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Licenciatura em Bioquímica

Pre-treatment of different types of lignocellulosic biomass using ionic liquids

Dissertação para obtenção do Grau de Mestre em Biotecnologia

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Resumo

A utilização de líquidos iónicos (LIs) no pré-tratamento de biomassa lenhocelulósica oferece novas possibilidades de fraccionamento de biomassa, permitindo a valorização de uma matéria-prima de baixo custo. Este trabalho tem como principal objectivo o estudo do pré-tratamento e fraccionamento de diferentes tipos de biomassa lenhocelulósica nas suas principais fracções constituintes (celulose, hemicelulose e lenhina), utilizando LIs. As biomassas utilizadas foram a palha de trigo, o bagaço de cana-de-açúcar, a palha de arroz e a triticale. Inicialmente procedeu-se ao desenvolvimento e optimização de uma metodologia de fraccionamento tendo como base duas metodologias descritas na literatura. O método desenvolvido permitiu obter amostras com elevada pureza e uma recuperação eficiente do LI. Este método permitiu ainda demonstrar a possibilidade de reutilização do LI, revelando o grande potencial deste método. O pré-tratamento de diferentes biomassas confirma a versatilidade e eficiência da metodologia optimizada, visto que não só permite uma dissolução macroscópica completa de cada biomassa, mas também permite efectuar um processo de fraccionamento eficaz. O pré-tratamento de bagaço de cana-de-açúcar e de triticale permitiram a obtenção de amostras ricas em celulose com um teor em carbohidratos de 90 % (p/p).

A fim de se verificar a potencial aplicabilidade das fracções ricas em carbohidratos, e avaliar a eficácia do pré-tratamento, as amostras ricas em celulose foram submetidas a uma hidrólise enzimática. Os resultados demonstraram uma elevada digestibilidade das amostras ricas em celulose, revelando um rendimento elevado de glucose para a metodologia de pré-tratamento desenvolvida. O bagaço de cana-de-açúcar e a triticale apresentaram o rendimento mais elevado de glucose com 79,9 % (p/p) e 78,5 % (p/p), respectivamente e o menor rendimento foi obtido para a palha de arroz, com 68,7 % (p/p).

As amostras obtidas após o pré-tratamento com LIs foram analisadas qualitativa e quantitativamente através de Infravermelho por Transformada de Fourier (FTIR). Após o pré-tratamento, a pureza dos LIs recuperados foi avaliada através de espectroscopia de ressonância magnética nuclear (RMN). Os resultados da hidrólise enzimática foram analisados através de HPLC (*High-Performance Liquid Chromatography*).

Palavras-Chave:

Líquidos Iónicos, Biomassa Lenhocelulósica, Pré-tratamento, Celulose, Hemicelulose, Lenhina

Abstract

The pre-treatment of biomass by ionic liquid (IL) is a method opening new possibilities of biomass fractionation for further valorisation of low value feedstock. This work is dedicated to study on the pre-treatment and fractionation of different types of lignocellulosic biomass into its major constituent fractions (cellulose, hemicellulose and lignin), using ILs. The biomass tested was: wheat straw, sugarcane bagasse, rice straw and triticale. Initially, the optimised methods were development basing on two methodologies described in the literature. This method allows the separation into high purity carbohydrate-rich (cellulose and hemicellulose) and lignin-rich fractions and permits an efficient IL recovery. The possibility of IL reuse was confirmed, demonstrating the great potential of the established method. The pre-treatment of various biomasses confirms the versatility and efficiency of the optimised methodology since not only the complete macroscopic dissolution of each biomass was achieved but also the fractionation process was successfully performed. Pre-treatment of sugarcane bagasse and triticale allowed to obtained cellulose samples rich in carbohydrate up to 90 wt %.

In order to verify the potential further applicability of the obtained carbohydrate-rich fractions, as well as to evaluate the pre-treatment efficiency, the cellulose-rich fraction resulting from 1-ethyl-3-methylimidazolium acetate ([emim][OAc]) pre-treatment was subjected to enzymatic hydrolysis. Results showed a very high digestibility of the cellulose-rich samples and confirmed a high glucose yield for the optimised pre-treatment methodology.

The samples obtained after the pre-treatment with ILs were qualitatively and quantitatively analysed by Fourier Transform Infrared Spectroscopy (FTIR). After the pre-treatment, the purity of the recovered ILs was evaluated through Nuclear Magnetic Resonance spectroscopy (NMR). The enzymatic hydrolysis results were analysed by High-Performance Liquid Chromatography (HPLC).

Keywords

Ionic liquids, Lignocellulosic biomass, Pre-treatment, Cellulose, Hemicellulose, Lignin

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Chapter 1

1. Introduction

1.1. Biorefinery concept

As a result of the increasing cost and diminishing supplies of fossil fuels, in addition to their damaging effects on the environment, there is currently a growing need to explore alternative energy sources.[1] Thus, is in this context that the concept of biorefinery emerges.

A biorefinery is an overall concept of an integrated and diversified processing plant that aims to make a sustainable and full use of biomass feedstocks to produce fuels, power, a wide range of value-added products and others materials, with a zero waste approach (figure 1.1).[2-5] The biorefinery concept is analogous to today's petroleum refineries, which produce multiple fuels and products from petroleum.[6] Overall profitability and productivity of all energy related products are potentially improved by integrating production of higher value bioproducts into biorefinery's fuel and power output.[2] In order to increase the productivity and efficiency it is also important to perform operations that decrease the overall energy intensity of biorefinery's unit operations, maximizing the use of all feedstock components, byproducts and waste streams, and using scale-up economies, common processing operations, materials, and equipment to drive down all production costs.[2,7,8]

The biorefinery platforms are defined according to the raw materials and the technological processes used as well as the products obtained.[2] Although biorefinery was divided into different platforms, these always end up interconnected. There are two main biorefinery platforms:

- Biochemical plataform - based on biochemical conversion processes and focuses on fermentation of sugars extracted from biomass feedstocks;[9]
- Thermochemical plataform - based on thermochemical conversion processes and focuses on gasification of biomass feedstocks and resulting by-products.[9]

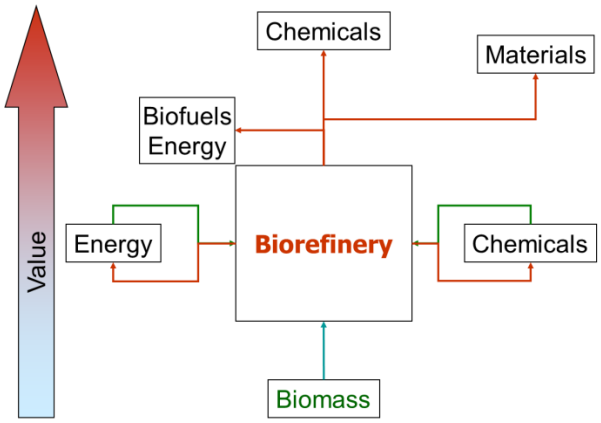

Figure 1.1. Biorefinery concept.[2]

Currently, in research and development are favored three biorefinery systems. First, the whole-crop biorefinery, which uses raw materials such as cereals or maize. Second, the green biorefinery, which uses naturally, wet biomass, such as green grass, lucerne, clover, or immature cereal. Third, the lignocellulose feedstock (LCF) biorefinery, which uses naturally dry raw materials such as cellulose-containing biomass and wastes.[5]

Among the potential large-scale industrial biorefineries, the LCF biorefinery will probably be the one with highest success. On the one hand, the raw material situation is optimal (straw, reed, grass, wood, paper-waste, etc.) and, on the other hand, conversion products have a good position within both the traditional petrochemical and the future biobased product markets. An important point for the utilization of biomass as a chemical raw material is the relatively low cost of raw materials. In figure 1.2 is illustrated an overview of the potential products of a LCF biorefinery.[5]

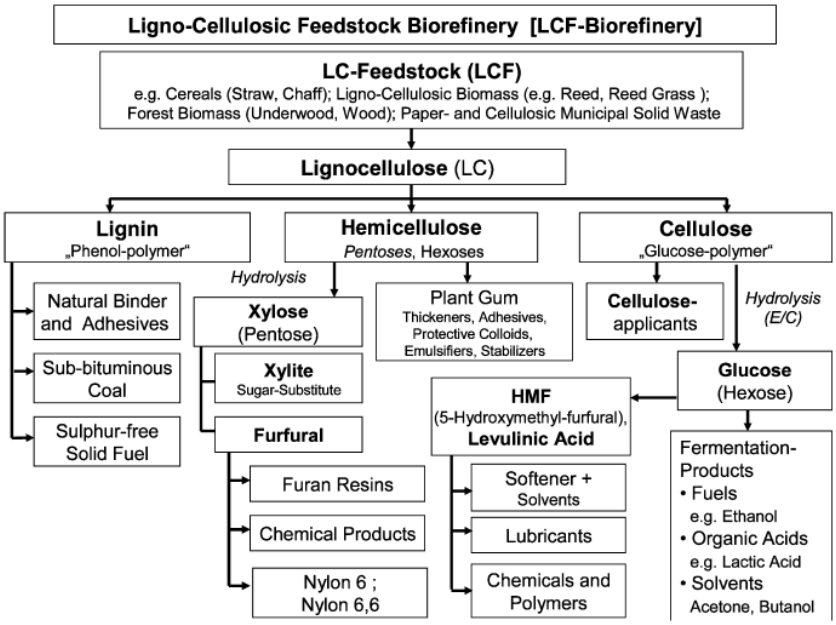

Figure 1.2. Lignocellulosic feedstock biorefinary (LCF biorefinary).[5]

1.2. Lignocellulosic biomass

Biomass can be defined as any organic matter that is available on a renewable or recurring basis (excluding old-growth timber), including dedicated energy crops and trees, agricultural food and feed crop residues, aquatic plants, wood and wood residues, animal wastes, and other waste materials.[5]

Lignocellulose is a class of biomass, relatively inexpensive and is the most abundant renewable resource on earth.[10] This biomass has a worldwide annual production of $1x10^{10}$ million tonnes and can be used in the production of biofuels and other valuable chemicals such as: proteins, enzymes, biopolymers, organic acids, furfural and its derivatives.[11-13] Lignocellulosic biomass is widely distributed and can be grown and harvested on a billion ton scale.[14] Contrary to starch-based substrates this biomass does not compete with the food chain and the production cost is lower.[12,14] Another important advantage is that the fuels and materials derived from it are potentially "carbon-neutral" or can even help to sequester carbon dioxide.[13] These are some advantages that make lignocellulose a suitable feedstock for future large-scale biorefineries. However, the extensive pretreatment required to release the carbohydrates and other components from the resistant cell wall matrix is the main disadvantage in using this feedstock, since it increases the process complexity and the costs.[14]

1.2.1.Composition

Lignocellulosic biomass is mainly composed by cellulose, hemicellulose, lignin and also by minor amounts of proteins, pectins, extractives and ash.[10] The typical percentages of dry weight are 35–50 % cellulose, 20–35 % hemicellulose, and 5–30 % lignin.[12] These percentages may vary from species to species, across different parts in the same plant and can also be influenced by geography or environmental factors.[1] All this components are intertwined in a complex matrix which results in the final structure.[1]

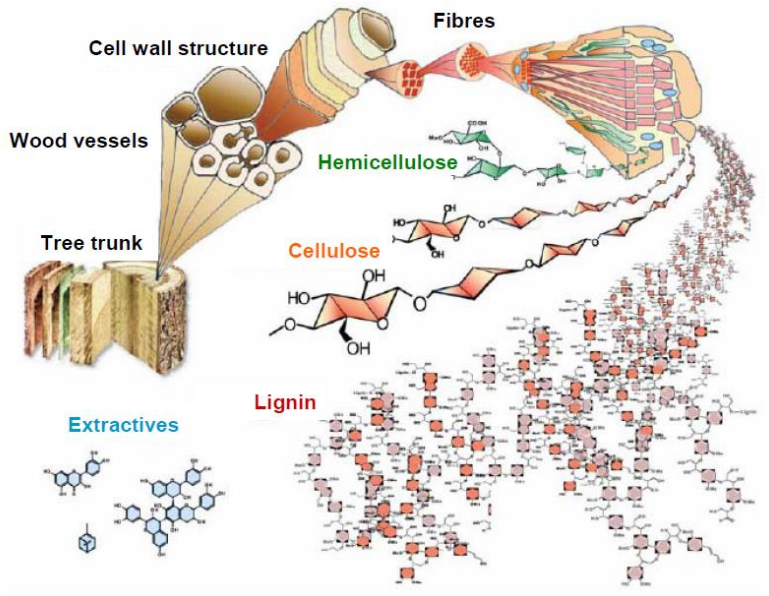

Figure 1.3. Representation of lignocellulosic biomass structure from wood.[15]

1.2.1.1. Cellulose

Cellulose is a homopolysaccharide composed of β-D-glucopyranose units which are linked together by (1→ 4)-glycosidic bonds, and is mainly located in the secondary cell wall.[16] Commonly, cellulose is considered as a polymer of glucose since cellobiose consists of two molecules of glucose. The chemical formula of cellulose is $(C_6H_{10}O_5)_n$.[11] Figure 1.4 presents the chemical structure of this polysaccharide. Cellulose exists in both the crystalline and the non-crystalline structure.[11] The crystalline structure of cellulose is obtained when the coalescence of several polymer chains leads to the formation of microfibrils, which in turn are united to form fibrils and finally cellulose fibers.[11,16] Cellulose fibers are surrounded by intra- and intermolecular hydrogen bonds which makes cellulose insoluble in water and in the most organic solvent.[2,4,5]

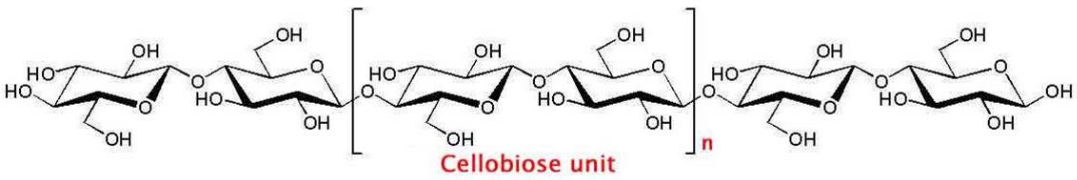

Figure 1.4. Chair conformation representation of the chemical structure of cellulose. As indicated the dimeric unit repeated is cellobiose.

The degree of polymerization (DP) of cellulose, i.e. the number of glucose units that make up one polymer molecule, has a great influence in many properties of this compound. This number differs depending on the cellulose origin. In general, this number can be between 800-10000 glucose units per cellulose chain.[17] The cellulose solubility is strongly affected by the DP which could become a drawback for industrials applications. Note that, although being insoluble in water, cellulose is a relatively hygroscopic material absorbing 8-14 % water under normal atmospheric conditions.[11]

1.2.1.2. Hemicellulose

Similarly to cellulose, hemicellulose function as supporting material in the cell walls and as a reserving substance. The main feature that differentiates this compound from cellulose is that, hemicellulose is a heteropolysaccharide, which contains shorter and amorphous branches consisting of different sugars. These monosaccharides include pentoses (D-xylose and L-arabinose), hexoses (D-glucose, D-mannose, and D-galactose), uronic acids (e.g., 4-O-methyl-D-glucuronic, D-glucuronic, and D-galactouronic acids) and small amounts of desoxyhexoses (L-rhamnose and L-fucose). Figure 1.5 illustrates the hexoses and pentoses found in hemicellulose. The backbone of hemicellulose consists of β-D- xylopyranose units, linked by (1→ 4)-bonds.[18,19]

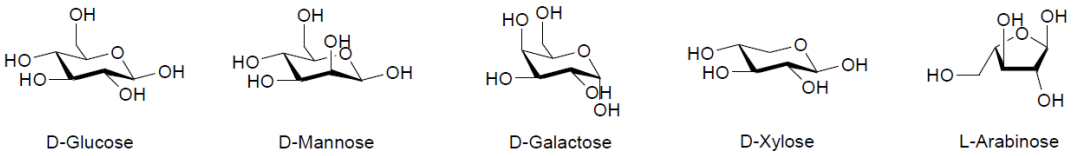

Figure 1.5. Chair conformation representation of the hexoses and pentoses typically found in hemicellulose.[19]

In contrast to cellulose, the polymers present in hemicelluloses are easily hydrolysed under mild acid or alkaline conditions. Note that, the amorphous nature of this compound makes it partially soluble in water at elevated temperatures, and the presence of an acid helps to greatly improve its solubility.[1,11,16,18]

Hemicellulose extracted from plants possesses a high degree of polydispersity, polydiversity and polymolecularity (a broad range of size, shape and mass characteristics) that may vary with the source material and the pre-treatment use. However, the degree of polymerization does not exceed the 200 monomers.[11]

1.2.1.3. Lignin

Lignin is the most complex natural aromatic polymer and in addition to providing mechanical strength to wood by holding the fibers together between the cell walls also provides a protective shield from enzymatic attack for cellulose and hemicelluloses.[1,11] It is an amorphous three-dimensional polymer, which predominant building blocks are phenylpropane units. These units are three monolignol precursors with various degrees of oxygenation/substitution on the aromatic ring, namely coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol, in order of abundance.[11,20] Once

incorporated into the lignin polymer, the units are identified by their aromatic ring structure and therefore called guaiacyl, syringyl and p-hydroxyphenyl units, respectively.[19,20] Figure 1.6 presents a chemical structure of the three monolignols involved in the lignin structure. Depending of the lignocellulosic material, the composition of lignin differs. For example, softwood consists almost exclusively of guaiacyl units while hardwood also contains a large number of syringyl units.[19]

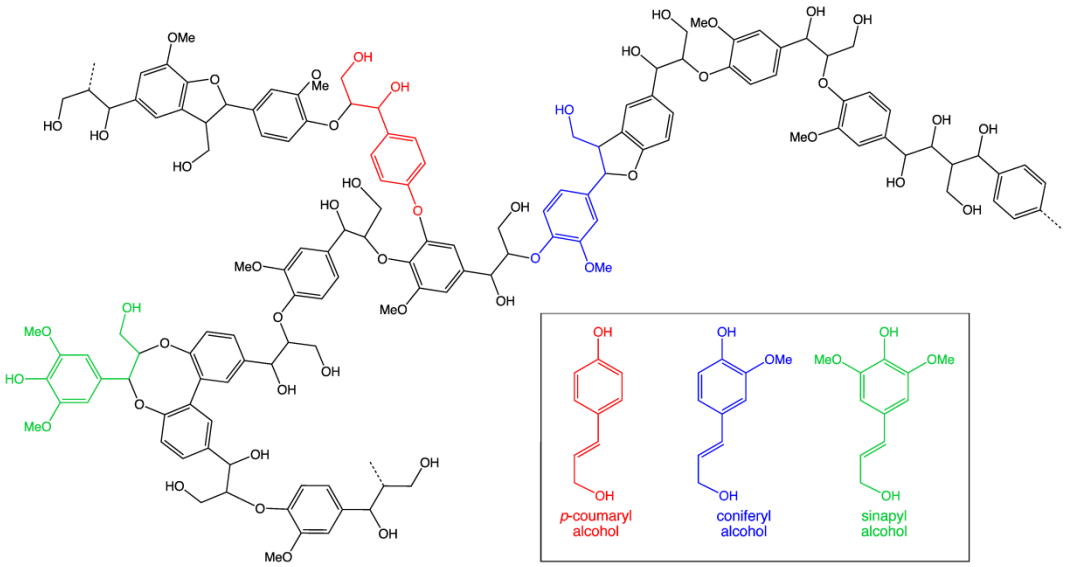

Figure 1.6. Structure of a lignin fragment with various C-O and C-C linkages. The chemical structure of the three monolignols that composed lignin is also illustrated.[21]

Lignin polymer contains a wide range of linkages. The most common linkage is the β-O-4 ether bond. Roughly 50 % of all inter-subunit bonds are of this type.[22] The β-O-4 ether bonds lead to a linear elongation of the polymer. Other C-O and C-C linkages are present in lower abundance, and branching occurs when lignification is advanced.[19]

Lignin is the most recalcitrant component of the plant cell wall, and the higher the proportion of lignin, the higher the resistance to chemical and enzymatic degradation. Generally, softwoods contain more lignin than hardwoods and most of the agriculture residues. There are chemical bonds between lignin and hemicellulose and even cellulose.[18] Lignin is one of the drawbacks of using lignocellulosic materials in fermentation, as it makes lignocellulose resistant to chemical and biological degradation.[22]

1.2.1.4. Extractives

Extractives constitute a large number of organic and inorganic compounds that can be extracted from the biomass by means of polar and nonpolar solvents such as hot or cold water, ether, benzene, methanol, or other solvents that do not degrade the biomass structure.[23,24] These compounds can be regarded as soluble nonstructural materials, almost exclusively composed of extracellular and low-molecular-weight compounds.[16,25] Terpenes, fats, waxes, proteins, phenolic compounds, hydrocarbons and sugars are examples of organic extractives. Inorganic extractives include, for example, certain sodium and potassium salts.[15] The amount and types of extractives present are entirely dependent upon the biomass nature.[24]

1.2.2.Types of biomass used

Any type of lignocellulosic biomass can be used. However, the biomasses selected will preferentially not compete with food and feed industries, will be those that are produced in large quantities and that are readily available (or can be made readily available) in the places with the greatest demand for the chemicals, biobased fuels and alternative fuels to be synthetised from the biomass.[26] In this work, the types of biomasses studied were wheat straw, sugarcane bagasse, rice straw and triticale.

1.2.2.1. Wheat straw

Wheat straw is an agricultural by-product that results from wheat production. Wheat (*Triticum* spp.) is a cereal grain from the Family of Poaceae and its cultivation has been made for more than 5000 years. This cereal is a major staple food crop in many parts of the world in terms of both cultivation area and prevalence as a food source.[27] It is widely grown throughout the temperate zones and in some tropical/sub-tropical areas. The main centres were wheat is produced are Europe, the former USSR, North America, China and India.[25]

In 2010, wheat was the third most-produced cereal with a world production of 651 million tonnes, after maize with 844 million tonnes and rice with 672 million tonnes.[28] During the period of 2001 – 2011, the world population increased from 6.16 to 6.92 billion (12.34 % increase). In this same period, although the global wheat production fluctuated and lacked behind the population growth, it increased from 589.3 to 694.5 million tonnes (17.84 % increase) as shown in figure 1.7.[29,30] For every 1.3 kg of wheat grain produced is generated about 1 kg of straw, hence the importance of valuing this residue.[31] According to more recent data, the world wheat production was 661.8 million tonnes, on 7 March 2013.[29] Figure 1.7 illustrates the world wheat production in million tonnes in the period 2002 – 2013.

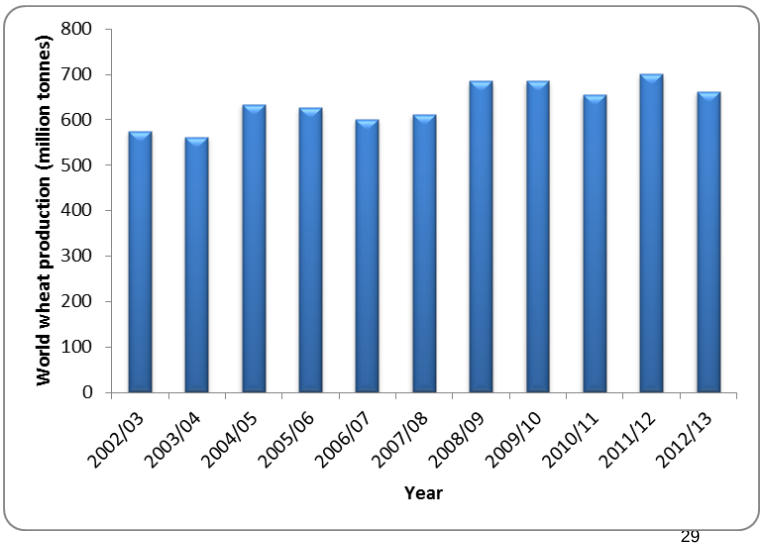

Figure 1.7. World wheat production, during 2002 - 2013.[29]

1.2.2.2. Sugarcane bagasse

Sugarcane bagasse is a fibrous residue obtained after the extraction of the juice from sugarcane (*Saccharum officinarum*) in the sugar production process.[32] This residue is one of the major lignocellulosic materials found in great quantities, especially in tropical countries such as Brazil,[33,34] India,[33,35] Cuba,[33] China,[33,36] México,[34] Indonesia[37] and Colombia.[38] In general, 1 ton of sugarcane produces 280 kg of bagasse, and 5.4×10^8 dry tons of sugarcane is processed annually throughout the world.[39] About 50 % of this residue is stockpiled and the remainder is used in distillery plants as a source of energy.[40] Sugarcane bagasse is mainly composed by 20 – 30 % of lignin, 40 – 45 % of cellulose and 30 – 35 % of hemicelluloses.[41] Due to its lower ash content (1.9 %),[42] bagasse offers numerous advantages compared with other agro-based residues such as rice straw (14.5 %)[43] and wheat straw (9.2 %).[44,45]

1.2.2.3. Rice straw

Rice straw is a by-product that results from the rice grain industry.[46] Rice is a type of grass and belongs to a family of plants that includes other cereals such as wheat and corn. It is commonly used as human food. The most important rice species used for human consumption are: *Oryza sativa*, grown worldwide; and *Oryza glaberrima*, grown in parts of West Africa. Relatively to the others cereals rice is unique because it can grow in wet environments that other crops cannot survive in. Across Asia, this wet environments where rice is grown, are very abundant. Irrigated lowland rice, which makes up three-quarters of the world rice supply, is the only crop that can be grown continuously without the need for rotation and can produce up to three harvests a year—literally for centuries, on the same plot of land. Farmers also grow rice in rainfed lowlands, uplands, mangroves, and deepwater areas.[47]

The growth of rice occurs in more than a hundred countries producing more than 700 million tonnes annually, with a total harvested area in 2009 of approximately 158 million hectares. Asia is the region with the highest production of rice in the world (about 90 % of rice, which corresponds at nearly 640 million tonnes). Sub-Saharan Africa had a production of about 19 million tonnes and Latin America some 25 million tonnes. Note that, in Asia and sub-Saharan Africa, almost all rice is grown on small farms of 0.5−3 hectares. Rice grows in a wide range of environments and in contrast with other crops is productive in many situations. Rice-growing environments are based on their hydrological characteristics and include irrigated, rainfed lowland, and rainfed upland. The estimative made until 7 March 2013 reveal a production of about 488.6 million tonnes of rice. In figure 1.8 is present the world rice production in million tonnes in the period 2002 – 2013.

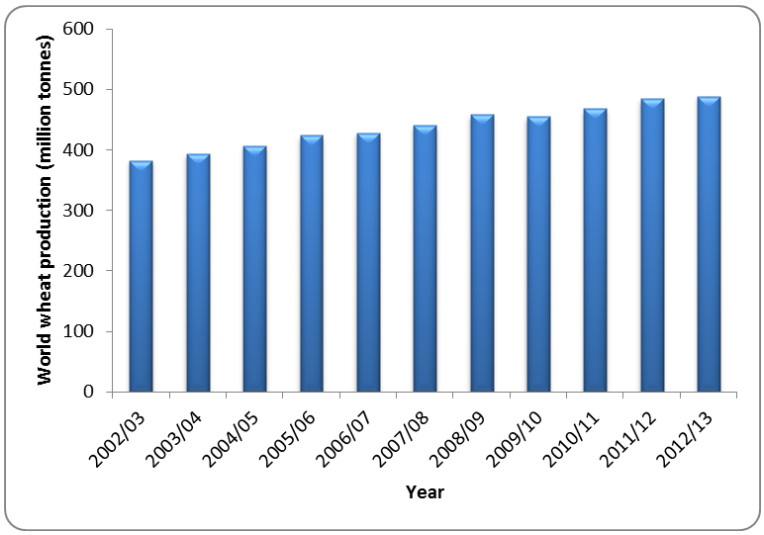

Figure 1.8. World rice production, during 2002 - 2013.[29]

1.2.2.4. Triticale

Triticale is a crop species produced in 1875 by crossing two distinct species: wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L). The name triticale combines the scientific names of the two genera involved. According to the vision of early scientists, triticale should combine the best characteristics of both parents. It presents the grain quality, productivity, and disease resistance of wheat and the robustness of rye for adaptability to difficult soils, drought tolerance, cold hardiness, disease resistance and low-input requirements. This is the first cereal produced by man, with significant economic impact.[48]

This cereal can be mainly used as a feed supplement in the dairy industry, as a component ingredient in beef feedlots and as a constituent of compound rations for intensive livestock (pigs and poultry) rations. Note that, as generating companies have identified triticale as an efficient source of energy, the rapidly expanding biofuel sector predicts an increase in demand for this crop. In addition to its high energy content, the high lysine content presented provides a distinct nutritional advantage over other cereals.

Until the middle of 1980s the evolution of triticale as a commercial crop was slow (figure 1.9). After this date, it was verify an increase in triticale production at an average rate of 150 000 tonnes/year (about 18 % increase per year), reaching nearly 11 million tonnes in 2002.[48] Comparatively, in this same year the world production of sorghum, oat, millet and rye was approximately 54, 25, 23 and 21 million tonnes, respectively. Although the world production of these crops is higher than triticale, they have decreased in the last fifteen years, and the trend seems to be continuing. Since 1985 the average annual increase in triticale production per hectare, at the world level, has been nearly 100 kg/ha/year, which is notable compared to 45, 39, 28 and 21 kg/ha/year for maize, rice, wheat and barley, respectively, in the same period.[48]

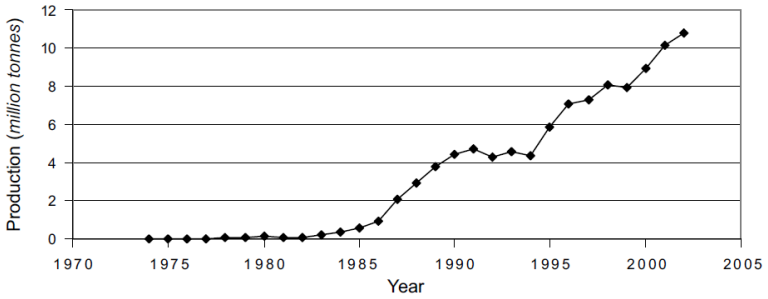

Figure 1.9. World triticale production, during 1974 - 2002 (adapted from *Mergoum M. et al, 2004)*.[48]

Triticale is cultivated in many countries of the world but the major producers are in Europe. In 2002, approximately 88 % of triticale was produced in Europe, 9 % in Asia and 3 % in Oceania. In Europe, the major producers were Germany, Poland and France, whereas most of the Asian production was in China. In this same year, the total hectares of triticale planted in the world were 75 % in Europe, 16 % in Asia and 9 % in Oceania, mostly in Australia.[48]

Concluding, the development of this crop may not appear be consistent with the initial expectations. However, comparing with the thousands years necessary to the present major crops (like wheat and rice) have evolved under domestication, the few years and modest effort devoted to triticale reveal that the results are quite notable.[48]

1.3. Ionic liquids

1.3.1. Definition and physicochemical properties

Ionic liquids (ILs) are salts with melting point below 100 ºC. Usually, they are constituted by an organic cation associated with an anion that can be organic or inorganic. The combination between cation and anion is vast, making possible to synthesize a wide diversity of ILs. There are 10^{18} possible combinations. The most common ILs studied are constituted by imidazolium, pyridinium, ammonium and phosphonium cations (figure 1.10).[49] Some examples of possible anions are presented in figure 1.11.

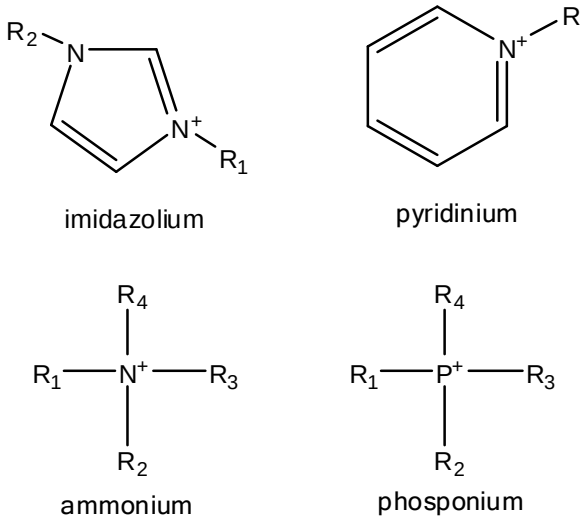

Figure 1.10. Chemical structure of imidazolium, pyridinium, ammonium and phosphonium cations.

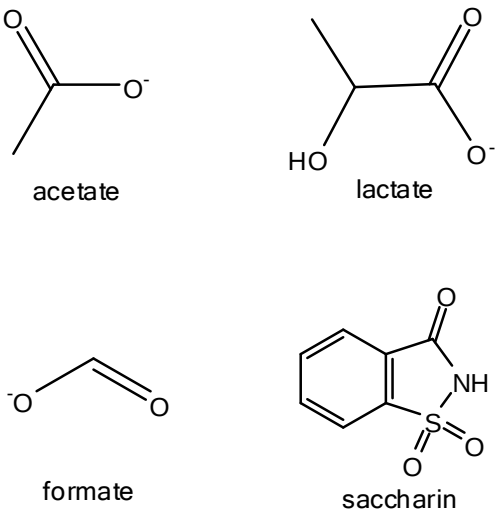

Figure 1.11. Chemical structure of some commonly used anions.

The main physicochemical properties that confers the typical unique characteristics of ILs are: negligible vapor pressure, high thermal stability and highly solvating capacity either for polar or nonpolar compounds, large electrochemical window and high conductivity.[50-52] Their very low vapour pressure reduces the risk of exposure that is a clear advantage relatively to the classical volatile organic compounds (VOCs). Since it is possible to perform a large number of cationic and anionic combinations, the physicochemical properties desired for a particular process can be easily tuned.[53] Simply by changing the structure of either the anion or the cation, properties such as solubility, density, refractive index, viscosity and others can be adjusted to meet the intended requirements.[54,55] For these reason, ILs are designated as "designer solvents".[56-58]

The information about the toxicity and biodegradability of ILs is scarce and therefore, they need to be treated with the same caution as any other chemical with a limited data about their properties. It is notable that toxicity of the ionic liquids is mainly ascribed to the alkyl chain and that the toxicity of imidazolium and pyridinium ILs increases with their cation chain length.[58]The water content in ILs can be considered as an impurity since it was found that decreases the solubility of carbohydrates.[59]

1.3.2.Main applications

The outstanding properties of ILs make them applicable to several areas. Properties such as non-flammability, high ionic conductivity, electrochemical and thermal stability of ILs make them ideal electrolytes in electrochemical devices like in batteries, capacitors, fuel cells, photovoltaics, actuators, and electrochemical sensors.[60] Besides these applications, ILs can also be used in various chemical processes such as in organic synthesis, in catalysis, extraction of heavy metals in water, in the field of effluent treatment and more recently in the areas of Physical Chemistry, Analytical Chemistry and Biotechnology. In this way, it is possible to reduce the amount of volatile organic compounds (VOC's) used in industry. In the context of green chemistry, the use of ILs has been increasing, not only due to their low vapor pressure, but also because of the possibility of being recycled, which makes them more environmentally clean and efficient.

1.4. Lignocellulosic biomass pre-treatments

In order to make full use of biomass, it must be selectively fractionated into its major constituent fractions (cellulose, hemicellulose and lignin). The main problem in using lignocellulosic biomass is due to not only the presence of covalent bonds between lignin and carbohydrates in the cell walls of plants but also the crystallinity of cellulose.[18] Therefore, lignin is the major barrier to enzymatic hydrolysis of cellulose, contributing to the recalcitrance of the lignocellulosic material. In this way, to access the carbohydrates in the biomass for biological conversion, an additional deconstruction step (also commonly called pre-treatment) is an essential prerequisite to bring the sugar polymers into a form suitable for hydrolysis and subsequent fermentation to convert lignocellulosic biomass into fuels and chemicals.[19] After the pretreatment the surface area available for enzyme binding and microbial attack greatly increases, making the transformation into fermentable sugars easier. An effective pre-treatment must meet the following requirements:

1) to improve subsequent biomass hydrolysis to liberate fermentable sugars;

2) to avoid significant degradation or loss of carbohydrates, and;

3) to avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes.[18]

Several pre-treatment technologies are currently employed to overcome the recalcitrance of lignocellulose, increase hydrolysis efficiency and improve the yields of monomeric sugars.[61] Note that, the result is a high recovery of all carbohydrates or/and require low capital and operational costs are some of the features, which should be taken into account in the selection of the pre-treatment method.[62],

In general, pre-treatment methods can be divided into conventional and alternative methods. However, since this thesis is based on alternative methods, namely ILs, only a brief introduction to conventional methods is presented below.

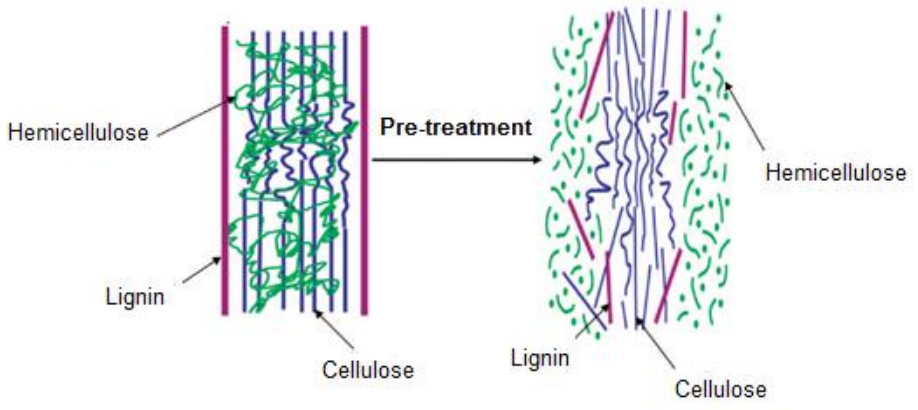

Figure 1.12. Schematic representation of the pre-treatment effect in lignocellulosic biomass.[18]

1.4.1. Conventional methods

Conventional pre-treatment methods are usually classified as physical (e.g. milling, grinding and irradiation), chemical (e.g. alkali, dilute acid, oxidizing agents and organic solvents), physicochemical (e.g. steam pretreatment/autohydrolysis, hydrothermolysis) and biological.[63] The selection of the feasible pre-treatment method for a specific process configuration take into account many factors and, which can be optimal for one process is not necessarily optimal for another process. It is also important to point out that none of these pre-treatments is highly selective and efficient. The main disadvantages of these methods are: insufficient separation of cellulose and lignin (which reduces the effectiveness of subsequent enzymatic cellulose hydrolysis), formation of by-products that inhibit ethanol fermentation (e.g. acetic acid from hemicellulose, furans from sugars and phenolic compounds from lignin), high use of chemicals and/or energy, and considerable waste production.[64,65] Therefore, the search for new green methodologies is crucial. A possible solution to overcome these problems may be the use of ILs. Table 1.1 shows the comparison between some conventional methods for the fractionation of lignocellulosic materials.

Table 1.1. Types of conventional pre-treatment methods (adapted from *Mohammad et al., 2008* [22]*; Carolina et al., 2012* [66]).

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

1.4.2. Alternative methods: ionic liquids

In the last decade, innumerable studies focused on the dissolution of biogenic polymers in ILs demonstrates a great potential of ILs as solvents.[67-69] Cellulose was one of the most studied biopolymers exhibiting a high solubility in a variety of ILs. A wide range of carbohydrate solubilities was scrutinised using different ILs, presenting that one of the main benefits of using ILs to dissolve carbohydrates is that ILs can be tailored to accomplish dissolution or functionalisation of these polymers.[70]

1.4.2.1. Influence of ionic liquids in the dissolution of biomass

The ability of ILs to dissolve carbohydrates and lignin is considered as an effective disruption of the intricate network of non-covalent interactions between these polymers. Remsing et al. using ^{13}C and $^{35/37}Cl$ NMR demonstrated that the interaction between the carbohydrate and the anion of an IL is predominant compared to the interactions of carbohydrate with the cation. It was reported that the dissolution of carbohydrates in [bmim][Cl] involves the formation of hydrogen bonds between chloride ions of the IL and hydroxyl protons of sugar units from carbohydrates in a 1:1 stoichiometry.[71] The IL cation has also some influence on the dissolution by interacting with cellulose hydroxyl oxygen groups.[34] In the case of lignocellulose dissolution the principle is the same, once the main fraction of these materials comprises carbohydrates.[72] However, the presence of lignin and extractives in lignocellulose restricts the solubility and the appearance of a brownish viscous mixture solution is observed during the process.[12] The selection of ILs for lignocellulosic biomass dissolution is difficult, due to the different physical and chemical properties that IL presents. It was referred that ILs constructed by bulky cation and halide anion may decrease the concentration of active chloride ion and thus the solvating capacity for both cellulose and lignin is reduced.[69] The comparison of the efficiency of [emim][Cl] and [bmim][Cl] in the dissolution of sugarcane bagasse allows to notice that [emim] cation as smaller sized than [bmim] cation might be more effective in interacting with cellulose macromolecule.[65] A smaller anion is also preferable to be able to diffuse faster within the lignocellulosic matrix as in the case of chloride anion. Nevertheless, the improvements in lignocellulose dissolution are related with the hydrogen bond basicity of the IL anion as referred above. ILs with a strong hydrogen bond basicity are effective in weakening the hydrogen-bonding network of the polymer chains.[14,73] For example the increased basicity of the [emim][OAc] anion makes it more efficient at disrupting the inter- and intramolecular hydrogen bonding in biopolymers than Cl anion.[74] Generally, increased hydrogen bond basicity of the anion leads to the incorporation of water molecules in the IL structure, which reduces the solubility of carbohydrates.[75] Therefore, drying of the IL prior to use is required. However, not only hydrogen basicity of IL is important but also its structure affects the dissolution process. The viscosity of ILs is also an important parameter, because it can impact the mixing and mass transfer of lignocellulose and IL itself. It was also reported that ILs with an adequate polarity and a low viscosity demonstrate good ability to extract polysaccharides in a short time. Abe et al. showed that the low viscosity and highly polar IL, [emim][$PO(O)H_2$], allows for rapid extraction of cellulose and other carbohydrates from bran under mild conditions.[73] The lower melting point of [emim][OAc] in comparison with [emim][Cl] and [bmim][Cl] also facilitate the dissolution of biomass and handling of the mixture, which makes [emim][OAc] a better solvent than chloride-based ILs in biomass processing.[74,76]

1.4.2.2. Biomass pre-treatment with ionic liquids

The subject of the biomass processing using ILs is very recent. The pre-treatment with ILs allows to: (i) alter the physicochemical properties of the biomass macromolecular components; (ii) extract a specific macromolecular component that is provided by the property of ILs; (iii) perform different fractionation approaches after biomass dissolution in ILs. The pre-treatment is dependent on

IL, lignocellulosic biomass (type, moisture, size and load), temperature, time of pre-treatment and precipitating solvent used.[76]

In relation to the particle size of lignocellulosic biomass, the results in literature are quite contradictory. In general, as reported by *Sun et al. (2009)*[74], smaller particles have larger superficial areas, which causes more efficient dissolution. However, *Viell et al. (2011)*[113] when studied the disintegration and dissolution kinetics of different particle sizes of beech and spruce wood in [emim][OAc], concluded that the dissolution is size-independent.

Generally, the temperature accelerates swelling and dissolution rates of lignocellulose in ILs.[12,14,74,77] This phenomenon is possible due to destabilisation of the hydrogen bonds by increasing the temperature that tightened the three-dimensional structure of cellulose.[20] In fact, some studies showed higher regeneration yields at higher temperatures.[78,79]

The pre-treatment duration time is related to the applied temperature in order to accomplish an efficient pre-treatment. It can be assumed that good results are expected with a simultaneous short duration of time and high temperatures or using prolonged treatments at relatively low temperatures.[76] *Yoon et al. (2012)*[78] pre-treated the sugarcane bagasse with [emim][OAc] and proposed a model based on Response Surface Methodology (RSM) to predict the reducing sugar yield by changing temperature, time and biomass loading. It was observed an improvement in reducing sugar yield at longer pre-treatment duration when lower temperature was applied (120 ^{o}C). However, prolonged pre-treatment used could lead to decrease in reducing sugar yield under higher temperatures (more than 135 ^{o}C), explained by depolymerisation process of biomass components.

The degree of biomass recalcitrance varies as a function of the biomass itself (i.e., grass, softwood, and hardwood), and is influenced by inherent variations in terms of age, harvest method, extent of drying and storage conditions.[80] Furthermore, the lignocellulosic biomass comprises different chemical and physical characteristics, such as composition of cellulose, hemicellulose and lignin, accessible surface area, crystallinity, degree of polymerisation, and others.[81] All these features affect the pre-treatment efficiency, thus a special attention is recommended regarding the type and concentration of biomass to be used in ILs to proceed with the pre-treatment.[76]

1.4.2.3. Ionic liquid used: [emim][OAc]

Until now, [emim][OAc] seems to be the most suitable IL for the pre-treatment of lignocellulosic biomass, since it possesses good solvent power for these materials and hence it is also referred to in the most studies of this research field. For example, several studies were performed focused on the evaluation of the pre-treatment behavior of different hardwood and softwood species with [emim][OAc].[57,74,82,83,113] More specifically Sun et al. (2009)[74] reports [emim][OAc] for dissolution of cellulose due to its desirable properties such as low toxicity, viscosity and corrosiveness, low melting point (< -20°C) and favorable biodegradability. In figure 1.13, the chemical structure of [emim][OAc] is presented.

In summary, as referred above the small size of both cation and anion as well as the high basicity of the anion of this IL contributes to its high suitability in the dissolution of lignocellulosic biomass.

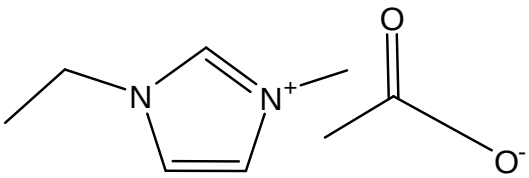

Figure 1.13. Chemical structure of [emim][OAc].

1.5. Enzymatic hydrolysis

The enzymatic hydrolysis consists in the conversion of the components (cellulose and hemicellulose) in the lignocellulosic materials to fermentable reducing sugars, after the pre-treatment step.[18] Note that, through enzymatic hydrolysis is possible to evaluate the efficiency of the pre-treatment process. The following criteria lead to an improvement in enzymatic hydrolysis of lignocellulosic material:

- Increase in the surface area and porosity;
- Modification of lignin structure;
- Removal of lignin;
- (Partial) depolymerization of hemicellulose;
- Removal of hemicellulose;
- Reduction of the crystallinity of cellulose.[11]

The pretreatment of cellulose using ILs has been shown to be an effective method to improve the enzymatic hydrolysis of cellulose. This technique affords a fast and complete saccharification of cellulose into reducing sugars.[1,84]

Chapter 2

2. Experimental

2.1. Materials

In this work, different types of lignocellulosic biomass, namely wheat straw, sugarcane bagasse, rice straw and triticale were tested. Wheat straw was supplied by Estação Nacional de Melhoramento de Plantas (Elvas, Portugal). The dry matter content was 92 % (w/w).[85] Sugarcane bagasse was provided by Inbicon in the framework of PROETHANOL2G project. The dry matter content was 92 % (w/w). Rice straw was acquired from Orivárzea, SA (Foros de Salvaterra) and the dry matter content determined was 95 % (w/w). Triticale was supplied by ICSO "Blachownia" Poland and the dry matter content was 94 % (w/w). All the feedstock material was grounded with a knife mill to particles smaller than 0.5 mm, homogenized in a defined lot, and stored in plastic containers at room temperature.

The IL ([emim][OAc]) used has a purity higher than 95 % and was purchased from Io-li-tec GmbH (Heilbronn, Germany). IL was prior to use in the pre-treatment, dried under (0.1 Pa) at room temperature for at least 24 h. The water content in [emim][OAc] was 2800 ppm and was determined by a volumetric Karl–Fischer titration.

In pre-treatment experiments the following reagents were used: 0.1 M and 3 % (w/w) NaOH aqueous solutions prepared from NaOH pellets (99 % purity) supplied by Eka Chemicals/Akzonobel (Bohus, Sweden), 1 M and 4 M HCl aqueous solutions prepared from fuming HCl 37 % (w/w) with a purity grade for analysis (Merck – Darmstadt, Germany). Ethanol 96 % (v/v) and acetonitrile of HPLC-gradient purity for analysis (Carlo Erba Group – Arese, Italy) and acetone (98 % purity) was supplied by Valente & Ribeiro, Ltda - Belas, Portugal. For the preparation of NaOH and HCl solutions distilled water (17 $M\Omega cm^{-1}$) and ultrapure water (18.2 $M\Omega cm^{-1}$) both produced by the PURELAB Classic of Elga system were used. For filtration, paper and glass microfiber filters (Whatman GE Healthcare Bio-Sciences Corp. – Piscataway, NJ, USA) and nylon filters, 0.45 µm HNPW (Merck Millipore – Billerica, MA, USA) were used.

The solution of sulfuric acid 1.2 % for acid hydrolysis of lignocellulossic materials was prepared with sulfuric acid 98 % (Merck – Darmstadt, Germany).

Acid hydrolysed wheat straw (130ºC, 150 minutes and 1.5 % H_2SO_4), with known composition (62.6 % glucan, 29.9 % lignin, 7.5 % ash and others content) was used for the purpose of FTIR calibration curves. All FTIR samples were prepared with KBr (≥ 99 % trace metals basis) purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The cellulose standard was Cellulose powder MN 300 acc. for thin layer chromatography bought from Macherey, Nagel & Co., Düren (Germany).

Samples of IL for NMR spectroscopy were prepared using chloroform-D (D, 99.8 %) + Silver Foil (Cambridge Isotope Laboratories, Inc. – Andover, MA, USA).

For the enzymatic hydrolysis experiments a 0.1 M sodium citrate buffer of pH = 4.8, prepared from citric acid monohydrate (99.7 % purity) and tris-sodium citrate (>99 % purity) (both from VWR International Ltd. - Leicester, England), a 2 % (w/w) sodium azide solution, prepared from sodium azide (99 % purity; Merck - Darmstadt, Germany) and the enzymes Celluclast® 1.5L (60 FPU g^{-1}; activity 100.57 FPU mL^{-1}) and β-glucosidase Novozym 188 (64 NPGU g^{-1}; activity 436.64 pNPGU mL^{-1}) both purchased from Novozymes (Bagsvaerd, Denmark) were used.

2.2. Equipment

The biomass was grounded to particles of 1.5 mm with the knife mill FRITSCH (Germany) and to particles ≤ 0.5 mm with the knife mill IKA® WERKE, MF 10 basic (Germany). The pH of the samples was adjusted with the pH meter CRISON, GLP 21 (Barcelona, Spain). The solvents were evaporated in the rotavapor Büchi R-210 (Switzerland). Centrifugations performed in B method were done in the Sigma 2-16K Sartorius, SciQuip centrifuge (Shropshire, UK). The oven used to dry all the samples obtained by the pre-treatment of biomass with [emim][OAc] was Cassel ES.6 (Amadora, Portugal). To calculate the moisture of the different biomass used it was used the oven Memmert UL-40 (Germany). Acid hydrolysis was performed in the autoclave A. J. Costa (Irmãos), LDA (Lisboa, Portugal). The ash of each types of untreated and biomass treated by acid hydrolysis was determined using the muffle Heraeus D-6450 (Germany). The total quantity of protein in the raw-material was determined using a protein semi-automatic analyser Kjeltec, Tecator (Sweden). For the quantitative acid hydrolysis it was used the thermostated bath Memmert (Germany). Enzymatic hydrolysis was made in the incubator Optic ivymen® system (Spain).

All spectra of samples were scanned using FTIR spectrometer Spectrum BX, Perkin Elmer, Inc. (San Jose, CA, USA). This instrument was equipped with DTGS detector and KBr beam splitter. The operating system used was Spectrum software (Version 5.3.1, Perkin Elmer, Inc., San Jose, CA, USA).

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker ARX of 400 MHz spectrometer at REQUIMTE, Associated Laboratory, Universidade Nova de Lisboa, Faculdade de Ciências e Tecnologia, Departamento de Química, Caparica, Portugal

The quantification of monosaccharides (D-glucose, D-xylose and L-arabinose) was done by HPLC using Agilent 1100 series HPLC system (Santa Clara, CA, USA) equipped with a Bio-Rad Aminex HPX-87H column (Hercules, CA, USA) using a 5 mM sulphuric acid mobile phase.

2.3. Biomass pre-treatment with [emim][OAc] and fractionation

The pre-treatment procedure was optimised based on two methodologies presented in the literature.[74,86,87] In this work, these two methods are denominated as the A and B methods. Note that the biomass used in the optimisation process was wheat straw and was already characterized. [85]

The A method allowed only the fractionation of biomass in carbohydrate- and lignin-rich materials through the addition of a 0.1 M NaOH aqueous solution after biomass dissolution in [emim][OAc]. In the B method, acetone was used instead of a NaOH aqueous solution in the regeneration step, which allowed to fractionate the regenerated material subsequently into cellulose-, hemicellulose- and lignin-rich fractions. From the liquid stream, acetone soluble lignin was extracted and recovered. From the combination of A and B methodologies an optimised pre-treatment and fractionation procedure, the C method was developed. According to the A method, a 0.1M NaOH aqueous solution was used to regenerate the carbohydrate-rich material, which was later fractionated by the same procedure as in the B method. Moreover, from the obtained liquid stream in the regeneration step (filtrate 1) not only lignin-rich material but also a residual hemicellulose-rich material, which was simultaneously extracted by the alkaline regeneration solution (0.1 M NaOH), was

recovered. In all experiments biomass/IL ratio was 5 % (w/w) except for the B method, where the ratio was 2 % (w/w). The studied methods are described in detail below.

The pre-treatment experiments made with the different types of biomasses were performed with the optimised C method and using the same IL ([emim][OAc]). All pre-treatment experiments were made at least in duplicate.

2.3.1. The A method

The A method was developed based on procedures described before.[74,86] A mixture of 5.00 g of [emim][OAc] and 0.25 g of wheat straw (5 % (w/w) of solid/liquid ratio) was heated at 120 ºC for 6 hours under continuous stirring. After complete dissolution 0.1 M NaOH was added and the mixture was stirred rigorously to precipitate the carbohydrate-rich material. The mixture was then transferred to a 100 mL Erlenmeyer. The total volume of 0.10 M NaOH used was 40 mL, which was added with continuous agitation. The carbohydrate-rich material was collected by vacuum filtration and washed with ultrapure water, until the pH of the washing water was neutral. The filtrate was acidified to pH = 2.0 with 1M HCl to precipitate the lignin-rich material. This solution was next heated at 70 ºC for 30 minutes to obtain further precipitation of lignin that was then separated by hot filtration. The recovered lignin was washed with 10 mL of ultrapure water. The carbohydrate- and lignin-rich materials were dried at 60 ºC for 24 hours. For [emim][OAc] recovery, the remaining filtrate was neutralised by the addition of NaOH pellets, then water was removed by evaporation and a solid containing NaCl and IL was formed. Subsequently, 130 mL of acetonitrile were added to dissolve the IL, leaving NaCl as an insoluble residue, which was later removed by filtration. Acetonitrile was evaporated under reduced pressure and the recovered IL was dried under vacuum for at least 24 hours. Figure 2.1 depicts a simplified schematic process of the A method.

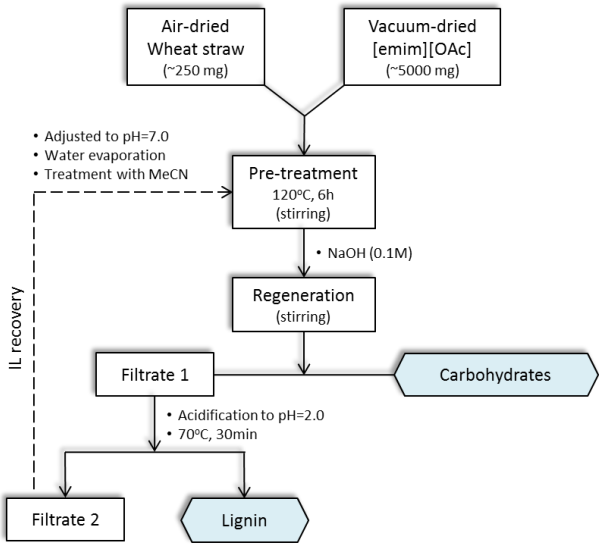

Figure 2.1. The A method pre-treatment procedure.

2.3.2. The B Method

The B method was based on the procedure presented by *Lan et al*.[87] with further optimisations performed. The complete dissolution of 0.10 g of wheat straw in 5.00 g of [emim][OAc] (2 % (w/w) of solid/liquid ratio) was performed at 110 ºC for 4 hours under continuous stirring. Then, an acetone/water mixture (9:1, v/v) was added and the solution was centrifuged at 4000 rpm, 22 ºC for 15 minutes. The total volume of acetone/water (9:1, v/v) used was 40 mL. After this centrifugation the carbohydrate-rich material (pellet) was washed with 35 mL of acetone/water (1:1, v/v) and then centrifuged at 9000 rpm, 4 ºC for 30 minutes. The resulting solid residue was washed with 35 mL of ultrapure water and centrifuged again at 9000 rpm, 4 ºC for 30 minutes. These two washes were necessary to eliminate [emim][OAc] efficiently. The supernatants were collected in the same flask and filtered to remove any traces of the solid fraction. Then, 5 mL of ultrapure water were added to the centrifuge tube with the pellet, which was also filtered to the same filtering flask. The solid carbohydrate-rich material was dried in the oven at 60 ºC for at least 18 h, before further use. The filtrate was concentrated under reduced pressure by removing acetone and pH was adjusted to 2.0 with 1M HCl, to precipitate the lignin-rich material (acetone soluble lignin). Subsequently, the latter was filtered and washed with 10 mL of HCl 0.01 mol L^{-1}. The dried carbohydrate-rich material was treated with a 3 % (w/w) NaOH aqueous solution with a solid/liquid ratio of 1:25 (g mL^{-1}), at 50 ºC for 45 minutes under continuous stirring. The insoluble residue (cellulose-rich fraction) was collected by filtration and washed with ultrapure water. The filtrate was adjusted to pH 6.8 with 4 M HCl and then precipitated with 3 volumes of 96 % (v/v) ethanol under continuous stirring. The resulting solid (hemicellulose-rich fraction) was filtered and repeatedly rinsed with 96 % (v/v) ethanol. The obtained filtrate (filtrate 4) was concentrated under reduced pressure in order to remove ethanol and then adjusted to pH 2.0 with HCl (0.01 mol L^{-1}) to precipitate the residual lignin. After filtration the residual lignin was washed with a 10 mL HCl (0.01 mol L^{-1}). All recovered solids were dried at 60 ºC for 24 hours. The IL recovery was performed as described in the A method, but pH of the liquid stream containing the IL was adjusted to 9.0. The schematic representation of the B method is shown in Figure 2.2.

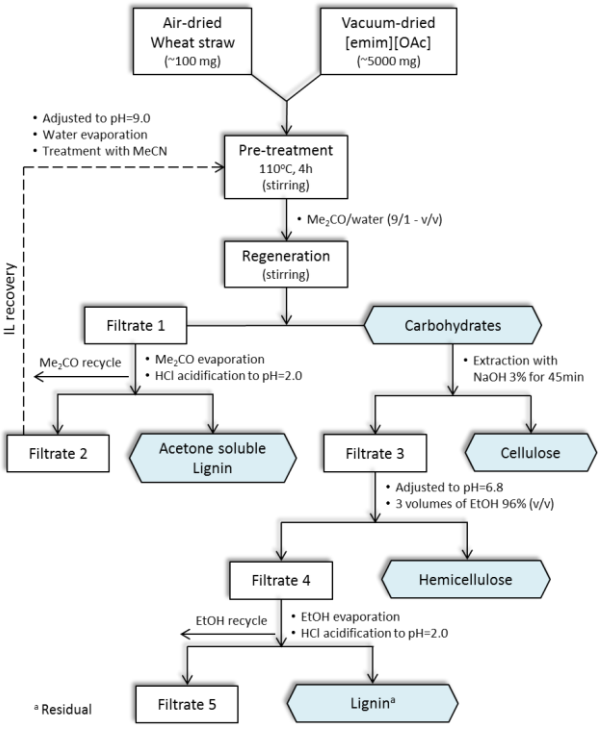

Figure 2.2. The B method pre-treatment procedure.

2.3.3. The C Method

A new methodology was developed based on the A and B methods, as referred above.[88,89]

A mixture of 5.00 g of [emim][OAc] and 0.25 g of wheat straw (5 % (w/w) of solid/liquid ratio) was heated at 120 ºC for 6 hours under continuous stirring. To regenerate the carbohydrate-rich material, 0.1 M NaOH was added. A total of 40 mL of 0.1 M NaOH was used, which was added under continuous stirring. The carbohydrate-rich material was collected by filtration and washed with distilled water, until the pH of the washing water was neutral. The solid material was dried at 60 ºC for at least 18 hours, before further use.

The remaining filtrate (filtrate 1) was reduced in volume by evaporation of water, the pH was then adjusted to 6.8 with 4 M HCl and 1 M HCl. The formed solid was precipitated in 3 volumes of 96 % (v/v) ethanol under continuous stirring. The resulting solid (residual hemicellulose-rich fraction) was filtered and repeatedly rinsed with distilled water. Ethanol from the filtrate (filtrate 2) was evaporated under reduced pressure and pH was adjusted to 2.0 with 4 M and 1M HCl to precipitate lignin-rich material. Subsequently, this solution was heated for 30 minutes at 70 ºC to precipitate further lignin and filtered without cooling. The filtered lignin was washed with HCl 0.01 mol L^{-1} (pH 2.0). The dried carbohydrate-rich material was treated with a 3 % (w/w) NaOH aqueous solution at a solid/liquid ratio of 1:25 (g mL^{-1}), and was kept at 50 ºC for 45 minutes under continuous stirring. The insoluble residue (cellulose-rich fraction) was separated by filtration and washed with distilled water. The filtrate (filtrate 4) was adjusted with an aqueous HCl to pH 6.8 and then three volumes of 96 % (w/w) ethanol were added to precipitate the hemicellulose. The recovery of the hemicellulose- and the residual lignin-rich fractions from the filtrates was carried out using the same procedure as described in the B method.

The IL recovery was performed as described in the A method. The schematic presentation of the C method is depicted in Figure 2.3.

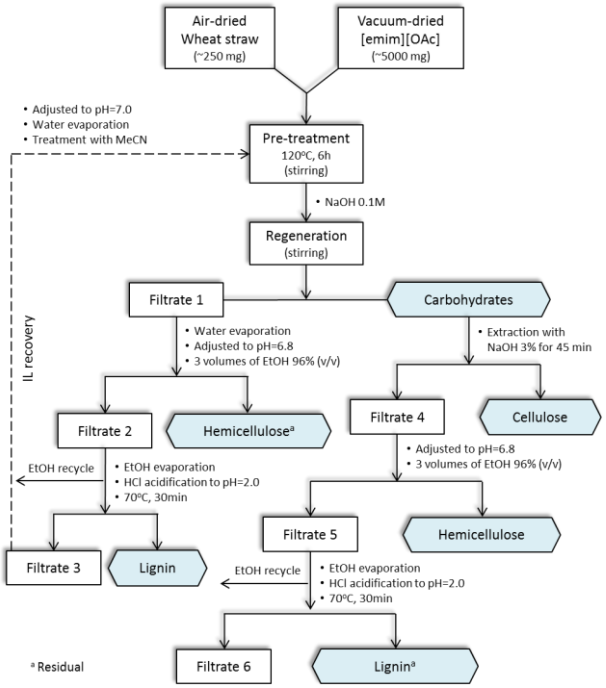

Figure 2.3. The C method pre-treatment procedure.

2.3.4. Reuse of [emim][OAc]

The reuse of the IL was examined using the A method. In the first process 5.00 g of pure [emim][OAc] were used and after the pre-treatment, the IL was recovered as described in the A method. The remaining volatile fractions were removed from the IL applying high vacuum and the obtained IL was reused in a new pre-treatment process maintaining a solid (biomass)/liquid (IL) ratio of 5 % (w/w). The same IL was used in seven pre-treatment processes (once pure and six times reused).

2.4. Conventional pre-treatment: dilute-acid hydrolysis

In order to compare the enzymatic digestibility of the biomass pre-treated with a conventional pre-treatment (acid hydrolysis) and using ILs it was necessary to do the acid hydrolysis of wheat straw, sugarcane bagasse, rice straw and triticale. In the case of wheat straw it was used a sample supplied by LNEG, in which the acid hydrolysis conditions used were 1.5 % (w/w) H_2SO_4, 150 minutes, 130 ºC, RLS = 7 (w/w). The remaining biomasses were subjected to the following acid hydrolysis conditions: 1.2 % (w/w) H_2SO_4, 150 minutes, 130 ºC, RLS = 7 (w/w).

The untreated material was placed inside the autoclave in 250 mL Schott flaks (Germany), closed with Schott screw caps GL45 (maximum allowable temperature 200 ºC). After the reaction time had elapsed, sample was cooled down to 90 ºC. The hydrolysate and the solid phase were recovered by filtration (Whatman no. 1 filter paper). The solid phase was washed with distilled water and dried at 60 ºC for at least 24 hours. The hydrolysate was stored in the refrigerator. All experiments were made in duplicate.

2.5. Enzymatic hydrolysis

The maximum possible extent of digestibility of original biomass, acid hydrolysed biomass and cellulose-rich materials from the IL pre-treatment was evaluated by enzymatic hydrolysis and subsequent HPLC sugar analysis of the hydrolysates, based on the standard NREL procedure.[90] Samples were prepared in 30 mL vials by adding 5.0 mL of 0.1 M sodium citrate buffer (pH 4.8) and 100 µl of a 2 % (w/w) sodium azide solution to prevent growth of organisms. Distilled water was added taking into account the volume of enzyme and sample needed to complete in each vial a total volume of 10.0 mL. Celluclast® 1.5L and Novozym 188 enzyme solutions were added at last. A reaction blank was prepared for the substrate. The substrate blank contains buffer, water, and the identical amount of substrate in 10.0 mL volume. Enzyme blanks were prepared for Celluclast® 1.5 L and Novozym 188 with buffer, water, and the identical amount of the enzyme. The enzymatic hydrolyses were performed in a shaking incubator at 150 rpm and 50 ºC for 72 hours. After hydrolysis the reaction vials were placed in an oil bath and boiled for 5 minutes. Each sample was filtrated through Millipore® filters with a pore diameter of 0.45 µm to remove insoluble solids and, stored in a refrigerator. Then, these filtrates were used to measure reducing sugar concentrations by HPLC analysis. The column temperature was 50 ºC, the flow rate 0.6 mL min^{-1}; the injection volume 5 µL and the acquisition time 15 minutes for standards and 30 minutes for samples. Glucose and xylose standards were prepared in distilled water at concentrations of 0.25, 0.5, 1.0, 2.5, 5.0 and 10 mg mL^{-1} to construct the calibration curve. The cellulose and xylan contents were calculated from glucose and xylose contents multiplied by conversion factors of 0.90 and 0.88, respectively.[90],[91] All samples were made at least in duplicate.

Note that all solutions and the biomass are assumed to have a specific gravity of 1.000 g mL^{-1}. Thus, if 0.200 g of biomass is added to the vial, it is assumed to occupy 0.200 mL and 9.733 mL of liquid is to be added, for example. The calculation of the water volume is done as follows:

Water = 10 mL – m (sample, g) – 0.1mL (azide) –x mL (Celluclast) – x mL (Novozym) – 5 mL (buffer)

2.6. Chemical characterization of the original and acid hydrolysed raw material

2.6.1. Determination of the moisture content

Firstly, 0.5 g of the raw material was weighted in the previously dried nickel plates. This sample was placed in the oven at 105 ºC ± 1 ºC for at least 16 hours, cooled down to room temperature in a desiccator for 1 hour and weighted on an analytical balance. This procedure was done in duplicate.

2.6.2. Determination of the polysaccharides (glucan, xylan and arabinan) and acetyl groups content

For the determination of polysaccharides, lignin and acetyl groups the samples were subjected to a quantitative acid hydrolysis according with the method described by Browning and according to the protocol of National Renewable Energy Laboratory.[92] The main objective of quantitative acid hydrolysis is to determine the composition of the feedstock. Therefore, differently from acid hydrolysis,

the conditions used are more severe in order to disrupt the lignocellulose structure converting polysaccharides in its monomeric subunits. In a test tube, 5 mL of H_2SO_4 72 % (w/w) was added to 0.5 g of raw-material and the mixture was incubated at 30 ± 1 ºC in a thermostated bath for 1 hour, with occasional stirring with a glass rod. Then, the entire content of the test tube was transferred to 250 mL Schott flaks with distilled water, in an amount sufficient to give a concentration of 4 % (w/w) H_2SO_4. This mixture was placed in the autoclave at 121 ºC for 1 hour. In order to determine if there were any losses during processing, the Schott flaks were weighted before and after the autoclave (note that after the autoclave, the Schott flaks were weighted after they were properly cooled to room temperature). The mixture was filtered through fritted filters (Schott) of porosity 3.

After the hydrolysis, the resulting solid residue corresponds to the Klason lignin and the aqueous phase corresponds to the hydrolysis products of polysaccharides. The solid residue was washed with distilled water (50 mL). The components of the aqueous phase were filtered through filters of 0.45 µm and were analysed by HPLC. The calculation of the percentage of polymers and acetyl groups was made considering the concentration of glucose, xylose, arabinose and acetic acid present in the hydrolysate.

2.6.3. Klason lignin and ash determination

1 g of the solid residue obtained after acid hydrolysis was placed in the previously dried porcelain crucibles. This sample was placed in the oven at 105 ºC ± 1 ºC for at least 16 hours. Subsequently, these crucibles were cooled in the dessicator for 1 hour and weighted on an analytical balance. The acid-insoluble residue was considered as Klason lignin, after correction for the acid-insoluble ash (determined by igniting the contents at 550 ºC for 5 hours). This procedure was made in duplicate.

2.6.4. Determination of the total quantity of protein

The total protein content in the feedstock was estimated according with Kjedahl method by a protein semi-automatic analyser. The conversion factor used was N x 6.25. This methodology is described in detail in A appendix.

2.7. Analytical methods

2.7.1. FTIR spectroscopy characterization

Calibration curve

For FTIR calibration curve it was used acid hydrolysed wheat straw (130 ºC, 150 minutes and 1.5 % (w/w) H_2SO_4), with known composition. In the case of the other lignocellulosic biomass it was used untreated biomass grounded with a knife mill to particles smaller than 0.5 mm.

Sample preparation

For the quantitative analysis, 1 mg of carbohydrate-rich material (regenerated material, hemicellulose- and cellulose-rich samples) or 0.5 mg of lignin-rich material were mixed with 50 mg of KBr and grinded in a mortar, until a homogeneous mixture was obtained. The milling time was 10 minutes and the samples were placed in the press with 8.5 tonnes for 5 minutes. This sample preparation methodology was performed for all the samples equally to minimise the experimental errors associated with the FTIR sample preparation.

FTIR Spectra Acquisition

FTIR spectra were acquired at region of 4000-400 cm^{-1}, with a total of 64 scans and a resolution of 4 cm^{-1} with strong apodization. These spectra were subtracted against the background of air spectrum and were recorded as absorbance values.

Lignocellulosic material quantification

The quantitative FTIR analysis was performed by the construction of two separate calibration curves, for each type of feedstock. One of this curves permits to quantify the carbohydrates content and the other permits to determine the lignin content. The calibration curve for wheat straw was prepared using acid hydrolysed pre-treated wheat straw with known composition as a standard.[85] For the others biomasses it was used an untreated sample of the raw material. To minimise the experimental error, samples were scanned at least three times and an average value was considered for the calibration curve. The obtained spectra exhibited a linearity of absorptions in characteristic regions of carbohydrates and lignin. Therefore, the spectrum regions with maximum linearity were selected for quantification. Namely, the band at 898cm^{-1} for carbohydrate and the range at 1503-1537cm^{-1} for lignin were selected. The quantification was made through the measurement of the total area (abs cm^{-1}) in the selected regions. Note that the measurement was made in absorbance instead transmittance. The others content present in the biomass was determined by difference since the carbohydrates and lignin content is known. In the calibration curve of carbohydrates the value on the x axis represents the integral of the band at 898 cm^{-1} (abs cm^{-1}) and the value on the y axis corresponds to the carbohydrate content (%). In the calibration curve of lignin the value on the x axis represents the integral of the range 1503-1537 cm^{-1} (abs cm^{-1}) and the value on the y axis corresponds to the lignin content (%).The calibration curves were validated regularly before each series of analysis. The quantification of the experimental samples was performed in the same way as described above.

2.7.2. NMR

To record the spectra, the IL was dried in vacuum pump (0.1 Pa) for at least 24 hours at room temperature and then, was weighed approximately 30 mg of IL for a NMR tube and it was added 500 µL of chloroform-D ($CDCl_3$).

2.7.3. HPLC

All samples were previously filtered through Millipore® filters with a pore diameter of 0.45 µm. D-glucose, D-xylose, L-arabinose and acetic acid was analysed by an Aminex HPX-87H (Bio-Rad, EUA) column with an IR detector. The determination of the concentration of the analysed compouds were made through calibration curves traced from standard solutions of 0.25, 0.5, 1, 2.5, 5, 7.5, and 10 g/L GXA (glucose, xylose, and arabinose).

Table 2.1. Characteristics of chromatograph Agilent 1100 Series.

Equipment	Name	Model
Automatic Injector	ALS	G1313
Quaternary pump	Quat Pump	G1311A
Degassing	Degasser	G1379A
Furnace	Colcom	G1316A
Diode array detector	DAD	G1315
Detector of refractive index	RID	G1362A

Table 2.2. Operating conditions for HPLC analysis

Designation	HPX-87H column
Mobile phase	H_2SO_4 5.0 mM
Flow	0.4 mL.min^{-1} (Characterization of the raw material and solid residues) 0.6 mL.min^{-1} (others)
Column temperature	50 ºC
IR detector temperature	45 ºC
Wavenumber	280 nm
Sample volume	20 µL (0.4 mL.min^{-1}) or 5 µL (0.6 mL.min^{-1})

2.7.4. FTIR measurement of cellulose crystallinity

To evaluate changes of cellulose structure in regenerated wheat straw as well as in cellulose-rich samples, two infrared ratios were calculated, namely crystallinity index (CI – $A_{1437\ cm\text{-}1}/A_{898\ cm\text{-}1}$) [93] also designated as lateral order index (LOI)[94] and total crystallinity index (TCI – $A_{1376\ cm\text{-}1}/A_{2900\ cm\text{-}1}$).[95] For the calculations, the total height of the band was considered.

2.8. Experimental error analysis

For all obtained results standard deviation errors (u) were determined. The applied temperature in pre-treatment experiments demonstrated an u(T) = 1 ºC. All mass determinations present an u(m) = 0.1 mg. The pre-treatment errors were given as total loss materials for each experiment. For FTIR quantitative analysis, an arbitrary error of 5 % of the experimental value was established.

Chapter 3

3. Results

3.1. Original lignocellulosic biomass composition

The chemical composition of a dry weight of original wheat straw, sugarcane bagasse, rice straw and triticale is shown in table 3.1. It is important to point out that the composition of each feedstock may vary depending on the variety and climacteric cultivation conditions.

Wheat straw

Wheat straw was selected for the pre-treatment optimisation with ILs. This biomass contains 8.0 % (w/w) of moisture. It contains 18.0 % (w/w) of Klason lignin and approximately 60.0 % (w/w) of total polysaccharides, among which 38.9 % (w/w) is cellulose and 23.5 % (w/w) is hemicellulose. The percentage of hemicellulose corresponds to the sum of acetyl groups, xylose and arabinose compounds.

Sugarcane bagasse

Sugarcane bagasse contains approximately 8.1 % (w/w) of moisture. The percentage of cellulose is similar to this of wheat straw (38.7 % (w/w)) but this biomass has more hemicellulose and lignin (30.5 % (w/w) and 20.1 % (w/w), respectively).

Rice straw

The moisture content of original rice straw is at the level of 4.8 % (w/w). This biomass has 40.9 % (w/w) of cellulose, 24.2 % (w/w) of hemicellulose and 14.4 % (w/w) of lignin. Comparing with the composition of the other biomasses used it can be seen that rice straw contains less lignin. The amount of hemicellulose is at the similar level as in wheat straw and slightly lower than in sugarcane bagasse. The content of cellulose is similar to two biomasses aforementioned.

Triticale

Triticale has a moisture content of about 6.2 % (w/w). It contains 41.7 % (w/w) of cellulose, 26.2 % (w/w) of hemicellulose and 20.5 % (w/w) of Klason lignin. Comparing with the other feedstocks, triticale contains similar quantity of cellulose of the other biomasses. Hemicellulose is similar to wheat straw and rice straw but is slightly lower than in sugarcane bagasse. The amount of Klason lignin is similar to sugarcane bagasse but in relation to wheat straw and rice straw this quantity is slightly higher.

Table 3.1. Average macromolecular composition of original wheat straw, sugarcane bagasse, rice straw and triticale (% of dry weight).

	Dry weight (% (w/w))			
Component	**Wheat straw[85]**	**Sugarcane bagasse**	**Rice straw**	**Triticale**
Cellulose[a]	38.9 ± 0.2	38.7 ± 0.4	40.90 ± 0.05	41.7 ± 0.3
Hemicellulose	23.5	30.5	24.2	26.2
Xylan	18.1 ± 0.3	21.2 ± 0.6	20.5 ± 0.2	20.1 ± 0.4
Arabinan	3.0 ± 0.2	2.7 ± n.d.	3.4 ± 1.0	2.2 ± 3.2
Acetyl groups	2.5 ± 0.1	6.6 ± 3.3	0.4 ± 5.6	3.9 ± 8.7
Klason lignin	18.0 ± 0.5	20.1 ± 0.2	14.4 ± 5.0	20.5 ± 0.3
Ash	9.70 ± 0.03	-	4.04 ± 0.03	-
Protein	4.5 ± 0.5	-	2.5 ± n.d.	-
Extractives	-	-	9.5	-
Others (by difference)	5.5		4.4	

[a] Measured as glucan ; n.d. – not determined

3.2. Acid hydrolysed lignocellulosic biomass composition

The chemical composition of dry weight of acid hydrolysed wheat straw, sugarcane bagasse, rice straw and triticale is presented in table 3.2.

Wheat straw

Acid hydrolysed wheat straw presents a moisture content of approximately 5.7 % (w/w). This biomass is free of hemicellulose and has 62.6 % (w/w) of cellulose and 29.9 % (w/w) of lignin.

Sugarcane bagasse

The moisture content of acid hydrolysed sugarcane bagasse is about 5.5 % (w/w). Acid hydrolysed sugarcane bagasse is mainly composed by 61.5 % (w/w) of cellulose, 6.7 % (w/w) of hemicellulose and 33.4 % (w/w) of lignin.

Rice straw

Acid hydrolysed rice straw has a moisture content of about 3.9 % (w/w). The cellulose, hemicellulose and Klason lignin content of this biomass is about 52.0 % (w/w), 7.0 % (w/w) and 24.0 % (w/w), respectively.

Triticale

The moisture content of acid hydrolysed triticale is 4.7 % (w/w). Triticale is mainly composed by 58.0 % (w/w) of cellulose, 8.0 % (w/w) of hemicellulose and 33.0 % (w/w) of Klason lignin.

Table 3.2. Average macromolecular composition of acid hydrolysed wheat straw, sugarcane bagasse, rice straw and triticale (% of dry weight).

	Dry weight (%(w/w))			
Component	**Wheat straw**	**Sugarcane bagasse**	**Rice straw**	**Triticale**
Cellulose[a]	62.6 ± 0.3	61.5 ± 0.2	52.4 ± 3.0	58.4 ± 0.2
Hemicellulose	0.0	6.7	7.3	8.0
Xylan	0.0	4.1 ± n.d.	5.6 ± n.d.	6.5 ± 4.5
Arabinan	0.0	1.4 ± n.d.	1.7 ± n.d.	1.5 ± n.d.
Acetyl groups	0.0	1.2 ± 8.5	0.0	0.0
Klason lignin	29.9 ± 0.6	33.4 ± 0.1	24.4 ± 0.7	33.0 ± 0.3
Ash	7.5 [b]	-	13.4	1.6
Protein	-	-	3.9	2.9

[a] Measured as glucan; [b] Ash & others; n.d. – not determined

3.3. Optimisation study of wheat straw pre-treatment using [emim][OAc]

The pre-treatment procedure was optimised based on two methodologies presented in the literature.[74,86,87] In this work these two methods are denominated as the A and B methods. For the optimisation of the pre-treatment time different reaction times (1, 6 and 16 hours) were tested at constant temperature (120 ºC) and biomass loading (5 % (w/w)). The complete macroscopic dissolution of wheat straw was observed for a 6-hour and 16-hour processing. Both pre-treatments produced dark brown solutions; however, an addition of antisolvent (0.1M NaOH) in the case of the 16-hour process caused formation of a jelly (regenerated) material and prolonged process time led to product and IL degradation. Therefore, the 6-hour process was found to be the best among the tested conditions as a complete macroscopic dissolution of biomass was guaranteed and an efficient recovery of the carbohydrate-rich fraction was achieved. The results are presented in Table 3.3.

Table 3.3. Results obtained for the study of the reuse of [emim][OAc] using A method.

				SF		LF		IL Recovery	
Exp.	**Time (h)**	**WS (mg)**	**Dried WS (mg)**	**RM (mg)**	**RY (% w/w)**	**Lignin[a] (mg)**	**ML (% w/w)**	**(% w/w)**	**pH**
1	1	103.2	94.9	63.0	66.4	2.6	30.9	-	-
3	16	250.4	230.4	119.2	51.7	6.6	45.4	76.3	7.0
4	6	250.5	230.5	120.8	52.4	4.8	45.5	88.2	7.0

WS – wheat straw; RM – regenerated material; RY – regeneration yield; ML – material lost; IL – ionic liquid

[a] Lignin-rich material

The A method

After the optimisation of the pre-treatment time it was made an experiment with different amounts of biomass weighed and with different volumes of 0.1 M NaOH. These results are shown in table 3.4. Comparing the results of A1 and A2 experiments, it was verified that is more advantageous to use 250 mg of biomass instead of 125 mg since the amount of the recovery lignin in A1 experiment is very low to perform the subsequent FTIR analysis. Hereafter, it was tested if increasing the volume of 0.1 M NaOH will improve the separation of carbohydrates from lignin. In A2 experiment was used

40 mL of 0.1 M NaOH and in A4 experiment was used 80 mL. As showed in table 3.4 the increased in the volume of 0.1 M NaOH does not improved the separation between carbohydrates and lignin because the A4 regeneration yield is lower than A2 regeneration yield. For that reason, in the next experiments it was used 40 mL of 0.1 M NaOH instead of 80 mL. The comparison between A3 and A1 experiments reveal that the use of 50 mL of 0.1 M NaOH is not advantageous since, although the A3 regeneration yield is slightly higher than A1 experiment, the material lost is very similar. The highest regeneration yield was observed for A2 experiment and the highest material lost was observed for A1 experiment. The recovery percentage of the IL in these experiments is about 71 – 77 % (w/w).

Table 3.4. Results obtained using A method with different amounts of biomass (A1, A2, A3) and different volumes of 0.1 M NaOH.

			Solid fraction		Liquid fraction		IL Recovery	
Exp.	**WS (mg)**	**Dried WS (mg)**	**RM (mg)**	**RY (% $_{w/w}$)**	**Lignin[a] (mg)**	**ML (% $_{w/w}$)**	**(% $_{w/w}$)**	**pH**
A1[b]	125.4	115.3	62.1	53.8	10.3	37.3	75.2	7.0
A2[b]	250.6	230.5	159.7	69.3	18.6	22.7	71.2	7.0
A3[c]	125.6	115.6	65.6	56.7	8.3	36.1	76.8	7.0
A4[d]	250.6	230.5	127.7	55.4	34.3	29.7	76.4	7.0

WS – wheat straw; RM – regenerated material; RY – regeneration yield; ML – material lost; IL – ionic liquid

[a] Lignin-rich material; [b] Addition of 40 mL of 0.1 M NaOH to precipitate the regenerated material; [c] Addition of 50 mL of 0.1 M NaOH to precipitate the regenerated material; [d] Addition of 80 mL of 0.1 M NaOH to precipitate the regenerated material

The B method

In this method it was used a pre-treatment time of 4 hours instead of 6 hours, a mixture of acetone/water (9:1, v/v) instead of 0.1 M NaOH to regenerate carbohydrate-rich material and a 2 % (w/w) solid/liquid ratio instead of 5 % (w/w). Contrary to A method, this method permits the separation of lignocellulosic biomass in cellulose-, hemicellulose- and lignin-rich materials. In table 3.5 and 3.6 the results for the two experiments performed are presented. The difference between these two experiments is in the regeneration step of carbohydrate-rich material. In B1 experiment it was used a mixture of acetone/water (9:1, v/v) and in B2 experiment besides this mixture it was also used an acetone/water (1:1, v/v) mixture and water to wash the precipitated fraction. This modification was made due to the low recovery percentage of IL obtained in B1 experiment. As shown in table 3.5, besides the improvement in the recuperation process of IL, the regeneration yield increased, it has been recovery a slightly higher quantity of lignin and the material lost decreased. Note that, B3 experiment is a duplicate of B2 experiment. B3 experiment has the highest regeneration yield and the lowest material lost. B2 experiment has the highest amount of lignin-rich material recovered and the highest percentage of IL recovered. The results obtained for the fractionation of the carbohydrate-rich material (solid fraction) are displayed in table 3.6. The percentage of cellulose-rich material recovered is very similar for the three experiments (between 61-63 % (w/w)). B2 experiment has the highest percentage of hemicellulose-rich material recovered (about 32 % (w/w)) and B1 experiment has the highest percentage of residual lignin-rich material recovered (about 6 % (w/w)). Note that these results

show that carbohydrate-rich material is namely composed by cellulose and is slightly contaminated with lignin.

Table 3.5. Results obtained using the B method for the liquid and solid fractions.

			Solid fraction		Liquid fraction		IL Recovery	
Exp.	**WS (mg)**	**Dried WS (mg)**	**RM (mg)**	**RY (% w/w)**	**Lignin[a] (mg)**	**ML (% w/w)**	**(% w/w)**	**pH**
B1	99.8	91.8	55.7	60.7	7.8	30.9	53.3	9.0
B2	100.8	92.7	64.5	69.6	10.4	19.2	93.6	9.0
B3	100.2	92.2	68.8	74.7	9.8	14.7	91.8	9.0

WS – wheat straw; RM – regenerated material; RY – regeneration yield; ML – material lost; IL – ionic liquid

[a] Lignin-rich material

Table 3.6. Results obtained with the B method for the fractionation of the regenerated material.

		RM fractionation			
Exp.	**RM load (mg)**	**Cellulose (mg)[a]**	**Hemicellulose (mg)[b]**	**Lignin[c] (mg)**	**ML (% w/w)**
B1	50.8	31.9	12.9	3.2	5.6
B2	60.4	36.8	19.0	2.9	2.8
B3	42.4	26.8	11.6	2.0	4.8

RM – regenerated material; ML – material lost

[a] Cellulose-rich material; [b] Hemicellulose-rich material; [c] Residual lignin-rich material

The C method

From the combination of A and B methodologies an optimised pre-treatment and fractionation procedure, the C method was developed. According to the A method, a 0.1M NaOH aqueous solution was used to regenerate the carbohydrate-rich material, which was later fractionated by the same procedure as in the B method. Moreover, from the obtained liquid stream in the regeneration step (filtrate 1) not only lignin-rich material but also a residual hemicellulose material, which was simultaneously extracted by the alkaline regeneration solution (0.1 M NaOH), was recovered. The results of the C pre-treatment method are indicated in table 3.7 and 3.8.

Comparing the results obtained for the three experiments (table 3.7), it was verified that the regeneration yield for C1 and C3 experiments are very similar. In the case of C2 experiment, it was not possible to determine the regeneration yield because the recovered carbohydrate-rich material was not dried in the oven. Instead, in this case was used wet regenerated material. As in C1 experiment the regenerated material was dried in the oven for 24 hours, an attempt was made to decrease the process time, which in turn would contribute to reduce the process energy costs. However, this modification has the disadvantage of increasing the volumes of solvents used, namely ethanol. Consequently, in C3 experiment instead of 24 hours, the regenerated material was dried for 18 hours since this time is sufficient to remove the water. Note also that, C1 experiment has the highest recovery of residual hemicellulose-rich material and has a negative value for the material loss percentage. The highest quantity of the recovered residual hemicellulose can be due to the combined precipitation of NaCl with hemicellulose, when ethanol was added to the liquid fraction. Therefore, in

this experiment was not obtained more hemicellulose, but a higher amount of salt has precipitated together with residual hemicellulose. As a result, a negative value of material loss was obtained since the total amount of the fractions recovered (residual hemicellulose and lignin) is higher than the real mass that could be recovered. In order to overcome this problem, in C2 and C3 experiments the precipitated residual hemicellulose was washed with distilled water. By this means, the addition of distilled water enables the NaCl solubilisation, remaining in the solid phase mainly the residual hemicellulose. The amount of residual hemicellulose-rich material is very similar for C2 and C3 experiments, as is given in table 3.7. Relatively to the percentage of the IL recovery, C1 experiment has the lowest percentage and the percentage for C2 and C3 experiments are very close.

Table 3.8 shows the results obtained after the fractionation of the regenerated material in cellulose-rich, hemicellulose-rich and residual lignin-rich materials. The amount of cellulose-rich material is very similar for the three experiments. Experiment C2 has the highest recovery of hemicellulose-rich material and has the lowest recovery of lignin-rich material. Note that, as mentioned above due to the precipitation of NaCl with hemicellulose, C1 experiment has the lowest percentage of material lost. The determination of the material lost percentage for C2 experiment was not possible because the weight of the regenerated material is not known since it was not dried in the oven.

Table 3.7. Results obtained using C method for the liquid and solid fractions.

			Solid fraction		Liquid fraction			IL Recovery	
Exp.	**WS (mg)**	**Dried WS (mg)**	**RM (mg)**	**RY (% w/w)**	**Lignin[a] (mg)**	**Hemicellulose[b] (mg)**	**ML (% w/w)**	**(% w/w)**	**pH**
C1	249.7	229.7	133.2	58.0	18.5	128.4	33.9	78.2	7.0
C2	250.4	230.4	-	-	15.3	38.9	-	89.1	9.0
C3	250.6	230.5	131.1	56.9	15.1	38.3	36.6	86.2	7.0

WS – wheat straw; RM – regenerated material; RY – regeneration yield; ML – material lost; IL – ionic liquid

[a] Lignin-rich material; [b] Residual hemicellulose-rich material

Table 3.8. Results obtained with C method for the fractionation of the regenerated material.

		RM fractionation			
Exp.	**RM load (mg)**	**Cellulose[a] (mg)**	**Hemicellulose[b] (mg)**	**Lignin[c] (mg)**	**ML (% w/w)**
C1	123.1	94	22.2	3.6	2.8
C2	-	83.5	30.2	1.2	-
C3	119.7	87.3	18.4	3.0	9.2

RM – regenerated material; ML – material lost

[a] Cellulose-rich material; [b] Hemicellulose-rich material; [c] Residual lignin-rich material

3.4. Different biomass pre-treatment using [emim][OAc]

In order to test the versatility and efficiency of the fractionation process of the optimised methodology development, pre-treatment of different types of biomasses has been performed. Besides wheat straw, sugarcane bagasse (CA), rice straw (CB) and triticale (CC) were tested. For all the experiments it was macroscopically verified the entire dissolution of biomass in the IL. The results

obtained for each biomass pre-treatment are shown in table 3.9 and 3.10. Note that, each experiment was made in duplicate but only the mean values are presented.

The highest regeneration yield was obtained for the pre-treatment of sugarcane bagasse (77.0 % (w/w)) and the regeneration yield for rice straw and triticale pre-treatment were at the similar level (70.8 % (w/w) and 70.1 % (w/w), respectively). Triticale was the biomass with the highest recovery of lignin and residual hemicellulose-rich materials. The experiment with the lowest percentage of IL recovery was triticale and the experiment with the highest was sugarcane bagasse (93.5 % (w/w)).

Results obtained after the fractionation of the regeneration material in cellulose-, hemicellulose- and lignin-rich materials are displayed in table 3.10. The biomass with the highest recovery of cellulose-rich material was sugarcane bagasse (121.1 mg) and rice straw presented the lowest one (106.3 mg). Rice straw shown the highest recovery of residual lignin-rich material (7.1 mg) and triticale presented the lowest (3.9 mg). Note that, triticale was the only biomass with the recovery of two fractions of hemicellulose because after the filtration, in the liquid fraction, remained a white precipitate that was filtered again for a new filter. Finally, rice straw revealed again the highest material loss in the fractionation process of the regenerated material (6.8 mg).

Table 3.9. Results obtained for the liquid and solid fractions from the pre-treatment of sugarcane bagasse (CA), rice straw (CB) and triticale (CC).

			Solid fraction		Liquid fraction			IL Recovery
Exp.	**WS (mg)**	**Dried WS (mg)**	**RM (mg)**	**RY (% w/w)**	**Lignin[a] (mg)**	**Hemicellulose[b] (mg)**	**ML (% w/w)**	**(% w/w)**
CA	250.4	210.1	161.7	77.0	11.2	19.9	8.2	93.5
CB	250.3	226.5	160.3	70.8	16.8	15.8	14.8	72.7
CC	250.4	219.2	153.6	70.1	25.3	27.0	6.1	73.5

WS – wheat straw; RM – regenerated material; RY – regeneration yield; ML – material lost; IL – ionic liquid

[a] Lignin-rich material; [b] Residual hemicellulose-rich material

Table 3.10. Results acquired for the fractionation of the regenerated material obtained after the pre-treatment of sugarcane bagasse (CA), rice straw (CB) and triticale (CC).

		RM fractionation				
Exp.	**RM (mg)**	**Cellulose[a] (mg)**	**Hemicellulose[b] (mg)**	**Hemicellulose[c] (mg)**	**Lignin[d] (mg)**	**ML (%w/w)**
CA	161.7	121.1	-	30.1	5.6	3.0
CB	140.0	106.3	-	17.2	7.1	6.8
CC	153.6	114.7	12.2	17.8	3.9	3.1

RM – regenerated material; ML – material lost

[a] Cellulose-rich material; [b] Residual hemicellulose-rich material; [c] Hemicellulose-rich material; [d] Residual lignin-rich material

3.5. FTIR qualitative and quantitative analysis

The FTIR spectroscopy was chosen to characterise all solid samples recovered from the biomass pre-treatment processes. The main chemical bond vibrations of lignocellulosic materials are detected in the region of 1800-800 cm^{-1}. Therefore, this region was selected for the analysis of all

samples considered in this work. The complete spectra are illustrated in B appendix. Note that, the identification of some absorption bands is a little controversial in literature. The characterization of all the absorption bands identified is summarized in C appendix.

3.5.1. FTIR characterization of wheat straw

The FTIR spectrum of untreated wheat straw is illustrated in figure 3.1. The bands at 1376, 1250, 1161, 1106, 1051 and 898 cm^{-1} are attributed to carbohydrates in native wheat straw. The band at 1376 cm^{-1} is related to O-H bending from hydroxyl groups. The broad absorption at 1250 cm^{-1} is originated by the C-O stretching of acetyl groups present in hemicellulose molecular chains. The C-O anti-symmetric bending is assigned to the band 1161 cm^{-1} and the arabinosyl side chains are assigned to 1051 cm^{-1}. The band at 898 cm^{-1} corresponds to the absorption of glycosidic C_1 – H deformation with ring vibration contribution, characteristic of β-glycosidic linkages between glucose in carbohydrates. Finally, the bands at 2852 and 2920 cm^{-1} are assigned to asymmetric and symmetric C-H stretching of CH, CH_2 and CH_3 groups. These bands can be seen in the complete spectrum present in B appendix and are also characteristic of carbohydrates.

The main characteristics bands of lignin are 1508, 1458 and 1420 cm^{-1} and are assigned to aromatic skeletal vibrations. The C=C stretching vibration is attributed to 1508 cm^{-1} band and the C-H deformations (CH and CH_2) in aromatic rings is attributed to 1458 cm^{-1} band. The symmetric bending vibrations of C-H bonds in methoxyl groups of syringil and guaiacyl units correspond to 1420 cm^{-1} band. The band at 1734 cm^{-1} is attributed to ester linkages in acetyl, feruloyl and p-coumaroyl groups between hemicellulose and lignin and the band at 1637 cm^{-1} is associated with the bending mode of absorbed water.

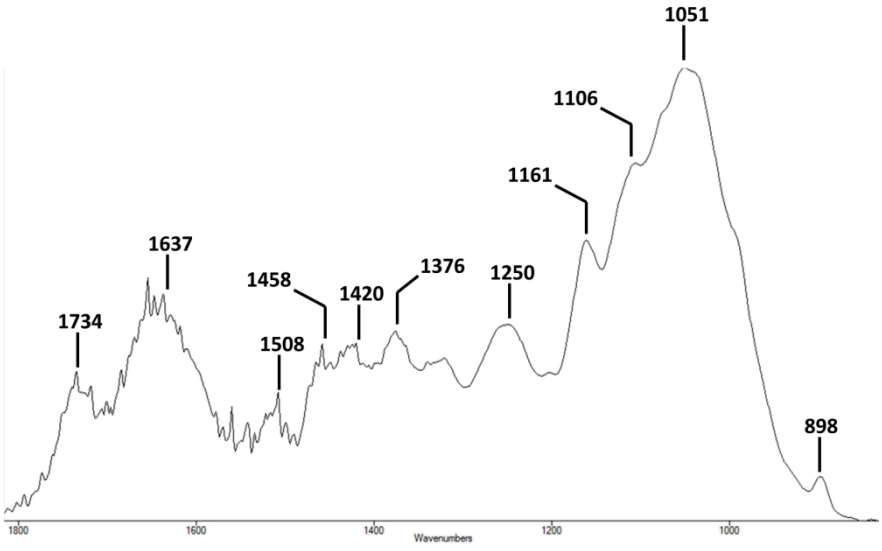

Figure 3.1. FTIR spectrum of original wheat straw.

3.5.2. FTIR characterization of fractions obtained by A, B and C methods

The A Method

This method allowed the fractionation of wheat straw into carbohydrate- and lignin-rich materials. Figure 3.2 illustrates the FTIR spectrum of carbohydrate-rich sample. In addition to the bands identified in the spectrum of wheat straw it is also possible to observe the appearance of new bands. The bands at 2918, 1637, 1376, 1161, 1066, 1046, 997 and 896 cm^{-1} are characteristic of carbohydrates. The new bands that appear at 1066 and 997 cm^{-1} are assigned to the ether linkage C-O-C skeletal vibration of both pentose and hexose unit contribution and to the arabinosyl side chains, respectively. Comparing this spectrum with wheat straw it is possible to observe a better resolution of the carbohydrates characteristic bands, present in the 1200 – 850 cm^{-1} region. Instead of the band at 1051 cm^{-1}, the bands at 1066 and 1046 cm^{-1} appears and the absorbance at 896 cm^{-1} increased. In the range of 1600 – 1300 cm^{-1}, it also can be observed a slight decrease in the absorbance bands at 1508, 1458 and 1420 cm^{-1}, revealing a lower contamination of the carbohydrate-rich material with lignin. It is important to note that, the band responsible for the hemicellulose-lignin interaction (1735 cm^{-1}) decrease considerably compared to the band present in wheat straw.

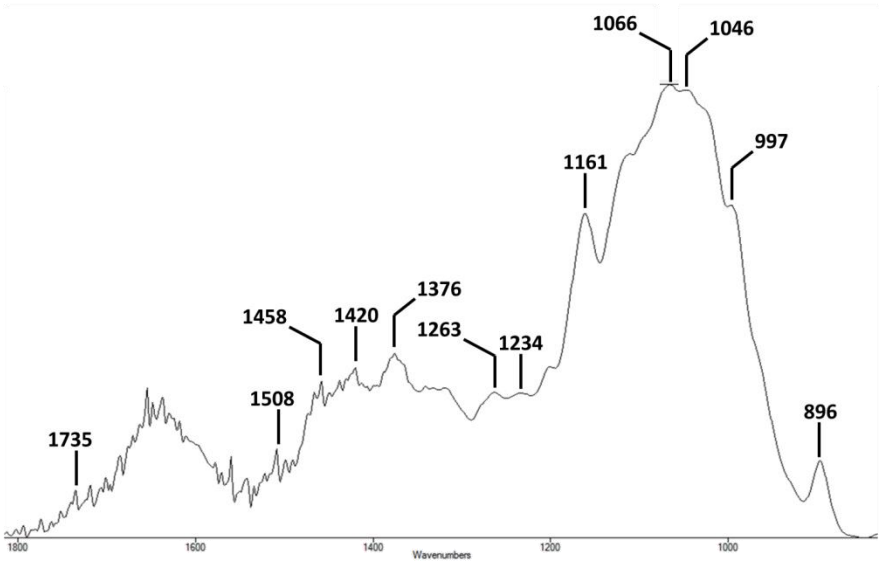

Figure 3.2. FTIR spectrum of regenerated material obtained by A method.

The spectrum acquired for lignin-rich material is illustrated in figure 3.3. In this figure it can be seen that the lignin characteristic bands are 3500-3100, 2928, 1596, 1508, 1458, 1420, 1364, 1340, 1262, 1228, 1157, 1125, 1083 and 1034 cm^{-1}. The band present in the range of 3500-3100 cm^{-1} is OH stretching vibrations. The symmetric and asymmetric ν_{CH} of methylene and methyl (methoxyl included) groups corresponds to the band at 2928 cm^{-1}. The band at 1596 cm^{-1} is assigned to aromatic skeletal vibrations and C=O stretching. Comparing with wheat straw spectrum the bands at 1508, 1458 and 1420 cm^{-1} have strong absorptions due to the higher purity of lignin-rich material. The symmetric deformation vibrations of C-H in metoxyl groups are attributed to the band 1364 cm^{-1}. The absorption at 1340 cm^{-1} is due to C-O and C-H deformation of syringy aromatic ring and phenolic hydroxyls. The bands at 1262 and 1228 cm^{-1} are attributed to C-O-C stretching vibration in methoxyl groups of guaiacyl and syringil ring. Deformation vibrations of the C-H bonds on benzene rings and C-O

asymmetric vibration in ester linkages are assigned to 1157 cm^{-1}. The absorption at 1125 cm^{-1} is typical of three types of vibrational absorptions, namely methoxyl group and C-H in-plane deformation in syringyl units as well as secondary alcohols present in lignin. The band at 1083 cm^{-1} corresponds to C-O deformations of secondary alcohols and aliphatic ethers linkages present in lignin and the band at 1034 cm^{-1} is assigned to aromatic C-H in-plane deformation for guaiacyl monomers.

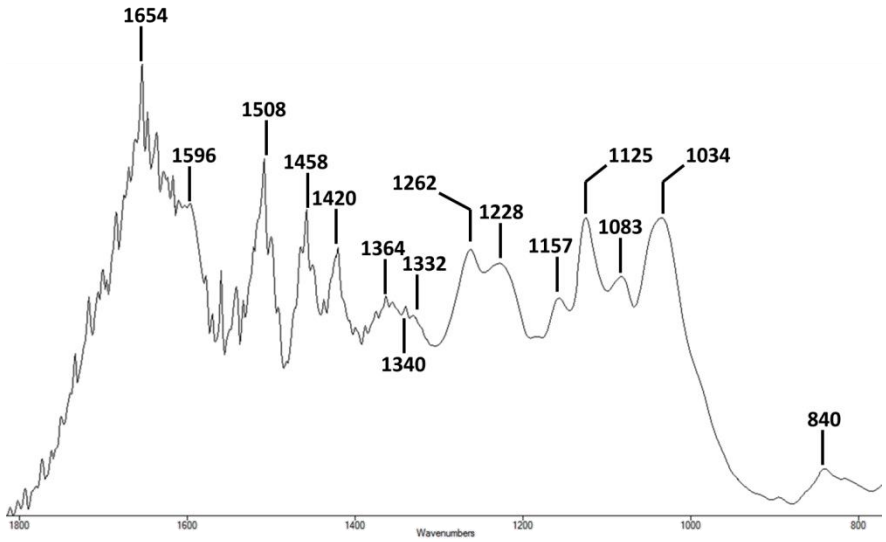

Figure 3.3. FTIR spectrum of lignin-rich sample obtained by A method.

The B method

This method allowed the fractionation of wheat straw in cellulose-, hemicellulose-, acetone soluble lignin- and alkaline lignin-rich (residual lignin) materials. Note that the spectra of the regenerated material and alkaline lignin-rich material obtained with B method are very similar to the spectra obtained using A method. The spectra of these compounds are present in B appendix. It can be verified some significant differences in the FTIR spectra range 1200 - 850 cm^{-1} that allows the characterization and differentiation of carbohydrate compounds. This region is dominated by ring vibrations overlapped with stretching vibrations of C–OH side groups and the C–O–C glycosidic bond vibration.

For cellulose-rich material spectra it was identified some bands that are described in literature, namely the bands at 1161, 1112, 1061 and 1035 cm^{-1}. All this bands are related to pyranosyl rings and are indicated in figure 3.4. The band 1112 cm^{-1} is assigned to C-OH skeletal vibration and 1035 cm^{-1} band is associated with C-O stretching vibration typical of cellulose. The existence of arabinose (arabinosyl side chains) is indicated by the band at 998 cm^{-1}, appearing as smooth shoulder. The band at 1319 cm^{-1} was produced by C-C and C-O skeletal vibrations. Note that the absorbance band at 1376 cm^{-1} is very pronounced compared with native wheat straw. The band 897 cm^{-1} is also present since it is very characteristic for carbohydrate. The absorbance bands at 1508, 1459 and 1421 cm^{-1} are less intense comparing to those of the spectrum of regenerated material obtained with A method, revealing the higher purity of this sample. Beside the three bands mentioned above, it could be also observed almost imperceptibles bands at 1264 and 1235 cm^{-1}.

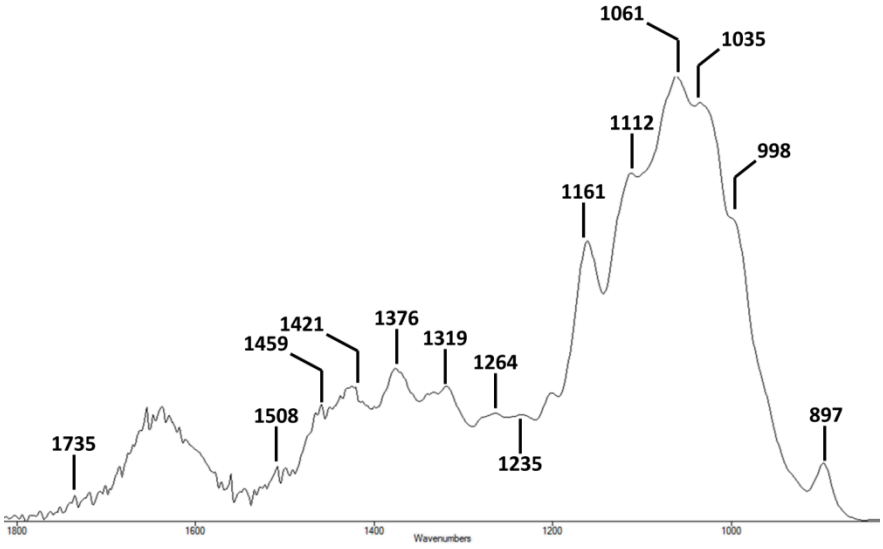

Figure 3.4. FTIR spectrum of cellulose-rich material obtained by B method

Comparing the spectrum of hemicellulose-rich material (figure 3.5) with the spectrum of cellulose-rich material (figure 3.4) it could be verified the existence of significant differences between them. The bands characteristic for hemicellulose-rich material are 1388, 1253, 1163, 1079, 1043 and 993 cm^{-1}. The strong band at 1043 cm^{-1} is associated to glycosidic linkages C-O-C contributions in xylans. The C-O stretching is assigned to the band 1388 cm^{-1} and the band at 1253 cm^{-1} reveals the presence of acetyl groups in hemicellulose structure. The presence of the arabinosyl side chains is characterized by two weak tails at 1163 and 993 cm^{-1} and the changes of intensity for these two bands suggested an arabinosyl substituent contribution. It is also possible to identify a less intense band at 1079 cm^{-1} associated to galactan side chains (figure 3.5).

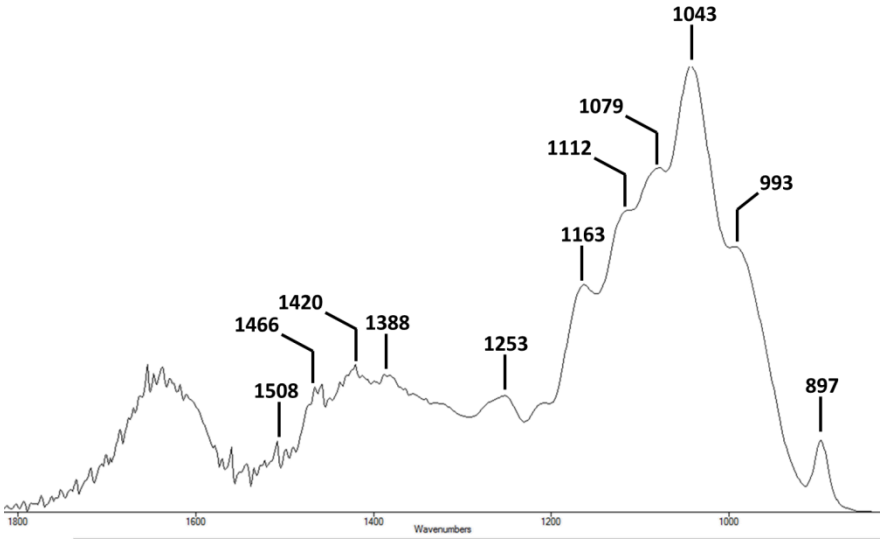

Figure 3.5. FTIR spectrum of hemicellulose-rich material obtained by B method.

The characteristics absorption bands of alkaline lignin-rich material are 2927, 1596, 1508, 1458, 1420, 1363, 1330, 1264, 1225, 1127, 1091 and 1032 cm^{-1}. The spectrum of this lignin is very similar to the spectrum of lignin obtained in the A method. The only difference is the absence of band 1157 cm^{-1}

in the spectrum of alkaline lignin-rich material of the B method and the presence of band 896 cm^{-1} in lignin-rich material obtained with the A method. In B appendix is illustrated the spectrum of this lignin.

For acetone soluble lignin-rich material, the characteristics bands are 2919, 1508, 1458, 1420, 1364, 1340, 1262, 1125 and 1080 cm^{-1}. Figure 3.6 illustrates the FTIR spectrum of this lignin. As for the alkaline lignin-rich material the bands attribution is similar to that of the A method. Acetone soluble lignin-rich material differs from alkaline lignin-rich material since the band at 1225 cm^{-1} is not present and the bands at 1125 and 1262 cm^{-1} are relatively smaller than the bands of alkaline lignin-rich material. In this spectrum is also possible to observe the presence of carbohydrates since the absorption bands at 1125, 1080, 1046 and 898 cm^{-1} are present.

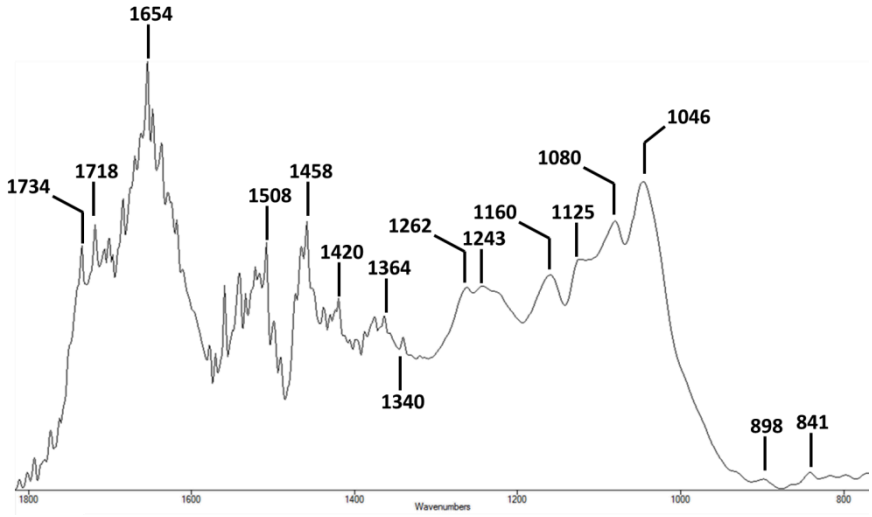

Figure 3.6. FTIR spectrum of acetone soluble lignin-rich material obtained by B method.

The C method

This method allowed the fractionation of wheat straw in cellulose-, hemicellulose-, residual hemicellulose-, alkaline lignin- and residual alkaline lignin-rich materials.

The spectrum of cellulose-rich material is presented in B appendix and is similar to the spectrum of cellulose-rich material fractionated by the B method, revealing however small differences.

Hemicellulose- and residual hemicellulose-rich materials spectra are identical to the spectrum of hemicellulose-rich material from the B method. The spectra of these samples are shown in B appendix.

Figure 3.7 illustrates the spectrum of alkaline lignin-rich material and figure 3.8 shows the spectrum of residual alkaline lignin-rich material obtained by the C method. These lignins are very similar. The main differences between them are the absence of the bands 1598, 1364 and 1091 cm^{-1} in residual lignin-rich material and, in general an increased intensity of all bands in residual lignin-rich material except in the case of band 1654 cm^{-1} that shows an decreased intensity relatively to lignin-rich material. Note also that the band 1702 cm^{-1} assigned to unconjugated C=O stretching (ketones and carbonyl groups) is only present in the spectrum of residual lignin-rich material.

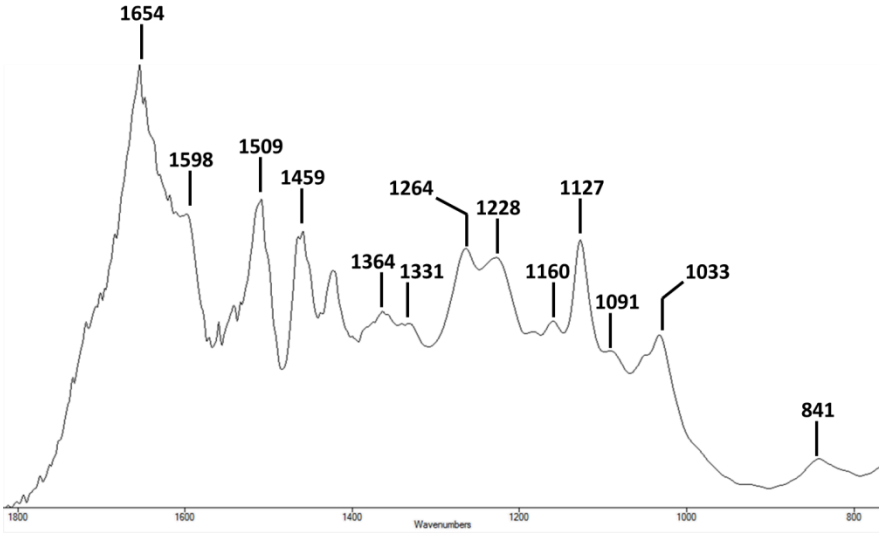

Figure 3.7. FTIR spectrum of alkaline lignin-rich material obtained by C method.

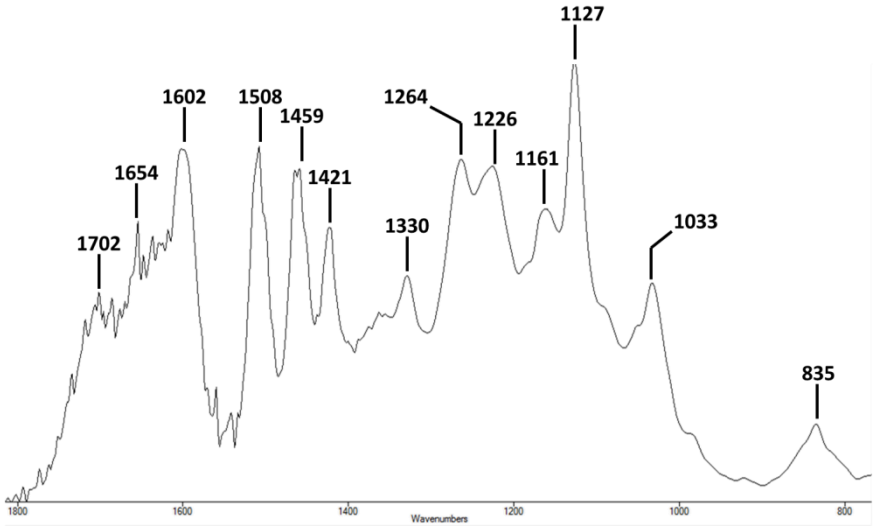

Figure 3.8. FTIR spectrum of residual alkaline lignin-rich material obtained by C method.

3.5.3. FTIR characterization of fractions obtained after the IL pre-treatment of different biomasses

As aforementioned in order to verify the efficiency of the optimised pre-treatment methodology, various types of biomasses namely sugarcane bagasse, rice straw and triticale were tested. The spectra of the samples obtained for each biomass were compared with the spectra of wheat straw.

The spectra of the regenerated material from the pre-treatment of rice straw and triticale are very similar. Figure 3.9 presents the spectrum of the regenerated material from rice straw and, in B appendix the spectrum of the regenerated material from triticale is present. Comparing this spectrum with the regenerated material obtained from wheat straw pre-treatment (figure 3.2) it is possible to observe significant differences. The shape of the bands and the presence of bands such as 1060 and 1036 cm^{-1} suggest that this sample is rich in cellulose. However the presence of a less intense band at 1253 cm^{-1} discloses also the presence of hemicellulose. The spectrum of the regenerated material from sugarcane bagasse (figure 3.10) has significant differences relatively to the spectra of other

biomasses and relatively to the spectrum of regenerated material from wheat straw. This spectrum reveals that the sample is rich in hemicellulose due to the presence of bands at 1044 and 994 cm^{-1}, for example. But this sample also contains cellulose due to the presence of a small band at approximately 1376 cm^{-1}.

The presence of bands at 1508, 1458 and 1420 cm^{-1} expose that sugarcane bagasse, rice straw and triticale are slightly contaminated with lignin.

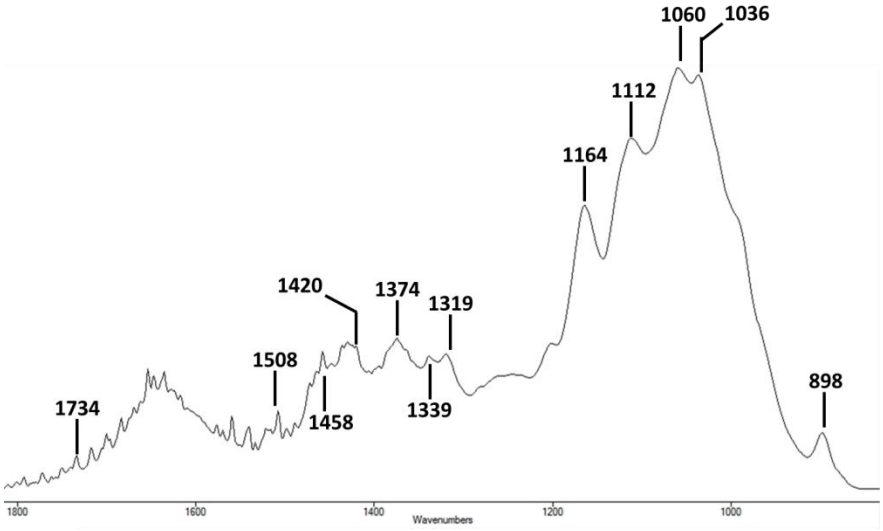

Figure 3.9. FTIR spectrum of regenerated material from rice straw pre-treatment.

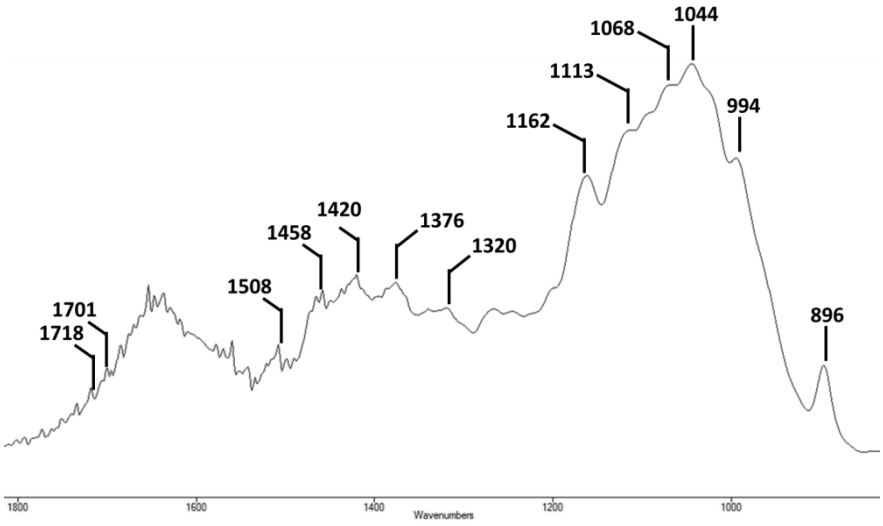

Figure 3.10. FTIR spectrum of regenerated material from sugarcane bagasse pre-treatment.

The spectra of cellulose-rich material from sugarcane bagasse, rice straw and triticale pre-treatments are shown in B appendix. The spectrum of cellulose-rich material from rice straw and triticale contains pronounced bands characteristic for cellulose and they are very similar to the spectrum of cellulose-rich material from wheat straw pre-treatment. The only significant difference is almost complete disappearance of the band at 998 cm^{-1}. In the case of cellulose from sugarcane bagasse, the bands are not as well defined as in the case of previous samples. The bands at 1060 and 1035 cm^{-1} detected in the cellulose-rich material of rice straw and triticale are slightly deviated in

the cellulose-rich material sample of sugarcane bagasse. In this sample the bands are 1064 and 1023 cm^{-1}. Note that all these samples are slightly contaminated with lignin due to the presence of a band at 1508 cm^{-1}. Comparing the spectrum of cellulose-rich material with the respective spectrum of regenerated material it can be seen that there is a slight increase in the purity but it is not as pronounced as in the case of wheat straw samples.

The spectra of hemicellulose- and residual hemicellulose-rich samples are presented in B appendix. Hemicellulose- and residual hemicellulose-rich materials from sugarcane bagasse are identical; the difference lies in the definition of the bands. Residual hemicellulose bands are better resolved. However, as in the filtrate remained some white flocks another filtration was made and the FTIR spectrum of the recovered solid was traced. In B appendix the spectrum of this compound is present. The absorption bands present in this spectrum indicate that this compound is rich in lignin instead of hemicellulose. FTIR spectra of hemicellulose- and residual hemicellulose-rich material from rice straw show some differences. The residual hemicellulose-rich sample spectrum is very similar to hemicellulose-rich samples from sugarcane bagasse. However, the presence of the high absorption bands at 1325 and 782 cm^{-1} in residual hemicellulose-rich sample reveal that this sample is contaminated with silica.[96] In the case of triticale three FTIR spectra were traced because, as in the case of sugarcane, in the filtrate remained some white flocks. All the three spectra are very similar. Hemicellulose rich-samples have the bands more defined but, in the case of residual hemicellulose-rich sample recovered after filtration, the bands between 1653-1300 cm^{-1} have a higher absorption relatively to the others hemicellulose-rich samples. Another difference between these samples is the absence of the 986 cm^{-1} band in residual hemicellulose-rich sample, recovered from the liquid fraction. The FTIR spectra of hemicellulose- and residual hemicellulose-rich materials from the different pre-treatments are similar to the hemicellulose samples from wheat straw. The differences that can be noticed are the disappearance of some bands. The three tested biomasses do not present the bands at 1112 and 993 cm^{-1}. Hemicellulose-rich material from rice straw and triticale do not present the band at 1163 cm^{-1} and rice straw does not present the band at 1253 cm^{-1}. Comparing all the hemicellulose spectra from the different biomasses, residual hemicellulose-rich material from triticale is the one with more defined bands.

The analysis of FTIR spectra of lignin-rich samples is a little more complex than the analysis of carbohydrate-rich samples due to the higher intricacy of this lignocellulosic component. In general, all the samples have the same bands but the intensity differs with the sample.

Lignin- and residual lignin-rich materials from sugarcane bagasse are very similar. Figure 3.11 shows the spectrum of residual lignin-rich material from sugarcane bagasse and the spectrum of lignin-rich material is present in B appendix. Comparing this spectrum to this of lignin-rich samples from wheat straw, the spectrum of residual lignin-rich material is very similar to the lignin samples of wheat straw but in the case of lignin-rich material some differences can be noticed. The most significant differences are much higher intensity of the band at 1654 cm^{-1} relatively to the spectra of lignin-rich samples from sugarcane bagasse and much higher intensity of the band at 1127 cm^{-1} comparing to lignin-rich material from wheat straw. The intensity of the rest of the bands of lignin-rich sample from wheat straw is lower than lignin-rich samples from sugarcane bagasse.

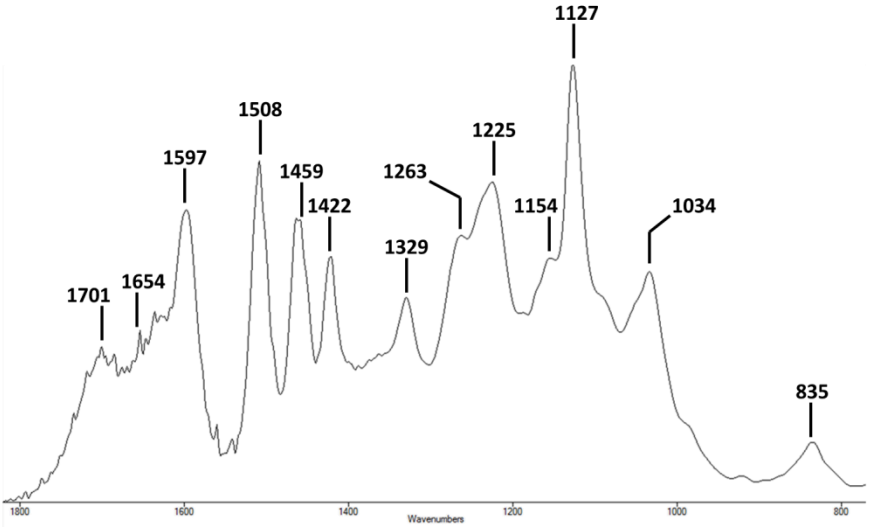

Figure 3.11. FTIR spectrum of residual lignin-rich material from sugarcane bagasse pre-treatment.

Lignin spectra from rice straw are very different between them. Figure 3.12 and 3.13 illustrates the spectrum of lignin-rich material and residual lignin-rich material from rice straw, respectively. Residual lignin-rich sample spectrum is totally different from lignin spectra from wheat straw and sugarcane bagasse. Although it is visible that this sample contains some lignin (band at 1508 cm^{-1}), the presence of a strong absorption band at 1094 cm^{-1} reveals that this sample was contaminated. Lignin-rich sample is more similar to the spectrum of lignin-rich sample from wheat straw, namely in the region between 1800-1250 cm^{-1}.

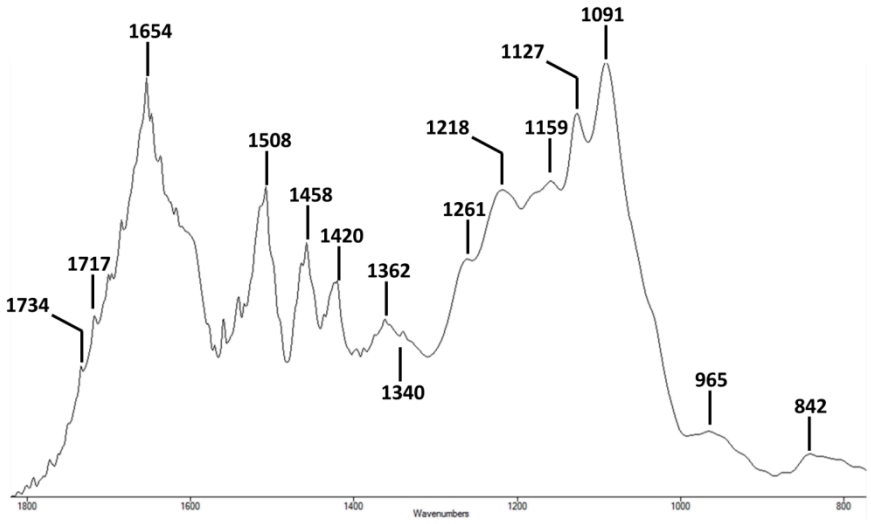

Figure 3.12. FTIR spectrum of lignin-rich material from rice straw pre-treatment.

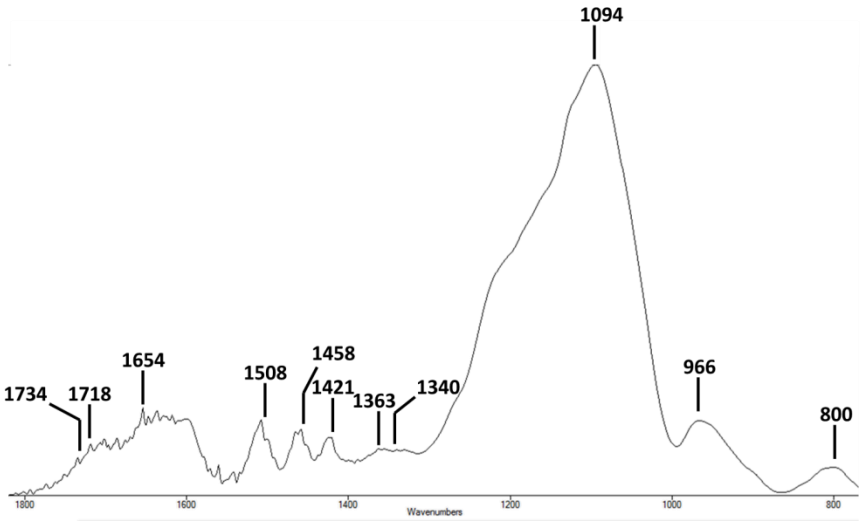

Figure 3.13. FTIR spectrum of residual lignin-rich material from rice straw pre-treatment.

Lignin spectra of residual lignin-rich and lignin-rich samples from triticale are practically identical. The spectrum of lignin-rich sample is present in figure 3.14 and the spectrum of residual lignin-rich sample is shown in B appendix. However, it is important to note that lignin-rich material obtained from the other duplicate pre-treatment of triticale has a different spectrum comparing with the above samples. The main differences can be seen in the region of 1300-900 cm^{-1} and the spectrum is present in B appendix. Comparing with the lignin-rich samples from the biomasses mentioned above, these lignin samples reveals more similarities with residual lignin-rich material from wheat straw.

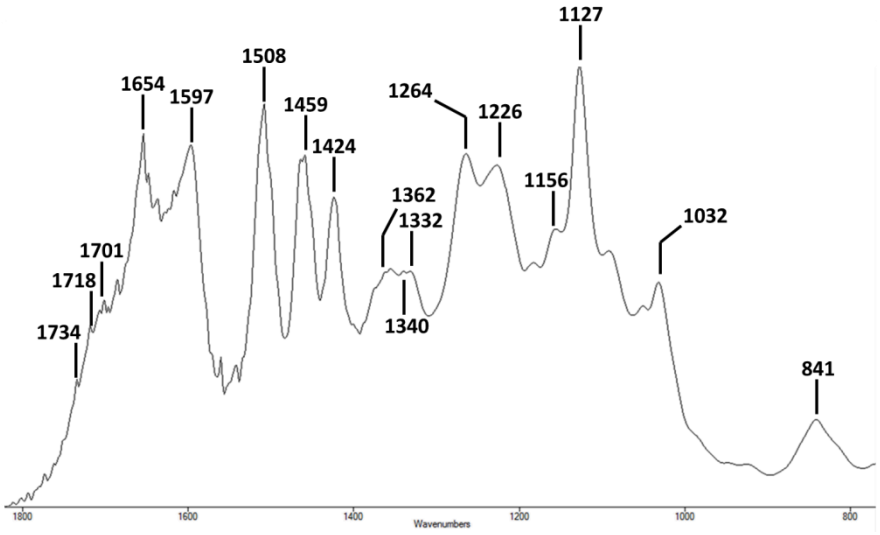

Figure 3.14. FTIR spectrum of lignin-rich material from triticale pre-treatment.

Note that in carbohydrate-rich samples it is important to analyse the presence of the band at 1734 cm^{-1} since when this band is present represents that some hemicellulose still remained bounded to lignin. As in all the samples rich in carbohydrates the band at 1734 cm^{-1} is too small, can be concluded that these samples do not have hemicellulose bounded to lignin and the separation was efficient. The small band at 1734 cm^{-1} can be attributed to noise of FTIR spectrometer.

The FTIR spectra of original and acid hydrolysed biomasses used are shown in B appendix. Comparing the four original biomasses tested it could be seen that they present some similarities namely they have identical absorption bands but their intensity may vary with the composition of each biomass. Another important comparison is between the original biomass and the samples obtained after IL pre-treatment. The differences are very visible and reveal that the separation of lignocellulosic biomass in their main constituents was achieved. Note also that original biomasses have a strong absorption band at 1734 cm^{-1}, revealing that in carbohydrate-rich samples practically all the linkages between hemicellulose and lignin were broken.

3.5.4. FTIR quantification of fractions obtained by A, B and C methods

The quantification of the composition of each sample obtained was made by measuring the total area of the absorbance bands characteristic of carbohydrates- and lignin-rich materials. In the case of carbohydrates the quantification is made in the band 898 cm^{-1} and for lignin is made in the range 1503-1537 cm^{-1}. In order to convert absorbance in concentration a calibration curve with acid hydrolysed wheat straw (130 ºC, 150 minutes and 1.50 % of H_2SO_4) was performed. Note that the composition of each sample was determined as carbohydrate (cellulose and hemicellulose together) and lignin content. Thus, in the case of cellulose- and hemicellulose-rich samples is not possible to determine the contamination of cellulose with hemicellulose and vice versa since the absorbance band is the same (898 cm^{-1}). The composition of other compounds that could be present was calculated by difference. FTIR quantification was done for a selected experiment of each method. The experiments selected were: A2, mean between B2 and B3 and C3. Table 3.11-3.13 shows the quantification results for the samples obtained by each method.

Note that the composition of dried acid hydrolysed wheat straw was determined by HPLC.

The A method

Table 3.11 shows the quantification results of the samples obtained using A method. After the pre-treatment of wheat straw it could be observed an enrichment of about 20 % in the carbohydrate content of regenerated material sample. However this sample is a little contaminated by lignin (9 % wt content) and by other compounds (12 % wt content). In the case of lignin-rich material the purity percentage is approximately 70 % wt. This sample is contaminated by 6 % wt of carbohydrates and by 24 % wt of other compounds. The percentage of other compounds is slightly higher comparatively to regenerated material sample and is similar to dried wheat straw.

Table 3.11. FTIR quantification of wheat straw pre-treated with A method.

	Total	Carbohydrates		Lignin		Others	
Sample	**mg**	**mg**	**wt%**	**mg**	**wt%**	**mg**	**wt%**
Dried WS	230.5	143.8	62	41.5	18	45.4	20
RM	159.7	126.4	79	13.9	9	19.3	12
Lignin[a]	18.6	1.1	6	13.1	70	4.42	24

WS – wheat straw; RM – regenerated material

[a] Lignin-rich material

The B method

The quantification results of wheat straw pre-treated using the B method is present in table 3.12. Contrary to the A method, the percentages of regenerated material sample and dried wheat straw are very similar. This method permits to separate regenerated material in cellulose-, hemicellulose- and residual lignin-rich materials. Cellulose-rich material has 82 % wt of carbohydrate content, showing a contamination with 10 % wt of lignin and 7 % wt of other compounds. The purity of hemicellulose-rich material is similar to the purity of cellulose-rich sample (80 % wt). The contamination with lignin is also similar (9 % wt) to cellulose-rich sample but the contamination with other compounds is slightly higher (11 % wt). The residual lignin contained in the regenerated material evidence a high purity (98 % wt), and it is free of carbohydrates and has a low contamination with other compounds (2 % wt). Unfortunately the quantity recovered of this lignin was very low. Although in the case of acetone soluble lignin the quantity recovered was higher, the purity percentage is very low (57 % wt).

Table 3.12. FTIR quantification of wheat straw pre-treated with B method.

			Total	Carbohydrates		Lignin		Others	
Sample			**mg**	**mg**	**wt %**	**mg**	**wt %**	**mg**	**wt %**
Dried WS			92.4	57.7	62	16.6	18	18.2	20
Solid fraction	**RM**		66.7	42.7	64	9.3	14	14.7	22
		Cellulose[a]	41.2	34.0	82	4.2	10	3.1	7
		Hemicellulose[b]	19.8	16.0	80	1.8	9	2.1	11
		Lignin[c]	3.2	0.0	0	3.1	98	0.1	2
Liquid fraction	**Lignin[d]**		10.1	0.8	8	5.8	57	3.5	35

WS – wheat straw; RM – regenerated material

[a] Cellulose-rich material; [b] Hemicellulose-rich material; [c] Residual lignin-rich material; [d] Lignin-rich material

The C method

The quantification of each sample obtained by C method is present in table 3.13. Comparing with A method is possible to see that the composition of regenerated material is, as expected, very similar since the fractionation process is the same. Note that in relation with B method, this method has an additional step to remove the residual hemicellulose that still connected to lignin. The cellulose- and hemicellulose-rich samples have a percentage of carbohydrates slightly higher than the samples obtained with B method, 85 and 86 % wt respectively. The contamination of these samples with lignin is about 4 % wt lower than those obtained with B method and the contamination with other compounds is similar. Unfortunately, was not possible to make a comparison between the residual lignin-rich samples because the sample had a different consistency that difficult its recuperation and therefore the quantity recovered was very low to perform FTIR quantification. The lignin-rich sample is free of carbohydrates and has a purity percentage much higher (87 % wt) than the lignin obtained using B method (57 % wt). Although, this sample is also contaminated with other compounds, the percentage is much lower (13 % wt) than the lignin of B method (35 % wt). Finally, the residual hemicellulose-rich

sample has a purity (71 **%** wt**)** lower than the hemicellulose present in the solid fraction of B and C methods (80 % and 85 % wt, respectively) and is less contaminated with lignin (3 % wt), showing a higher contamination with other compounds (26 % wt).

Table 3.13. FTIR quantification of wheat straw pre-treated using C method.

			Total	Carbohydrates		Lignin		Others	
Sample			**mg**	**mg**	**wt %**	**mg**	**wt %**	**mg**	**wt %**
Dried WS			230.5	142.9	62	41.5	18	46.1	20
Solid fraction	**RM**		131.1	106.2	81	7.9	6	17.0	13
		Cellulose[a]	95.6	82.2	86	5.7	6	7.6	8
		Hemicellulose[b]	20.2	17.2	85	1.0	5	2.0	10
		Lignin[c]	3.2	NQ	NQ	NQ	NQ	NQ	NQ
Liquid fraction	**Lignin**[d]		15.1	0.0	0	13.1	87	2.0	13
	Hemicellulose[e]		38.3	27.2	71	1.1	3	10.0	26

WS – wheat straw; RM – regenerated material

[a] Cellulose-rich material; [b] Hemicellulose-rich material; [c] Residual lignin-rich material; [d] Lignin-rich material; [e] Residual hemicellulose-rich material

3.5.5. FTIR quantification of fractions obtained after the pre-treatment of different biomasses

All the samples obtained after the pre-treatment of the different biomasses with the optimised method were also quantified using FTIR spectroscopy. Table 3.14-3.16 illustrates the results obtained for sugarcane bagasse, rice straw and triticale pre-treatment, respectively.

After the pre-treatment of sugarcane bagasse, the regenerated material obtained has much lower lignin content than the original biomass (6 % wt) but the content of other compounds increased for 25 % wt and the content of carbohydrates is the same. The fractionation process allowed to obtain cellulose- and hemicellulose-rich samples with high purity (approximately 90 % wt). Although lignin-rich samples do not present carbohydrates, the purity percentage is low (about 66 % wt) because the contamination with others compounds is relatively high (approximately 34 % wt).

Table 3.14. FTIR quantification of fractionated samples from sugarcane bagasse pre-treated using the C method.

			Total	Carbohydrates		Lignin		Others	
Sample			**mg**	**mg**	**wt %**	**mg**	**wt %**	**mg**	**wt %**
Dried SB			230.2	158.8	69	46.0	20	25.3	11
Solid fraction	**RM**		161.7	111.6	69 ± 4	9.7	6 ± 0.5	40.4	25
		Cellulose[a]	121.1	109.0	90 ± 9	8.5	7 ± 0.4	3.6	3
		Hemicellulose[b]	30.1	28.0	93 ± 9	1.8	6 ± 0.5	0.3	1
		Lignin[c]	5.6	-	-	3.7	65 ± 5	2.0	35
Liquid fraction	**Lignin[d]**		11.2	-	-	7.4	66 ± 5	3.8	34
	Hemicellulose[e]		19.9	17.9	90 ± 8	1.2	6 ± 0.5	0.8	4

SB – sugarcane bagasse; RM – regenerated material

[a] Cellulose-rich material; [b] Hemicellulose-rich material; [c] Residual lignin-rich material; [d] Lignin-rich material; [e] Residual hemicellulose-rich material

FTIR quantification of fractionated samples from rice straw pre-treatment revealed that FTIR method although very reliable presents some errors. An example of this fact is that purity of some samples were higher than 100 % however, lower than 110 % therefore it can be acceptable due to errors associated to the used method. The purity of all carbohydrate-rich samples is relatively high. Regenerated material-rich sample has a carbohydrate content higher than 91 % wt and a lignin content of about 10 % wt. Comparing with the original biomass an enrichment in carbohydrates and a decrease in lignin content was obtained. The fractionation process permits to obtain a cellulose-rich material with more than 95 % wt of carbohydrates and approximately 6 % wt of lignin. Hemicellulose-rich samples have more than 83 % wt of carbohydrates and between 3 and 7 % wt of lignin. Note that from the two hemicellulose-rich samples recovered, residual hemicellulose-rich material is the one with higher purity. Unfortunately, FTIR quantification of lignin-rich samples was not possible, because the samples were contaminated.

Table 3.15. FTIR quantification of fractionated samples from rice straw pre-treated using C method.

			Total	Carbohydrates		Lignin		Others	
Sample			**mg**	**mg**	**wt %**	**mg**	**wt %**	**mg**	**wt %**
Dried RS			238.4	154.9	65	33.4	14	47.7	21
Solid fraction	**RM**		160.3	161.9	101 ± 10	16.0	10 ± 0.2	-	-
		Cellulose[a]	121.8	129.1	106 ± 11	7.3	6 ± 0.6	-	-
		Hemicellulose[b]	19.7	17.9	91 ± 8	1.2	6 ± 0.6	0.6	3 ± 9
		Lignin[c]	8.1	NQ	NQ	NQ	NQ	NQ	NQ
Liquid fraction	**Lignin[d]**		16.8	NQ	NQ	NQ	NQ	NQ	NQ
	Hemicellulose[e]		15.8	15.8	100 ± 10	0.5	3 ± 1	-	-

RS – rice straw; RM – regenerated material

[a] Cellulose-rich material; [b] Hemicellulose-rich material; [c] Residual lignin-rich material; [d] Lignin-rich material; [e] Residual hemicellulose-rich material

In the case of triticale pre-treatment, the regenerated material obtained has a relatively high purity. The regenerated material was enriched in approximately 16 % wt of carbohydrates and the lignin content decreased in about 13 % wt. In general, cellulose- and hemicellulose-rich fractions have a high content in carbohydrates (about 90 % wt) and the lignin content in general decreased comparatively to original biomass and regenerated material sample. The only exception is the hemicellulose-rich sample derived from regenerated material with approximately 10 % wt of lignin. Note that residual hemicellulose-rich samples have the carbohydrate content lower than regenerated material, cellulose- and hemicellulose-rich samples but residual hemicellulose separated from the liquid fraction has the lowest lignin content. The lignin-rich samples do not present carbohydrates in its constitution but the contamination with others compounds is relatively high (29 % wt) and have a lignin content of about 71 % wt.

Table 3.16. FTIR quantification of fractionated samples from triticale pre-treated using C method.

			Total	Carbohydrates		Lignin		Others	
Sample			**mg**	**mg**	**wt %**	**mg**	**wt %**	**mg**	**wt %**
Dried Triticale			234.8	159.6	68	49.3	21	25.8	11
Solid fraction	**RM**		153.6	129.0	84 ± 7	12.3	8 ± 2	12.3	8 ± 9
		Cellulose[a]	114.7	103.3	90 ± 8	6.9	6 ± 2	4.6	4 ± 10
		Hemicellulose[b]	17.8	16.1	90 ± 8	1.8	10 ± 1	-	-
		Hemicellulose[c]	12.2	9.4	77 ± 5	0.9	7 ± 2	2.0	16 ± 7
		Lignin[d]	3.9	-	-	2.9	75 ± 12	1.0	25 ± 12
Liquid fraction	**Lignin[e]**		25.3	-	-	18.0	71 ± 12	7.3	29 ± 12
	Hemicellulose[f]		27.0	18.9	70 ± 4	1.1	4 ± 3	7.0	26 ± 7

RM – regenerated material

[a] Cellulose-rich material; [b] Hemicellulose-rich material; [c] Residual hemicellulose-rich material; [d] Residual lignin-rich material; [e] Lignin-rich material; [f] Residual hemicellulose-rich material

3.5.6. FTIR evaluation of cellulose crystallinity of carbohydrate-rich fractions

Cellulose crystallinity of fractions obtained by the A, B and C methods

The crystallinity indexes TCI and LOI of fractions obtained with A, B and C methods are present in table 3.17. These indexes reveal that a crystallinity change in cellulose structure after pre-treatment with [emim][OAc] occurs. The decrease of LOI value of samples obtained after IL pre-treatment relatively to standard cellulose, original and acid hydrolysed wheat straw is more evident than the decrease of TCI value. The sample with the highest LOI and TCI values is original wheat straw and cellulose-rich material from C method has the lowest LOI and TCI values.

Table 3.17. Crystallinity indexes of original and acid hydrolysed wheat straw, standard cellulose, regenerated material from A, B and C methods and cellulose-rich samples from B and C methods.

	Crystallinity index	
Sample	**LOI**	**TCI**
Original wheat straw	1.74	1.13
STD cellulose	1.69	1.12
AH wheat straw	1.68	1.07
RM A	1.38	1.02
RM B	1.40	1.07
RM C	1.36	1.02
Cellulose B	1.41	1.05
Cellulose C	1.34	1.02

LOI - lateral order index; TCI - total crystallinity index; STD – standard;
AH - Acid hydrolysed; RM - regenerated material

Cellulose crystallinity of fractions obtained from the pre-treatment with different biomasses

Table 3.18 illustrates the crystallinity indexes TCI and LOI of the fractions obtained from sugarcane bagasse, rice straw and triticale IL pre-treatment. As expected, the samples with the highest LOI and TCI values are the original biomasses. Regenerated material and cellulose-rich samples have similar LOI and TCI values but for regenerated material samples these values are relatively lower.

Table 3.18. Crystallinity indexes of original and acid hydrolysed biomasses (sugarcane bagasse, rice straw and triticale), regenerated material and cellulose-rich samples obtained from different biomasses IL pre-treatments.

	Crystallinity index	
Sample	**LOI**	**TCI**
Sugarcane	1.57	1.18
Rice straw	1.76	1.15
Triticale	1.74	1.14
Sugarcane AH	1.58	1.05
Rice straw AH	1.60	1.10
Triticale AH	1.81	1.01
RM CA	1.27	1.04
RM CB	1.45	1.10
RM CC	1.55	1.08
Cellulose CA	1.31	1.07
Cellulose CB	1.46	1.11
Cellulose CC	1.59	1.10

LOI - lateral order index; TCI - total crystallinity index;
AH - Acid hydrolysed; RM - regenerated material; CA – sugarcane bagasse; CB – rice straw; CC - triticale

3.6. Study of the reuse of the IL: [emim][OAc]

In order to verify the potential of IL reuse, seven consecutive experiments were performed. In these experiments, wheat straw was pre-treated with [emim][OAc] using the A method. Note that as some quantity of IL is lost in the experimental process, the initial biomass weighted was determined so that the solid/liquid ratio was 5 % (w/w) for all the experiments. Table 3.19 illustrates the results obtained in this study.

The regeneration yields are very similar, approximately 60 % (w/w) for all the pre-treatments. This study shows that the percentage of the IL recovered is always above 80 % (w/w) and the maximum recovery percentage that was achieved was approximately 95 % (w/w).

Table 3.19. Results obtained for the study of the reuse of [emim][OAc] using A method.

			Solid fraction	Liquid fraction		
Exp.	WS (mg)	**Dried WS (mg)**	**RY (% w/w)**	**Lignin[a] (mg)**	**ML (% w/w)**	**IL recovered (% w/w)**
1	250.1	230.1	58.1	6.0	39.3	85.2
2	203.8	187.5	57.5	13.0	35.6	79.5
3	159.7	146.9	62.9	6.0	33.0	94.9
4	143.7	132.2	61.2	8.9	32.1	92.8
5	127.6	117.4	60.3	8.2	32.7	90.8
6	103.0	94.8	64.3	6.0	29.4	86.9
7	77.3	71.1	63.0	4.2	31.1	83.7

WS – wheat straw; RM – regenerated material; RY – regeneration yield; ML – material lost; IL – ionic liquid

[a] Lignin-rich material

3.7. Enzymatic Hydrolysis

The enzymatic digestibility of each sample was determined as glucose yield (% $w/w_{biomass}$) and total sugar yield (% w/w). Note that the calculation of glucose yield corresponds to the ratio of the mass of cellulose digested and the mass of biomass weighed. On the other hand, the total sugar yield corresponds to the ratio of the sum of total sugars (glucose and xylans) and the total sugars present in the weighed biomass. The determination of total sugars present in each sample was made through FTIR quantification.

The A, B and C methods

To evaluate the enzymatic digestibility of the samples obtained by the three pre-treatment methods, the enzymatic hydrolysis of regenerated material from the A method and cellulose from the B and C methods was made. Enzymatic hydrolysis of original wheat straw, acid hydrolysed wheat straw and standard cellulose was also performed to comparison. In table 3.20 are displayed the enzymatic hydrolysis results.

The sample with the highest glucose yield was pure cellulose (97.2 % ($w/w_{biomass}$)) and the sample with the lowest glucose yield was original wheat straw (19.7 % ($w/w_{biomass}$)). Acid hydrolysed wheat straw has a glucose yield (37.7 % ($w/w_{biomass}$)) higher than original wheat straw and lower than

the regenerated material from A method (49.1 % (w/$w_{biomass}$)) and cellulose from the B and C methods (70.2 % (w/$w_{biomass}$) and 76.0 % (w/$w_{biomass}$), respectively).

Cellulose samples obtained with the B and C methods as well as pure cellulose sample had a complete enzymatic hydrolysis of carbohydrates (set of cellulose and hemicellulose). Only 41.9 % (w/w) of carbohydrates were hydrolysed in original wheat straw sample and 64.0 % (w/w) was achieved for acid hydrolysed wheat straw. For the A method regenerated material the total sugar yield obtained was 89.9 % (w/w).

Table 3.20. Enzymatic hydrolysis results for original wheat straw, acid hydrolysed wheat straw, standard cellulose, regenerated material obtained with A method and cellulose obtained from B and C methods.

Sample	Biomass weighed (mg)	Total sugars (mg)	[Glucose] (mg/mL)	Cellulose digested (mg)	[Xylose] (mg/mL)	Xylans digested (mg)	Glucose yield (% w/$w_{biomass}$)	Total sugar yield (% w/w)
WS	150.0	93.6	3.3	29.5	1.1	9.7	19.7	41.9
WS "AH"	149.9	93.9	6.3	56.6	0.4	3.5	37.7	64.0
RM A	29.7	23.5	1.6	14.5	0.8	6.6	48.8	89.9
Cellulose B	29.6	24.3	2.3	20.8	0.4	3.8	70.2	101.4
Cellulose C	30.3	26.1	2.6	23.0	0.4	3.5	76.0	101.7
Cellulose STD	30.1	30.1	3.2	29.2	0.2	1.5	97.2	102.1

Pre-treatment of different biomasses

The results for the enzymatic hydrolysis of samples obtained after pre-treatment of the different biomasses studied are presented in table 3.21. Enzymatic hydrolysis of original and acid hydrolysed biomasses was also performed for comparison and the results as well displayed in table 3.21. As expected the samples with the lowest glucose and total sugar yield were native biomasses and those with the highest were cellulose-rich samples. Between original and acid hydrolysed biomasses, sugarcane bagasse is the one with the lowest glucose and total sugar yield (glucose yield of 4.6 % (w/$w_{biomass}$) and 19.4 % (w/$w_{biomass}$) and total sugar yield of 8.5 % (w/w) and 32.1 % (w/w) for original and acid hydrolysed biomass, respectively) and triticale present the highest values (glucose yield of 11.9 % (w/$w_{biomass}$) and 37.2 % (w/$w_{biomass}$) and total sugar yield of 23.3 % (w/w) and 64.2 % (w/w) for original and acid hydrolysed biomass, respectively). However, after IL pre-treatment cellulose-rich material from rice straw has the lowest glucose and total sugar yield (68.7 % (w/$w_{biomass}$) and 75.8 % (w/w), respectively), cellulose-rich material from sugarcane bagasse has the highest glucose yield (79.9 % (w/$w_{biomass}$)) and cellulose-rich material from triticale has the highest total sugar yield (103.2 % (w/w)).

Table 3.21. Enzymatic hydrolysis results for original and acid hydrolysed sugarcane bagasse, rice straw, triticale and cellulose-rich samples obtained after the IL pre-treatment of the different biomasses aforementioned.

Sample	Biomass weighed (mg)	Total sugars (mg)	[Glucose] (mg/mL)	Cellulose digested (mg)	[Xylose] (mg/mL)	Xylans digested (mg)	Glucose yield (% $w/w_{biomass}$)	Total sugar yield (% w/w)
SB	149.9	95.3	0.8	6.9	0.1	1.2	4.6	8.5
RS	149.7	92.8	1.7	15.6	0.0	0.0	10.4	16.8
Triticale	150.2	95.7	2.0	17.9	0.5	4.4	11.9	23.3
SB "AH"	150.1	94.2	3.2	29.1	0.1	1.1	19.4	32.1
RS "AH"	150.3	86.3	5.9	53.3	0.0	0.0	35.5	61.8
Triticale "AH"	150.0	94.9	6.2	55.8	0.6	5.1	37.2	64.2
Cellulose CA	30.0	27.3	2.7	24.0	0.3	2.8	79.9	98.1
Cellulose CB	28.5	30.2	2.2	19.6	0.4	3.3	68.7	75.8
Cellulose CC	30.0	27.0	2.6	23.5	0.5	4.3	78.5	103.2

3.8. NMR analysis

The purity of the IL after the different pre-treatment procedures was verified using ^{1}H- and ^{13}C-NMR techniques. The determined chemical shifts of pure [emim][OAc] were as follows: [emim][OAc] ^{1}HNMR (400 MHz; $CDCl_3$) δ(ppm): 1.54 (t, 3H, NCH_2CH_3); 1.92 (s, 3H, CH_3COO); 4.05 (s, 3H, NCH_3); 4.36 (q, 2H, NCH_2CH_3); 7.29 (d, 2H, NCHCHN); 10.63 (s, 1H, CH_3COOH). ^{13}CNMR ($CDCl_3$) δ(ppm): 15.58 (NCH_2CH_3); 24.92 (CH_3COO); 36.39 (NCH_3); 45.07 (NCH_2CH_3) 121.50 (NCHCHN); 123.36 (NCHCHN); 138.26 (NCHN) and 177.57 (CH_3COO). The NMR spectra of ILs are illustrated in D appendix.

Chapter 4

4. Discussion

4.1. Optimisation study of wheat straw pre-treatment using [emim][OAc]

Study of pre-treatment conditions

In this work, three methods were performed in order to achieve an optimised pre-treatment methodology.

Initially it was tested the influence of time and volume of antisolvent (0.1 M NaOH) used in the first precipitation step. These tests were realised using A method. After 1 hour pre-treatment time it was verified an incomplete dissolution of the biomass since biomass particles were still observed. The complete dissolution of lignocellulosic material was verified after 6 hours and 16 hours pre-treatment times. However, after 16 hours pre-treatment time the addition of 0.1 M NaOH results in the formation of a dark brown and very viscous gum. This gum difficult the separation between carbohydrates and lignin. For this reason, 16 hour pre-treatment time is not advantageous. On the other hand, after 6 hour pre-treatment time it was verified a complete dissolution of biomass and there was no formation of a viscous gum, after the addition of NaOH 0.1 M. Comparing with literature, for longer pre-treatment time, similar results are obtained.[78] Note that it is also reported that long pre-treatment times contribute to degradation of biomass compounds and of IL.[77] However, some results are contradictory with the literature. *Li et al.* affirmed that 1 hour pre-treatment time of wheat straw with [emim][OAc] was sufficient to achieve macroscopic complete dissolution. But *Singh et al.* based on microscopic observations, reported that [emim][OAc] was capable to dissolve switchgrass completely after 3 hours at 120 ºC.[72] Relatively to the increased of the volume of 0.1 M NaOH used it was verified that the duplication of the volume, does not improved the separation of carbohydrates from lignin since the regeneration yield obtained is practically the same. This mean that higher volumes of 0.1 M NaOH contribute to a higher concentration of dissolved compounds, which in turn results in lower regeneration yields. The volume of 0.1 M NaOH used has also impact in the amount of lignin and IL recovered. Higher quantities of 0.1 M NaOH results in higher amount of lignin recovered (A4 experiment). However, as illustrate in figure 4.1, this lignin is more contaminated by hemicellulose relatively to the lignin recovered from A2 experiment. The bands at 897, 1042, 1079 and 1158 cm^{-1} are characteristic of the presence of hemicellulose. These bands are also present in the spectrum of lignin-rich sample from A2 experiment but the bands intensity is lower. For this reason, the use of 40 mL 0.1 M NaOH solution is more advantageous since the regeneration yield of carbohydrate-rich sample and the purity of lignin-rich sample are higher than those obtained when 80 mL 0.1 M NaOH solution is used. Relatively to the IL recovery percentage, it was verified that A2 experiment has a lower recovery percentage than A4 experiment. Probably, the higher viscosity and concentration of the IL solution may contribute to the IL entrapment and loss. Therefore, for the A method the optimised conditions defined was 6 hours pre-treatment time and 40 mL of 0.1 M NaOH.

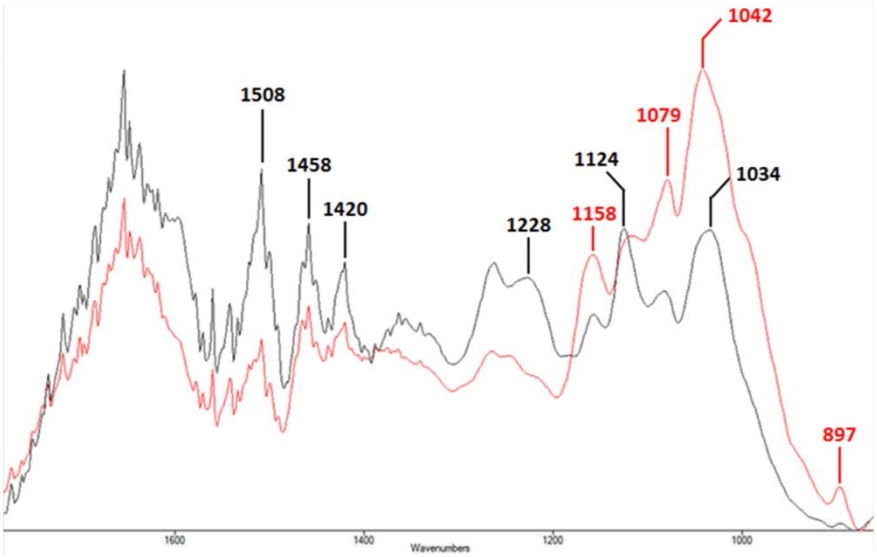

Figure 4.1. FTIR spectra of lignin-rich samples from A2 and A4 experiments.

Besides the pre-treatment time and antisolvent, another factor that affects the dissolution process is the biomass loading. In this work, the complete macroscopic dissolution was achieved at 120 ºC after 6 hours with 5 % (w/w) biomass loading (A and C methods) as well as at 110 ºC in a 4-hour process with a 2 % (w/w) biomass loading (B method). Analysis of the obtained results reveals a relation between the regeneration yield, applied conditions (biomass loading, temperature and time) and the used antisolvent. In the case of A and C methods, the regeneration yields observed are very similar (60.9 % (w/w) and 57.5 % (w/w), respectively) since the applied conditions and the antisolvent (NaOH) used were the same. For the pre-treatment B method the much higher regeneration yield (72.1 % (w/w)) can be attributed to the use of a different antisolvent (acetone/water mixture) and partially to differences in temperature, pre-treatment time and biomass loading. Thus, it can be concluded that the antisolvent used in the pre-treatment is an important factor that affects the yield of the regenerated material.[74,82,97,98] On the other hand, NaOH provided a lower regeneration yield, which means that it is inferior in this respect to the acetone/water antisolvent. However, the purity of the carbohydrate fractions from the regenerated material obtained with the antisolvent acetone/water was generally lower relatively to the antisolvent NaOH. This indicates that NaOH is a more selective antisolvent than the acetone/water solution used in the B method. Similar observations were reported for 0.1M NaOH and acetone/water antisolvents.[74,86,87] Using 0.1M NaOH as antisolvent after pre-treatment of sugarcane bagasse with [emim][Abs] (1-ethyl-3-methylimidazolium alkylbenzenesulphonate) resulted in 46-55 % of regenerated material.[86] Rice straw pre-treatment with cholinium lysinate and 0.1M NaOH gave 55.9 % of regenerated material. In the case of acetone/water solution in sugarcane bagasse pre-treatment with 1-butyl-3-methylimidazolium chloride the regeneration yield was higher (84.34 % (w/w)) and acetone soluble lignin constituted only 6.54 % (w/w) of the used biomass.[87] Note that, as initially the B method was based on the methodology described by *Lan W. et al*., the antisolvent used was only acetone. However, it was verified that [emim][OAc] was not miscible with this solvent, what has caused the low recovery of the IL in B1 experiment. Therefore, to improve the percentage of IL recovered, in the regeneration step of B2 and

B3 experiments was added a mixture of 9/1 (v/v) of acetone/water, followed by a mixture of 1/1 (v/v) acetone/water and finally a solution of water.

4.2. FTIR qualitative and quantitative analysis

All the samples obtained by each method were analysed by FTIR spectroscopy. This technique permits to realise a qualitative and quantitative analysis. However, note that for quantitative analysis, this technique is not rigorous and the results obtained are seen as estimated values.

A method only permits the separation of lignocellulosic material in carbohydrate- and lignin-rich materials. B and C methods allow the fractionation in cellulose-, hemicellulose- and lignin-rich materials.

A method permits to fractionate wheat straw into a 54.8 % (w/w) carbohydrate-rich sample and a 5.7 % (w/w) lignin-rich sample. In the case of the B method, the wheat straw was fractionated into a 44.4 % (w/w) cellulose-rich material, a 21.3 % (w/w) hemicellulose-rich material and a total of 13.5 % (w/w) of lignin-rich materials (acetone soluble lignin + residual lignin). Better results of fractionation with lower losses of initial biomass were obtained in this work when compared to the available literature data.[87] With the C method, an optimised process based on the A and B methods, the overall fractionation of wheat straw gave 41.8 % (w/w) cellulose, a 25.4 % (w/w) of total hemicellulose (hemicellulose + residual hemicellulose), and 8.0 % (w/w) of total lignin (lignin + residual lignin). The obtained results show that the recovery of the residual hemicellulose from the liquid stream increased the total hemicellulose content which is counterbalanced by a lower recovery of cellulose and lignin compared to the results of the B method. It is interesting to point out that a similar fractionation process to as the C method was performed by Yang et al. before,[64] although only the regenerated product was fractionated, leaving the liquid stream only for IL recovery. In this process, the overall recovery of biomass (cellulose, two fractions of hemicellulose and two fractions of lignin) was only 40.26 % (w/w) of the initial biomass input. It can be concluded that the C method, although characterised by a slightly lower biomass recovery than the B method, provide much higher recovery of biomass than other similar methods reported in the literature[64] with higher purity as discussed above. Furthermore, the results of the C method demonstrate the importance to fractionate the liquid stream after the regeneration process as there is still a significant amount of hemicellulose and lignin that can be recovered, which is essential for the development of an industrial feasible pre-treatment and fractionation process. A big amount of biomass is dissolved in the IL, but it is also soluble in the antisolvent. Therefore, this biomass can later be recovered and the contamination of the recovered IL can be simultaneously reduced.

In order to facilitate the comparison between the fractions obtained by each method, the spectra of some samples were superimposed.

Figure 4.2 depicts the FTIR spectra of standard cellulose (spectrum a) and original wheat straw (spectrum d) in comparison to the regenerated material (spectrum e), cellulose (spectrum b) and hemicellulose (spectrum c) fractions obtained after the IL pre-treatment. Qualitatively it is visible that the regenerated material obtained from the A method (spectrum e) consists in a mixture of cellulose and hemicellulose and is slightly contaminated with lignin. The presence of the bands at 1066 and

1046 cm^{-1} are indicative of this mixture. Comparing with the spectrum of original wheat straw, the similarities are evident. The difference is that, the bands of regenerated material from A method are more defined and this sample contains less lignin than original wheat straw. Comparing with the spectra of cellulose- and hemicellulose-rich samples from the C method, it can be seen that, in the case of the regenerated material, the bands present in the region of 1250-835 cm^{-1}, are not as well defined as those of cellulose- and hemicellulose-rich samples and the contamination with lignin is higher. The spectra of standard and fractionated sample of cellulose demonstrated great similarities especially for the characteristic region 1035-1061 cm^{-1}. Therefore, it is possible to affirm that no cellulose derivatisation occurred during the pre-treatment with [emim][OAc] as often reported.[99] For the hemicellulose spectrum there are clearly visible differences in the carbohydrate finger print region presenting a very strong absorption band at 1043 cm^{-1}. This vibration which is not observed in the cellulose spectrum is characteristic of the presence of xylans. Furthermore, the hemicellulose spectrum demonstrated the absence of the band at 1734 cm^{-1}, indicating the successful cleavage of ester linkages between hemicellulose and lignin in the pre-treatment process. A very low lignin content or almost complete absence of lignin in both cellulose- and hemicellulose-rich fractions could be confirmed by the negligible band at 1508 cm^{-1}. Furthermore, the absence of the characteristic cellulose band at 1320 cm^{-1} confirms the purity of the hemicellulose-rich fraction. Similarly, the cellulose fraction was found to be of a high purity too, because the acetyl groups characteristic of hemicellulose (1251 cm^{-1}) were not observed, and a less pronounced arabinan band at 993 cm^{-1} was detected. Additionally, it is important to emphasise that the acetyl groups (1251 cm^{-1}) in the hemicellulose-rich fraction were less noticeable. This indicates that, acetyl groups from the hemicellulose chains were partial degraded e.g. hydrolysed in the pre-treatment.

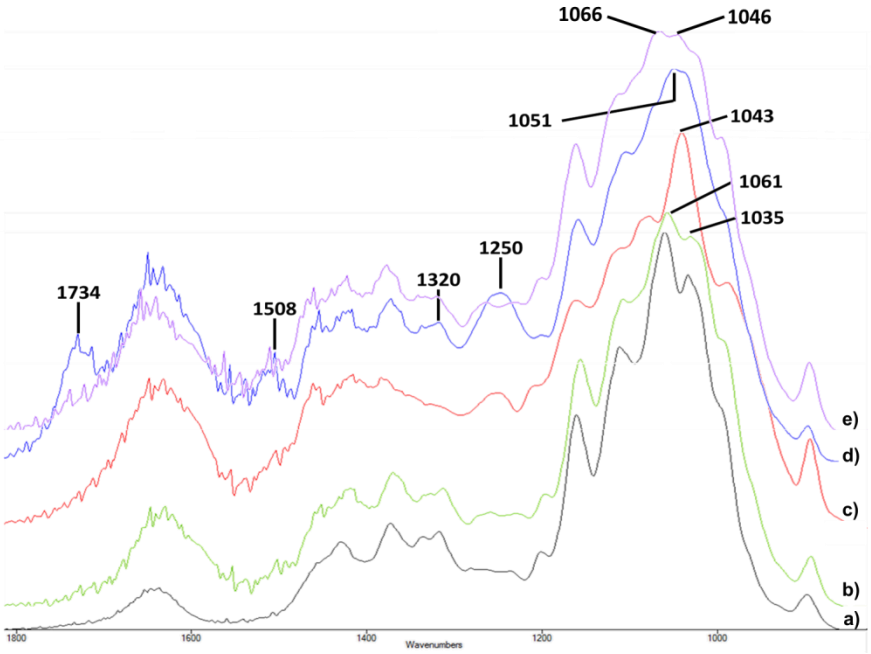

Figure 4.2. FTIR spectra (1800-800cm^{-1}) of standard cellulose (spectrum a), original wheat straw (spectrum d), regenerated material (spectrum e), cellulose- (spectrum b) and hemicellulose-rich (spectrum c) fractions obtained after the IL pre-treatment.

Lignin as a more complex compound can give different products depending on the pre-treatment process. The obtained lignin samples also demonstrated successful fractionation. The figure 4.3 depicts the comparison of spectra of the lignin-rich material from acetone/water mixture from the B method (spectrum a), lignin-rich material from C method (spectrum c) and residual lignin-rich material from C method (spectrum b). Significant differences between the three lignin samples presented here were noticed. First, a less purified lignin was obtained by acetone/water (B method) extraction, which can be determined by the presence of carbohydrates and confirmed by the vibrational absorptions at 898, 1046 and 1080 cm^{-1}. Furthermore, the multiple small absorptions in region 1200-1600 cm^{-1} indicated the presence of other compounds. In fact, acetone as a hydrophobic organic solvent is able to dissolve long chain hydrocarbons[100] that can lead to a significant increase of absorption intensities at 2852 and 2920 cm^{-1} as observed in the complete spectrum of acetone soluble lignin-rich material (see B appendix). These absorptions are attributed to C-H stretching vibrations that are characteristic of CH, CH_2 and CH_3 groups present in hydrocarbon molecules. The two other lignin-rich samples (b and c spectra) can be considered as carbohydrate-free lignin since characteristic bands at 898, 1046 and 1080 cm^{-1} were not observed. One of main differences between these three lignin spectra is with respect to the band at 1127cm^{-1} that was not detected for acetone soluble lignin spectrum and appeared as strong absorption bands in the two other spectra. As described in literature this band corresponds to C=O stretching of syringyl units as well as secondary alcohols present in lignin.[101] Therefore, it can be assumed that acetone soluble lignin was found to be free from the syringil unit. Furthermore, the reduction of the intensity of the band at 1654 cm^{-1} can be noticed in the presented figures. This band characteristic of conjugated para-substituted aryl ketones was observed in the residual lignin-rich material spectrum. This reduction can be caused by a stronger deformation of the carbonyl group existing in the side chains of lignin structural units and by modification of functional groups in the side chains. Additionally, the band at 1330 cm^{-1} indicates the condensation in lignin structure (syringyl and guaiacyl). The lignin condensation phenomenon is usually generated by high heating temperatures during the pre-treatment.

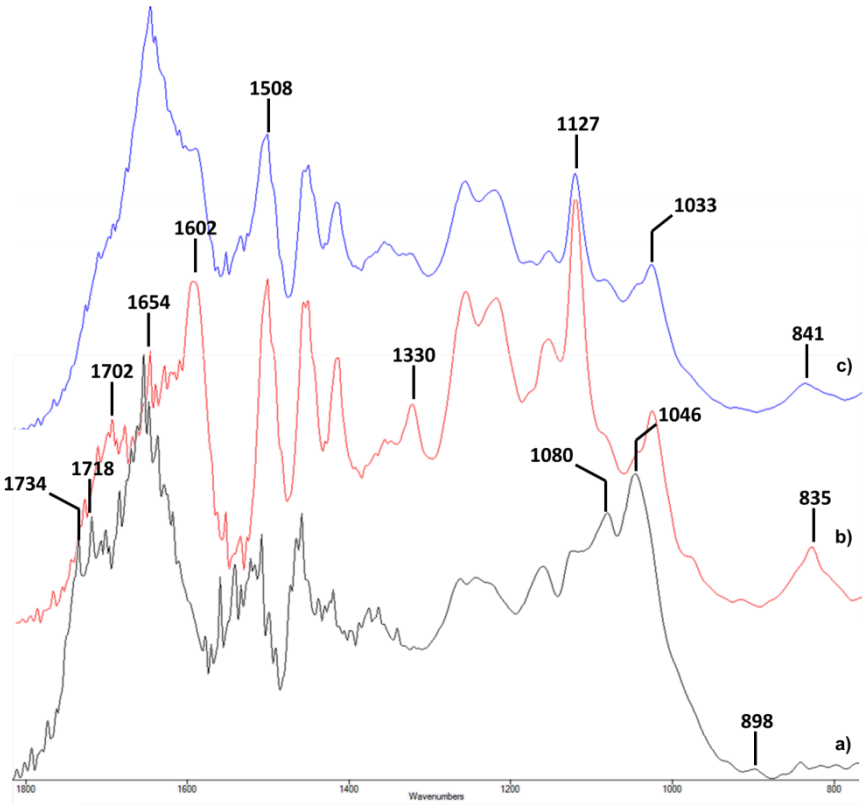

Figure 4.3. FTIR spectra (1800-800cm^{-1}) of acetone soluble lignin-rich material from the B method (spectrum a), lignin-rich material from the C method (spectrum c) and residual lignin-rich material from the C method (spectrum b) pre-treatment experiments.

The FTIR quantitative analysis permitted to have a clear perspective of sample purities and allowed to compare not only the recovery of each compound but also the efficiencies of the tested methodologies. Figures 4.4, 4.5 and 4.6 shows the FTIR quantification results obtained with A, B and C methods, respectively.

With the A method, a carbohydrate content of 79 % wt was obtained for the regenerated material, although the extracted lignin with a 70% wt purity still contained 6 % wt of carbohydrates (Figure 4.4). Regarding a maximal exploitation of biomass in the biorefinery concept it can be stated that this methodology has a limited utilisation due to a relatively poor fractionation of the original biomass compared to other presented methods.

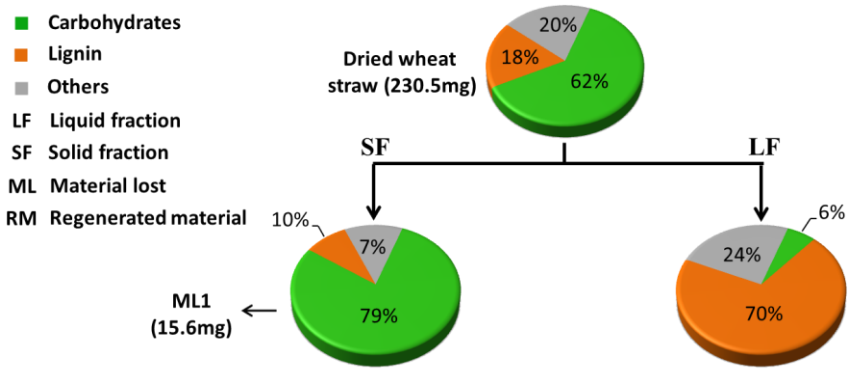

Figure 4.4. Quantitative FTIR results for fractionation of wheat straw with [emim][OAc] using A method.

The B method make possible to overcome the problem raised by A method since permits the fractionation of the regenerated material into cellulose- and hemicellulose-rich fractions. Furthermore, the residual lignin retained in carbohydrate-rich material after the regeneration process was recovered ensuring a more efficient fractionation process. In fact, cellulose- and hemicellulose-rich materials were recovered with a high carbohydrate content reaching 82 % wt and 80 % wt, respectively (figure 4.5). Simultaneously, the reduction of lignin content was observed from 18 % wt of dried wheat straw to 14 % wt in the regenerated material, followed by cellulose and hemicellulose fractionation containing a 10 % wt and 9 % wt lignin content, respectively (figure 4.5). Additionally, it was possible to obtain an extremely high pure residual lignin (98 % wt purity). However, it has to be pointed out that the main lignin fraction coming from the liquid stream in the fractionation process was strongly contaminated, showing only 57 % wt purity. Secondly, the obtained regenerated material had a similar composition to the original biomass what affected the following fractionation.

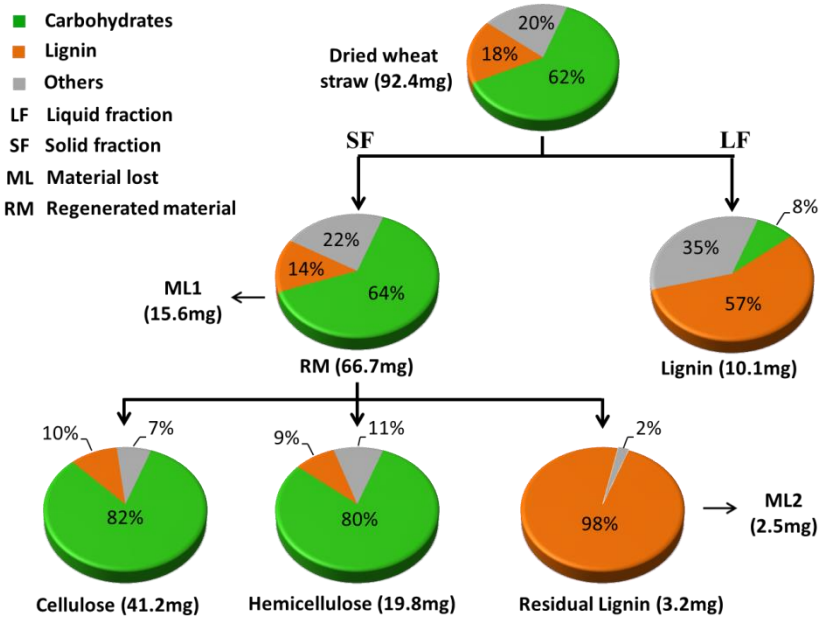

Figure 4.5. Quantitative FTIR results for fractionation of wheat straw with [emim][OAc] using B method.

The optimised C method proved to be the most efficient pre-treatment process as it produced fractions with the highest purity among the studied methods. The fractionation process realised with this method provided a reduction from 18 % wt lignin content in the initial biomass to 6 % wt for the regenerated material just in a one-step extraction. The regenerated material was then fractionated and the lignin content was maintained in the cellulose fraction and decreased to 5 % wt in the hemicellulose fraction. Herein the lignin extracted by a 0.1M NaOH antisolvent demonstrated to be carbohydrate-free. For the treatment of southern yellow pine (68.2 % wt carbohydrates and 31.8 % wt lignin) with [emim][OAc] (16 hours, 110 ^{o}C) a fractionation into a nearly pure lignin (~100 % wt) and a regenerated carbohydrate-rich material with 76.5 % wt carbohydrate and 23.5 % wt lignin contents was reported.[74] FTIR analyses confirmed that the lignin was carbohydrate-free, but the presence of other components expected to be in sample was not studied. In contrast, the results obtained in this

study with the C method resulted also in a carbohydrate-free lignin but with the contamination of nearly 13 % wt of other compounds. Another literature example, that can be mentioned, is the pre-treatment of switchgrass, composed of 64.5 % wt carbohydrate and 21.8 % wt lignin, with [emim][OAc] (3 hours, 160 ^{o}C) permitted to obtain a carbohydrate-rich material with 79.5 % wt carbohydrates, 13.6 % wt lignin plus a remaining content of ash and other compounds.[80] As it is depicted in Figure 4.6 the regenerated material from the C method shows a higher carbohydrate content than that for switchgrass with only 6 % wt lignin. It should also be emphasised that a better delignification occurred with the C method that is associated with the use of NaOH which has a well-known good potential for lignin extraction.[82] Considering the presented results it can be concluded that NaOH is superior to deionised or distilled water commonly used for lignin extraction.[64,78]

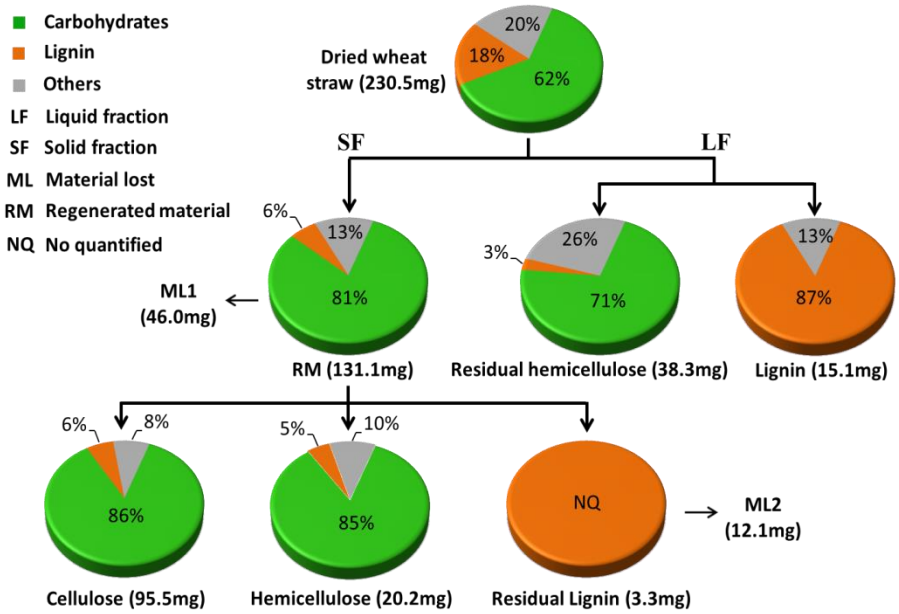

Figure 4.6. Quantitative FTIR results for fractionation of wheat straw with [emim][OAc] using C method.

4.3. Different biomass pre-treatment using [emim][OAc]

The regeneration yields obtained for each one of this pre-treated biomass are relatively higher than the regeneration yield obtained for wheat straw. This can be explained by the fact that depending on the type of lignocellulosic material, the composition of biomass varies (table 3.1). Therefore, as wheat straw has the lowest carbohydrate content (62.4 % (w/w)), the regeneration yield is also the lowest (table 3.10). The biomass with the highest regeneration yield is sugarcane bagasse since it presents the highest carbohydrate content (69.2 % (w/w)). Rice straw was the biomass with the highest material loss. Depending of the nature of biomass, the precipitation of carbohydrates-rich material with 0.1 M NaOH and the precipitation of lignin-rich material with HCl can be more or less easier. If the linkages between the compounds of the sample are relatively stronger is normal that the fractionation process became more difficult. As stated by *Taherzadeh M. et al*. the best method and conditions of pre-treatment depend greatly on the type of lignocellulosic biomass.[22] For example, the pre-treatment with a dilute-acid process of bark from poplar tree or corn leaf seems to be promising,

but in the case of the pre-treatment of bark from sweetgum or corn stalks this method is not as efficient.[22,58,102] Relatively to materials recovered from the liquid fraction, triticale presented the highest recovery of lignin and residual hemicellulose-rich materials (table 3.10). This lignin regeneration yield is consistent with the fact that triticale is composed with the highest percentage of lignin comparing to other tested biomasses. However, triticale is not the one composed with the highest amount of hemicellulose. An explanation could be in the quantity of hemicellulose that remains bounded to lignin after the precipitation with 0.1 M NaOH. In this case, more hemicellulose could remain bounded to the lignin which contributes to the increase of this material in the liquid fraction. The results obtained after the fractionation of the regeneration material into cellulose-, hemicellulose- and lignin-rich materials (table 4.1) reveal that sugarcane bagasse and wheat straw have the highest amounts of recovered cellulose-rich material. However, of the four tested biomasses this two show the lowest cellulose percentage in their composition. This make evident that the cellulose-rich sample recovered is not pure and other compounds precipitated together with cellulose. The biomass with the lowest recovery of cellulose-rich material is rice straw. Again this result is not consistent with the composition of original rice straw since, this biomass is the second with the highest percentage of cellulose. Relatively to the hemicellulose-rich sample, triticale was the only biomass with the recovery of two fractions of hemicellulose due to remain in the liquid fraction, a white precipitate that was filtered again to a new filter. This additional recovered hemicellulose makes the total quantity of hemicellulose recovered similar to the one recovered from sugarcane bagasse. Sugarcane bagasse is the biomass with the highest hemicellulose percentage in their composition and triticale has the second highest percentage. Therefore, in this case the amounts recovered are relatively consistent with the biomass composition. The biomass with the highest recovery of residual lignin-rich material was rice straw and the one with the lowest was triticale. This reveal that in case of triticale the initial fractionation process was efficient since the major amount of lignin remains in the liquid fraction and a little part remained bounded with carbohydrates, precipitating together when 0.1 M NaOH was added. For rice straw the fractionation process was not as efficient since a relatively high amount of lignin remained bounded with carbohydrates. Another fact that supports this is the highest material loss after the fractionation of regenerated material presented by rice straw.

The FTIR qualitative analysis of the samples obtained after the pre-treatment of each biomass shows that although the similarities between most samples, some of them reveal some differences comparatively to the samples obtained from wheat straw. The spectra of the regenerated material from rice straw and triticale are very similar. However, when compared with regenerated material spectrum from wheat straw, they are different. The presence of the bands such as 1060 and 1036 cm^{-1} suggest that this sample is rich in cellulose. But hemicellulose is also present due to the presence of a small band at 1253 cm^{-1}. The regeneration yield of the samples obtained from the fractionation of the regenerated material shows that the regenerated material derived from rice straw and triticale have a higher percentage of cellulose (76.0 % and 78.6 %, respectively) relatively to the regenerated material from wheat straw (72.9 %). Comparatively to the percentage of hemicellulose in the regenerated material, triticale has a percentage almost identical to wheat straw (15.3 % and 15.4 %, respectively) but rice straw has a lower percentage of hemicellulose (12.3 %). Therefore, the higher

percentage of cellulose in the regenerated material from rice straw and triticale can contribute to the appearance of bands that are more characteristic of cellulose. Contrary, the spectrum of the regenerated material from sugarcane bagasse presented bands, such as 1044 and 994 cm^{-1}, which indicates that this sample is rich in hemicellulose. Comparatively, with wheat straw, rice straw and triticale, the regenerated material of this biomass has more percentage of hemicellulose than the others. This fact can justify the predominance of this compound in the FTIR spectra. Note that, the small band at approximately 1376 cm^{-1} shows that this sample also contains cellulose. The spectra of all the regenerated material from sugarcane bagasse, rice straw and triticale are slightly contaminated with lignin, since the bands at 1508, 1458 and 1420 cm^{-1} are present. The regeneration yield of residual lignin-rich material from sugarcane bagasse, rice straw and triticale are 3.5 %, 5.1 % and 2.3 %, respectively.

Table 4.1. Regeneration yield (% w/w) of the samples obtained from the fractionation of regenerated material for sugarcane bagasse (CA), rice straw (CB) and triticale (CC) pre-treatments.

	RY (% w/w)		
Experiment	**Cellulose**	**Hemicellulose**	**Lignin[a]**
CA	74.9	18.6	3.5
CB	76.0	12.3	5.1
CC	78.6	15.3	2.3

[a] Residual lignin-rich material

After the fractionation process of regenerated material from each biomass, the qualitative and quantitative analysis of the spectrum of each sample reveals that the separation was successful and cellulose-rich sample is predominantly composed by cellulose, hemicellulose-rich sample is predominantly composed by hemicellulose and lignin-rich sample is predominantly composed by lignin. The quantitative results determined by FTIR spectroscopy for sugarcane bagasse and triticale are illustrated in figure 4.7 and 4.8, respectively. The results for rice straw were not presented since the quantification of cellulose-rich sample was higher than 100%.

The spectrum of cellulose from rice straw and triticale evidence pronounced bands characteristic of cellulose and are very similar between them. On the other hand, cellulose from sugarcane bagasse is very similar to the spectrum of cellulose from wheat straw pre-treatment. But in the case of these two last samples, the bands are not as well defined as the previous samples. These are supported by the quantitative results determined. The purity percentage of cellulose-rich material from rice straw (106 ± 11 % wt) is the highest and therefore the bands in the spectra are more defined. However, in the case of cellulose-rich sample from triticale this percentage (90 ± 8 % wt) is similar to the cellulose-rich sample from sugarcane bagasse (90 ± 9 % wt) whose bands are less defined. The only way to clarify this situation is through enzymatic hydrolysis. Cellulose-rich material from wheat straw and sugarcane bagasse has lower purity percentages (86 % and 90 ± 9 % wt, respectively), and then the spectra bands are less defined. All these samples are slightly contaminated with lignin due to the presence of a small band at 1508 cm^{-1}. The FTIR quantification reveals that the percentage of lignin present in each sample is 6 % wt for wheat straw, rice straw and triticale samples and 7 % wt for

sugarcane bagasse sample. Note also that the spectrum of cellulose-rich material has a slightly increase in the purity comparatively with the respective spectrum of regenerated material, but it is not as pronounced as in the case of wheat straw samples. This is due to the regenerated material from sugarcane bagasse, rice straw and triticale presented bands that are indicative of the predominance of hemicellulose (sugarcane bagasse) or cellulose (rice straw and triticale) instead of bands that reveal a mixture of cellulose and hemicellulose (wheat straw).

Hemicellulose- and residual hemicellulose-rich materials from sugarcane bagasse are identical, but the bands of residual hemicellulose are more defined. The purity percentage of these samples is relatively similar. Hemicellulose-rich sample presents 93 ± 9 % wt of hemicellulose and residual hemicellulose-rich sample presents 90 ± 8 % wt. This result may appear a little contradictory because normally when the bands are more defined, the purity is higher. However, as the FTIR quantification method of hemicellulose and cellulose samples is made in the same band, this method does not differentiate these two samples. Therefore, if the sample contains a little more cellulose, and even the characteristic bands of hemicellulose are less defined, the purity percentage could increase relatively to other hemicellulose-rich samples that have the bands more defined. As in the filtrate, resulting after the filtration of residual hemicellulose-rich material, remained some white flocs another filtration was made. The FTIR analysis of the recovered material reveals that this precipitate is rich in lignin instead of hemicellulose. The FTIR spectra of hemicellulose and residual hemicellulose from rice straw present some differences. The bands of the hemicellulose-rich fraction are more defined than residual hemicellulose-rich fraction. Besides, the presence of the high absorption bands at 1325 and 782 cm^{-1} in residual hemicellulose-rich sample reveals that this sample is probably contaminated with silica.[96] Again comparing with the FTIR quantitative results, the sample with the bands less defined presents a higher purity percentage. Hemicellulose-rich sample has 91 ± 8 % wt of hemicellulose and residual hemicellulose-rich sample has 100 ± 10 % wt. Similarly to what was explained above, the relatively high absorption of another compound (silica) can contribute to increase the absorption of the band used in the quantification of carbohydrates. For triticale three FTIR spectra were traced, since some white flocs remained in the filtrate, after filtration of hemicellulose-rich fraction. The spectra of these samples are very similar but, hemicellulose-rich sample has the bands more defined. Therefore, this sample is the purest but has more lignin than the others. The other two samples have a smaller purity, and as are less contaminated with lignin which means that the contamination with other compounds is higher. Note that from these two residual hemicellulose-rich materials, the one recovered from the regenerated material has a higher purity. The FTIR quantification results supports this since the hemicellulose-rich sample is composed by 90 ± 8 % wt of carbohydrates and 10 ± 1 % wt of lignin, residual hemicellulose-rich samples recovered from the liquid fraction has 70 ± 4 % wt of carbohydrates and 4 ± 3 % wt of lignin and residual hemicellulose-rich samples recovered from the regenerated material has 77 ± 5 % wt of carbohydrates and 7 ± 2 % wt of lignin. Comparing with the FTIR spectrum of hemicellulose samples from wheat straw, the spectra of hemicellulose- and residual hemicellulose-rich materials from the different pre-treatments are relatively similar. The quantification results for hemicellulose- and residual hemicellulose-rich materials from wheat straw are 85 % wt of carbohydrates and 5 % wt of lignin and 71 % wt of carbohydrates and 3 % wt of lignin, respectively.

Comparing all the hemicellulose spectra from the different biomasses, residual hemicellulose-rich material from triticale is the one with more defined bands and as reveal the FTIR quantification results is the one with the highest purity.

The FTIR spectra of the recovered lignin-rich samples are relatively similar but the intensity of some absorption bands may differ from sample to sample. These differences can occur due to the difference in the local of the cleavage of the chemical bonds of samples.[103] Analysing each spectra it can be seen that all the lignin-rich samples recovered are free of carbohydrates due to the absence of the band at approximately 898 cm^{-1}. Note that the residual lignin-rich sample from rice straw is contaminated with a compound with a high absorption band at 1094 cm^{-1}. In this spectrum, the region characteristic of lignin has an absorption relatively low comparatively to the others lignin-rich samples. Note also that both lignin-rich samples from triticale and rice straw are a little different from the others lignin samples.

Contrary to carbohydrates-rich samples, compare purities of lignin-rich samples analysing only qualitatively the spectra is very difficult because, most samples are free of carbohydrates and is not possible to see the contamination with other compounds unless the contamination is too high. Therefore, this comparison is only possible after FTIR quantification. The quantitative results obtained reveal that lignin-rich sample from wheat straw is the purest (87 % wt) and the residual lignin-rich sample from sugarcane bagasse has the lowest percentage purity (65 % wt). Unfortunately, was not possible to determine the purity of lignin samples from rice straw because the samples were highly contaminated with other compound. The presence of the bands at 1094, 966, 800 and 468 cm^{-1} in the residual lignin-rich sample, and the bands at 1091, 965 and 468 cm^{-1} in the lignin-rich sample may reveal that this compound could be silica.[96] An possible explanation to this occur could be that, as original rice straw is composed by a higher amount of extractives comparatively to the other biomasses, the step of water washing does not allow the complete removal of this compound that seems to precipitates with the addition of ethanol. It is noteworthy that rice straw was the only biomass that reveals this problem in the FTIR spectra and consequently the quantification results were affected. Note also that, in the case of residual lignin-rich sample from wheat straw, the quantification was not possible since the quantity recovered was too low.

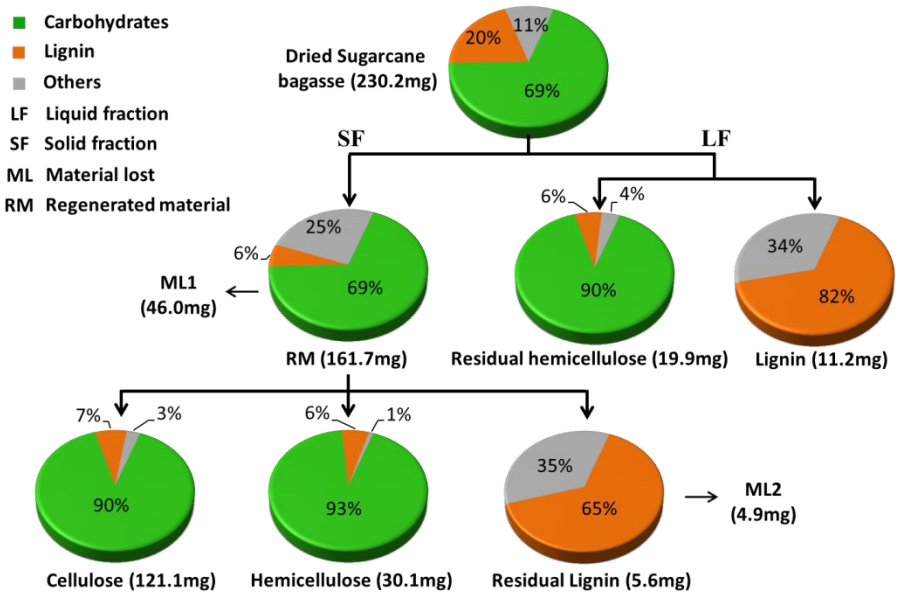

Figure 4.7. Quantitative FTIR results for fractionation of sugarcane bagasse with [emim][OAc] using C method.

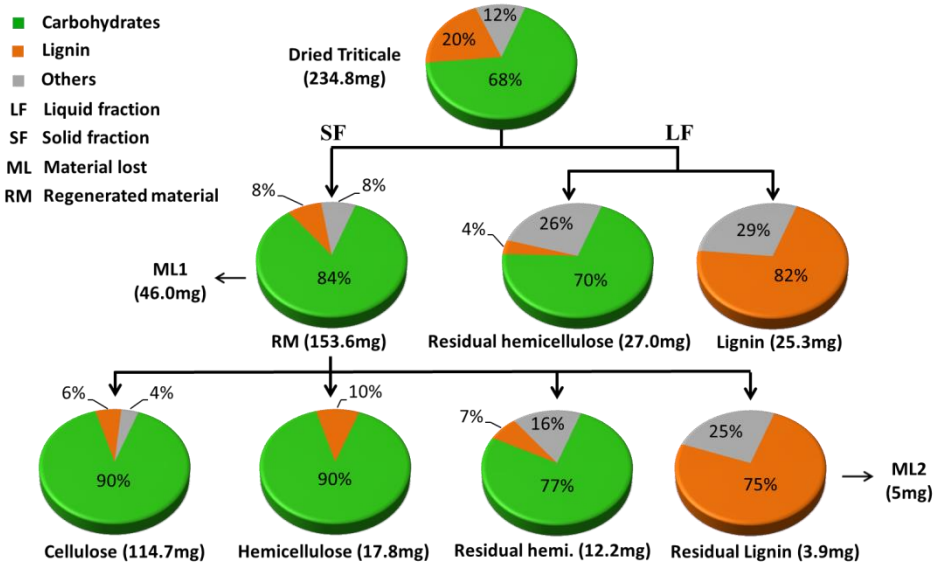

Figure 4.8. Quantitative FTIR results for fractionation of triticale with [emim][OAc] using C method.

4.4. [emim][OAc] recovery and reuse and NMR analysis

The IL recovery studied in the three pre-treatment methods was found to be 92.7 % (w/w) for the B method compared to those from the A (71.2 % (w/w)) and C (86.2 % (w/w)) methods (table 4.2). The principle difference observed is a modestly higher recovery of IL with the B method where an acetone/water solution was used as an antisolvent instead of NaOH used in A and C methods. In the experiments performed with NaOH as antisolvent it was visually observed that a higher quantity of NaCl salt was generated as the neutralisation is needed for the recovery of IL. The acetone/water solution leads to an extensive precipitation of carbohydrates and all impurities and consequently results in a lower amount of impurities present in the liquid stream (confirmed by NMR analysis) and a

high recovery of IL. Summarising, yields of IL recovery obtained in this work are similar to those published in the literature.[100,104] It is crucial to state that although various studies are performed, a more deep analysis and research are needed to develop a reliable and versatile method for IL recovery and to enhance the economic viability of the IL-based processes.

Considering this aspect, the feasibility of IL for the reuse in the further pre-treatments was examined in this work. Seven consecutive reactions were performed. The results obtained for the study of the reuse of [emim][OAc] are presented in table 3.19. As the regeneration yield was approximately the same in each experiment, means that the reused [emim][OAc] still able to fractionate efficiently wheat straw in carbohydrates- and lignin-rich materials. The ^{1}H- and ^{13}C- NMR spectra of pure and recovered [emim][OAc] is shown in D appendix. Comparing the spectra is possible to see that in the spectrum referred to the IL reuse appear new peaks. These peaks demonstrate that some contaminants accumulate in the IL during the pre-treatment. However, as the peaks are so small, this contamination is negligible.

Table 4.2. IL recovery percentage for pre-treatments performed using A, B, and C methods.

Method	% IL
A	71.2
B	92.7
C	86.2

4.5. Enzymatic hydrolysis

The A, B and C methods

To evaluate the pre-treatment efficiency of the developed methods using the IL the enzymatic hydrolysis of the untreated wheat straw, acid hydrolysed wheat straw, pure cellulose, carbohydrate-rich material obtained with the A method and cellulose-rich fractions obtained with B and C methods (table 3.20) was performed. The worst result was observed for the hydrolysis of native wheat straw (19.7 % $w/w_{biomass}$), which has to be attributed to the low accessibility of the carbohydrates within the lignocellulosic matrix of wheat straw since this material does not suffer any pre-treatment. The native intricate structure of wheat straw as well as the presence of lignin, hemicellulose and other compounds are known to hinder the access of cellulases to the cellulosic substrate, resulting thus in poor hydrolysis performance.[72,44,105] As expected the enzymatic hydrolysis of a high purity standard cellulose without pre-treatment resulted in complete hydrolysis (97.2 % $w/w_{biomass}$). Comparing the results of the regenerated material from A method with cellulose from B and C methods we see that the glucose yield of the regenerated material is the lowest (49.1 % $w/w_{biomass}$) not only due to the presence of hemicellulose but also due to the higher amount of lignin present. This result demonstrates the importance to fractionate the regenerated material further into a more cellulose enriched fraction to achieve higher glucose yields after the enzymatic hydrolysis step. The highest glucose yield (76.0 % $w/w_{biomass}$) after hydrolysis was obtained with the optimised pre-treatment C method, which allowed a more efficient fractionation of cellulose than the B method. Finally, the results of enzymatic hydrolysis permits to confirm the higher efficiency of IL pre-treatment relatively to acid

hydrolysis. Although the glucose and total sugar yield of acid hydrolysed wheat straw is higher than the untreated feedstock, revealing the importance of a previous biomass pre-treatment, these values are lower than the carbohydrate-rich samples obtained after the IL pre-treatment. The analysis of the hydrolysates of cellulose hydrolysis demonstrated that in apart from glucose also xylose was detected. This result indicates that the cellulase mixture Celluclast 1.5L plus β-glucosidase Novozyme 188 exhibits along with cellulose activities also xylanase and β-xylosidase activities, which is in agreement with previous reports.[106] For the cellulose standard negligible although detectable quantities of xylose were released during the enzymatic hydrolysis and a complete carbohydrate hydrolysis was observed for cellulose samples reaching 100 % (w/w) of total sugar yield. In the case of the native wheat straw hydrolysis, as expected, less than 50 % of the carbohydrates were converted to sugars. As mentioned before, for the regenerated material an incomplete hydrolysis was observed, which is attributed to the still relatively high hemicellulose content in the sample (after native wheat straw is the sample with the higher content of xylans) that could hinder the enzyme activity.[107] This limitation could be overcome by the supplementary addition of xylanases and β-xylosidases with a higher activity in order to achieve complete conversion; however, it would make the process more costly.[108] The degree of conversion obtained in enzymatic hydrolysis experiments allows to estimate the cellulose purity of the carbohydrate-rich fractions. Thus, cellulose obtained by the B and C methods showed cellulose contents of 85.6 % wt and 88.4 % wt from the total carbohydrate content quantified by FTIR, respectively. These data indicate that the C method is a more adequate procedure for biomass pre-treatment and fractionation process.

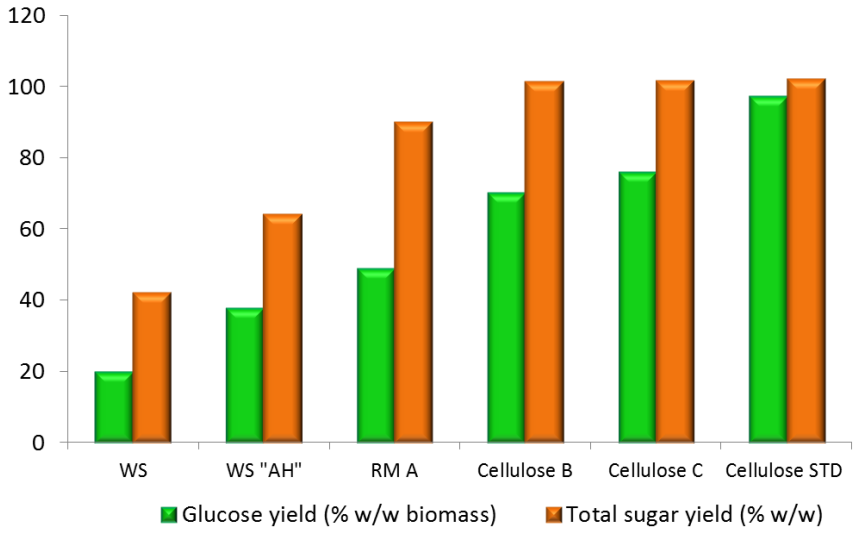

Figure 4.9. Glucose and total sugar yield of untreated wheat straw, acid hydrolysed wheat straw, carbohydrate-rich material obtained with the A method, cellulose-rich fractions obtained with B and C methods and pure cellulose.

Ionic liquid pre-treatment of various biomasses

In order to evaluate the enzymatic digestibility of cellulose-rich samples obtained after the pre-treatment of the different biomasses as well as of original and acid hydrolysed biomasses, the enzymatic hydrolysis of these samples was performed. In figures 4.10, 4.11, 4.12 is illustrated the glucose and total sugar yield for sugarcane bagasse, rice straw and triticale samples, respectively. As

expected the samples with the lowest glucose and total sugar yield are the original biomasses. From the biomass tested, sugarcane bagasse is the one with the lowest percentages (4.6 % w/$w_{biomass}$ of glucose yield and 8.5 % w/w of total sugar yield) and wheat straw is the one with the highest percentage (19.7 % w/$w_{biomass}$ of glucose yield and 41.9 % w/w of total sugar yield). This means that the cellulose from native wheat straw is more easily accessible to the enzyme. As expected as well, the samples with the highest glucose and total sugar yield are cellulose-rich samples recovered after the IL pre-treatment. Cellulose-rich sample from sugarcane bagasse has the highest glucose yield (79.9 % w/$w_{biomass}$) and cellulose-rich sample from triticale has the highest total sugar yield (103.2 % w/w). Although this last sample has less cellulose, the content in xylans is higher, which contributes to the higher total sugar yield. Note that, the untreated and acid hydrolysed sugarcane bagasse presents the lowest glucose yield but the IL pre-treatment increased considerably this percentage. These mean that the IL made possible the transformation of a difficult digestible feedstock into an easily digestible material. However, depending on the nature of the lignocellulosic material the interaction with the IL and the consequent transformation can be more or less efficient. The results for acid hydrolysed samples indicate that the IL pre-treatment is more efficient than acid hydrolysis. Relatively to the untreated biomasses, the glucose and total sugar yields of acid hydrolysed samples increase, but comparatively to the IL pre-treated samples, the percentages are lower. From the acid hydrolysed samples, wheat straw and triticale presents the highest glucose and total sugar yields (in the case of wheat straw 37.7 % w/$w_{biomass}$ and 64.0 % w/w and for triticale 37.2 % w/$w_{biomass}$ and 64.2 % w/w, respectively). On the other hand, acid hydrolysed sugarcane bagasse has the lowest glucose and total sugar yields (19.4 % w/$w_{biomass}$ and 32.1 % w/w, respectively). Therefore, this pre-treatment is less selective than the IL pre-treatment since like is illustrated in B appendix, the sample recovered after acid hydrolysis has a relatively high quantity of lignin. The presence of lignin limits the enzymatic susceptibility of cellulose and hemicellulose components of the feedstock.[109] Comparing these results with the results from FTIR quantification it can be seen that they seem to be a little contradictory. According with FTIR quantification, rice straw is the sample with the highest percentage of cellulose. But, as said before, the FTIR quantification does not allow the differentiation between cellulose and hemicellulose. Therefore, the absorption of the both compounds will contribute to increase the purity percentage. The enzymatic hydrolysis reveals that this sample presents more hemicellulose than the cellulose-rich sample from sugarcane bagasse, which can justify partially the higher purity percentage determined by FTIR spectroscopy. However, cellulose-rich sample obtained from triticale has a higher amount of hemicellulose than rice straw, and contrary to the expected, the glucose and total sugar yield are higher than rice straw. Therefore, this explanation does not totally justify the results obtained by the two quantification methods used. Another justification may be in the homogeneity of the sample prepared for FTIR analysis. If the sample is not sufficient homogenous, the form of the spectrum can be influenced.[110] For this reason, the FTIR quantification methodology defined is not versatile enough when certain parameters are different.

The cellulose purity of cellulose-rich fractions obtained after sugarcane bagasse, rice straw and triticale pre-treatment can be estimated by knowing the degree of conversion obtained in enzymatic hydrolysis experiments. Thus, cellulose-rich sample obtained by sugarcane bagasse, rice straw and

triticale showed cellulose contents of 87.9 wt %, 64.9 wt % and 87.0 wt % from the total carbohydrate content quantified by FTIR, respectively. These data indicate that cellulose-rich fraction derived from sugarcane bagasse is the purest.

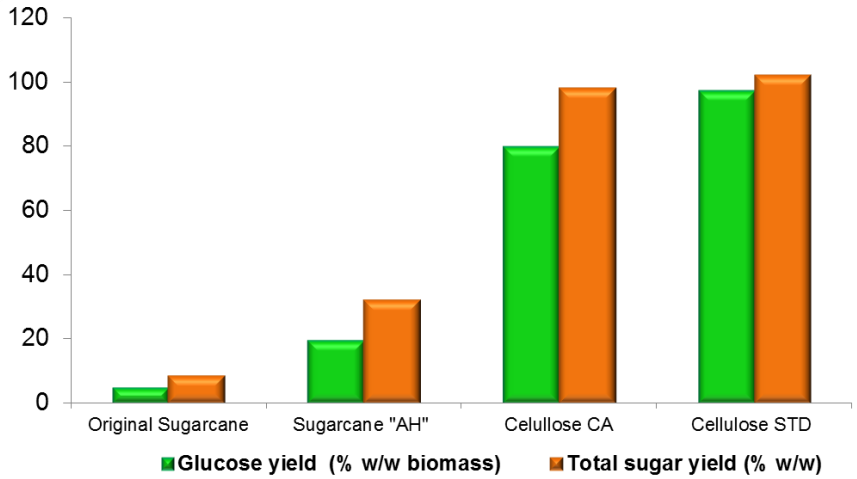

Figure 4.10. Glucose and total sugar yield of untreated sugarcane bagasse, acid hydrolysed sugarcane bagasse and cellulose-rich sample obtained from sugarcane bagasse fractionation.

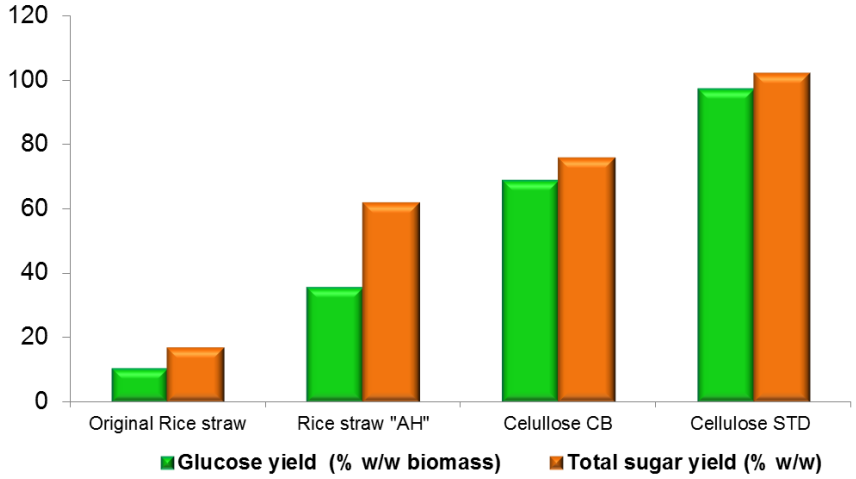

Figure 4.11. Glucose and total sugar yield of untreated rice straw, acid hydrolysed rice straw and cellulose-rich sample obtained from rice straw fractionation.

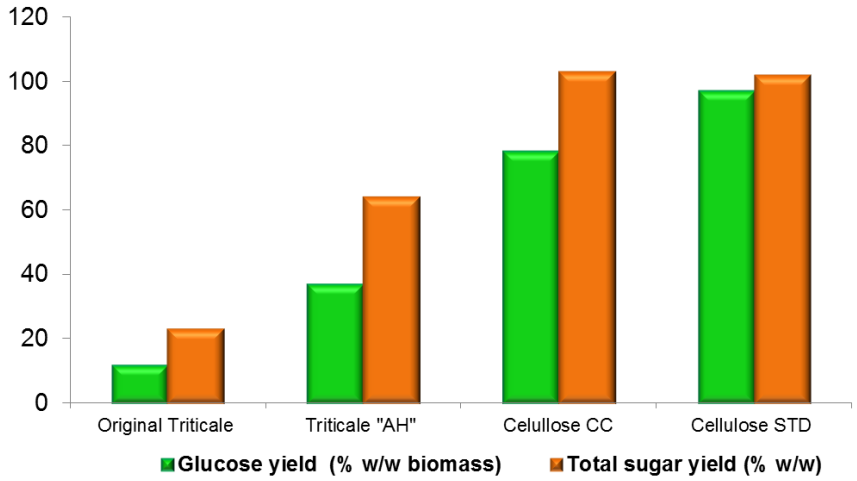

Figure 4.12. Glucose and total sugar yield of untreated triticale, acid hydrolysed triticale and cellulose-rich sample obtained from triticale fractionation.

4.6. Crystallinity of regenerated cellulose fractions

Cellulose crystallinity is considered to be an important factor to evaluate the accessibility of cellulose to cellulase enzymes. In general, a lower crystallinity index seems to be related to an enhancement of the enzymatic hydrolysis of cellulose.[104,105] Among a limited set of ILs tested, [emim][OAc] was found to be highly selective for the extraction of lignin from lignocellulosic biomass and reduce simultaneously the crystallinity of cellulose.[82] It is known that lignin hinders the enzymatic hydrolysis and an extensive delignification should be attained to improve hydrolysis.[111,112] In the wood flour pre-treatment with [emim][OAc], 40 % of lignin was removed and the cellulose crystallinity index decreased, resulting in more than 90 % of the cellulose to be hydrolysed by cellulase.[82] In the presented work, the total sugar released for all tested samples was higher than 90 % and the lignin content decreased drastically in the pre-treated samples. The regenerated material from the A method contained 50 % less lignin than the original feedstock and with B and C methods 44 % and 67 % of lignin removal was obtained, correspondingly. Beside the partial delignification achieved with methods used in this work, an additional reduction in cellulose crystallinity was achieved. The crystallinity index LOI decreased in all pre-treated samples in comparison to native and acid hydrolysed wheat straw as well as to standard cellulose. The crystallinity index TCI also decreased but the change was less substantial. These results are in agreement with the results obtained by the enzymatic hydrolysis of these substrates as the reduction in cellulose crystallinity led to a better performance of the enzymatic hydrolysis of cellulose. However, standard cellulose, that was completely hydrolysed (100 % total sugar yield), presented a crystallinity index similar to native wheat straw, that was only partially hydrolysed (45 % total sugar yield; table 3.17). Moreover, incomplete carbohydrate hydrolysis was verified for regenerated material from the A method even with crystalline structures similar to cellulose-rich fractions obtained from pre-treatments with B and C methods. Therefore, it may be concluded that besides the cellulose crystallinity, the presence of lignin and hemicellulose also affects the results of enzymatic hydrolysis. High purity cellulose-rich samples such as those obtained with the pre-treatment C method, are highly desirable for further processing to bioethanol or conversion into added value products within the biorefinery concept.

Relatively to the different biomass pre-treated, the regenerated material and cellulose-rich samples have similar LOI and TCI values but for regenerated material samples these values are relatively lower. Once again, the samples with the highest LOI and TCI values are the original biomasses. Relatively to the acid hydrolysed samples it can be seen that the LOI and TCI values are lower than the original biomasses values and higher than the regenerated material and cellulose-rich samples values. Comparing with the enzymatic hydrolysis results, the samples with the lowest crystallinity index, namely cellulose-rich samples has a higher enzymatic digestibility. On the other hand, the samples with the highest crystallinity index have a lower enzymatic digestibility, as is the case of original biomasses. The enzymatic digestibility of acid hydrolysed samples is higher than original biomasses and is lower than cellulose-rich samples, agreeing with the determined crystallinity indexes. As the enzymatic hydrolysis of the regenerated material was not realised, a comparison with the crystallinity indexes is not possible.

Chapter 5

5. Conclusions

In this work, the pre-treatment of wheat straw using [emim][OAc] was successfully performed by three different methods. The A method allowed the fractionation of wheat straw into a carbohydrate- and lignin-rich portions. The B and C methods allowed the fractionation into cellulose-, hemicellulose- and lignin-rich fractions. The maximal exploitation (maximum biomass recovery) of the wheat straw feedstock was achieved by the B method. However, the C method, which was developed and optimised based on the procedures of A and B methods, afforded regenerated solid fractions of higher purity. The improved performance of this method was demonstrated by the high carbohydrate content of the cellulose and hemicellulose fractions determined to be 86 % wt and 85 % wt, respectively. Additionally, lignin was recovered after extraction by an aqueous 0.1M NaOH solution and with 87 % wt purity.

The studies on the IL recovery and reuse performed with the A method, confirm that IL can be reused without losses in the biomass pre-treatment efficiency. Therefore, pre-treatment with ILs could be advantageous when the feasibility of the process is guaranteed.

Relatively to the results of the pre-treatment of different types of lignocellulosic biomass is important to point out that, several factors can influence the efficiency of the process. The main factors are: the chemical composition of each biomass and the nature of the linkages between the compounds of the feedstock. In terms of global mass balance, the biomass with the higher amount of total mass recovered was triticale, with 6.1 % of material loss. On the other hand, rice straw was the biomass with the highest material loss (14.8 %). The analysis of FTIR results reveals that, sugarcane bagasse and triticale presents similar quantification results namely, in terms of carbohydrate- and lignin-rich fractions. These two biomasses have the highest purity percentages of recovered fractions. Note that, the results obtained for rice straw do not permit the comparison between the others biomasses since they are over-quantified.

The enzymatic hydrolysis of the carbohydrate- and cellulose-rich materials regenerated after IL pre-treatment resulted in total sugar release. The pre-treatment with [emim][OAc] was capable to perform a partial although sufficient delignification of wheat straw and caused changes in the cellulose structure that facilitated the access of the enzymes to its substrates, enhanced hydrolysis and led complete hydrolysis into reducing sugars within 72 hours. Comparing the results of enzymatic hydrolysis of the samples from the A, B and C methods it can be seen that, the C method is the most efficient since its cellulose-rich sample presents the highest glucose yield. The enzymatic hydrolysis results of cellulose-rich samples from the different biomasses pre-treatment support the FTIR quantification results. The biomasses with the highest content of carbohydrates in cellulose-rich samples (sugarcane bagasse and triticale) also present the highest glucose and total sugar yield.It can be concluded that the quantitative analysis of relatively pure compounds by FTIR spectroscopy is viable. However, when contaminants are present, the results could be over-quantified. The enzymatic hydrolysis results also reveal that the IL pre-treatment is more efficient than the conventional acid hydrolysis pre-treatment. Thus, hydrolysed reducing sugars from cellulose-rich samples could be further applied in fermentation systems to produce bioethanol and other value added products within the frame of the biorefinery concept.

The crystallinity indexes were also determined in this work. In general, lower crystallinity indexes are associated with to an enhancement of the enzymatic hydrolysis of cellulose. However, in some cases the presence of lignin and hemicellulose also affects the results of enzymatic hydrolysis..

Chapter 6

6. Perspectives

The IL technology on biomass processing is relatively recent and has demonstrated several advantages relatively to the conventional pre-treatments methods. However, a vast research is still strongly required in this field since the majority of the studies are relatively to the dissolution of carbohydrates in ILs and the majority of the biomass pre-treatments only permits to obtain carbohydrate- and lignin-rich fractions. Only few studies report the complete separation of lignocellulosic biomass constituents, namely cellulose, hemicellulose and lignin.

In this work, it was possible to fractionate various lignocellulosic biomass into cellulose, hemicellulose and lignin samples characterized by high purities.

In order to verify the feasibility for a future application in industry, the scale-up of the optimised method should be achieved. The major limitation in the use of ILs in an industrial scale is associated with their high cost, comparatively to the conventional pre-treatment agents (e.g. ammonia and sulphuric acid). Therefore, the optimisation of the recovery process of ILs is a crucial factor for the economic feasibility of the pre-treatments with ILs.

Another important study that should be performed is the fermentation of the reducing sugars released after enzymatic hydrolysis and the evaluation of needs of hydrolysate detoxification to confirm the influence of the IL on the production of bioethanol and value added products after pre-treatment and enzymatic hydrolysis.

Note also that, due to the price of ILs it would be more advantageous and feasible to focus the study on the production of value added products. The majority of works dealing with this subject focuses on the further processing of cellulose that can be easily converted to cellulosic ethanol widely used as biofuel. However, the two other fractions, hemicellulose and lignin are even more rarely considered as important to treat. The probable reason for this is a great diversity of both fractions that on one hand makes process more difficult but on the other opens the room for variety of commodities that can be obtained. The valorisation of these two diverse fractions constituted by different compounds depending on the raw material is especially important as it allows obtaining products with high commercial value (e.g. xylitol, oligosaccharides, polyphenols, etc.) which may contribute to the economic feasibility of the whole process.

Chapter 7

7. Bibliography

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Appendix

A appendix: determination of the total quantity of protein

The total protein content was estimated according with Kjedahl method. Kjeldatherm digestion block was preheated to 390 º. Initially, 50 mL of sample, 10 mL of H_2SO_4 and one piece of Kjeltabs were added to digestion tubes. The digestion tubes were placed in Kjeldatherm digestion block and digested until white smokes come off (approximately 1 hour). The digestion tubes were removed from Kjeldatherm digestion block and cooled for 1 hour. Then, 15 ml of deionized water was added to dissolve all crystallized material. The digestion tubes were attached to distillation head of Vapodest VAP 30. The delivery tubes were placed in 250 mL Erlenmeyer flask which contains 25 mL of boric acid indicator solution. Next, 60 mL of NaOH solution was added to the digestion tubes and the distillation process started (10 minutes at 75 % steam pressure). When distillation was complete, a burette to the mark was filled with HCl 0.2 N and titrated to purple endpoint. Finally, is necessary to do the correction with the reagent blank. For the blank test, the same procedure was made, replacing the sample mass with distilled water. In table A1, the reagents used are described. Note that, all the reagents should be reagent grade and nitrogen-free.

Table A1. Reagents used for the determination of the total quantity of protein.

Reagents	
Sulfuric acid concentrated (H_2SO_4)	95-98 % (w/w)
Sodium hydroxide solution (NaOH)	50 % (w/v)
Hydrochloric acid (HCl)	0.2 N
Methylene blue indicator	200 mg of methyl red were dissolved in 100 mL of ethyl alcohol. In a separate beaker, 200 mg of methylene blue were dissolved in 100 mL of ethyl alcohol and 2 volumes of methyl red were mixed with 1 volume of methylene blue.
Boric acid indicator solution (H_3BO_3)	20 g of H_3BO_3 were dissolved in 800 mL of deionized water. 10 mL of methyl red/methylene blue indicator solution were added and diluted to one liter with deionized water.
Kjeltabs	-

The total percentage of nitrogen is determined using the following expression:

$$\text{Total nitrogen } (\%) \quad = 0.14 \times \frac{(V - V0)}{A}$$

Where,

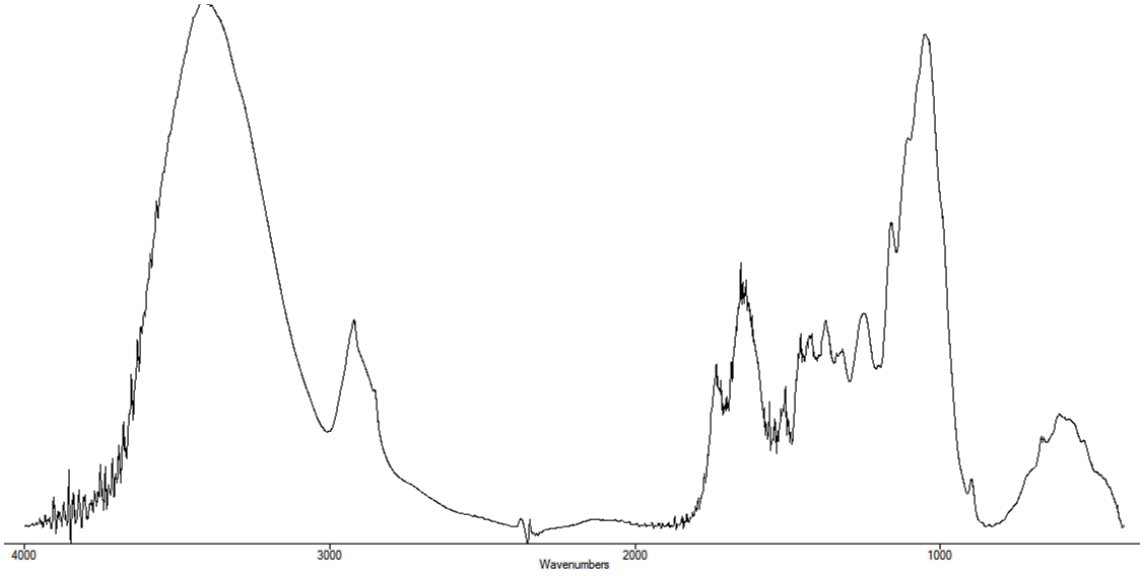
V = Volume (mL) of 0.1N HCl solution used in the titration.

V0 = Volume (mL) of 0.1N HCl solution used in the titration of the blank test.

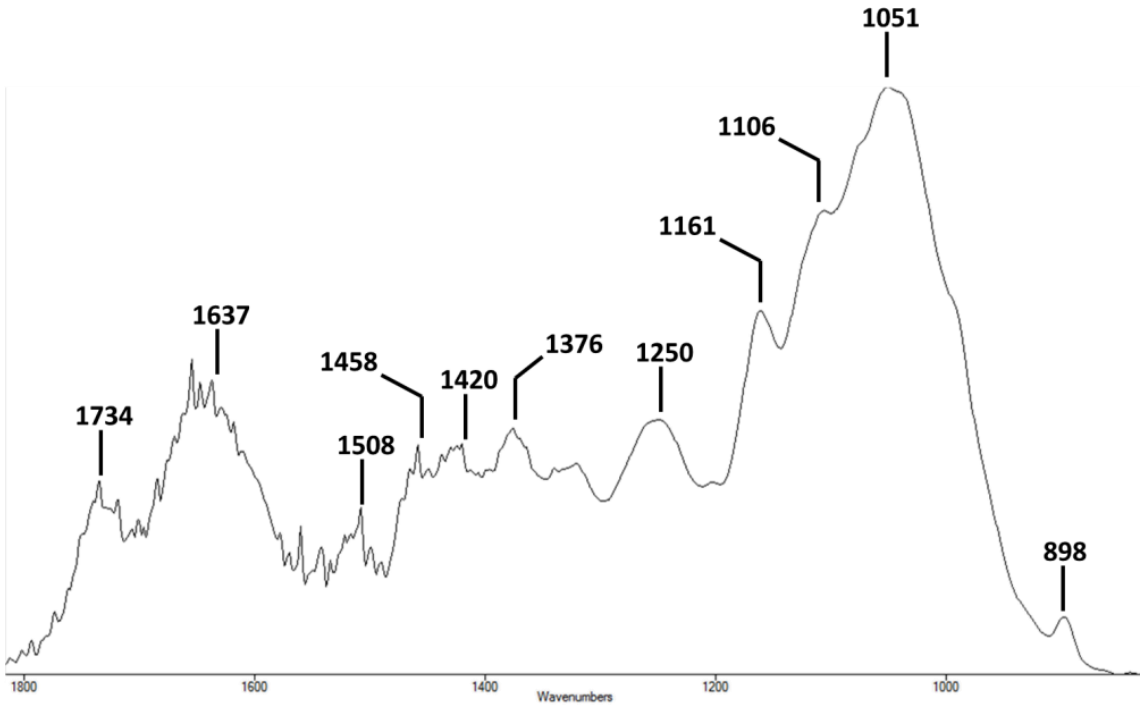
A = Mass of sample (dry mass).

B appendix: FTIR spectra

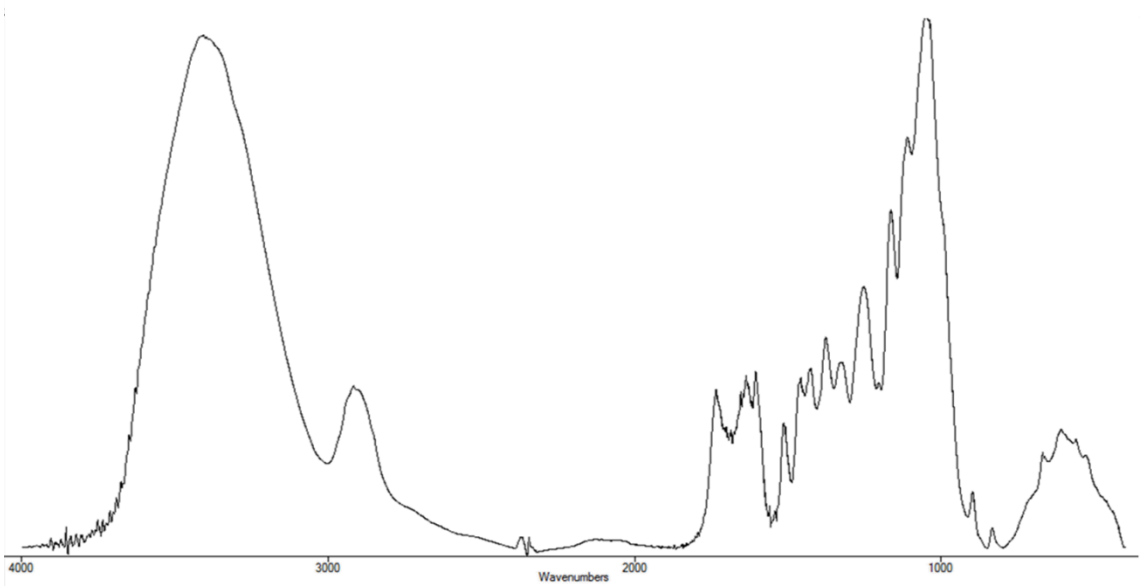
Original wheat straw (4000-400 cm^{-1})



Original wheat straw (1800-800 cm^{-1})



Original sugarcane bagasse (4000-400 cm^{-1})



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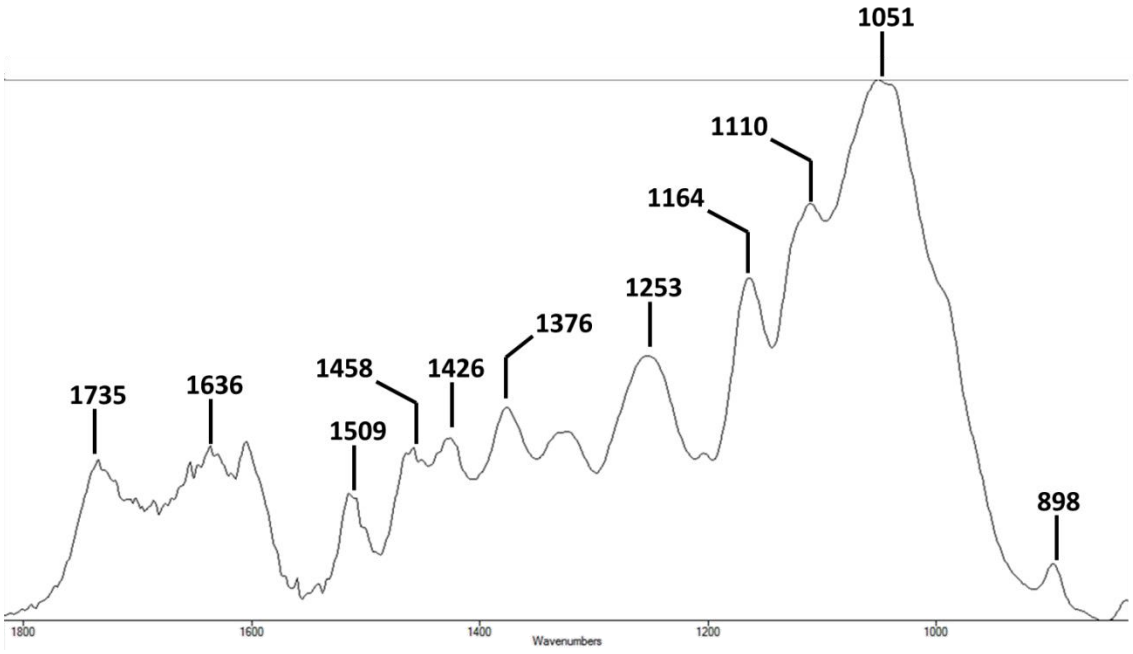
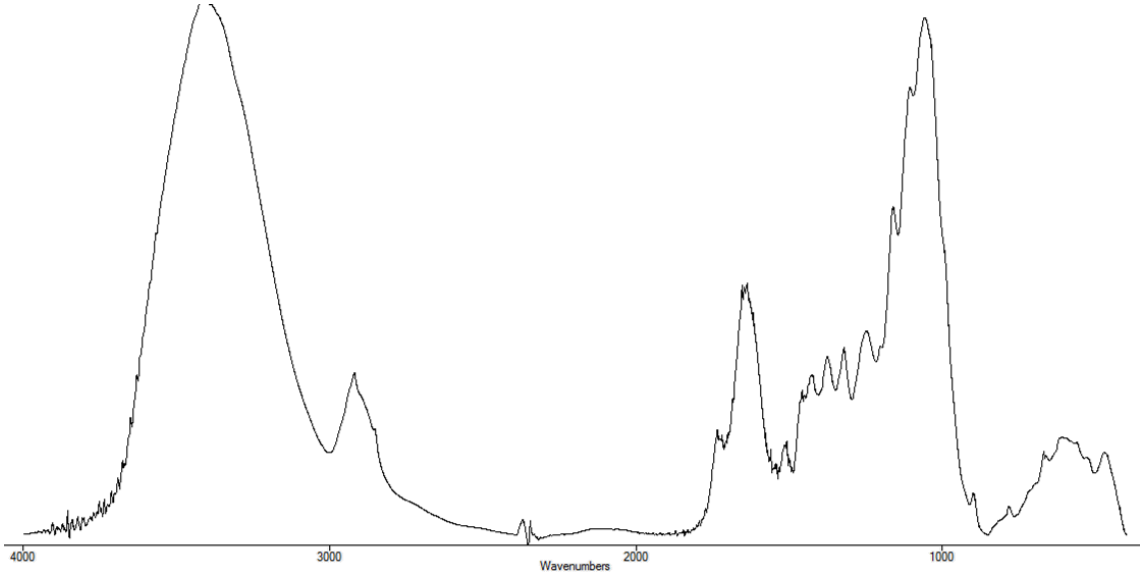
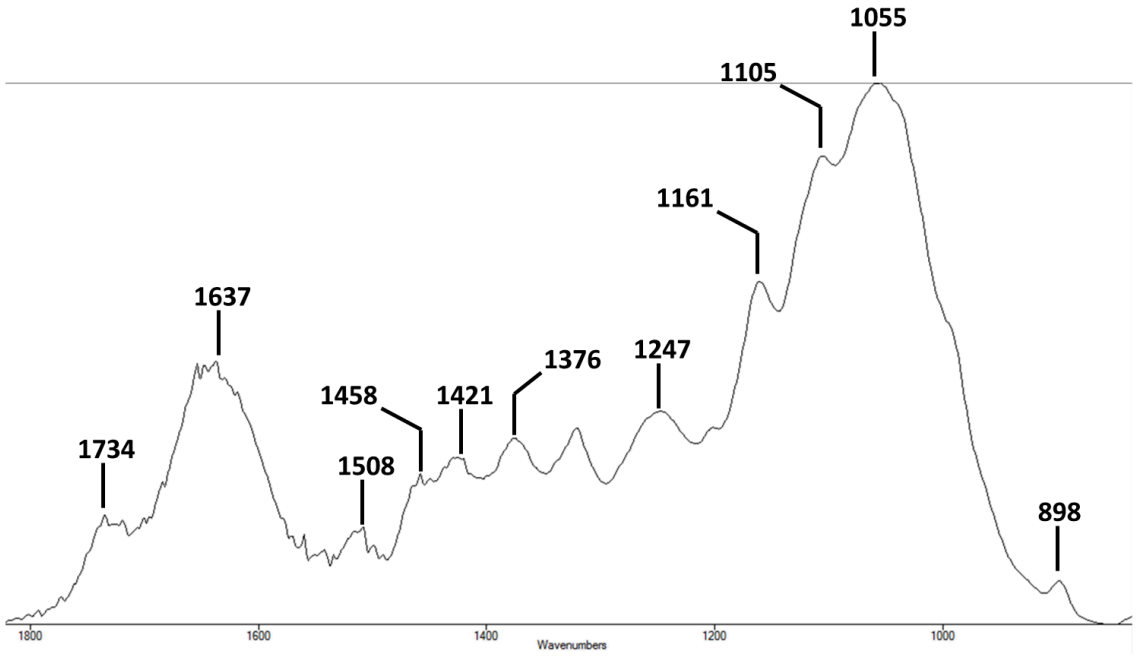


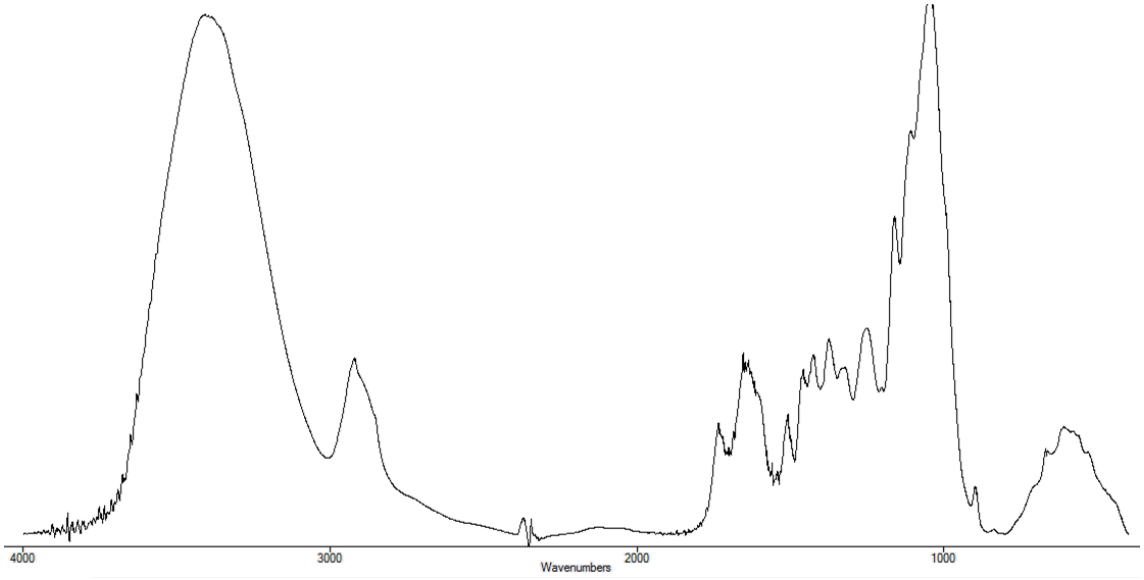
Acid hydrolysed rice straw (4000-400 cm^{-1})



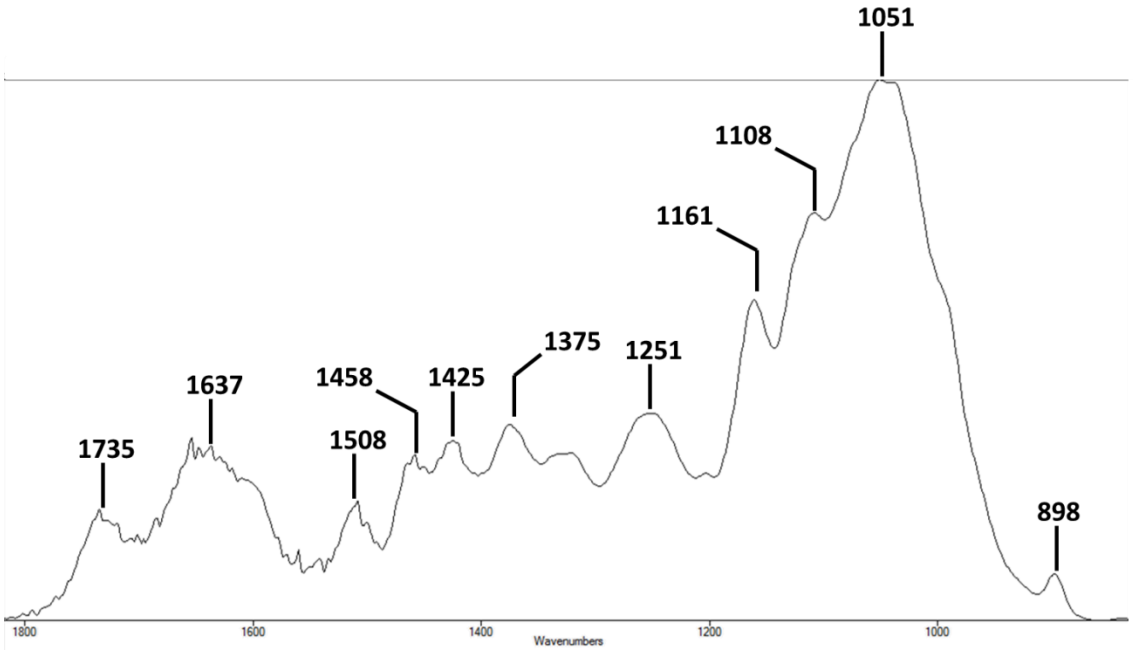
Acid hydrolysed rice straw (1800-800 cm^{-1})



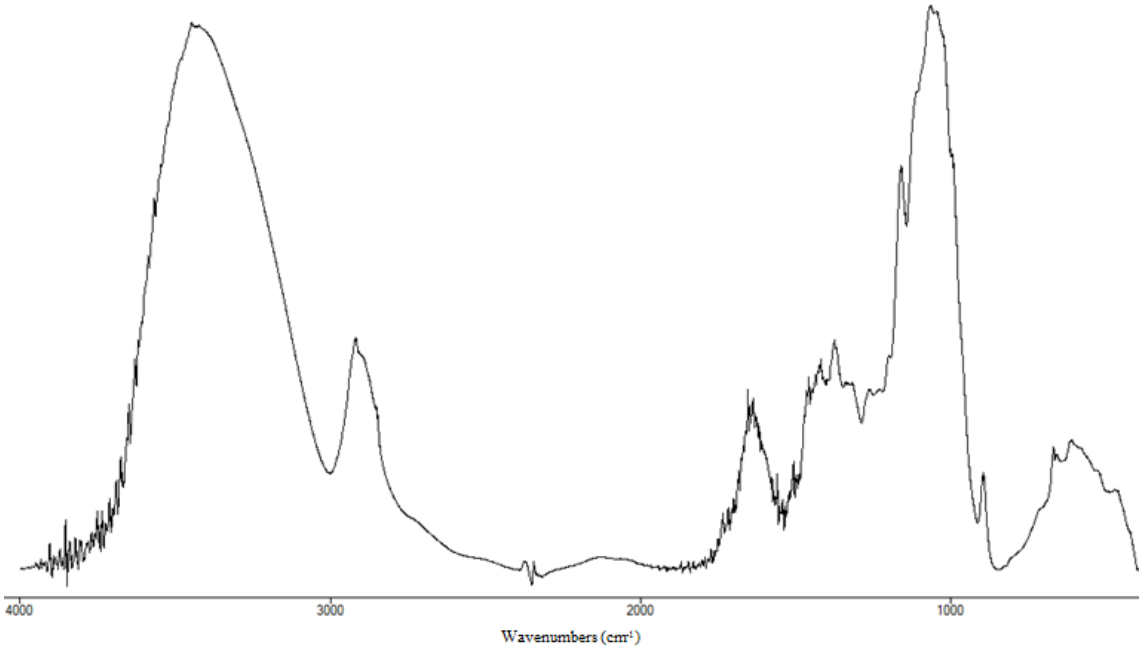
Acid hydrolysed triticale (4000-400 cm^{-1})



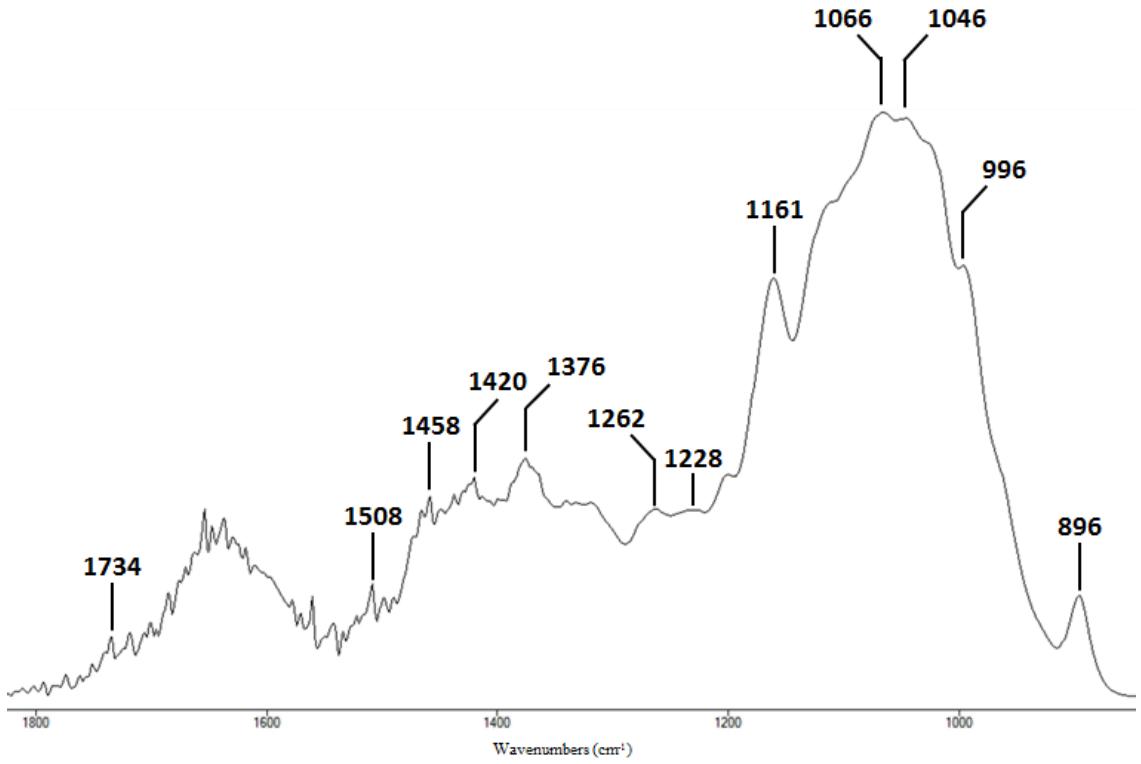
Acid hydrolysed triticale (1800-800 cm^{-1})



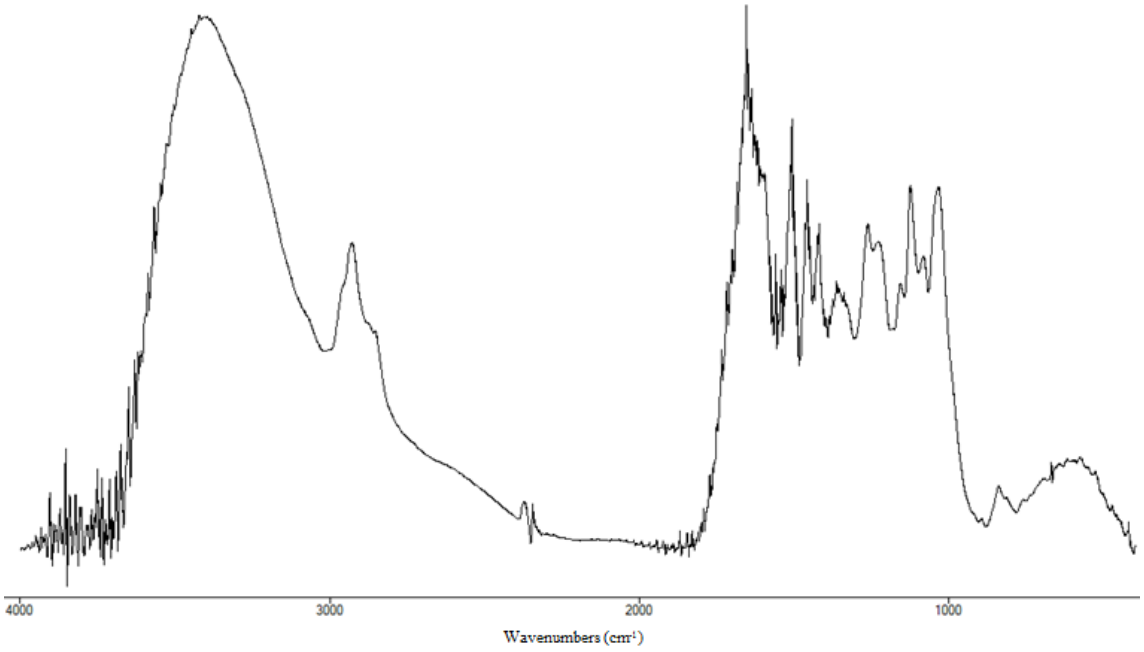
Regenerated material from A method (4000-400 cm^{-1}) – wheat straw



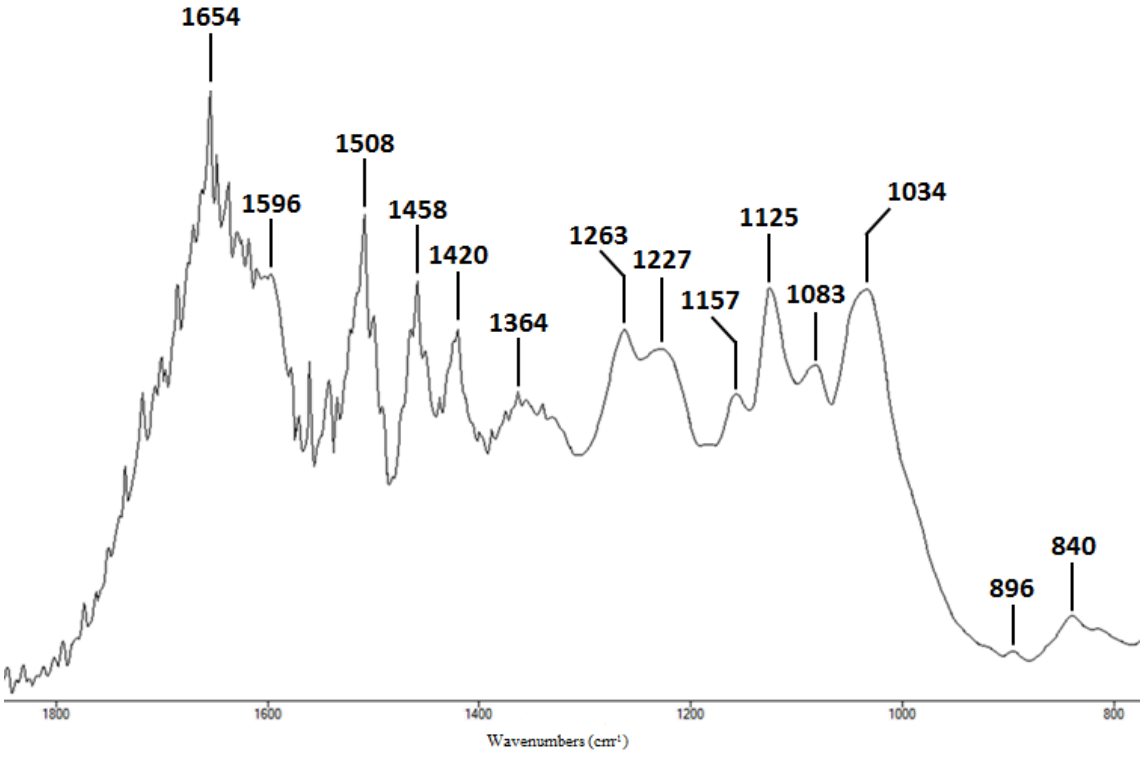
Regenerated material from A method (1800-800 cm^{-1}) – wheat straw



Lignin-rich material from A method (4000-400 cm^{-1}) – wheat straw



Lignin-rich material from A method (1800-800 cm^{-1}) – wheat straw



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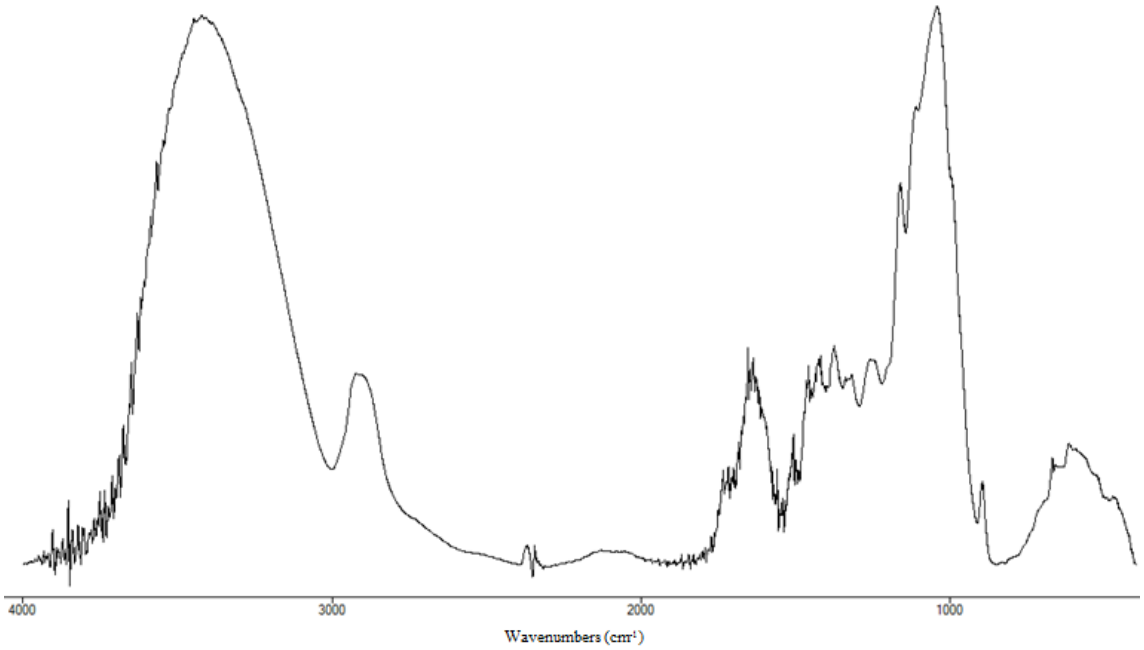

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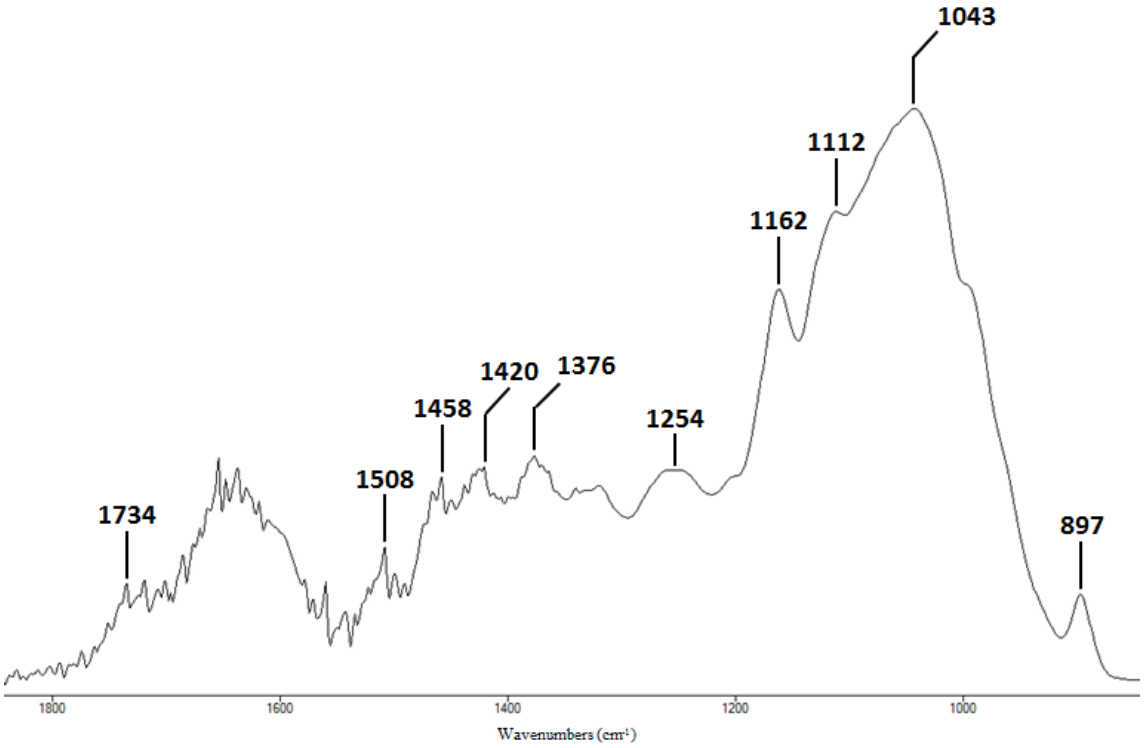
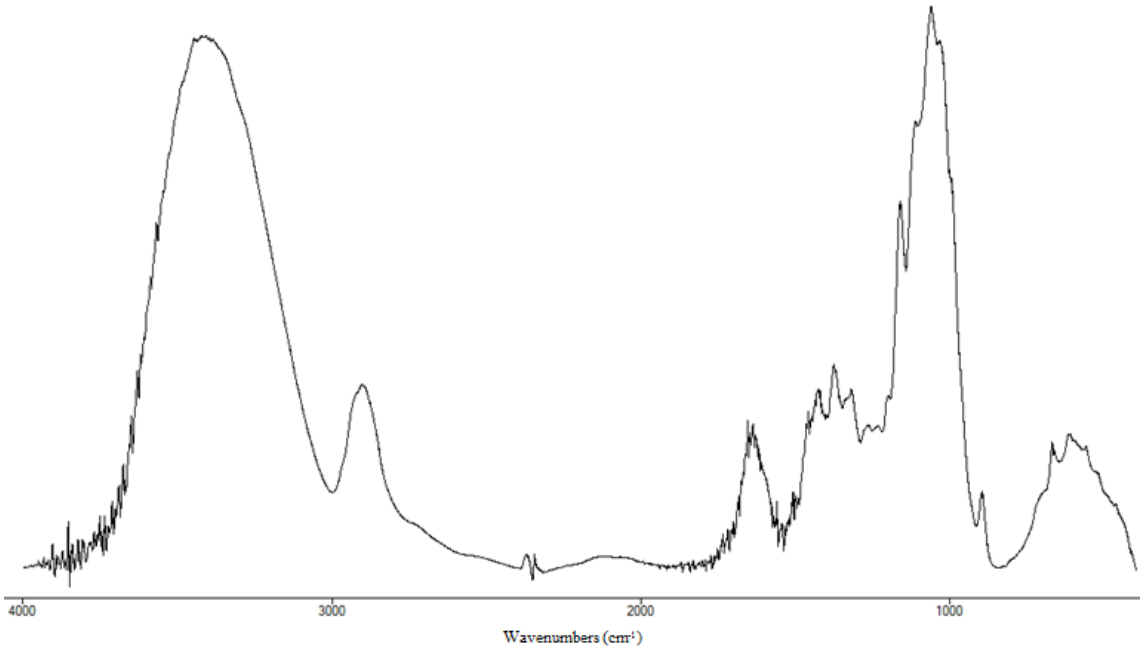


Cellulose-rich material from B method (4000-400 cm^{-1}) – wheat straw



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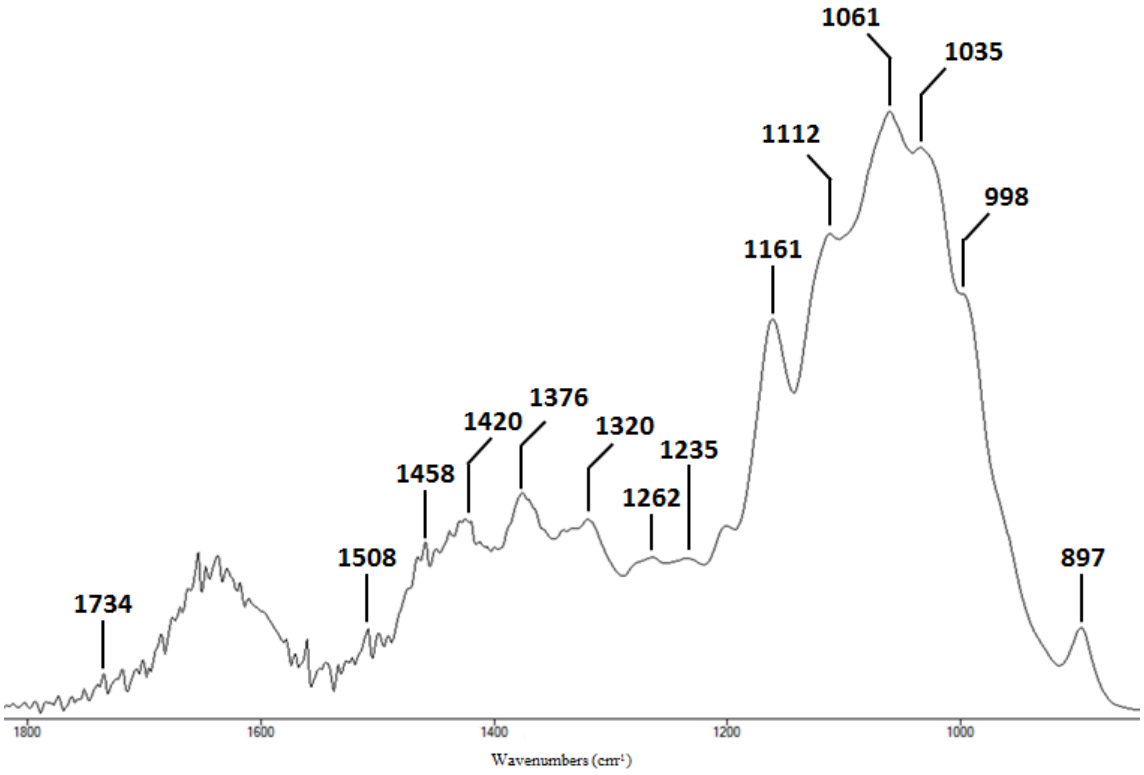
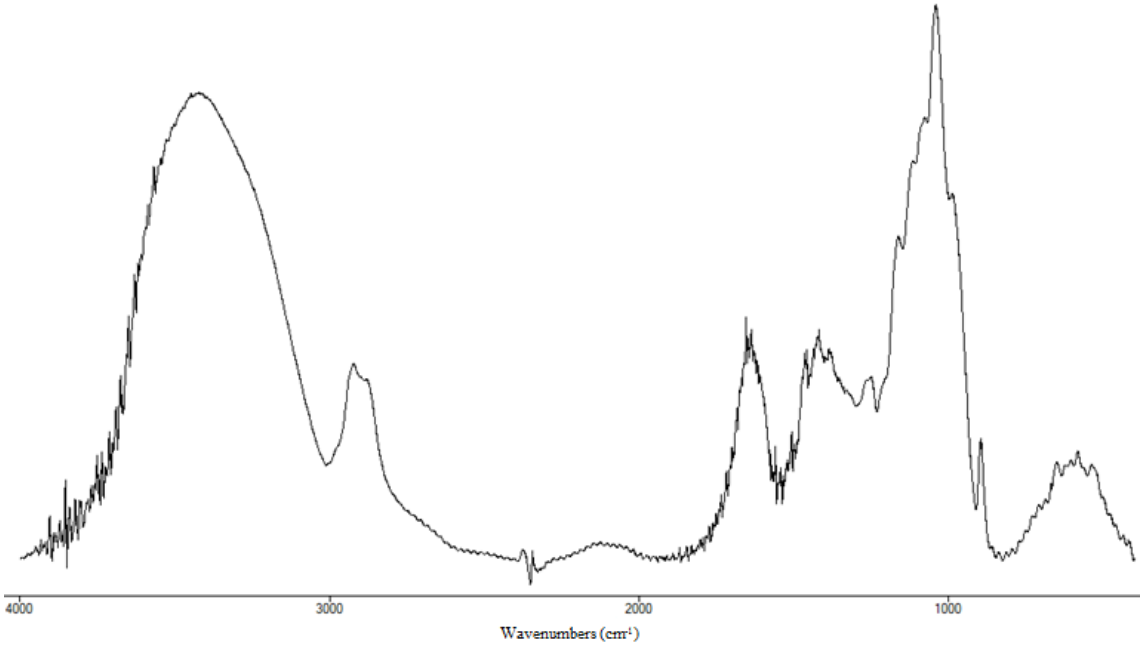


Hemicellulose-rich material from B method (4000-400 cm^{-1}) – wheat straw



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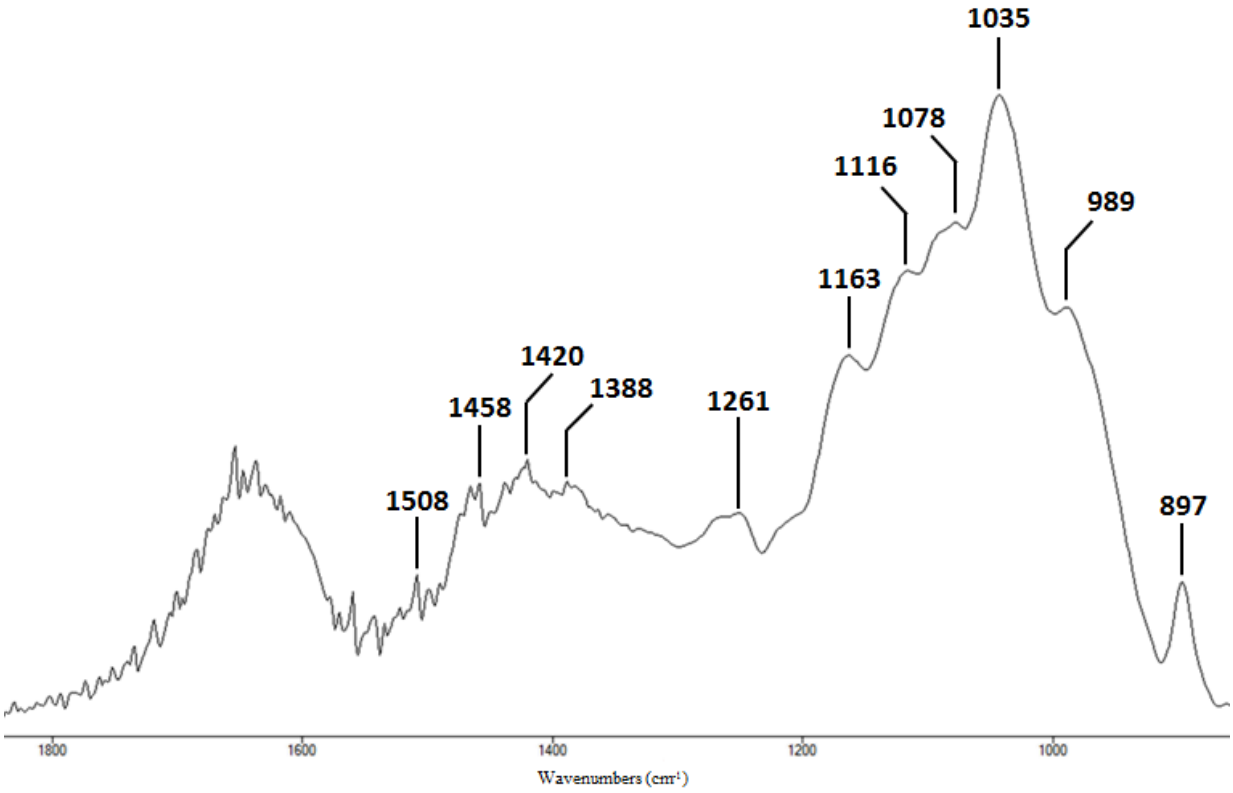
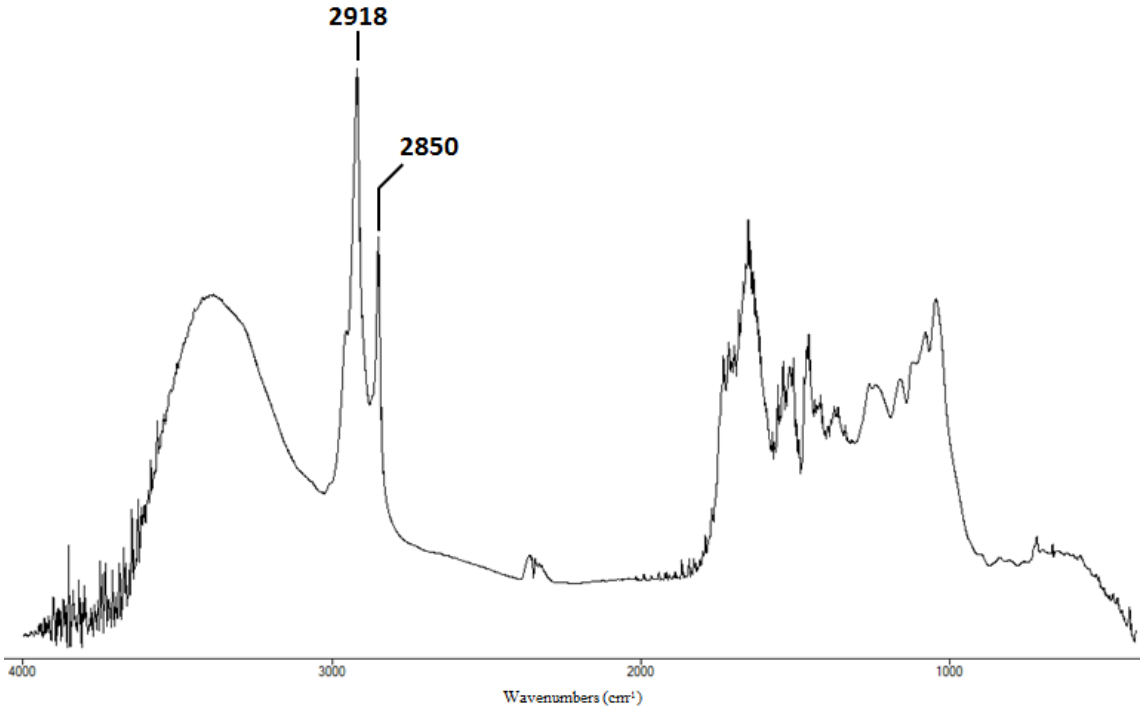
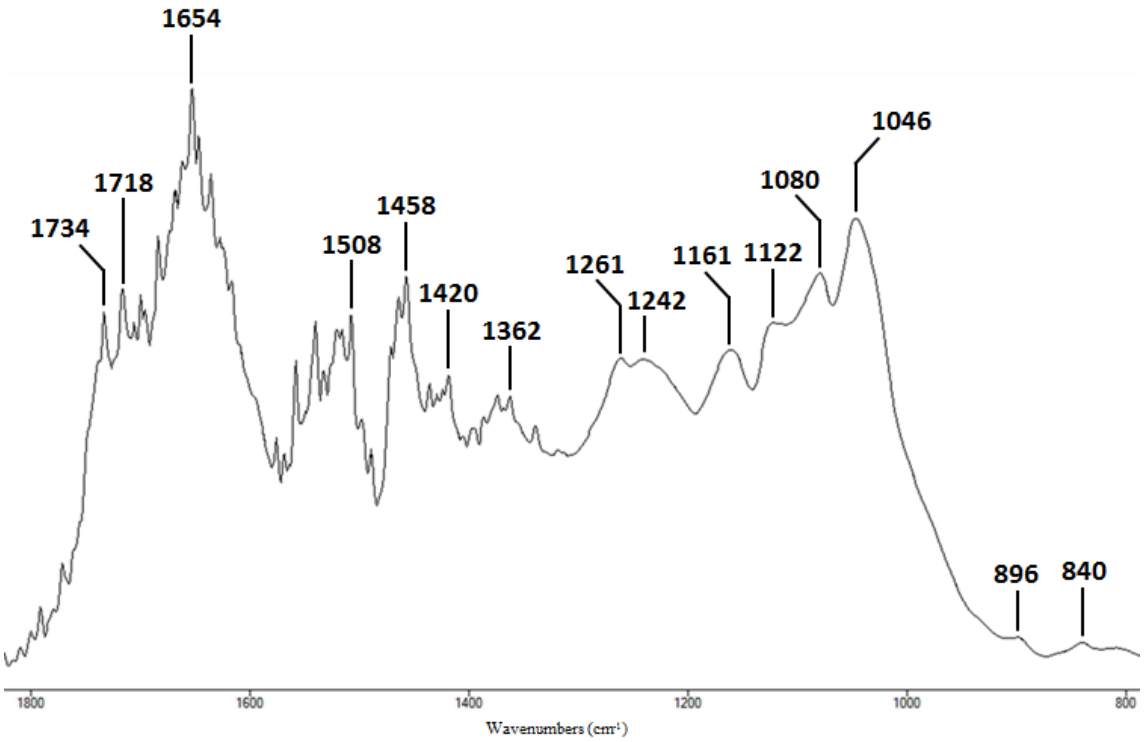


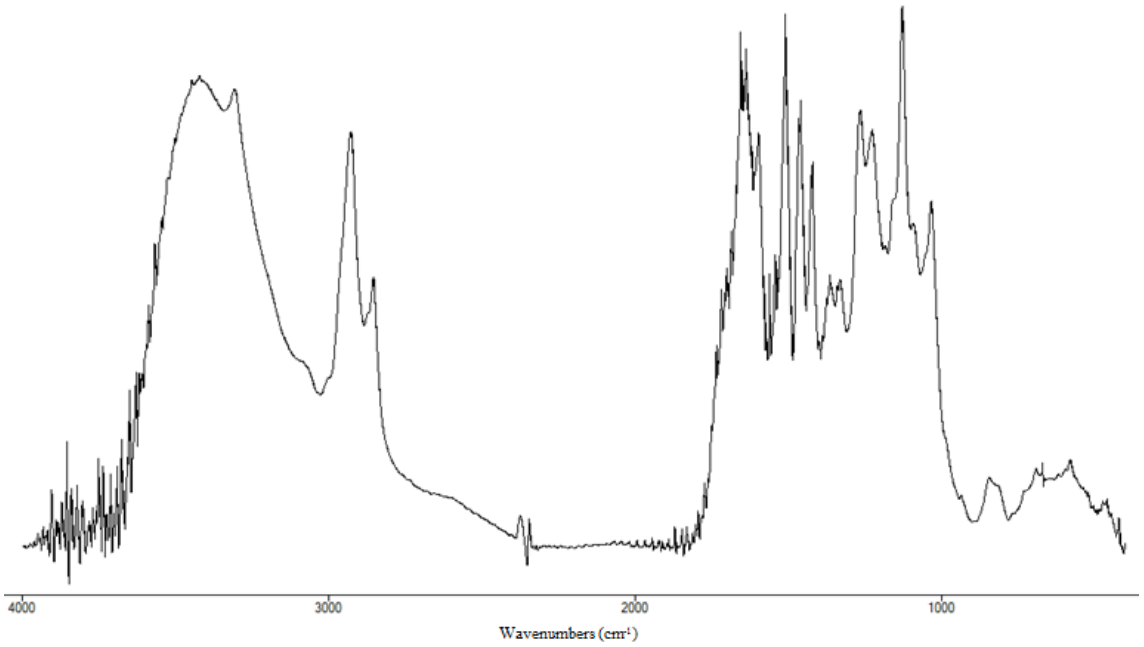
Acetone soluble lignin-rich material from B method (4000-400 cm^{-1}) – wheat straw



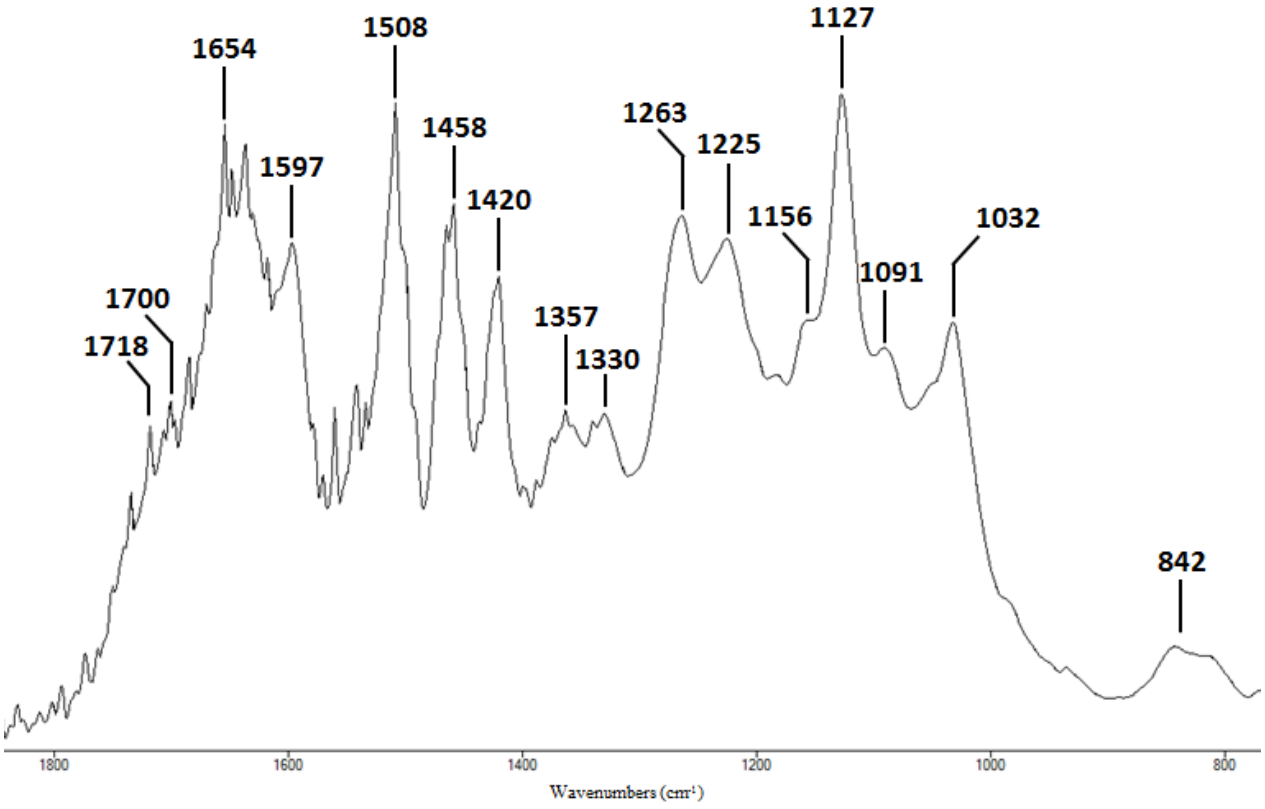
Acetone soluble lignin-rich material from B method (1800-800 cm^{-1}) – wheat straw



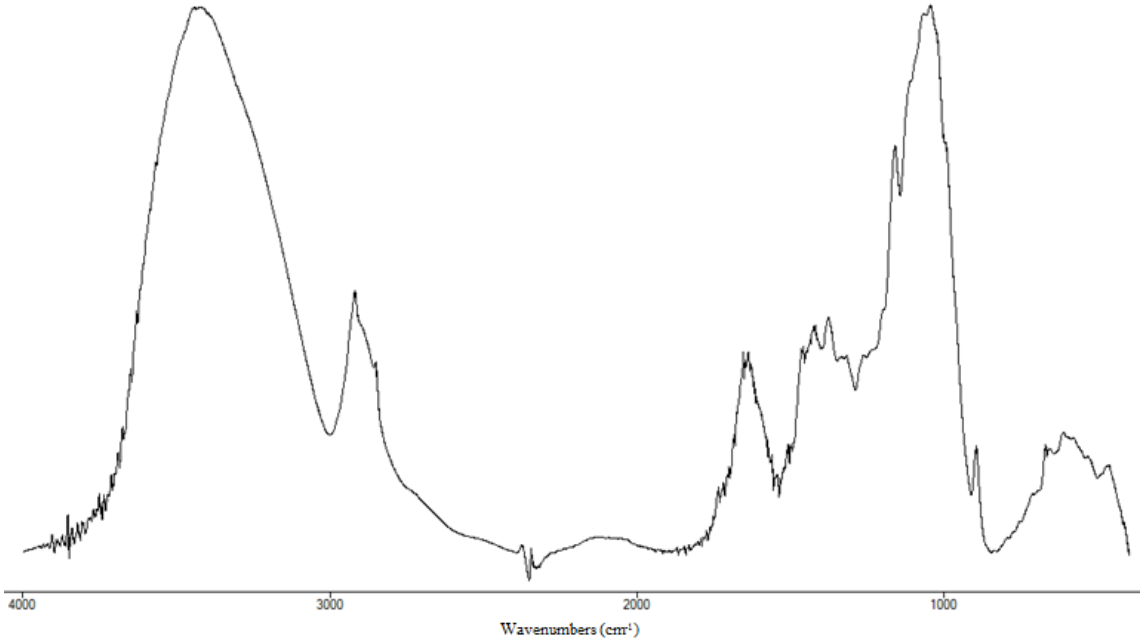
Residual lignin-rich material from B method (4000-400 cm^{-1}) – wheat straw



Residual lignin-rich material from B method (1800-800 cm^{-1}) – wheat straw



Regenerated material from C method (4000-400 cm^{-1}) – wheat straw



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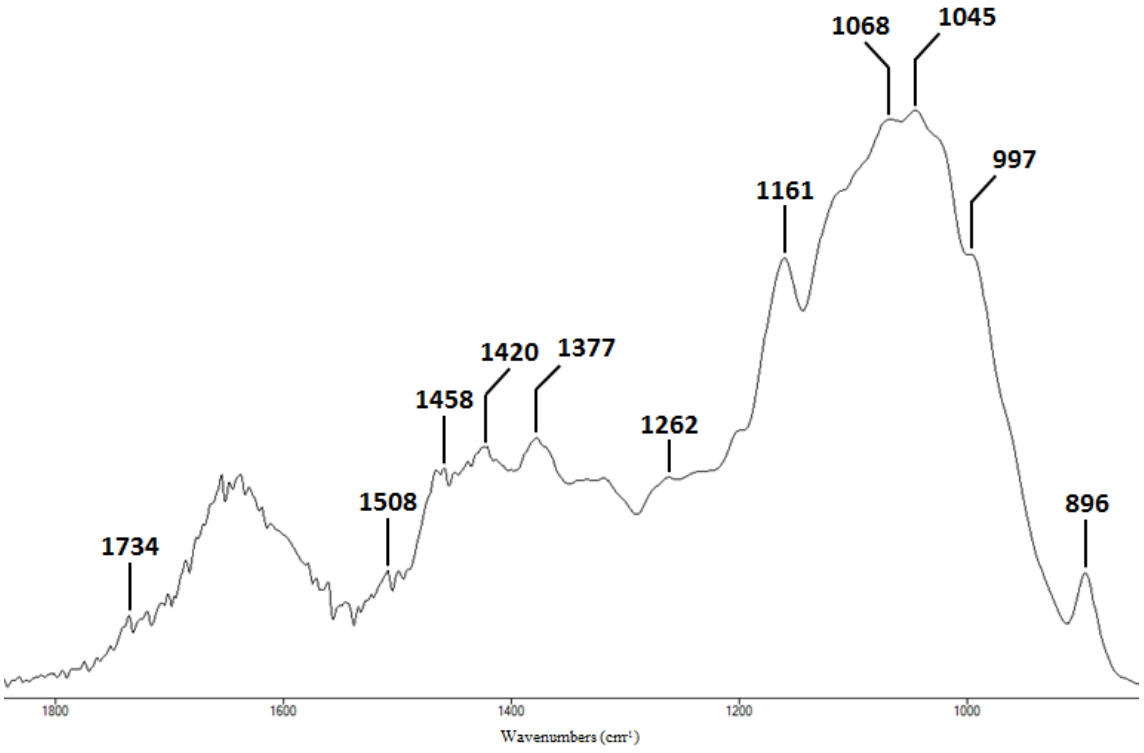
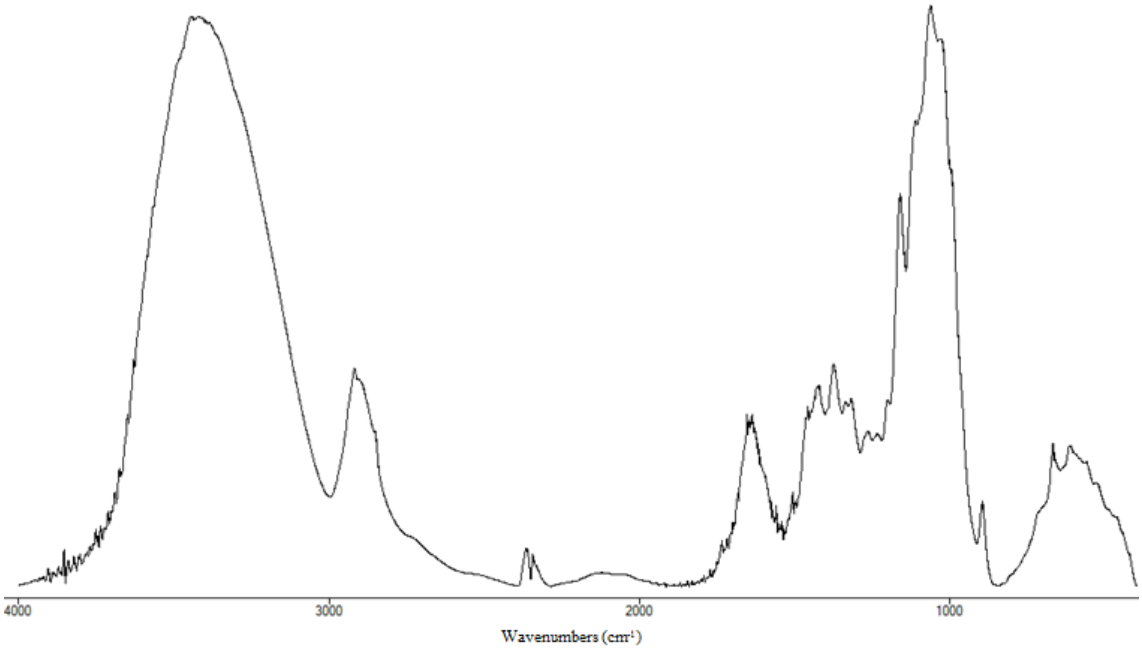


Cellulose-rich material from C method (4000-400 cm^{-1}) – wheat straw



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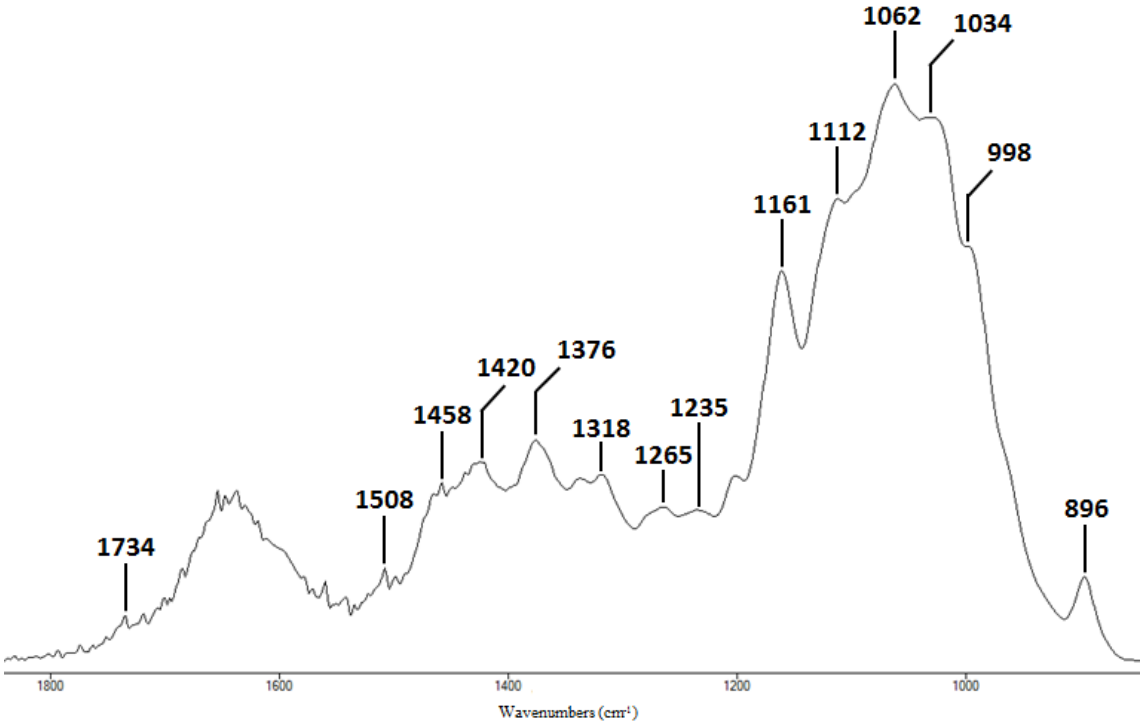
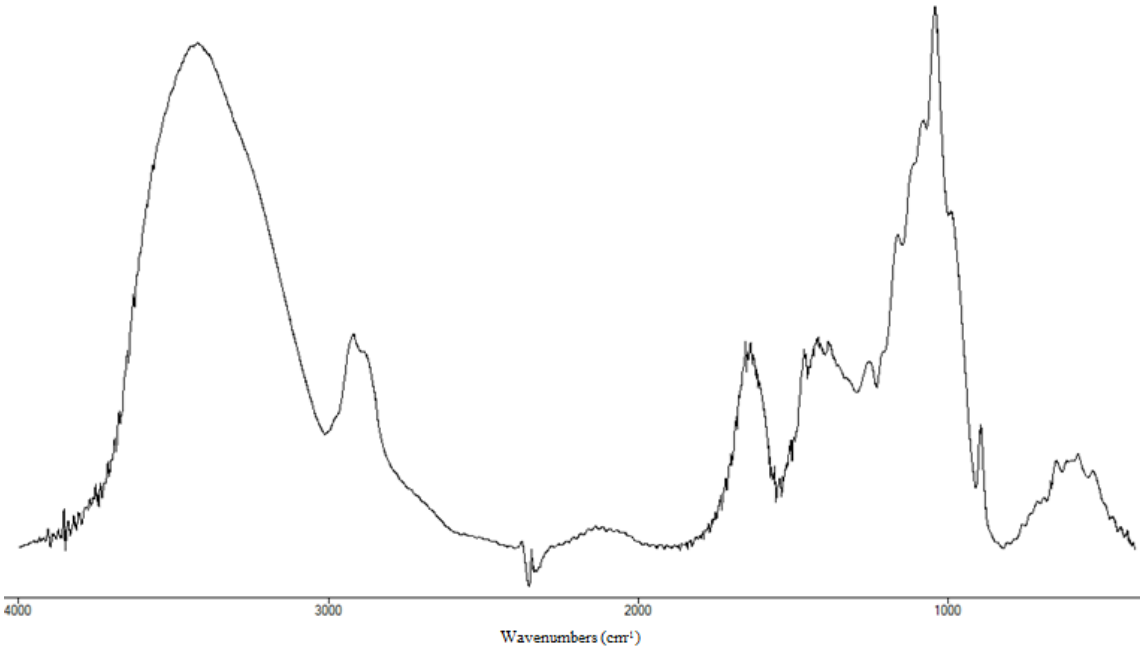


Hemicellulose-rich material from C method (4000-400 cm^{-1}) – wheat straw



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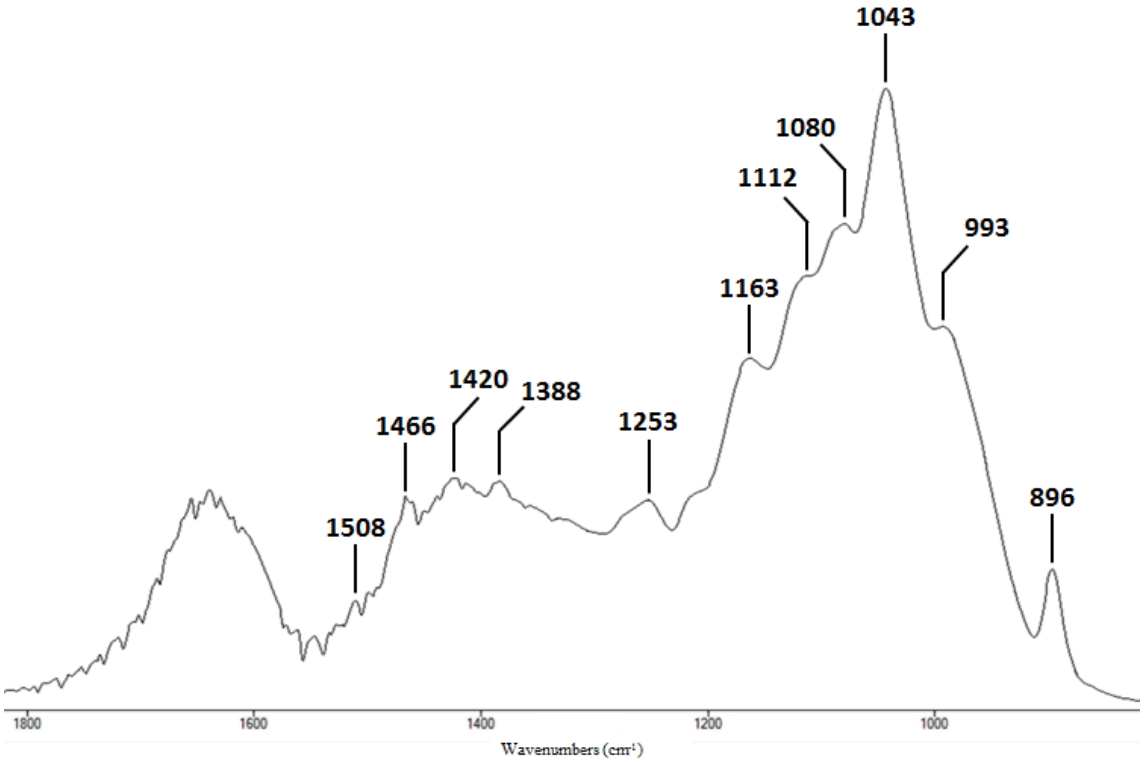
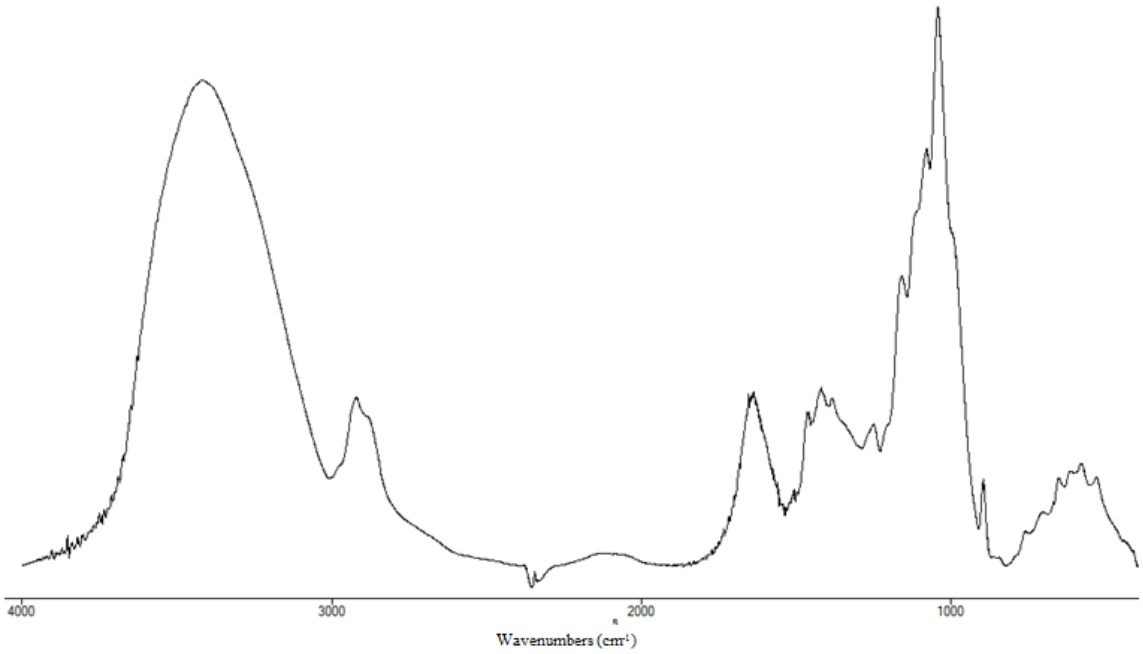
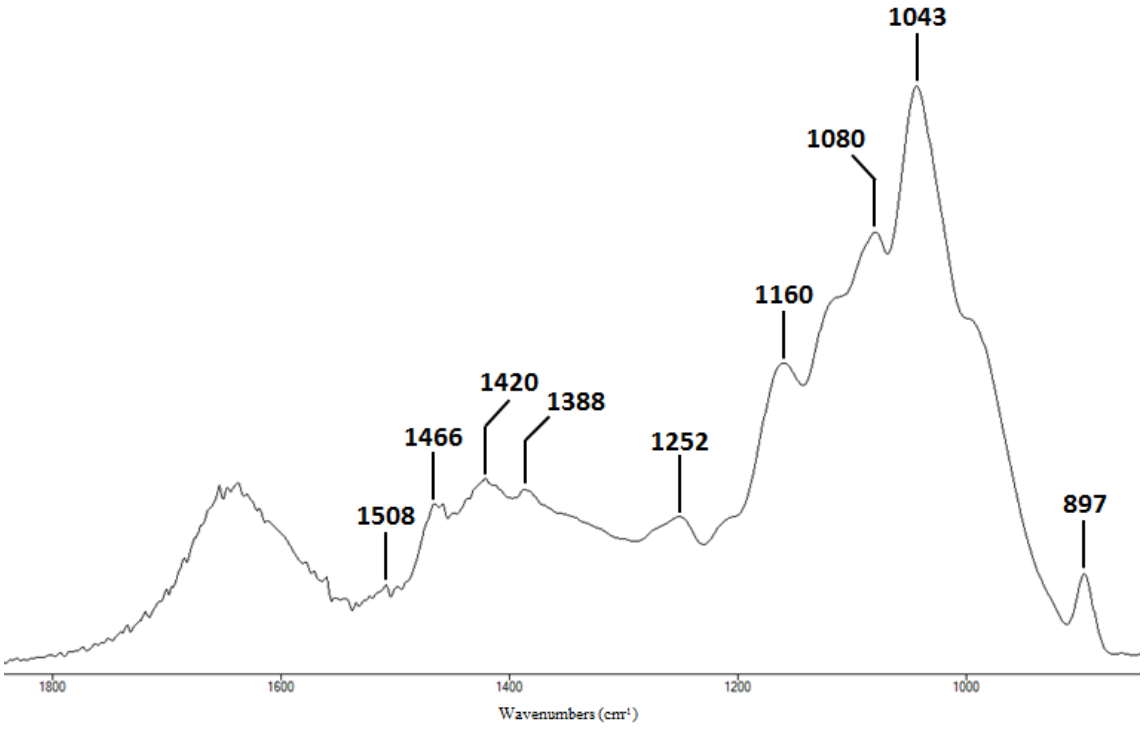


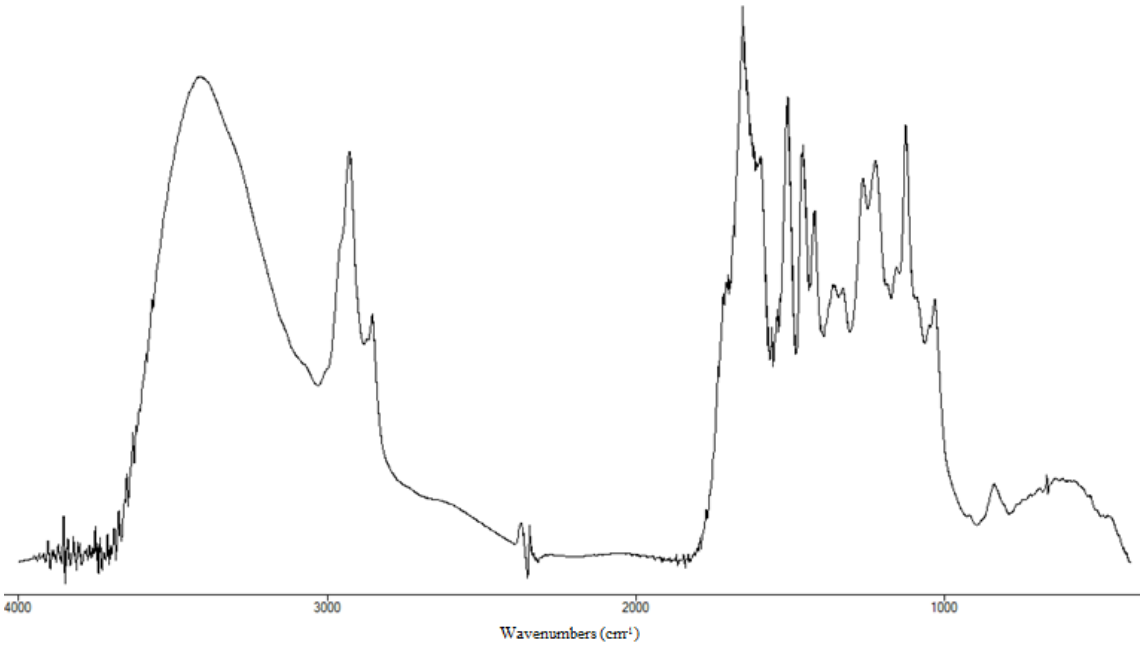
Residual hemicellulose-rich material from C method (4000-400 cm^{-1}) – wheat straw



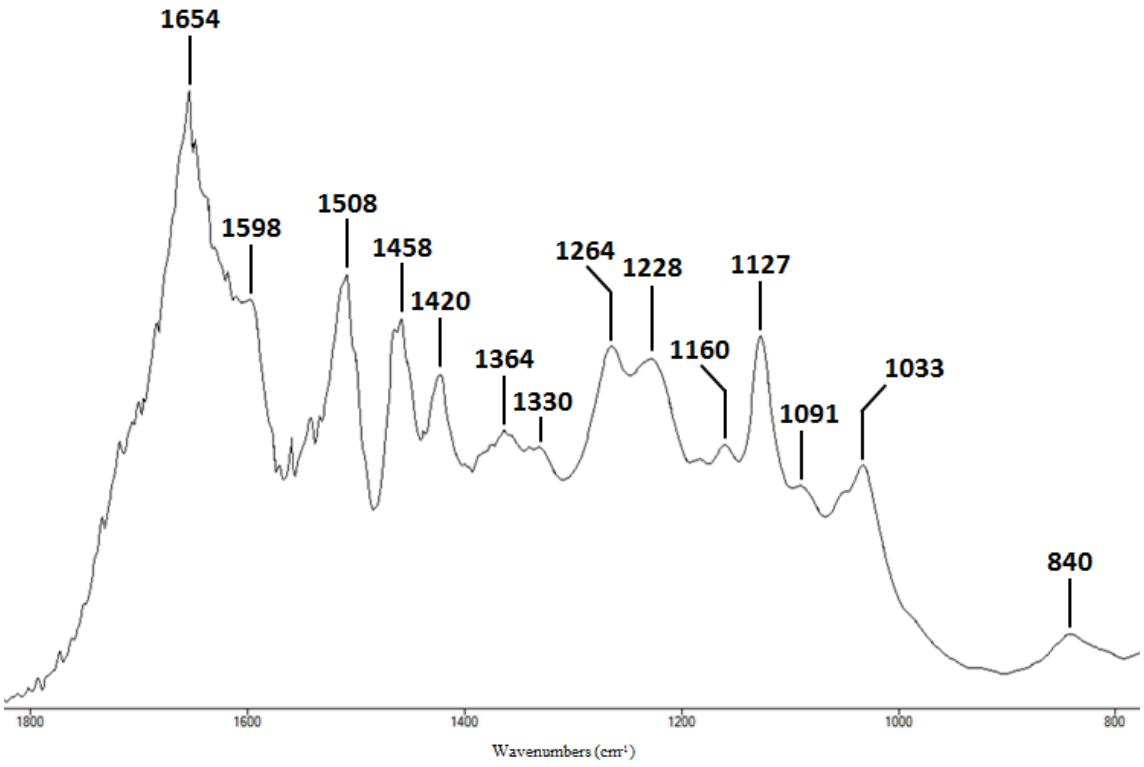
Residual hemicellulose-rich material from C method (1800-800 cm^{-1}) – wheat straw



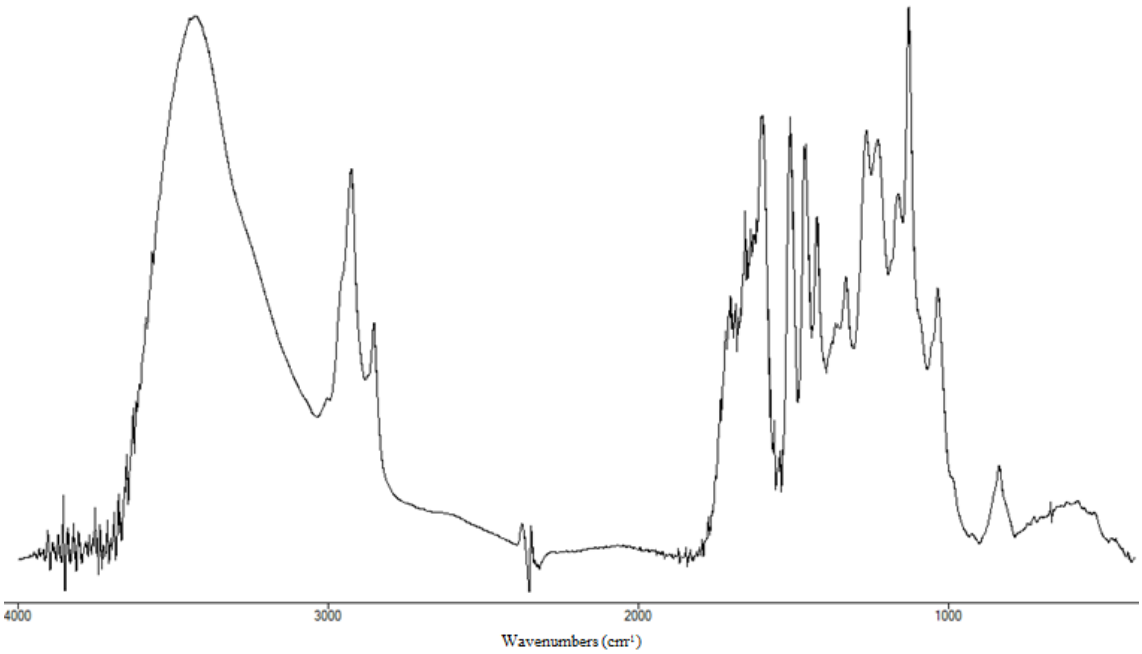
Lignin-rich material from C method (4000-400 cm^{-1}) – wheat straw



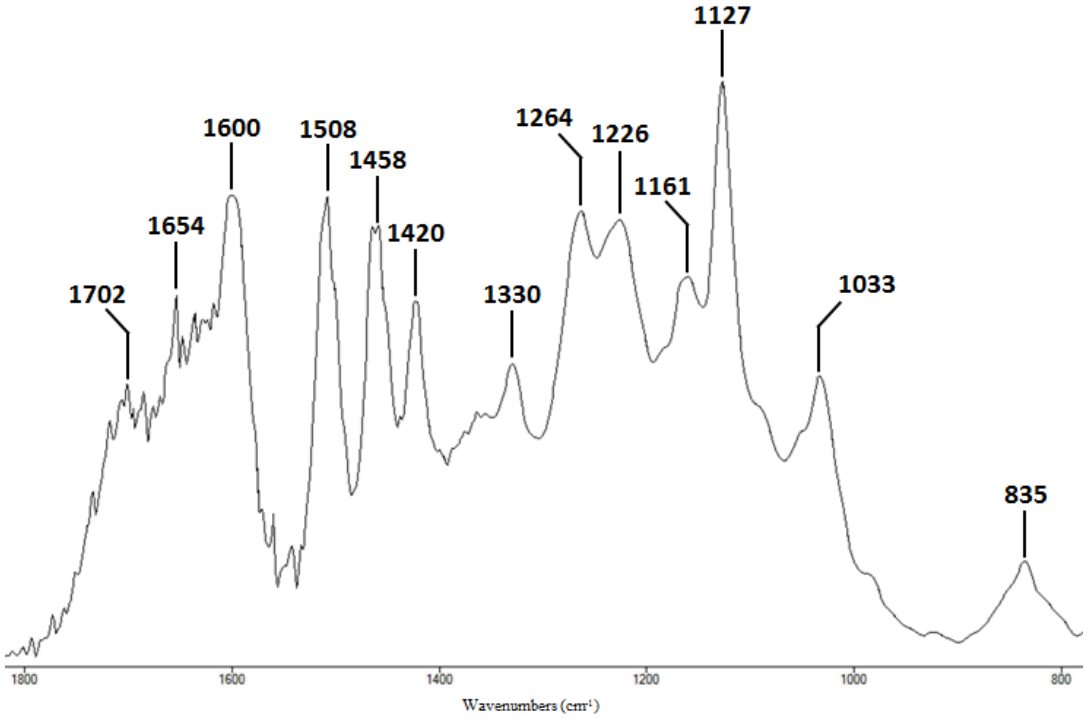
Lignin-rich material from C method (1800-800 cm^{-1}) – wheat straw



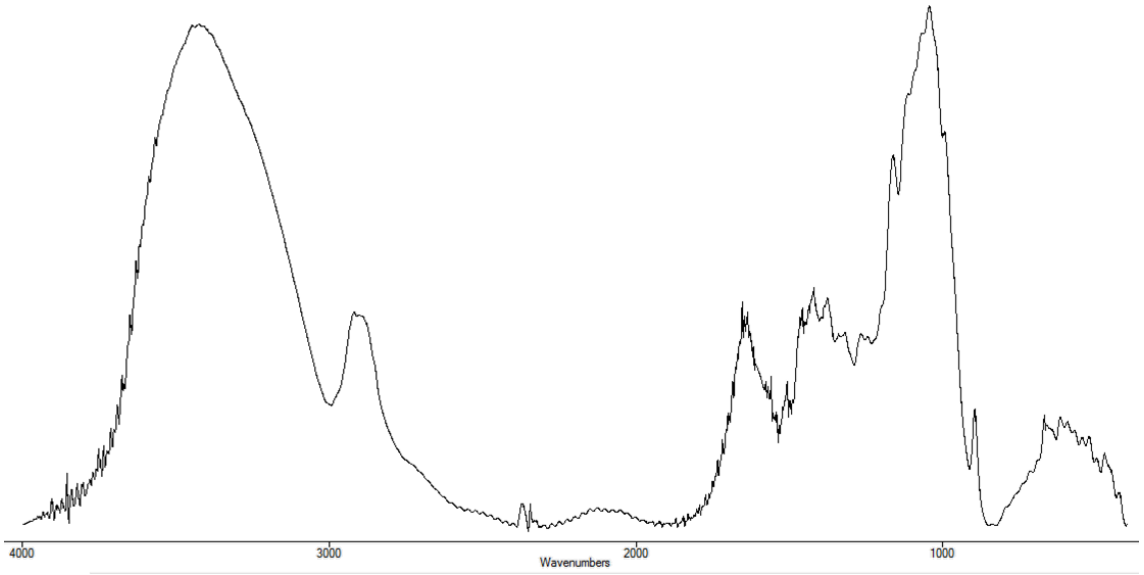
Residual lignin-rich material from C method (4000-400 cm^{-1}) – wheat straw



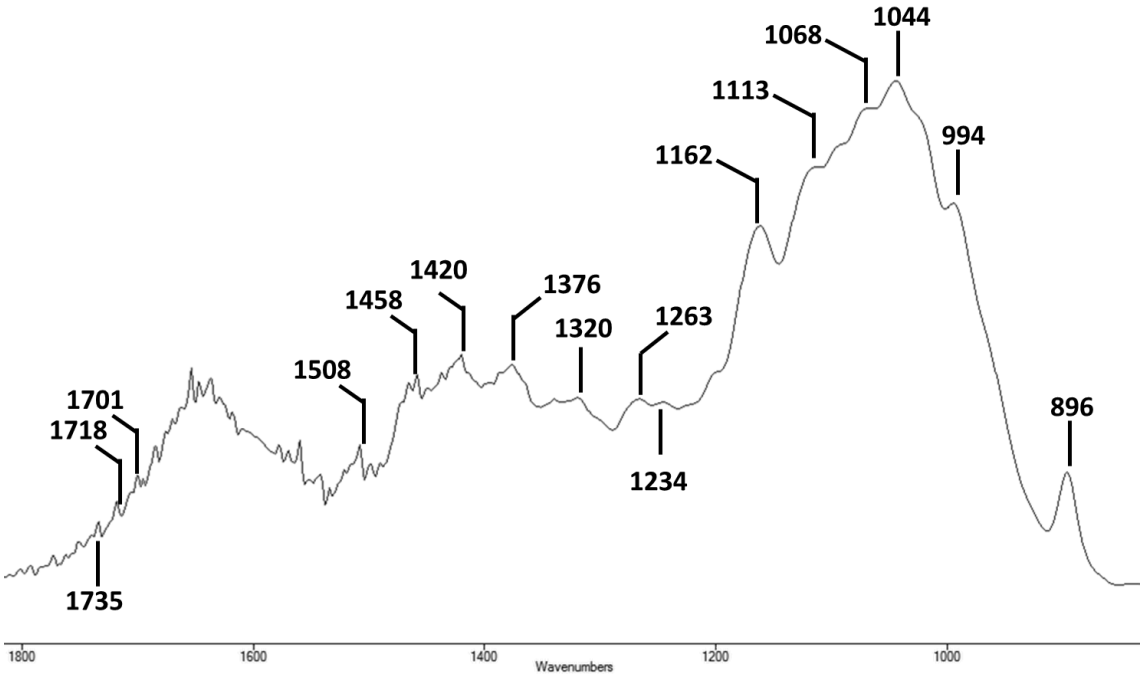
Residual lignin-rich material from C method (1800-800 cm^{-1}) – wheat straw



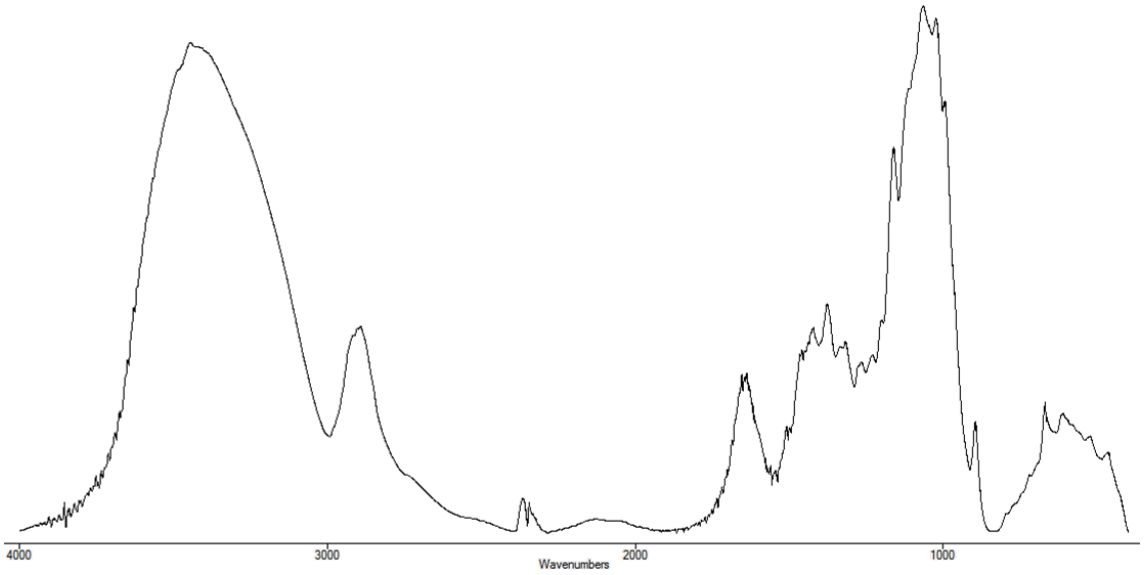
Regenerated material from C method (4000-400 cm^{-1}) – sugarcane bagasse



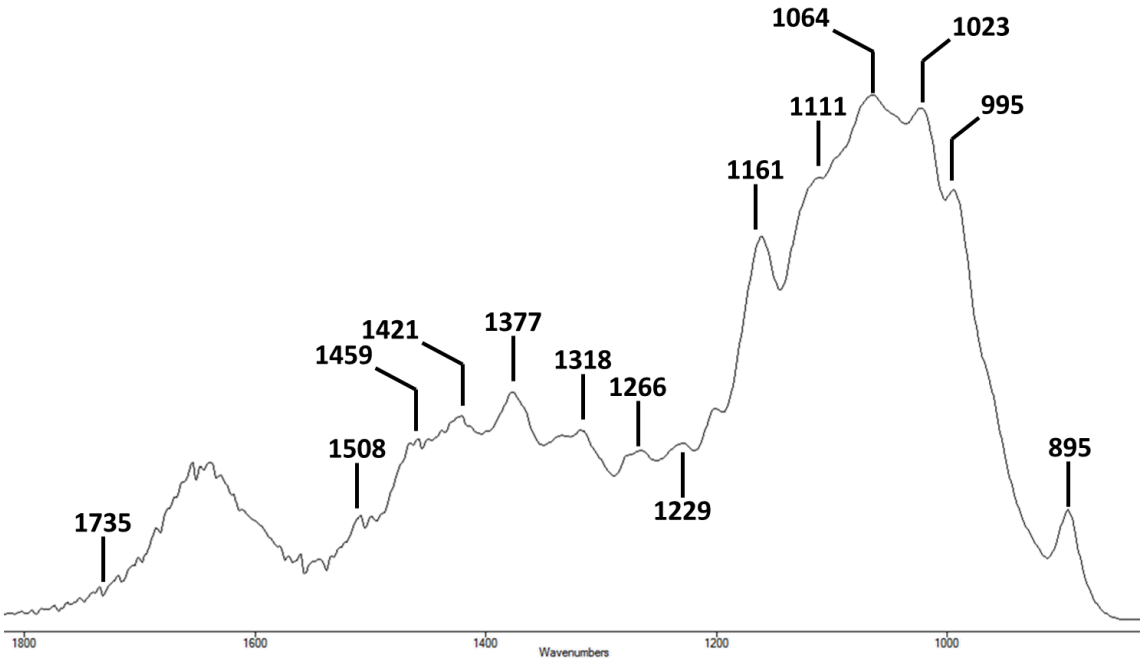
Regenerated material from C method (1800-800 cm^{-1}) – sugarcane bagasse



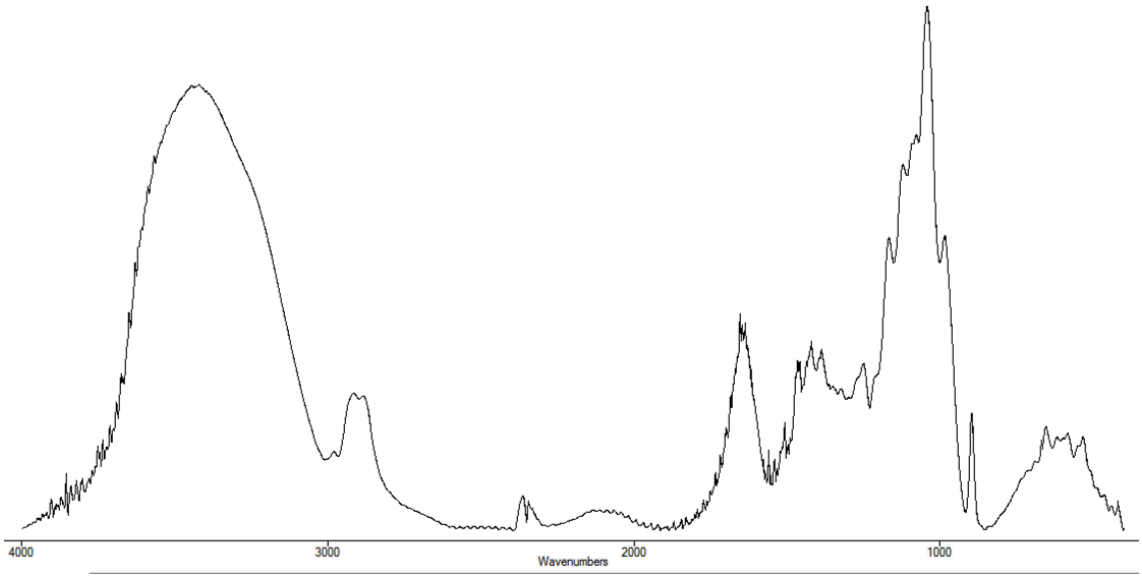
Cellulose-rich material from C method (4000-400 cm^{-1}) – sugarcane bagasse



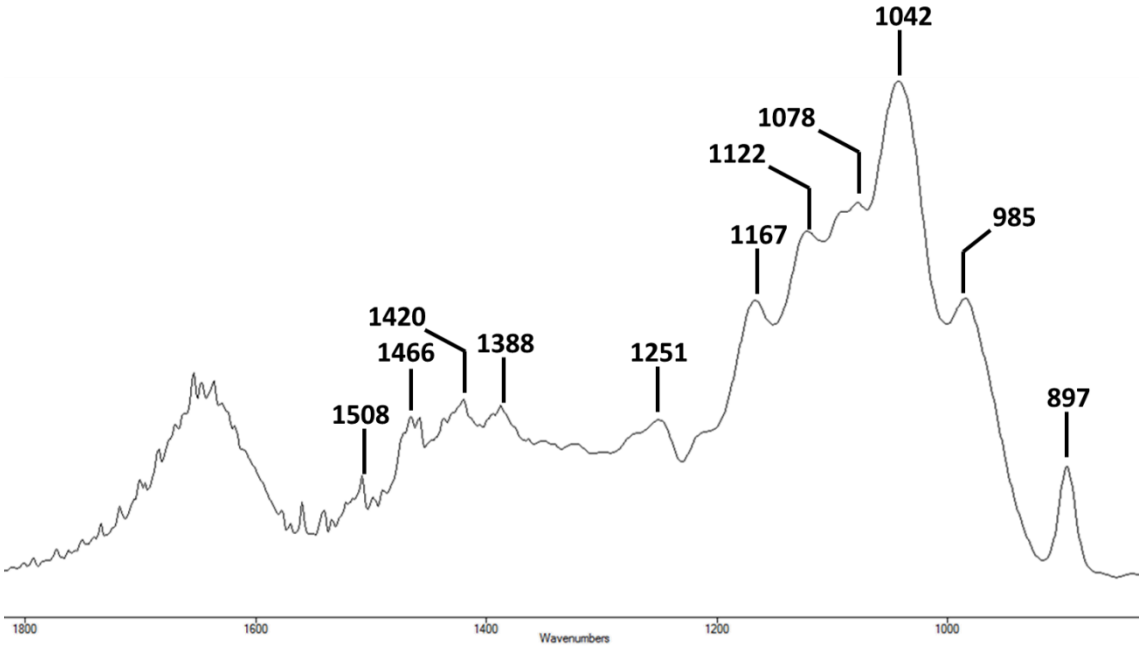
Cellulose-rich material from C method (1800-800 cm^{-1}) – sugarcane bagasse



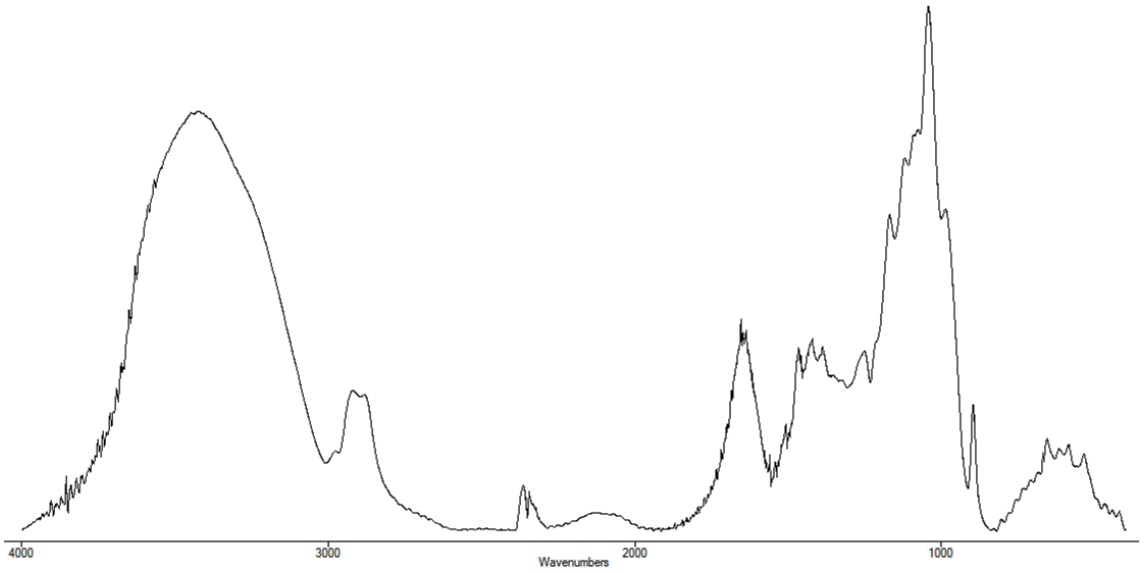
Hemicellulose-rich material from C method (4000-400 cm^{-1}) – sugarcane bagasse



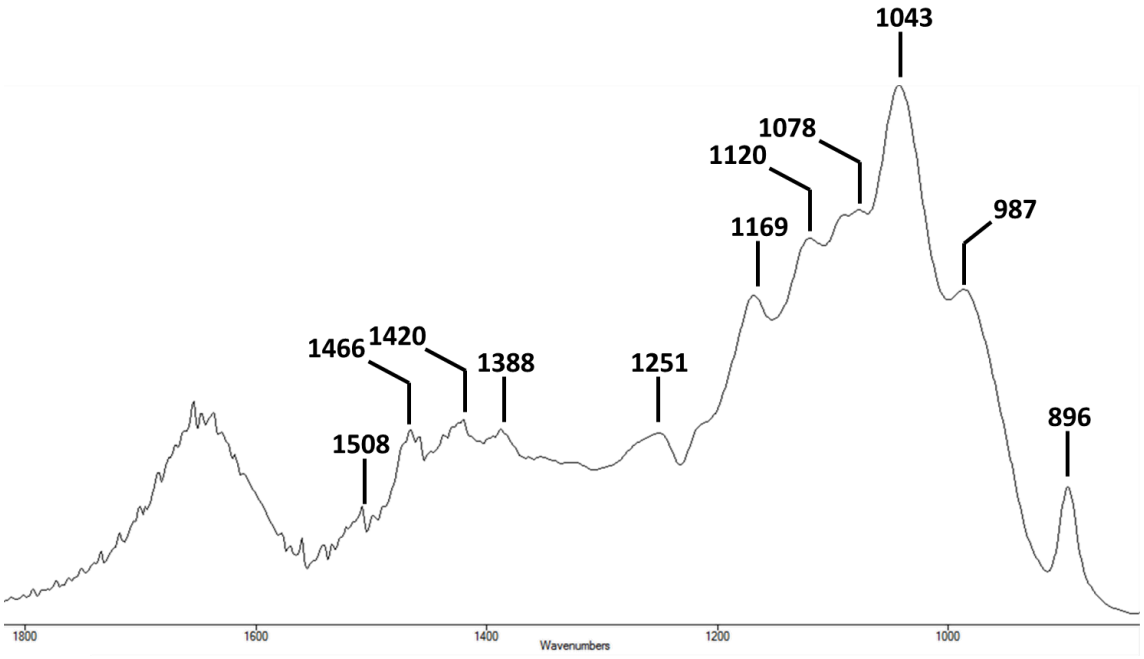
Hemicellulose-rich material from C method (1800-800 cm^{-1}) – sugarcane bagasse



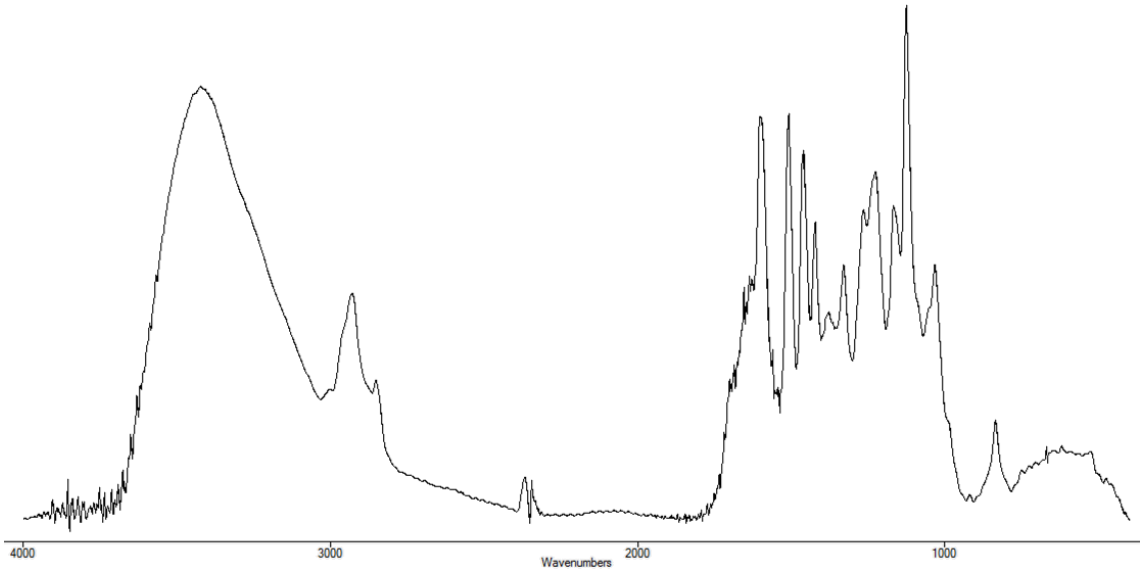
Residual hemicellulose-rich material from C method (4000-400 cm^{-1}) – sugarcane bagasse



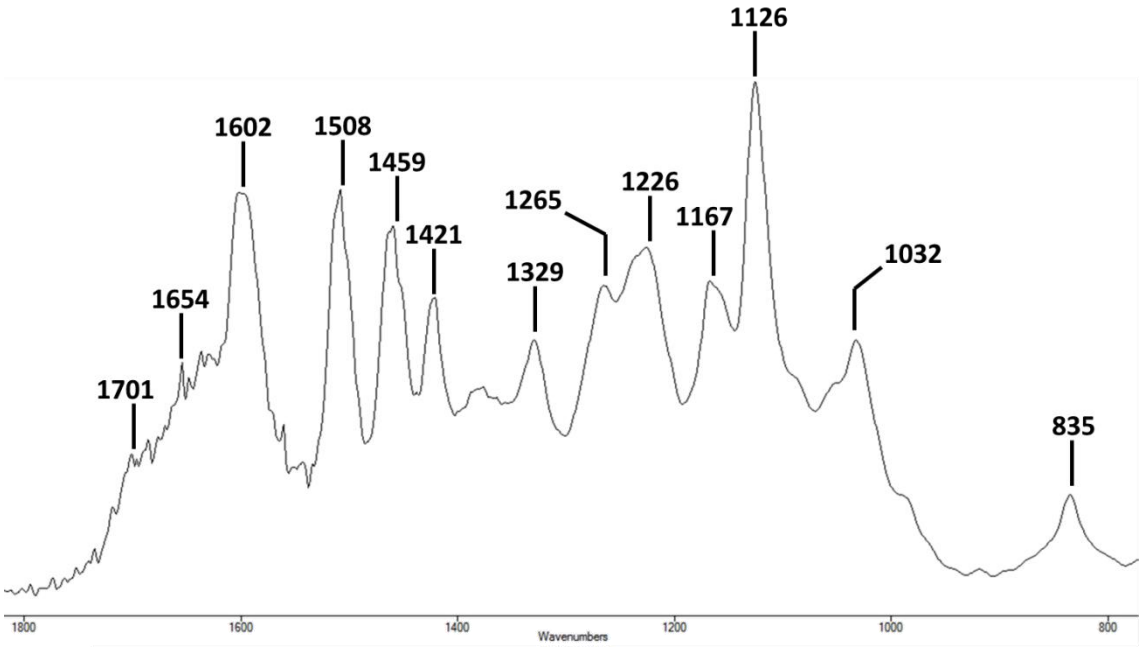
Residual hemicellulose-rich material from C method (1800-800 cm^{-1}) – sugarcane bagasse



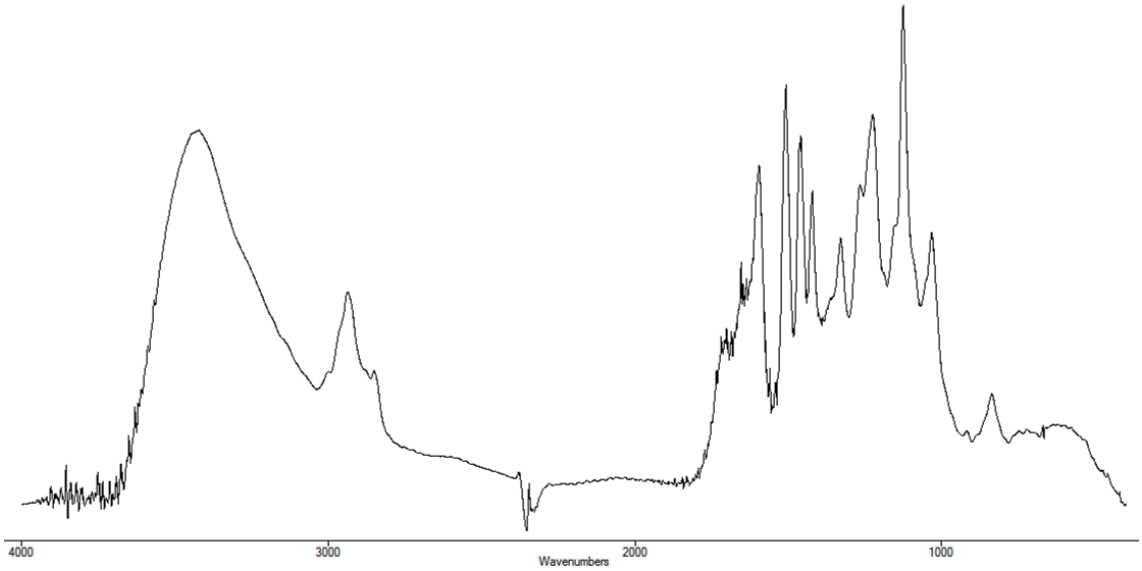
Residual lignin-rich material (solid recovered after the filtration of residual hemicellulose-rich material) from C method (4000-400 cm^{-1}) – sugarcane bagasse



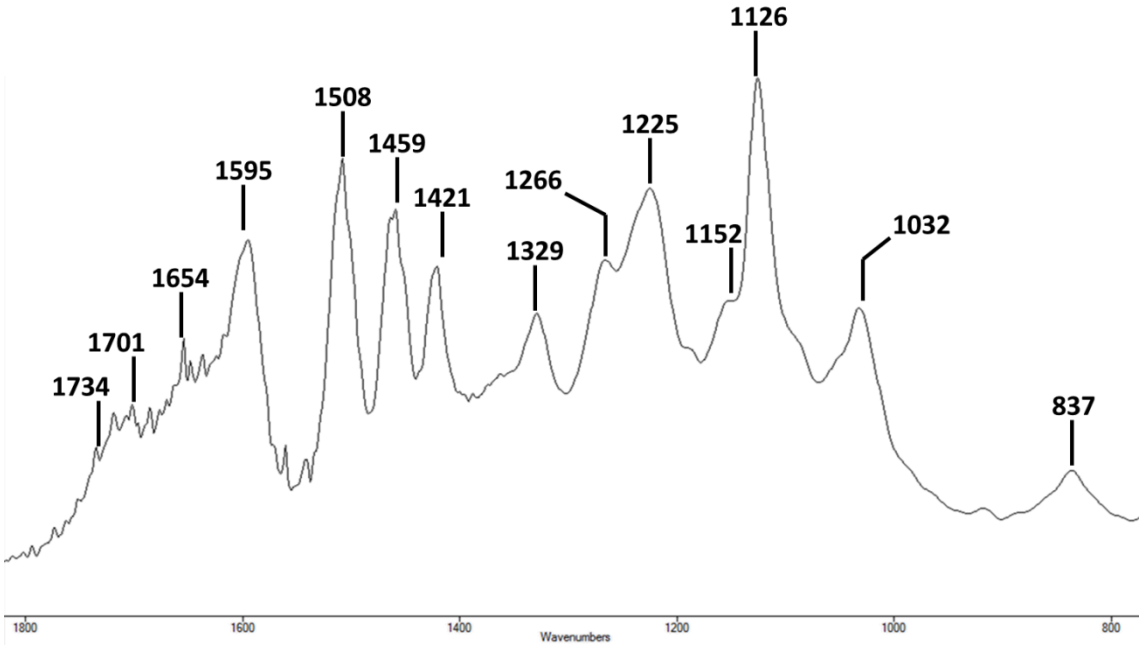
Residual lignin-rich material (solid recovered after the filtration of residual hemicellulose-rich material) from C method (1800-800 cm^{-1}) – sugarcane bagasse



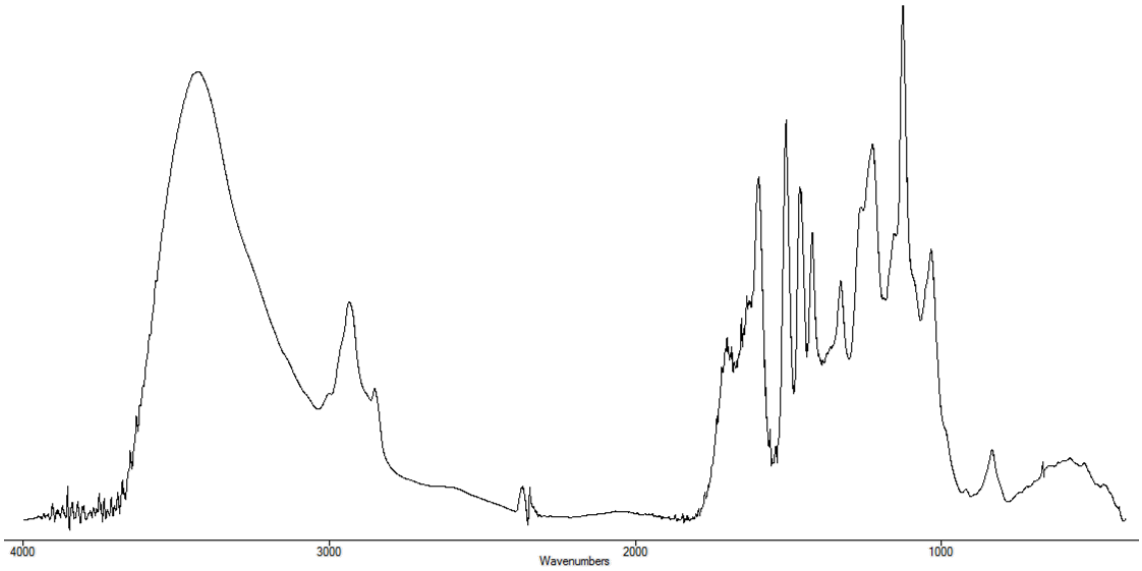
Lignin-rich material from C method (4000-400 cm^{-1}) – sugarcane bagasse



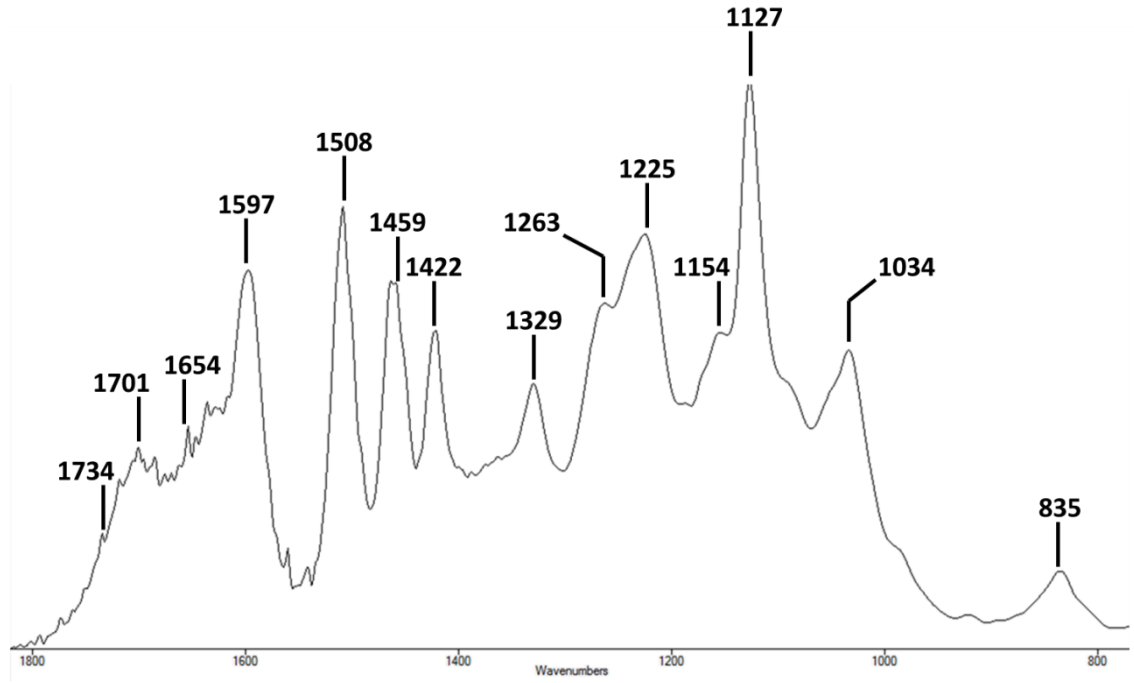
Lignin-rich material from C method (1800-800 cm^{-1}) – sugarcane bagasse



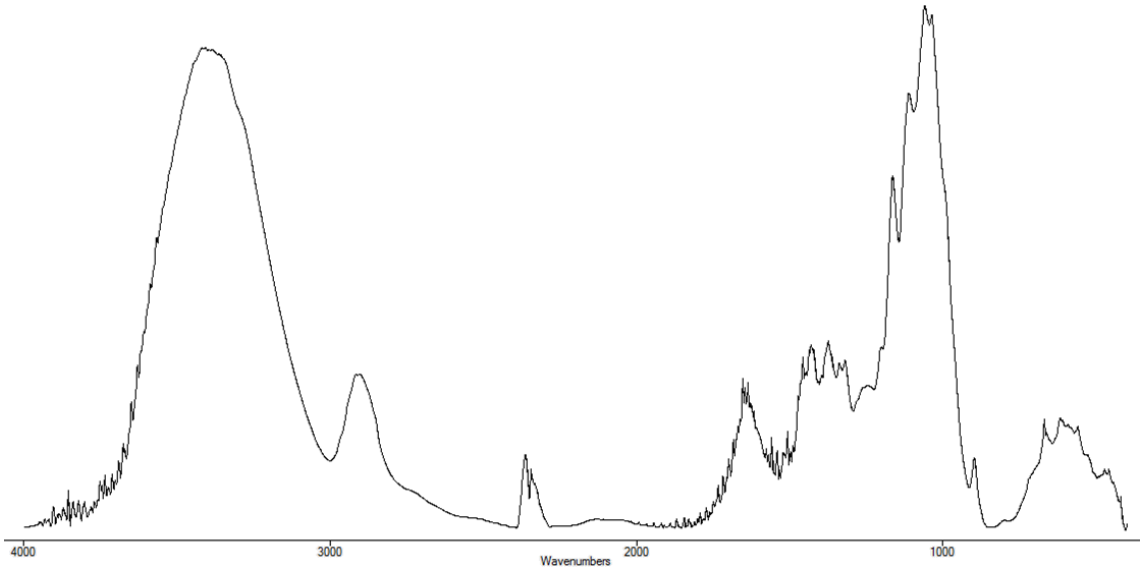
Residual lignin-rich material from C method (4000-400 cm^{-1}) – sugarcane bagasse



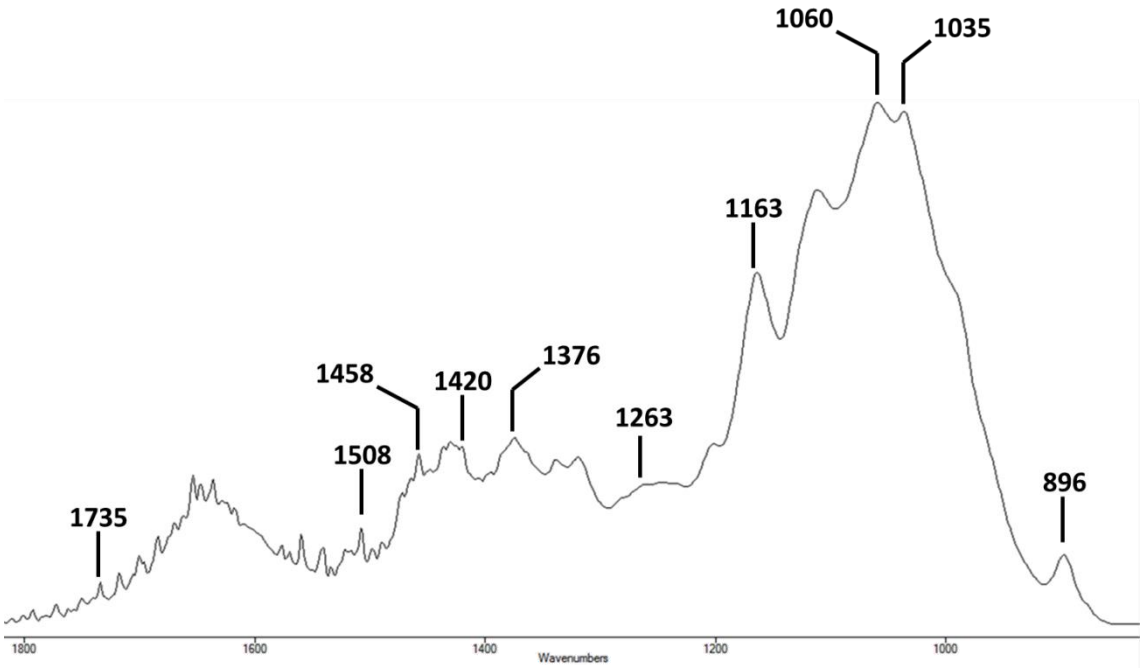
Residual lignin-rich material from C method (1800-800 cm^{-1}) – sugarcane bagasse



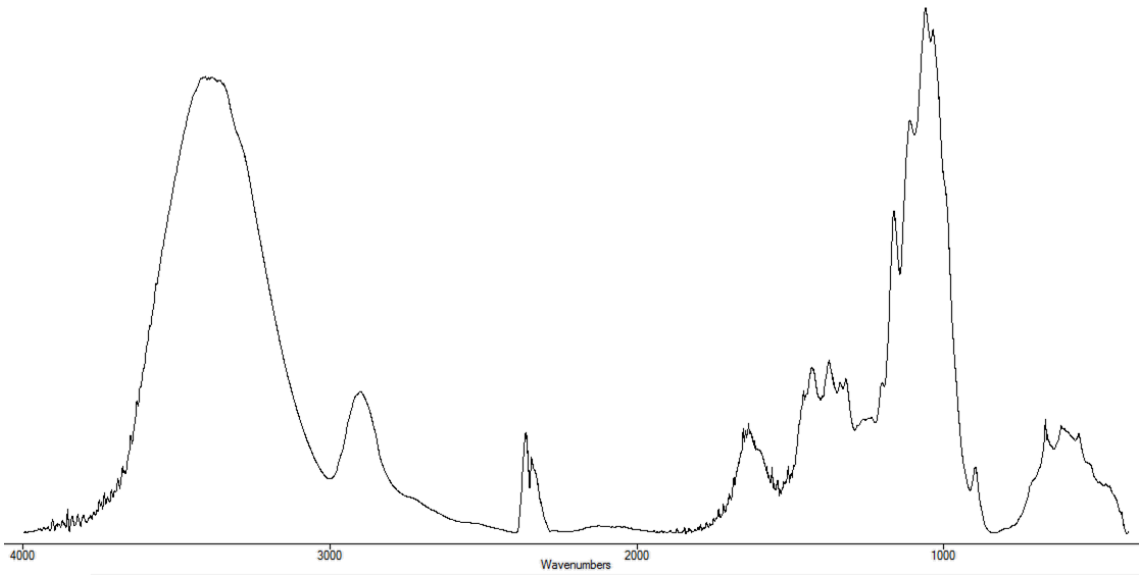
Regenerated material from C method (4000-400 cm^{-1}) – rice straw



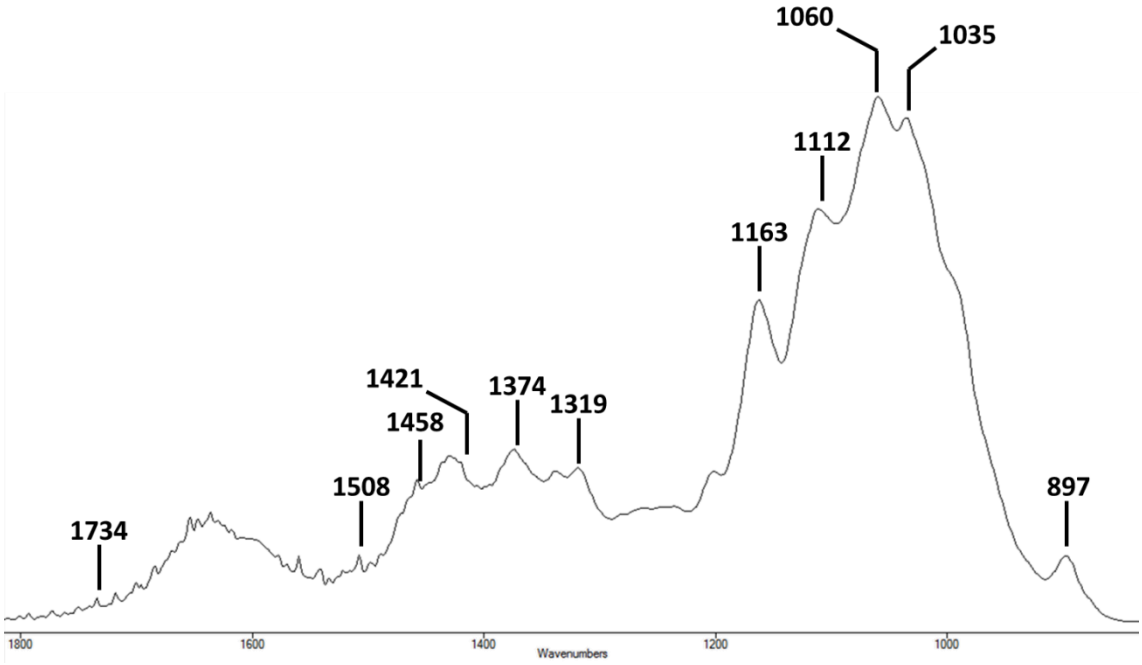
Regenerated material from C method (1800-800 cm^{-1}) – rice straw



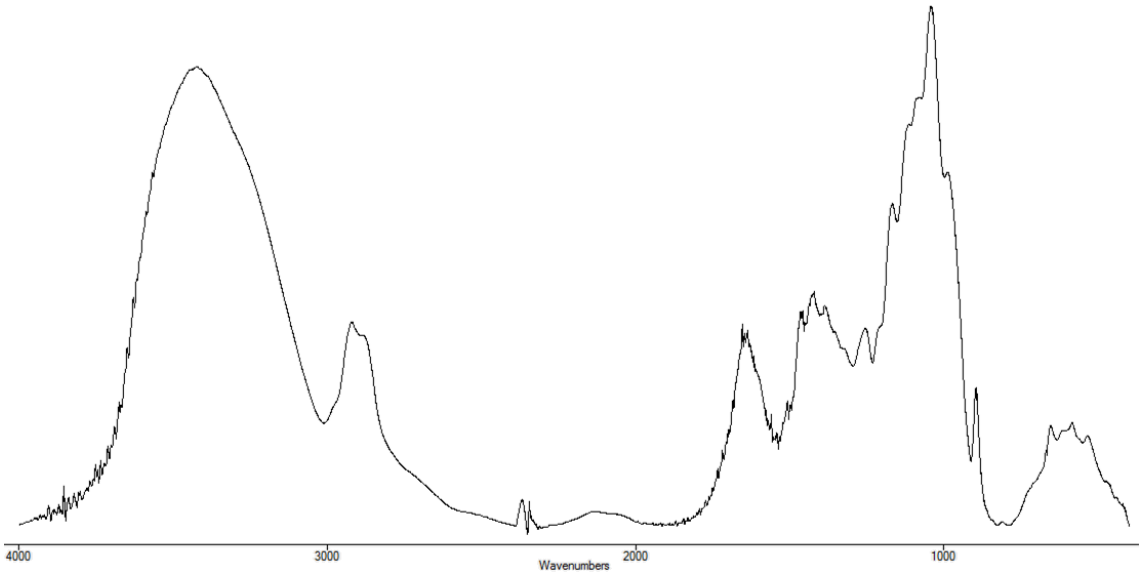
Cellulose-rich material from C method (4000-400 cm^{-1}) – rice straw



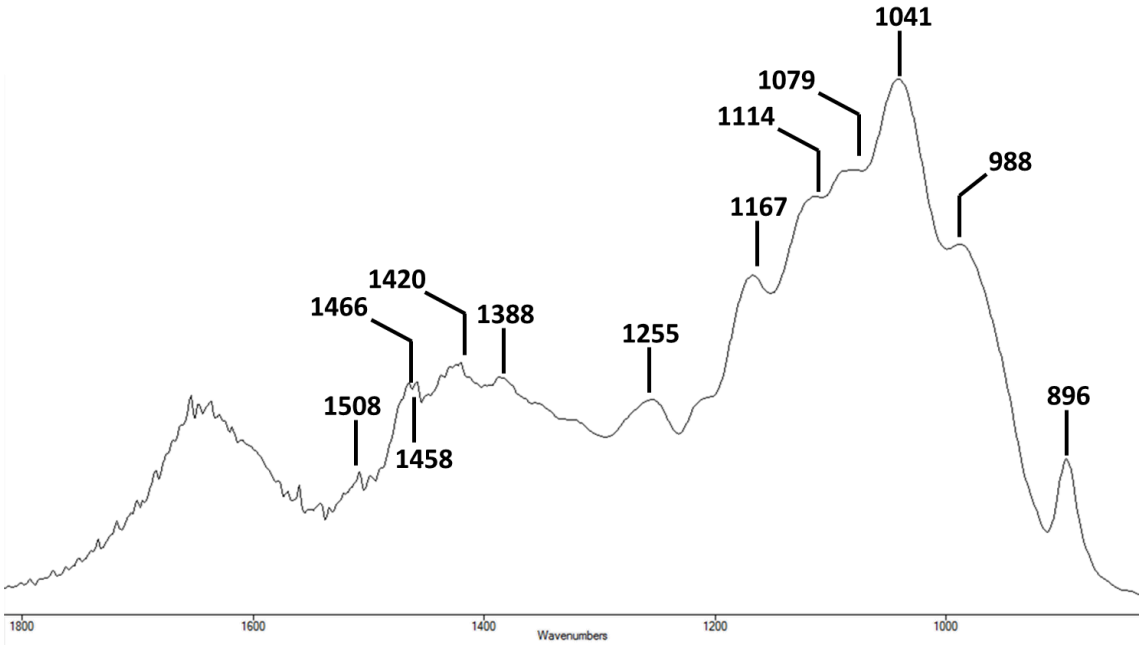
Cellulose-rich material from C method (1800-800 cm^{-1}) – rice straw



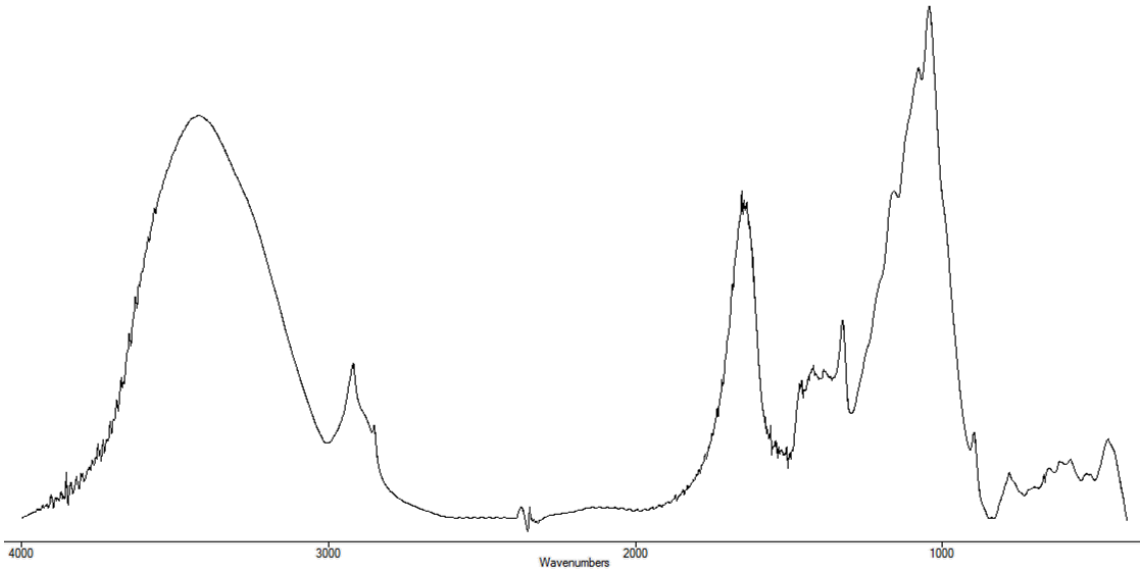
Hemicellulose-rich material from C method (4000-400 cm^{-1}) – rice straw



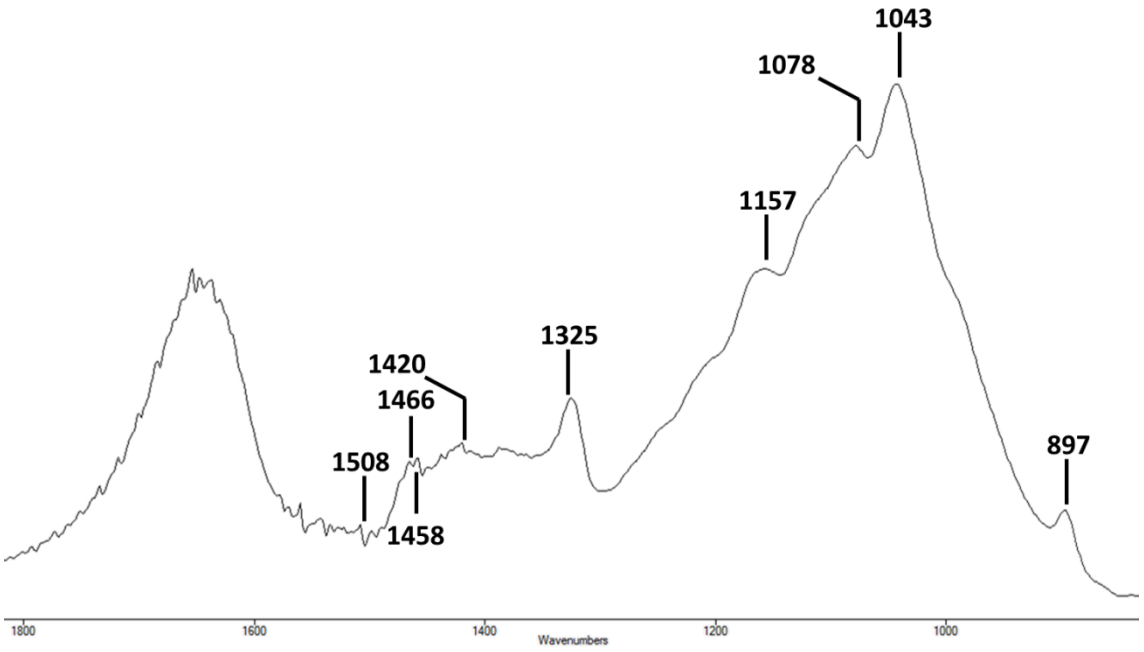
Hemicellulose-rich material from C method (1800-800 cm^{-1}) – rice straw



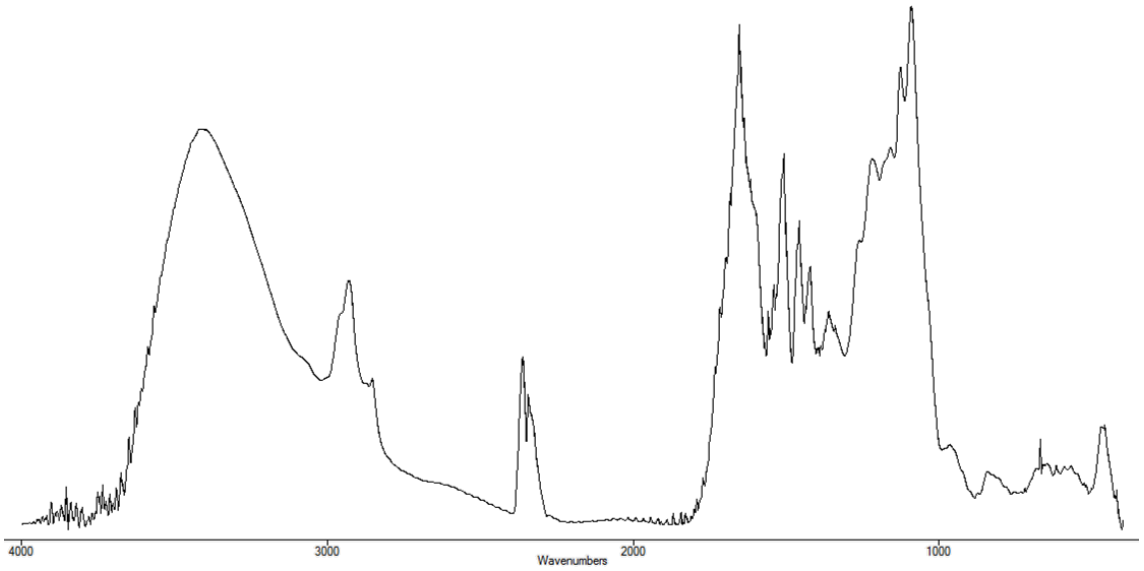
Residual hemicellulose-rich material from C method (4000-400 cm^{-1}) – rice straw



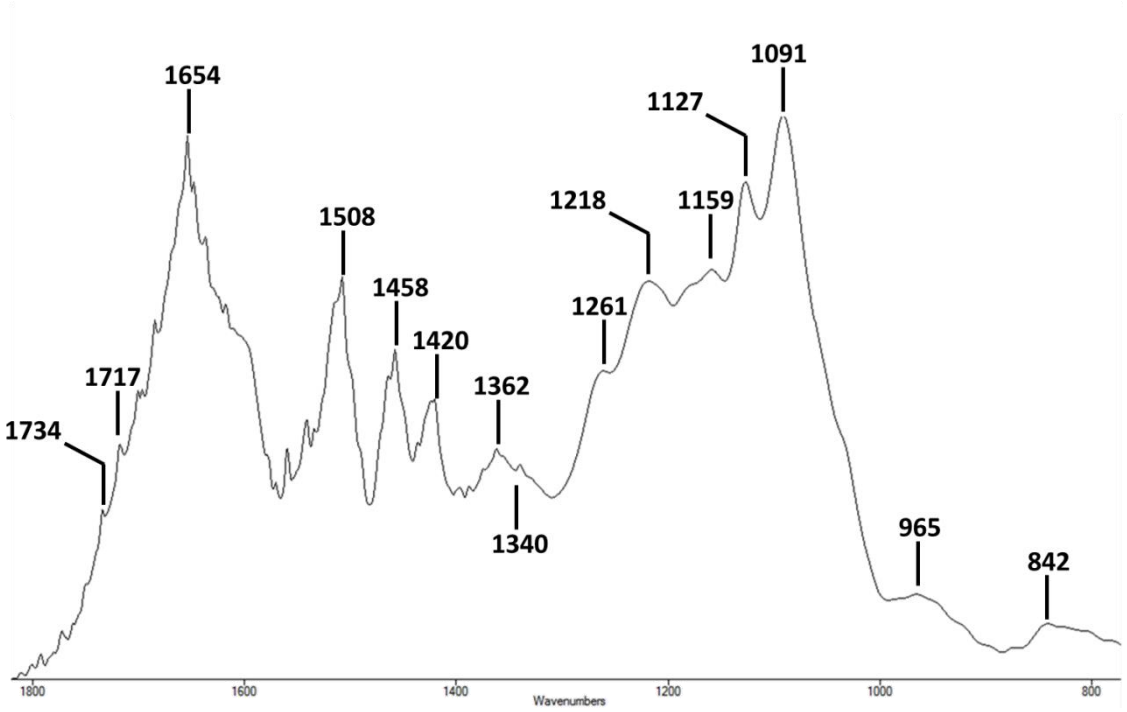
Residual hemicellulose-rich material from C method (1800-800 cm^{-1}) – rice straw



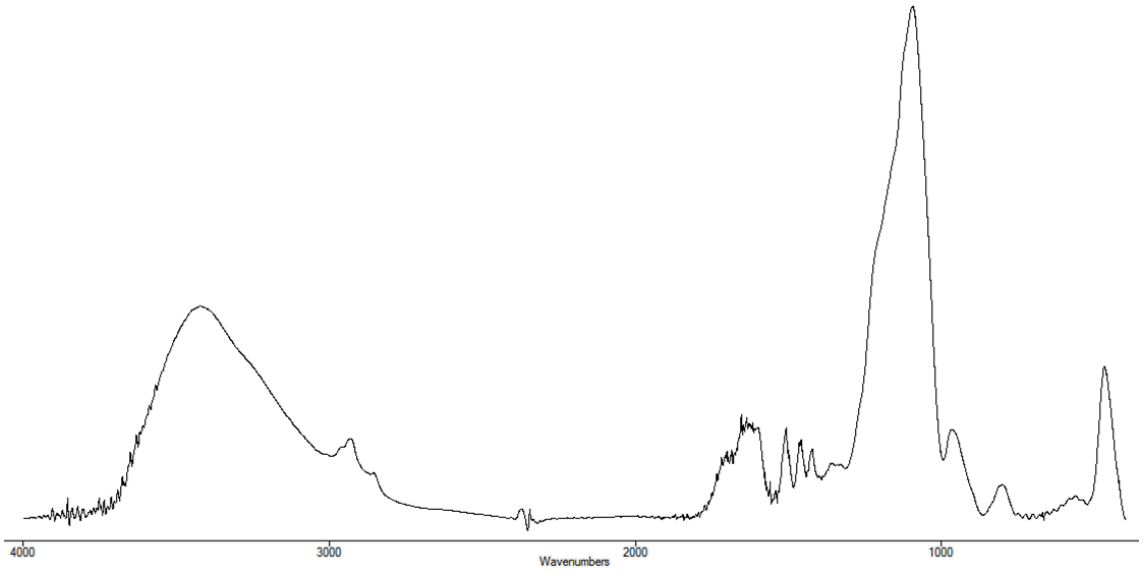
Lignin-rich material from C method (4000-400 cm^{-1}) – rice straw



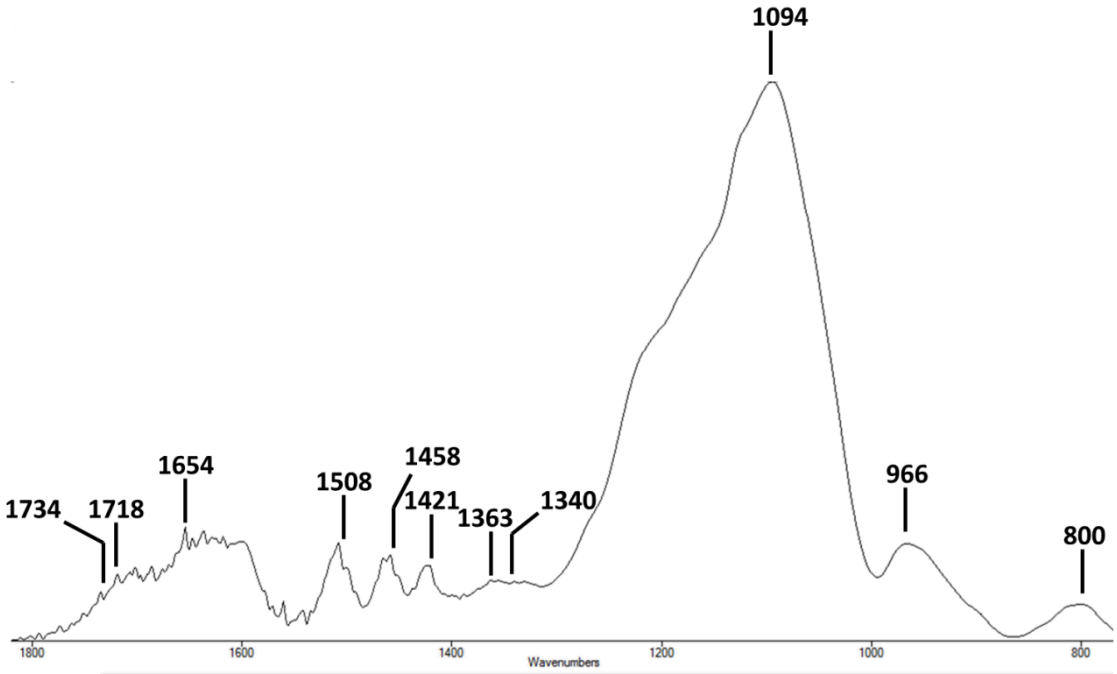
Lignin-rich material from C method (1800-800 cm^{-1}) – rice straw



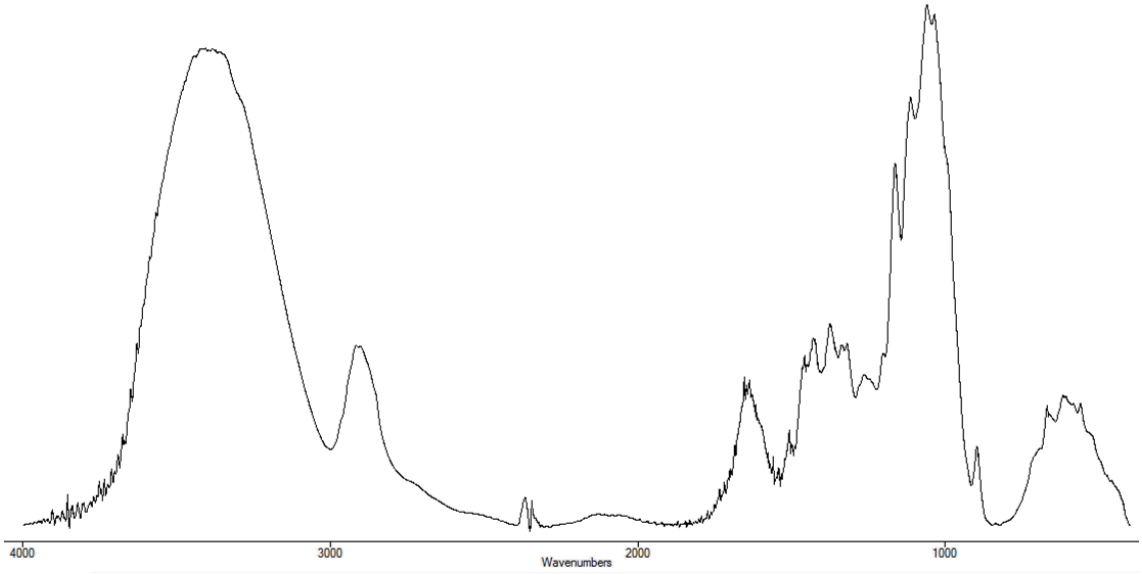
Residual lignin-rich material from C method (4000-400 cm^{-1}) – rice straw



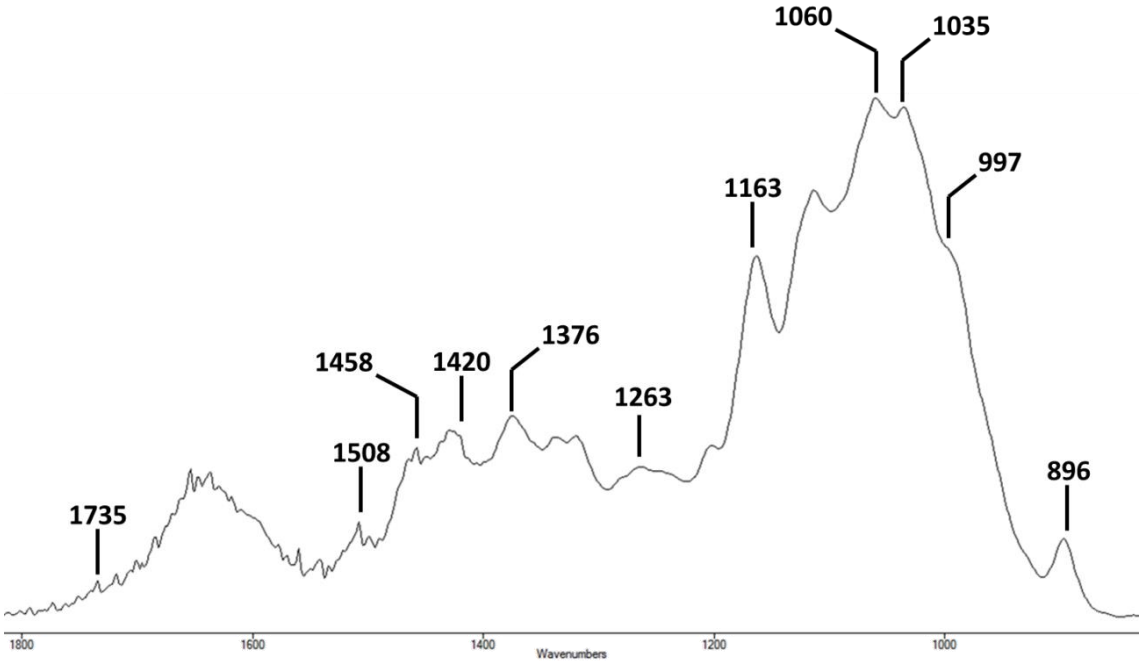
Residual lignin-rich material from C method (1800-800 cm^{-1}) – rice straw



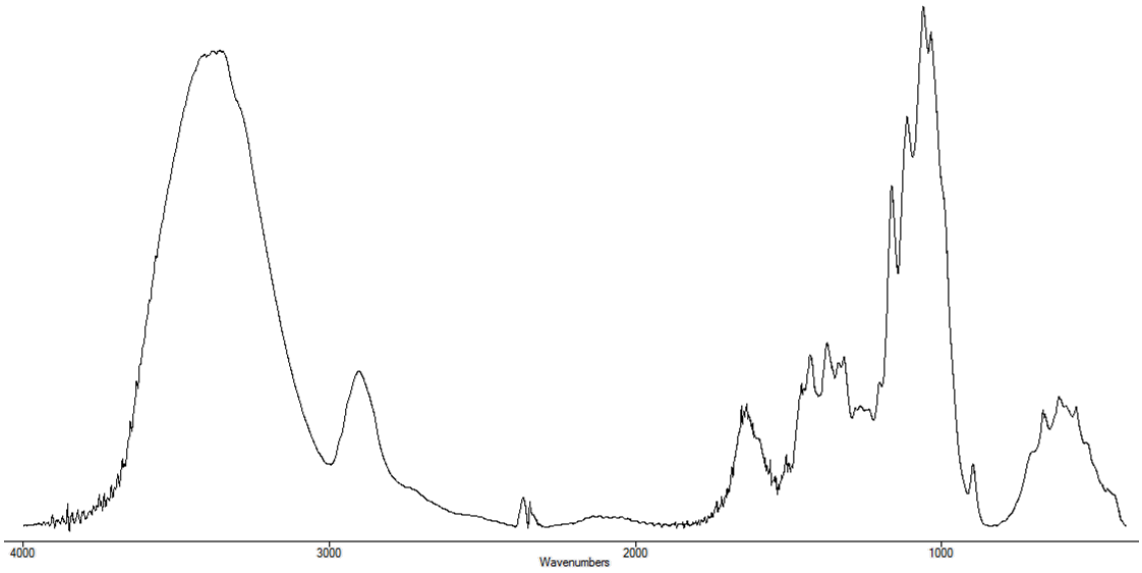
Regenerated material from C method (4000-400 cm^{-1}) – triticale



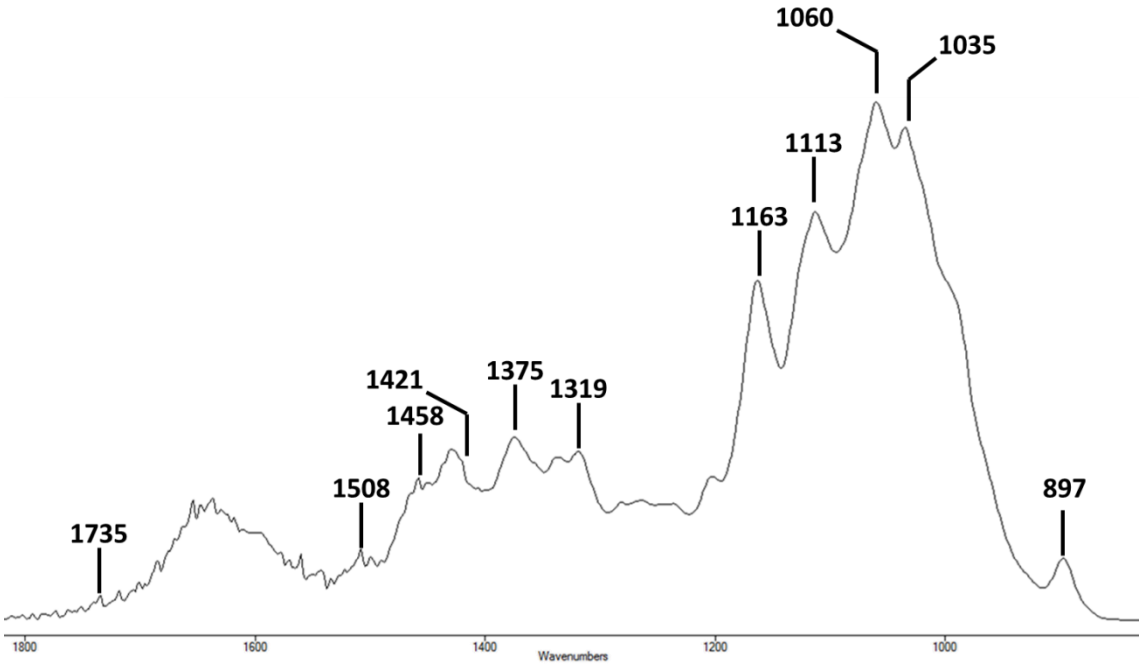
Regenerated material from C method (1800-800 cm^{-1}) – triticale



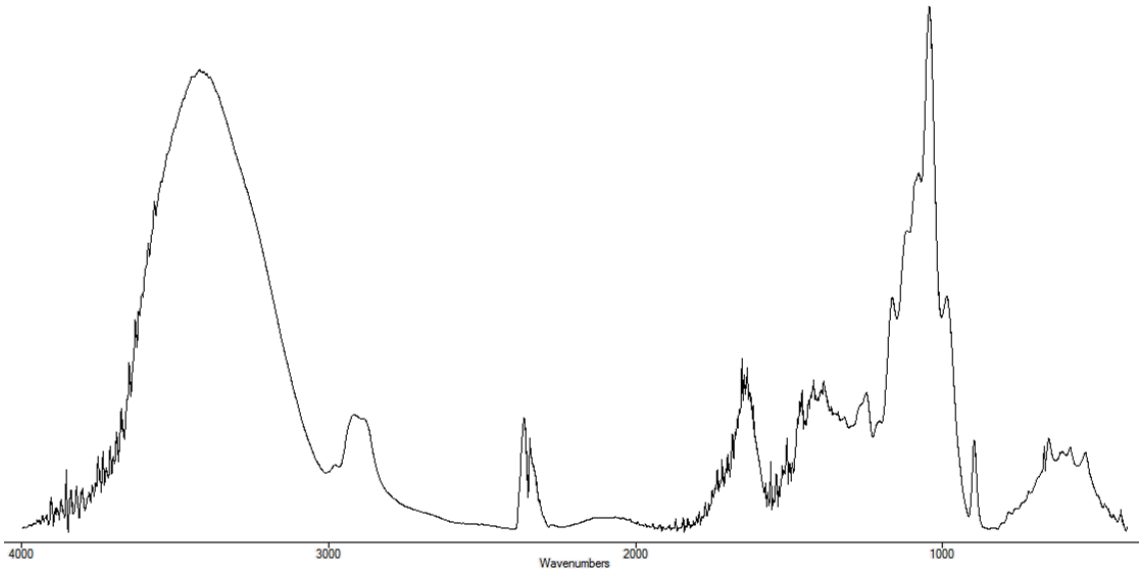
Cellulose-rich material from C method (4000-400 cm^{-1}) – triticale



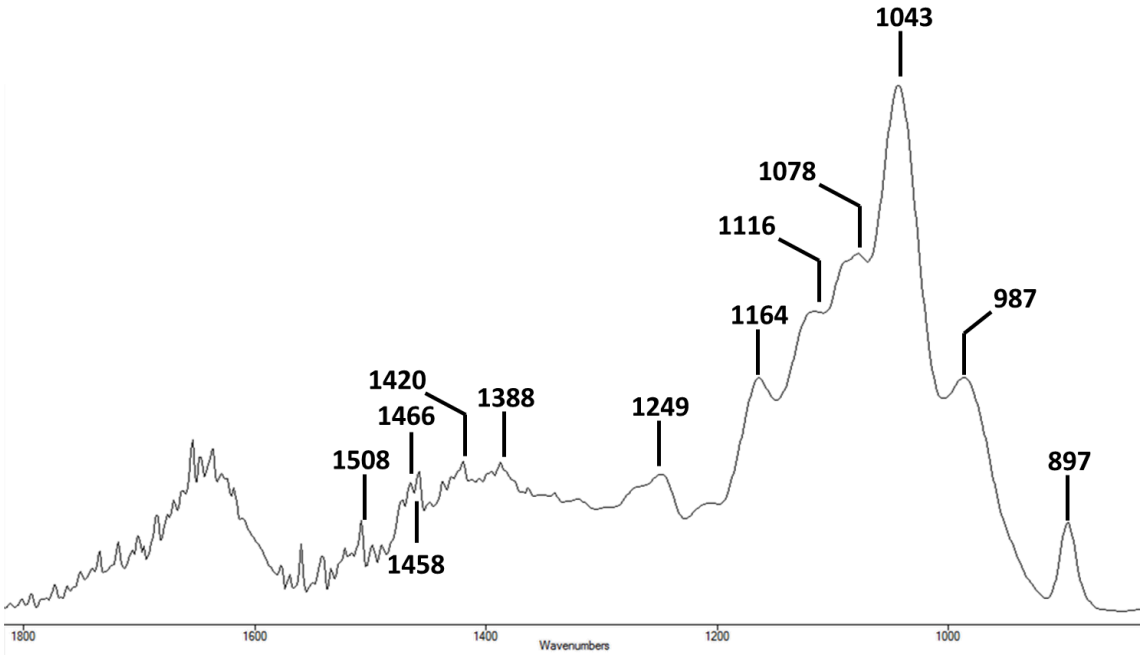
Cellulose-rich material from C method (1800-800 cm^{-1}) – triticale



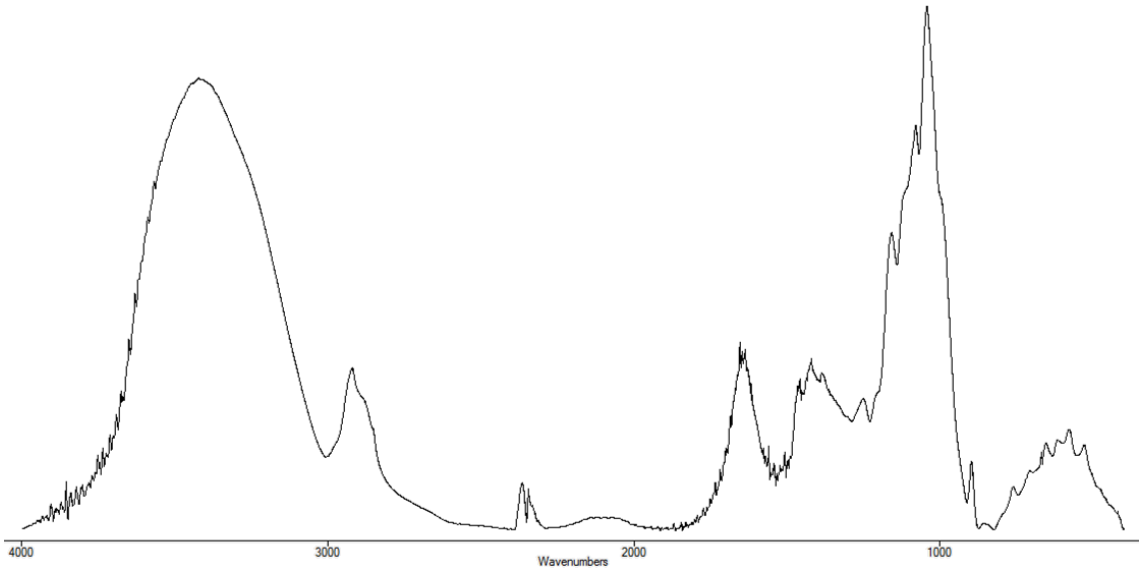
Hemicellulose-rich material from C method (4000-400 cm^{-1}) – triticale



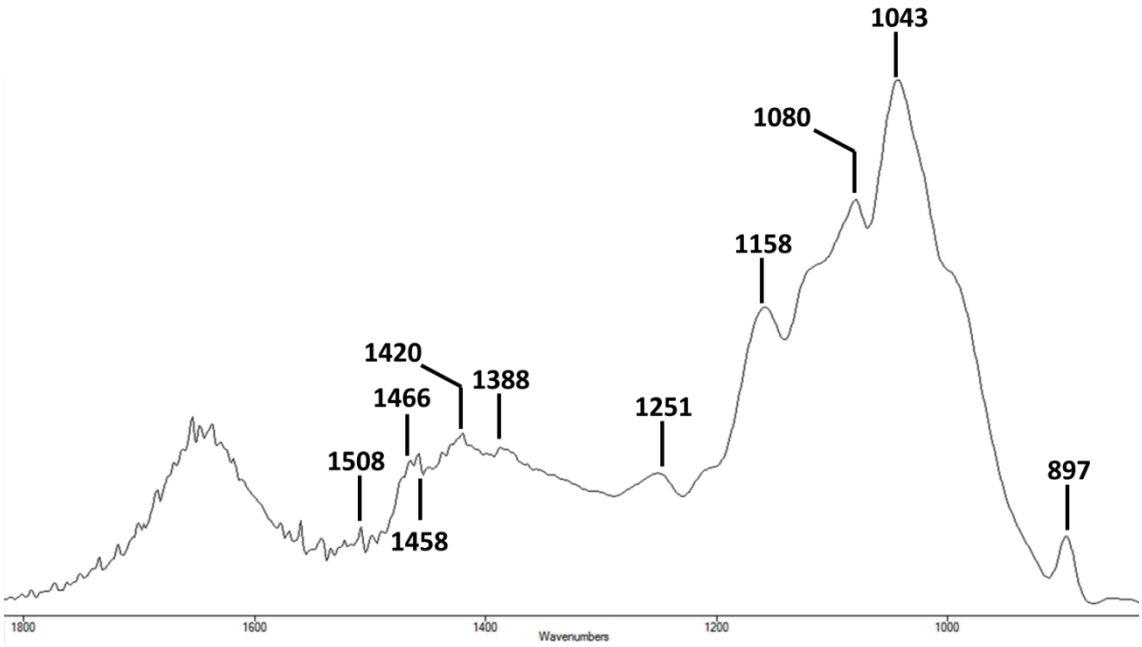
Hemicellulose-rich material from C method (1800-800 cm^{-1}) – triticale



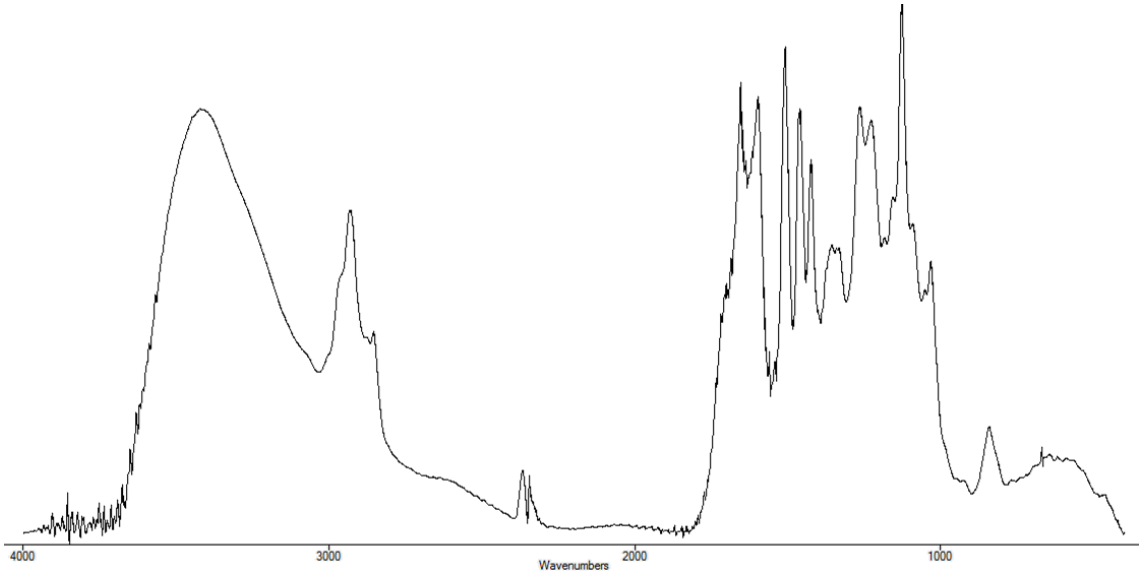
Residual hemicellulose-rich material from C method (4000-400 cm^{-1}) – triticale



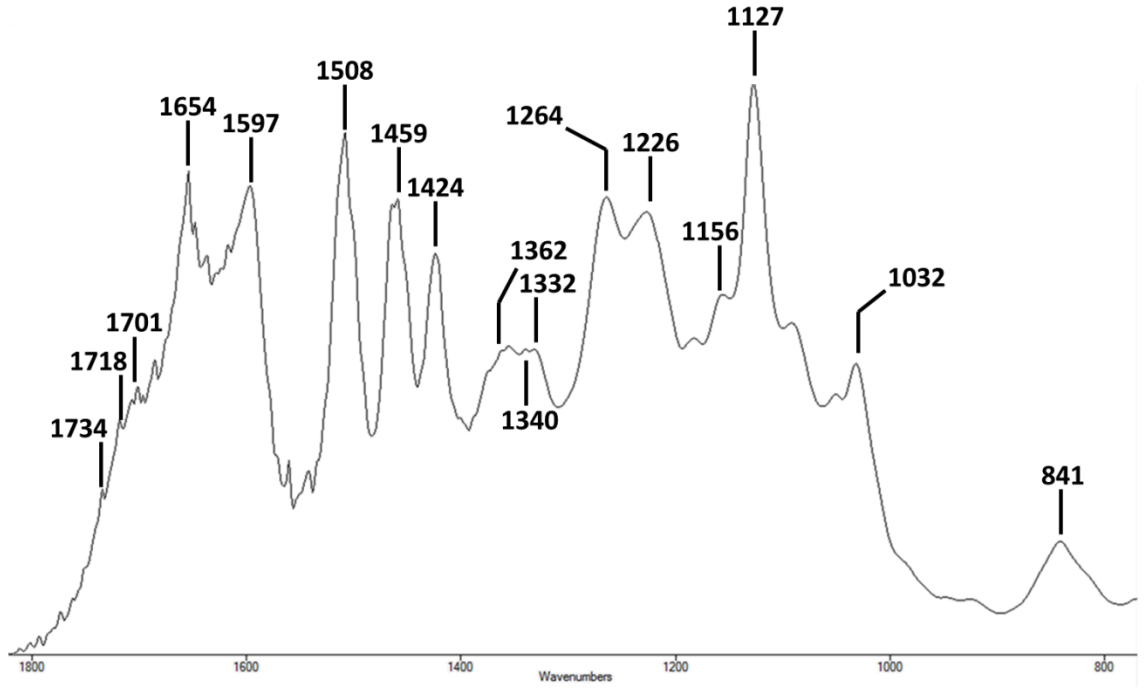
Residual hemicellulose-rich material from C method (1800-800 cm^{-1}) – triticale



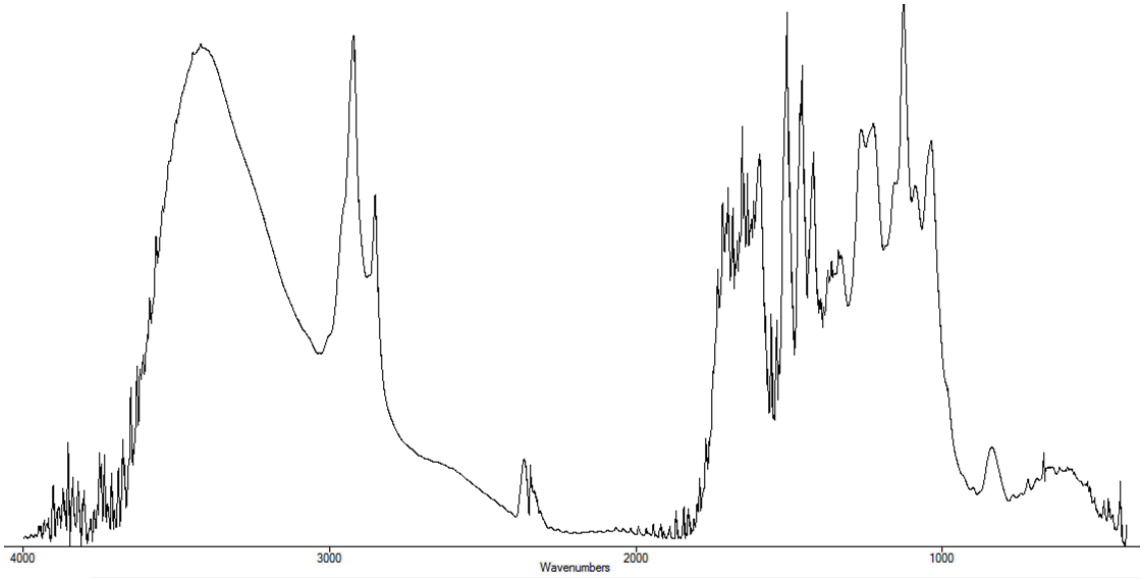
Lignin-rich material from C method (4000-400 cm^{-1}) – triticale



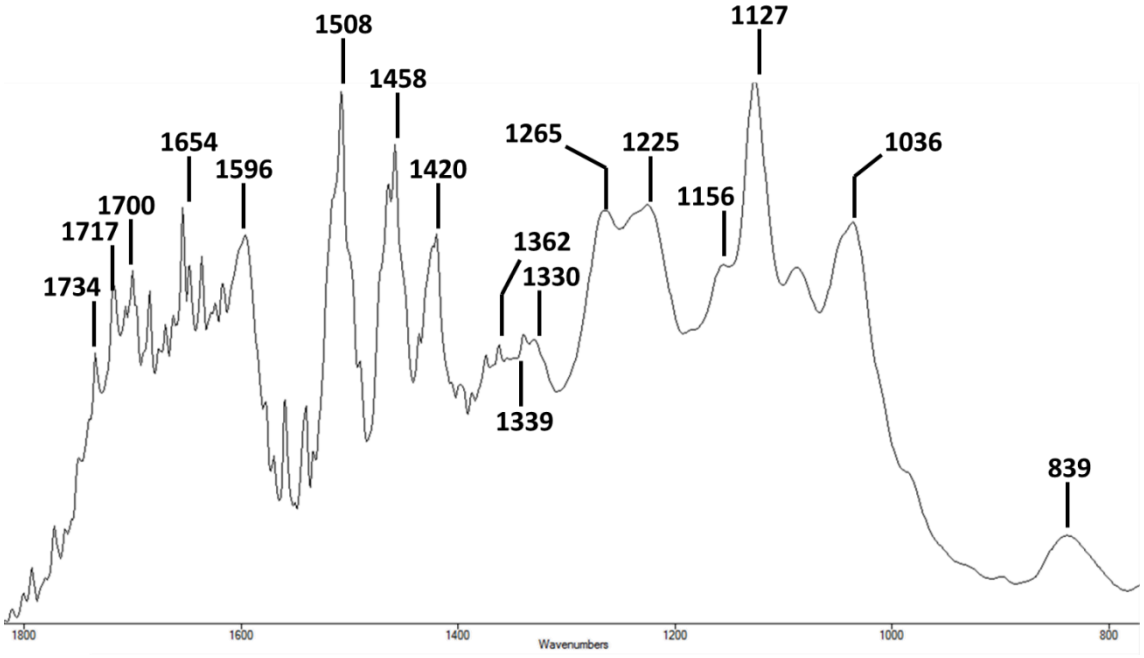
Lignin-rich material from C method (1800-800 cm^{-1}) – triticale



Residual lignin-rich material from C method (4000-400 cm^{-1}) – triticale



Residual lignin-rich material from C method (1800-800 cm^{-1}) – triticale



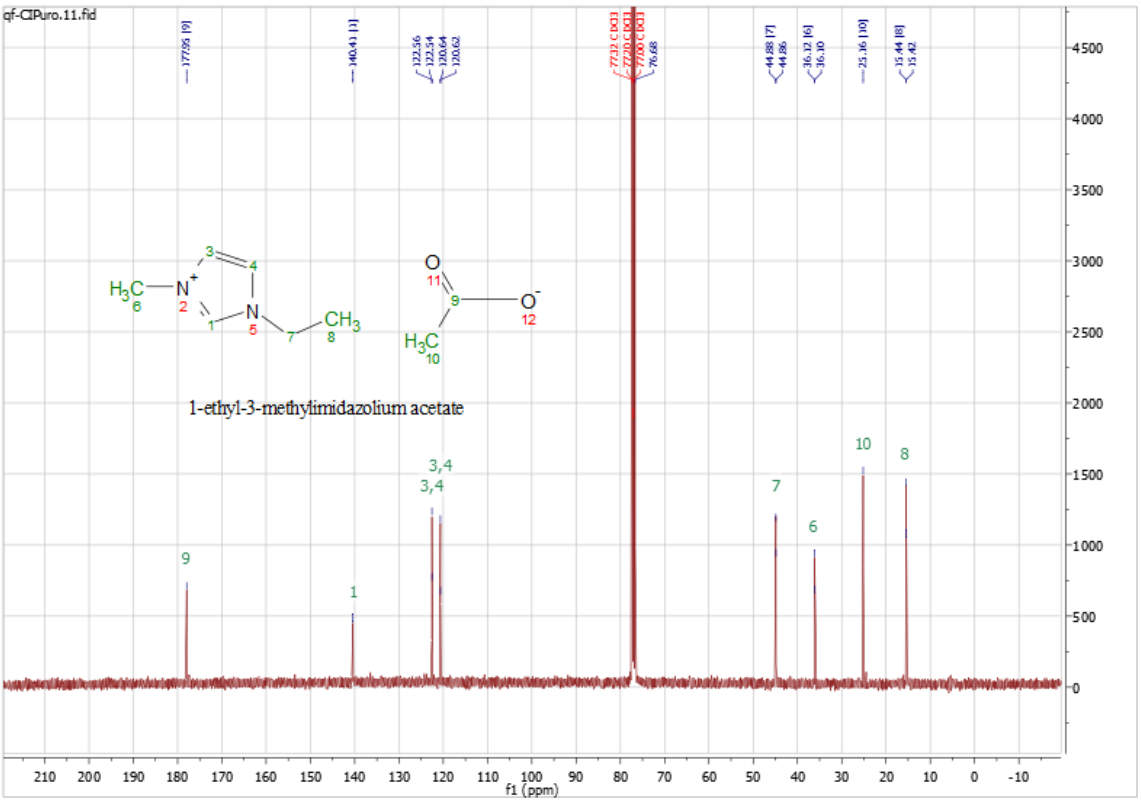
C appendix: FTIR characterization of the absorption bands

Table C1. Characteristic FTIR absorption bands for cellulose, hemicellulose and lignin.[76]

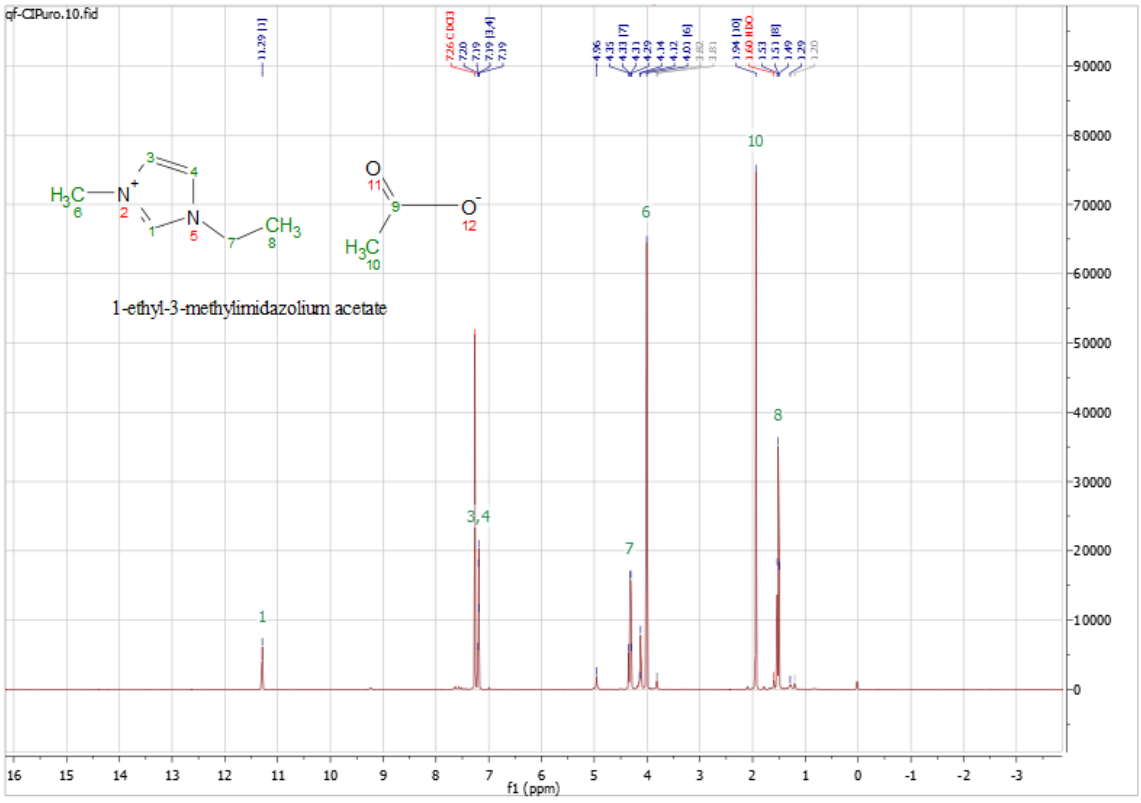
Absorptions /cm^{-1}	Description	Cellulose	Hemicellulose	Lignin
2918-2920	Asymmetric and symmetric C-H stretching of CH, CH_2 and CH_3	✓	✓	✓
2900	CH and CH_2 stretching	✓	-	-
2850-2852	Asymmetric and symmetric stretching of CH, CH_2 and CH_3	✓	✓	✓
1734	Ester-linked acetyl, feruloyl and p-coumaroyl groups between hemicellulose and lignin	-	✓	✓
1718	C=O stretching in unconjugated ketone, carbonyl and ester groups	-	-	✓
1654	C=O stretching in conjugated para-substituted aryl ketones	-	-	✓
1597-1598	Contribution of the aromatic skeletal and C=O vibrations	-	-	✓
1508	C=C stretching vibration in phenol rings	-	-	✓
1458	C-H deformations (CH and CH_2) in phenol rings	-	-	✓
1437	CH_2 scissoring motion	✓	-	-
1420	C-H deformations (CH and CH2) in phenol rings	-	-	✓
1388	C-O stretching	-	✓	-
1376	Bending of C-H	✓	-	-
1320	C-C and C-O skeletal vibrations	✓	-	-
1262-1265	Guaiacyl methoxyl groups	-	-	✓
1251	C-O stretching of acetyl groups	-	✓	-
1228-1235	C-O stretching vibrations of syringil	-	-	✓
1161	C-O asymmetric band	✓		-
1127	Contribution of C-H in a plane deformation, C=O stretching of syringyl units and secondary alcohols	-	-	✓
1107-1112	C-OH skeletal vibration in pyranosyl ring	✓	✓	-
1091	C-O deformations of secondary alcohols and aliphatic ether linkages	-	-	✓
1080	Galactan side chains	-	✓	-
1061-1066	C-O-C ether linkage of the skeletal vibration of both pentose and hexose unit contribution	✓	✓	-
1158	C-O asymmetric bridge stretching in ester linkages	-	-	✓
1043-1049	Contribution of C-O stretching and C-O-C glycosidic linkage in xylan	-	✓	-
1035	C-O stretching vibration characteristic for cellulose	✓	-	-
1033-1034	Aromatic C-H in-plane deformation for guaiacyl units	-	-	✓
993-998	Arabinosyl side chains	-	✓	-
896-898	Vibration of β-glycosidic C-H deformation with a ring vibration contribution (hexoses/pentoses) characteristic of glycosidic bonds	✓	✓	-
840	Out-of-plane deformation vibrations of C-H bond	-	-	✓

D appendix: NMR spectra

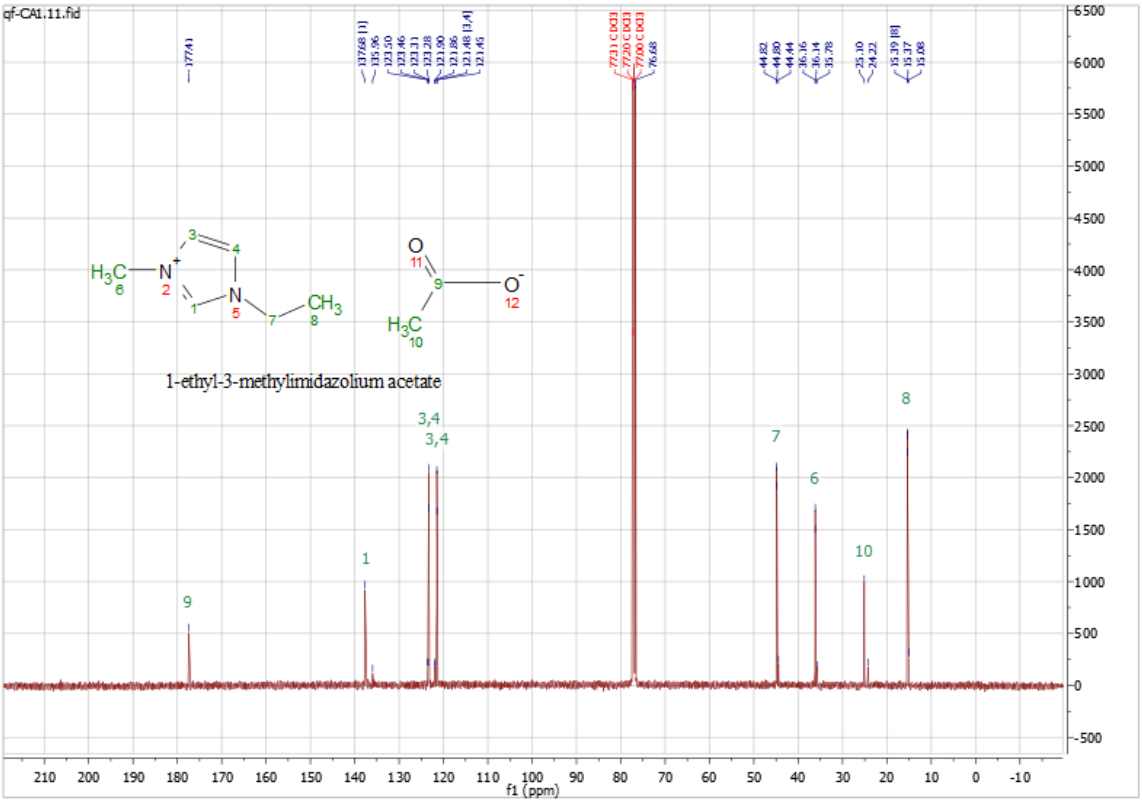
^{13}C NMR Pure [emim][OAc]



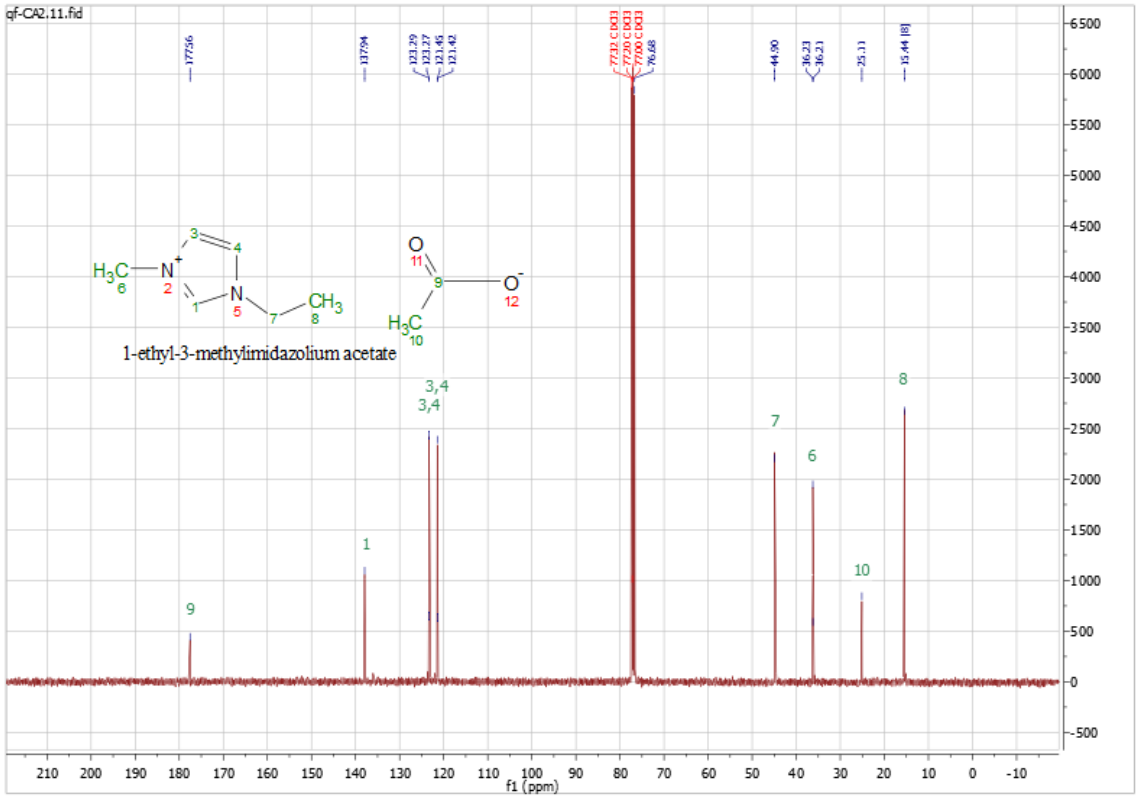
^{1}H NMR Pure [emim][OAc]



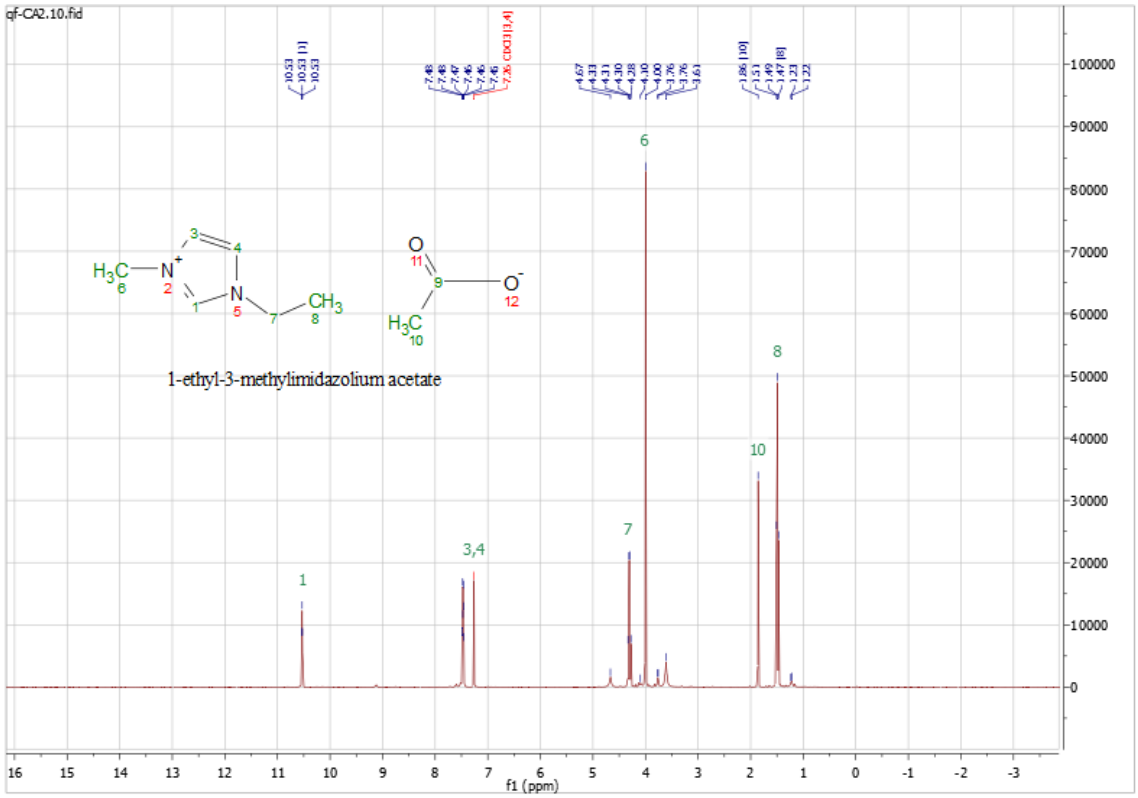
^{13}C NMR [emim][OAc] – Method C_{A1}



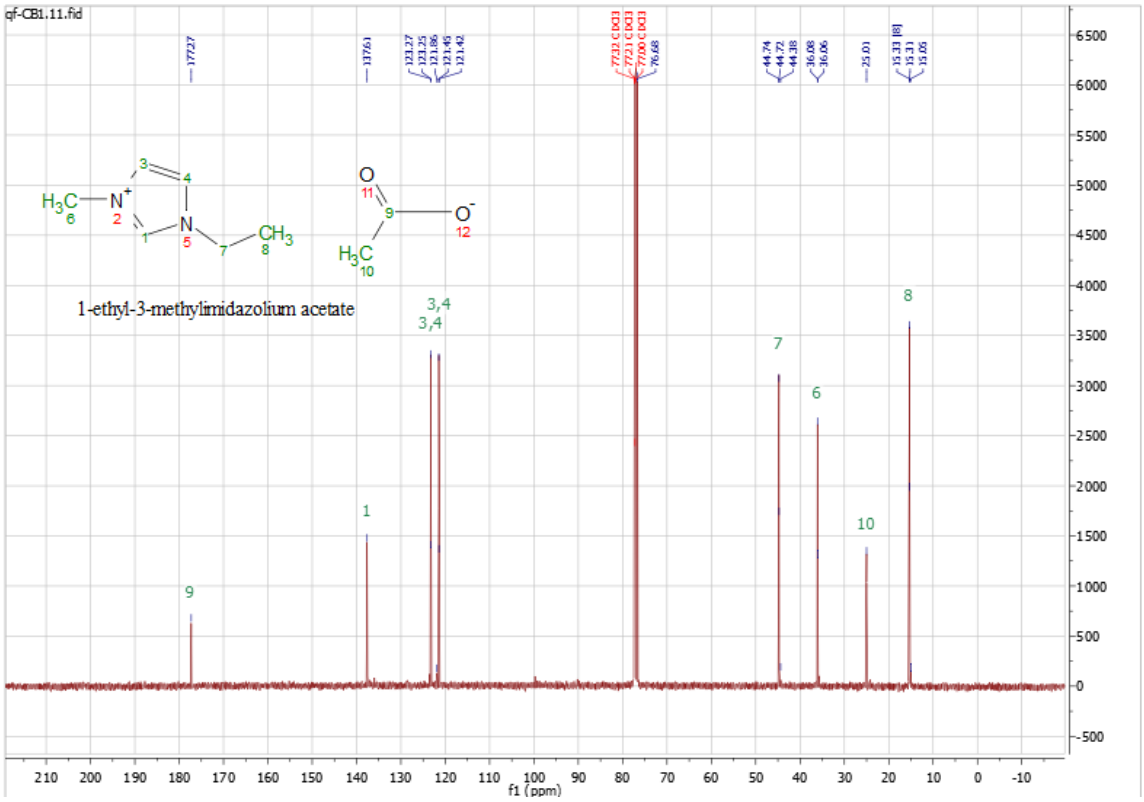
^{13}C NMR [emim][OAc] – Method C_{A2}



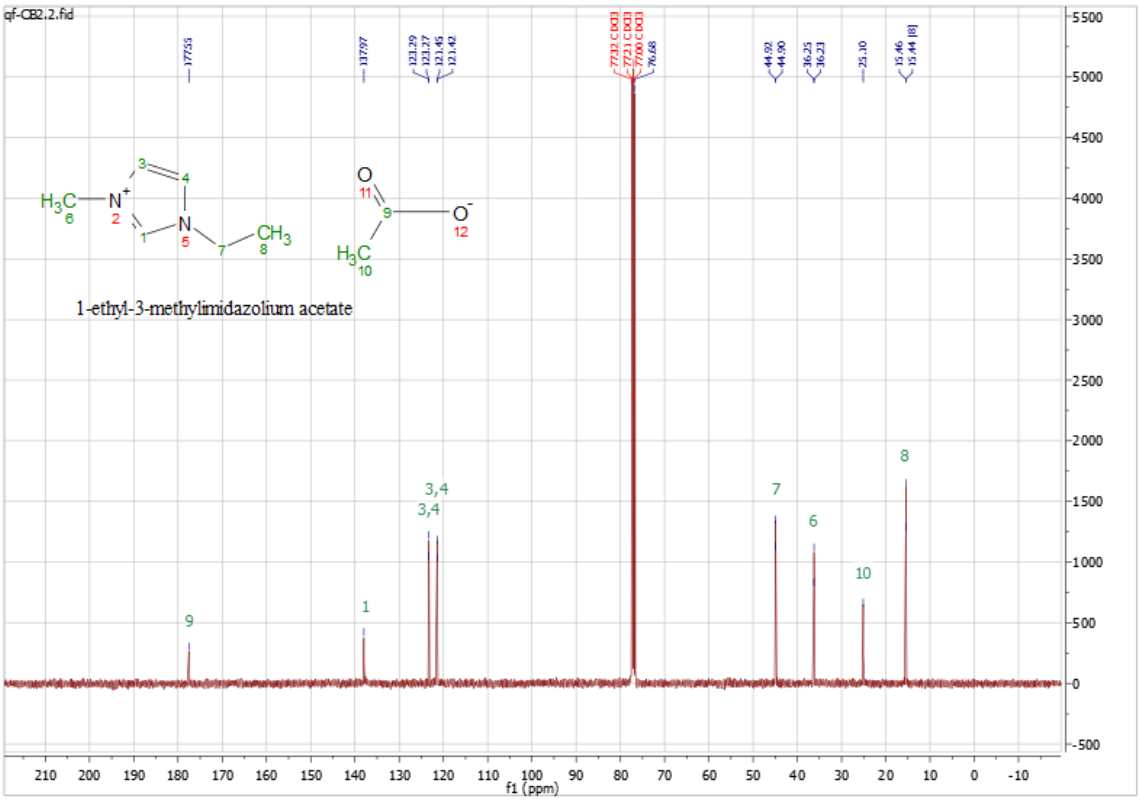
^{1}H NMR [emim][OAc] – Method C_{A2}



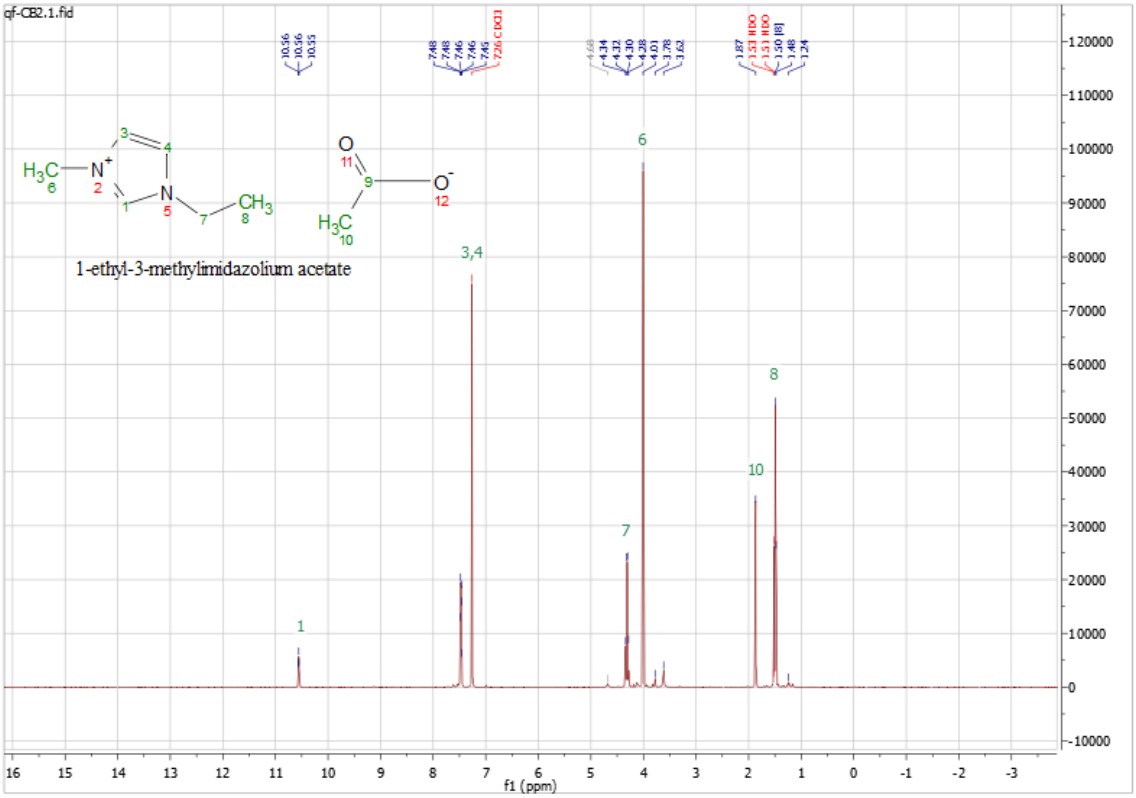
^{13}C NMR [emim][OAc] – Method C_{B1}



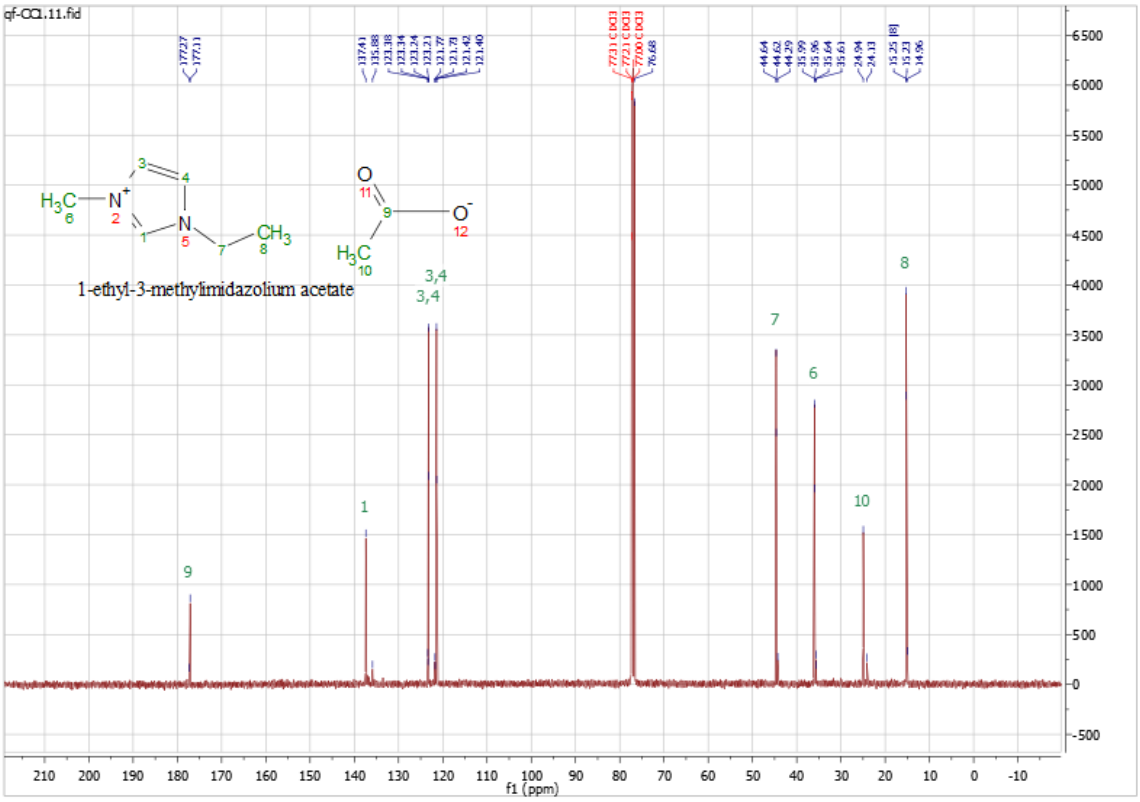
^{13}C NMR [emim][OAc] – Method C_{B2}



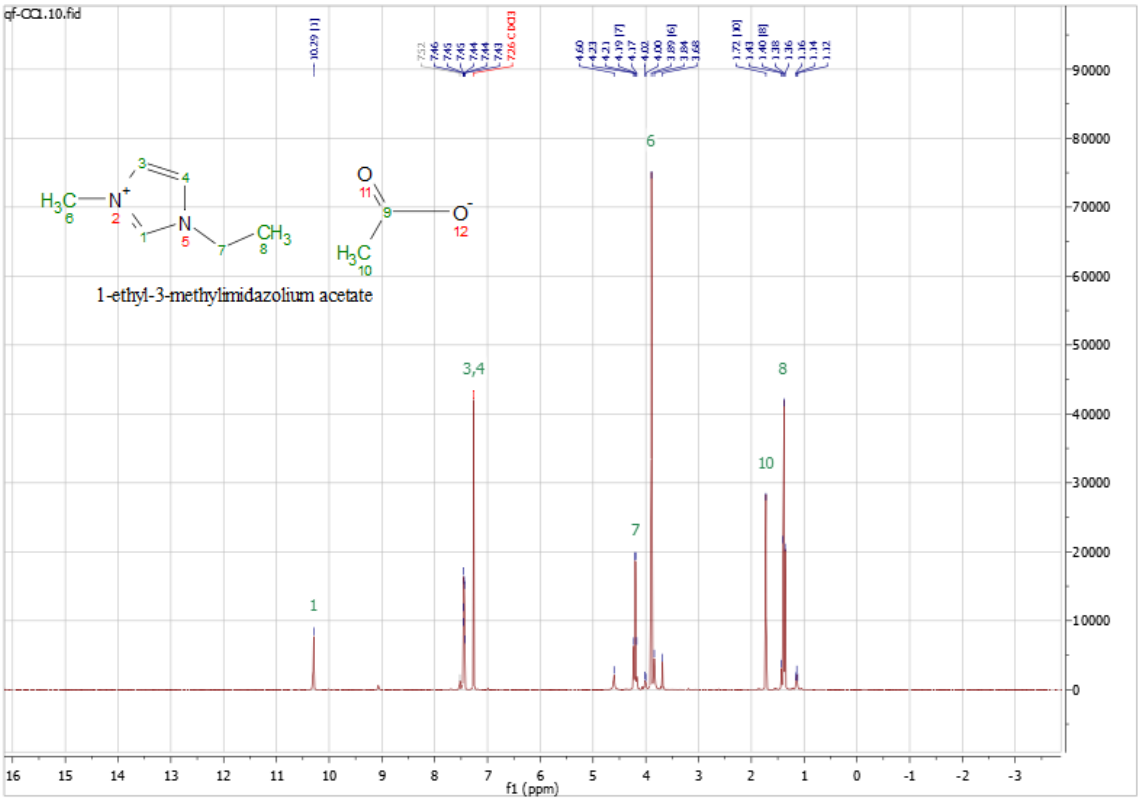
^{1}H NMR [emim][OAc] – Method C_{B2}



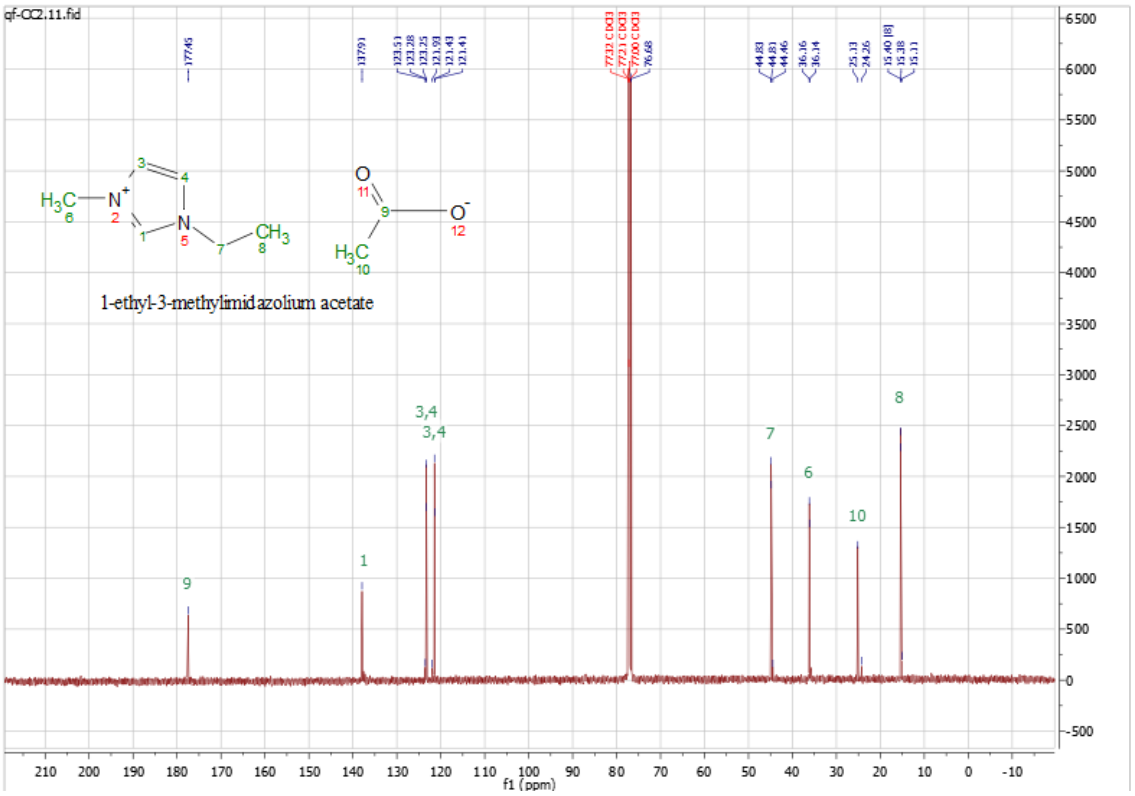
^{13}C NMR [emim][OAc] – Method C_{C1}



^{1}H NMR [emim][OAc] – Method C_{C1}



^{13}C NMR [emim][OAc] – Method C_{C2}



^{1}H NMR [emim][OAc] – Method C_{C2}