6	68.6

Analysing the recovery of polysaccharide fractions showed that in case of hemicellulose a continuous decrease of recover yield with the increase of temperature is observed hence cellulose recovery yield is less susceptible for reaction conditions and thus, the recovery yield decreases much slower than this for hemicellulose (Figure 4.4).

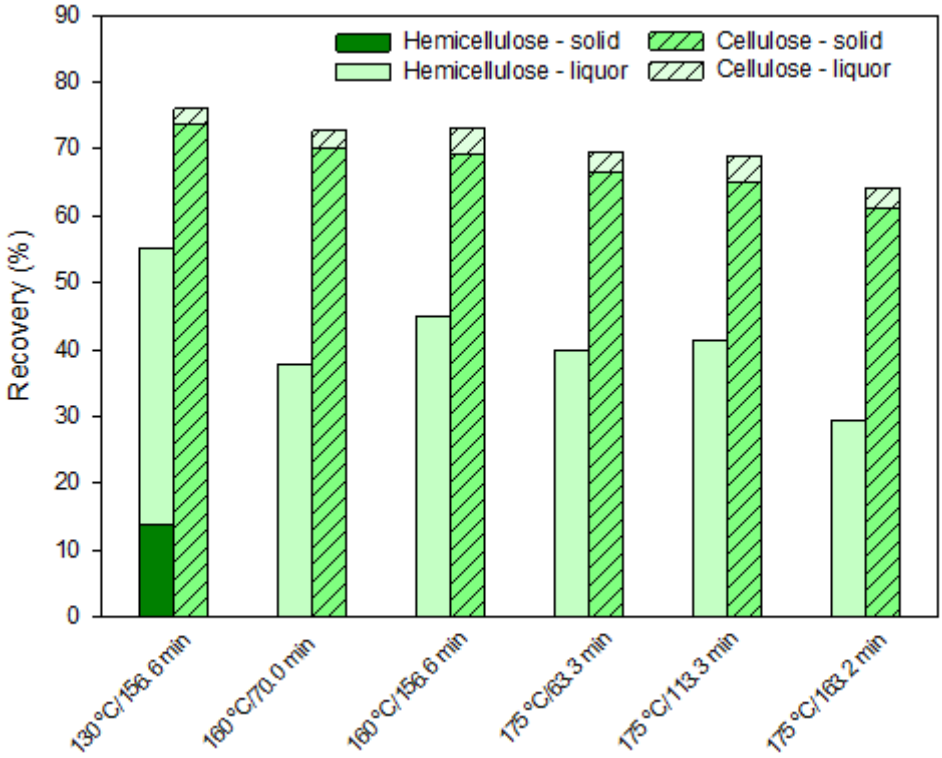


Figure 4.4 Total recovery of hemicellulose and cellulose in pre-treatment of wheat straw with [bmim][HSO_4] for furfural production conditions

4.3. Statistical modelling

The pre-treatment conditions for production of xylose and furfural undergo statistical modelling. In order to optimise the effect of the principal independent variables (temperature (X_1) and time (X_2)) on efficiency of xylan hydrolysis into xylose (Y_1) and to furfural (Y_2), the following Doehlert experimental designs were employed (Table 3.2, Table 4.5 and Table 4.6).

Table 4.5 Doehlert experimental design applied for the corresponding experimental responses Y_1 (xylan hydrolysis to xylose)

Run	Coded variables		Response
	X_1	X_2	Y_1
A	-0.33	0.00	1.9
B	0.33	0.00	18.9
C	0.00	0.87	15.4
D	0.00	-0.87	4.5
E	-0.67	0.87	0.0
F	0.67	-0.87	12.9
G	0.67	0.87	3.8

Table 4.6 Doehlert experimental design applied for the corresponding experimental responses Y_2 (xylan conversion to furfural)

Run	Coded variables		Response
	X_1	X_2	Y_2
A	-0.50	-0.87	14.3
B	-1.00	0.00	3.1
C	0.00	0.00	26.1
D	1.00	0.00	34.4
E	0.50	0.87	36.2
F	0.50	-0.87	30.7
G	-0.50	0.87	23.4
H	1.00	1.00	15.6
I	1.00	-1.00	30.6

The statistical significance of estimated effects on both Y_1 and Y_2 responses was checked by analysis of variance (ANOVA) (Table 4.7). The p-values of the main effects indicated the statistical significance (p>0.05) of the estimated relations between variables within a 95 % confidence interval for some coefficients. Model analysis by the coefficient of multiple determinations (R^2) indicated that the relevance of the dependent variables in the model was well fitted to explain the behaviour variation (R^2 value is near the unity).

Table 4.7 Parameters of the polynomial models representing the studied response Y_1 (xylan hydrolysis to xylose) and Y_2 (xylan hydrolysis to furfural); The adequacy of the models to fit the sets of data was performing using *Fisher* test (F-test) for the effectiveness of the factors.

Model parameters (MP)	Y_1		Y_2	
	MP	*p*	*MP*	*p*
β_0	13.82	0.01	30.16	0.00
β_1	19.77	0.02	12.89	0.01
β_2	7.04	0.04	2.81	0.40
β_{12}	-18.29	0.03	-7.94	0.12
β_{11}	-32.00	0.03	-13.70	0.05
β_{22}	-5.19	0.16	-3.36	0.49
F-test				
Effectiveness of the parameters	16.23		5.49	
Significance level	0.06		0.06	
R^2	0.99		0.93	

The 3D response surfaces based on the statistical modelling are illustrated in Figure 4.5. The figures show the modelled effects of independent variables, temperature and residence time, on the reaction outputs, Y_1 and Y_2.

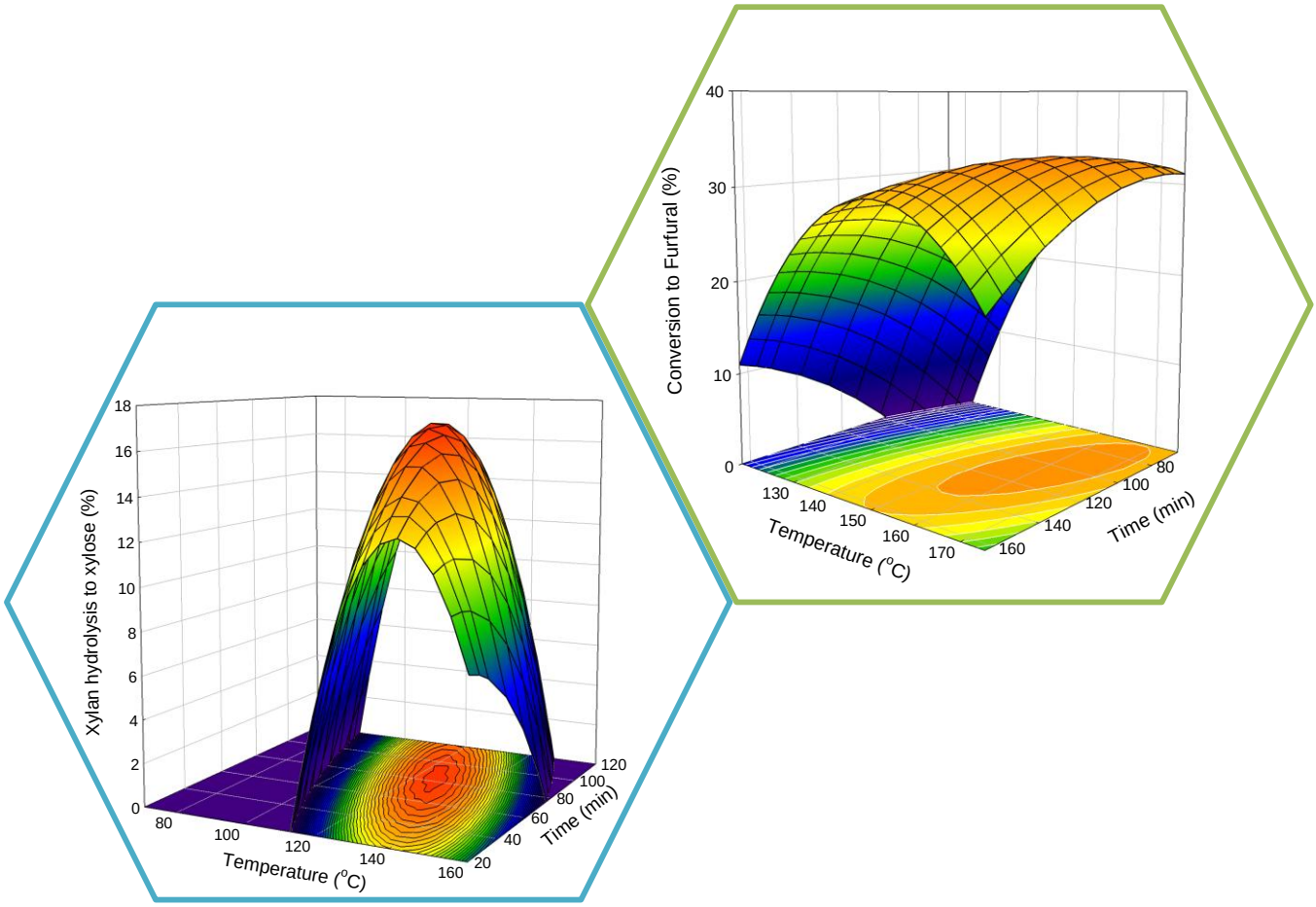


Figure 4.5 Response surface and contour plot of modelled xylan hydrolysis into xylose (bottom figure), and hemicellulose conversion to furfural (upper figure) as a function of reaction time (min) and temperature (°C)

The optimum condition set obtained after the statistical modelling analysis for Y_1 was 125 °C/82.1 min with a statistical response estimated at the level of 17.1 % hydrolysis. The optimum condition for the response Y_2 was 161 °C/104.5 min with 33.3% of hydrolysis to furfural. The model was validated after performing the experimental pre-treatment at the optimised conditions. The analysis results of liquid and solid fractions are represented in Table 4.8 and Table 4.9. The experimental value for the xylose production obtained was 16.7 % hydrolysis. In case of xylan to furfural conversion the experimental validations allowed to obtain 32.2 % of conversion to furfural

Table 4.8 Analysis of the liquid fraction produced in experiments performed at the optimum conditions for xylose and furfural production

T (°C)	*t* (min)	Xylose		Arabinose		Furfural		Glucose		HMF		Acetic acid		Formic acid		Levulinic acid	
		mg	% (w/w)[a]	mg	% (w/w)[a]	mg	% (w/w)[b]	mg	% (w/w)[a]	mg	% (w/w)[b]	mg	% (w/w)[c]	mg	% (w/w)[c]	mg	% (w/w)[c]
125	82.1	11.7	16.7	1.2	10.9	6.2	7.6	1.3	0.9	1.5	1.1	4.0	40.8	0.0	0.0	0.0	0.0
161	104.5	0.0	0.0	0.0	0.0	24.9	30.7	1.3	0.9	2.0	1.4	6.7	67.9	2.1	0.9	0.7	2.1

[a]Xylose, arabinose, glucose and acetic acid production as percentage of initial concentration of xylan, arabinan, glucan and acetyl groups present in untreated biomass, respectively; [b]Furfural and HMF conversion percentage were calculated based on xylan and glucan present in untreated biomass, respectively; [d]Formic acid as percentage of initial concentration of xylan, arabinan and glucan; [e]Levulinic acid as percentage of initial concentration of glucan.

Table 4.9 Analysis of the solid produced from wheat straw pre-treatment at optimum conditions for xylose and furfural production obtained from statistical modelling

T (°C)	*t* (min)	Solid characterization (% w/w)							Total recovery yield (%)	
		Xylan	Arabinan	Acetyl	Glucan	Lignin	Ash	Y (%)	Hemicellulose	Cellulose
125	82.1	12.4	0.3	0.5	55.3	20.1	6.1	64.4	54.5	81.1
161	104.5	0.0	0.0	1.0	47.5	40.5	6.4	62.8	38.4	71.4

5. Discussion

Various studies about the pre-treatment and fractionation of biomass using ILs have been made in the last few years.[45, 46, 48, 49, 64, 73] Recently, the ILs containing the anion [HSO_4] became an appealing option to be used in the biomass pre-treatment, once the acidic properties of this IL allows the catalytic conversion of biomass.[48, 74-77] This work is focused on the study of [bmim][HSO_4] IL as a tool for hydrolysis and conversion of wheat straw hemicellulosic fraction during the pre-treatment process. In fact, the acidity of this IL not only allows the hydrolysis of hemicellulose into monosaccharides, such as xylose and arabinose, but also converts those monosaccharides into further degradation products such as furfural.[78] The examined processes resulted in the liquid phase containing mainly hemicellulose hydrolysis products and a processed solids constituted by cellulose and lignin. After each pre-treatment both liquid and solid fractions were duly analysed. The analysis was focused on the detection and quantification of compounds that are directly obtained from the hydrolysis and/or conversion of wheat straw hemicellulose. Therefore, CE was employed to analyse monosaccharides (xylose, arabinose and glucose) and furans (furfural and HMF), while HPLC allowed to identify the organic acids present in the sample. The main reason to use CE is the capacity of this technique to detect and separate analytes in the sample with higher tolerance for IL concentration than HPLC.[71] Unfortunately, CE was could not be used for the organic acid analysis, due to the direct interference of the IL on the separation of organic acids.

The applied methodology to study temperature and time of the pre-treatment on the reaction effectiveness does not allow for direct analysis of these parameters. Thus, for comparison purposes a severity factor ($logR_0$) defined by Overend and Chornet[79, 80] normally used to measure the effects of exactly these two parameters according to equation below can be used. A severity factor is described by the equation below:

$$R_0 = \int_0^t e^{\frac{T(t)-100}{14.75}} dt \quad (2)$$

where *t* is time expressed in minutes, T relates to temperature in °C, 100 is the reference temperature (100 °C) and 14.75 is an empirical constant. Furthermore considering a strongly acidic character of some pre-treatments the combined severity factor described by the following below should be applied.

$$CSF = \log(R_0) - pH \quad (3)$$

A close inspection of the equation depicting the severity factor reveals that the reference temperature and empirical factors are related to temperature at which water starts to act as a catalyst in classical pre-treatment processes (e.g. autohydrolysis or acid catalysis).[81] However this is not the case in the processes with acidic ionic liquid, thus new parameters were established following the methodology presented by Chum et la.[82] Shortly, the percentage of hemicellulose hydrolysis attained in this

work was used to estimate the values of reference temperature and empiric factor. The details of the severity factor determination are shown in Appendix 3. Therefore applying the methodology proposed by Chum et al.,[82] the reference temperature established is 88.28 and empirical factor equals 6.47. All CSF obtained are depicted in Table 5.1.

Table 5.1 Combined severity factor for all conditions performed

T (°C)	85	100	115	115	145	130	130	145	160	160	175	175	175
t (min)	113.3	70.0	26.7	113.1	26.7	70.0	156.6	113.3	70	156.6	63.3	113.3	163.3
CSF	0.80	1.56	1.89	2.79	3.52	3.52	3.94	4.77	5.46	5.93	6.34	6.74	6.95

5.1. The effect of temperature and pre-treatment time on biomass conversion

The wheat straw pre-treatment with [bmim][HSO_4] was studied at fixed IL/biomass ratio equals 10 (w/w) and at constant water content of 1.25 % (w/w). Two other parameters namely temperature and residence time, which have a direct impact on the hydrolysis and conversion performance of the IL, were studied in a broad range. The pre-treatment was carried out at six different temperatures (85, 100, 115, 130, 160 and 175 °C) and six residence times (26.7, 63.3, 70.0, 113.3, 156.6, 163.3 min) resulting in a diverse range of pre-treatment conditions. Among all examined pre-treatment conditions, the hydrolysis of hemicellulose occurred, even at the lowest temperature examined. At 85 °C/ 113.3 min (CSF = 0.80) the presence of arabinose in the liquid phase (20.3 % (w/w)) without xylose presence indicates that arabinan is the first and the most susceptible fraction for hydrolysis. Similar behaviour was found in literature for other types of pre-treatments. For example, Carvalheiro et al. studied the kinetics of brewery's spent grain autohydrolysis and verified that highest concentrations of arabinose oligomers were obtained for shorter reaction times than xylose oligomers.[83] This phenomenon finds also an explanation in the chemical structure of hemicellulose, which consists of arabinan branches in xylopyranosyl backbone that makes arabinan more susceptible to the hydrolysis than xylan polymer. Hence results attained for experiment performed at temperature of 85 °C shows clearly that selective hydrolysis of arabinan in detriment of xylan is possible. Furthermore, it is important to notice that arabinose content in the liquid phase decreases with the increase of pre-treatment severity. On the contrary, yield of xylose increases and reaches maximum at CSF=3.52 (130 °C/70 min). Further increase of severity (CSF > 3.94) guides to complete disappearance of xylose observed in case of pre-treatments performed at CSF = 5.46 or in other words for temperatures equal or higher than 160 °C. As expected, the discussed decrease of monosaccharide yields is accompanied by the increase of furfural content in the liquid phase. The achieved results permit to conclude that temperature seems to have a crucial effect on favouring the hydrolysis of hemicellulose, which for more severe conditions (CSF > 3.52) guides the conversion of monosaccharides into furfural. Analogous behaviour was also

reported in the literature for the pre-treatment of Miscanthus biomass using the same IL.[76] As mentioned before arabinose is firstly formed, but next is rapidly converted into furfural. Xylose follows this pathway and undergoes a quick conversion to furfural too. Hence for CSF = 6.34 degradation of furfural was observed, suggesting that the used conditions were too severe and furfural may suffers a further degradation.[9, 84] Similarly to both hemicellulosic saccharides, the increase of acetic acid production was observed in the liquid phase meaning a continuous hydrolysis of acetyl groups attached to hemicellulose structure. In respect to cellulose hydrolysis and conversion with [bmim][HSO_4], the results show that glucose is barely produced and the same occurs to its conversion into HMF. This is an unique characteristic of [bmim][HSO_4], which demonstrates to selectively act over hemicellulose and hemicellulose derivative products. Figure 5.1 illustrates all the discussed behaviours summarising the main results obtained in the liquid phase after each pre-treatment.

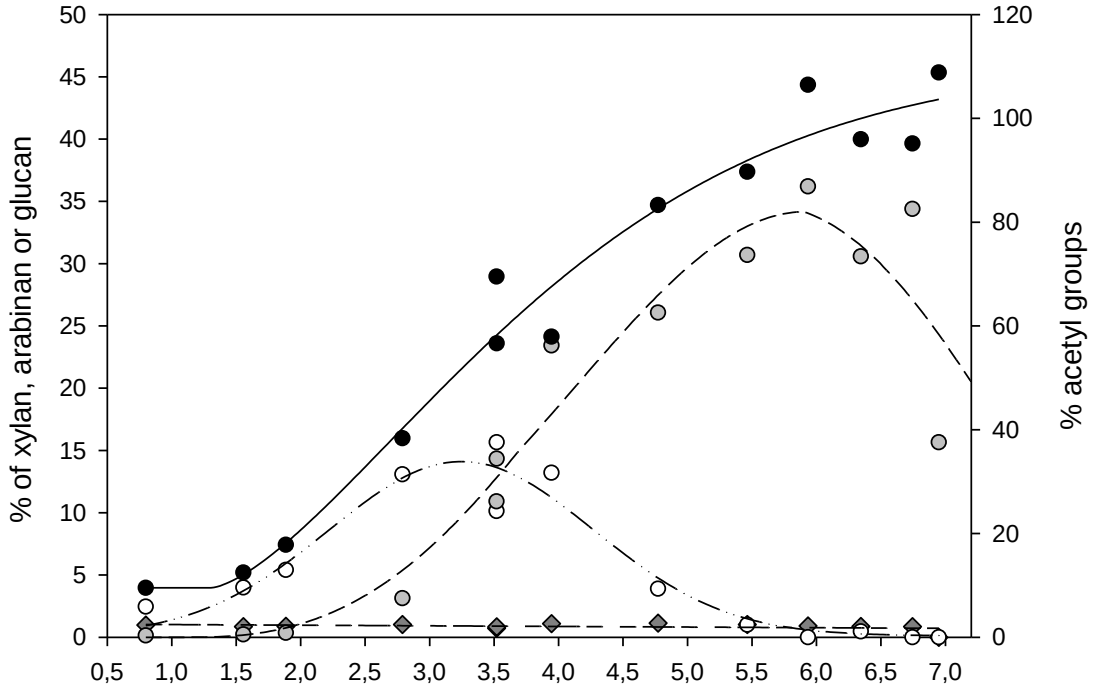


Figure 5.1 Glucose (♦), arabinose and xylose (○), furfural (◍) and acetyl groups (●) found in [bmim][HSO_4] liquors after pre-treatment. Solid/dashed/dashed-dot-dot and short-long-short dashed lines are designed by polynomial adjustment to the experimental points and serve as guide for the eye

Regarding the obtained solid, the chemical characterisation of the pre-treated solids (Table 4.2 and Table 4.4) shows an enrichment of glucan and lignin contents with the severity of the performed reactions. It is caused by the extensive hydrolysis of hemicellulose and total hemicellulose hydrolysis was achieved for pre-treatments with CSF > 4.77 (temperature above 145 °C).

The main fractions of solid are cellulose and lignin. The maximum cellulose content (58.2 % (w/w)) in the solid was reached at CSF = 3.52 (130 °C/70.0 min), but for more severe conditions, a cellulose content decreases and the lowest cellulose content (38.6 % (w/w)) in the solid was found after the

reaction at the most severe conditions (CSF = 6.95). Surprising is that this loss is not reflected in the liquid phase, where glucose, HMF and levulinic acid contents are very low (4.9 % (w/w) on the glucan basis). Actually, regarding to the mass balance of the process, around 34.0 % (w/w) cellulose was lost for the most severe reaction conditions (Figure 4.4). The reason for this might be extensive hydrolysis caused by [bmim][HSO_4]. In spite of high cellulose lost, the hemicellulose mass loss is even higher. Hemicellulose had the lowest recovery value at CSF = 6.95 (29.3 %). Equally to cellulose, the total mass of hemicellulose quantified in the liquid phase does not correspond to the mass solubilised/removed from the solid. Thus, it can be concluded that both biopolymers are strongly affected by hydrolysis potential of [HSO_4]-based IL at more severe conditions and once the hydrolysis of xylan or glucan to xylose or glucose occurs, next steps are very rapid guiding to completely degraded products. It is also important to underpin that the mass balance depends on the products' identification which might be limited by the analytical techniques. Hence to identify more products besides these analysed by CE (reducing sugars, furans) and HPLC (organic acids) other techniques might be necessary. Another explanation of the cellulose and hemicellulose disappearance is a possibility to form humins (pseudo-lignins).[9, 84] The literature reports state that released sugars and produced furans may react in the liquid phase and form insoluble carbon-enriched compounds termed as chars or pseudo-lignin (humins). The method for compositional analysis of lignocellulosic biomass, developed by the NREL, does not distinguish between Klason lignin, naturally present in the biomass, and pseudo-lignin resulting from sugars degradation.[84] Therefore, the lignin recovery higher than 180 % obtained in this work as shown in Figure 5.2 may justify partial cellulose and hemicellulose mass loss. The humin creation normally occurs for temperature superior to 160ºC[84] and Kumar et al. found that at more severe pre-treatment temperatures (180 °C) carbohydrate-derived pseudo-lignin can achieve even 94.4 % (w/w).[84]

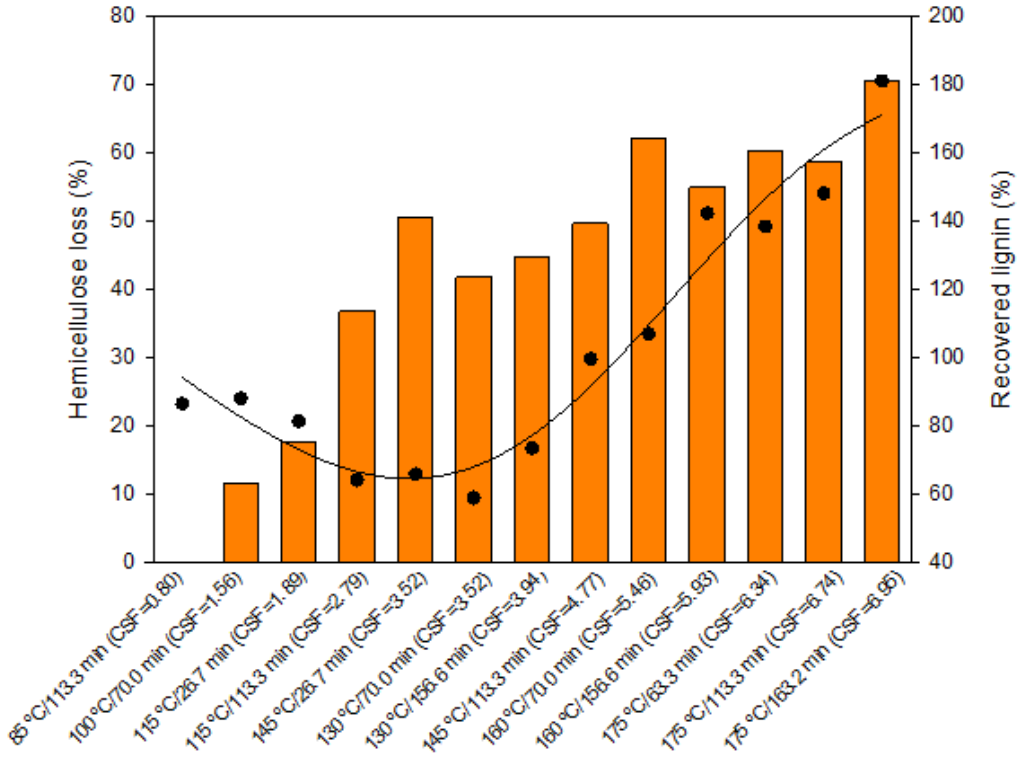


Figure 5.2 Total hemicellulose loss (bars) and recovered lignin (●) in the course of the pre-treatments executed. Solid line is designed by polynomial adjustment to the experimental points and serve as guide for the eye

One more obtained result confirms the veracity of these observed phenomena. Looking for the solid yield presented in Table 4.2 and Table 4.4 it can be stated that for less severe reaction conditions, especially those performed at lower temperature, the solid yield is decreasing with an increase of reaction severity. This behaviour is typical as great part of hemicellulosic fraction became hydrolysed.[81, 85] Furthermore, as stated above, the lack of proportional increase of hemicellulose-origin products in liquor might be the effect of formation of degradation products which in fact are not detected by the employed analytical techniques. However, a close inspection of solid yield of processes carried out at high temperatures shows the increase of solid yield with the increase of reaction temperature. Therefore, it is contradictory to this stated above and it can be expected that some compounds analysed as lignin, as its concentration increase, are formed for more severe reaction conditions. Consequently, it can be summarised that for less severe conditions, an extensive degradation of products occur, while for more severe conditions, humins formation contributes to increase of solid yield and extraordinary amount of lignin recovery.

5.2. Optimisation of xylose and furfural production

Optimisation of hemicellulose hydrolysis to sugars, in particular to xylose, was one of the goals of this work. The collected data for xylose production was submitted to statistical modelling (Table 4.7) and using the statistically significant regression coefficients (p<0.05) the following model equation was found:

$$Y_1 = 13.82 + 19.77X_1 + 7.04X_2 - 18.29X_1X_2 - 32.00{X_1}^2 \quad (4)$$

According to the positive linear coefficient in Eq. (4) for X_1 and X_2, it can be concluded that the amount of xylose obtained increases with the increase of temperature and reaction time. The absolute values of the coefficients β_1 and β_2 show that the factor X_1 (temperature) has stronger influence (almost 3-fold) on xylose production than X_2 (time). However, the negative value for β_{11} implies that the quadratic interaction of X_1 effects negatively the production of xylose. The negative value of β_{12} coefficient indicates that the interaction of X_1 and X_2 is not proportional and the increase of temperature and, subsequent, reaction time has a negative effect on xylose yield.

The optimum conditions to attain maximal xylose yield was identified to be at 125 °C/82.1 min. The xylose yield obtained was 16.7 % (w/w) and 7.6 % (w/w) conversion to furfural. The TRS (total reducing sugar) yield, for this condition was 12.5 % (w/w). Li et. al also explored the use of [HSO_4] based ILs on the pre-treatment of corn stalk.[77] They attained a maximum 23 % and 15 % TRS yield at 5 and 2 min using [bmim][HSO_4] and [C_4SO_3Hmim][HSO_4], correspondingly for 100 ºC. Nevertheless, longer reaction times produced even lower TRS yield.[77]

The chemical analysis of solid fraction obtained for optimum conditions demonstrated that xylan was still present in the recovered biomass (Table 4.9). In other words, at these conditions an incomplete hydrolysis of hemicellulose was not attained. However, as it was found for other conditions, more severe conditions favours furfural production thus it can be stated that [bmim][HSO_4] converts xylan to xylose and next a quick conversion to furfural occur.

The production of furfural with [bmim][HSO_4] was also studied considering higher biomass conversion provoked by this IL. The experimental data submitted to Doehlert model design presented low statistical importance after evaluating statistically significant regression coefficients (p<0.05). The following model equation was obtained:

$$Y_2 = 30.16 + 12.89X_1 - 13.70{X_1}^2 \quad (5)$$

Eq. (5) showed that in the model obtained for xylan conversion to furfural, only the variation of temperature has statistical significance (linear and quadratic). As such, it can be concluded that variation of time is statistically insignificant for furfural production. Nevertheless, the negative value of the β_{11} coefficient translates into a decrease of furfural for more severe processes. This can be

observed at CSF = 6.95 (175 °C/163.3 min) were a minimum of furfural was noted. The optimum condition for furfural formation was found to be at 161 °C/104.5 min. At this condition, the conversion of hemicellulose to furfural was 30.7 % (w/w) and xylose was not present in the pre-treatment liquor. Brandt et al. verified that at 120 ºC, using 80 vol.% of the IL [bmim][HSO_4] and 20 vol.% water in the pre-treatment of Miscanthus for 22 h, the resulting liquor contained approximately 39 % of furfural.[76] They also reported that using [bmim][$MeSO_3$] at the same conditions 14.8 % furfural yield was accomplished. Thus comparing of the obtained results to these presented in this work, it can be stated that similar conversion to furfural 30.7 % vs 39 % was achieved during shorter processes without the need of water presence in the system.

5.3. Hemicellulose product selectivity

The selectivity of [bmim][HSO_4] to produce xylose or furfural was screened for each studied pre-treatment as shown in Figure 5.3. For the lowest pre-treatment severity conditions studied, CSF = 0.80 (85 °C/113.3 min), no xylose was found in the liquor however, the degradation of released arabinose produces furfural giving very high selectivity but with very low furfural yield equals 0.1 % (w/w). For more severe reaction conditions, the selectivity to xylose is very high and reaches a maximum (93% xylose selectivity) at CSF = 2.79 (115 °C/26.7 min). With the increase of severity, more extended conversion of xylose to furfural occurs guiding to complete disappearance of xylose and hence at these conditions process is 100% selective towards furfural production.

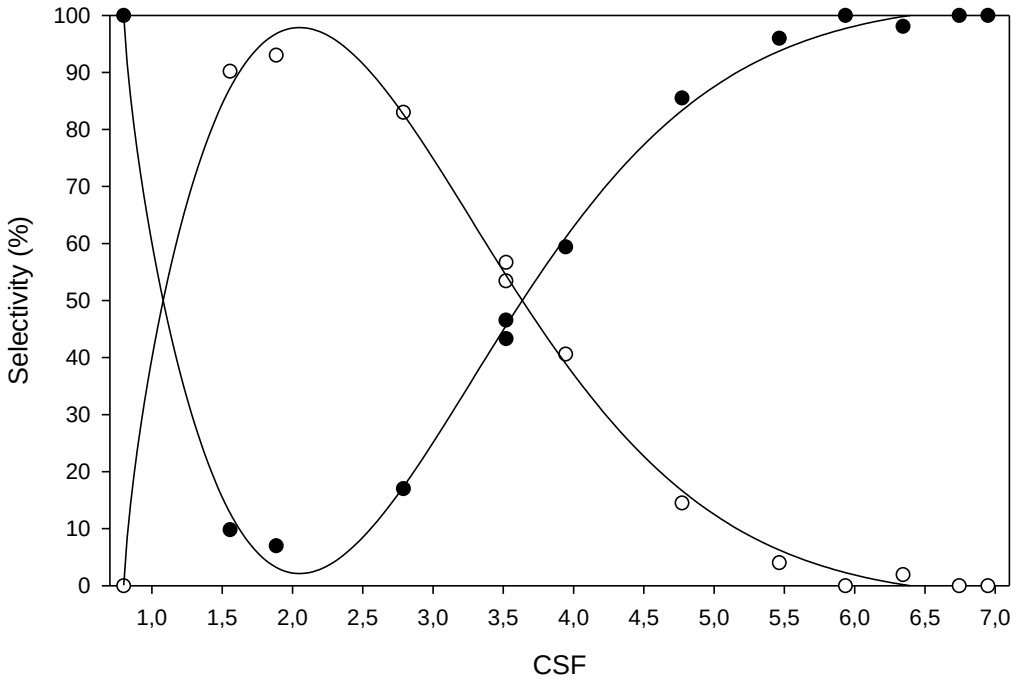


Figure 5.3 Selectivity of [bmim][HSO_4] for xylose (○) or furfural (●) production after wheat straw pre-treatment. Solid lines are designed by polynomial adjustment to the experimental points and serve as guide for the eye

5.4. Effect of water content on the biomass pre-treatment

As it has been reported water content has a great influence on the efficiency of the biomass pre-treatments using ILs.[43, 47, 63, 86] Generally, water acting as an anti-solvent affects negatively cellulose and lignocellulose dissolution in ILs.[47] Furthermore, considering the selectivity discussed above, it is important to understand the mechanism driving the xylose conversion to furfural. The mechanism is given in Figure 5.4.

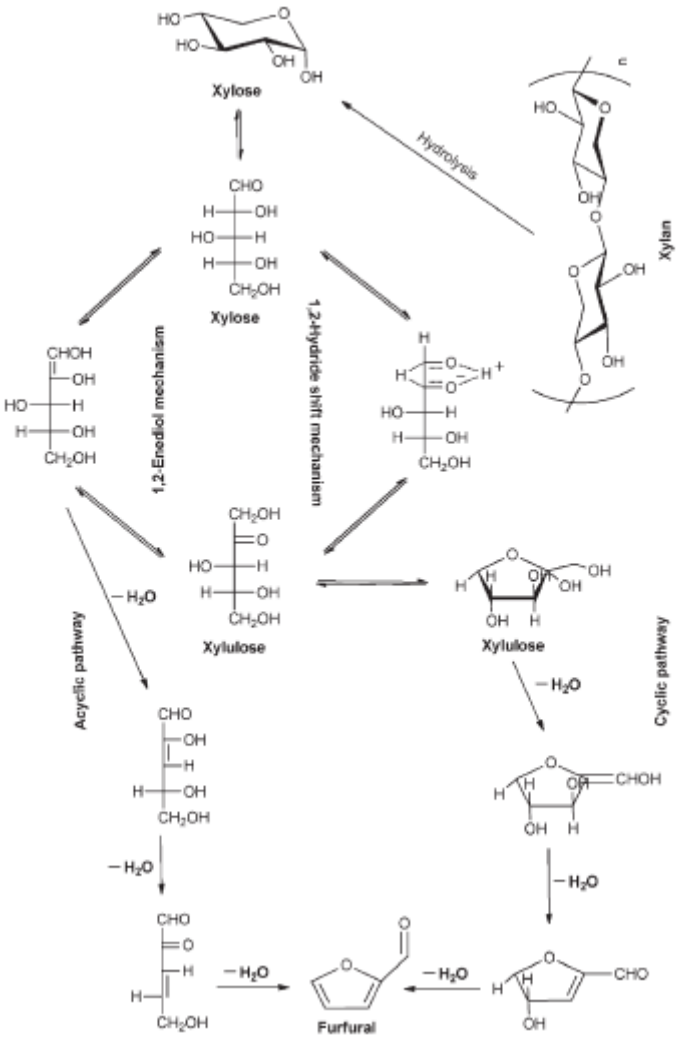


Figure 5.4 The proposed mechanism of furfural formation from biomass (adapted from [87-89]).

As it can be observed, the formation of furfural occurs by the dehydration of xylose molecule. Therefore, the equilibrium of this reaction must be sensitive on the water content in the reaction mixture. Considering that in the examined system only 1.25 % (w/w) (5385 ppm of water in IL and 8.3 % (w/w) humidity of biomass) is present it can be explained why in the hydrophilic ionic liquid such as [bmim][HSO_4], the hemicellulose undergoes hydrolysis to xylose and later rapidly, kinetically

favoured, conversion to furfural occurs. Following this postulation, it can be assumed that addition of water could have a positive "protecting" effect on inhibition of xylose conversion to furfural. In addition, the extra presence of water could enhance the hydrolysis of hemicellulose to monosugars.

To validate the veracity of this hypothesis, other experiments were performed. Two additional pre-treatments at the optimum conditions achieved for xylose production (125 °C/82.1 min) were performed using 5.00 % and 10.00 % (w/w) water content in the pre-treatment system. Liquid phase analysis demonstrated that by increasing the water content from 1.25 % to 5.00 % (w/w) the xylan hydrolysis into xylose duplicated. The xylose hydrolysis yield obtained in the initial pre-treatment was 16.7 % (w/w) and at 5.00 % (w/w) water content the value increased to 33.4 % (w/w) (Figure 5.5). Appling 10.00 % (w/w) water content into the pre-treatment system showed another increase of xylan hydrolysis to xylose, however less pronounced (Figure 5.5) to 40.1 % (w/w). The yield of hemicellulose monomers available in the liquor reached 28.3 % and 35.9 % (w/w) for 5 % and 10 % (w/w) water content, respectively. Similar fact was observed by Brandt et al. who found an optimum amount of hemicellulose monomers (around 15%) after the hydrolysis of Miscanthus biomass using [bmim][HSO_4] with 20% (w/w) water in the system.[76] Other important aspect, which must be mentioned, is that a continuous increase of water content can have a negative effect on the xylose formation due to the anti-solvent effect of water which is expected to compete with the biomass to form hydrogen bonds with IL.[43, 47, 63] Important is also to mentioned that the amount of water does not alter significantly the furfural presence in the liquor and furfural yield was observed to change within the experimental error.

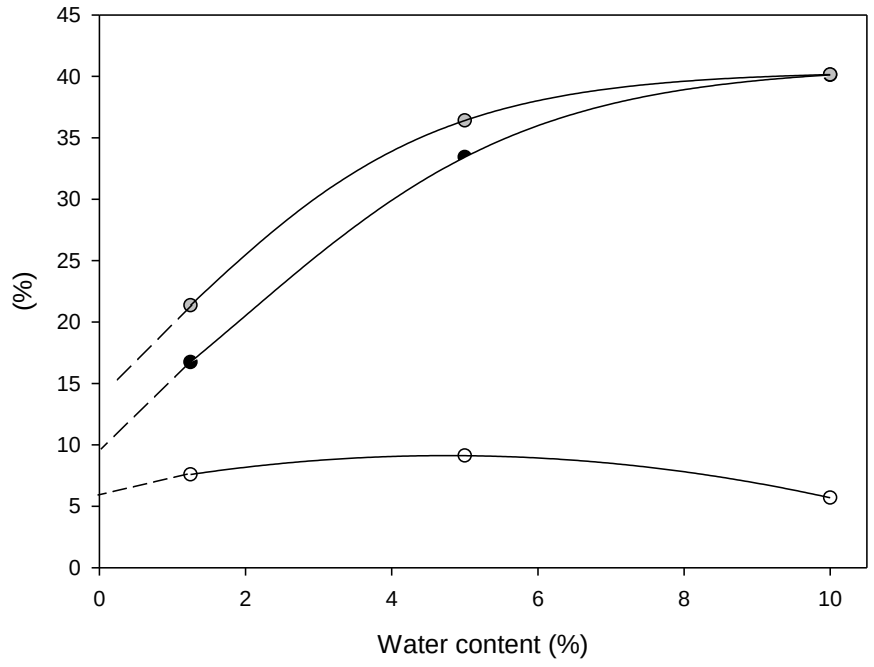


Figure 5.5 Effect of water content in hemicellulose hydrolysis (arabinoxylan (◎)), xylan hydrolysis to xylose (●) and hemicellulose conversion to furfural (○) at 120 °C and 82.1 min; Solid/dashed lines are designed by polynomial adjustment to the experimental points and serve as guide for the eye.

The obtained results drive to one more interesting finding. Based on the fitting of the experimental data, the IL capacity to hydrolyse hemicellulose can be estimated. Therefore, for theoretically anhydrous conditions, approximately 14 % (w/w) of arabinoxylan among which 9 % (w/w) is xylan, and 7 % furfural could be found in the pre-treatment liquor. This shows that although ionic liquid even acidic is interesting medium for catalytic conversion of biomass, but some amount of water is needed. Furthermore, this shows also that in some situations, the extensive drying of biomass might be unnecessary.

6. Conclusions

Exploitation of lignocellulosic residues' potential has become an important issue in the context of green chemistry and biorefinery concept. In this work a method of wheat straw pre-treatment using the acidic IL [bmim][HSO_4] was employed. Two independent parameters were optimised using Doehlert statistical model design aiming the maximal xylose and furfural production. For comparison of the set of experimental data, the severity factor for [bmim][HSO_4] was proposed. Less severe reaction conditions favour xylose formation and maximum yield of xylose 16.7 % (w/w) attained 125 °C/82.1 min. The contrary is true to furfural formation where a maximum of 30.7% (w/w) was accomplished at 161°C/104.5 min. By changing the reaction conditions, 100 % selectivity towards xylose or furfural production can be achieved. Variation of water content was studied for the optimum conditions found for xylose formation. An increased of water content to 5 % (w/w) lead to a xylose yield of 33.4 % (w/w). Pre-treatment process using a 10 % (w/w) content resulted in a xylose yield of 40.1 % (w/w) showcasing an increased by 140 % of the xylose concentration in the liquor. At the same time, conversion to furfural was maintained.

The performed experiments allowed for selective removal of hemicellulose from processed biomass confirming that the acidic IL is capable to selectively hydrolyse hemicellulose; however, higher temperatures also lead to higher cellulose hydrolysis. Nevertheless, the cellulose hydrolysis is always minor and in the best case, the processed solid contained more than 50 % (w/w) with no signs of other polysaccharides.

Analytical and chemical techniques employed in the analysis of the executed processes such as regression analysis, HPLC, CE and qualitative acid hydrolysis were used in this work proving their versatility and effectiveness in the executed works.

7. Perspectives

Among several intriguing results some of them required more detailed analysis and future study. For example, the study of water content in the context of necessity of biomass and ionic liquid drying would be interesting to perform. In addition, biomass/IL ratio could be also studied to maximise the potential of the developed results. The solid phase obtained, rich in cellulose and lignin, has a room to exploitation to produce glucose by enzymatic hydrolysis as well as valorise the produced lignin. A recover and reuse of IL should also be study to reduce the investment cost of the process. Last, a plausible scale up of the process might be considered.

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9. Appendix

Annex A – Determination of sugars and building blocks present in liquid fraction

Liquid phase of all the pre-treatment executed was analysed by CE and HPLC. Monosaccharides, furfural and HMF were identified by CE, while organic acids were quantified by HPLC. Samples analysed with CE were diluted (for analysis proposes and with the addition of the anti-solvent), and as such all results were multiplied by a dilution factor of 32.87. Organic acids analysed in HPLC also contained a dilution factor of 3.88.

$$Xyl\ (\%) = \frac{Xyl \times V_{IL}}{Xn_{theoric}} \times 32.87 \times \frac{132}{150} \times 100 \quad (6)$$

$$Ara\ (\%) = \frac{Ara \times V_{IL}}{Arn_{theoric}} \times 32.87 \times \frac{132}{150} \times 100 \quad (7)$$

$$Glu\ (\%) = \frac{Glu \times V_{IL}}{Gln_{theoric}} \times 32.87 \times \frac{162}{180} \times 100 \quad (8)$$

$$Fur\ (\%) = \frac{Fur \times V_{IL}}{Arn_{theoric} + Xn_{theoric}} \times 32.87 \times \frac{132}{96} \times 100 \quad (9)$$

$$HMF\ (\%) = \frac{HMF \times V_{IL}}{Gln_{theoric}} \times 32.87 \times \frac{162}{126} \times 100 \quad (10)$$

$$Ac\ (\%) = \frac{Ac \times V_{IL}}{AcG_{theoric}} \times 3.88 \times \frac{60}{61} \times 100 \quad (11)$$

$$For\ (\%) = \frac{For \times V_{IL}}{Arn_{theoric} + Xn_{theoric} + Gln_{theoric}} \times 3.88 \times \frac{462}{116} \times 100 \quad (12)$$

$$Lev\ (\%) = \frac{Lev \times V_{IL}}{Gln_{theoric}} \times 3.88 \times \frac{162}{46} \times 100 \quad (13)$$

where,

- $Xyl, Ara, Glu, Fur, HMF, Ac, For, Lev$ are concentrations of xylose, arabinose, glucose, furfural, HMF, acetic acid, formic acid and levulinic acid in the liquor, respectively expressed in g/L;
- $Xn_{theoric}$, $Arn_{theoric}$, $Gln_{theoric}$ and $AcG_{theoric}$ are the theoretical xylan, arabinan, glucan and acetyl groups present in the initial dry biomass expressed in mg;
- V_{IL} is the volume of [bmim][HSO_4] used in the pre-treatment.
- $(132/150), (162/180), (60/61), (462/116)$ and $(162/46)$ are stoichiometric factors for the conversion of xylose and arabinose, glucose, acetic acid, formic acid and levulinic acid to xylan, arabinan, glucan and acetyl groups, respectively.

Oligosaccharides content was calculated in the subsequent manner:

$$ArnXn = \left[(Xyl + Ara) \times \frac{132}{150} + Fur \times \frac{132}{96} + For \times \frac{132}{116}\right] \times V_{IL} \quad (14)$$

$$Gln = \left[Glu \times \frac{162}{180} + For \times \frac{162}{116} + Luv \times \frac{162}{46}\right] \times V_{IL} \quad (15)$$

$$AcG = Ac \times \frac{60}{61} \times V_{IL} \quad (16)$$

where,

- $ArnXn, Gln$ and AcG are masses of arabinoxylan, glucan and acetyl groups present in the liquor in mg;
- $Xyl, Ara, Glu, Fur, Ac, For$ and Luv are concentrations of xylose, arabinose, glucose, acetic acid, formic acid and levulinic acid in the liquor, respectively expressed in g/L;
- V_{IL} is the volume of IL in mL.
- $(132/150), (132/96), (132/46), (162/180), (162/116), (162/46)$ and $(60/61)$ are stoichiometric factors for the conversion of xylose and arabinose, glucose, acetic acid, formic acid and levulinic acid to arabinoxylan, glucan and acetyl groups, respectively.

Total hemicellulose was calculated by the sum of the arabinoxylan and acetyl groups' content. Glucan content was assigned to cellulose content.

Recovered hemicellulose and cellulose in the liquor

Hemicellulose and cellulose present in the liquor were calculated in the following manner:

$$Hemi_{liquor}(\%) = \frac{MHemi_{liquor}}{Hemi_{theoric}(\%) \times M_{inicial,bio} \times (1 - H_{bio})} \times 100 \quad (17)$$

$$Cell_{liquor}(\%) = \frac{MCell_{liquor}}{Cell_{theoric}(\%) \times M_{inicial,bio} \times (1 - H_{bio})} \times 100 \quad (18)$$

where,

- $MHemi_{liquor}$ and $MCell_{liquor}$ are the hemicellulose and cellulose-origin products in the liquor, respectively in mg;
- $Hemi_{theoric}$ and $Cell_{theoric}$ are the percentages of hemicellulose and cellulose present in the untreated wheat straw;
- $M_{inicial,bio}$ is the mass of the initial biomass used in the pre-treatment in mg;
- H_{bio} is the humidity present in the untreated biomass in %.

Annex B – Determination of sugars present in solid fraction

The characterisation of the recovered solids was performed. In order to perform this, xylan, glucan, arabinan, Klason lignin and ash content were identified and calculated as presented below. It is assumed that negligible amount of sugars in the biomass are degraded by the sulphuric acid (H_2SO_4) and as such, correction factors, related to those losses are used.[90] A loss of 2.6 % for glucose, 8.8 % for xylose and 4.7 % for arabinose are assumed.[90] Basing on these losses the correction factor *F* for each fraction was calculated as in the example below:

$$F_{glucose} = \frac{1}{1-0{,}026} \tag{19}$$

Xylan, glucan, arabinan and acetyl groups are calculated by HPLC analysis of the resulting liquor after quantitative acid hydrolysis. The equations used are:

$$Xn = \frac{Xyl \times P_{sol}}{A} \times \frac{100}{1005} \times F \times \frac{132}{150} \tag{20}$$

$$Arn = \frac{Ara \times P_{sol}}{A} \times \frac{100}{1005} \times F \times \frac{132}{150} \tag{21}$$

$$Gln = \frac{Glu \times P_{sol}}{A} \times \frac{100}{1005} \times F \times \frac{162}{180} \tag{22}$$

$$AcG = \frac{Ac \times P_{sol}}{A} \times \frac{100}{1005} \times \frac{60}{61} \tag{23}$$

$$KL = \frac{CIM \times CAsh}{A} \times 100 \tag{24}$$

$$Ash = \frac{DC \times CAsh}{A} \times 100 \tag{25}$$

where,

- Xn, Arn, Gln, AcG, KL and Ash are the percentages of xylan, arabinan, glucan, acetyl groups, Klason lignin and ash present in the recovered solid, respectively;
- $Xyl, Ara, Glu,$ and Ac are concentrations of xylose, arabinose, glucan and acetic acid identified in the resulting liquid, correspondingly in g/L;
- $CIM, CAsh$ and DC are masses of acid-insoluble solid and ash in the sample and the dry crucible mass expressed in g;
- P_{sol} is the mass of the solution (g) and A is the mass of the sample (g) used in the experiment;
- $(132/150), (162/180)$ and $(60/61)$ are stoichiometric factors for the conversion of xylose and arabinose, glucose and acetic acid to xylan and arabinan, glucan and acetyl groups, respectively.

Recovered hemicellulose and cellulose

Hemicellulose and cellulose present in the solid were calculated in a similar manner than the ones depicted in Eq.17 and Eq. 18. However, in this case, the humidity considered was the humidity of the recovered biomass. Total recovery of hemicellulose and cellulose was the sum of both the solid and liquid fractions.

Annex C – Severity factor

The severity factor, R_0, given by Eq. 4 can also be presented by the following equation:

$$R_0 = e^{\left(\frac{T_r - T_b}{\omega}\right)} \times \Delta t \quad (26)$$

where,

- T_r and T_b are absolute reaction temperature and reference temperature when hydrolysis initiates, respectively expressed in °C;
- and ω is an adimensional constant that translates the effect of the temperature in the conversion.

Percentages of hemicellulose hydrolysis attained in this work were used to estimate the values of T_b and ω. The value of T_b was attained by applying the Doehlert design for all the hemicellulose hydrolysis values obtained with the variation of time and temperature. The point (x,0,0) represents the value of T_b and as such, by resolving the equation obtained from experimental design, T_b= 88.28 °C.

$$Y = 64.0048 + 74.5027X_1 + 19.4424X_2 - 19.9022X_1X_2 - 36.3594{X_1}^2 \quad (27)$$

where,

- Y is the percentage of hemicellulose hydrolysis;
- and X_1, X_2 are the temperature (°C) and time (min) of the pre-treatment, respectively.

The value of ω was attained by representation of the equation $Y = mX + B$, where

$$Y = \ln(-\ln(1-\alpha)) \quad (28)$$

and α is the hydrolysis of hemicellulose, X is the combined severity factor calculated in the following manner:

$$X = CSF = log_{10}\left(R_{0_{aquecimento}} - R_{0_{isotérmico}}\right) - pH \quad (29)$$

- $R_{0_{aquecimento}}$ is the severity factor where variation of temperature is taking place, and Δt considers the time to reach T_r and the time to reach T_b;
- $R_{0_{isotérmico}}$ is the severity factor where no variation of temperature occurs;
- pH is the pH of the IL [bmim][HSO_4] and is equal to 1.0.

Both $R_{0_{aquecimento}}$ and $R_{0_{isotérmico}}$ are a function of ω. The value of ω is obtained by maximization of R^2. This methodology was analogous as the one used by Chum et al.[82] Chum et al. suggested that the best transformation is capable when using a limited range of conversion (0.2-0.8). This limitation was verified and employed in the estimation of ω. The function *SOLVER* in Microsoft Excel package was used and an $R^2 = 0.99$ was achieved, resulting in a value of ω equals to 6.47. As a result the CSF used had the following formula:

$$CSF = log_{10}\left(e^{\left(\frac{T_r - 88.28}{6.47}\right)} \times \Delta t\right) - pH \qquad (30)$$