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M. VINCENT ORIEZ

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Laboratoire de Chimie Agro-Industrielle (L.C.A.)

**Directeur(s) de Thèse :**

M. PIERRE YVES PONTALIER

M. JEROME PEYDECASTAING

**Rapporteurs :**

Mme VIOLAINE ATHES, AGROPARISTECH

M. NICOLAS BROSSÉ, UNIVERSITÉ LORRAINE

**Membre(s) du jury :**

M. PIERRE AIMAR, CNRS TOULOUSE, Président

M. ABDELLATIF BARAKAT, INRA MONTPELLIER, Invité

M. JEROME PEYDECASTAING, INP TOULOUSE, Membre

Mme HÉLÈNE CARRERE, INRA NARBONNE, Membre

Mme MARLENE BEYERLE, NOVASEP SAS, Invité

M. PIERRE YVES PONTALIER, INP TOULOUSE, Membre



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## FOREWORD

Fossil hydrocarbons (petroleum, gas, charcoal) are intensively used by human beings to provide them energy, fine chemicals and materials. However, their rate of consumption largely exceed their rate of formation: complete consumption of the fossil hydrocarbons may happen in a few centuries, whereas they were formed from biomass in several dozens of million years. Moreover, this creates imbalances in the carbon cycle, the carbons trapped in the lithosphere are released in the atmosphere (mainly under the form of carbon dioxide) some part being dissolved in the oceans, leading to potential life threatening effects such as global warming and ocean acidification. For these reasons, alternatives to fossil hydrocarbons need to be found.

Nowadays, lignocellulosic biomass valorization into some form of energy (e.g., liquid fuels) and more importantly into molecules (synthons and biomaterials) in biorefineries represents the only available option. Human beings have been using some part of the biomass for their food (the edible parts of the plants, i.e., non-lignocellulosic compounds) and biomass as a whole for their shelters (e.g., timber) or energy by burning it. The challenge of using lignocellulose for liquid fuels, synthons and biomaterials production is that it requires extensive processing. Lignocellulosic biomass is essentially made of cellulose, hemicelluloses and lignin. Fractionation and purification of these three compounds are necessary for their valorization as substitutes to molecules obtained from fossil hydrocarbons. Collaboration between Novasep, the joint research unit Agropolymer Engineering and Emerging Technologies (IATE), and the Laboratory of Agro-industrial Chemistry (LCA) within LigNov project was created to explore promising fractionation and purification pathways on lignocellulosic biomass. Novasep is a French company specialized in purification processes by chromatography, membrane filtration and electro dialysis and IATE provided its expertise on electrostatic separation pretreatments.

This PhD thesis was done within the framework of the French projet Lignov (ANR-14-CE06-0025-01), financed by the French National Research Agency (ANR). The investigations were carried out in the Laboratory of Agro-industrial Chemistry (LCA)

located in Toulouse, France, under the direction of Doctor Pierre-Yves Pontalier and Doctor Jérôme Peydecastaing.

Six chapters compose this manuscript:

The first chapter presents the concept of lignocellulosic biorefinery, the potential molecules of interest that can be generated, and the two main chemical fractionation processes existing, acid and alkaline, and their respective associated purification pathways under the form of a review soon to be submitted.

In chapter 2, the characterization of the two lignocellulosic biomasses used in this project, sugarcane bagasse and sunflower oil cake, is exposed. Acid and alkaline extractions were carried out on these materials, the composition of the obtained fractions and the yield of the different compounds in these fractions are thoroughly reported.

Chapter 3 is composed of one publication under minor revision and two other parts that will be submitted shortly for publication, about membrane filtration as a separation technique for the compounds contained in sugarcane bagasse mild alkaline extract. The first one deals with a membrane screening and a study on the influence of the filtration parameters on separation performance. Based on this publication, the best membrane was used for further test in concentration and diafiltration modes which is reported in second time. The last part tackles resistances due to fouling during filtration and cleaning procedures.

Chapter 4 presents the results of batch column elution chromatography tests mainly applied to the purification of sugarcane bagasse mild alkaline extract which was published.

Chapter five corresponds to a publication soon to be submitted about an integrated process to obtain purified compounds from sugarcane bagasse mild alkaline extract. The purification steps include membrane filtration, concentration by evaporation, batch column chromatography and precipitation by the addition of acid and ethanol.

Finally, even if analytical methods were described throughout this manuscript every time an experiment is reported, chapter six details the development of the analytical methods and the potential analytical issues faced during this work.

## Chapter 1:

# THE LIGNOCELLULOSIC BIOREFINERY

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### 1.1. The lignocellulosic biorefinery concept

#### 1.1.1. Introduction

The biorefinery concept is the generation of a variety of goods (fuels, power, materials, chemicals) from different biomass feedstocks through a combination of technologies (FitzPatrick et al., 2010). The term biorefinery is derived both from the raw material feedstock which is renewable biomass and also from the bioconversion processes often applied in the treatment and processing of the raw materials (FitzPatrick et al., 2010). Besides, products from biorefineries often present a lesser environmental impact compare to traditional refineries (Ma et al., 2015). For instance the use of high concentration of ethanol in ethanol:gasoline blends in engine reduces drastically emission of CO, CO<sub>2</sub> and NO<sub>x</sub> (Koç et al., 2009). In the last decades, biorefineries have also been gaining interest in the scientific, industrial and political communities as petroleum, coal and gas prices keep on increasing as demand stresses supply (Thorp, 2010; Tuck et al., 2012). The forecast studies about the price evolution of these fossil resources all agreed on a rise in the future years, the only differences are the chronology and the intensity of the increase (Shafiee and Topal, 2009; Capellán-Pérez et al., 2014). In 2016, 4 billion tons of pretroleum were produced worldwide (<https://www.eia.gov/> US Energy Information Administration) and in parallel, on average during the three-year period 2006-2008, 3.7 billion tons of dry residues from six crops only (barley, maize, rice, soybean, sugarcane and wheat) were produced worldwide (Bentsen et al., 2014), showing the tremendous potential of lignocellulosic biomass (other crops and forestry residues contributing as well) to replace petroleum based on the quantity consumed.

Fully replacing fuels from fossil sources by fuels from biomass seem challenging regarding the surface available (Hill et al., 2006), but chemicals production requires far lower volumes of biomass to satisfy demand (FitzPatrick et al., 2010). For example, in the United States, the chemicals production consumed just over 3% of the total US petroleum consumption in 2007, whereas the transportation segment accounted for over 70%, for an equivalent value-added worth (FitzPatrick et al., 2010).

Aromatic compounds are key building blocks for the chemical industry (Holladay et al., 2007; Haveren et al., 2008), but from now on, they are still mainly issued from

petroleum. Aromatic compounds are found in nature in various forms - anthocyanin, flavonoids, and tannins - but only in limited amount. Lignin is the only significant source of aromatic compounds in nature and the second most abundant terrestrial biopolymer, after cellulose, accounting for approximately 30% of the organic carbon in the biosphere (Boerjan et al., 2003). The biorefineries generate a tremendous amount of lignin, and its valorization into products will significantly improve the economic feasibility and sustainability of biofuel production from renewable biomass (Ma et al., 2015). Aside from the substitution of fossil aromatics, lignin can be used for new applications where its polymeric structure is of interest such as bioplastics, composites, nanoparticles, adsorbents, dispersants and carbon fibers (Norgren and Edlund, 2014). However, lignin is not easily accessible in the plants, making its extraction and purification costly and often non-environmental friendly.

Generally, the fractionation of lignocellulosic biomass is very difficult because of its recalcitrant structure (crystallinity, low porosity, high molecular weights). Nowadays, three main processes are used at pilot or industrial scale: dilute or concentrated inorganic acids, alkaline solution in severe conditions inspired from the pulp and paper industry, and organosolv processes using organic solvents such as acetone, ethanol, acetic acid, formic acid. Pretreatment of the lignocellulosic biomass can be carried out to increase the efficiency of these fractionation processes, such as mechanical size reduction of the lignocellulosic biomass in order to increase the surface of particles in contact with the acid, the base or the solvent.

### 1.1.2. Second generation biorefinery

First generation biorefineries use edible parts of the plants – sugars, starch, oils - for the production of fuel and chemicals (Naik et al., 2010). Second generation biorefineries are defined in opposition with the first generation biorefineries, as they use the non-edible parts of the plants (stem, leaves, roots) made of lignocellulose. In these biorefineries, agricultural by-products or forest biomass are processed to produce energy and a wide variety of precursor chemicals and bio-based materials, similar to the modern petroleum refineries. Among the variety of possible products manufactured in second generation biorefinery, liquid transportation fuels mainly in the form of ethanol is rapidly gaining significance (Huang et al., 2008).

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When producing first generation ethanol from maize or sugarcane for instance, the raw material constitutes about 40–70% of the production cost (Cardona et al., 2010). The use of forestry and agriculture by-products can reduce the cost of the feedstocks drastically. However, the savings realized through the raw material are compensated by the complexity and thus the price of the processes implied in second generation ethanol production.

Lignocellulosic materials do not contain monosaccharides readily available for bioconversion. First, they contain polysaccharides, such as cellulose and hemicelluloses, which have to be hydrolyzed, by means of acids or enzymes, to fermentable sugars. Secondly, cellulose in plants is closely associated with hemicelluloses and lignin, preventing the access of hydrolytic agents to cellulose. Thirdly, the crystalline structure of cellulose itself represents an extra obstacle to hydrolysis. Therefore, a pretreatment is required for removing lignin and hemicelluloses, reducing cellulose crystallinity and increasing the porosity of the cellulose (Cardona et al., 2010). The yield of cellulose hydrolysis into glucose by acid or enzyme is generally less than 20% when pretreatment is not carried out, whereas the yield after pretreatment often exceeds 90% (Sánchez and Cardona, 2008; Hayes, 2009). In order to increase the sugars yields and reduce the ethanol production cost, efficient hydrolysis and valorization of hemicellulosic sugars has become important (Alvira et al., 2010). Once the sugars are under their monomeric form they can be fermented or chemically converted into molecules of interest such as alcohol (ethanol, butanol, xylitol, arabinol), carboxylic acids (succinic acid, lactic acid, levulinic acid) or other molecules (Werpy et al., 2004; Ragauskas et al., 2006). After the pretreatment leading to the fractionation of the lignocellulosic biomass and according to the process selected the conversion into new molecules of interest, the molecules undergo a purification step. Among the different processes in biorefineries the pretreatment and the purification steps represent usually the most expensive stages and high technical challenges (Ragauskas et al., 2006; Hayes, 2009). In the literature about lignocellulosic ethanol biorefineries, the notion of pretreatment of the lignocellulosic biomass comes out of the main treatment which the cellulose conversion to ethanol via enzymatic saccharification then fermentation. In this manuscript, to describe this step, instead of pretreatment, the notion of fractionation will be favored since the three components of the lignocellulose are of interest.

Second generation process cost-effectiveness can be improved by the development of lignin refining procedure for their further valorization (Alvira et al., 2010; Ragauskas et al., 2014). Aromatic compounds (e.g., phenol, benzene) are key building blocks for the chemical industry (Holladay et al., 2007). From now on, they are currently issued from non-renewable fossil hydrocarbons. Aromatic compounds are found in nature (anthocyanin, flavonoids, tannins...) but only in limited amount, lignin is the only significant source of renewable aromatic compounds. Lignin is, after cellulose, the second most abundant terrestrial biopolymer, accounting for approximately 30% of the organic carbon in the biosphere (Boerjan et al., 2003). However, lignin is not easily accessible in the plants, making its extraction and purification costly and often non-environmental friendly. But petroleum constantly rising price coupled with research and development efforts on lignocellulosic conversion processes will make fractionation and purification processes of sugars and phenolic compounds cost-efficient.

No cost-effective industrial lignocellulosic fractionation process has emerged, they all present some drawbacks such as the formation of fermentation inhibitors, high use of energy or chemicals, waste production, expensive equipment. However, acid and alkaline fractionation processes are the mostly used (Alvira et al., 2010; Cardona et al., 2010; Anwar et al., 2014; Ragauskas et al., 2014). The following reviews (1.3. Acid fractionation process & 1.4. Alkaline fractionation process) will focus on acid and alkaline fractionation of lignocellulosic biomass and their associated purification steps.

### 1.1.3. Third generation biorefinery

In the second generation biorefineries the valorization of the whole plant is targeted with a focus on the lignocellulosic material. Third generation biorefinery notion usually refers to the use of microalgae as a raw material, but here we will briefly introduce a new kind of third biorefinery that still focus on lignocellulose valorization but differs from the second generation biorefineries regarding the process used. In second generation biorefineries, the lignocellulose usually undergoes a pretreatment or fractionation before conversion processes where significant loss of organic carbons can happen during the fermentation step, for example. Indeed, when glucose is fermented into ethanol, one third of the organic carbons are converted into inorganic carbons of carbon dioxide, and when xylose is fermented, 60% of the organic carbons are converted into inorganic carbons

(Yie et al., 2013). Besides, the dehydration of ethanol in order to use it as a fuel constitute an expensive process step.

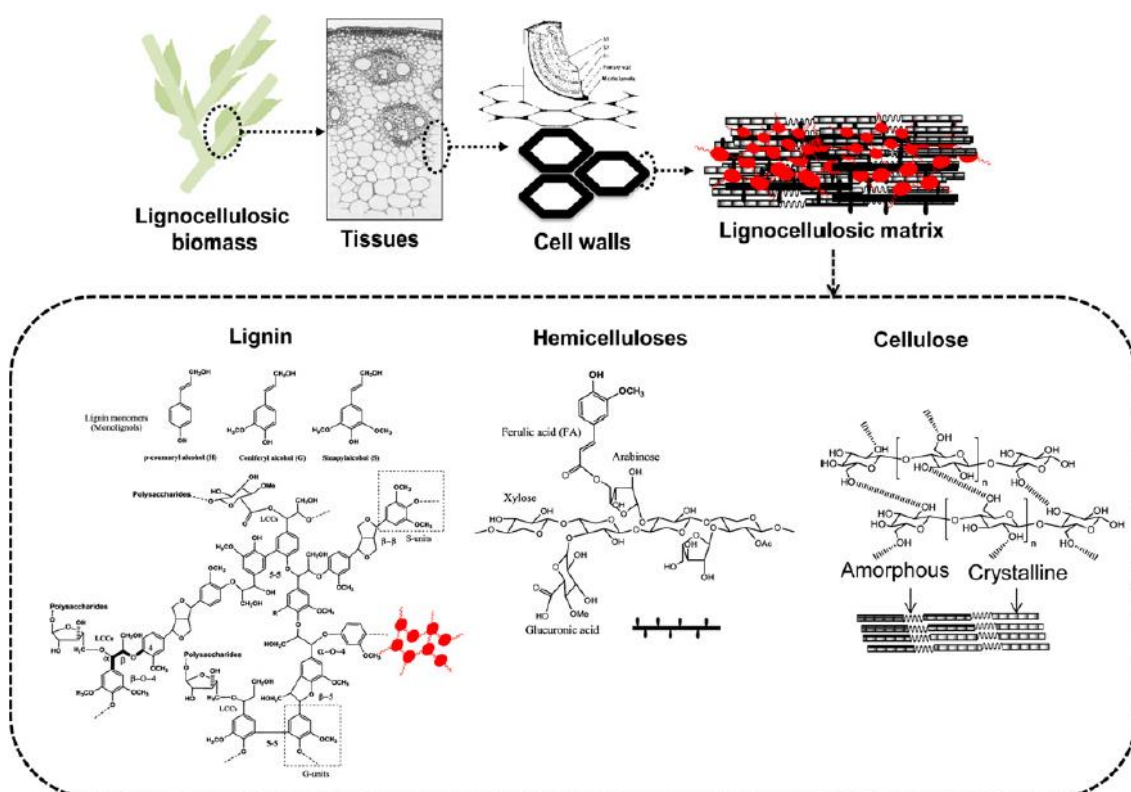
A new concept of biorefinery has been developed where a one-step reaction would convert all the lignocellulosic biomass in small molecules, either carbohydrates and their derivatives such as organic acids, or aromatic compounds (Zhu, 2013). The small organic acids derived from carbohydrates can be formic acid, acetic acid, glycolic acid and lactic acid. These organic acids are useful organic chemicals, classified as key building block by the US Department of Energy (Werpy et al., 2004). Based on an oxygen content analysis, lignin has extra oxygen regarding its possible application (phenolic resin for instance), and carbohydrates needs extra oxygen to form organic acids. In the coming decades, R&D efforts could lead to the development of a catalytic system that could transfer oxygen from lignin to carbohydrates (Zhu, 2013).

So far, a process has been reported to directly produce hydroxymethylfurfural (HMF) and furfural from corn stover using chromium as a catalyst. Under the best conditions, 19% of the dry weight of corn stover was transformed into HMF and furfural in one step. For comparison, optimized cellulosic ethanol process enables the conversion of 24% of the dry weight of corn stover into ethanol in a complex process involving multiple steps (Binder and Raines, 2009). In another process using sodium anthraquinone-2-sulfate as a catalyst, all the lignin was converted quantitatively into small molecular aromatics compounds and all the cellulose and hemicelluloses were converted quantitatively into small organic acids, lactic acid accounting for 50% (Zhu and Zhu, 2015).

Black liquor from pine wood was also studied as a reaction media for the third generation biorefinery (Zhu, 2013). Sodium hydroxide was added to the black liquor and the mixture was contacted with lignocellulosic biomass at 250 °C for 1 h, resulting in the conversion of cellulose and hemicelluloses into organic acids such as lactic acid (about 50%), formic acid, acetic acid, glycolic acid, succinic acid, and lignin into small molecular aromatics.

## 1.2. Lignocellulose structure and targeted molecules

A major portion of lignocellulosic biomass comprises cellulose, hemicelluloses and lignin. They form a complicated network through weak bonds such as H linkages and covalent bonds such as ester and ether linkages (**Fig. 1.1**). Lignocellulose is mainly present in the cell walls of plant cells bringing structural support and protection against external aggressions (e.g., attacks from microorganisms) to the cells.



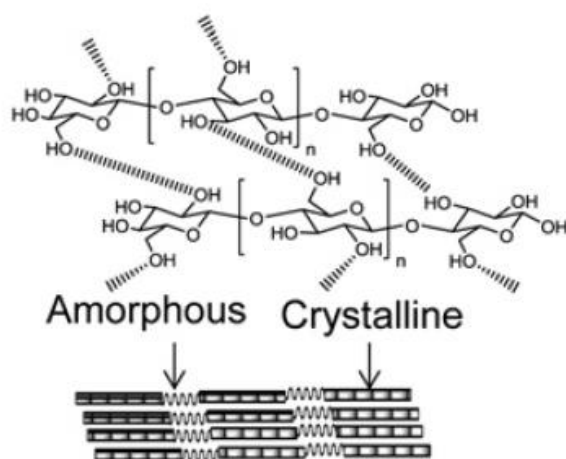
**Fig. 1.1** Composition, structure and organization of lignocellulosic biomass (Barakat et al., 2013).

### 1.2.1. Cellulose

Cellulose is a polymer of glucose units linked by  $\beta$ -1,4-glucosidic bonds, that aggregates to one another through H bonds to form fibers. The fibers present some amorphous and crystalline areas (**Fig. 1.2**). Cellulose constitutes 35–50% of the dry weight in agricultural and forestry lignocellulosic biomass.

Cellulose can be interesting in its monomeric form (glucose) or its polymeric form (glucan). Glucose can be transformed in a tremendous variety of key building block molecules (i.e., synthons) by chemical conversions (e.g., sorbitol, glucaric acid) or

enzymatic reactions (e.g., succinic acid, lactic acid) (Werpy et al., 2004). Currently, cellulose valorization as a biofuel, via the fermentation of glucose into ethanol, is one of the main process studied by scientists and industrials working on lignocellulosic biorefineries.



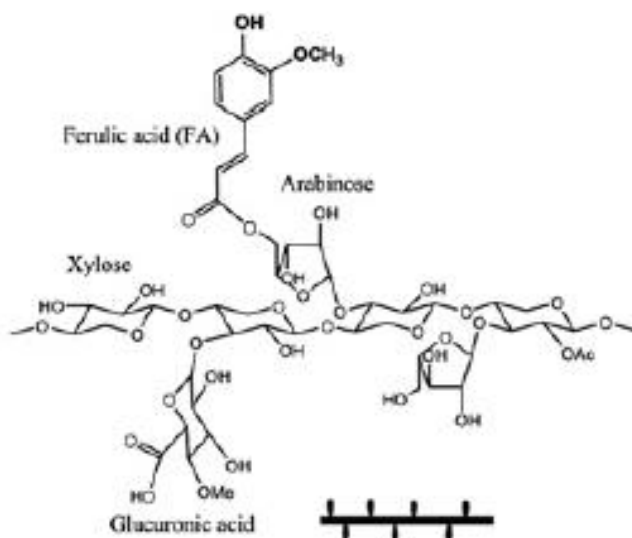
**Fig. 1.2** Composition and structure of cellulose (Barakat et al., 2013).

Cellulose can also be valorized under its polymeric form, without chemical modification, historically for paper production more recently as food additives (for its emulsifying, thickening, stabilizing properties); but also for new biomaterials application with chemical modification usually esterification or etherification on the hydroxyl groups, for example to produce cellulose acetate or hydroxyethyl methyl cellulose, that are used for cellulose film production.

### 1.2.2. Hemicelluloses

Hemicelluloses are polymers of sugars with 5 carbons (xylose, arabinose) and 6 carbons (glucose, galactose, mannose, rhamnose), with contents varying from one biomass to another (**Fig. 1.3**). The structure of hemicelluloses is generally based on a backbone substituted with side chains. Various organic acids can be bound to hemicelluloses through ester linkages on the hydroxyl groups of the sugars: acetic acid, forming acetate groups and uronic acid (mainly galacturonic acid) forming pectins. As for cellulose, hemicelluloses present interest in their monomeric form (e.g., xylose, arabinose, galactose) or their polymeric form.





**Fig. 1.3** Composition and structure of hemicelluloses (Barakat et al., 2013).

#### 1.2.2.1. Monomeric form

Like for glucose, C5 sugars are of great interest as their alcohol (e.g., xylitol and arabinitol) and carboxylic acid (e.g., xylaric acid, arabinoic acid) derivatives were targeted as key building blocks for the chemical industry by the 2004 report from US Department of Energy (Werpy et al., 2004). Monomeric C5 sugars from hemicelluloses can also be fermented to ethanol, but microorganism selection still need to be optimized to improve the yields (Valinhas et al., 2018).

#### 1.2.2.2. Acetic acid

Acetate groups are linked to hemicellulosic sugars and under some conditions the ester bond can be broken releasing acetic acid. Acetic acid present less added value (0.40-0.45 \$/kg), but has a broad spectrum of applications: polymers (vinyl acetate, cellulose acetate), solvent, reagent (Cheung et al., 2011).

#### 1.2.2.3. Uronic acid

Uronic acids are also linked to hemicelluloses with ester bonds, and are forming the pectins. The main uronic acid present in pectin is galacturonic acid. Pectins are mainly

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used as food additives for their gelling, thickening and stabilizing properties. They are also used in cosmetics and pharmaceuticals application.

### 1.2.2.4. Polymeric form

Hemicelluloses under their polymeric form can form hydrogels thanks to their numerous properties such as adsorption capacity, mechanical strength, hydrophilicity, biodegradability, biocompatibility, transparency, low cost, and non-toxicity which find application in various fields such as water depollution, food additives, food packaging, cosmetics and pharmaceuticals (Werpy et al., 2004; Ruiz et al., 2013; Hu et al., 2017). Hemicelluloses use under polymeric form is not commercially as advanced as their use under monomeric form.

### 1.2.3. Sugar degradation products

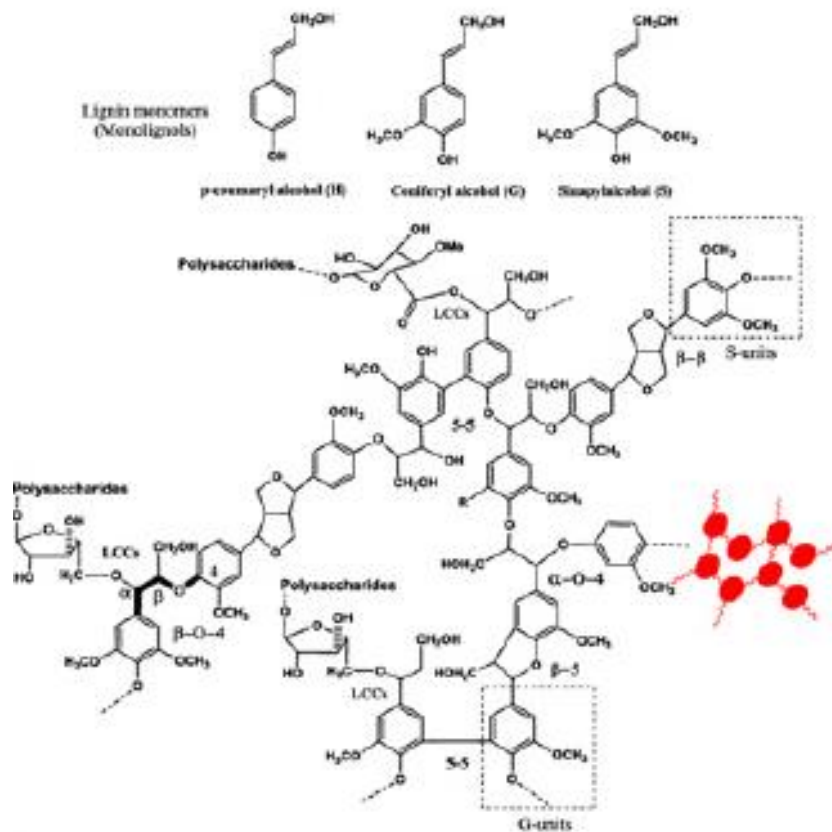
Under some fractionation conditions, carbohydrates are degraded into furans: furfural for C5 sugars and HMF for C6 sugars. Furfural can be the starting material for the synthesis of a series of derivatives including furfuryl alcohol, furoic acid, furan, tetrahydrofuran, 2-methyl-tetrahydrofuran, and related resins. HMF is an even more attractive building block molecule. It can easily be converted into dimethylfuran, which has applications as both solvent and transportation fuel. It can also be converted to furan dicarboxylic acid, which has the potential to become a major bulk chemical because it can be copolymerized with ethylene glycol to make a renewable polymer with properties similar to those of the PET polyesters used for textiles and packaging (Tuck et al., 2012). HMF may be considered an excellent platform molecule which can be converted to energy products (2,5-dimethylfuran, an octane booster), monomers for high-value polymers (2,5-carboxyfuran and 2,5-hydroxymethylfuran) and valuable intermediates for fine chemistry (Werpy et al., 2004; Lanzafame et al., 2011).

### 1.2.4. Lignin and phenolic derivatives

Lignin binds the cell wall components together, giving lignocellulosic biomass its mechanical strength and protecting parietal carbohydrates from degradation by fungi and bacteria (Chaturvedi and Verma, 2013). Lignin is a complex aromatic polymer composed of phenylpropanoid units, linked through a complex network of ether and carbon-carbon

bonds (**Fig. 1.4**). Lignin from annual plants contains guaiacyl, syringyl and p-hydroxyphenyl units (Jönsson and Martín, 2016). Other phenolic compounds, strictly not considered as lignin, are typically found in grass such as p-coumaric and ferulic acids and contribute to crosslinking with hemicelluloses. They are esterified to arabinoxylans and ether- or ester-linked to lignin (Jönsson and Martín, 2016).

In 2007, the report from US Department of Energy (Holladay et al., 2007) underlined the different opportunities that arise from utilizing lignin: (1) power, fuel and syngas (short term opportunities), (2) macromolecules (medium term opportunities), (3) aromatics and miscellaneous monomers (long-term opportunities). We will focus on the medium-term and long-term opportunities in this document.



**Fig. 1.4** Composition and structure of lignin (Barakat et al., 2013).

### 1.2.4.1. Monomers

First the molecules making the links between lignin and the carbohydrates can be valorized quite directly as fractionation process can lead to their release. In some conditions, ferulic and *p*-coumaric acid, forming bridges between lignin units and carbohydrates can be broken. They are used as food additives, in cosmetic and pharmaceuticals products due to their antioxidant activity, cholesterol-lowering activity, prevention against thrombosis and atherosclerosis, antimicrobial and anti-inflammatory activity, and anticancer effect (Ou et al., 2007, 2009; Tilay et al., 2008). Ferulic acid can also be used to produce vanillin, another important synthon, by microbial transformation (Torre et al., 2008).

Considering the phenylpropanoid units of lignin, aggressive (i.e., non-selective) depolymerization in the form of C-C and C-O bond rupture, can produce aromatics for instance in the form of benzene, toluene, xylene or phenol and aliphatics in the form of C1 to C3 fractions (Holladay et al., 2007). These products have to be purified and then they can be easily and directly used by conventional petrochemical processes. Temperatures above 300 °C and high pressure in alkaline conditions are required to depolymerize lignin and thus to obtain high added value monomers (Wang et al., 2013). For instance, hemp treated by steam explosion with 5% NaOH (w/w) at temperature between 300 and 330 °C under pressure ranging from 90 to 130 bar produced phenols and phenol derivatives (Lavoie et al., 2011). 26 compounds were identified, guaiacol, catechol, and vanillin being the most abundant. The mechanism was the cleavage of the aryl-alkyl bond ( $\beta$ -O-4 bond being the most abundant in lignin), which occurred above 270 °C. However, selectivity is low and difficult to control and the severe reaction conditions (high pressure, high temperature, and extreme pH) resulted in requirement of specially designed reactors, which led to high costs of facility and handling.

Vanillin was the first phenolic monomer synthesized from lignin and today vanillin is the world's most widely used flavor and fragrance ingredient and its market value is around 13 \$/kg (Holladay et al., 2007). Vanillin has also interesting chemical properties and is used as a synthon in agrochemicals and pharmaceuticals (Walton et al., 2003). Historically, vanillin was extracted from vanilla beans, but the demand for vanillin has long exceeded the supply of vanilla beans. In 2010, the annual demand for vanillin was

higher than 15,000 tons, but about 2,000 tons of natural vanillin were produced ([http://www.solvay.com/fr/binaries/GPS\\_2011\\_12\\_v2\\_Vanillin\\_gb-139567.pdf](http://www.solvay.com/fr/binaries/GPS_2011_12_v2_Vanillin_gb-139567.pdf)).

Oxidation of lignin, particularly issued from black liquor, to obtain vanillin has been extensively studied (Bryan, 1954; Bjørsvik and Liguori, 2002; Araújo et al., 2010). Nowadays, 15% of the vanillin is produced from lignin which requires very alkaline pH (close to 14), high temperature (130 °C) and high oxygen pressure (3 bar) (Araújo et al., 2010).

Catechol, another chemical potentially produced from lignin (Amen-Chen et al., 2001; Toledano et al., 2012), is used as a synthon for the production of numerous molecules found in perfumes, drugs, pesticides, dyes, photographic developers, or as a deoxygenating agent and analytical reagent (Fiege et al., 2000). The market price of catechol is around 3-5 \$/kg (Fiege et al., 2000; Holladay et al., 2007).

### 1.2.4.2. Polymeric form

Complete lignin depolymerization is an energy-negative process aimed at undoing what nature has done during biosynthesis (Holladay et al., 2007). Contrary to hemicelluloses, all current commercial uses of lignin, except burning and production of synthetic vanillin, take advantage of lignin's polymer and polyelectrolyte properties (Holladay et al., 2007). Currently, the main applications include dispersants, emulsifiers, binders and sequestrant. These applications requires little or no modification other than sulfonation or thio hydroxymethylation, but they represent relatively low value and market volume (Holladay et al., 2007). Lignin can be used for new applications having higher value and larger market such as bioplastics, composites, nanoparticles, adsorbents, resins (specially for formaldehyde free resins) and carbon fibers (Holladay et al., 2007; Norgren and Edlund, 2014). To facilitate lignin modification, such as polymerization to form resins, sulfur-free lignins obtained through some processes (e.g., organosolv, soda pulping) are more interesting than the thio-lignin obtained through the traditional pulp and paper processes (i.e., sulfite and sulfate processes) (El Mansouri and Salvadó, 2006).

The use of lignin for chemical production has so far been limited due to contamination from salts, sugars, particulates, volatiles, and the molecular weight distribution in

lignosulfate (Holladay et al., 2007; Higson, 2011). Important research effort are still required regarding the purification of lignin.

### 1.2.5. Conclusion

The conversion of lignocellulose to the targeted molecules implies the selection of fractionation and purification processes. Four main fractionation processes are currently co-existing at the lab scale: acid fractionation, alkaline fractionation, organosolv fractionation and steam explosion. The fractionation processes have to be selected carefully since they influence the structure of the extracted molecules. For instance, steam explosion pretreatment lead to lower molecular weight lignin than Kraft process (Higson, 2011).

### 1.3. Acid fractionation process

Vincent Oriez\*, Jérôme Peydecastaing, Pierre-Yves Pontalier\*

Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, 4 allée Emile Monso, 31030 Toulouse, France

\*Corresponding authors at: Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France

*E-mail addresses:* [vincentoriez@yahoo.fr](mailto:vincentoriez@yahoo.fr), [vincent.oriez@ensiacet.fr](mailto:vincent.oriez@ensiacet.fr) (V. Oriez), [pierreyyves.pontalier@ensiacet.fr](mailto:pierreyyves.pontalier@ensiacet.fr) (P.Y. Pontalier)

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#### 1.3.1. Introduction

Second generation biorefineries aim essentially at valorizing sugar polymers - cellulose and hemicelluloses. The first process step of such biorefineries is to fractionate biomass into cellulose, hemicelluloses and lignin. However, biomasses from plants are naturally recalcitrant, and therefore in order to increase the accessibility of cellulose and hemicelluloses, the hemicelluloses-lignin complex cross-links must be broken (Balan, 2014; Ragauskas et al., 2014). The major pretreatment studied are biological, physical and chemical sometimes with some combinations (Alvira et al., 2010). Among the chemical treatment - hot water, steam explosion, acid, alkaline, organosolv and ionic liquid – acid pretreatment is still the method of choice in several model processes (Cardona et al., 2010).

#### 1.3.2. Acid extraction

##### 1.3.2.1. Effect and mechanism

Acid media usually acts on lignocellulose by breaking glycosidic bonds and solubilizing hemicelluloses under mild conditions (Barberousse et al., 2008) and both

hemicelluloses and cellulose under severe conditions (Moe et al., 2012). The breakdown of biomass during pretreatment under acid conditions facilitates downstream enzymatic hydrolysis by disrupting cell wall structures, driving some lignin into solution, and reducing cellulose crystallinity and chain length (Humbrid et al., 2011). Polysaccharides are sequentially dissolved, then converted into monomeric sugars and finally the sugar monomers can be degraded in HMF for C6 sugars and furfural for C5 sugars according to the conditions of the reaction (Clausen and Gaddy, 1993).

The acid pretreatment is especially suitable for biomass with low lignin content (Harmsen et al., 2010), as most of the lignin remained in a solid residue. A small part of the lignin is solubilized (about 5-10% of the total lignin) and qualified as acid-soluble lignin (Saha et al., 2005a; Sluiter et al., 2008). Depending on the severity of the acid conditions employed, ester and ether bounds can be broken in the lignin and between the lignin and hemicelluloses under acid conditions (Sun et al., 2002), but acidic media also lead to the precipitation of the lignin and phenolic monomers (Sarkanen et al., 1984). Phenol monomers commonly formed when annual biomass undergo acid treatment are *p*-coumaric acid and ferulic acid, as for wood biomass, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, vanillin, dihydroconiferyl alcohol, coniferyl aldehyde, syringaldehyde, syringic acid are the most commonly produced (Jönsson and Martín, 2016).

The only exception regarding the effects described so far of mineral acid catalyst is sulfurous acid, which solubilized lignin and hemicelluloses, without impacting cellulose (Shi et al., 2012; Svetlitchnyi et al., 2013).

### 1.3.2.2. Nature of the acid

Sulfuric acid is the most preferred acid catalyst based on its price, corrosivity, toxicity and efficiency but other acids were also studied such as hydrochloric acid, nitric acid or phosphoric acid (Cardona et al., 2010; Kanchanalai, 2015). Addition of sulfuric acid has been initially applied to remove hemicelluloses either in combination with breakdown of cellulose to glucose or prior to acid hydrolysis of cellulose since the middle of the 20<sup>th</sup> century (Mosier et al., 2005). Under the same hydrolysis conditions, higher yield of monomeric sugars were obtained with sulfuric acid compare to hydrochloric, nitric or



phosphoric acids (Aguilar et al., 2002; Bustos et al., 2003; Rodríguez-Chong et al., 2004; Gámez et al., 2006; Sindhu et al., 2011).

Hydrochloric acid leads to high yield of sugars at high concentration (Israilides et al., 1978; Goldstein et al., 1983). However, more recently, it was demonstrated that in equivalent conditions hydrochloric acid is less efficient than sulfuric acid (Lavarack et al., 2002). Besides, its environmental impact, corrosive properties and price compare to sulfuric acid strongly limits its application.

Nitric acid has not been widely studied. It reduces containment costs relative to sulfuric acid, but its higher cost counterbalances this benefit (Mosier et al., 2005). Under optimized conditions, nitric acid was less efficient than sulfuric acid to convert hemicelluloses into monomeric sugars (Aguilar et al., 2002; Rodríguez-Chong et al., 2004). Nitric acid (2%) combined with acetic acid (35%) at 100 °C for 30 min was found to increase drastically the lignin removal (up to 80%) from newsprint, whereas no lignin was solubilized with acetic acid alone even under elevated temperature (Xiao and Clarkson, 1997).

Not many data can be found on the use of phosphoric acid for lignocellulosic biomass pretreatment (Israilides et al., 1978; Gámez et al., 2006; Idrees et al., 2013), though phosphoric acid is promising. Phosphoric acid was more efficient at producing monomeric sugars under the same optimized conditions (concentration, time, temperature) than sulfuric acid with a yield of 79.9% of monomeric sugar against 75.9% with sulfuric acid on water hyacinth biomass (Idrees et al., 2013). On sugarcane bagasse, the reverse result was obtained with sulfuric acid leading to more monomeric sugar than phosphoric acid with even milder conditions (Aguilar et al., 2002; Gámez et al., 2006). After neutralization of phosphoric acid hydrolysates with sodium hydroxide, the salt formed is sodium phosphate. This salt can remain in the hydrolysates because it is used as nutrient by microorganisms during the following monosaccharides fermentation step. This has two advantages: no filtration step to remove the precipitated salt is required and it decreases the addition of nutrients to run the fermentation (Cardona et al., 2010).

### 1.3.2.3. Conditions and yields

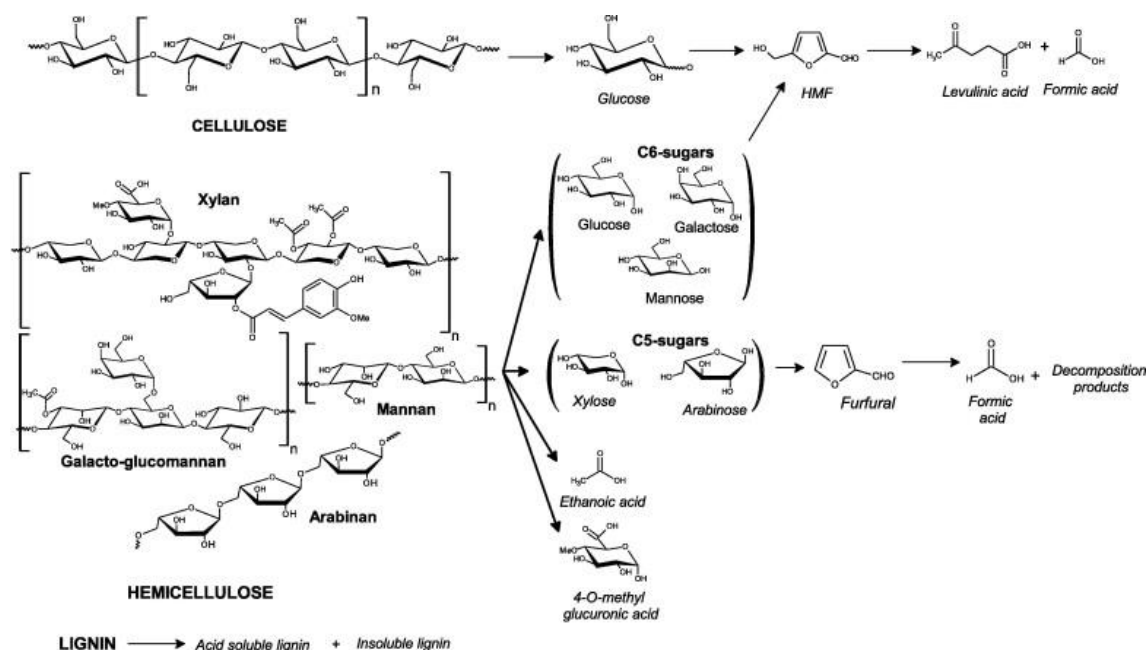
An optimal balance in the hydrolysis conditions has to be found to maximize the hydrolysis of polysaccharides to obtain monomeric sugars and prevent further degradation of these monomeric sugars into degradation products (Lee et al., 1999; Lavarack et al., 2002). For the conditions of pretreatment, four main parameters vary: the concentration of the acid, the solid:liquid ratio (S:L ratio), the temperature and the reaction time. Other parameters, such as the biomass particle size or the agitation, are also important but more standard in the different studies found in the literature. Acid treatment is carried out in the presence of high and low concentrations of acids, and at high and low temperatures (Chaturvedi and Verma, 2013). Low concentration are often associated with high temperature, whereas high concentration of acid are carried out at low temperature with a higher solid/liquid ratio.

#### a) Low concentration of acid and high temperature

Dilute acid pretreatment are usually in the acid concentration range of 0.5 to 8% (w/w), S:L ratio range of 1:5 to 1:20 (w/v), temperature range of 100 to 200 °C, and reaction time range of 5 to 300 min.

Dilute sulfuric acid pretreatment generally lead to the solubilization of the hemicelluloses and a small fraction of the lignin, the hydrolysis of the solubilized hemicelluloses, and the decrease of cellulose crystallinity (Huang et al., 2008; Saha et al., 2005a). Therefore, dilute acid pretreatment eliminates or reduces the need for hemicellulase enzyme mixtures for hemicellulose saccharification (Saha et al., 2005a). In parallel, during dilute acid pretreatment, the majority of lignin remains as a solid residue, only some ether and ester linkages are cleaved, generating low molecular-weight lignin fragments with increased hydroxyl group content (Ma et al., 2015). When dilute acid hydrolysis is run at high temperatures or for long period of time, it can break down cellulose too (Girisuta et al., 2013; Kanchanalai, 2015). During the acid hydrolysis of lignocellulosic biomass at given acid concentration, S:L ratio and temperature, reaction time monitoring is important. Indeed, under acidic conditions, cellulose is first hydrolyzed under glucose which in turn can be converted into HMF, and HMF can finally be degraded into levulinic acid and formic acid (**Fig. 1.5**). In parallel, hemicelluloses are

hydrolyzed under monomeric sugars too (xylose, arabinose, galactose, mannose, glucose...), acetate groups attached to the hemicelluloses are released as well as uronic groups producing acetic acid, glucuronic acid and galacturonic acid. Monomeric C5 sugars are then converted into furfural, which in turn can be converted into formic acid and other degradation products (Almeida et al., 2007; Girisuta et al., 2013). There is an optimum time after which the hydrolysis of more hemicelluloses and cellulose into monomeric sugars do not compensate the loss of monomeric sugars being converted in furan degradation products (Saha et al., 2005a). Variation of one of the three parameters - acid concentration, S:L ratio or temperature - affects the optimum time duration of the hydrolysis. For instance, at 1% sulfuric acid, the higher the temperature the faster the maximum yield of glucose was reached, from 50 min at 170 °C to 2 min at 220 °C (Lee et al., 1999). Longer reaction time led to a decrease of glucose concentration due to its degradation (Lee et al., 1999). Similarly, with all other hydrolysis variables constant, an increase of acid concentration led to a faster rate for the hemicelluloses hydrolysis (Lavarack et al., 2002).



**Fig. 1.5** Degradation products formation from lignocellulosic biomass under dilute acid pretreatment at high temperature (Girisuta et al., 2013).

Inspired from the pulp and paper industry, a combined severity factor (CSF) was developed by Chum et al. (1990) for dilute acid treatment taking into account temperature, acid concentration and time of the pretreatment as detailed in Eq. (1) (Chum et al., 1990). On corn stover, a CSF in the range of 1.4-1.8 was optimal for the xylose yield in the acid hydrolysate, lower or higher CSF reduced this yield due to incomplete solubilization and hydrolysis or due to monomeric sugar degradation, respectively (Lee et al., 2015).

$$\text{CSF} = \log \left\{ t \cdot \exp \left( \frac{T_H - T_{\text{Ref}}}{14.75} \right) \right\} - \text{pH} \quad (1)$$

where  $t$  is the reaction time in min,  $T_H$  is the reaction temperature in °C,  $T_{\text{Ref}}$  is the reference temperature, most often 100 °C, and  $\text{pH}$  is the initial pH value (calculated from the mineral acid concentration).

The efficiency of the pretreatment has sometimes been defined by the sum of the monomeric sugars concentrations (glucose, xylose, arabinose) divided by the sum of the fermentation inhibitors concentrations (furfural, HMF, acetic acid) (Rodríguez-Chong et al., 2004; Gámez et al., 2006; Patra et al., 2008). It is interesting to anticipate the yield of the following fermentation step based on this ratio and in this way to compare the pretreatment conditions efficiency. However, different inhibitors have different inhibition threshold according the fermentation enzymes or microorganisms used, for instance furans usually decrease their activity at lower concentration than acetic acid (Mussatto and Roberto, 2004; Almeida et al., 2007). Besides, the final concentration of monomeric sugars is highly dependent on the S:L ratio used for the dilute-acid hydrolysis.

Studies also presented the yield of monomeric sugars after dilute acid pretreatment and enzymatic saccharification or the yield of ethanol after fermentation of the sugars (Saha et al., 2005b; Sun and Cheng, 2005). Comparison between the different pretreatments is valid only if the same enzymatic saccharification or fermentation conditions were employed, but as there is no standard process, it is rarely the case. Besides, some studies focused on the production of other molecules than ethanol from the enzymatic conversion of monomeric sugars such as hydrogen (Patra et al., 2008) or xylitol (Rocha et al., 2014). For these reasons, to compare the different acid pretreatments, the yields of monomers obtained after the dilute-acid hydrolysis (monomeric sugars production over their total potential regarding the polysaccharides content in the initial biomass) appeared to be of

interest. As purification is often required between the saccharification step and the valorization of the monomeric sugars, after the pretreatment step it is valuable to look for the highest yield possible for the monomeric sugars and not consider their purity or concentration. The results of some studies are gathered in **Table 1.1**, with a focus on sugarcane bagasse for the lignocellulosic biomass and sulfuric acid for the acid used, for easier comparison between the hydrolysis conditions.

Sulfuric acid was found to be the most efficient to yield monomeric sugars among all the acid tested (**Table 1.1**). The lignocellulosic biomass treated has a significant impact on the monomeric sugars yields (**Table 1.1**). For instance, with the same pretreatment conditions, glucose yield reached 33% for bermudagrass whereas it was 10% for rye straw (Sun and Cheng, 2005).

Concentration lower than 1% present the advantage of being more cost efficient (Sun and Cheng, 2005). Short treatment time (less than 60 min) and relatively high temperature (at least 120 °C) are necessary for an efficient pretreatment. Concentration can be reduced to level as low 0.07% w/w of sulfuric acid using a flow-through reactor configuration at high temperature (140-204 °C) while still obtaining 83-100% recovery for the hemicelluloses, 80 to 95% being under monomeric form, and 26-53% lignin recovery. However, this pretreatment presented the major drawbacks of high water and energy consumption (Mosier et al., 2005).

Dilute acid treatments are considered as cheap regarding the low cost of acids, are relatively efficient regarding the hydrolysis of hemicelluloses into monomeric sugars (generally about 90% yield) and the yield of the following step - the enzymatic hydrolysis of cellulose. They are also fast regarding other pretreatments, for instance alkaline pretreatment. However, they are carried out at high S:L ratio and at high temperatures which impact the cost efficiency of the process. Besides, acids under high temperature are corrosive, so resistant reaction vessels are required. This process also generates fermentation inhibitors (furans, carboxylic acids, phenol derivatives) that has to be removed. Removing these molecules is usually a necessity for the following enzymatic reaction step, and purifying them for further valorization can enhance the profitability of the process.

**Table 1.1**

Yields of sugar solubilization and sugar monomers produced after treatment on lignocellulosic biomass under optimized acid conditions

<b>Biomass</b>	<b>Variable</b>	<b>Optimized conditions</b>	<b>Monomer yield</b>	<b>Author</b>
SCB	0.25-7% H <sub>2</sub> SO <sub>4</sub> (v/v)	0.5% H <sub>2</sub> SO <sub>4</sub> (v/v)	44% glucose	(Pattra et al., 2008)
	No variation of S:L ratio	S:L ratio 1:15 (w/v)	74% hemicelluloses	
SCB	No variation of temperature	121 °C	62% xylose	(Canilha et al., 2011)
	Time: 15-240 min	60 min		
	0-3% H <sub>2</sub> SO <sub>4</sub> (w/v)	1.5% H <sub>2</sub> SO <sub>4</sub> (w/v)		
	No variation of S:L ratio	S:L ratio 1:6.7 (w/v)		
SCB	112.5-157.5 °C	135 °C	80% xylose	(Lavarack et al., 2002)
	5-35 min	20 min		
	0.25-8% H <sub>2</sub> SO <sub>4</sub> (w/w)	4% H <sub>2</sub> SO <sub>4</sub> (w/v)		
	S:L ratio 1:5-1:20 (w/w) DS	S:L ratio 1:20		
	80-200 °C	120 °C		
	10-2000 min	60 min		
SCB	2-6% H <sub>2</sub> SO <sub>4</sub> (w/w)	2% H <sub>2</sub> SO <sub>4</sub> (w/w)	5% glucose	(Aguilar et al., 2002)
	No variation of S:L ratio	S:L ratio 1:10 (w/w)	92% xylose	
	100-128 °C	122 °C		
	0-300 min	24.1 min		
SCB	2-6% HCl (w/w)	2% HCl (w/w)	8% glucose	(Bustos et al., 2003)
	No variation of S:L ratio	S:L ratio 1:10 (w/w)	100% xylose	
	100-128 °C	128 °C	36% arabinose	
	0-300 min	51.1 min		

SCB	2-6% HNO <sub>3</sub> (w/w) No variation of S:L ratio 100-128 °C 0-300 min	6% HNO <sub>3</sub> (w/w) S:L ratio 1:10 (w/w) DS 122 °C 9.3 min	7% glucose 85% xylose 32% arabinose	(Rodríguez-Chong et al., 2004)
SCB	2-6% H <sub>3</sub> PO <sub>4</sub> (w/w) No variation of S:L ratio 100-128 °C 0-300 min	4% H <sub>3</sub> PO <sub>4</sub> (w/w) S:L ratio 1:8 (w/w) DS 122 °C 300 min	6% glucose 60% xylose 33% arabinose	(Gámez et al., 2006)
Rye straw	0.6-1.5% H <sub>2</sub> SO <sub>4</sub> (w/w) No variation of S:L ratio No variation of temperature 30-90min	1.5% H <sub>2</sub> SO <sub>4</sub> (w/w) S:L ratio 1:10 (w/v) DS 121 °C 90 min	10% glucose 65% xylose 67% arabinose	(Sun and Cheng, 2005)
Bermudagrass	0.6-1.5% H <sub>2</sub> SO <sub>4</sub> (w/w) No variation of S:L ratio No variation of temperature 30-90min	1.5% H <sub>2</sub> SO <sub>4</sub> (w/w) S:L ratio 1:10 (w/v) DS 121 °C 90 min	33% glucose 59% xylose 65% arabinose	(Sun and Cheng, 2005)
Sweet sorghum bagasse		0.5% H <sub>2</sub> SO <sub>4</sub> (w/w) S:L ratio 1:20 (w/v) 170 °C 20 min	85% xylose	(Wu et al., 2011)

The size of the biomass has a strong influence on the hydrolysis yield. It is not mentioned here as it was usually not studied. (Sindhu et al., 2011)

Here are some explanations of explanations for the understanding of **Table 1.1**. SCB is one of the most studied lignocellulosic biomass it was taken as the model biomass, besides, it contains mainly 3 sugars - glucose, xylose, arabinose – facilitating the comparison. Other biomass are presented as reference. H<sub>2</sub>SO<sub>4</sub> was taken as the reference for the acid, as it was the most studied and employed industrially.

For SCB, glucose was supposed to come from cellulose exclusively, and xylose and arabinose were supposed to be the only components of the hemicelluloses

When the results of the hydrolysis is given in g/L in the literature, it was converted by calculation to yield of monomeric sugar following Eq. (2) and (3):

$$Y_{glucose} = \frac{[glucose]}{C \cdot 1.11 \cdot r_{S:L}} \quad (2)$$

With [glucose], the glucose concentration in the acid hydrolysate (g/L), C the cellulose content in the initial biomass (%DS), r<sub>S:L</sub> the solid/liquid ratio of the hydrolysis (g/L).

C has to be corrected by a 1.11 factor to represent the initial potential in glucose of the biomass as a molecule of water is added to glucose during the hydrolysis of cellulose.

$$Y_{hemicelluloses} = \frac{[xylose] + [arabinose]}{H \cdot 1.14 \cdot r_{S:L}} \quad (3)$$

With [xylose] and [arabinose], the xylose and arabinose concentration in the acid hydrolysate (g/L), H the hemicelluloses content, the addition X the xylan and A the arabinan in the initial biomass (%DS), r<sub>S:L</sub> the solid/liquid ratio of the hydrolysis (g/L).

X and A has to be corrected by a 1.14 factor to represent the initial potential in xylose and arabinose of the biomass, respectively, as a molecule of water is added to xylose and arabinose during the hydrolysis of hemicelluloses.



Dilute acid process can be used to produce the degradation products of sugars under acid conditions which presents high value, some being referred as platform chemicals by the US Department of Energy such as levulinic acid or furfural (Werpy et al., 2004). Biofine process developed by Fitzpatrick in the 1990s produce levulinic acid and furfural from a dilute acid treatment of lignocellulosic biomass without enzymatic hydrolysis or fermentation step (Fitzpatrick, 1990, 1997). Sulfuric acid at concentration between 1 and 5% is contacted with biomass for short period of time (from a few seconds to a few minutes) in a two-reactor system at high temperature (in the range of 195-230 °C) to obtain high yields of levulinic acid and furfural from the degradation of the hexoses and pentoses (Fitzpatrick, 1990, 1997). It leads to the conversion of approximately 50% of the mass of 6-carbon sugars to levulinic acid, with 20% being converted to formic acid and 30% being incorporated in the residual “char” material which also contains all of the Klason lignin and 50% of the pentoses that do not convert to furfural (Hayes, 2009). A commercial facility run by GF Biochemicals in Caserta, Italy, process 50 tons per day of waste paper, municipal wastes and agricultural residues to produce levulinic acid and furfural based on Biofine process (Hayes, 2009).

### b) High concentration and low temperature

The concentrated acid hydrolysis process appeared to be an interesting process for saccharification of lignocellulosic biomass, as it leads to high sugar yields, low levels of fermentation inhibitors and good flexibility regarding the different raw material treated compare to the dilute acid pretreatment (Moe et al., 2012). Low temperatures (typically under 60 °C) and high S:L ratio (from 1:2.5 to 1:10 (w/v)) are generally used, improving significantly the cost effectiveness of the treatment (Harmsen et al., 2010; Chaturvedi and Verma, 2013). No enzymatic saccharification is required to reach the same monomeric yield as with dilute acid hydrolysis, for instance, rice hulls treated with concentrated H<sub>2</sub>SO<sub>4</sub> 67% (w/w) at 25 °C for 3 h gave higher monomeric sugar yield (62%) than dilute acid treatment (H<sub>2</sub>SO<sub>4</sub> 1% (v/v), 121 °C, 15 min) followed by enzymatic saccharification (60% yield) (Saha et al., 2005b). Concentrated mineral acids such as sulfuric acid and hydrochloric acid are widely used for treating lignocellulosic materials because they are powerful agents for both hemicelluloses and cellulose hydrolysis. The major drawback of concentrated acid is their corrosive nature and the need to recycle acids to lower the

cost of pretreatment (Harmsen et al., 2010; Chaturvedi and Verma, 2013). Special acid resistant material for the vessels are to be investigated such as such as ceramic or carbon-brick lining (Anwar et al., 2014).

As for dilute acid treatment, the four main parameters (acid concentration, S:L ratio, temperature and experiment duration) have to be selected carefully in order to maximize the yield of the polysaccharides solubilization and hydrolysis. For instance, from corn stover treated under 70% H<sub>2</sub>SO<sub>4</sub> at 50 °C for 20 min, 90% yield for the monomeric sugars was achieved with a S:L ratio of 1:50 (w/v), but when the S:L ratio was increased to 1:10, then the monomeric sugar yield dropped to 65% (Clausen and Gaddy, 1993). With the same sulfuric acid concentration, an intermediate S:L ratio of 1:20 (w/v) and an increased temperature to 70 °C, it was possible to achieve total conversion into monomeric sugars (Clausen and Gaddy, 1993).

In order to be competitive with the fossil fuels, the main challenge for the use at industrial scale of the concentrated acid pretreatment of lignocellulosic biomass to produce ethanol is the recycling of the acid, that have risen from 80 to 97% in the process in the last 50 years, but the cost of the recycling process remain high (Hayes, 2009; Moe et al., 2012). In theory, sulfuric acid acts during lignocellulosic biomass hydrolysis as a catalyst, so in principle no sulfuric acid should be consumed during this process (Cheng et al., 2008). However, some acid still have to be reintegrated in the process in addition to the recycled acid due to unavoidable losses (for instance through absorption by the biomass or salification of inorganic cations).

#### 1.3.2.4. Industrial applications

Dilute sulfuric acid pretreatment under fairly mild conditions seem to receive the biggest focus from industrial, as for instance the National Renewable Energy Laboratory (NREL), from the US department of Energy, established an exhaustive public report with technical and economic feasibility of such pretreatment (Humbrid et al., 2011). The process described uses co-current dilute-acid pretreatment of lignocellulosic biomass (corn stover) to liberate the hemicelluloses, followed by enzymatic hydrolysis (saccharification) of the remaining cellulose, followed by fermentation of the resulting glucose and xylose to ethanol (Humbrid et al., 2011). Overall, the total acid loading is

22.1 mg/g dry biomass, and the effective sulfuric acid concentration in the pretreatment reactor was estimated at 0.3–0.4% w/v, which may allow for the use of lower-cost metallurgies in the reaction zone (Humbrid et al., 2011). All acetate groups bound to the hemicelluloses were released under the form of acetic acid, 5% of the xylose was converted to furfural and 5% of the lignin was solubilized (**Table 1.2**) (Humbrid et al., 2011). Ammonia gas was used to adjust the pH of the acid hydrolysate from 1 to 5-6 to enable further enzymatic saccharification and fermentation. (Humbrid et al., 2011)

**Table 1.2**

Pretreatment hydrolysis reactions and assumed conversions (Humbrid et al., 2011)

Reaction	Reactant	% Converted to Product
$(\text{Glucan})_n + n \text{H}_2\text{O} \rightarrow n \text{Glucose}$	Glucan	9.9%
$(\text{Glucan})_n + n \text{H}_2\text{O} \rightarrow n \text{Glucose Oligomer}^a$	Glucan	0.3%
$(\text{Glucan})_n \rightarrow n \text{HMF} + 2n \text{H}_2\text{O}$	Glucan	0.3%
$\text{Sucrose} \rightarrow \text{HMF} + \text{Glucose} + 2 \text{H}_2\text{O}$	Sucrose	100%
$(\text{Xylan})_n + n \text{H}_2\text{O} \rightarrow n \text{Xylose}$	Xylan	90.0%
$(\text{Xylan})_n + m \text{H}_2\text{O} \rightarrow m \text{Xylose Oligomer}^a$	Xylan	2.4%
$(\text{Xylan})_n \rightarrow n \text{Furfural} + 2n \text{H}_2\text{O}$	Xylan	5.0%
$\text{Acetate} \rightarrow \text{Acetic Acid}$	Acetate	100%
$(\text{Lignin})_n \rightarrow n \text{Soluble Lignin}$	Lignin	5.0%

<sup>a</sup> Sugar oligomers are considered soluble but not fermentable.

Industrial development of the concentrated acid pretreatment is at its beginning. Some companies such as Biosulfurol Energy attempted to commercialize concentrated acid process for the pretreatment of lignocellulosic biomass in order to produce ethanol (van Groenestijn et al., 2006) but eventually failed (the company was dissolved in 2016). The market appears to be led by three companies: BlueFire Renewables, Virdia, and Renmatix (Tuck et al., 2012). The companies claim to be economically competitive with the production of sugars from traditional agricultural sources such as sugarcane, for instance, the process from Virdia claim to produce sugars at 0.25 \$/kg from lignocellulosic wastes compared to 0.45 \$/kg from sugarcane (Tuck et al., 2012).

Arkenol gave detailed conditions in two patents regarding the pretreatment with concentrated acid followed by dilute acid hydrolysis. Sulfuric acid 70-77% should be added to the biomass in order to achieve a ratio of acid to cellulosic and hemicellulosic materials of at least 1.25:1 (w/w) (Farone and Cuzens, 1993, 1997). After the decrystallization or solubilization stage, if the concentrated acid pretreatment did not lead

to a 100% monomeric sugar yield, the concentrated acid can be diluted, to a concentration of about 20-30% for a second hydrolysis to completely convert the solubilized cellulose and hemicelluloses into monomeric sugars (Farone and Cuzens, 1997). BlueFire Renewable process is based on Arkenol patent (Farone and Cuzens, 1997), their plant in operation since 2002 in Izumi, Japan was already producing over 80,000 L of ethanol 95% in 2004 (<https://bfreinc.com/production-plant/>). Another patent gave similar hydrolysis conditions, and provided detailed solubilization yield of polymeric sugars and conversion yield into their monomeric forms according to the conditions (acid concentration, solid:liquid ratio, temperature and experiment duration) employed during the concentrated acid hydrolysis then the dilute acid hydrolysis (Clausen and Gaddy, 1993). Similar processes have been employed in other studies, for example Sun et al. (2011) produced an acid hydrolysate from bamboo with successive concentrated acid then dilute acid hydrolysis and obtained a monomeric sugar yield of 81.6% (Sun et al., 2011).

Combination of concentrated acid hydrolysis and diluted acid hydrolysis for the saccharification of cellulose and hemicelluloses has been optimized and proved to be so efficient that it became a standard method for the characterization of lignocellulosic biomass carbohydrates and lignin, developed by NREL (Sluiter et al., 2008). A first hydrolysis with concentrated sulfuric acid (72% w/w) and a S:L ratio of 1:10 (w/v) at 30 °C for 1 h, followed by a dilution to reach 4% (w/w) sulfuric acid to run a second hydrolysis at 121 °C for 1 h, lead to a complete saccharification of the cellulose and hemicelluloses. Uncomplete saccharification, detected by the presence of cellobiose, can be corrected by a longer diluted-acid hydrolysis, while over time hydrolysis can be detected by furfural and HMF follow-up (Sluiter et al., 2008).

### 1.3.3. Purification routes applied to acid hydrolysates

Monomeric sugars are the target molecules to valorize from lignocellulosic acid hydrolysates. Depending on the operational conditions, the lignocellulosic acid hydrolysates are also constituted of other molecules which are growth fermentation inhibitors such as acetic acid (generated from the hydrolysis of acetyl groups linked to hemicelluloses), furans and other decomposition products from monosaccharides (e.g., levulinic acid, formic acid), phenolic monomers and acid soluble lignin.

Purification requirements (purity, yield and cost) have to be considered regarding the final objective of the process as for some application, for instance xylitol production from a hemicellulosic extract, fermentation inhibitors removal prior to fermentation, by several methods (overliming, adsorption on charcoal or resins) did not lead to higher xylitol yield compared to a simple pH adjustment to 5.5 (Carvalho et al., 2005). For other fermentation reactions, glucose or xylose to ethanol for instance, furan concentration greater than 1 g/L strongly inhibit the enzymes (Weil et al., 2002), so lower concentration for these molecules are targeted after the purification step. Since the detoxification process can be expensive and take a large portion of the whole ethanol production cost, selection of detoxification method is of major importance (Huang et al., 2008).

However, this cost can be compensated by the value of these undesired molecules such as furfural, HMF and phenolic monomers. Their valorization would contribute to the efficiency of the lignocellulosic biorefinery.

### 1.3.3.1. Alkalinisation/overliming

Alkali treatment is suitable for dilute-acid hydrolysates because of the high quantity of base required to adjust the pH. It leads to great improvement in fermentability of the hydrolysates by chemically converting fermentation inhibitors (Persson et al., 2002) or precipitating and removing them by centrifugation or filtration (Alriksson et al., 2006; Sánchez and Cardona, 2008; Lemaire et al., 2016). Sodium hydroxide, potassium hydroxide, calcium hydroxide and ammonia have already been tested for neutralization or alkalization of lignocellulosic acid hydrolysate (Persson et al., 2002).

The best conditions to use sodium hydroxide are high temperature in combination with moderate pH or moderate temperature in combination with high pH. For instance, a pH adjustment to 9 at 80 °C has been found to be the best condition, with an increase of 110% in ethanol production compared to the value of the reference (Alriksson et al., 2006). However, under similar conditions sodium hydroxide treatment has so far been less efficient than overliming (Alriksson et al., 2006).

Overliming, i.e., the addition of calcium hydroxide, is considered as one of the most efficient process for the removal of fermentation inhibitors (Alriksson et al., 2006; Huang et al., 2008). In the overliming process, the hydrolysate is detoxified by the addition of

calcium hydroxide to adjust the pH to 9–10, leading to the precipitation of some of the furans and phenolic monomers which are recovered by centrifugation or filtration (Carvalho et al., 2005; Chandel et al., 2007). The resulting hydrolysate is then readjusted to 5.5 with dilute sulfuric acid in order to carry out fermentation. Overliming treatment (pH adjustment to 10.5, 90 °C, 30 min) on acid- and enzyme-treated rice hull hydrolysate not only increased the maximum ethanol yield but also reduced the duration required for maximum ethanol production in the case of simultaneous saccharification and fermentation process (Saha et al., 2005b). However, overliming lead to drastic reduction of potential fermentable sugars, both at pH 5.5 or 10, with 28 and 47% sugar elimination, respectively (Mateo et al., 2013).

However, overliming may be associated with problems that are not acceptable for industrial implementation, such as simultaneous degradation of fermentable sugars, resulting in moderate ethanol yield, and the formation of large amounts of gypsum (Alriksson et al., 2006; Huang et al., 2008; Mateo et al., 2013). In NREL technical report (2011), it is reported that a significant amount of sugar in the liquor (as much as 13%) could be lost to side reactions occurring at high pH or pressed out with the wet gypsum (Humbrid et al., 2011).

Alkalinisation with ammonia is an alternative to overliming as it presents the advantage of reducing the precipitate formed compared to the amount of gypsum formed using calcium hydroxide. Besides, milder conditions (pH 9 and 55 °C) can be used with ammonia, the removal of furan aldehydes and phenols is relatively extensive and precipitation is not prerequisite for an efficient detoxification (Alriksson et al., 2006). Detoxification with ammonia was found to be more efficient than with sodium hydroxide and calcium hydroxide regarding ethanol productivity and ethanol yield (Persson et al., 2002; Alriksson et al., 2006). Less drastic conditions of detoxification with ammonia was even taken as reference for a model 2<sup>nd</sup> generation ethanol lignocellulosic biorefinery by NREL (Humbrid et al., 2011). In their process, ammonia was added to the lignocellulosic acid hydrolysate to raise its pH from about 1 to 5 for the following enzymatic hydrolysis. No precipitation occurred and fermentation studies have indicated that there was no benefit to over-conditioning at high pH when using ammonia, so the hydrolysate was simply adjusted to enzymatic hydrolysis pH in one step. Ammonia is more expensive than lime, but the economic benefits of reduced sugar loss and reduced capital cost make

ammonia the most economical alternative. It can also possibly reduce nitrogen requirements during the fermentation step, but it has not been demonstrated yet (Humbrid et al., 2011).

Despite the optimization realized, alkanisation processes present some drawbacks including the large amount of base required, the impossibility to recycle the acid catalyst for the hydrolysis step, their lower efficiency compare to other purification techniques and the acetic acid remaining fully in the hydrolysates (Chandel et al., 2007; Lemaire et al., 2016). Nevertheless, most industrial purification methods of acid hydrolysates begin with a partial or complete neutralization of the inorganic acid then other methods such as ion-exchange, adsorption, chromatography or crystallization are used to purify the sugars (Lemaire et al., 2016).

### 1.3.3.2. Evaporation

Evaporation is a simple procedure to remove acetic acid, furfural and other volatile components in the hydrolyzates, but phenolic monomers and lignin degradation products cannot be removed (Wilson et al., 1989). It can decrease the concentrations of acetic acid and furfural below their inhibitory level for some application such as the fermentation of xylose to xylitol, for instance, the boiling of *Eucalyptus globules* wood dilute-acid hydrolysate for 160 min decrease the concentration of acetic acid and furfural by evaporation from 31.2 to 1.0 g/L and from 1.2 to 0.5 g/L, respectively (Huang et al., 2008). Evaporation under acid conditions (pH = 1) favors the evaporation of acetic acid, which is volatile only under its protonated form, but at the opposite, acidic conditions is less favorable for HMF removal (only 4.5% removed at 70 °C under vacuum) (Mateo et al., 2013).

Association of alkanisation and moderate heat treatment (90 °C during 30 min) was already employed leading to the removal of volatile compounds along with the precipitation induced by the alkanisation (Palmqvist and Hahn-Hägerdal, 2000) Evaporation at lower temperature (55 °C) under vacuum was efficient to remove about half of the acetic acid and all the furfural from a hemicelluloses acid hydrolysate but not the acid soluble lignin (Wilson et al., 1989).

### 1.3.3.3. Liquid/liquid extraction

Several organic solvents (chloroform, n-hexane and ethyl acetate) have been tested for the removal of fermentation inhibitory compounds from lignocellulosic acid hydrolysates under various hydrolysate:solvent ratio (2:1, 1:1, 1:2, 1:3, (v/v)) (Mateo et al., 2013). Overall, ethyl acetate was the most efficient solvent with removal rates of 50% for phenolic compounds, 94% for furfural and 40% for HMF. Increasing the hydrolysate:solvent ratio improved the efficiency of the fermentation inhibitors removal until 1:2 (v/v), further increase did not produce noticeable changes. The sugar loss (8%) was found to be less important than with alkalinasation/overliming or evaporation as purification processes.

Ethyl acetate is usually the organic phase used for the liquid/liquid extraction. Four extractions with an ethyl acetate:lignocellulosic acid hydrolysate ratio of 1:1 (v/v) enabled to remove all the phenolic monomers and the furfural and led to the same removal level of acetic acid as evaporation (2.7 g/L left in the solvent extracted hydrolysate) (Wilson et al., 1989). Based on ethanol yield in the following fermentation stage liquid:liquid extraction was far more efficient than evaporation. However, the level of acid acetic left in the solvent extracted acid hydrolysate was still too high as it led to longer fermentation than acid hydrolysate free of acetic acid.

Prefiltrated *Eucalyptus globules* dilute-acid from hydrolysate was extracted once with ethyl acetate using a hydrolysate:ethyl acetate ratio of 1:3 (v/v) (González et al., 2004). The resulting solvent extracted hydrolysate had a higher acetic content (5 g/L) than the process with four extractions at a ratio of 1:1, and some furfural (0.5 g/L) and HMF (0.1 g/L) were detected as well.

The high consumption of solvent and the necessity to recycle it are the main limiting factor for the economic efficiency of the process.

### 1.3.3.4. Adsorption

Purification through adsorption processes is based on the difference of affinity among the different molecules from a mixture with a sorbent. Two sorbents were mainly studied to separate lignocellulosic alkaline extract components: activated charcoal and resins. The



aim of using these sorbents on lignocellulosic acid extracts is to adsorb sugar fermentation inhibitors such as phenolic compounds, furans and in a lower extent acetic acid.

### a) Activated charcoal

The most important parameter for efficient adsorption is the hydrolysate :AC ratio (Parajó et al., 1996a), optimum ratio to ensure good impurity removal while not adsorbing sugar appeared to be about 200:1 to 50:1 (w/w) depending on the concentration of the hydrolysate components (Parajó et al., 1996b; Mussatto and Roberto, 2004; Chandel et al., 2007). pH also strongly influences adsorption process, weak organic acids (phenols, acetic acid) being most readily adsorbed in the non-ionized state and consequently a low pH favors adsorption, whereas the ionized form of the weak acids are poorly adsorbed at high pH (Mussatto and Roberto, 2004; Mateo et al., 2013). Contact time between activated charcoal and hydrolysates for optimizing adsorption was found to be at least 20 min, equilibrium was found to be about 60 min (Parajó et al., 1996a). From room temperature to 80 °C, adsorption of phenolic compounds increased drastically (Mussatto and Roberto, 2004).

Addition of AC to neutralized *Eucalyptus globulus* wood acid hydrolysates with hydrolysate/AC ratio of 200:1 (w/w) at 40 °C led to about 80% lignin adsorption while xylose is almost totally recovered in the hydrolysate (less than 2% adsorption) (Parajó et al., 1996b). Lignin adsorption improved by 28% the xylose consumption during the downstream fermentation step (Parajó et al., 1996b). When ethanol production is the target of the whole process, the lignocellulosic acid hydrolysates are often pH adjusted to 5.5 prior to AC adsorption as the fermentation of the sugars occurred at this pH (Carvalho et al., 2005; Chandel et al., 2007). This raise in pH of the hydrolysates can also be beneficial for the adsorption of the inhibitory compounds, as low pH can lead to adsorption of sulfuric acid that makes the surface of the AC less hydrophobic, which reduces adsorption of the inhibitory compounds (Sainio et al., 2011).

Comparison of alkanisation, overliming, evaporation, liquid/liquid extraction and adsorption with AC showed that adsorption with AC was the most efficient method for the removal of fermentation inhibitors while minimizing sugar losses (Mateo et al., 2013).

The use of adsorption also minimize the cost of the process as low temperature (30 °C) is used as well as low ratio of hydrolysate:charcoal (50:1 (w/w)).

On a synthetic solution of 20% (w/w) sulfuric acid, containing glucose, furfural, HMF and acetic acid, activated charcoal was found to adsorb furans and acetic acid more effectively than a cation exchange resin from (Sainio et al., 2011). However, in order to give value to the adsorbed fermentation inhibitory compounds, desorption of these compounds is important as well. Usually, in adsorption/desorption process, regeneration or desorption time is comparable to the duration of the loading or adsorption step. Unlike cation exchange resin, the regeneration of GAC with water was not feasible, ethanol 50% (v/v) was required to desorb furans and acetic acid in a limited duration. Overall, AC yielded highest process performance compare to resins when high purity is required and ethanol can be used to regenerate the adsorbent. (Sainio et al., 2011)

### b) Resin

Various resins were studied in the literature to separate the components of lignocellulosic acid extracts: neutral resin, anionic exchanger and cationic exchanger.

Adsorption by resins can also be used to remove fermentation inhibitors (phenolic and furanic compounds) by hydrophobic interactions as well as ionic bonds in the case of anion- or cation-exchange resin (Nilvebrant et al., 2001). Desorption of these inhibitors also presents the advantage of producing a fraction with high added value molecules that can be further valorized. Many examples are found in the literature for the adsorption of these fermentation inhibitors on nonionic resin, cation- and anion-resin, their best removal rate and the ethanol yield at the following fermentation step of the process are usually compared.

When the pH is adjusted to 5.5 prior to adsorption step, anion resin presented the best removal of fermentation inhibitors and the best ethanol yield at the following fermentation step compared to overliming or adsorption on activated charcoal or nonionic resin or cation resin (Nilvebrant et al., 2001; Carvalheiro et al., 2005; Chandel et al., 2007). A higher pH (for instance, pH 10) before the adsorption on anionic resin, increased the adsorption of aliphatic acids and phenol by making them negatively charged, it also increased the adsorption of furan derivatives (Nilvebrant et al., 2001). The

hydrolysate:resin ratio also had a substantial impact on the adsorption of fermentation inhibitors and the ethanol yield of the following fermentation step (Nilvebrant et al., 2001). Removal rate reached 96% of aliphatic acids, 68% of furfural and 65% HMF, 81% of phenolic compounds with a hydrolysate:anionic resin ratio of 25:8 (v/w) (Nilvebrant et al., 2001). Increasing the pH before the adsorption was also interesting to minimize the sugar loss by adsorption (no loss at pH 10) pH, as ionized aliphatic acids, phenols, and inorganic ions such as sulfate efficiently competed for the positive sites in the anion-exchange resin (Nilvebrant et al., 2001).

In order to reduce the use of chemicals, adsorption can be run without neutralization of lignocellulosic acid extract. Without neutralization, anion-exchange resin present less interest as such resins adsorb preferably sulfate ions instead of anions of weak organic acids (aliphatic acids or phenolate ions), and thus give low removal percentage (Sainio et al., 2011). On cation resin and nonionic resins (resins without charged groups), acidic hydrolysates (i.e., no pH adjustment) were more favorable to sugar purification as sugar was very weakly adsorbed whereas adsorption of furfural and HMF were more favorable (Schwartz and Lawoko, 2010), phenolic compounds adsorption was high as these molecules were uncharged at low pH (Nilvebrant et al., 2001; Weil et al., 2002; Schwartz and Lawoko, 2010), however acetic acid was not separated from the sugars (Nilvebrant et al., 2001; Sainio et al., 2011). Furfural loading capacity was higher on hydrophobic polymeric adsorbent for instance nonionic resin made of polystyrene–divinylbenzene (PS-DVB) than on more hydrophilic adsorbent such as methacrylic ester resin suggesting than the predominant mechanism of attraction between the resin and the furfural is hydrophobic attraction (Weil et al., 2002). Besides, adsorption on cation or nonionic resin is higher in 20% (w/w) sulfuric acid than in water for acetic acid, furfural and HMF. This is due to the “salting out effect” corresponding to an increase of the adsorption of hydrolysate components with increasing ionic strength of the liquid phase (Sainio et al., 2011). Indeed, in an aqueous solution, water molecules preferably solvate sulfuric acid molecules instead of the neutral molecules that are “salt out” (i.e., adsorbed) onto the resin (Sainio et al., 2011).

When the focus is put on the capacity of the resin to adsorb fermentation inhibitors during the feed loading of the adsorption process, it appeared that the loading capacity of a cation resin was 3 times less than the capacity of a nonionic resin and 14 times less of

the capacity of AC (Sainio et al., 2011). During the loading step, temperature affects the loading capacity for furfural on a nonionic resin, low temperature (30 °C) gave better results, increasing the temperature to 50 °C and to 70 °C led to decrease in loading capacity of 22% and 39%, respectively (Weil et al., 2002). A sorbent has to be selected not only on his ability to adsorb the fermentation inhibitors from a lignocellulosic acid hydrolysate but also on its ability to desorb them with the appropriate solvent. The increase of concentration of a desorbed molecule in the eluent during desorption step compare to its concentration during the loading step in the feed, called overshoot, decrease the necessity to further concentrate the molecule before the following purification and valorization steps. The regeneration of a strong cation resin can be carried out with water alone, with a comparable duration of the loading step and with a small overshoot (concentration of furans is higher in the desorption solvent than in the initial solution to purify, due to lower BV required to desorb the furans than the BV of the solution fed on the column), making the use of strong cationic resin interesting (Sainio et al., 2011). Water can desorb the adsorbed fermentation inhibitors due to the salting out phenomena vanishing as the ionic strength of the eluent is reduced (Sainio et al., 2011). Contrary to cation-exchange resin, regeneration of nonionic resin and GAC with water is not feasible, an organic solvent is required, like ethanol or acetone. All organic solvents were not suitable for the desorption of fermentation inhibitors from nonionic resin and GAC, some like of *n*-propanol and *n*-butanol can be possibly significantly adsorbed on the hydrophobic adsorbent (Weil et al., 2002). Batch desorption with an ethanol:resin ratio of 15:1 at 50 °C with stirring for 90 min led to 95% furfural desorption (Weil et al., 2002). Regeneration of nonionic resin in a column performed with 75% acetone at room temperature desorbed 85% of the acid-soluble lignin (Schwartz and Lawoko, 2010). Finally, 50% ethanol run at 15 BV/h was efficient (3 times less BV required for the desorption than for the breakthrough during the adsorption step) to desorb furans from a nonionic resin or GAC in a column and led to an overshoot of furans during the desorption (Sainio et al., 2011).

### 1.3.3.5. Low pressure chromatography

Several eluents and a process set-up with numerous steps are used for adsorption: feed loading, rinsing, desorption, regeneration, equilibration; chromatography requires only

one eluent and an easier process set-up - feed loading, elution – which generally leads to lower economical and environmental cost (Ladisch, 2001). If two types of particles differ in their adsorption rate, separation of them may be accomplished by selectively adsorbing one species on the sorbent. Reversibility of adsorption allow chromatographic separation of particles contained in a solution (Ruckenstein and Prieve, 1976).

After acid extraction on lignocellulosic biomass, chromatographic resin process can be used to separate acid and sugars on a first step as demonstrated by Arkenol patents for instance (Clausen and Gaddy, 1993; Farone and Cuzens, 1997). Gel type PS–DVB strong acid cation exchange resin with  $H^+$  as counter ion are used during this step under chromatographic conditions with water as eluent (Farone and Cuzens, 1993; Heinonen and Sainio, 2010). The water flow rate on the resin bed is about 2 to 5 m/h and the temperature is kept between 40 and 60 °C. Sugars were slowed down by the resin while acid is not retained. On batch elution, according to the different conditions tested it was possible to reach 90-93% sugar purity and 90-96% sugar recovery, and 95-96% acid purity and 97-99% acid recovery (Farone and Cuzens, 1997). A plant using this technology is runnin by BlueFire Renewable in Izumi, Japan, with a capacity of 80,000 L ethnol 99.5% (v/v) (<https://bfreinc.com/>). The performance of the chromatographic separation was found to decrease with increasing concentration of sulfuric acid from 20% to 70% (Heinonen and Sainio, 2012), besides as explained previously lignocellulosic concentrated acid extraction is often followed by a more diluted second acid hydrolysis in order to optimize the monosaccharide recovery (1.3.2.4. Industrial applications). Therefore, lignocellulosic acid hydrolysates containing 20%  $H_2SO_4$  were often studied as the feed of the chromatographic step for the monosaccharides/acid separation. A process was developed to recycle the acid after the chromatographic separation and sent it back to the beginning of the process for the lignocellulosic biomass hydrolysis, 92% of the acid needed for initial hydrolysis was obtained through recycling (Heinonen and Sainio, 2012). Moreover, the water removed from the acid can be reused as eluent for the chromatographic, decreasing the water consumption of the whole process by 60% (Heinonen and Sainio, 2012).

Some fermentation inhibitors (acetic acid, furans) can be removed from lignocellulosic acid hydrolysates along with sulfuric acid by chromatographic process. The use of a cation-exchange resin with a PS-DVB matrix induced the elution of sulfuric acid first,

then the monomeric sugars and finally acetic acid and furans whereas the use of an anion-exchange resin with a polyvinylpyrrolidone matrix led to the elution of monomeric sugars first followed by the fermentation inhibitors and sulfuric acid (Wooley et al., 1998; Xie et al., 2005). After fermentation of the resulting purified sugar mixtures, the ethanol yield were as good as on synthetic sugar solution and better than the acid hydrolysates purified by overliming (Xie et al., 2005).

Resins were also studied for the separation of the monomeric sugars constitutive of lignocellulosic acid extract after acid removal. Adsorption behavior of glucose, xylose and arabinose has been tested on several strong acid cationic exchanger with various cross linking degree (DVB content of 4, 6 and 8%) and different counterions ( $K^+$ ,  $Ca^{2+}$ ,  $Fe^{3+}$ ) for the separation of glucose, xylose and arabinose (Lei et al., 2010). Resins with  $Ca^{2+}$  as counterions were the most suitable for the monomeric sugars separation after adsorption isotherms determination tests; 6% DVB resin being more efficient for arabinose/xylose separation and 8% DVB resin being more efficient for xylose/glucose separation (Lei et al., 2010). Other adsorption isotherms experiments confirmed the higher potential of  $Ca^{2+}$  form strong acid exchange resin over  $Na^+$  form to separate glucose, xylose and arabinose (Chen et al., 2018). With Amberlite IRP69- $Ca^{2+}$  packed column, high recovery and purity for glucose, xylose and arabinose were obtained by batch column chromatography (i.e., pulse test) from both a synthetic solution and a pine branches hydrothermal liquefaction extract (Chen et al., 2018). Continuous chromatography via multi-column system such as simulated moving bed (SMB) is a classic industrial method to separate sugars and can be completed by crystallization to reach high level of purity for the different sugars (Lemaire et al., 2016).

### 1.3.3.6. Cross-flow membrane filtration

Membrane filtration was extensively studied on spent liquor in sulphite pulp mill to remove lignosulphonates (Jönsson and Wallberg, 2009) and in a more limited extent on lignocellulosic dilute acid hydrolysate, as sugar cannot be purified in one membrane filtration step. First, membranes with molecular weight cut-off (MWCO) of 10-50 kDa can be used to separate lignin or proteins in the retentate from the monomeric sugars, sulfuric acid and the other impurities (acetic acid, furans) (Lemaire et al., 2016; Blanc et al., 2017). Then in a second time, membranes with MWCO of 150-300 Da can retain

monomeric sugars (glucose, xylose, arabinose), while acetic acid and furans pass through the membrane (Weng et al., 2010).

Organic flat sheet membranes of 10, 20 and 50 kDa (Alfa Laval) were efficient to retain totally macromolecules (lignin and proteins) from a wheat bran acid extract, at the opposite of ceramic tubular membranes with lower MWCO 8 and 15 kDa (Tami) where the retention of macromolecules was not total (Lemaire et al., 2016). Several successive concentrations by a volumic reduction factor (VRF) of 3.6 of wheat bran acid extract were run on the 10 kDa organic membrane (UFX10 pHt, Alfa Laval) and the flux was on average about 10 L/h/m<sup>2</sup> showing good reproducibility (Blanc et al., 2017). Afterward, diafiltration with 2.5 diavolumes was required in order to maximize sugar recovery in the permeate (99% recovery). Filtration of rice straw dilute acid hydrolysate adjusted to pH 3 on an organic spiral wound membrane with a MWCO of 150–300 Da (Desal-5 DK, GE-Osmonic) led to total retention of glucose and very high retention of xylose and arabinose (over 94%) while acetic acid and furans totally passed through the membrane (Weng et al., 2010). The separation performance decreased when the operating temperature was increased from 25 to 40 °C (Weng et al., 2010).

During the filtration of lignocellulosic hydrolysates, fouling appears on the membrane changing the initial properties of the membrane (membrane permeability and selectivity), membrane cleaning is necessary to recover its initial properties. Membrane cleaning with 0.01 N of sodium hydroxide and rinsing with water was enough to recover the initial water flux after the filtration of rice straw dilute acid hydrolysates (Weng et al., 2010).

### 1.3.3.7. Electrodialysis

Electrodialysis (ED) has been applied to monomeric sugars purification from lignocellulosic acid hydrolysates, to remove sulfuric acid and acetic acid but partial or complete neutralization prior to ED was still required to eliminate macromolecules (lignins and proteins) which could precipitate during ED and damage the membrane (Cheng et al., 2008; Lemaire et al., 2016).

A study on sugarcane bagasse dilute acid hydrolysate treated by ED lead to a sulfuric acid recovery of 88% the loss of sugar was not more than 5% (Cheng et al., 2008). Successive ED run on wheat bran dilute acid hydrolysates showed good reproducibility

and led to similar results with most of sulfuric acid removed (80-87%) without losing sugars (<1%) with a faradic yield of about 70-80% in 7-20 min (Lemaire et al., 2016; Blanc et al., 2017).

### 1.3.3.8. Combination of different techniques

The technologies mentioned previously have been sometimes combined in integrated purification processes of lignocellulosic acid extracts.

For instance, a monomeric sugars acid hydrolysate obtained from bamboo was treated by AC to remove color compounds (e.g., phenolic compounds, furanic compounds) then by simulated moving bed (SMB), a continuous chromatography set-up, to separate the sulfuric acid from the sugars. Under the best conditions, AC led to the removal of 93% of the color while no sugars were adsorbed and the SMB led to the recovery on one side of 90.5% of the sulfuric acid and the acetic acid and on the other side of 99.9% of xylose and 97.4% of glucose (Sun et al., 2011).

Another combination of different purification techniques involved the use of ultrafiltration to remove macromolecules such as lignin or proteins from a wheat bran acid extract, then electrodialysis to recover the acid and finally ion exchange to complete the demineralization (conductivity <10  $\mu\text{S}/\text{cm}$ ) (Lemaire et al., 2016; Blanc et al., 2017). Overall, the sugars recovery was 90% and their purity close to 100%.

### 1.3.3.9. Conclusion

Purification after acid treatment implies mainly to remove the fermentation inhibitors and the acid to potentially recycle it and send it back to the extraction step. The removal of the inorganic acid from lignocellulosic acid hydrolysates is already carried out industrially using low pressure chromatography. Once the purified extract contains only monomeric sugars, sugar/sugar separation can be carried out before further valorization of the individual sugars or fermentation can be made directly on the sugar mixture with the appropriate enzymes for the production of ethanol for instance.



## 1.4. Alkaline fractionation process

Vincent Oriez\*, Jérôme Peydecastaing, Pierre-Yves Pontalier\*

Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, 4 allée Emile Monso, 31030 Toulouse, France

\*Corresponding authors at: Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France

*E-mail addresses:* [vincentoriez@yahoo.fr](mailto:vincentoriez@yahoo.fr), [vincent.oriez@ensiacet.fr](mailto:vincent.oriez@ensiacet.fr) (V. Oriez), [pierreyves.pontalier@ensiacet.fr](mailto:pierreyves.pontalier@ensiacet.fr) (P.Y. Pontalier)

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### 1.4.1. Pulp and paper industry

Papermaking industry is the first historical lignocellulosic biorefinery, where fractionation is applied to lignocellulosic biomass then valorization of each fraction is carried out. The first patents where strong alkaline solutions were used to produce cellulose from wood were recorded in the second half of the 19<sup>th</sup> century (Dixon, 1865; Dahl, 1884).

Commercial pulping processes include the soda, the sulfite and the sulfate (also known as Kraft) processes. These processes induce cellulose fibers dissociation from lignin and hemicelluloses by the cooking chemicals (Eckert and Abdullah, 2008; Cardoso et al., 2009). Kraft and soda are alkaline processes, the former being mainly used for wood hydrolysis, while the latter is commonly applied to non-wood biomass, such as bagasse, straw, grass or bamboo (Cardoso et al., 2009). In both processes, lignin, low molecular weight hemicelluloses and other extractives from the wood are dissolved in what is called black liquor (Christensen, 1982).

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In the Kraft process, a solution containing sodium hydroxide and sodium hydrosulfide is contacted with lignocellulosic material at 150 to 170 °C, under pressure during a few hours (Christensen, 1982; Wu et al., 2011). The process yields high lignin removal rate and high mechanical strength for the pulp. The products from the reaction are the cellulose pulp in the solid residue and the black liquor (Cardoso et al., 2009). In the black liquor from the Kraft process lignins contain a small number of aliphatic thiol groups, because of sodium sulfide, and are usually called thio-lignin (Lora and Glasser, 2002). The black liquor is usually burnt for steam and electricity generation. To transform Kraft pulping process into a complete biorefinery concept separation of black liquor compounds (mainly lignin, hemicelluloses and pulping chemicals) to further valorize them is required (Huang et al., 2008; Sixta and Schild, 2009). Hemicelluloses have a lower heating value than lignin, so their separation from lignin prior to combustion would enable a more efficient use for the production of fuels (e.g., ethanol) or higher value chemicals (e.g., polyesters), for example (FitzPatrick et al., 2010). Another option could be to extract hemicelluloses in a first step, for instance with dilute acid, and then run Kraft extraction (Huang et al., 2008). Lignin recovery from black liquors has also been studied as instead of burning it, higher economic value can be achieved based on lignin derivatives in application such as biofuel, chemical phenolic platform or carbon fibers can (Abels et al., 2013; Haddad et al., 2017). Kraft lignins are water-insoluble but dissolve in alkali solution owing to their high concentration of phenolic hydroxy groups (Lora and Glasser, 2002).

Pretreatment using sodium hydroxide alone, referred as soda process, is a technically a feasible pretreatment method but lead to lower yield than the Kraft process (Kim et al., 2016). Addition of small quantities of redox catalyst anthraquinone (AQ) can significantly increase the pulping rate of wood without any adverse effect on strength properties (El-Saied et al., 1984). The increased delignification rate in soda-AQ process is due to extensive  $\beta$ -O-4 ether cleavage of the lignin molecule. Kraft process remains more efficient in terms of delignification rate and pulp properties than soda-AQ on wood biomass (Sixta and Schild, 2009), but soda-AQ pulping eliminates air pollution arising from the organic sulfur-containing compounds generated by the Kraft process (El-Saied et al., 1984). The following pulping conditions were reported on soda-AQ treatment of cotton stalks: NaOH: fiber ratio of about 1:6 (w/w), AQ: fiber ratio of 1:1333, S:L ratio of

1:4 and a maximum temperature of 165 °C (Ali et al., 2001). As for Kraft process, pre-extraction of hemicelluloses prior to soda-AQ was investigated (Sixta and Schild, 2009). Alkaline pre-extraction resulted in substantial extraction of xylan in polymeric form while preserving the pulp yield and quality. Lignin from black liquor, obtained by the treatment of oil palm empty fruit bunch fiber by soda-AQ, were all free of sugar, the linkages between lignin and polysaccharides were completely cleaved (Sun et al., 1999). On softwood black liquor, successive acid precipitation experiments showed that lignin had at most 2% bound sugar (Alekhina et al., 2015).

The third process in the paper making industry is the sulfite process, developed by Tilghman (Phillips, 1943). The wood chips are cooked in a mixture of sulfurous acid and bisulfide ions which dissolve lignin and hemicelluloses (Pokhrel and Viraraghavan, 2004). Sulfite pulps account for less than 10% of the total chemical pulp production. (Biermann, 1993). An aliphatic sulfonic acid function becomes part of the lignin backbone making them water-soluble in the presence of a suitable counter ion (e.g., Na<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>) (Lora and Glasser, 2002). The lignosulfates from the sulfite process are byproducts already commercialized, the global market in 2011 was 1 million tons, as a comparison only 100,000 tons of lignin from the Kraft pulping process were produced (Higson, 2011).

### 1.4.2. Mild alkaline extraction

#### 1.4.2.1. Effect and mechanism

The main features of alkaline extraction, similarly to Kraft or soda-AQ pulp and paper processes, are that it solubilizes both lignin and hemicelluloses without degrading cellulose, and it increases the porosity and surface area of cellulose, thereby enhancing potential enzymatic hydrolysis of cellulose (Hayes, 2009; Ragauskas et al., 2014; Kim et al., 2016). The solid residue (mainly cellulose) can be used in its polymeric form in application such as paper and cellulose derivatives (e.g., cellulose acetate), or in its monomeric form (glucose), after acid or enzymatic hydrolysis, in application such as biofuels (ethanol) and chemical intermediates (Cardona et al., 2010; Kim et al., 2016). Less than 3% degradation of glucan (accounting for cellulose) was reported on mild alkaline extraction of SCB (Chang et al., 2017; Oriez et al., 2018). Hemicelluloses are

usually dissolved to a lower extent than lignin (Hayes, 2009; Cardona et al., 2010). Conditions are milder than with acid pretreatments which can eliminate the need for expensive materials and special designs to cope with corrosion of the vessels (Kim et al., 2016). However, reaction times are usually longer and unlike acid pretreatments, a limitation occurs because some of the alkali is converted to irrecoverable salts or incorporated as salts into the biomass by the pretreatment reactions (Mosier et al., 2005). Mild alkaline pretreatments and the Kraft and soda-AQ pulping processes share the same fundamental principles; therefore, the mature techniques and equipment used in the pulping process, for instance to recover the reaction chemicals as well as energy, are applicable to the mild alkaline pretreatment process (Wu et al., 2011). The activation energy (50–54 kJ/mol) used for delignification of herbaceous species (e.g., bagasse and corn stover) by mild alkaline pretreatment is lower than that required for delignification of wood by the Kraft process (Kim and Holtzapfle, 2006), making mild alkaline pretreatment particularly suitable for herbaceous biomass.

The treatment of lignocellulosic biomass by alkaline solution induces several mechanisms. Cellulose swells due to disruption of inter-molecular hydrogen bonds which bind cellulose molecules together (Sun et al., 1995). In parallel, some alkali-labile linkages between lignin monomers or lignin and polysaccharides are broken. The ester-linked substituents of the hemicellulose (acetate groups, uronic molecules) are also broken, as long as ester-linked ferulic acid (FA), *p*-coumaric acid (*p*-CA) and sinapic acid (Bunzel et al., 2003; Hayes, 2009; Harmsen et al., 2010). This improves the digestibility of the undissolved hemicelluloses recovered in the solid residue and the dissolved hemicelluloses are quite similar to the native polysaccharides, except for the removed groups (acetate) or molecules (uronic acids, phenolic acids) (Sun et al., 1998; Sun, 2000). The hemicelluloses contain a relatively small amount of bound lignin (0-5%) (Sun et al., 2004). Xylans undergo only partial hydrolysis in alkaline solution at room temperature. Increasing the severity of the treatment (temperature, base concentration) produce smaller oligomers (Sun et al., 2004; El Mansouri and Salvadó, 2006).

Lignin is insoluble under neutral or acidic conditions, its solubilization in alkaline conditions comes from acidic moieties such as carboxylic or phenolic groups that are ionized in alkaline solution, and its hydrolysis comes from the cleavage of  $\beta$ -O-4 ether bonds in poly-phenolic units (Sun et al., 1995; Lora and Glasser, 2002). Molecules linked

to lignin by their carboxyl group via ester bonds such as uronic acids, *p*-CA, FA are cleaved in mild alkaline media whereas the ferulic acid molecules linked by their phenolic group via ether bond requires stronger alkaline conditions to be cleaved (Sun et al., 1999, 2002; Xu et al., 2005). Lignin extracted under mild alkaline pretreatments contain very low level of bound sugars (1-3%) (Sun et al., 1998). Alkaline solutions are a better reaction media than acidic or neutral media, for the valorization of lignin for the synthesis of phenolic resins (Hu et al., 2011).

Lignin issued from alkaline fractionation is sulfur-free, unlike that produced by pulping processes which is a great advantage for further chemical activation opening up valorization pathways for instance as fuel additives or bio-based polymers (adhesives and asphalt extenders) (Kim et al., 2016).

### 1.4.2.2. Nature of the base

Biomass can be treated with alkali such as sodium, potassium, calcium and ammonium hydroxides at normal temperature and pressures (Chaturvedi and Verma, 2013). Among alkali pretreatment sodium hydroxide has received the most attention (Mosier et al., 2005), and are typically preferred because of the high extraction yields for lignin (60-80%) and hemicelluloses (50%) (Peng et al., 2012; Kim et al., 2016).

Calcium hydroxide or lime is also commonly employed for pretreatment under alkaline conditions (Chaturvedi and Verma, 2013) because it has lower cost, less safety requirements, it is less corrosive and can be recovered from hydrolysate by reaction with CO<sub>2</sub>, so that the carbonate formed, can then be reconverted to lime (Chang et al., 1998). Lime pretreatment leads to the extraction of lignin and hemicelluloses, because it cleaves  $\alpha$ - and  $\beta$ -ether bonds in phenolic units and  $\beta$ -ether linkages in non-phenolic units (Grimaldi et al., 2015) and to the removal of acetyl groups from hemicelluloses (Kim et al., 2016). However, its fractionation effect is not as strong as with sodium hydroxide or ammonia (Peng et al., 2012). For instance, under similar conditions the use of sodium hydroxide led to lignin and hemicelluloses removal yields of 70 and 22%, respectively, whereas the use of calcium hydroxide led to lignin and hemicelluloses removal yields of 28 and 8% , respectively (Chang et al., 2017).

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Aqueous ammonia treatment differs from other alkali pretreatments as it is run at elevated temperature or high pressure or long period of time (e.g., over 170 °C for 1h30 at atmospheric pressure, or 100 °C for 5 min at 20 bar, or 75 °C for 48 h, at atmospheric pressure) (Kim et al., 2008; Chaturvedi and Verma, 2013). These conditions correspond to three types of ammonia pretreatments: ammonia recycle percolation (high temperatures), ammonia fiber explosion (high pressure) and soaking in aqueous ammonia (long duration). The three processes sufficiently reduces lignin content (65 to 75% delignification) and removes some hemicelluloses (up to 92%), while cellulose is decrystallized, leading to improved enzymatic saccharification yield (Kim et al., 2008; Chaturvedi and Verma, 2013). The use of ammonia requires recycling to lower the cost of the pretreatment as ammonia is expensive, and special care as ammonia is toxic for environment.

### 1.4.2.3. Conditions and yields

Unlike pulp and paper industry, where extraction conditions are drastic regarding base concentration, temperature or pressure (Christensen, 1982; da Silva et al., 2013), pretreatment conditions studied in the frame of the lignocellulosic ethanol biorefinery are milder (Saha and Cotta, 2007, 2008).

Mild alkali treatment was shown to be more effective on agricultural residues than on wood materials (Alvira et al., 2010). Mild reaction conditions prevent condensation of lignin leading to its high solubility and greater removal (Chaturvedi and Verma, 2013). Alkali pretreatment conditions of 0.5-10.0% NaOH, 60–180 °C, 5–60 min, and solid loading of 10–30% give generally 50% hemicellulose dissolution, 60–80% delignification. (Kim et al., 2016). On sweet sorghum bagasse, the delignification rate during mild alkaline treatment presented high correlation with the ethanol yield at the following enzymatic saccharification and fermentation steps (Wu et al., 2011). In the frame of second generation ethanol biorefinery, comparing efficiency of various mild alkaline pretreatments based on the dissolution of lignin and hemicelluloses is more relevant than comparing yields of saccharification or fermentation at the following process steps since saccharification and fermentation conditions differ from one study to another. The results of some studies are gathered in **Table 1.3**, with a focus on sugarcane bagasse for the lignocellulosic biomass and sodium hydroxide for the base used, for an

easier comparison between the mild alkaline conditions and the dissolution yields obtained.

Similarly to acid pretreatments, four main parameters influence the hemicelluloses and lignin removal during alkaline pretreatments: base concentration, S:L ratio, temperature and experiment duration. Increasing one parameter value increase the solubilization for lignin and hemicelluloses, or it can be compensated by the decrease in another parameter value while maintaining the solubilization yields at the same values. For instance, on apple tree pruning residues, increasing the S:L ratio from 1:18 to 1:10 (w/v) during mild alkaline extraction (7.5% NaOH (w/w), at 90 °C for 90 min) led to an increase in lignin solubilization of 53% (García et al., 2012). Optimal conditions for delignification (up to 60%) and dissolution of hemicelluloses (up to 80%) of wheat bran in mild alkaline conditions were obtained with pretreatment with 1.5% sodium hydroxide with a S:L ratio of 1:40 (w/v) at 20 °C for 144 h (Sun et al., 1995). Similar solubilization yields were achieved by increasing the temperature to 80 °C and decreasing experiment duration to 6 h. The same phenomenon was observed on SCB with alkaline pretreatment using calcium hydroxide, high temperature (85-135 °C) coupled with short experiment duration (1-3 h) gave similar glucose yield at the following saccharification step than pretreatment with lower temperature (50-65 °C) and longer experiment duration (24 h) (Chang et al., 1998).

With low S:L ratio (1:40-1:30 (w/v)), low temperature (20-60 °C) and short experiment duration (2-6 h), increasing the sodium hydroxide concentration increase the dissolution rate for lignin and hemicelluloses first rapidly, until a concentration of about 1.5%, then further increase in concentration led to smaller increase in dissolution rates (Sun et al., 1995, 2003). During mild alkaline treatment, hemicelluloses and lignin removal rates vs. time are different depending on the lignocellulosic feedstock. Hemicelluloses removal rate was quicker than lignin removal rate on wheat bran (Sun et al., 1995), but the reverse trend was observed on sweet sorghum (Wu et al., 2011).

**Table 1.3**

Yields of lignin and hemicelluloses solubilization after treatment on lignocellulosic biomass under optimized mild alkaline conditions

<b>Biomass</b>	<b>Variable</b>	<b>Optimized conditions</b>	<b>Solubilization yield</b>	<b>Author</b>
SCB (dewaxed)	1-3% NaOH (w/v)	3% NaOH (w/v)	54% lignin	(Peng et al., 2009)
	No variation of other parameters	S:L ratio of 1:25 50 °C 3 h	75% hemicelluloses	
SCB (dewaxed)	No variation of parameters value	2% NaOH (w/v)	55% lignin	(Sun et al., 2003)
		S:L ratio of 1:30 55 °C 2 h	48% hemicelluloses	
SCB	2-6% NaOH (w/v)	4% NaOH	43% hemicelluloses	(Yao et al., 2015)
	No variation of S:L ratio 150-190 °C 40-80 min	S:L ratio 5:1 170 °C 1 h		
SCB	No variation of parameters values	1.5% NaOH (w/v)	46% lignin	(Oriez et al., 2018)
		S:L ratio of 1:20 (w/v) 60 °C 6 h	22% xylan 50% arabinan	
SCB	No variation of parameters values	2% NaOH (w/v)	70% lignin	(Chang et al., 2017)
		S:L ratio 1:10 (w/v) 80 °C 2 h	22% xylan 22% arabinan	



SCB	No variation of parameters values	2% Ca(OH) <sub>2</sub> (w/v) S:L ratio 1:10 (w/v) 80 °C 2 h	28% lignin 9% xylan 6% arabinan	(Chang et al., 2017)
SCB	No variation of parameters value	1% Ca(OH) <sub>2</sub> (w/v) S:L ratio of 1:10 120 °C 1 h	14% lignin 0% hemicelluloses	(Chang et al., 1998)
SCB	No variation of parameters value except for experiment duration 7-60 min	1% Ca(OH) <sub>2</sub> (w/v) S:L ratio of 1:100 120 °C 1 h	30% lignin 5% hemicelluloses	(Grimaldi et al., 2015)
SCB	0.03-0.3% NH <sub>3</sub> (w/w) No variation of S:L ratio and temperature values 0-40 day	0.3% NH <sub>3</sub> (w/w) S:L ratio of 1:8 (w/v) 30 °C 40 day	46% lignin 27% hemicelluloses	(Kim et al., 2010)
SCB	20-28% NH <sub>3</sub> (v/v) No variation of S:L ratio 50-70 °C 8-24 h	20% NH <sub>3</sub> (v/v) S:L ratio of 1:10 (w/v) 70 °C 24 h	42% lignin 69% hemicelluloses	(Chandel et al., 2013)
Wheat straw	0.5-10% NaOH (w/v) No variation of S:L ratio 0-80 °C 0-144 h	1.5% NaOH (w/v) S:L ratio 1:40 (w/v) 20 °C 144 h	59% lignin 83% hemicelluloses	(Sun et al., 1995)

Wheat straw	0.5-10% NaOH (w/v) No variation of S:L ratio 0-80 °C 0-144 h	1.5% NaOH (w/v) S:L ratio 1:40 (w/v) 80 °C 6 h	62% lignin 75% hemicelluloses	(Sun et al., 1995)
Wheat straw	0.5-10% NaOH (w/v) No variation of S:L ratio 0-80 °C 0-144 h	10% NaOH (w/v) S:L ratio 1:40 (w/v) 20°C 6 h	47% lignin 81% hemicelluloses	(Sun et al., 1995)
Wheat straw	3% NaOH (w/v) S:L ratio of 1:26.6 (w/v) 45 °C 2-15 h	3% NaOH (w/v) S:L ratio of 1:26.6 (w/v) 45 °C 15 h	70% lignin	(Sun et al., 1998)
Maize stems	No variation of parameters	4% NaOH (w/v) S:L ratio of 1:100 30 °C 18 h	78% lignin 72% hemicelluloses	(Xiao et al., 2001)
Rye straw	No variation of parameters	4% NaOH (w/v) S:L ratio of 1:100 30 °C 18 h	79% lignin 73% hemicelluloses	(Xiao et al., 2001)
Rice straw	No variation of parameters	4% NaOH (w/v) S:L ratio of 1:100 30 °C 18 h	82% lignin 85% hemicelluloses	(Xiao et al., 2001)

Sorghum bagasse	2-10% NaOH (w/v)	4% NaOH (w/v)	76% lignin	(Wu et al., 2011)
	No S:L ratio and temperature variations 0.5-2 h	S:L ratio of 1:20 (w/v) 25 °C 2 h	60% xylan	
Sorghum bagasse	2-10% NaOH (w/v)	10% NaOH (w/v)	80% lignin	(Wu et al., 2011)
	No S:L ratio and temperature variations 0.5-2 h	S:L ratio of 1:20 (w/v) 25 °C 2 h	81% xylan	

The size of the biomass has a strong influence on the hydrolysis yield. It is not mentioned here as it was usually not studied. (Sindhu et al., 2011)

As SCB is one of the most studied lignocellulosic biomass it was taken as the model biomass, besides, it contains mainly 3 sugars - glucose, xylose, arabinose – facilitating the comparison. Other biomass are presented as comparison with the reference. Sodium hydroxide was taken as the reference for the base, as it was the most studied.

For SCB, glucose was supposed to come from cellulose exclusively, and xylose and arabinose were supposed to be the only components of the hemicelluloses

As for acid pretreatment, comparison between the efficiency of the different mild alkaline pretreatment based on the saccharification yield on the next step cannot be done as enzymatic or acid saccharification conditions change from one study to another. **Table 1.3** enables comparison between the pretreatment efficiencies based on the solubilization of lignin and hemicelluloses. High solubilization rates, particularly for lignin, are targeted as they are coupled with high saccharification yield at the next step (Vancov and McIntosh, 2011; Wu et al., 2011; Chang et al., 2017) and they allow the purification and valorization of larger quantities of lignin and hemicelluloses.

The alkaline conditions for pretreatment have an impact on the size and the functional groups of the dissolved lignin oligomers (Holladay et al., 2007). Soft alkaline extraction conditions helped producing large oligomers (Sun et al., 2004; El Mansouri and Salvadó, 2006). These polymers could be interesting for some applications where long chain of lignin or hemicelluloses are looked for, as non-exhaustively in coatings, surfactants, adhesives and cosmetics applications. (Werpy et al., 2004; Holladay et al., 2007).

Along with lignin oligomers and hemicelluloses, phenolic monomers are released during mild alkaline treatment of lignocellulosic biomass. The content and nature of the phenolic monomers vary from one biomass to another, but the major phenolic monomers released are *p*-CA and FA with about 1 g for each compound extracted from 100 g of biomass (Sun et al., 2001; Tilay et al., 2008; Buranov and Mazza, 2009).

The solubilization yields of lignin and hemicelluloses from lignocellulosic biomass after mild alkaline pretreatment with sodium hydroxide and the ethanol yield after the saccharification and fermentation steps can be increased with the addition of different chemicals during the pretreatment (Chaturvedi and Verma, 2013). For instance, the solubilization yield of lignin and hemicelluloses after peroxide-alkaline pretreatment (4% H<sub>2</sub>O<sub>2</sub> (w/v), 0.25% MgSO<sub>4</sub> (w/v), pH of 11.6 adjusted with NaOH, S:L ratio of 1:20 (w/v), 40 °C, 10 h) on SCB reached 88% and 95%, respectively (Brienzo et al., 2009). As for the other pretreatment conditions already reported, a change of one parameter value influence the solubilization yields for lignin and hemicelluloses, for instance a decrease in the S:L ratio from 1:10 to 1:30 (w/v) increased the lignin and hemicelluloses removal rates from 66% to 72% and from 79 to 85%, respectively (Sun et al., 2003). However, high cost of hydrogen peroxide and requirement for reactions vessels that can withstand

oxidative conditions are important drawbacks regarding the use of hydrogen peroxide (Chaturvedi and Verma, 2013).

Overall, several studies showed that mild alkaline treatments combined with enzymatic saccharification led to much higher yield in monomeric sugars than acid treatments sometimes followed by enzymatic saccharification, and therefore to higher ethanol yield in the following fermentation step. For instance, on agave bagasse and sugarcane bagasse a dilute acid treatment (1.2% HCl (v/v), S:L ratio of 1:15, 121 °C, 4 h) yielded 10 and 37% reducing sugars, respectively, whereas an alkaline treatment (2% NaOH (w/v), S:L ratio of 1:5, 121 °C, 4 h) followed by enzymatic saccharification yielded other 50% reducing sugars for both materials (Hernández-Salas et al., 2009). However, the choice of the enzymes for the saccharification is key parameter to reach high yields in reducing sugars. On sorghum bagasse, alkaline treatment (2% NaOH (w/v), S:L ratio of 1:20, 25 °C, 2 h) was more efficient to produce monomeric sugars than acid pretreatment (0.5% H<sub>2</sub>SO<sub>4</sub> (w/w), S:L ratio of 1:20, 170 °C, 0.5 h) with yield of 92% and 70%, respectively (Wu et al., 2011). Increasing sodium hydroxide concentration to 10% (w/v) led to a yield of 99%. On rice hull, alkaline peroxide treatment (7.5% H<sub>2</sub>O<sub>2</sub> (v/v), pH 11.5 adjusted with NaOH, S:L ratio of 1:6.7, 35 °C, 24 h) and saccharification yielded 90% monomeric sugars (Saha and Cotta, 2007), whereas dilute acid treatment (1% H<sub>2</sub>SO<sub>4</sub> (v/v), S:L ratio of 1:6.7, 121 °C, 1 h) and saccharification yielded 60% monomeric sugars (Saha et al., 2005b). On the same biomass, lime treatment (1.5% Ca(OH)<sub>2</sub> (w/v), S:L ratio of 1:6.7, 121 °C, 1 h) and saccharification yielded even less monomeric sugars (32%) (Saha and Cotta, 2008). The better enzymatic saccharification yields following alkaline pretreatments of lignocellulosic biomass compared to acid pretreatments are supported by the fact that lignin removal has a stronger effect on enzymatic saccharification than the removal of hemicelluloses (Kim et al., 2003).

#### 1.4.2.4. Industrial applications

In the frame of 2<sup>nd</sup> generation ethanol production some companies developed alkaline pretreatment such as SuGanit. The company set a sequential treatment of lignocellulosic biomass with ionic liquid pretreatment followed by mild alkaline treatment for efficient generation of cellulosic material and lignin fractions (Paripati and Dadi, 2014). This is a two-step process: first lignocellulosic biomass are contacted with an ionic liquid for a

sufficient time and temperature to swell the lignocellulosic biomass without dissolution of the lignocellulosic biomass in the ionic liquid; and secondly, the swelled lignocellulosic biomass is treated under mild alkaline conditions with 5% NaOH for about 1 h at about 75 °C to separate the lignin from the cellulose and hemicellulose. Lignocellulosic alkaline hydrolysate from GreenValue, a Swiss company, were used to study the recovery of lignin by flocculation (Piazza et al., 2015, 2017).

Mild alkaline extraction can also be adapted to existing industrial pulp and paper process. For instance, mild alkaline pre-extraction (10% NaOH (w/v), 90 °C, 1 h) prior to soda-AQ pulping largely preserved the pulp yield, while a substantial amount of xylan was pre-extracted in polymeric form (about 15 kDa), allowing specific valorization where long oligomeric chains are required (Sixta and Schild, 2009).

### 1.4.3. Purification routes applied to alkaline hydrolysates

Mild alkaline pretreatment on lignocellulosic biomass coupled with enzymatic saccharification and fermentation of the solid residue containing cellulose is more efficient than acid pretreatment for the production of cellulosic ethanol. However, the cost of bases, such as sodium hydroxide, is high, making mild alkaline pretreatments uncompetitive for large scale plants (Sánchez and Cardona, 2008). Mild alkaline hydrolysates are composed of lignin oligomers, hemicelluloses oligomers, phenolic monomers, acetic acid and salts. Some of these compounds present high added value, but their purification is required to make their valorization possible and improve the economic efficiency of biorefineries using mild alkaline pretreatment process (Wu et al., 2011). The purification usually focus on hemicelluloses oligomers or lignin oligomers and consists in removing inorganic salts (sodium hydroxide used in the process and potentially solubilized silica from the biomass), acetate and phenolic monomers. However, purifying and valorizing, inorganic salts (to reuse them in the process), acetate or phenolic monomers can bring additional economic value to the process. Purification can also occur within a given pool of molecules, for instance, lignin oligomers can be further separated depending on their size or their functional groups for various application (Holladay et al., 2007). Research on the separation of the components of mild alkaline hydrolysates is very recent. Since their composition is similar to those of lignocellulosic

alkaline hydrolysates obtained in severe conditions, the purification routes described here include both types of alkaline hydrolysates.

### 1.4.3.1. Flocculation

Lignin can be flocculated from sodium hydroxide pretreatment hydrolysates, but not from calcium hydroxide pretreatment hydrolysates (Chang et al., 2017). With the addition of calcium chloride in sodium hydroxide hydrolysate, calcium ion can replace the sodium ion to bridge the negative charges of lignin components and induce the flocculation of lignin. The best lignin recovery (23% of the DS of the hydrolysate) by flocculation was obtained with a loading of calcium hydroxide:hydrolysate of 1:11 (w/v).

### 1.4.3.2. Precipitation

#### a) Acidification

Acidification of lignocellulosic alkaline extract lead to the precipitation of lignin. At high pH, phenol, carboxyl and hydroxyl groups within the lignin are deprotonated making them negatively charged. The acidic environment tend to neutralize through protonation the anionic charges of lignin (Sarkanen et al., 1984; Shi et al., 2011). The neutralization of the charge, prevent repulsion from the different lignin molecules and allow the interaction between aromatic moieties of the different compounds leading to precipitation. When the pH is decreased, high molecular weight lignin molecules precipitate first, smaller molecules precipitate at lower pH (Sarkanen et al., 1984; Wang and Chen, 2013; Alekhina et al., 2015).

This process has been widely studied in the pulp and paper industry and many patents are reported to specify the conditions of lignin precipitation from black liquor (Axegard et al., 2006; Littorin et al., 2010; Oehman et al., 2006; Wallmo and Wimby, 2014). By extension, lignin precipitation via acidification was applied to lignocellulosic mild alkaline extract produced (Minu et al., 2012).

Sulfuric acid or carbon dioxide are usually used for acidification of Kraft black liquor (Uloth and Wearing, 1989; Eckert and Abdullah, 2008). Other inorganic acids have also been studied for the precipitation of lignin from lignocellulosic alkaline hydrolysates,

such as phosphoric, hydrochloric or nitric acid (Minu et al., 2012). Phosphoric acid was found to give the higher lignin yield but its price and the larger volume required for the pH adjustment compare to sulfuric acid, made sulfuric acid more efficient (Minu et al., 2012). The precipitation is run at temperature ranging from 60 to 85 °C, then the precipitate is recovered by filtration (filter press industrially), centrifugation or decantation (Axegard et al., 2006; Eckert and Abdullah, 2008). Temperature influence the yield of the precipitate, temperatures higher than 50 °C are required to reach higher yields (Minu et al., 2012). The temperature also affects the size of lignin flocks which affect the precipitate/supernatant separation by filtration, good filterability occurring above 70 °C (Glasser and Wright, 1998). However, at temperatures above 85 °C, the acid precipitated lignin become soft and tacky and large clumps of lignin bound together making the mixing difficult (Uloth and Wearing, 1989). For the acidification, sulfuric acid can be added at high concentration (about 72% (v/v)) (García et al., 2012) or diluted (about 2% (v/v)) (Mousavioun and Doherty, 2010; Minu et al., 2012).

When black liquor (pH 13.8) from oil palm empty fruit bunch was precipitated with phosphoric acid, pH 2 was found to be the optimum for lignin recovery, further acidification did not improve the yield (Sun et al., 1999). On centrifuged wheat straw black liquor, an optimal pH of 3.5 was determined for the acidification of the black liquor based on lignin precipitation yield (80%) and sulfuric acid consumption (100 mEq/L of black liquor) (Gilarranz et al., 1998). Differential acid precipitation of lignin on alkaline hydrolysate (7.5% NaOH (w/w), 90 °C, 90 min) from apple tree pruning waste showed that 20% of the lignin was recovered with acidification until pH 6-5 while about 80% of lignin recovery was achieved when pH was further adjusted to 2 (García et al., 2012). When sulfuric acid is added at 80 °C to softwood black liquor until the pH is decreased to 9, 67-77% of the lignin were recovered and when pH was decreased to 3, up to 93-95% of the lignin were recovered with the sodium content of the precipitate decreasing with decreasing pH (Uloth and Wearing, 1989). Another study by Alekhina et al. (2015) on softwood black liquor with sequential precipitation at pH 10.5, 5 and 2.5, showed that majority of the lignin (74-89%) was precipitated at pH 5 whereas further pH decrease to 2.5 increased the precipitation yield only by 4-5% (Alekhina et al., 2015).

Acid precipitated lignin usually showed very high ash contents (up to 55% (w/w)) (García et al., 2012) that requires extensive washing with dilute sulfuric acid and water



to eliminate the salts (Uloth and Wearing, 1989; Eckert and Abdullah, 2008). Sugars are also recovered in the precipitate and decrease lignin purity. SCB black liquor obtained from soda-AQ pulping process, precipitated by sulfuric acid until pH 3 at 65 °C, and then, washed with hot water (50 °C) led to lignin purity of 70%, with the 30% remaining being carbohydrates (da Silva et al., 2013). To increase lignin purity some studies proposed a two-step precipitation process as carbohydrates, silica and other inorganic salts tend to precipitate together with lignin at pH about 7 to 5, then lignin with higher purity were precipitated between pH 5 and 3 (Mousavioun and Doherty, 2010; Minu et al., 2012). However another study on softwood black liquor, with sequential precipitation at pH of 10.5, 5 and 2.5 showed opposite results since lignin precipitated at lower pH had a lower purity due to more co-precipitated sugar (Alekhina et al., 2015). Extensive wash of the lignin did not remove the hemicellulosic sugars probably due the polymeric form of xylan and its linear structure making it insoluble in water (Alekhina et al., 2015).

Metso published several patents about lignin separation from black liquor by acid precipitation, among which, WO 2006/031175 (Axegard et al., 2006) discloses the basic two stage acidic process and WO2006/038863 (Oehman et al., 2006) discloses an improvement of the process where sulphate ions are added to the process. pH is adjusted to 1-3.5 with carbon dioxide or sulfuric acid, then a press filter is used to separate the lignin precipitate from the black liquor, and the cake is rinsed with the acid solution (pH from 1 to 3.5) (Axegard et al., 2006). Addition of sulphate ion (e.g., in the form of sodium sulfate) into the black liquor before precipitation, enable to increase lignin yield (Oehman et al., 2006). For instance, addition of Na<sub>2</sub>SO<sub>4</sub> to black liquor with a ratio of 1:20 (w/v), prior to acidification with carbon dioxide until pH 9.6 at 80 °C enable to increase the lignin yield from 60.5 to 66.8%. Innventia, a Swedish company, is precipitating lignin from softwood kraft black liquor (pH 13) by the addition of carbon dioxide or mineral acid at 80 °C until pH 8 only (Eckert and Abdullah, 2008).

### b) Ethanol addition

Ethanol addition to lignocellulosic alkaline extract lead to the precipitation of the hemicelluloses. Ethanol is the solvent most commonly used, but other organic solvents have also been applied for the precipitation of hemicelluloses (Sun, 2000). Hemicelluloses, as polysaccharides, contained many hydroxyl groups which form

hydrogen bonds with the water molecules. When ethanol is added, it adheres to the polysaccharides through hydrophobic interaction and rearranges hydrogen bonds between water and ethanol, resulting in the possibility for the polysaccharide chains to set hydrogen bonds between each other (Umemura and Yuguchi, 2009; Sedlmeyer, 2011).

Final ethanol concentration is the operating condition having the most influence on the hemicellulose precipitation yield (Xu et al., 2014). Increasing volume of ethanol leading to higher hemicelluloses precipitation, classic yield of hemicelluloses precipitated ranges from 70-80% with an ethanol concentration at 70% or above, and 80 to 95% with concentration of ethanol at 80% or above, respectively, depending on the initial biomass and the hemicelluloses extraction conditions (Brillouet et al., 1982; Bian et al., 2010; Peng et al., 2011; Xu et al., 2014). The structural features of a polysaccharides (e.g., nature of the sugar or ramifications) also impact the precipitation behavior and yield. Higher arabinose/xylose ratios were obtained in the isolated hemicelluloses, with increasing concentration of ethanol (Peng et al., 2009, 2011). The undissolved hemicelluloses at high concentration of ethanol are short-chained polysaccharides (Sun, 2000). For synthetic glucans, as the molecular size increased from 1 kDa to 270 kDa, the precipitate yield increased from 10% to 100% in 80% ethanol (Xu et al., 2014).

Addition of ethanol 95% (v/v) at room temperature with constant stirring for a few minutes to an hour or initial stirring associated with sedimentation at lower temperature (4-6 °C) for a few hours to 12 hours are the main process described, then the precipitated hemicelluloses are recovered by centrifugation or by filtration on 0.45 µm nylon (Buranov and Mazza, 2009; Peng et al., 2011; Sun et al., 2004; Zeitoun et al., 2010).

Hemicelluloses precipitation from wheat straw mild alkaline extract by ethanol, with an ethanol:extract ratio of 4:1 (v/v), led to the recovery of 38% of the lignin in the precipitated hemicellulosic fraction (Sun et al., 1998). Two precipitation steps can be done sequentially to increase lignin purity from a lignocellulosic alkaline hydrolysate. First pH is adjusted to 5-7, then 3-4 volumes of ethanol are added to precipitate the hemicelluloses, and finally the ethanol is evaporated and the pH is lowered down to 1.5-2 to precipitate the lignin (Lan et al., 2011; Sun et al., 1998, 1999). This process applied on corncob mild peroxide-alkaline hydrolysate led to 89% recovery for the hemicelluloses after the first precipitation step and 78% recovery for the lignin after the

second precipitation step (Su et al., 2015). A pH adjustment to 5-7 with acid addition before ethanol addition increase the hemicelluloses precipitation yield but led to co-precipitation of lignin and therefore decrease hemicelluloses purity and lignin yield (Sun and Tomkinson, 2001).

Both ethanol and acid additions have been coupled to sequentially precipitate the polysaccharides at pH 6 with 3 volumes of ethanol, then the lignin at pH 2 (Sun et al., 1999). It led to higher lignin purity than direct pH adjustment to 2, but lower lignin yield was obtained.

### 1.4.3.3. Adsorption

#### a) Activated charcoal

Activated charcoal, also known as activated carbon, is usually used to adsorb high molecular weight lignin while carbohydrates remained mainly unadsorbed, and phenolic monomers present different behaviors (Zhao et al., 2011; Shen et al., 2013). The adsorption of lignin onto activated charcoal is an endothermic and spontaneous process, and at least two layers of lignin can be adsorbed (Andersson et al., 2011). Adsorption of phenol molecules on activated charcoal is controlled by the dispersion force between the  $\pi$ -electrons in activated charcoal, under the form of carboxyls, lactones, aldehydes and ketones groups among others, and those in phenol molecules (Jung et al., 2001).

Adsorption via activated charcoal is carried out in batch on lignocellulosic alkaline extracts in order to pretreat the extract before further purification, by adsorption on anion exchange resins (Ou et al., 2007; Zhao et al., 2011; Shen et al., 2013). Adsorption of lignin on activated charcoal before adsorption on resin enable the recycling of the anionic resin (Zhao et al., 2011). The targeted molecules can be adsorbed on the activated charcoal like ferulic acid (Ou et al., 2007), or the molecules considered impurities are adsorbed while the targeted molecules are not like coumaric acid (Zhao et al., 2011), and hemicelluloses (Shen et al., 2013). When activated charcoal is added to a SCB alkaline extract, coumaric acid is weakly adsorbed (14%) and can be separated from lignin and ferulic acid that are adsorbed at 80% (Zhao et al., 2011).

Ferulic acid adsorption from a neutralized SCB alkaline extract is strongly influenced by the ratio of activated charcoal:extract (w/v), a ratio of 1:100 enabled the adsorption of 48% ferulic acid, whereas a ratio of 3:100 enabled the fixation of 98% ferulic acid (Ou et al., 2007). Sequential desorption can help purifying ferulic acid, indeed water at 90 °C or acetic ether can desorb most of the color adsorbed on the charcoal and only a small part of the ferulic acid, then 2% NaOH (w/v) can desorb 94% of the adsorbed ferulic acid (Ou et al., 2007).

### b) Resin

Adsorption of phenol from a synthetic solution is affected by the pH of the solution and the resin used (Caetano et al., 2009). A non-functionalized resin with a polystyrene–divinylbenzene (PS-DVB) matrix, reported maximum loading capacity under acidic conditions, where undissociated phenol form predominates. In contrast, anion exchange resins, also with a PS-DVB matrix, reported higher loading capacity than non-functionalized resin but under alkaline conditions, where phenoxide form predominate, and thus, a combined effect of both adsorption and ion exchange mechanisms occur. Desorption of phenol with sodium hydroxide on the non-functionalized resin was inefficient, but a solution of methanol:water 1:1 (v/v) yielded 90% recovery. On the anion exchange resin, desorption with 4% NaCl (w/v) at pH 12 yielded a 90% recovery for phenol (Caetano et al., 2009). Polyvinyl polypyrrolidone matrix was tested for the adsorption of FA, involving hydrogen binding with phenolic and carboxyl groups, but adsorption on PS-DVB resins, involving hydrophobic interaction with the aromatic ring of FA, led to higher binding capacity (Tilay et al., 2008). Desorption was made with an ethanol:NH<sub>3</sub> solution at a ratio of 1000:1 (v/v), the purity of FA increased by a 1.35-fold factor and its recovery was 58% (Tilay et al., 2008).

Macroporous-type anion exchange PS-DVB resins were used on lignocellulosic alkaline extract to adsorb and purify phenolic monomers such as ferulic acid (Ou et al., 2007) or coumaric acid (Ou et al., 2009); desorption involved water:ethanol:HCl solution at a ratio of 36:60:4 (v/v/v). For the purification of hemicelluloses from a lignocellulosic mild alkaline extract, macroporous-type strong base anion (SBA) exchange resin with an acrylic DVB matrix exhibited better adsorption capacity for the color (phenolic compounds) than a macroporous-type SBA exchange resin with a PS-DVB matrix and a

macroporous-type non-functionalized resin with an aliphatic-DVB matrix (Zeitoun et al., 2010). Desorption of color compounds was carried out with 4% NaOH (w/v). Ion exchange resin were also used to purify hemicelluloses by adsorbing organic acids (mainly acetic acid); sulfuric acid was suggested for the desorption of acetic acid (Shen et al., 2013).

#### 1.4.3.4. Low pressure chromatography

Some chromatographic processes using ion exchange resins on lignocellulosic alkaline extracts have been reported. Pine soda-AQ hydrolysate was acidified until pH 1.2 to precipitate the lignin, then complete separation of aliphatic carboxylic acids and sodium sulfate from the acidified hydrolysate was achieved using chromatography on strong acid cation (SAC) exchange resin and water as eluent (Alén et al., 1991). More recently, chromatography on SAC exchange resins with PS-DVB matrix using water as eluent applied on sugarcane bagasse mild alkaline extract has been reported (Oriez et al., 2018). Depending on the size of the resin pores different separations were obtained by pulse chromatography, on a gel-type resin phenolic monomers with a carboxyl group (e.g., ferulic acid) were recovered at 75% in a fast eluted fraction and phenolic monomers without carboxyl group (e.g., vanillin) were recovered in a fraction eluted later at 75%. On a macroporous-type resin, a fraction containing the largest oligomers of lignin (14% recovery) and hemicelluloses (30% recovery) was obtained free from salts, phenolic monomers and acetic acid.

#### 1.4.3.5. Cross-flow membrane filtration

Membrane filtration has been used in the pulp and paper industry to concentrate black liquor (hemicelluloses and lignin) and to remove some of the salts (Abels et al., 2013; Haddad et al., 2017). Hemicelluloses have a low heating value and can be used for other valuable application, so membrane filtration was studied to concentrate and purify hemicelluloses from black liquor and by extension from other lignocellulosic alkaline extracts (Huang et al., 2008; Sixta and Schild, 2009; Persson and Jönsson, 2010; Toledano et al., 2010). Lignin has a higher heating value than hemicelluloses, but it can be the starting point of a chemical phenolic platform, therefore their concentration and purification from lignocellulosic strong alkaline extract has also been widely studied

(Wallberg and Jönsson, 2006; Jönsson and Wallberg, 2009; Abels et al., 2013). Ultrafiltration, for instance on ceramic membranes (with MWCO values of 0.8  $\mu\text{m}$ , 0.2  $\mu\text{m}$  and 50 nm) at 30-60 °C, can be used on black liquor from raw materials with high content in silica (e.g., rice straw) to retain lignin (75%) and silicate (80%), while cooking chemicals are recovered in the permeate (Liu et al., 2004).

Ultrafiltration has been compared to precipitation to recover kraft lignin as it present the advantage of not altering the pH or the temperature of the black liquor (Uloth and Wearing, 1989; Jönsson and Wallberg, 2009). Kraft lignin obtained via ultrafiltration are more contaminated by ash than lignin obtained via acidification (Uloth and Wearing, 1989). However lignin obtained from soda pulping process of *Miscanthus sinensis* (7.5% NaOH (w/w), 90 min, 90 °C) and then passed through 5, 10 or 15 kDa membranes are less contaminated by hemicelluloses than acid precipitated lignin (Toledano et al., 2010b). Ultrafiltration has been used at one Scandinavian mill to produce Karatex®, a kraft lignin used as an extender for phenol formaldehyde resin in the manufacture of plywood (Uloth and Wearing, 1989).

Retention of hemicelluloses from black liquor, while lignin are recovered in the permeate, have also been demonstrated by membrane filtration and the most efficient membranes have usually an MWCO between 1 and 15 kDa (Uloth and Wearing, 1989; Wallberg and Jönsson, 2006; Jönsson and Wallberg, 2009; Persson and Jönsson, 2010; Singh and Murthy, 2017). However, the MWCO of the membranes has to be adapted for every alkaline lignocellulosic extract as the raw material and extraction conditions variabilities lead to different size and configuration of hemicelluloses and lignin oligomers. For instance, the concentration of black liquor from hardwood by a VRF of 3 was carried out on 15 kDa ceramic membrane (Orelis, now Novasep) at 1 bar, 5.0 m/s, 90 °C and resulted in an average flux of 33 L/h/m<sup>2</sup>, in the retention of 15–25% lignin and the retention of 75–95% of hemicelluloses, while cooking chemicals (sodium hydroxide and sodium sulphide) were not retained (Jönsson and Wallberg, 2009). Membranes with even smaller MWCO, for instance about 200-400 Da, can retain more than 97% of the hemicelluloses from a wood steam hydrolysate, but are not suitable for purifying hemicelluloses extracts as small molecules present high retention rate as well, i.e., acetic acid (70%), furfural (70%) or HMF (85%) (Ajao et al., 2015).

Concentration by a volumic reduction factor (VRF) of 3.4 of *Eucalyptus globulus* cold caustic extract on flat-sheet polyethersulfone membrane of 10 kDa (UP010, from Microdyn-Nadir) at 40 °C with cross flow velocity changing in the range of 1 to 3 L/min and PTM in the range of 2 to 8 bar, led to a xylan concentration increase up to 67.4 g/L from 22.0 g/L, while their concentration in permeate was lower than 1 g/L. Meanwhile, sodium hydroxide concentration was maintained in the retentate around 80 g/L, so xylans/NaOH ratio was increased from 0.28 to 0.84 (Sixta and Schild, 2009). Purification can be improved if the filtration is carried out in diafiltration mode (Uloth and Wearing, 1989), 90% of the impurities can be removed when 2.3 diavolumes of water are used before concentration by VRF 2 of softwood black liquor on 25 kDa polysulfone membrane (GR60PP from Danish Sugar Company, now Alfa Laval) at 60 °C. The permeate flux for dialysis and post-concentration were 90 and 70 L/h/m<sup>2</sup>, respectively, with a global lignin recovery of 54%.

Polysulfone membranes were used successfully in order to recover caustic silicate in the ultrafiltration permeate from herbaceous alkaline extract (50% w/w NaOH, 50 °C, 2 h) (Lucas and Martin, 1998). Ceramic membranes can be used at higher temperatures, and therefore higher flux can be achieved, but a side effect is the lower lignin retention. Filtration of black liquors at 145 °C and pH of 13-14 at 4 bars on 15 kDa and 5 kDa ceramic membranes (Orelis, now Novasep) led to fluxes of 100 and 50 L/h/m<sup>2</sup> but low retention of lignin with 20% and 30%, respectively (Wallberg and Jönsson, 2006).

The initial water flux of the membrane, used to check the efficiency of a cleaning procedure after the filtration of an alkaline hydrolysate, should be measured after the rinsing of the new membrane with an alkaline solution (e.g., sodium hydroxide). Indeed, an alkaline solution increase the hydrophilicity and the flux, by the swelling of the membrane (Nilsson et al., 2008; Sixta and Schild, 2009). Cleaning is usually performed at low TMP in order to avoid compression of a possible cake formed at the membrane surface (Wallberg and Jönsson, 2006). Care is needed when membranes are cleaned after treatment of kraft cooking liquors because the solubility of lignin decreases when the pH decreases. This means that if water is used for rinsing, lignin will precipitate and foul the membranes. A cleaning method based on the use of collected permeate as the first rinsing solution, followed by synthetic alkaline solution cleaning was successful (Wallberg and Jönsson, 2006).

### 1.4.3.6. Electrodialysis

Electrodialysis was studied to acidified black liquor to recover lignin (1.4.3.2.a)Acidification) and at the same time recover NaOH from the black liquor (Haddad et al., 2017). Hydrogen ions are produced in the black liquor stream and replace the sodium ions that migrate into the sodium hydroxide stream. The results have indicated that the implementation of electrodialysis led to a lower chemical consumption than the chemical acidification method.

### 1.4.3.7. Combination of different purification techniques

The technologies mentioned previously have been sometimes combined in integrated purification processes of lignocellulosic alkaline extracts. Here are a few examples of integrated process.

Purification of *p*-CA from SCB mild alkaline extract involved ultrafiltration, adsorption on activated charcoal, adsorption on anion exchange resin and finally crystallization (Zhao et al., 2011). Ultrafiltration on a 3 kDa hollow fiber membrane produced a permeate free of hemicelluloses and lignin oligomers, but still containing phenolic monomers responsible of a brown color and considered impurities. Addition of activated charcoal in the permeate with a ratio of 3:100 (w/v), was the optimal ratio and removed 78% of the color whereas 14% of the *p*-CA was adsorbed as well. The removal of these phenolic compounds improved the adsorption and desorption performance of anion exchange resins after several adsorption-desorption cycles. *p*-CA was crystallized from the desorption solution (water:ethanol:HCl at a ratio of 36:60:4 (v/v/v)), by evaporating the ethanol and the resulting crystal had a purity of 95.2% for *p*-CA. Overall, 8 g of *p*-CA was formed from 1 kg of SCB.

Another method was developed by Buranov and Mazza (2009) to purify ferulic acid and hemicelluloses from lignocellulosic mild alkaline hydrolysates, using a combination of neutralisation, ethanol precipitation, ultrafiltration and a second ethanol precipitation step (Buranov and Mazza, 2009). After neutralization of the alkaline extract and addition of ethanol 95% (v/v) to reach an extract:ethanol ratio of 65:35 (v/v), wax and glucomannans were precipitated. They were separated by centrifugation and the supernatant was ultrafiltrated on a 30 kDa PS membrane resulting in the separation of



high polymeric hemicelluloses in the retentate from oligomeric hemicelluloses and ferulic acid in the permeate. Oligomeric hemicelluloses were precipitated from the permeate by the addition of ethanol and ferulic acid was recovered after evaporation of the ethanol. However, no purity and recovery values for hemicelluloses and ferulic acid were reported.

An integrated process to produce purified hemicelluloses from a wheat bran alkaline extract has been developed including ultrafiltration and adsorption steps (Zeitoun et al., 2010). The alkaline extract was first separated from the solid residue of the extraction by centrifuge filtration with a 1  $\mu\text{m}$  mesh. A 30 kDa polyethersulfone hollow fiber membrane was used in concentration mode with a VRF of 1.8 and led to the removal of 65% of the initial salts. The retentate containing hemicelluloses was treated on an anion exchange resin, to remove color compounds by adsorption on the resin, while only 8% of the xylan was lost by adsorption.

### 1.5. Combination of acid and alkaline extraction

The alkaline treatment induces the extraction of many valuable molecules like lignin and hemicelluloses oligomers, phenolic monomers such as coumaric and ferulic acid. Nevertheless, they are obtained in a mixture solution requiring several purification steps to obtain pure fractions. Another process set-up, including a sequential acid pretreatment where hemicelluloses are mainly extracted followed by an alkaline pretreatment where lignin and other valuable compounds are extracted, has been studied. This process does not require special purification step to remove fermentation inhibitors and yield high saccharification yields.

Empty palm fruit bunch fiber was treated first by dilute acid (4% H<sub>2</sub>SO<sub>4</sub> (v/v), S:L ratio of 1:5 (w/v), 121 °C, 1 h) and washed, then treated by a highly concentrated alkaline solution (40% NaOH (w/v), 25 °C, 4 h) and washed and finally the solid residue was enzymatically saccharified (Kim et al., 2012; Kim and Kim, 2013). Acid pretreatment removed 88% of the hemicelluloses and 30% of the lignin, and the combination of acid and alkaline pretreatments removed 70% of the lignin and about 96% of the cellulose was preserved in the solid residue. Eventually, about 98% of the cellulose contained in the solid residue was saccharified into glucose, and simultaneous enzymatic saccharification and fermentation converted about 84% of the cellulose into ethanol.

Corn stover was treated with a similar process, sequential dilute acid extraction (0.5% H<sub>2</sub>SO<sub>4</sub> (w/v), S:L ratio of 1:10 (w/v), 160 °C, 10 min) and alkaline extraction (2% NaOH (w/v), S:L ratio 1:20 (w/v), 80 °C, 1 h) led to even higher lignin yield despite less drastic alkaline conditions than in the previous study (Lee et al., 2015). Dilute acid treatment produced yields of xylose of 71% and glucose of 19%, and removed about 15% of the lignin. With the following alkaline treatment, overall removal yields were 89% for lignin, 91% for xylan and 22% for glucan, leading to an increase of glucan content in the solid residue of 51% (w/w). The enzymatic saccharification yield of the solid residue after the two pretreatments was 97%.

A patent has been developed based on sequential acid (carbonic acid at pH 4, 50 °C, 60 min) and alkaline (50% NaOH (w/w), 50 °C, 2 h) extraction on counter-current extractors to produce ethanol, sulfur-free lignin, and potentially waterglass (caustic silicate solution), when raw materials have a high content of silica (Lucas and Martin,

1998). The acid extract, containing 5-carbon sugars, soluble salts, soluble plant proteins, and soluble polypeptides, is sent to fermentation. The strong alkaline extract, containing lignin and potentially silica, is ultrafiltrated to purify and concentrate lignin up to 40% dry solid (w/w) while the permeate is fed back to the alkaline extraction solution or sold as waterglass. The solid residue is washed, pH adjusted and sent to fermentation. On rice hull, the authors claimed that 98.0% of the initial hemicelluloses are recovered in the acid hydrolysate, 97.0% of the initial lignin is covered in the alkaline hydrolysate and the solid residue contained 100% of the initial cellulose. A company, Colusa Biomass Energy Corporation, in California, has been using this patent.

In the previous studies, water consumption of the washing step is not mentioned, but might be significant as neutral pH of the effluent is looked for after the dilute acid extraction and the alkaline extraction, and then after the alkaline treatment and before the enzymatic saccharification. Extraction of the hemicelluloses in the first stage can also be performed by steam explosion, before extraction of the lignin in a second stage by alkaline treatment ( NaOH S:L ratio of 1:10 (w/v), NaOH, 80 °C, 1 h), and therefore the water consumption would be reduced (Glasser and Wright, 1998). The remaining solid residue, supposed to contain mainly cellulose, contain 7-10% lignin according the initial biomass treated, whereas the lignin fraction has a purity up to 90-95% and represent 15-20% (w/w) of the initial biomass.

Finally, sequential treatment with alkaline extraction first is also interesting in a characterization prospect (Barberousse et al., 2008). Mild alkaline hydrolysis releases ester-bonded ferulic acid and other monomer phenolic acid from lignocellulosic biomass, then acid hydrolysis can be used to cleave the remaining alkyl aryl ether bonds. It has also been applied at pilot scale as presented in the exhaustive process proposed by NREL (Humbrid et al., 2011). Deacetylation in mild alkaline conditions (NaOH:dry biomass ratio of 17:1000 (w/w), 80 °C, 1 h) is carried out before dilute acid pretreatment, to enhance downstream enzymatic hydrolysis and potentially decrease enzyme loading requirements.

### 1.6. Conclusion

The fractionation processes have to be selected carefully according the molecules targeted in the biorefinery, since they influence the structure of the extracted molecules, for instance acid extraction leads to carbohydrate hydrolysis whereas alkaline extractions do not. The bibliographic study shows that nowadays, simple industrial processes using resins, low pressure chromatography, are used for the purification of lignocellulosic acid extract. The aim of the project was to define a similar simple process for the recovery of the various fractions contained in lignocellulosic mild alkaline hydrolysates. Our idea was to explore two low-energy and low-chemical consuming processes - resin in chromatographic mode and membrane filtration - to purify model mild alkaline extracts.

## Chapter 2:

# CHEMICAL FRACTIONATION

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### 2.1. Introduction

Two model raw materials were studied in this work: sugarcane bagasse (SCB) and sunflower oil cake (SuOC). SCB has been already studied extensively to find other economical and environmental interesting valorizations than just burning it to provide power. SCB can be considered a model lignocellulosic biomass due to its high production worldwide and its composition as it contains only little amount of non-lignocellulosic compounds (e.g., ash, proteins, wax). SuOC is currently used for its high protein content in animal feed but also contains a large amount of lignocellulose that reduces its digestibility for the ruminant. The lignocellulose fraction could be valorized for higher added value products but its recovery might be hindered by the proteins. SuOC has been chosen as a model lignocellulosic biomass that contains proteins and because it is a typical crop of the South West of France, where this work was carried out.

Traditional routes coming from the papermaking industry or from the developing second generation ethanol production from lignocellulosic biomass were reviewed. The most relevant in terms of technical feasibility, economic aspect and environmental awareness were selected and implemented. Optimization of the selected processes was not carried out, efforts were focused on the purification of the extracts.

### 2.2. Raw materials characterization and pre-treatments

#### 2.2.1. Sugarcane bagasse (SCB)

##### 2.2.1.1. Production, current and potential uses

Sugarcane was the most produced crop in the world in 2013 with 1.9 billion tons (FAO, 2015). In 1998, the average yield was about 65 tons per hectare, but yields as high as 90-105 tons per hectare can be obtained in some areas (Pandey and Soccol, 1998). Sugarcane has been described as a rich solar energy reservoir due to its high yields, in comparison to yield of wheat (1 ton/Ha), grass (2 ton/Ha) and trees (20 ton/Ha), for example (Pandey and Soccol, 1998). On average, 1 ton of sugarcane generates 140 to 280 kg of bagasse, the fibrous by-product remaining after sugar extraction from sugarcane (Sun et al., 2004; Chandel et al., 2012; Melati et al., 2017), so about 300 million tons of sugarcane bagasse (SCB) is produced annually. More than 50% of the SCB is used by the sugar factories themselves as a fuel for the boilers to generate steam and the rest is burnt in biomass power plant to generate electricity (Pandey & Soccol, 1998; Boussarsar, 2008; Liu et al., 2008).

Instead of burning the SCB to generate electricity, its use for the production of fuels and chemicals could offer economic, environmental, and strategic advantages (Cardona et al., 2010). The high added value of C5 sugar derivatives (e.g., 2-3 \$/kg for xylitol) or phenolic compounds under monomeric form (e.g., 15 \$/kg for vanillin) (Holladay et al., 2007) or under oligomeric form (lignin price ranging from 0.35 to 3 \$/kg depending on its form) (Higson, 2011) could justify the extra steps required for their extraction and purification compare to the price of sugar (0.25 to 0.35 \$/kg).

The sugarcane bagasse used in this work was provided by eRcane, a research institute based in La Réunion. In 2004, sugarcane covered 26,500 hectares in La Réunion (54% of the agricultural land) and the production was around 2 million tons of sugarcane (average yield of 75 ton/Ha). Two mills process the sugarcane on the island, providing 22% of the island's electricity (775,000 inhabitants) (Lejars and Siegmund, 2004).



## 2.2.1.2. Composition

## a) In literature

The composition of SCB has been reported on numerous studies and limited variability was observed when the analyses were performed using the methodology developed by National Renewable Energy Laboratory (NREL) on SCB from different origins (**Table 2.1**). Some results presented the carbohydrates cell wall as cellulose and hemicelluloses whereas other studies used the polymeric chains (e.g., xylan) related to the sugar monomers quantified by HPLC (e.g., xylose) as expressed by NREL methodology. For an easier comparison, the results expressed with the polymeric chains were reported in **Table 2.1** as cellulose for glucan, and hemicelluloses for all the other polymeric sugars. The following composition for SCB has been commonly observed: cellulose 38-45% (w/w), hemicelluloses 25-35% (w/w), lignin 18-25% (w/w) and ash 2-5% (w/w).

In the study by Chen et al. (2011) and Okano et al. (2006) the SCB composition assessed by ADF-NDF method was significantly different from the SCB composition reported by NREL method. Even if, the SCB composition depends on its species and growth conditions, we may suggest that ADF-NDF method tends to underestimate the lignin content and overestimate the cellulose content (Chen et al., 2011) or the hemicelluloses content (Okano et al., 2006). The ADF-NDF and NREL protocols are detailed in Chapter 6.

Hemicelluloses are mainly composed of xylose 78-92%, that constitute a backbone on which arabinose and uronic acids (galacturonic and glucuronic acids) are branched (Lavarack et al., 2002). By increasing the severity of successive alkaline extractions, Sun et al. (2004) confirmed that SCB hemicelluloses are made from a xylose backbone with branched portion containing with decreasing extent xylose, arabinose, glucose, galactose, mannose and rhamnose (Sun et al., 2004). However, the content of glucose in hemicelluloses is hard to quantify as it may come from cellulose degradation during alkaline hydrolysis. Biomasses containing hemicelluloses with a xylan backbone like SCB generally contain high level of bound acetate groups on the xylose unit through ester linkage (Sluiter et al., 2008).

**Table 2.1**

Composition of the sugarcane bagasse in different studies. Composition of the hemicelluloses (X for xylose, A for arabinose, G for glucose, UA for uronic acids) and of lignin (AIL for acid insoluble lignin and ASL for acid soluble lignin) is reported when the study mentioned it.

<b>SCB origin</b>	<b>Water solubles</b>	<b>Wax</b>	<b>Cellulose</b>	<b>Hemicelluloses</b>	<b>Lignin</b>	<b>Ash</b>	<b>Others</b>	<b>References</b>
Unknown	4.0	39.2	28.7	19.4	5.1	3.6	(Sun and Tomkinson, 2000)	
Guangzhou, China	0.8	43.6	33.5	18.1	2.3	1.7	(Sun et al., 2003)	
Pradópolis, Brazil		44.5	32.8	23.8		NA	(Grimaldi et al., 2015)	
Unknown		Remo- ved	42.4	25.2 (X 82.6%, A 6.2%, G 5.6% (**), UA 5.2%)	19.6	1.6	11.2	(Brienzo et al., 2009)
Tamaulipas, Mexico		38.9	26.2 (X 78.6%, A 11.4%)	23.9		11.0	(Aguilar et al., 2002)	
La Réunion, France		45	26 (X 92%)	20	2.1	7	(Boussarsar et al., 2009)	
Tainan, Taiwan		52.5	26.0	12.7	1.0	7.8	(Chen et al., 2011) (*)	
Kagoshima, Japan		31.7	46.1	10.5		11.7	(Okano et al., 2006) (**)	

Results are expressed on dry solid basis (w/w).

(\*) Analyzed by ADF-NDF

(\*\*) We do not agree with the glucose being reported as part of the hemicelluloses since it could be from some cellulose being co-extracted.

Ferulates and coumarates are particularly abundant in the cell walls of grasses (Ragauskas et al., 2014). After an alkaline treatment, Xu et al. (2005) reported that SCB contains about 1.8% *p*-CA (w/w) and 1.3% FA (w/w) as well as minor quantities of related phenols such as *p*-hydroxybenzaldehyde, vanillin, syringaldehyde and vanillic acid (Xu et al., 2005). *p*-CA has more ester bounds (69–76%) than ether bounds to the cell wall components, mainly lignin, whereas about half of FA (44-55%) is esterified to the hemicelluloses, the other half being etherified through the phenolic oxygen to lignin.

SCB contains lower ash content (2-5%) than other crop residues (e.g., rice straw and wheat straw contains about 14-18% and 9-11% ash, respectively) (Pandey and Soccol, 1998; Cardona et al., 2010). It is in advantage for instance for the productivity of a given process as it means more fibers and thus more sugars are available per mass of SCB treated. It also increases the efficiency of some chemical fractionation process where metal ions can decrease the catalytic activities of acid or base chemicals.

b) In our project

Dry SCB (1 m<sup>3</sup>) was provided by eRcane (La Réunion, France). Because of the visual heterogeneity of the raw SCB, a sample of raw SCB (146 g) was fractionated depending on the size of the particles by an analytical vibratory sieve shaker (AS200 basic, Retsch) with three grids of 2, 1 and 0.5 mm. Most of the particles were less than 0.5 mm (35.5% (w/w)) and the ash content increased with decreasing size of particles (**Table 2.2**). Fibers larger than 2 mm had an ash content of 2.4% (w/w) similar to what is reported in the literature, whereas smaller particles had a higher ash content (e.g., 17.0% (w/w) for particles smaller than 0.5 mm) with a higher heterogeneity in their ash content (higher standard deviation). According to Arnaud Petit (eRcane, La Réunion), small particles are likely to be from parenchyma (filler cells) that can still contain minerals if the lysis was not complete during the crushing prior to the sugar extraction step, whereas particles longer than 2 mm are structural fibers, made essentially from lignocellulose.

**Table 2.2**

Fractions obtained after the sieving of raw SCB on grids of 2, 1 and 0.5 mm

<b>Fraction (F)</b>	<b>F &gt; 2 mm</b>	<b>2 mm &gt; F &gt; 1 mm</b>	<b>1 mm &gt; F &gt; 0.5 mm</b>	<b>0.5 mm &gt; F</b>
Portion ( $w_F/w_i$ ) <sup>(*)</sup>	19.1	26.0	15.8	35.5
Ash content (%)	2.4 ( $\pm$ 0.1)	5.0 ( $\pm$ 2.1)	11.7 ( $\pm$ 3.3)	17.0 ( $\pm$ 1.7)

Ash content values are calculated based on the percentage of dry solid. All the analyses were run in triplicate.

(\*) with  $w_F$  the weight of the fraction and  $w_i$  the initial weight of the SCB fractionated

In order to homogenize the raw SCB and increase the extraction yields at the following chemical fraction steps, the SCB was ground on a 2 mm mesh by a knife mill (Mill F6 N V, Electra). The ground SCB was analyzed by two techniques to determine its content in cellulose, hemicelluloses and lignin: the technique commonly called ADF-NDF (Van Soest and Wine, 1967, 1968; Van Soest et al., 1991) (**Table 2.3**) and the protocol developed by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2005, 2008) (**Table 2.4**), inspired from the TAPPI procedure from the pulp and paper industry.

The removal of the extractives (Sluiter et al., 2005) by Soxhlet with water then ethanol is interesting when the biomass to analyze contains high level of ash, proteins or lipophilic substances (e.g., lipids, pigments, wax). These compounds have low contents in the SCB but we run this procedure in order to get a precise composition of our raw material. The removal of the extractives reduced the amount of acid soluble lignin (ASL) and it also increased the standard deviation of the sugar contents revealing less reliable results for both ADF-NDF and NREL protocols (**Table 2.3** & **Table 2.4**). Besides, the mass balance established during the following chemical fractionation steps would not be accurate if the SCB free from extractives was taken as a reference since no extraction procedure with water then ethanol was run on SCB before its chemical fractionation. The water and ethanol extractions by NREL protocol removed 8.2% ( $\pm$  1.7%) and 3.8% ( $\pm$  0.3%) of the initial DS content, and 9.1% ( $\pm$  3.7%) and 2.7% ( $\pm$  1.9%) of the initial ash content of the SCB, respectively.

**Table 2.3**

Cellulose, hemicelluloses and lignin determination on SCB ground on a 2 mm mesh by ADF-NDF procedure and the influence of extractives removal (ER) by NREL protocol (Sluiter et al., 2005).

	Without ER	With ER
Cellulose	35.5 ± 0.7	43.0 ± 1.2
Hemicelluloses	21.7 ± 1.1	19.6 ± 2.2
Lignin	14.1 ± 0.5	15.7 ± 1.1
Others	28.7	21.7

All values are calculated based on the percentage of dry solid. All the analyses were run in triplicate.

The SCB was also ground to 50 µm to test electrostatic separation, it was also analyzed as reported in **Table 2.4**. Analyses run by two different operators with slightly different protocols between LCA and IATE laboratories showed similar results. The difference in results between the SCB ground on 2 mm mesh and on a 100 µm mesh by an impact mill ( $D_{50} = 50 \mu\text{m}$ ) could be due to an uncomplete degradation of sugars with the SCB 2 mm, but also to a different sampling method since the ash content of the SCB 50 µm (6.1% and 5.8% according to LCA and IATE, respectively) was lower than the ash content of SCB 2 mm (9.9%).

Results obtained by ADF-NDF protocol showed higher standard deviation for sugar polymers content values than results obtained by NREL protocol, and a lower lignin content as it was also found in the literature, confirming that ADF-NDF minimize the content of lignin in biomass.

HPLC analysis of raw SCB on the RPM Rezex column revealed the presence of three main sugars: glucose, xylose and arabinose. Traces of galactose and mannose were detected but their concentrations were too low to enable a reliable follow-up at the extraction and purification stages. In other studies on SCB from China, galactose and mannose have been quantified in hemicelluloses at levels of about 2 to 3% and traces to 1%, respectively (Sun et al., 2004; Cheng et al., 2008). Fructose was not found in the raw SCB.

**Table 2.4**

Cellulose, hemicelluloses and lignin determination on SCB ground on a 2 mm mesh by NREL procedure (Sluiter et al., 2008) and the influence of extractives removal (ER) by NREL protocol (Sluiter et al., 2005) and the use of RPM and RHM HPLC column for the quantification of monomeric sugars.

Raw material	SCB 2 mm LCA	SCB 50 µm LCA	SCB 50 µm ER LCA	SCB 50 µm ER Rezex RPM <sup>(*)</sup>	SCB 50 µm ER LCA	SCB 50 µm ER Rezex RHM	SCB 50 µm IATE
Place of analyses							
HPLC column used for the monomeric sugar quantification	Rezex RHM	Rezex RHM	Rezex RHM	Rezex RPM <sup>(*)</sup>	Rezex RHM	Rezex RHM	Aminex HPX-87H
Acid insoluble lignin (AIL)	21.6 ± 0.3	23.2 ± 0.1	22.5 ± 0.6	22.5 ± 0.6	22.5 ± 0.6	22.5 ± 0.6	
Acid soluble lignin (ASL)	5.5 ± 0.2	5.0 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	
Lignin (AIL + ASL)	27.1 ± 0.1	28.2 ± 0.3	26.2 ± 0.4	26.2 ± 0.4	26.2 ± 0.4	26.2 ± 0.4	28.4
Glucan	35.9 ± 1.2	39.2 ± 0.2	43.3 ± 3.7	43.3 ± 3.7	44.0 ± 3.5	44.0 ± 3.5	37.5
Xylan	19.4 ± 0.8	21.8 ± 0.1	20.6 ± 2.9	20.6 ± 2.9	21.6 ± 2.0	21.6 ± 2.0	20.0
Arabinan	2.3 ± 0.1	2.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.1
Hemicelluloses (Xylan + Arabinan)	21.8 ± 0.8	24.3 ± 0.1	21.9 ± 2.9	21.9 ± 2.9	23.6 ± 2.1	23.6 ± 2.1	23.9
Others	15.2	8.3	8.6	8.6	6.2	6.2	13.0

All values are calculated based on the percentage of dry solid. All the analyses were run in triplicate.

(\*)filtration on SPE cartridge Strata ABW before injection on HPLC column.

The composition of the SCB ground on 2 mm mesh and analyzed without extractives removal (ER) (**Table 2.5**) was taken as a reference in the rest of this work to calculate extraction yields at the following fractionation steps (except for the electrostatic separation). 3% of the SCB dry solid content was not determined, it could be compounds such as wax or pigments.

**Table 2.5**

Composition of the SCB ground on a 2 mm sieve without NREL water and ethanol Soxhlet extractions and analyzed on Rezex RHM HPLC column. Reference for the rest of this work.

Dry solid	92.5 ± 0.2
Ash	9.9 ± 0.7
Acid insoluble lignin (AIL)	21.6 ± 0.3
Acid soluble lignin (ASL)	5.5 ± 0.2
Lignin (AIL + ASL)	27.1 ± 0.1
Glucan	35.9 ± 1.2
Xylan	19.4 ± 0.8
Arabinan	2.3 ± 0.1
Hemicelluloses (Xylan + Arabinan)	21.8 ± 0.8
Galacturonic acid	0.5 ± 0.1
Protein	1.8 ± 0.1
Others	3.0

All values are calculated based on the percentage of dry solid. All the analyses were run in triplicate.

## 2.2.2. Sunflower oil cake (SuOC)

### 2.2.2.1. Production, current and potential uses

The worldwide production of sunflower oil for 2009 was 32.8 million tons, with about 13.4 million tons of sunflower oil cake (SuOC) generated as a by-product (De la Rubia et al., 2011). In 2011, the production of SuOC was estimated at about 14.9 million tons (Barakat et al., 2015).

SuOC has been mainly used as a protein complement in animal feed rations and sometimes as a fertilizer, a combustible source or a substrate for white biotechnology laboratory studies (De la Rubia et al., 2011; Lomascolo et al., 2012). However, its high lignocellulose content limits its efficiency as feed for ruminants and poultry. The

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separation of the protein and the lignocellulose would improve the animal feed application with the protein-enriched fraction of the SuOC and in parallel it would generate a lignocellulose-enriched fraction interesting for green chemistry applications (Bautista et al., 1990).

### 2.2.2.2. Composition

#### a) In literature

The composition of SuOC depends on the seed (species and growing conditions) but also on the oil extraction process from which they are obtained. Indeed, factors such as the amount of hulls removed before the oil extraction, or the process used for the oil extraction (e.g., mechanical- or solvent-extraction) influences its composition (Bautista et al., 1990). Removing the hull before the extraction increases the protein content of the SuOC and mechanical oil extraction leads to higher residual oil content in the SuOC than solvent extraction process.

SuOC typically contain 28-40% crude protein and 15-25 % crude fiber (Bautista et al., 1990; Barakat et al., 2015). Due to this composition, no work has been focusing on the lignocellulosic fraction of SuOC. But in a biorefinery concept all the fractions must be valorized. As massively cultivated in South West of France it was interesting to work on this raw material.

#### b) In our project

SuOC came from sunflower seeds, harvested in the South West of France, and processed by Grandes Huileries Médiaco, in Béziers. The process consists in seeds grinding, cold press for oil extraction, heating and hot press for a second oil extraction. The resulting specks are extracted with hexane to complete the lipids removal. Finally, the specks are compacted into pellets with steam under pressure.

The water and ethanol extractions by NREL protocol removed 17.3% ( $\pm$  2.0%) and 7.7% ( $\pm$  0.9%) of the initial DS content, and 43.5% ( $\pm$  4.5%) and 5.9% ( $\pm$  2.6%) of the initial ash content of the SuOC, respectively. SuOC contains about twice more water and



ethanol extractives than SCB (8.2% and 3.8%, respectively) and its ash were removed more easily than the ash from the SCB (9.1% and 2.7%, respectively).

The SuOC used for electrostatic separation pretreatment and alkaline extraction without running extractives removal before the analysis were characterized by IATE and the LCA (**Table 2.6**). The analyses conducted by IATE showed similar results to those we obtained for the contents of ash, celluloses (i.e., glucan), hemicelluloses and proteins, only the lignin content differed significantly in the analyses from the LCA and IATE.

**Table 2.6**

Composition of the SuOC ( $D_{50} = 50 \mu\text{m}$ ) without NREL Soxhlet extraction with water and ethanol and analyzed on Rezex RPM HPLC column with filtration on SPE cartridge Strata ABW prior to injection (sugars quantification) and Rezex RHM HPLC column (galacturonic acid and acetic acid quantification). Reference for the rest of this work.

Compounds	LCA	IATE
Dry solid	$93.5 \pm 0.2$	93.7
Ash	$5.7 \pm 0.2$	5.4
Acid insoluble lignin (AIL)	$20.5 \pm 1.2$	
Acid soluble lignin (ASL)	$12.3 \pm 0.4$	
Lignin (AIL + ASL)	$32.8 \pm 1.1$	26.4
Glucan	$17.0 \pm 1.4$	18.6
Xylan	$7.0 \pm 0.3$	
Arabinan	$3.9 \pm 0.3$	
Galactan	$3.0 \pm 0.1$	
Mannan	$1.9 \pm 0.2$	
Hemicelluloses (Xylan + Arabinan + Galactan + Mannan)	$15.7 \pm 0.2$	14.0
Galacturonic acid	$2.4 \pm 0.7$	
Protein	$27.4 \pm 0.3$	28.4
Glycerol (*)	$0.4 \pm 0.1$	
Others	NA	7.0

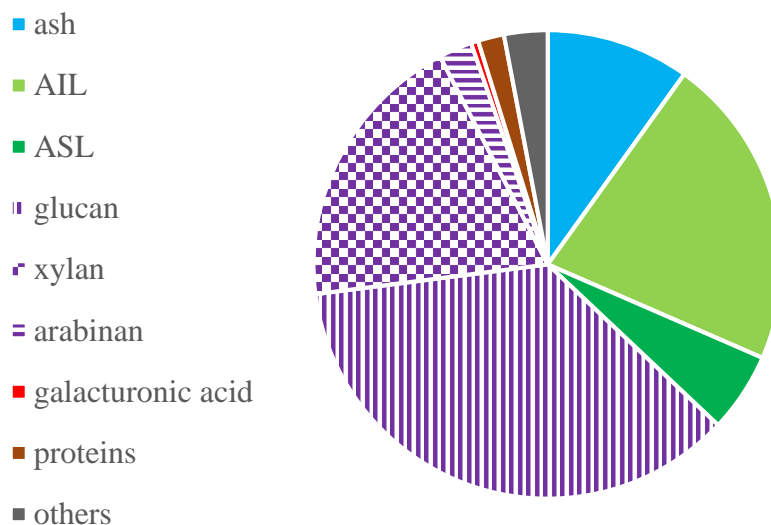
All values are calculated based on the percentage of dry solid. All the analyses were run in triplicate.

(\*) Glycerol was coming from the hydrolysis of triglycerides hydrolysis during NREL acid hydrolyses. It is an indicator of the leftover lipids in the SuOC.

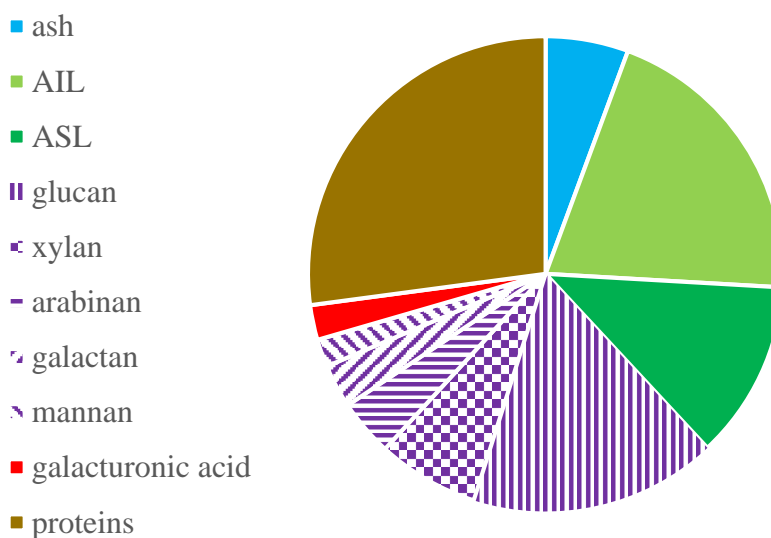
Major differences are noticeable between SCB and SuOC compositions in **Fig. 2.1** and **Fig. 2.2**, SCB is essentially composed of lignocellulose, with a high percentage of

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cellulose whereas SuOC has significantly lower lignocellulose content, with a high share of lignin.



**Fig. 2.1** Schematic composition of SCB.



**Fig. 2.2** Schematic composition of SuOC.

As a comparison, SuOC was also analyzed by ADF-NDF methodology, results are presented in **Table 2.7**. As for SCB, the content of lignin was much lower with ADF-NDF procedure than with NREL procedure. Moreover, the standard deviation appeared to be higher with ADF-NDF procedure on this biomass.

**Table 2.7**

Composition of the SuOC ( $D_{50} = 50 \mu\text{m}$ ) following analysis by NREL and ADF-NDF procedures

<b>Compounds</b>	<b>NREL</b>	<b>ADF-NDF</b>
Cellulose	$17.0 \pm 1.4$	$21.2 \pm 1.3$
Hemicelluloses	$15.7 \pm 0.2$	$17.8 \pm 2.7$
Lignin	$32.8 \pm 1.1$	$10.9 \pm 2.0$
Others	34.5	50.1

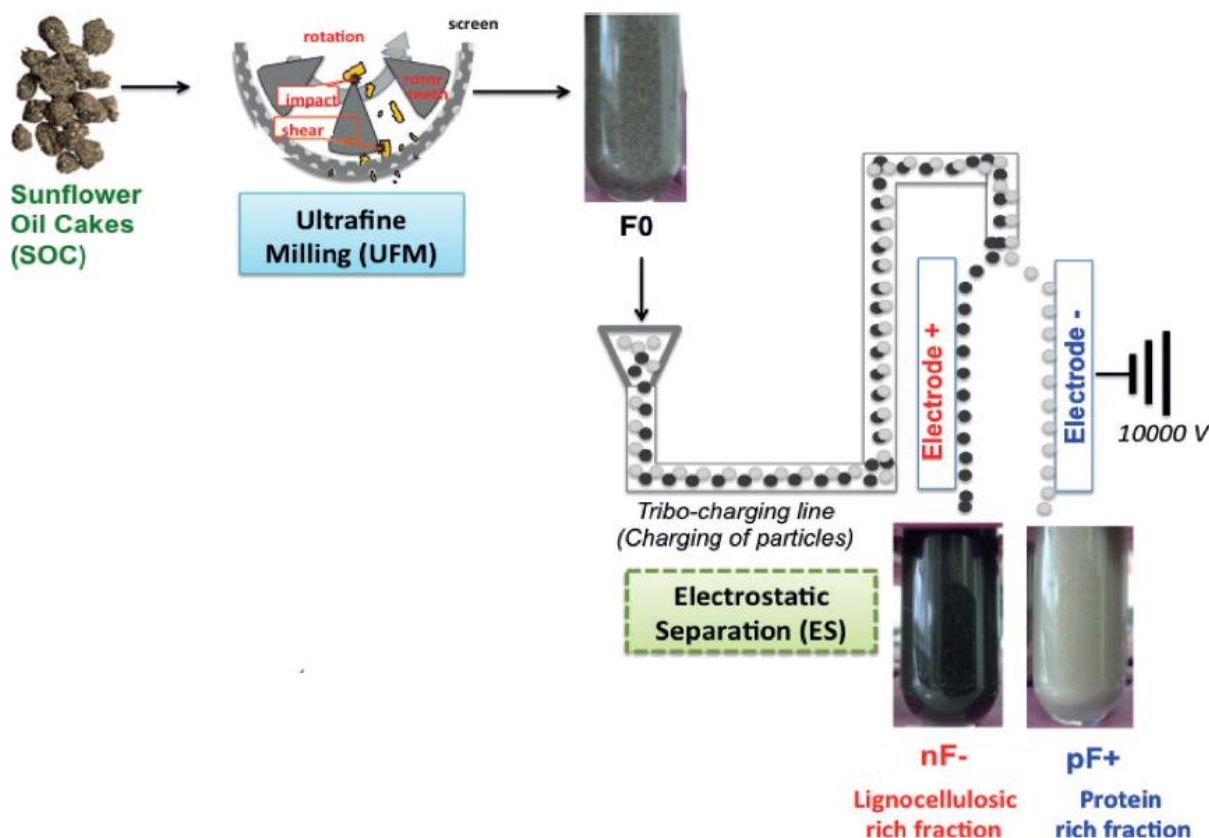
All values are calculated based on the percentage of dry solid. All the analyses were run in triplicate.

### 2.2.3. Pretreatment by electrostatic separation

Electrostatic separation experiments on SCB and SuOC presented bellow were carried by the joint research unit IATE, based in Montpellier. Their process was patented under the number US20160310957A1 (Barakat and Rouau, 2016).

#### 2.2.3.1. Protocol by IATE

The raw material was first ground by a knife mill on 1 mm mesh then by an impact mill equipped with a  $100 \mu\text{m}$  screen and operating at ambient temperature and at a speed of 12,000 rpm. The size of the resulting particles ranged from 20 to  $80 \mu\text{m}$ , with a  $D_{50}$  (i.e., median diameter) of  $50 \mu\text{m}$ , this fraction was called F0 and constituted the feed of the electrostatic separation. F0 fraction was conveyed by compressed air in a charging line, a tube made of polyvinyl chloride with a 2 m length and a 1 cm internal diameter, where the particles were charged by tribo-electricity, by impacting each other and impacting against the walls of the charging line (**Fig. 2.3**) At the outlet of the tube, the charged particles passed through a separation chamber containing two high voltage electrodes (10,000 V) with height of 20 cm and a width 5 cm, where the positively charged particles (pF+) are separated from the negatively charged particles (nF-). The two fractions were separately recovered in two cyclones and analyzed (Barakat et al., 2015).



**Fig. 2.3** Sunflower oil cake pretreatment by ultrafine milling and electrostatic separation (Barakat et al., 2015).

### 2.2.3.2. Results

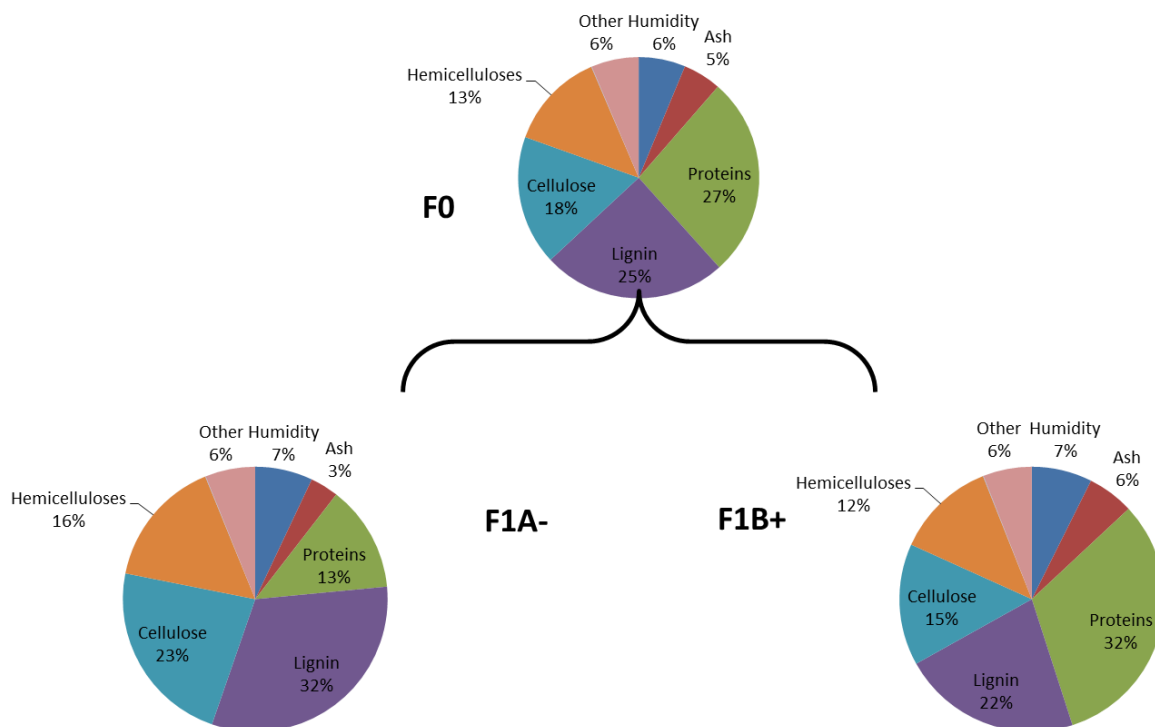
#### a) Sugarcane bagasse

On SCB, no significant difference in composition was noticed after a single-pass electrostatic separation between positively charged particles (F1+) and negatively charged particles (F1-). The low content of proteins in SCB (less than 2% (w/w)) may explain the low efficiency of the process on this biomass.

#### b) Sunflower oil cake

On SuOC (different batch than the one used for the following alkaline extraction), important differences in composition were observed after a single-pass between positively charged particles (F1A+) and negatively charged particles (F1B-) (**Fig. 2.4**). In F1A- fraction, the protein content was reduced from 27% to 13% whereas the content of

lignin, cellulose and hemicelluloses increased from 25% to 32%, 18% to 23% and 13% to 16%, respectively.



**Fig. 2.4** Composition of the SuOC initial fraction (F0) and the fractions obtained after single-pass (F1A- and FB1+) electrostatic separation. Figure from IATE.

On the SuOC used for the alkaline extraction, a second pass of electrostatic separation was run on the fraction F1A- forming the fraction F2A-. 4.8 kg of fraction F0 were used to produce 1 kg fraction F2A- in 20 h, meaning that the mass yield was 21%. Based on IATE analyses, the content of proteins decreased from 27% to 7%, whereas the lignin content increased from 25% to 37%. The analyses run in LCA showed slightly different compositions for the fractions F0 (**Table 2.12**) and F2A- (**Table 2.13**), partially because unlike IATE, the values provided are on dry solid basis, with the content of proteins and carbohydrates decreasing from 31% to 13% and from 33% to 28% whereas the content of lignin increased from 20% to 33%.

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### 2.2.3.3. Conclusion

Electrostatic separation presented no noticeable fractionation on SCB, due to the low content in proteins of this biomass. At the opposite, fractionation by electrostatic separation of SuOC, a protein rich biomass, showed interesting results with the production of protein-enriched and lignin-enriched fractions. Alkaline extraction on the lignin-enriched fraction (F2A-) was studied in the next part and compared to alkaline extraction on the initial SuOC (fraction F0).

## 2.3. Alkaline extraction

### 2.3.1. Introduction

The extraction conditions were based on Sun *et al.* study (1995) on wheat straw to optimize the extraction yield of lignin and hemicelluloses (Sun et al., 1995). Alkaline extractions were carried out on SCB and SuOC, and both the solid residues and the alkaline extracts were characterized to know their precise composition and how the different compounds were separated between these two fractions. The identification of alkaline extracts major compounds was a key step to establish purification pathways in the next chapters.

SCB was extracted at three different scales, i) 10 g were extracted for a preliminary study, ii) 150 g were extracted to produce the feed (alkaline extract) for the chromatography study, i.e., pulse tests (Chapter 4), and iii) 3 kg were extracted to test the different ultrafiltration membranes to avoid biased results due to variability in the feed composition (Chapter 3). The later extract was also used for the integrated purification process study (Chapter 5). As for SuOC, the effect of the electrostatic pretreatment on the alkaline extraction of SuOC was assessed.

### 2.3.2. Materials and methods

#### 2.3.2.1. Chemicals

Sodium hydroxide ( $\geq 98.5\%$ ), sulfuric acid 72% for analytical hydrolysis, sulfuric acid 95% and acetonitrile ( $\geq 99.9\%$ ) to prepare HPLC eluents, and methanol ( $\geq 99.8\%$ ) used as a tracer for column void volume, were purchased from VWR. Calcium carbonate ( $\geq 98.5\%$ ) was purchased from Merck. HPLC standards: D-(+)-cellobiose ( $\geq 98\%$ ), D-(+)-glucose ( $\geq 99.5\%$ ), D-(+)-galactose, L-(+)-arabinose (99%), D-(+)-xylose (99%), D-(+)-mannose ( $\geq 99\%$ ), fructose ( $\geq 99\%$ ), acetic acid ( $\geq 99\%$ ), furfural (99%), 5-hydroxymethyl-2-furfuraldehyde (99%), gallic acid (97%), 4-hydroxybenzoic acid ( $\geq 99\%$ ), caffeic acid ( $\geq 98\%$ ), vanillic acid (97%), syringic acid ( $\geq 95\%$ ), 4-hydroxybenzaldehyde (98%), vanillin (99%), *p*-coumaric acid ( $\geq 98\%$ ), syringaldehyde (99%), *trans*-ferulic acid

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( $\geq 99\%$ ), sinapic acid ( $\geq 98\%$ ), trans-3-hydroxycinnamic acid (99%), were all purchased from Sigma Aldrich.

### 2.3.2.2. Alkaline extraction on SCB

Alkaline extraction on dry SCB ground on a 2 mm mesh by a knife mill (IKA MF 10 basic) was run at three different scales: 200 mL for preliminary test, 3 L for the production of the feed for the preparative chromatography tests and 60 L for the production of the feed for the membrane filtration tests and integrated process assessment. Comparison between the different scales would have been interesting since the temperature control, the agitation and the solid/liquid separation at the end of the filtration differed from one step to another. Unfortunately, comparison between these different scales are hard to make since the solid residue was rinsed and the rinsing solution incorporated in the alkaline extract only for the larger scale (60 L).

Two preliminary tests were carried out on 10 g of SCB in 200 mL of sodium hydroxide solution at 1.5% (w/v) in a 250 mL Erlenmeyer flask, leading to a solid:liquid ratio of 1:20 (w/v) and a NaOH:SCB ratio of 0.3:1 (w/w), under continuous magnetic stirring (600 rpm). The first test was run at 60 °C for 5 h, and the solid residue was removed from the alkaline extract using a cellulose filter (90 mm diameter) on a Büchner filtration device. The second test was run at 80 °C for 6 h and the solid residue was removed from the alkaline extract using a Whatman filter grade 52 (with a porosity of 7  $\mu\text{m}$  and 90 mm diameter). The solid residues were rinsed with water, the rinsing solutions were collected with the alkaline extracts. The solid residues were then dried at 50 °C for 48 h and finally ground using a microfine grinder (IKA MF 10 basic) on a 1 mm sieve prior to analyses. The alkaline extract obtained at 60 °C for 5 h was precipitated by the addition of sulfuric acid at 72% (w/w) until pH 2.3, the precipitate was recovered using a cellulose filter (90 mm diameter) on a Büchner filtration device dried at 50 °C for 48 h and finally ground using a microfine grinder (IKA MF 10 basic) on a 1 mm sieve prior to analyses. The alkaline extract obtained at 80 °C for 6 h was precipitated by the addition of sulfuric acid at 95% (w/w) until pH 2.3. The precipitate was recovered using a Whatman filter grade 3 (porosity of 6  $\mu\text{m}$  and 90 mm diameter) on a Büchner filtration device dried at 50 °C for 48 h and finally ground using a microfine grinder (IKA MF 10 basic) on a 1 mm sieve prior to analyses. The supernatants were qualified as purified alkaline extract.



The alkaline extraction at the medium scale was carried out on 150 g of SCB in 3 L of sodium hydroxide solution at 1.5% (w/v) in a 4 L jacketed glass reactor (**Fig. 2.5**), leading to a solid:liquid ratio of 1:20 (w/v) and a NaOH:SCB ratio of 0.3:1 (w/w), under continuous stirring (200 rpm) for 6 h at 60 °C. The SCB solid residue was removed from the alkaline extract on Whatman filters grade 3 (150 mm diameter) on a Büchner filtration device, then dried at 50 °C for 48 h and finally ground using a microfine grinder (IKA MF 10 basic) on a 1 mm sieve prior to analysis.



**Fig. 2.5** Set-up for the alkaline extraction on SCB in a 4 L jacketed glass reactor.

The alkaline extraction at the largest scale was carried out on 3 kg of dry SCB ground on a 2 mm mesh with 60 L of 1.5% NaOH (w/v) in a stainless steel-lined vessel (De Dietrich) (**Fig. 2.6**), with a solid:liquid ratio of 1:20 (w/v) and a NaOH:SCB ratio of ratio of 1:3 (w/w), with continuous mechanical stirring (200 rpm) for 6 h at 60 °C. The solid residue was removed from the alkaline extract using a top-discharge vertical basket centrifuge (RC 50 PX R, Rousselet) equipped with a 5 µm polypropylene bag (**Fig. 2.7**). This residue was rinsed with distilled water, dried at 50 °C for 48 h and ground using microfine grinder (IKA MF 10 basic) on a 1 mm sieve before analysis. The filtered SCB

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alkaline extract and the filtered solution used to rinse the solid residue were mixed and analyzed.



**Fig. 2.6** Set-up for the alkaline extraction on SCB in 100 L stainless steel-lined vessel.



**Fig. 2.7** Solid residue/alkaline extract separation using a top-discharge vertical basket centrifuge (RC 50 PX R, Rousselet) equipped with a 5 µm polypropylene bag.

### 2.3.2.3. Alkaline extraction on SuOC

The conditions were the following: 10 g of SuOC ( $D_{50} = 50 \mu\text{m}$ ) in 200 mL of sodium hydroxide solution at 1.5% (w/v) in a 250 mL Erlenmeyer flask, leading to a solid: liquid ratio of 1:20 (w/v) and a NaOH:SCB ratio of 0.3:1 (w/w), under continuous magnetic stirring (600 rpm) for 6 h at 60 °C. The SuOC solid residue was removed from the alkaline extract on Whatman filters grade 3 (90 mm diameter, 6  $\mu\text{m}$  pore size) on a Büchner filtration device, then dried at 50 °C for 48 h and finally ground by a microfine grinder (IKA MF 10 basic) on a 1 mm sieve before analysis.

### 2.3.2.4. Analytical methods

#### a) Dry solid and ash

Dry solid (DS) content was gravimetrically determined at 103 °C for 12 h and ash content at 500 °C for 12 h.

#### b) Carbohydrates and lignin

Based on Laboratory Analytical Procedure of the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008), Acid Insoluble Lignin (AIL) was gravimetrically quantified and Acid Soluble Lignin (ASL) was determined at 240 nm using an absorptivity constant of 25 L/g/cm. High Performance Liquid Chromatography (HPLC) on a Rezex RHM-Monosaccharide H<sup>+</sup> 300 x 7.8 mm column (Phenomenex) in conjunction with a Rezex RHM-Monosaccharide H<sup>+</sup> 50 x 7.8 mm guard column (Phenomenex) was used to quantify glucose, xylose, arabinose, acetic acid, furfural and HMF (Sluiter et al., 2006). Isocratic conditions were applied with 5 mmol/L H<sub>2</sub>SO<sub>4</sub> at 0.6 mL/min, the injection volume was 50  $\mu\text{L}$ , the column was maintained at 65 °C and the RI detector at 50 °C. Since solid residue, alkaline extract and purified fractions were very alkaline, the NREL protocol was adapted to ensure total hydrolysis of the sugar oligomers under acidic conditions. 150 mg of solid residue were analyzed instead of 300 mg (Sluiter et al., 2008) and liquid samples (Sluiter et al., 2006) were diluted by 4 with distilled water before acid hydrolysis.

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### c) Monomeric sugars and hemicelluloses acetyl groups

Sulfuric acid was added to the alkaline extract to adjust its pH to 2, corresponding to the pH of HPLC eluent with RHM column, then the extract was analyzed on RHM column without running NREL protocol (Sluiter et al., 2006). pH adjusted samples directly injected on HPLC enabled the quantification of monomeric sugars and free acetic acid, whereas samples analyzed through NREL protocol gave the total amount of sugars (monomeric and oligomeric forms) and acetic acid (free and bound to hemicelluloses).

### d) Phenolic monomers

Quantification of twelve phenolic monomeric compounds potentially present in SCB alkaline extract (Xu et al., 2005; Capriotti et al., 2015) - gallic acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid (VA), syringic acid, 4-hydroxybenzaldehyde (4HBA), vanillin, *p*-CA, syringaldehyde, FA, sinapic acid and hydroxycinnamic acid - was studied by HPLC on an OmniSpher 3 C18 100 x 4.6 column (Agilent Technologies). The gradient was as follow: 91% acidified water (1% acetic acid (v/v)) and 9% acetonitrile for 25 min, from 9 to 90% acetonitrile in 5 min, kept constant for 5 min, then decreased back to 91% acidified water in 5 min and the column was equilibrated for 7 min between runs. The flow rate was 0.5 mL/min, the injection volume was 10  $\mu$ L and the column temperature was maintained at 25 °C. The UV detector was set at 280 nm. Concentrations for the calibration curves ranged between 0 and 200 mg/L. Standard and process samples were diluted in acetonitrile:water at a ratio of 50:50 (v/v) prior to injection.

## 2.3.3. Results and discussion

### 2.3.3.1. SCB

After the alkaline extractions at the smallest scale (preliminary experiments), the filtration flow rate on a Büchner device for the separation of the solid residues and the alkaline extracts was very slow. Instead of cellulose filter, or Whatman grade 52 filter, a Whatman grade 3 filter was used for the extraction at medium scale.

The results of the SCB alkaline extraction at the smallest scale are reported in **Table 2.8** and **Table 2.9**. Surprisingly, longer reaction time and higher temperature led

to lower lignin extraction with 72% at 60 °C during 5 h and 56% at 80 °C during 6 h. Moreover, higher temperature also led to a lower yield closure for glucan and xylan indicating potential degradation of these sugars. Chromatograms of the analyses are reported in Chapter 6:.

**Table 2.8**

SCB, solid residue, alkaline extract (purified extract and precipitate) compositions and extraction yields after extraction on 10 g of SCB at 60 °C for 5 h

Compounds	Alkaline extract						
	SCB	Solid residue		Purified extract		Precipitate	
	Content	Content	Yield	Content	Yield	Content	Yield
DS	92.5	98.3	48	0.8		96.7	
Ash	9.9	15.5	23	71.2		3.0	
Glucan	35.9	51.6	92	1.4	3	1.8	1
Xylan	19.4	17.5	57	6.9	24	10.4	9
Arabinan	2.3	2.2	59	1.8	50	2.4	18
Hemicellulose	21.8	19.7	58	8.7	27	12.7	10
AIL	21.6	11.9	35	2.1	6	77.9	63
ASL	5.5	3.4	39	5.5	66	5.2	16
Total lignin	27.1	15.3	36	7.6	19	83.1	53
Mass closure	94.7	102.1		88.8		100.6	

All the content values are calculated based on the percentage of dry solid. All the analyses were run in triplicate, standard deviation was at most 1%.

The solid residues obtained after extraction at 60 °C and 80 °C were enriched in cellulose from 35.9% in the initial SCB to 51.6% and 42.3%, whereas the hemicelluloses and lignin contents decreased from 21.8% to 19.7% and 17.8% and from 27.1% to 15.3% and 16.6%, respectively. This fraction was not further studied as many papers already tackles its valorization for instance for cellulosic ethanol production via saccharification and fermentation (Chapter 1).

The acid precipitation on the alkaline extract was carried out in order to facilitate the analysis by decreasing the pH of the liquid fraction to perform its analysis by HPLC on H<sup>+</sup> column (eluent pH is around 2). It also confirmed the efficiency of the acid addition as a technique to separate lignin and hemicelluloses from a lignocellulosic alkaline extract. Indeed, from the alkaline extracts, the precipitates obtained by acid addition contained mainly AIL, 77.9% and 81.4% in the two tests, and 91% and 87% of the AIL

were recovered in the precipitates. However, hemicellulosic sugars were also co-precipitated, with potentially some sugars being bound to lignin too, but it should not represent more than 2-5% of the lignin mass (Chapter 1).

**Table 2.9**

SCB, solid residue, alkaline extract (purified extract and precipitate) compositions and extraction yields after extraction on 10 g of SCB at 80 °C for 6 h

Compounds	SCB Content	Solid residue		Alkaline extract			
				Purified extract		Precipitate	
		Content	Yield	Content	Yield	Content	Yield
DS	92.5	96.5	54	1.5		94.3	
Ash	9.9	24.1	41	70.2		5.9	
Glucan	35.9	42.3	84	0.9	2	0.5	0
Xylan	19.4	15.8	58	6.4	20	3.3	2
Arabinan	2.3	1.9	59	2.0	52	1.0	6
Hemicellulose	21.8	17.8	58	8.4	23	4.3	2
AIL	21.6	12.5	41	2.4	7	81.4	47
ASL	5.5	4.1	53	4.9	54	4.6	11
Total lignin	27.1	16.6	44	7.3	16	86.0	40
Mass closure	94.7	100.8		86.8		96.8	

All the content values are calculated based on the percentage of dry solid. All the analyses were run in triplicate, standard deviation was at most 1%.

Acid addition leading to precipitation was not carried out on the medium and large scale alkaline extracts, the adaptation of the NREL protocol by diluting the alkaline extracts prior to acid hydrolysis was sufficient to obtain relevant results, mass closure of the fractions and yield closure of the compounds close to 100%, as presented in **Table 2.10** and **Table 2.11**.

Most of the glucan was recovered in the solid residue (95%), which had a yellowish color (**Fig. 2.8**) and its purity increased from 35.9% to 43.6%, whereas the content of hemicelluloses and lignin decreased. 35% of the DS was recovered in the alkaline extract, the major fraction of the DS being composed of inorganic salts (56.1%). Xylan mass closure (89%) could indicate potential degradation of xylose during the alkaline reaction. 25% of the hemicelluloses and 46% of the lignin were recovered in the mild alkaline extract, hemicelluloses accounting for 11.9% and lignin for 27.3% of the composition of the extract. Phenolic monomers were the fourth main pool of molecules with 4.1% of the

extract composition, *p*-CA being the main extracted phenolic monomers (3.6% of the DS).

**Table 2.10**

SCB, solid residue, alkaline extract and concentrated alkaline extract composition and extraction yields after extraction on 150 g of SCB

Compounds	SCB	Solid Residue		Alkaline Extract		Yield closure
	Content	Content	Yield	Content	Yield	
DS	92.5	96.4	59	2.6	35	94
Ash	9.9	19.7	36	56.1	61	97
Glucan	35.9	43.6	95	1.5	2	97
Xylan	19.4	16.6	67	9.4	22	89
Arabinan	2.3	1.6	53	2.5	50	103
Hemicelluloses	21.8	18.2	65	11.9	25	90
AIL	21.6	13.8	50	21.1	45	95
ASL	5.5	3.8	54	6.2	52	106
Total Lignin	27.1	17.7	51	27.3	46	97
VA				traces		
4HBA				0.1		
Vanillin				0.1		
<i>p</i> -CA				3.6		
FA				0.4		
Total phenolic monomers				4.1		
Mass closure	94.7	99.1		100.9		

All the values are calculated based on the percentage of dry solid. All the analyses were run in duplicate, standard deviation was at most 1%.



**Fig. 2.8** Solid residue of the SCB acid extraction before drying and grinding.

**Table 2.11**

SCB, solid residue, alkaline extract and concentrated alkaline extract composition and extraction yields after extraction on 3 kg of SCB

Compounds	SCB	Solid residue		Alkaline extract		Yield
	Content	Content	Yield	Content	Yield	closure
DS	92.5	97.5	48	3.4	49	97
Ash	9.9	17.1	26	56.0	85	111
Glucan	35.9	48.2	86	1.4	3	88
Xylan	19.4	18.1	60	8.8	29	89
Arabinan	2.3	2.0	55	2.2	59	114
Hemicelluloses	21.8	20.1	59	13.3	40	99
AIL	21.6	14.9	44	16.6	49	94
ASL	5.5	3.8	44	8.2	96	140
Total Lignin	27.1	18.7	44	24.6	58	102
VA				traces		
4HBA				0.1		
vanillin				0.1		
<i>p</i> -CA				3.3		
FA				0.4		
Total phenolic monomers				3.9		
Mass closure	94.7	104.2		97.0		

All these values were calculated as a percentage of dry solid. All analyses were run in duplicate, standard deviation was at most 1%.

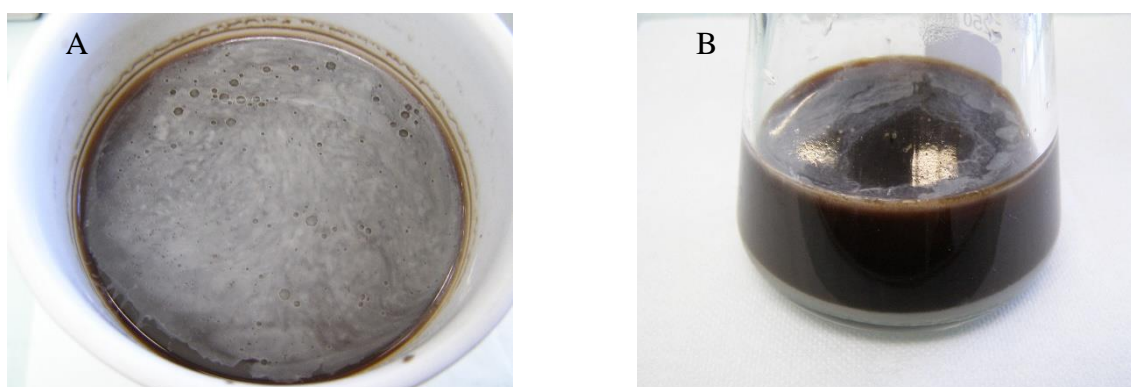
The recovery of ash (85%), lignin (58%) and hemicelluloses (40%) were higher in the alkaline extract at the largest extraction scale (3 kg of SCB treated) compared to the medium extraction scale (150 g of SCB treated). This can be due to a different technique for the solid/liquid separation after the extraction –Büchner filtration at small and medium scale vs. centrifuge filtration at the largest scale - and the rinsing of the solid residue with distilled water at the end of the solid residue/alkaline extract separation. A better mechanical agitation might have also influenced the extraction yields at the largest scale. The composition of the alkaline extract remained mostly unchanged with about 56% of inorganic salts, 1% of glucan, 9% of xylan, 2% of arabinan, 4% of phenolic monomers, only the AIL differed with 21.1% at medium scale and 16.6% at the largest scale. At the largest scale, the alkaline extract contained less AIL but more ASL, suggesting that more AIL was converted to ASL probably due to the better agitation or temperature control.



## 2.3.3.2. SuOC

After the alkaline extraction on SuOC fractions F0 and F2A-, the solid was removed by Büchner filtration with a Whatman grade 3 filter (porosity of 6  $\mu\text{m}$  and diameter of 150 mm) and a low filtrate flow was obtained probably because of some fouling of the filter generated by the extracted proteins. Indeed, the proteins were poorly recovered in the solid residue (22%) so even if their analysis was not possible on the alkaline extract (solid samples are required for Kjeldahl method) we can assume that about 78% of the proteins were recovered in the alkaline extract (**Table 2.12**). The same trend was observed on the fraction enriched in lignocellulose after electrostatic fractionation (F2A-), 20% of the proteins were recovered in the solid residue (**Table 2.13**).

Overall, the analyses are quite unreliable due to yield closure far from 100% for most of the compounds. This can be due to the high content of protein of the biomass and the few lipids left that interfere with the analyses. In the alkaline extracts for both F0 and F2A-, a white precipitate, probably proteins, occurred and oily droplets were floating (**Fig. 2.9**). The alkaline extracts were centrifuged and the white precipitates accounted for 31.2% and 31.7% DS of the F0 and F2A- alkaline extracts, respectively. The quantity of dried precipitates did not enable Kjeldahl analysis to confirm its high content in proteins. The glycerol content found after the alkaline extraction in the solid residue and the extract largely exceeded its initial quantity in SCB probably because acid conditions (used in the NREL protocol for the analysis) lead to uncomplete hydrolysis of triglycerides.



**Fig. 2.9** (A) SuOC F0 alkaline extract during the filtration on the Buchner device. (B) SuOC alkaline extract after the filtration on Whatman filter grade 3.

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The recovery rates of hemicellulosic sugars and lignin in the F0 and F2A- alkaline extracts were very low with 3% and 10% for xylan, 9% and 13% for galactan, 7% and 12% for arabinan and 2% and 3% for mannan, 9% and 7% for AIL, 46% and 45% for ASL, respectively. Proteins were preferably extracted compared to hemicelluloses and lignin, even when the protein content of the SuOC was decreased from 30.8% to 13.3% by using F2A- instead of F0 as raw material.

**Table 2.12**

Native SuOC (fraction F0), solid residue, alkaline extract compositions and extraction yields after alkaline extraction

Compounds	SuOC F0	Solid residue		Alkaline extract		Yield closure
	Content	Content	Yield	Content	Yield	
DS	93.5	98.6	53	2.7	45	98
Ash	5.7	20.7	38	58.5	92	130
Proteins	27.4	8.9	22	NA	NA	NA
Glucan	17.0	16.9	69	1.0	3	73
Xylan	7.0	7.5	74	0.4	3	78
Galactan	3.0	2.1	50	0.5	9	59
Arabinan	3.9	3.5	63	0.5	7	70
Mannan	1.9	2.7	48	0.1	2	50
Glycerol	0.4	1.3	209	0.2	28	237
AIL	20.5	24.7	84	3.0	9	93
ASL	12.3	6.6	37	9.7	46	84
4HBA				1.8		
caffeic acid				5.1		
vanillin				0.7		
<i>p</i> -CA				0.7		
FA				1.1		
sinapic acid				0.6		
Total phenolic monomers				9.9		
Mass closure	101.5	94.8		83.4		

All values are calculated based on the percentage of dry solid. All the analyses were run in triplicate. The value in red for the protein content of the alkaline extract are deducted from the solid analysis.

**Table 2.13**

SuOC treated by electrostatic fractionation, lignocellulose enriched fraction (fraction F2A-), solid residue, alkaline extract compositions and extraction yields after alkaline extraction

Compounds	SuOC F2A-	Solid residue		Alkaline extract		Yield closure
	Content	Content	Yield	Content	Yield	
DS	94.7	97.3	55	2.2	41	96
Ash	3.8	12.0	24	64.3	99	123
Proteins	13.3	3.6	20	NA	NA	NA
Glucan	16.5	27.9	121	0.7	2	124
Xylan	5.8	12.5	155	1.1	10	165
Galactan	1.9	1.6	61	0.4	13	73
Arabinan	2.2	3.0	100	0.5	12	111
Mannan	1.2	2.7	89	0.1	3	92
Glycerol	0.3	0.8	177	0.2	35	212
AIL	32.6	31.2	69	4.3	7	76
ASL	9.1	10.4	82	7.5	45	127
4HBA				5.5		
caffeic acid				6.3		
Vanillin				3.1		
<i>p</i> -CA				1.4		
FA				2.8		
sinapic acid				0.8		
Total phenolic monomers				19.9		
Mass closure	88.4	105.7		98.8		

All values are calculated based on the percentage of dry solid. All the analyses were run in triplicate. The value in red for the protein content of the alkaline extract are deducted from the solid analysis

SuOC (F0) mild alkaline extract contained more phenolic monomers than SCB mild alkaline extract with 9.9% of the DS and also presented a different composition for the phenolic monomers, with caffeic acid being the main one 5.1% of the DS, then 4HBA (1.8%) FA (1.1%), *p*-CA (0.7%), vanillin (0.7%) and sinapic acid (0.6%). In the fraction enriched in lignocellulose (F2A-), the content of phenolic monomers in the alkaline extract increased by a two-fold factor with 19.9% of the DS. The same phenolic monomers were detected with a similar order of importance even if caffeic acid content (6.3%) increased less than the contents of the other phenolic monomers.

### 2.3.4. Conclusion

Mild alkaline extraction on SCB led to the production of an extract containing inorganic salts, lignin and hemicelluloses oligomers, phenolic monomers (five of them were detected) and acetic acid. The next chapters focus on the separation of these compounds by membrane filtration, chromatography and precipitation by acid or ethanol addition.

Alkaline extract from native SuOC and SuOC treated by electrostatic fractionation presented low solubilization rates for hemicellulosic sugars and lignin, proteins appeared to be preferably solubilized. The electrostatic fractionation before alkaline extraction did not improve the solubilization of hemicelluloses and lignin, but it had a positive impact on the content of phenolic monomers that increased by a two-fold factor. The composition of the alkaline extracts in phenolic monomers was noticeably different depending on the material extracted (SCB vs. SuOC).

## 2.4. Acid extraction

### 2.4.1. Introduction

Since based on the review in Chapter 1, the conditions for complete solubilization and hydrolysis of cell wall carbohydrates combining concentrated acid then dilute acid treatment are well-known and since single-step concentrated acid fractionation present a renewed interest with acid emerging recycling process, this last process was tried on SCB.

### 2.4.2. Materials and methods

#### 2.4.2.1. Chemicals

Sulfuric acid 72% for acid extraction and analytical hydrolysis, sulfuric acid 95% and acetonitrile ( $\geq 99.9\%$ ) to prepare HPLC eluents, were purchased from VWR. Calcium carbonate ( $\geq 98.5\%$ ) was purchased from Merck. HPLC standards: D-(+)-cellobiose ( $\geq 98\%$ ), D-(+)-glucose ( $\geq 99.5\%$ ), D-(+)-galactose, L-(+)-arabinose (99%), D-(+)-xylose (99%), D-(+)-mannose ( $\geq 99\%$ ), fructose ( $\geq 99\%$ ), acetic acid ( $\geq 99\%$ ), furfural (99%), 5-hydroxymethyl-2-furfuraldehyde (99%), gallic acid (97%), 4-hydroxybenzoic acid ( $\geq 99\%$ ), caffeic acid ( $\geq 98\%$ ), vanillic acid (97%), syringic acid ( $\geq 95\%$ ), 4-hydroxybenzaldehyde (98%), vanillin (99%), *p*-coumaric acid ( $\geq 98\%$ ), syringaldehyde (99%), *trans*-ferulic acid ( $\geq 99\%$ ), sinapic acid ( $\geq 98\%$ ), *trans*-3-hydroxycinnamic acid (99%), were all purchased from Sigma Aldrich.

#### 2.4.2.2. Acid extraction

5 g of SCB ground on a 2 mm mesh by a knife mill was treated in 95 g of 72% H<sub>2</sub>SO<sub>4</sub> (w/w) in a 250 mL Erlenmeyer flask, leading to a solid:liquid ratio of 1:20 (w/w) (equivalent to 1:12.6 (w/v)) and a H<sub>2</sub>SO<sub>4</sub>:SCB ratio of 14.7:1 (w/w), under continuous magnetic stirring (500 rpm) for 1 h at 50 °C. In order to stop the reaction and facilitate the filtration at the next step, 1,628.6 g of water was added to the SCB/acid mixture, the sulfuric acid content became 4% (w/w). The SCB solid residue was removed from the alkaline extract on Whatman filters grade 50 (porosity of 2.7 μm, 90 mm diameter) on a Büchner filtration device, then dried at 50 °C for 48 h and finally ground with a mortar and pestle prior to analysis. The filtrated acid extract was injected with and without

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running the second hydrolysis step of the NREL protocol (4% H<sub>2</sub>SO<sub>4</sub> (w/w) at 121 °C for 1 h) on HPLC column Rezex RHM.

### 2.4.2.3. Analytical method

#### a) Dry solid and ash

Dry solid content (DS) and ash content analyses cannot be carried out on acid extract since sulfuric acid can evaporate at high temperature (boiling point: 337 °C).

#### b) Carbohydrates and lignin

Based on Laboratory Analytical Procedure of the National Renewable Energy Laboratory (Sluiter et al., 2008), acid-insoluble lignin (AIL) was quantified gravimetrically and acid-soluble lignin (ASL) was determined spectrophotometrically, at a wavelength of 240 nm using an absorptivity constant of 25 L/g/cm. High-performance liquid chromatography (HPLC) on a Rezex RHM-Monosaccharide H<sup>+</sup> 300 x 7.8 mm column (Phenomenex), used in conjunction with a Rezex RHM-Monosaccharide H<sup>+</sup> 50 x 7.8 mm guard column (Phenomenex) was performed to quantify glucose, xylose, arabinose, acetic acid, furfural and hydroxymethylfurfural (HMF) (Sluiter et al., 2006). Isocratic conditions were applied, with 5 mmol/L H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.6 mL/min; the injection volume was 50 µL, the column was maintained at 65 °C and the RI detector was maintained at 50 °C.

#### c) Monomeric sugars, hemicelluloses acetyl groups, furfural and HMF

The extract was analyzed on the Rezex RHM column without running the NREL protocol. The direct injection of the acid extract onto the HPLC column made it possible to quantify monomeric sugars and free acetic acid, whereas the analysis of samples with the NREL protocol provided data for total sugars (monomeric and oligomeric forms) and acetic acid (free and bound to hemicelluloses). The concentrations of HMF and furfural were also determined by direct injection of the acid extract onto the HPLC column without running the NREL protocol.

## d) Phenolic monomers

Twelve phenolic monomeric compounds potentially present in SCB alkaline extract (Xu et al., 2005; Capriotti et al., 2015) - gallic acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid (VA), syringic acid, 4-hydroxybenzaldehyde (4HBA), vanillin, *p*-CA, syringaldehyde, FA, sinapic acid and hydroxycinnamic acid – were quantified by HPLC on an OmniSpher 3 C18 100 x 4.6 column (Agilent Technologies). The gradient was as follows: 91% acidified water (1% acetic acid (v/v)) and 9% acetonitrile for 25 min, acetonitrile concentration increasing from 9 to 90% over 5 min, then kept constant for 5 min, before decreasing back to 91% acidified water over 5 min, with column equilibration for 7 min between runs. The flow rate was 0.5 mL/min, the injection volume was 10 µL and the column temperature was maintained at 25 °C. The UV detector was set at 280 nm as it corresponds to a maximum in the absorbance of phenolic monomers such as *p*-CA and FA (Holser, 2014). The concentrations used to plot the calibration curves ranged from 0 to 200 mg/L. Standards and process samples were diluted in acetonitrile:water at a ratio of 50:50 (v/v) before injection.

## 2.4.3. Results and discussion

The results of the extraction of SCB in concentrated acid conditions are reported in **Table 2.14**.

Glucan was fully recovered in the concentrated acid extract. Glucan was also converted into monomeric sugar, i.e., glucose, into a large extent (45%) (**Fig. 2.10**). No HMF was detected in the extract, showing that no glucose was degraded during the reaction.

The yield closure of AIL reached 121% whereas the yield closure for ASL reached 89%. It can be assumed that the acidic media led to the precipitation of some phenolic monomers or small lignin oligomers accounted in ASL in the raw SCB but accounted in AIL after the acid extraction. The solid residue of the extraction had a very dark color (**Fig. 2.11**) and contained mainly AIL (63.4%) and salts (22.0%).

## Chapter 2: CHEMICAL FRACTIONATION

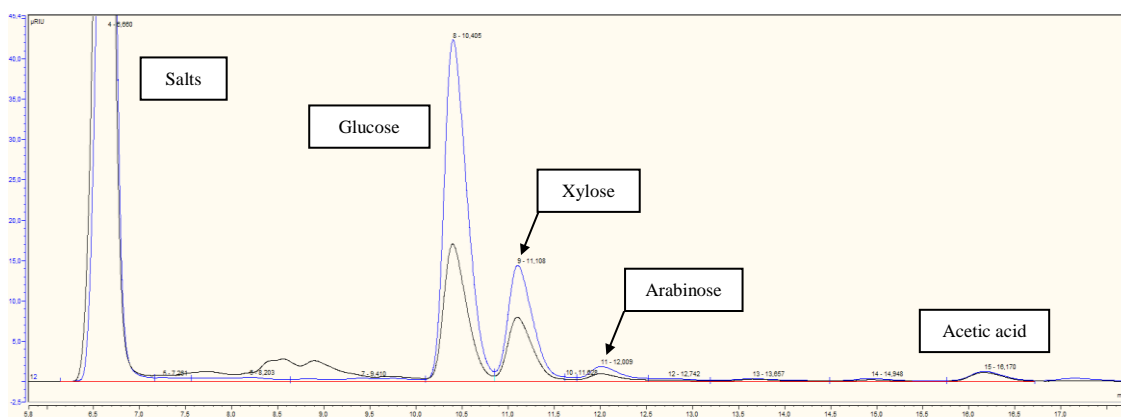
**Table 2.14**

SCB, solid residue, acid extract (4% H<sub>2</sub>SO<sub>4</sub> (w/w)) composition and extraction yields

Compounds	SCB	Solid residue		Acid extract		
	Content	Content	Yield	g/L	%	Yield
DS	92.5	90.5	40	NA		
Ash	9.9	22.0	88	NA		
Glucan (total)	35.9	1.2	1	1.04	64.2	105
Glucan (monomeric)		0.1	0	0.45	27.6	45
HMF				0.00	0.0	ND
Xylan (total)	19.4	0.6	1	0.39	24.0	73
Xylan (monomeric)				0.23	14.3	43
Arabinan (total)	2.3	0.1	4	0.04	2.7	67
Arabinan (monomeric)				0.03	1.9	49
Furfural eq. Xylose				0.05	3.1	9
AIL	21.6	63.4	117	0.02	1.5	4
ASL	5.5	1.3	10	0.12	7.5	79
Total lignin	27.1	64.8	95		0.0	
VA				0.0004	0.0	
4HBA				0.0006	0.0	
vanillin				ND	NA	
p-CA				0.0017	0.1	
FA				0.0002	0.0	
Total phenolic monomers				0.0029		
Mass closure	94.7	88.7		1.62	100	

All the content values are calculated based on the percentage of dry solid. All the analyses were run in triplicate, standard deviation was at most 1%.





**Fig. 2.10** HPLC chromatograms on Rezez RHM column of SCB concentrated acid extract, analyzed after only a pH adjustment to 2 (black line) and analyzed by NREL protocol on liquid fractions (blue line).



**Fig. 2.11** Solid residue of the SCB acid extraction after drying and grinding.

Acetic acid was used to correct xylan concentration as explained in NREL protocol (Sluiter et al., 2008) and was thus not reported directly in **Table 2.14**. Xylan and arabinan were recovered in the acid extract at 73% and 67%, respectively. 43% of the original xylan was converted into monomeric xylose, and 47% of the original arabinan was converted into monomeric arabinose. Some C5 sugars were converted into furfural, the equivalent of 9% of xylose was converted to furfural. In **Table 2.14**, in order to figure out the quantity of xylose transformed into furfural, the concentration of furfural was converted with Eq. (4) into a concentration equivalent to xylose (g/L), since one molecule of xylose is converted to one molecule of furfural.

$$C_{\text{Furfural eq. C5 sugar}} = \frac{C_{\text{Furfural}} \cdot M_{\text{Xylose}}}{M_{\text{Furfural}}} \quad (4)$$

With  $C_{\text{Furfural}}$  the concentration of furfural measured by HPLC (g/L),  $M_{\text{Xylose}}$  the molecular mass of xylose (150 g/mol) and  $M_{\text{Furfural}}$  the molecular mass of furfural (96 g/mol).

Even when furfural expressed in xylose equivalent is accounted in the yield of xylan (74% + 9%) the mass balance for xylose was still far from 100%. Furfural might have been degraded as well in formic acid and other degradation products (Girisuta et al., 2013), making the mass balance for xylose and arabinose unbalanced. The follow-up of formic acid would have been interesting.

Out of the twelve phenolic monomers analyzed, only four were detected: VA, 4HBA, *p*-CA and FA. Like for alkaline extract *p*-CA was the main phenolic monomer extracted with 1.7 mg/L followed by 4HBA (0.6 mg/L), VA (0.4 mg/L) and FA (0.2 mg/L). The proportionally lower content of FA in acid extract compare to alkaline extract is unexplained since concentrated acid should have broken ether bondage of FA with lignin, and FA has a higher solubility in acidic conditions than *p*-CA, meaning that it is less prone to precipitation in such media.

Composition of the extract before dilution (i.e., at 72% H<sub>2</sub>SO<sub>4</sub> (w/w)) was: 18.7 g/L glucan (8.0 g/L under monomeric form), 7.0 g/L xylan (4.2 g/L under monomeric form), 0.8 g/L arabinan (0.6 g/L under monomeric form), 0.4 g/L AIL, 2.2 g/L ASL, 0.6 g/L furfural, 1.1 g/L acetic acid, 6.5 mg/L VA, 11.2 mg/L 4HBA, 0.7 mg/L vanillin, 31.0 mg/L *p*-CA, 3.1 mg/L FA.

The concentration of acetic acid was higher after NREL protocol (66 mg/L) suggesting that a small fraction of acetic acid was still bound to xylose after the concentrated acid extraction. The HMF and furfural concentrations in the diluted acid extract increased to 12 mg/L and 55 mg/L, respectively, when the NREL protocol was applied compared to direct injection of the acid extract. It justified the necessity to analyze acid extract with direct injection otherwise the dilute acid hydrolysis (4% H<sub>2</sub>SO<sub>4</sub> (w/w) at 121 °C for 1 h) of NREL protocol increased the degradation of monomeric sugars and the formation of degradation furan degradation products.

### 2.4.4. Conclusion

In the selected conditions (72% H<sub>2</sub>SO<sub>4</sub> (w/w), S:L ratio of 1:20 (w/w), 50 °C, 1 h), concentrated acid extraction on SCB was efficient to fully solubilize cellulose and hemicelluloses. Cellulose was also converted to monomeric glucose in a large extent (45%), however, these conditions caused the degradation of C5 sugars into furfural, and thus decreased the yield of C5 sugars (73% for xylose and 67% for arabinose).

### 2.5. Conclusion

Electrostatic fractionation had little influence on the separation of SCB components but produced significantly protein-enriched and lignin-enriched fractions from SuOC. However, electrostatic fractionation led to limited improvements of compounds extraction on SuOC when it was submitted to mild alkaline extraction, since proteins were mainly extracted at the expense of lignin or hemicelluloses, only phenolic monomers extraction was improved. Mild alkaline extraction on SCB produced an extract composed of inorganic salts, lignin and hemicellulosic sugars oligomers, phenolic monomers and acetic acid, that could be interesting to purify. Concentrated acid extraction on SCB resulted in the total solubilization and the partial hydrolysis of cellulose and hemicelluloses, the degradation of hemicellulosic sugars whereas phenolic compounds were mostly unaffected.

The production of both monomeric sugars and polymeric sugars in a single step acid fractionation raised the issue of an extra purification step to separate them. A process combining concentrated and dilute acid treatment as presented in Chapter 1 and leading to a complete sugar polymer hydrolysis into monomers, seems more appropriate.

For application where sugar oligomers are of interest, alkaline treatment should be selected. Application where monomeric sugars are of interest (e.g., ethanol production), based on the results we obtained a single step concentrated acid extraction should be avoided, and a combined concentrated then dilute-acid treatment should be favored as detailed previously. In this case, alkaline extraction is also of interest due to the high yield of monomeric sugars after an enzymatic saccharification step on the solid residue and the opportunity to valorize the solubilized lignin.

## Chapter 3:

# PURIFICATION BY MEMBRANE FILTRATION

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### 3.1. Membrane filtration introduction

#### 3.1.1. General

The general principle is to separate a fluid (feed) containing molecules into two different streams using a membrane: the retentate, which is retained on the feed side of the membrane and enriched into large molecules and the permeate, which passes through the membrane and does not contain large molecules retained by the membrane. This process can be used to purify molecules by separating them from other molecules and/or to concentrate molecules in the retentate stream.

The size of the pores, i.e., molecular weight cut-off (MWCO) of the membrane, characterizes four types of filtration: microfiltration, ultrafiltration, nanofiltration and reverse osmosis (**Table 3.1**). The pore size of microfiltration is usually expressed in micrometers and ranges from about 0.1  $\mu\text{m}$  to 10  $\mu\text{m}$ , for ultrafiltration pore size unit commonly used is Dalton (Da) and it ranges from about 1 kDa to 500 kDa, nanofiltration pore size is expressed in Dalton or in terms of divalent ions rejection, e.g.,  $\text{MgSO}_4$ , and ranges from about 100 Da to 1 kDa, and reverse osmosis pore size is expressed by monovalent ions rejection, e.g., NaCl, and ranges about 70% rejection to 99.9% rejection.

**Table 3.1**

Typical characteristics of microfiltration, ultrafiltration, nanofiltration and reverse osmosis

	<b>Microfiltration</b>	<b>Ultrafiltration</b>	<b>Nanofiltration</b>	<b>Reverse Osmosis</b>
Retained molecules	Bacteria, yeast cells, enzymes suspended solid	Virus, proteins, macromolecules, colloidal silica	Multivalent salts, amino acids, sugars	Monovalent salts
Pore size ( $\mu\text{m}$ )	0.1 – 10 $\mu\text{m}$	0.005 – 0.1 $\mu\text{m}$	0.001 – 0.005 $\mu\text{m}$	< 0.001 $\mu\text{m}$
Pore size (Da)	> 500 kDa	1 – 500 kDa	100 – 1000 Da	< 100 Da
Pore size (ion rejection)			75 – 99% $\text{MgSO}_4$	75 – 99.9% NaCl
Range of pressure	0.5 – 3 bar	1 – 5 bar	5 – 20 bar	20 – 80 bar

Separation by membrane filtration is essentially based on size difference between molecules, i.e., sieving mechanism, but not only. Donnan exclusion, particularly on low

## Chapter 3: PURIFICATION BY MEMBRANE FILTRATION

MWCO membrane (nanofiltration and reverse osmosis), i.e., charged molecules are more retained than uncharged molecules, has been demonstrated on acetic acid with pH variation experiments (Liu et al., 2004; Weng et al., 2009, 2010). Moreover, hydrophobic interactions can also be involved in the separation mechanism during membrane filtration (Maartens et al., 2002; Liu et al., 2004).

### 3.1.2. Membranes

There are four configurations for the membrane: hollow fiber, spiral wound, flat sheet and tubular and they are made of two main types of material: organic and inorganic molecules. Inorganic membranes are made only in tubular configuration, they present the highest chemical and thermal resistance.

Tight channels induce a high compaction (filtration area by volume of the installation) and reduces the cost of the installation via less space occupied and smaller pump for equivalent shear rate (see below), but more open channels allow the filtration of viscous fluids and thus higher volume concentration factors (VCF) can be reached than with tight channels.

Hollow fiber, spiral wound and flat sheet membranes are made of organic polymers. The organic membranes can be homogeneous in their composition such as hollow fiber made of polysulfone (e.g., the membranes used in this work). They can also present a heterogeneous structure with a support usually made of polyester or polypropylene and a filtration layer in contact with the feed that has the effective MWCO of the membrane and which is as thin as possible to reduce the resistance of the membrane and thus increase the flux. This filtration layer can be made of various materials such as polysulfone, polyethersulfone, polyamide, polyvinylidene fluoride, cellulose acetate.

### 3.1.3. Transmembrane pressure

The separation with a membrane takes place under a gradient of pressure. The smaller the MWCO of the membrane, the higher the pressure to be applied on the membrane (**Table 3.1**).



The transmembrane pressure  $TMP$  is given by Eq. (5):

$$TMP = \frac{P_{inlet} + P_{outlet}}{2} - P_{permeate} \quad (5)$$

where  $P_{inlet}$  and  $P_{outlet}$  are the pressures (bar) at the inlet and the outlet of the membrane, and  $P_{permeate}$  the pressure on the permeate stream. No back pressure was applied on the permeate side in this work, so  $P_{permeate} = 0$ .

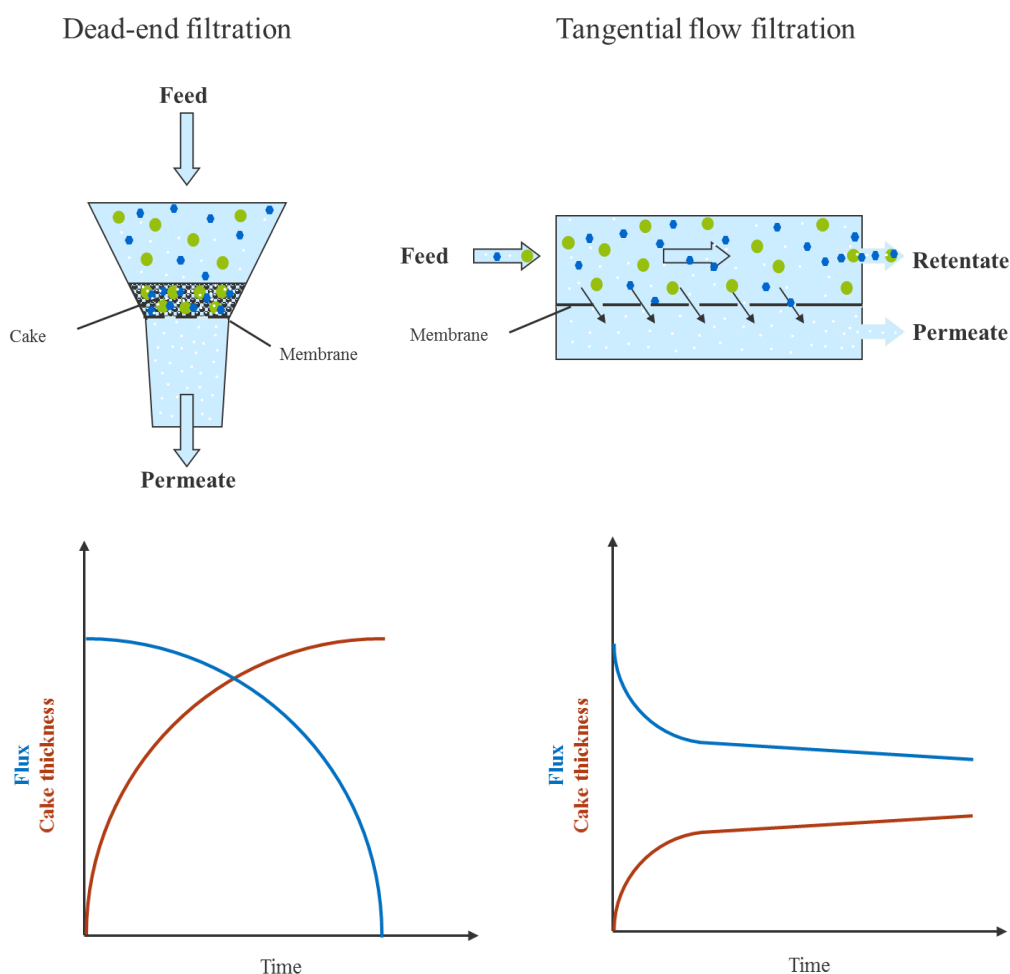
#### 3.1.4. Shear rate and cross-flow velocity

Dead-end filtration, e.g., filtration on a Buchner device or filter press, rapidly leads to the formation of a cake that slows down the flux through the membrane (**Fig. 3.1**). Shear rate and cross-flow velocity notions exist only with tangential flow filtration. Tangential flow filtration prevents the formation of a cake at the surface of the membrane because of the turbulence; its increase usually leads to higher flux and lower molecule retention.

For circular section (hollow fiber and tubular membranes), the shear rate  $\gamma$  ( $s^{-1}$ ) is given by Eq. (6):

$$\gamma = \frac{4Q}{nr^3} \quad (6)$$

where  $Q$  is the flow rate ( $m^3/s$ ),  $n$  is the number of channels or fibers and  $r$  is the radius (m) of a channel or fiber.



**Fig. 3.1** Principles of dead-end filtration and tangential flow filtration

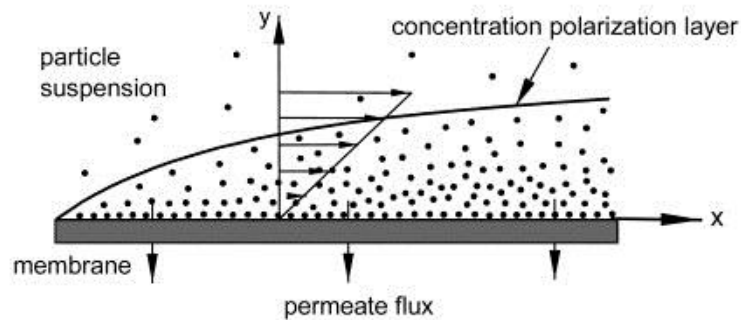
### 3.1.5. Fouling and cleaning

Fouling is a pollution of the membrane that occurs during the filtration, causing decrease in productivity (i.e., flux decreases over time during an experiment and from one experiment to another, see **Fig. 3.1**), modified selectivity and shorter membrane life span. Fouling can be prevented by pretreating the solution to filtrate, modifying the surface properties of the membrane or by optimization of the operational conditions. The later solution was studied in this work. However, fouling may occur anyway, various types of fouling have been identified, and cleaning procedures were adapted to solve them.

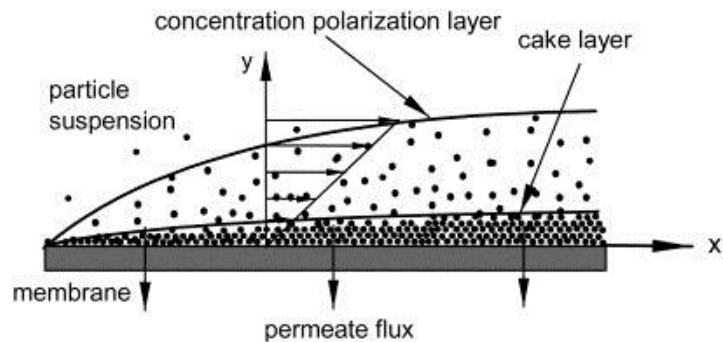
Concentration polarization is the result of the pressure gradient and flux through the membrane, small molecules pass through the membrane whereas larger molecules are

retained and their concentration increases close to the membrane surface, and can even reach 20-50 times their concentration in the bulk solution (Shi et al., 2014) (**Fig. 3.2**). This phenomenon is inevitable, but it is reversible with a reduction of the TMP and thus a reduction of the flux (Bacchin et al., 2006).

(a) Concentration Polarization ( $N_F < N_{F_c}$ )



(b) Cake Formation ( $N_F > N_{F_c}$ )



**Fig. 3.2** Schematic description of concentration polarization and cake formation over a membrane surface in crossflow filtration. (a) Below the critical filtration number,  $N_{F_c}$ , a pure concentration polarization layer exists. (b) Above the critical filtration number,  $N_{F_c}$ , particles accumulate and form a cake layer. Source: (Chen et al., 2004).

The other types of fouling may cause irreversible loss of flux and modification of selectivity of the membrane despite the appropriate cleaning procedure.

Concentration polarization triggers the cake formation, corresponding to the deposit of particles larger than the membrane pores growing progressively at the membrane surface and compressed with increasing pressure. Cake formation can be anticipated by the calculation of the critical number  $N_{F_c}$  that enables the calculation of a critical pressure above which the cake formation begins (**Fig. 3.2**).

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Particles close to the size of membrane pores can cause pore blocking by entering and being trapped in the membrane pores. This phenomena usually occurs at the beginning of the filtration of the feed when cake formation has not happened yet and the membrane pores are easily accessible (Shi et al., 2014).

Adsorption is another form of fouling occurring when bonds can be formed between the molecules from the feed and the membrane. Various interactions are responsible for these bonds such as van der Waals forces, electrostatic attraction and chemical linkage. A monolayer of adsorbed compounds can form a deposit at the membrane surface even without TMP.

Cost analysis of the membrane filtration process showed that the cost of the membrane, the membrane replacement frequency and the power are the major factors influencing a filtration unit (Owen et al., 1995). Increasing the cross flow velocity has a significant impact on the cost while an increase in TMP barely affects the overall cost of the unit (Owen et al., 1995). Increasing the cross flow velocity is required when fouling issues appear during the filtration but it is kept as low as possible to reduce the energy consumption of the process. Similarly, a TMP optimum has also to be set, and it is usually defined as the TMP before the critical flux is reached in order to maximize the flux while limiting the membrane fouling (Bacchin et al., 2006).

Membrane cleaning efficiency is assessed by the comparison of the initial water flux (IWF) measured on the new membrane and the water flux measured after the filtration of the solution to purify/concentrate, a rinsing step and a given cleaning procedure. It is commonly admitted that a membrane is cleaned when at least a recovery of 80% of the flux after the first cycle of production/rinsing/cleaning compare to the IWF is achieved. The cleaning procedure depends on the molecules responsible for the pollution on the membrane, general procedures are presented in **Table 3.2**. Other types of cleaning exist, mainly classified as physical cleaning such as reversing the TMP, known as backwashing. Physical cleaning can be efficient to remove cake at the membrane surface or particles blocking the pores, but not on fouling due to adsorption that requires chemical cleaning.

**Table 3.2**

Common cleaning agents and possible interactions between cleaning agents and foulants (Shi et al., 2014).

<b>Family</b>	<b>Examples</b>	<b>General functions</b>
Acids	Strong: HCl, HNO <sub>3</sub> Weak: H <sub>3</sub> PO <sub>4</sub> , Citric	pH regulation, dissolution of inorganic precipitates, acidic hydrolysis of certain macromolecules
Alkalis	Strong: NaOH, KOH Weak: Na <sub>2</sub> CO <sub>3</sub>	pH regulation, alteration of surface charges, alkaline hydrolysis of proteins, catalyzing saponification of fats
Oxidants	NaClO, H <sub>2</sub> O <sub>2</sub>	Oxidation of organics; disinfection
Surfactants	Anionic: SDS Cationic: CTAB Nonionic: Tween 20	Dispersion/suspension of deposits
Chelants	EDTA	Complexion with metals, removal of mineral deposits
Enzymes	Proteases, lipases	Catalyzing lysis of specific substrates (e.g., proteins, lipids)

### 3.2. Membrane screening and effect of filtration parameters

Vincent Oriez\*, Jérôme Peydecastaing, Pierre-Yves Pontalier\*

Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, 4 allée Emile Monso, 31030 Toulouse, France

\*Corresponding authors at: Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France

*E-mail addresses:* [vincentoriez@yahoo.fr](mailto:vincentoriez@yahoo.fr), [vincent.oriez@ensiacet.fr](mailto:vincent.oriez@ensiacet.fr) (V. Oriez), [pierreyves.pontalier@ensiacet.fr](mailto:pierreyves.pontalier@ensiacet.fr) (P.Y. Pontalier)

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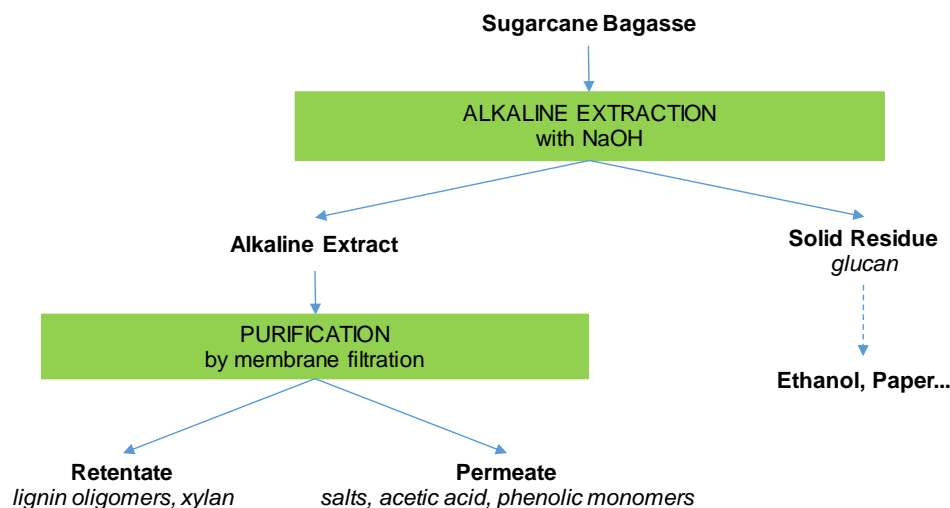
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#### Abstract

Mild alkaline treatment (1.5% NaOH solution, solid:liquid ratio of 1:20, 60 °C, 6 h) of sugarcane bagasse (SCB) extracts hemicelluloses, lignin, phenolic monomers and acetic acid. The purification of the resulting alkaline extract, usually considered a by-product, is of major importance to give value to the whole mild alkaline fractionation process. Ultrafiltration was assessed to separate the components of the alkaline extract. The permeate flux and the retention rates of the extract components were studied on seven membranes (polysulfone hollow fiber and ceramic tubular) with different molecular weight cut-offs, under various operating conditions. On all the membranes tested, oligomers of lignin and hemicelluloses were separated from salts, phenolic monomers and acetic acid. The 10 kDa polysulfone hollow fiber membrane presented the highest lignin and hemicelluloses retention, exceeding 85 and 90%, respectively, regardless of shear rate and with a limited influence of transmembrane pressure. For salts, acetic acid and phenolic monomers, retention levels of about 0-10% were recorded for this membrane. At 2.8 bar and at 20 °C, the permeate flux reached 16 L/h/m<sup>2</sup> and the critical flux was not reached.

Keywords: *Sugarcane bagasse fractionation; alkaline extraction; lignin; hemicelluloses; membrane filtration*

Graphical abstract



### 3.2.1. Introduction

Lignocellulosic or second generation biorefineries transform agricultural by-products or forest biomass into energy, various chemicals and materials (Huang et al., 2008). Most lignocellulosic biorefineries currently subject the raw material to pretreatment with a mineral acid, usually sulfuric acid, to produce monomeric sugars from the cellulose and the hemicelluloses, with lignin mostly recovered in the solid residue of this extraction. Another process gaining importance is the dissolution of the lignin and the hemicelluloses in mildly alkaline conditions, with the recovery of cellulose in the solid residue (Ragauskas et al., 2014). Mild alkali-based pretreatments originated in the pulp and paper industry, but use less harsh conditions. They yield higher delignification rates and higher total monomeric sugar rates after enzymatic saccharification of the cellulose than acid pretreatments (Saha et al., 2005b; Xu et al., 2016).

The separation/purification steps are of crucial importance in lignocellulosic biorefineries as they account for 60-80% of the production cost of the end products (Ragauskas et al., 2006). For lignocellulosic acid extracts, the monomeric sugars are purified by two chromatographic steps, the first involving the separation of sulfuric acid from the monomeric sugars (Heinonen and Sainio, 2010), and the second involving

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separation of the different sugars from each other (Chen et al., 2018). This process is already used industrially by several companies, including BlueFire Renewables (<https://bfireinc.com>).

For mild alkaline pretreatments, the cellulose remains in the solid residues and the alkaline extracts are considered to be the by-products of the biorefinery. Lignocellulosic alkaline extracts contain lignin and hemicelluloses in an oligomeric form, salts, acetic acid and phenolic monomers (Kim et al., 2016). The separation of these compounds and their further valorization would provide added value for the biorefineries using mild alkaline pretreatment processes. The purification of some of these compounds has been studied with the use of several different methods including acid precipitation (García et al., 2012), ethanol precipitation (Zeitoun et al., 2010), adsorption (Ou et al., 2007) and membrane filtration (Li et al., 2015), but none of these methods has yet been adopted by industry.

Membrane filtration is of particular interest because of its low levels of chemical and energy consumption (He et al., 2012). It can be performed on various streams of the lignocellulosic biorefinery: suspended solids can be retained by microfiltration (MF), or macromolecules, such as hemicelluloses and lignin, can be concentrated by ultrafiltration (UF) (Jönsson, 2013). Monosaccharides, low-molar mass lignin and phenolic monomers can be concentrated by nanofiltration (NF) and salts can be removed by reverse osmosis (RO) for water recycling.

Membrane filtration has been studied for the purification of hemicelluloses and lignin, mostly from the black liquors obtained in the strongly alkaline conditions used in the pulp and paper industry. Hemicelluloses retention rates of 69 to 81% have been achieved by the ultrafiltration of black liquor on inorganic membranes (Wallberg and Jönsson, 2006), and hemicelluloses retention rate of 70% was obtained with a spiral wound membrane following the pulp-steeping of viscose (Singh and Murthy, 2017). High hemicelluloses retention rates (over 90%) and intermediate lignin retention rates (30-50%) have been reported for pulp mill process water (Persson et al., 2010; Persson and Jönsson, 2010). Some studies have reported lignin retention rates of up to 75% (Liu et al., 2004) or 80% (Wallberg et al., 2003) with total recovery in the permeate of black liquor salts. Compare to the severe alkaline conditions employed in the pulp and paper industry, the mild



alkaline conditions studied in the second generation biorefinery produce a different extract, notably with shorter hemicelluloses and lignin oligomers (Sun et al., 2004; El Mansouri and Salvadó, 2006) or the presence of lignosulphonates and NaSH salts. Only a few studies have investigated the membrane filtration of lignocellulosic mild alkaline extracts (Toledano et al., 2010a; Zeitoun et al., 2010). However, these studies did not report retention rate for all the components typically found in lignocellulosic mild alkaline extracts and flux behavior was not discussed.

Retention rates and permeate flux (reflecting productivity) depend on the molecular weight cut-off (MWCO) and type of membrane, and the hydrodynamic conditions used. In studies on various hemicelluloses-lignin strong alkaline extracts, the best MWCOs for high rates of hemicelluloses and lignin retention associated with low rates of retention for smaller molecules were found to be in the range of 1 to 50 kDa (Wallberg and Jönsson, 2006; Singh and Murthy, 2017; Persson and Jönsson, 2010; Wallberg et al., 2003; Uloth and Wearing, 1989). Among the different membrane configurations, hollow fiber membranes present the best filtration area:volume ratio ( $\text{m}^2/\text{m}^3$ ) (Ladisich, 2001). Polysulfone (PS) is a common organic polymer used in hollow fiber membranes, with good mechanical, chemical and thermal stability (Scott, 1995). Conversely, ceramic membranes have a longer life span than organic membranes (Owen et al., 1995), are more resistant to temperature and chemicals, and back washing is possible to remove fouling (pore blocking and cake formation). PS hollow fiber and ceramic tubular membranes are both suitable for use with alkaline extracts at pH values of up to 13.

In terms of hydrodynamic conditions, shear rate can be increased by raising the flow rate (or cross-flow velocity or Reynolds number) and, thus, the energy consumption of the filtration unit. But the shear rate must be high enough to minimize the polarization layer and guarantee efficient cake removal, thereby maintaining a high permeate flux (Rossi et al., 2008). The transmembrane pressure (TMP) applied to the membrane must also be optimized. Increasing the TMP increases flux, thus increasing the productivity of the filtration unit, linearly at first, but the slope then becomes more shallow and a plateau known as the limiting flux is eventually reached, due to pore blocking, cake formation and/or increases in the polarization layer. The inflection point of the curve after its linear region is known as the critical flux, and increasing the TMP above this point is generally not economically favorable (Bacchin et al., 2006). Besides, increasing the TMP can also

affect the retention of molecules in various ways, either reducing retention due to higher solution diffusion through the membrane below the critical flux, or increasing retention due to membrane fouling above the critical flux (Persson and Jönsson, 2010).

The aim of this work was to investigate in detail the influence of operating conditions (TMP, shear rate, temperature) on the performances of PS hollow fiber and ceramic tubular membranes for separating all the components of a model lignocellulosic mild alkaline extract. Sugarcane bagasse was used for this study, as it is one of the most produced and studied lignocellulosic biomasses for second generation biorefineries worldwide.

### 3.2.2. Materials and methods

#### 3.2.2.1. Chemicals

Sodium hydroxide ( $\geq 98.5\%$  purity) for the alkaline extraction, sulfuric acid 72% for NREL hydrolysis, 95% sulfuric acid and acetonitrile ( $\geq 99.9\%$ ) for HPLC eluents were purchased from VWR, and calcium carbonate (NREL protocol) was obtained from Merck. The following HPLC standards were purchased from Sigma Aldrich: D-(+)-cellobiose ( $\geq 98\%$ ), D-(+)-glucose ( $\geq 99.5\%$ ), D-(+)-galactose ( $\geq 99\%$ ), L-(+)-arabinose ( $\geq 99\%$ ), D-(+)-xylose ( $\geq 99\%$ ), D-(–)-fructose ( $\geq 99\%$ ), acetic acid ( $\geq 99\%$ ), furfural (99%), 5-hydroxymethyl-2-furfuraldehyde (99%), gallic acid (97%), 4-hydroxybenzoic acid ( $\geq 99\%$ ), caffeic acid ( $\geq 98\%$ ), vanillic acid (97%), syringic acid ( $\geq 95\%$ ), 4-hydroxybenzaldehyde (98%), vanillin (99%), *p*-coumaric acid ( $\geq 98\%$ ), syringaldehyde (99%), *trans*-ferulic acid ( $\geq 99\%$ ), sinapic acid ( $\geq 98\%$ ), *trans*-3-hydroxycinnamic acid (99%).

#### 3.2.2.2. Alkaline extraction

The alkaline extraction was carried out on 3 kg of SCB with 60 L of 1.5% NaOH (w/v) in a stainless steel-lined vessel (De Dietrich), with a solid:liquid ratio of 1:20 (w/v) and a NaOH:SCB ratio of 1:3 (w/w), with continuous mechanical stirring (200 rpm) for 6 h at 60 °C. These are optimized conditions reported by Sun *et al.* (1995), for maximizing the recovery of hemicelluloses and lignin by the mild alkaline pretreatment of wheat straw (Sun *et al.*, 1995). The solid residue was removed from the alkaline extract using

a top-discharge vertical basket centrifuge (RC 50 PX R, Rousselet) equipped with a 5  $\mu\text{m}$  polypropylene bag. This residue was rinsed with distilled water, dried at 50 °C for 48 h and ground by microfine grinder (IKA MF 10 basic) on a 1 mm sieve before analysis. The filtered SCB alkaline extract and the filtered solution used to rinse the solid residue were mixed, analyzed and used as the feed for the membrane filtration experiments (mixture referred to hereafter as the SCB alkaline extract).

### 3.2.2.3. Membrane filtration

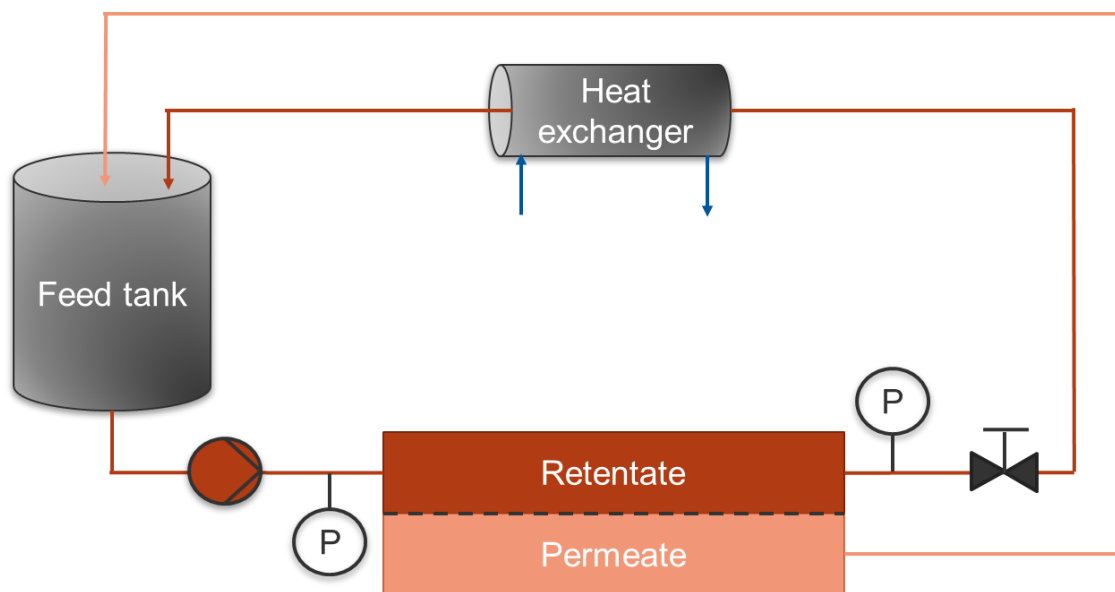
#### a) Set-up

Membrane filtration was carried out on the filtered SCB alkaline extract. Five new PS hollow fiber membranes (GE Healthcare) and two Kerasep ceramic tubular membranes (Novasep Process) were tested (**Table 3.3**). Reproducibility was assessed with another new 10 kDa PS hollow fiber membrane and a new 3 kDa PS hollow fiber membrane. The feed tank contained 5 L of water or filtered SCB alkaline extract. The feed was circulated in the membrane with a gear pump (Johnson Pump, model 10/0005). Feed flow was measured with a flowmeter (Rosemount, Mexico). Permeate flux was assessed by collecting permeate over a given time period and weighing the sample collected. TMP was set with a valve on the retentate stream and checked with two manometers (Tecsis), one on either side of the membrane. The temperature was maintained at 20 °C during the experiments, with a monotube heat exchanger located in the retentate flow. When the effect of temperature was studied, the feed tank was heated to 40 °C with a hot plate (Heidolph). Experiments were run in recycling mode, with both the retentate and the permeate recirculated to the feed tank (**Fig. 3.3**).

**Table 3.3**

Characteristics of the membranes used for the filtration of sugarcane bagasse mild alkaline extract

	<b>UFP-1-C- 4X2M</b>	<b>UFP-5-E- 4X2MA</b>	<b>UFP-10-E- 4X2MA</b>	<b>UFP-30-E- 4X2MA</b>	<b>UFP-50-E- 4X2MA</b>	<b>K1335</b>	<b>K927</b>
Module configuration	Hollow fiber	Hollow fiber	Hollow fiber	Hollow fiber	Hollow fiber	Tubular	Tubular
Membrane material	Polysulfone	Polysulfone	Polysulfone	Polysulfone	Polysulfone	ZrO <sub>2</sub> /TiO <sub>2</sub>	ZrO <sub>2</sub> /TiO <sub>2</sub>
Membrane area (cm <sup>2</sup> )	1400	850	850	850	850	75	75
Number of fibers/channels	140	50	50	50	50	1	1
Channel inner diameter (mm)	0.5	1	1	1	1	6	6
Molecular weight cut-off (kDa)	1	5	10	30	50	5	15
Range of feed pH	2-13	2-13	2-13	2-13	2-13	0-14	0-14
Range of feed temperature (°C)	0-80	0-80	0-80	0-80	0-80	0-80	0-80
Range of feed pressure (bar)	0-3.1	0-3.1	0-3.1	0-3.1	0-3.1	0-6	0-6
Initial water flux (L/h/m <sup>2</sup> /bar) at 20 °C	21.3	40.7	47.5	76.3	113.2	38.1	57.0



**Fig. 3.3** Set-up of the filtration system used for the screening of membranes for the filtration of sugarcane bagasse mild alkaline extract.

Before each experiment, the new membrane (PS hollow fiber membranes were stored in glycerol), was washed several times with an ethanol/water solution (1:1, v/v), rinsed with water and the initial water flux (IWF) was measured at 20 °C and various TMP values. Water was drained from the installation and the SCB alkaline extract was loaded into the feed tank and recirculated at a TMP of 0.8 bar until the flux was stable over time (about 15 min) and a quasi-stationary state was reached. Permeate flux was measured at different TMP values, from 0.8 to 2.8 bar, and different shear rates: 1966, 3408 and 4587 s<sup>-1</sup> for ceramic tubular membranes (corresponding to cross-flow velocities of 1.5, 2.6 and 3.4 m/s, respectively) and 3396, 6791 and 10,187 s<sup>-1</sup> for PS hollow fiber membranes (corresponding to cross-flow velocities of 0.4, 0.8 and 1.3 m/s, respectively). Nine permeate samples were collected for analysis at three different TMP values (0.8, 1.6 and 2.4 bar) and at the three shear rates tested. The feed volume was large enough relative to the total volume of permeates collected for analysis to assume that the composition of the SCB alkaline extract remained constant throughout each experiment. The final retentate was collected for analysis, to confirm that there had been no change in the composition of the SCB alkaline extract during the filtration process. At the end of the

experiment, the SCB alkaline extract was drained and the membrane was washed several times with water.

### b) Theoretical notions

In the publication, there was a reminder for the TMP and shear rate given by Eq. (5) and (6).

The rejection rate  $R$  is given by Eq. (7):

$$R = 1 - \frac{C_P}{C_R} \quad (7)$$

where  $C_P$  and  $C_R$  are the solute concentrations (g/L) in the permeate and the retentate streams, respectively.

### 3.2.2.4. Analytical methods

The following analytical methods were applied to the initial SCB, the extract obtained following alkaline pretreatment (to determine its composition and extraction yields), and the various permeates obtained during the filtration experiments (to assess retention rates for the various components of the SCB alkaline extract).

#### a) Dry solid and ash

Dry solid (DS) content was determined gravimetrically by heating at 103 °C for 12 h and ash content was determined at 500 °C for 12 h: 1 g was used for solid samples, 1 mL was used for alkaline extract and retentate samples, and 5 mL was used for permeate samples.

#### b) Carbohydrates and lignin

Based on Laboratory Analytical Procedure of the National Renewable Energy Laboratory (Sluiter et al., 2008), acid-insoluble lignin (AIL) was quantified gravimetrically and acid-soluble lignin (ASL) was determined spectrophotometrically, at a wavelength of 240 nm using an absorptivity constant of 25 L/g/cm. High-performance liquid chromatography (HPLC) on a Rezex RPM-Monosaccharide Pb<sup>+2</sup> 300 x 7.8 mm column (Phenomenex), used in conjunction with a Rezex RPM-Monosaccharide Pb<sup>+2</sup>

50 x 7.8 mm guard column (Phenomenex) was performed to quantify the cellobiose, glucose, xylose, galactose, arabinose, mannose and fructose released by the acidic hydrolysis of cellulose, hemicelluloses or residual sucrose. Before injection, the samples were filtered on an ABW solid phase extraction (SPE) cartridge (Phenomenex) to remove salts and prevent interference with the sugar peaks. Isocratic conditions were used with Milli-Q water at a flow rate of 0.6 mL/min; the injection volume was 20  $\mu$ L, the column was maintained at 80 °C and the RI detector was maintained at 50 °C. For the alkaline extract and purified samples, HPLC on a Rezex RHM-Monosaccharide H<sup>+</sup> 300 x 7.8 mm column (Phenomenex), used in conjunction with a Rezex RHM-Monosaccharide H<sup>+</sup> 50 x 7.8 mm guard column (Phenomenex) was performed to quantify glucose, xylose, arabinose, acetic acid, furfural and hydroxymethylfurfural (HMF) (Sluiter et al., 2006). The salts did not interfere with the sugar peaks on the RHM column, so, by contrast to the RPM column, no desalting of the samples was required before their injection. Isocratic conditions were applied, with 5 mmol/L H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.6 mL/min; the injection volume was 50  $\mu$ L, the column was maintained at 65 °C and the RI detector was maintained at 50 °C. The SCB alkaline extract and permeates collected were diluted by four-fold with distilled water before the NREL protocol.

#### c) Monomeric sugars and hemicelluloses acetyl groups

Sulfuric acid was added to the alkaline extract to adjust its pH to 2, corresponding to the pH of the RHM column HPLC eluent. The extract was then analyzed on the RHM column without running the NREL protocol. The direct injection of pH-adjusted samples onto the HPLC column made it possible to quantify monomeric sugars and free acetic acid, whereas the analysis of samples with the NREL protocol provided data for total sugars (monomeric and oligomeric forms) and acetic acid (free and bound to hemicelluloses).

#### d) Phenolic monomers

Twelve phenolic monomeric compounds potentially present in SCB alkaline extract (Xu et al., 2005; Capriotti et al., 2015) - gallic acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid (VA), syringic acid, 4-hydroxybenzaldehyde (4HBA), vanillin, *p*-CA, syringaldehyde, FA, sinapic acid and hydroxycinnamic acid – were quantified by HPLC

## Chapter 3: PURIFICATION BY MEMBRANE FILTRATION

on an OmniSpher 3 C18 100 x 4.6 column (Agilent Technologies). The gradient was as follows: 91% acidified water (1% acetic acid (v/v)) and 9% acetonitrile for 25 min, acetonitrile concentration increasing from 9 to 90% over 5 min, then kept constant for 5 min, before decreasing back to 91% acidified water over 5 min, with column equilibration for 7 min between runs. The flow rate was 0.5 mL/min, the injection volume was 10  $\mu$ L and the column temperature was maintained at 25 °C. The UV detector was set at 280 nm as it corresponds to a maximum in the absorbance of phenolic monomers such as *p*-CA and FA (Holser, 2014). The concentrations used to plot the calibration curves ranged from 0 to 200 mg/L. Standards and process samples were diluted in acetonitrile:water at a ratio of 50:50 (v/v) before injection.

### 3.2.3. Results and discussion

#### 3.2.3.1. Alkaline extraction

Glucan, xylan and arabinan were the only sugars detected in significant amounts in the SCB. The SCB alkaline extract contained no monomeric sugars (glucose, xylose, arabinose); all the extracted sugars were under oligomeric form. No sugar degradation products (furfural and HMF) were detected and the acetate groups bound to hemicelluloses were completely hydrolyzed in the alkaline extract. Five of the 12 phenolic monomers tested, (VA, 4HBA, vanillin, *p*-CA, FA) were present in detectable amounts in the alkaline extract.

After alkaline pretreatment, only very small amounts of glucan (3%) were recovered in the extract (**Table 3.4**). Xylan, arabinan, AIL and ASL were recovered at levels of 29, 59, 49 and 96%, respectively. Most of the salts (85%) were recovered in the alkaline extract.

The SCB alkaline extract used for all the membrane experiments consisted of six major pools of molecules: 19.4 g/L inorganic salts, 6.2 g/L AIL, 3.1 g/L ASL, 5.3 g/L oligomeric sugars (3.8 g/L xylan, 0.9 g/L arabinan, 0.6 g/L glucan), 1.5 g/L acetic acid and 1.3 g/L phenolic monomers.



**Table 3.4**

Initial sugarcane bagasse (SCB) composition, SCB mild alkaline extract composition and yield of the various components in the SCB mild alkaline extract

Components	SCB	Alkaline extract	
	Composition	Composition	Yield
Dry solid	92.5	3.4	49
Ash	9.9	56.0	85
Glucan	35.9	1.4	3
Xylan	19.4	8.8	29
Arabinan	2.3	2.2	59
Acid-insoluble lignin	21.6	16.6	49
Acid-soluble lignin	5.5	8.2	96
Total lignin	27.1	24.6	58
Vanillic acid		traces	
4-hydroxybenzaldehyde		0.1	
Vanillin		0.1	
<i>p</i> -coumaric acid		3.3	
Ferulic acid		0.4	
Total phenolic monomers		3.9	
Mass closure	94.7	97.0	

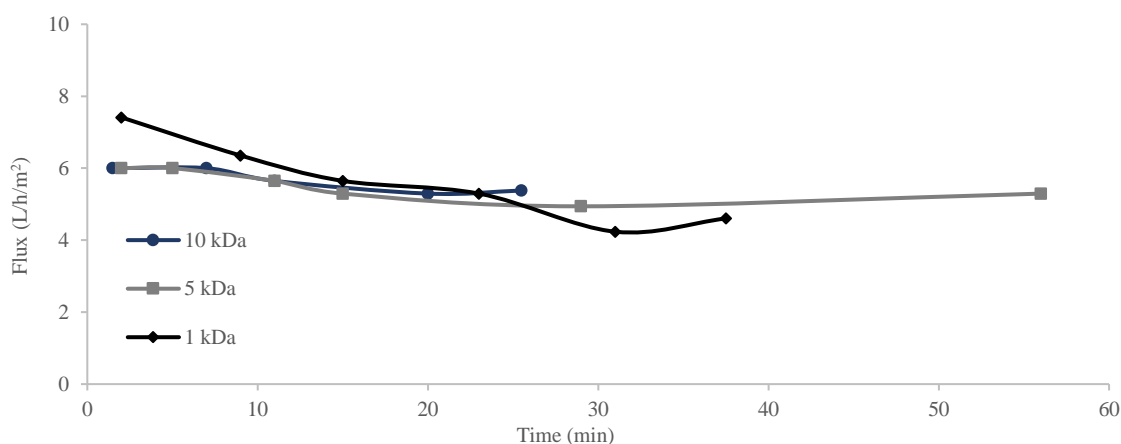
All these values were calculated as a percentage of dry solid. All analyses were run in duplicate.

### 3.2.3.2. Membrane filtration

The effects of MWCO, TMP, shear rate, temperature and the nature of the membrane on permeate flux and the rejection rates of the various compounds present in the SCB alkaline extract were evaluated in recycling mode at quasi-steady state. In most of the permeates analyzed, glucan and arabinan were barely detectable by HPLC. Xylan was, therefore, the only sugar oligomer studied in analyses of the permeate composition. Mass closure for each permeate composition was between 89 and 101%, so we can assume that the main compounds of the collected permeates were analyzed.

## a) Effect of time: quasi-stationary state study

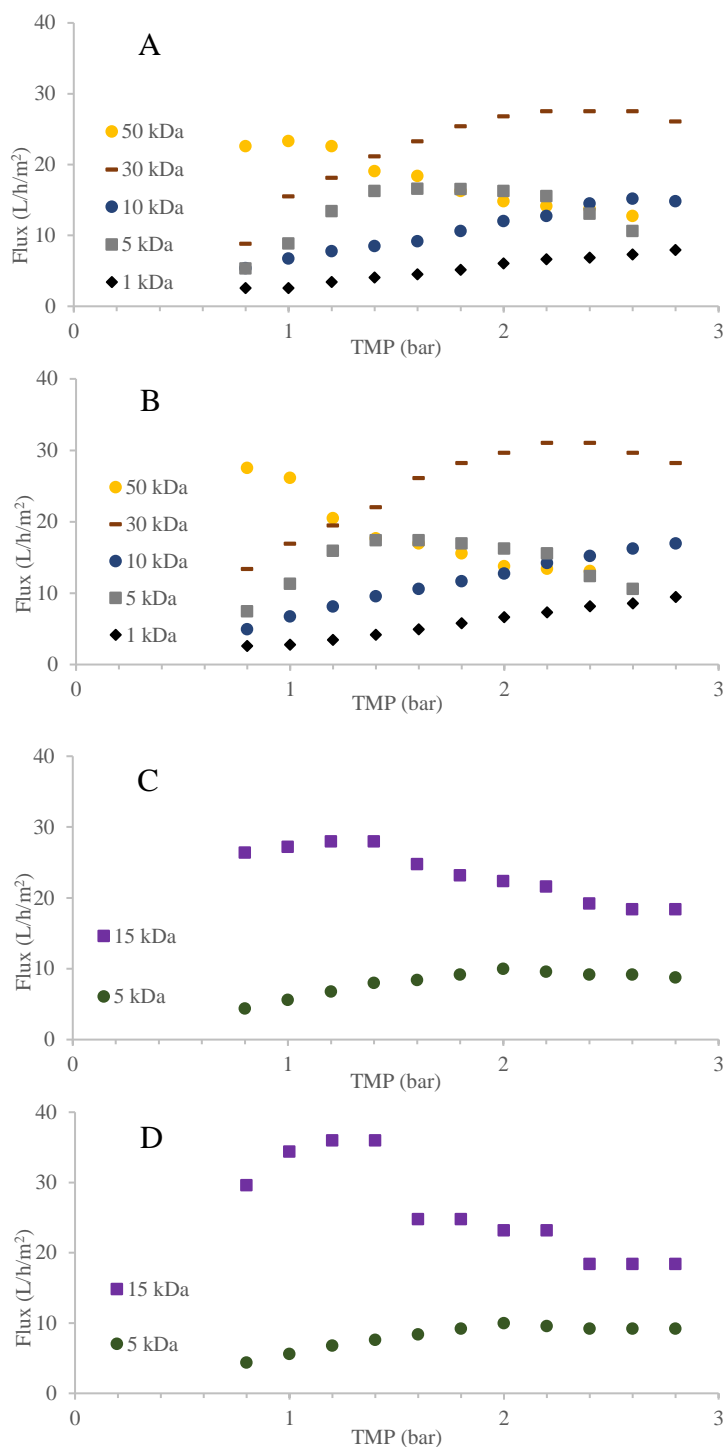
The effect of the experiment duration on the flux was investigated by recirculating the SCB alkaline extract at the lowest TMP (0.8 bar) and shear rate ( $3396 \text{ s}^{-1}$ ) (**Fig. 3.4**). On all three membranes tested, flux slowly decreased with a small change in magnitude, for instance, from 6 to  $5 \text{ L/h/m}^2$  after 1 h for the 5 kDa PS hollow fiber membrane. Zeitoun *et al.* (2010) also observed a very small decrease in flux over time during the filtration of a wheat bran mild alkaline extract with a 30 kDa PS hollow fiber membrane from the same supplier but with a 0.5 mm fiber lumen (Zeitoun *et al.*, 2010).



**Fig. 3.4** Changes in permeate flux over time during the filtration of the sugarcane bagasse mild alkaline extract at 0.8 bar and  $3396 \text{ s}^{-1}$  on three polysulfone hollow fiber membranes.

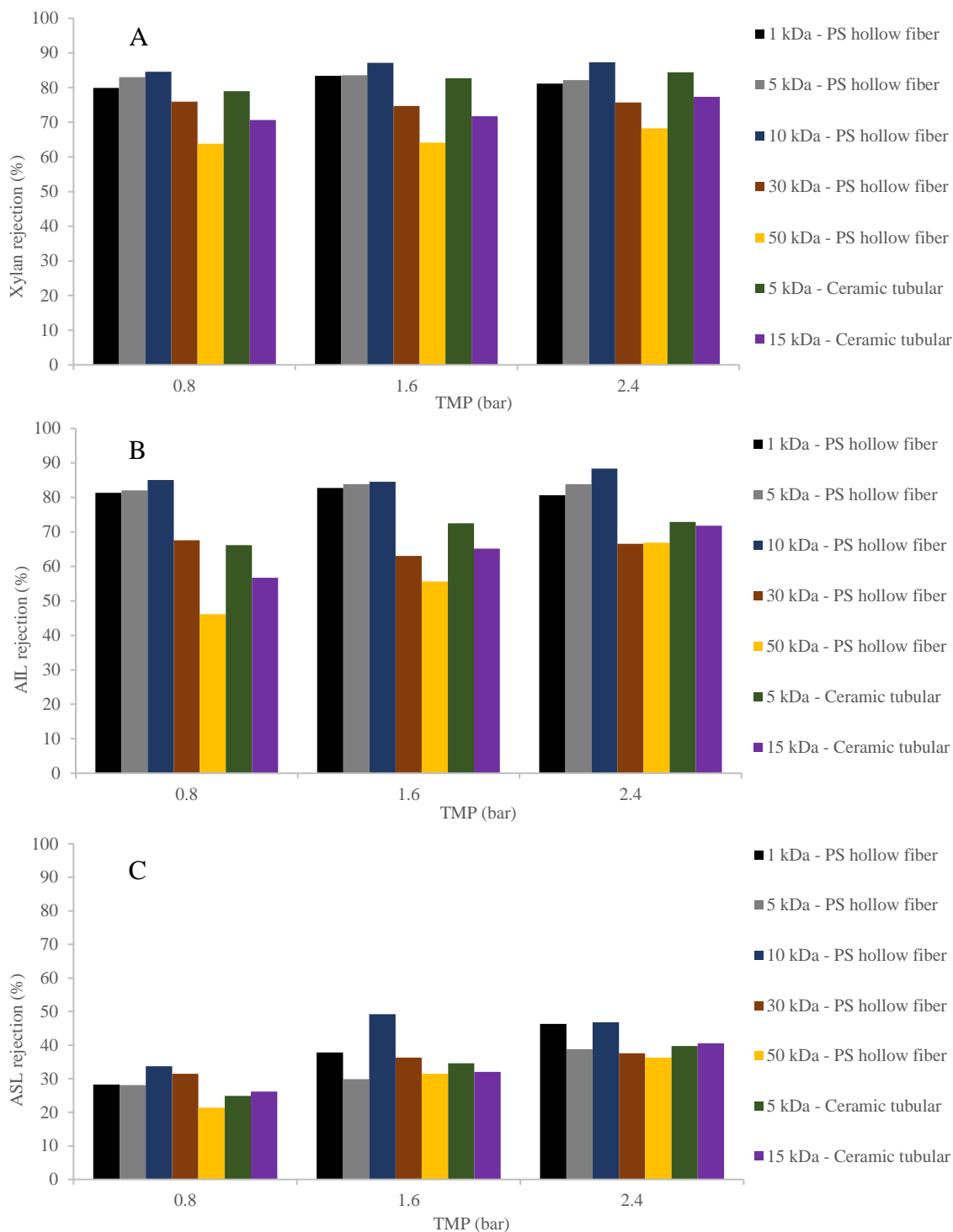
## b) Effect of MWCO

IWF increased with MWCO, from 21 to  $113 \text{ L/h/m}^2$  for the PS hollow fiber membranes, confirming that, for a given type of membrane, higher MWCO is associated with a higher water flux (**Table 3.3**). A similar trend was observed when SCB alkaline extract was recirculated at low TMP and low shear rate (**Fig. 3.5A**). At 0.8 bar and  $3396 \text{ s}^{-1}$ , the flux gradually increased with increasing MWCO for the PS hollow fiber membranes, from  $3 \text{ L/h/m}^2$  for the 1 kDa membrane to  $28 \text{ L/h/m}^2$  for the 50 kDa membrane. Only the 5 kDa membrane behaved differently with a flux similar to that for the 10 kDa membrane.

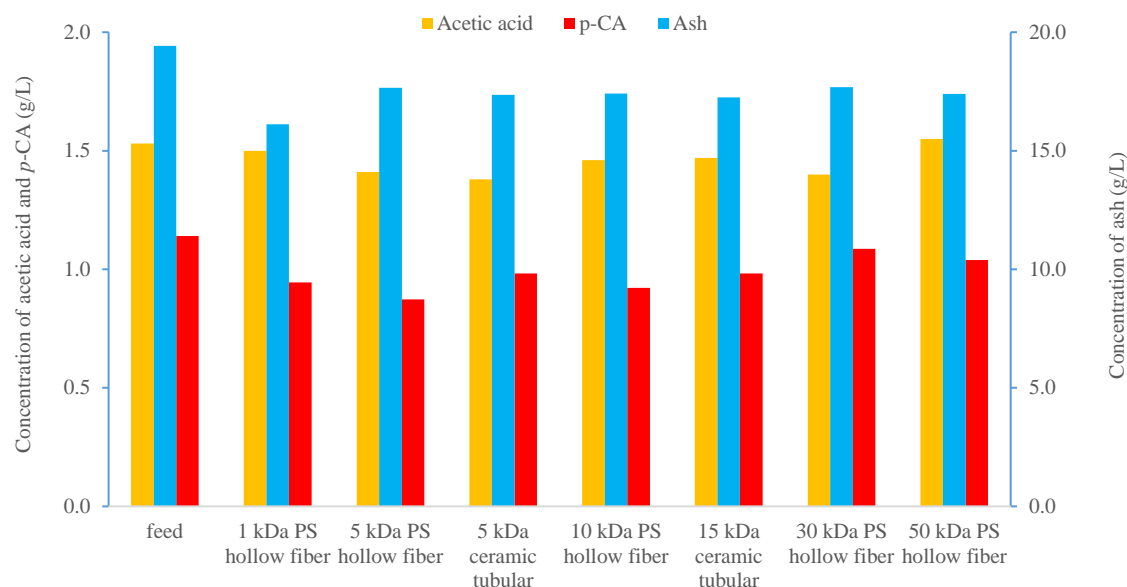


**Fig. 3.5** Influence of the molecular weight cut-off, transmembrane pressure (TMP) and shear rate on the permeate flux during the filtration of sugarcane bagasse mild alkaline extract. (A) Polysulfone hollow fiber membranes with a shear rate of 3396 s<sup>-1</sup>, (B) polysulfone hollow fiber membranes with a shear rate of 10,187 s<sup>-1</sup>, (C) ceramic tubular membranes with a shear rate of 1966 s<sup>-1</sup>, (D) ceramic tubular membranes with a shear rate of 3408 s<sup>-1</sup>.

On PS hollow fiber membranes, at 0.8 bar and  $3396\text{ s}^{-1}$ , the rejection rates xylans, AIL and the ASL increased from 1 kDa to 10 kDa then decreased from 10 kDa to 50 kDa (**Fig. 3.6**). The highest rejection rates were recorded for the 10 kDa PS hollow fiber membrane with values of 85%, 86% and 36% obtained for xylan, AIL and ASL, respectively. The change in ASL rejection rates with MWCO was less marked than those for xylans and AIL, probably because phenolic monomers, which are accounted for in ASL (and also followed via HPLC on C18 columns) pass through all the membranes (**Fig. 3.7**, only data for *p*-CA are presented). The concentration of *p*-CA in the various permeates ranged from 0.9 to 1.1 g/L, whereas that in the retentate/feed was 1.1 g/L. Similar trends were observed for vanillic acid, vanillin, 4HBA and FA. Likewise, acetic acid and ash concentrations did not differ significantly between the retentate and the various permeates, resulting in a rejection rate close to 0%.



**Fig. 3.6** Effect, during the filtration of sugarcane bagasse mild alkaline extract, of the molecular weight cut-off, the transmembrane pressure (TMP) and the nature of the membrane on the rejection rates of (A) xylans, (B) acid insoluble lignin (AIL) and (C) acid soluble lignin (ASL). Shear rate of  $3396 \text{ s}^{-1}$  for PS hollow fiber membranes and  $3408 \text{ s}^{-1}$  for ceramic tubular membranes.



**Fig. 3.7** Concentration of acetic acid, *p*-coumaric acid (*p*-CA) and ash in the feed and permeate of the seven membranes tested at 0.8 bar and shear rates of  $3396 \text{ s}^{-1}$  and  $3408 \text{ s}^{-1}$  for PS hollow fiber and ceramic membranes, respectively. The same trend was observed for vanillic acid, 4-hydroxybenzaldehyde, vanillin and ferulic acid but these molecules are not presented on this graph for the sake of clarity.

With the MWCO values tested, it was possible to separate xylans and AIL from phenolic monomers, acetic acid and ash, whereas ASL was moderately retained by the membranes. The rejections rates of both xylans and AIL decreased with increasing MWCO (**Fig. 3.6A&B**), from 85 and 86% on the 10 kDa PS hollow fiber membrane to 64 and 46% on the 50 kDa PS hollow fiber membrane, respectively. Xylans and AIL from SCB alkaline extract could not be separated by filtration through PS hollow fiber membranes under the conditions tested.

### c) Effect of TMP

On the 1 and 10 kDa PS hollow fiber membranes, permeate flux increased linearly with TMP (**Fig. 3.5**). On the 1 kDa PS hollow fiber membrane with a shear rate of  $3396 \text{ s}^{-1}$ , flux increased from  $3 \text{ L/h/m}^2$  at 0.8 bar to  $8 \text{ L/h/m}^2$  at 2.8 bar. With 10 kDa PS hollow fiber membranes at the same shear rate, flux displayed the same linear behavior but with a steeper slope, increasing from  $5 \text{ L/h/m}^2$  at 0.8 bar to  $15 \text{ L/h/m}^2$  at 2.8 bar. The linear

increase in flux with TMP for these two membranes showed that the critical flux was not reached in the TMP range tested.

On the 5 kDa PS hollow fiber membrane, flux increased linearly with TMP up to 1.4 bar, at which the critical flux ( $16 \text{ L/h/m}^2$ ) was reached. Critical flux and limiting flux merged into the same point on this membrane. For filtration with 30 kDa PS hollow fiber membrane, the critical flux ( $25 \text{ L/h/m}^2$ ) was reached at 1.8 bar and the limiting flux ( $28 \text{ L/h/m}^2$ ) at 2.2 bar. With the 50 kDa PS hollow fiber membrane, flux steadily decreased with increasing TMP. Rossi *et al.* (2008) also reported a decrease in flux with increasing TMP during the ultrafiltration of microalgae, probably due to polysaccharides retention, resulting in membrane fouling (Rossi *et al.*, 2008). Here, the retention of xylans or AIL were most likely accounted for the decrease in flux with increasing TMP for the PS hollow fiber membranes with higher MWCO. The limiting flux was reached within the range of TMPs used in the experiment, and the critical and limiting fluxes must therefore have occurred at TMP values below 0.8 bar. With the filtration system used, it was not possible to have a TMP lower than 0.8 bar at the shear rates tested, so it was not possible to check the critical point on this membrane.

During this study, critical fluxes appeared at different TMP values for PS hollow fiber membranes differing only in terms of their MWCO values (under critical flux at 2.8 bar for 1 and 10 kDa membranes, critical flux at 1.4 bar for the 5 kDa membrane critical flux, at 1.8 bar for the 30 kDa membrane, and over critical flux and even limiting flux at 0.8 bar for the 50 kDa membrane). TMP critical flux decreased with increasing MWCO for all the membranes other than the 5 kDa PS hollow fiber membrane. In another study, Wu *et al.* (1999) observed a similar phenomenon when a colloidal silica suspension was filtered on polyethersulfone flat sheet membranes with an MWCO of 50 or, 100 kDa and a PS flat sheet membrane with an MWCO of  $0.2 \mu\text{m}$  (Wu *et al.*, 1999).

An increase in TMP from 0.8 to 2.4 bar at a shear rate of  $3396 \text{ s}^{-1}$ , resulted in a slight increase in the xylan rejection rate for all membranes tested, for instance, from 85 to 87% for the 10 kDa PS hollow fiber membrane and from 64 to 68% for the 50 kDa PS hollow fiber membrane (**Fig. 3.6A**). Persson and Jönsson (2010) observed that the rejection rate for hemicelluloses decreased with increasing TMP below the critical flux, but increased with increasing TMP above the critical flux, during the filtration of pulp mill process

water with 1 and 10 kDa polyvinylidene fluoride spiral-wound membrane (Persson and Jönsson, 2010). In this study, the rejection rate for xylans increased with increasing TMP independently of critical flux.

Increase in TMP led to a moderate increase in AIL retention for all PS hollow fiber membranes except for the 50 kDa membrane, where AIL rejection rate varied more significantly from 46% at 0.8 bar to 67% at 2.4 bar (**Fig. 3.6B**). The increase in ASL retention rates with TMP was clearly observed for all the PS hollow fiber membranes (**Fig. 3.6C**). Slightly higher retention rates for small molecules (inorganic salts, acetic acid, phenolic monomers) were also observed, but these retention rates did not exceeded 20%.

Overall, the change in rejection rates with TMP was lower for PS hollow fiber membranes with smaller MWCOs (1, 5 and 10 kDa) than for those with larger MWCOs (30 and 50 kDa). As for the lowest TMP (0.8 bar), the 10 kDa PS hollow fiber membrane presented the highest rejection rate for xylans (87%) and AIL (88%) at the highest TMP (2.4 bar).

#### d) Effect of shear rate

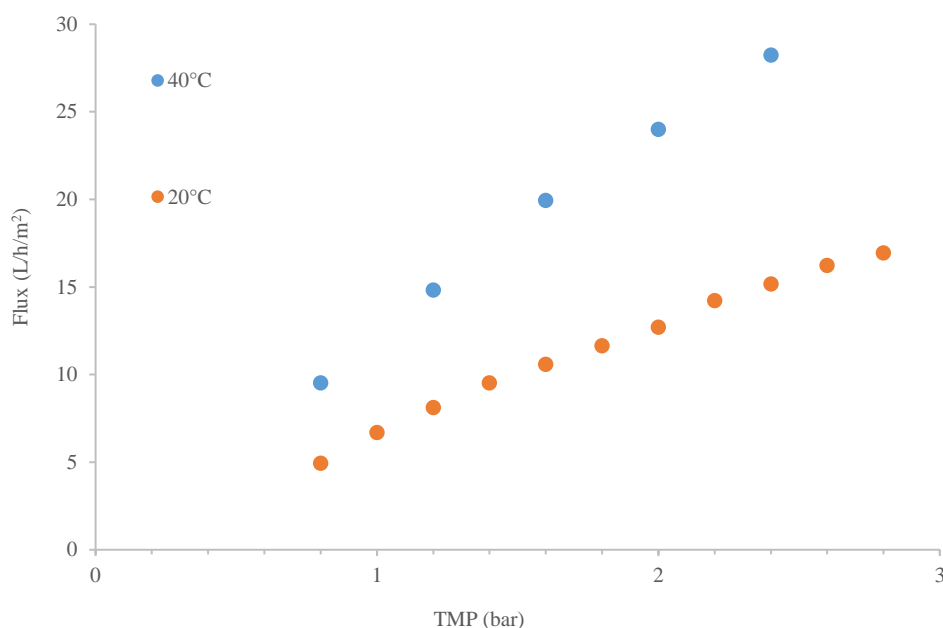
For the three shear rates tested, fluxes increased on all the PS hollow fiber membranes, but only to a limited extent (**Fig. 3.5A&B**, only the two extreme shear rates are presented). On the 1 kDa and 10 kDa PS hollow fiber membranes, a three-fold increase in shear rate led to an increase of about 10% in flux increase on the TMP range tested. Behavior was different on the other PS hollow fiber membranes. On the 5 kDa PS hollow fiber membrane, the highest shear rate had a positive impact on flux but only below the critical point at 1.2 bar. On this membrane, for the three shear rates tested (**Fig. 3.5A&B**), the limiting flux was 17 L/h/m<sup>2</sup>, and the flux began to decrease at 2 bar. On the 30 kDa PS hollow fiber membrane, the opposite effect was observed. Shear rate variations had no impact below the critical point, but above it, the lowest shear rate was associated with a lower flux. On the 50 kDa PS hollow fiber membrane, the highest shear rate increased the flux from 23 to 28 L/h/m<sup>2</sup> at the beginning of the TMP range, but at 2.6 bar, flux had fallen to 13 L/h/m<sup>2</sup> for all shear rates. Overall, a large increase in shear rate, from 3396 to 10,187 s<sup>-1</sup>, triggered a limited increase in flux on all the PS hollow fiber membranes



tested. A low shear rate may be sufficient to guarantee slow cake formation and/or minimization of polarization layer during the filtration. Shear rate had no significant effect on the rejection rates of the molecules on any of the PS hollow fiber membranes.

e) Effect of temperature

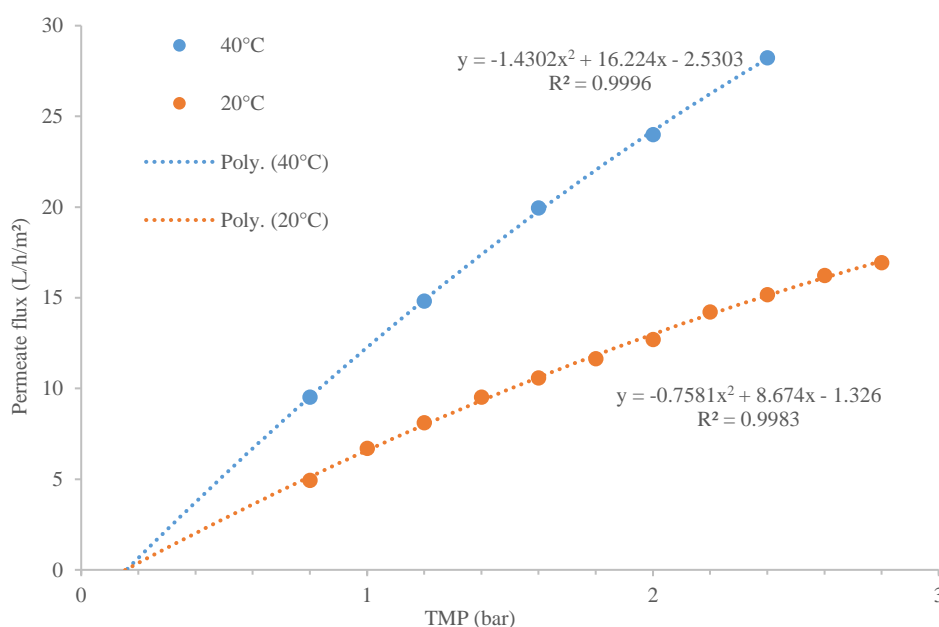
Two temperatures (20 and 40 °C) were tested on the 10 kDa PS hollow fiber membrane at a shear rate of  $10,187 \text{ s}^{-1}$  (**Fig. 3.8**). At both temperatures, the retention rates for molecules passing through the membrane (inorganic salts, acetic acid, phenolic monomers) remained close to 0%. The increase in temperature (+ 20 °C) led to a doubling of the flux, for instance, from 5 to 10 L/h/m<sup>2</sup> at 0.8 bar and from 15 to 28 L/h/m<sup>2</sup> at 2.4 bar. In parallel, the retention rates of xylans and AIL decreased with increasing temperature, from 87 to 81% and from 88 to 79%, respectively. The decrease in the viscosity of the SCB alkaline extract and the dilation of the pores of the membrane may account for the increase in flux and the lower retention of large molecules at high temperature.



**Fig. 3.8** Change in permeate flux evolution with transmembrane pressure (TMP) during the filtration of sugarcane bagasse mild alkaline extract on 10 kDa polysulfone hollow fiber membrane, with a shear rate of  $10,187 \text{ s}^{-1}$ , and temperatures of 20 °C and 40 °C.

These reflections were not part of the publication, but we assessed they were worth being reported here. An increase in temperature led to a lower viscosity of the solution which increased the flux. This modification should be linear if the comportment of the system was Newtonian. This result confirms that even for the 10 kDa membrane, a fouling layer could have been created modifying membrane performances.

The evolution of the flux with the TMP did not look linear but seemed to follow a polynomial equation of the 2<sup>nd</sup> order (**Fig. 3.9**). The extrapolations of the curves showed that the flux is 0 when the TMP is 0.16 bar that might correspond to the osmotic pressure of the SCB alkaline solution on the 10 kDa PS hollow fiber membrane.



**Fig. 3.9** Permeate flux evolution with TMP at 10,187 s<sup>-1</sup> shear rate at 20 °C and 40 °C.  $y=0$  when  $x=0.16$  for  $y=-0.76x^2+8.67x-1.33$  (20 °C).  $y=0$  when  $x=0.16$  for  $y=-1.43x^2+16.22x-2.53$  (40 °C).

f) Effect of the nature of the membrane

Membranes made of different materials and with different configurations presented similar IWFs for the same MWCO. IWF was 38 L/h/m<sup>2</sup>/bar for the 5 kDa ceramic tubular membrane and 41 L/h/m<sup>2</sup>/bar for the 5 kDa PS hollow fiber membrane (**Table 3.3**). The IWF for the 15 kDa ceramic tubular membrane, 57 L/h/m<sup>2</sup>/bar, was intermediate between

the IWF values for the 10 and 30 kDa PS hollow fiber membranes (48 and 76 L/h/m<sup>2</sup>/bar, respectively).

During filtration of the SCB alkaline extract, change in flux with TMP for the ceramic tubular membranes (**Fig. 3.5C&D**) was similar to that for PS hollow fiber membranes (**Fig. 3.5A&B**). The 5 kDa ceramic tubular membrane behaved similarly to the 5 kDa PS hollow fiber membrane, with a linear increase in flux with TMP up to a critical flux merging with the limiting flux, although the critical flux was lower (10 L/h/m<sup>2</sup>) and was reached at a higher TMP (2.0 bar) for the 5 kDa ceramic tubular membrane. The shear rates tested did not affect flux on this membrane. The 15 kDa ceramic tubular membrane had a similar flux versus TMP profile to the 50 kDa PS hollow fiber membrane. At the lowest shear rate (1966 s<sup>-1</sup>) and TMP (0.8 bar), the critical flux had already been reached and the limiting flux (28 L/h/m<sup>2</sup>) was reached at 1.4 bar. On this membrane, an increase in shear rate had a significant impact on the flux before the limiting flux, as limiting fluxes were 28, 36 and 48 L/h/m<sup>2</sup> for shear rates of 1966, 3408 and 4587 s<sup>-1</sup> shear rates, respectively. For TMP values above that at which the limiting flux was achieved, shear rate had no influence on flux. Membranes of different composition and configuration yielded similar results in terms of the effects of MWCO, TMP and shear rate on flux.

For xylans, the 5 kDa ceramic tubular membrane and 5 kDa PS hollow fiber membrane had similar rejection rates, ranging from 79 to 84% at 0.8 and 2.4 bar, respectively (**Fig. 3.6A**). On the 15 kDa ceramic tubular membrane, xylan rejection rates (71-77%) were lower and close to the values observed for the 30 kDa PS hollow fiber membrane (**Fig. 3.6A**). As for PS hollow fiber membranes, xylan rejection rate on the ceramic tubular membranes increased significantly with MWCO and TMP, but was not influenced by shear rate.

AIL rejection rates were significantly lower on the ceramic tubular membranes than on the PS hollow fiber membranes. For instance, on the 5 kDa ceramic tubular membrane, the AIL rejection rate was between 66 and 73% depending on TMP, whereas rejection rates of 82-84% were obtained with the 5 kDa PS hollow fiber membrane. Ceramic materials are less hydrophobic than PS, potentially accounting for the lower levels of the hydrophobic AIL by ceramic membranes and the potential adsorption of AIL on the PS

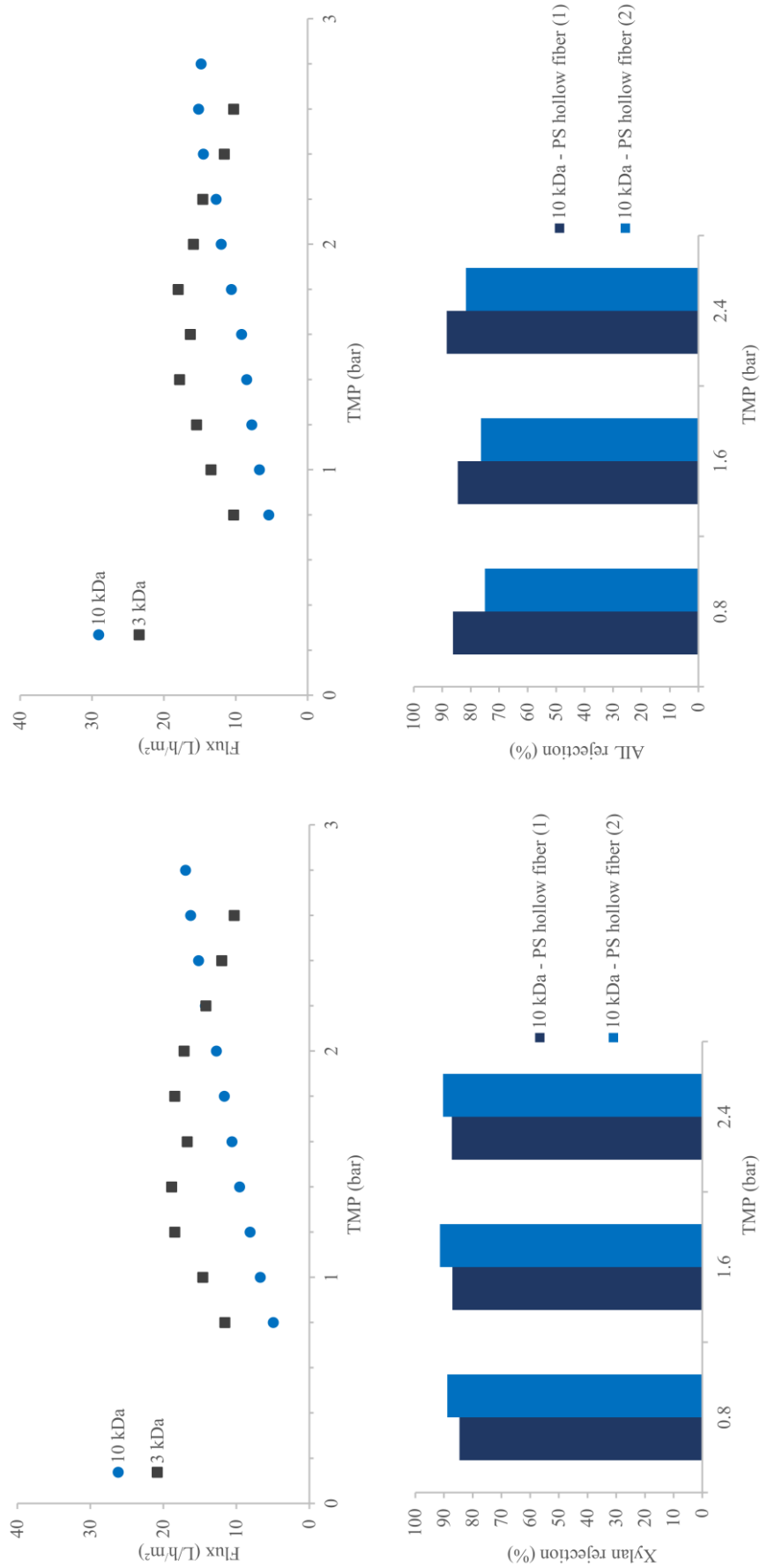
membranes. Inorganic salts, acetic acid and phenolic monomers were not retained on the ceramic tubular membranes either (**Fig. 3.7**).

As observed for PS hollow fiber membranes, an increase in PTM led to an increase in rejection rates for xylans, AIL and ASL on the ceramic tubular membranes, and shear rate had no significant effect on the rejection of SCB alkaline extract components.

### g) Repeatability study

Repeatability is often assessed on the same membrane, with successive filtration runs. The flux usually decrease over the filtration experiments, whereas the retention rates of the compounds gradually increase (Singh and Murthy, 2017). In this study, the repeatability test was run on a new 10 kDa PS hollow fiber membrane, to determine whether the selectivity and flux of the membrane presenting the best separation performances could be extrapolated to another new membrane. The two 10 kDa PS hollow fiber membranes presented similar linear flux behavior, with a slightly lower flux on the new membrane, with increases from 3 L/h/m<sup>2</sup> at 0.8 bar to 16 L/h/m<sup>2</sup> at 2.8 bar at 10,187 s<sup>-1</sup> (**Fig. 3.10**). Rejection rates for the various compounds were also similar in the different conditions tested, values of 91% for xylans and 82% for AIL at 2.8 bar on the second membrane tested (**Fig. 3.10**).

Concerning the different flux versus TMP profiles of the 5 kDa PS hollow fiber membrane, and the 1 and 10 kDa PS hollow fiber membranes, changes in flux with TMP were assessed on a new 3 kDa PS hollow fiber membrane from the same supplier. The same flux behavior as reported for the 5 kDa membrane was observed, with merging critical and limiting fluxes (18 L/h/m<sup>2</sup> at low shear rate and 19 L/h/m<sup>2</sup> at high shear rate) reached at 1.4 bar, regardless of shear rate (**Fig. 3.10**). The difference in flux behavior on these two membranes relative to the 1 and 10 kDa PS hollow fiber membranes showed that the results obtained for a given MWCO cannot be extrapolated to other membranes of the same type with different MWCO values for the filtration of SCB alkaline extract.



**Fig. 3.10** Repeatability study on new 3 and 10 kDa polysulfone hollow fiber membranes for the filtration of sugarcane bagasse mild alkaline extract. (A) Flux vs transmembrane pressure (TMP) profile at a shear rate of 3,396 s<sup>-1</sup>, (B) flux vs. TMP profile at a shear rate of 10,187 s<sup>-1</sup>, (C) xylan rejection rate on the two new 10 kDa polysulfone (PS) hollow fiber membranes, (D) acid-insoluble lignin (AIL) rejection rate on the two new 10 kDa polysulfone hollow fiber membranes.

### 3.2.4. Conclusion

During the ultrafiltration of SCB alkaline extract, xylans and AIL were retained in the retentate to different extents, depending on the membrane used, whereas almost no retention of salts, acetic acid and phenolic monomers (vanillic acid, 4HBA, vanillin, *p*-CA, FA) was observed. Sugar oligomers and lignin oligomers from SCB alkaline extract cannot be separated by membrane filtration. The retention of these large molecules was not completely inversely proportional to the MWCO of PS hollow fiber membranes. A maximum retention rate close to 90% was obtained for xylans and AIL from a SCB alkaline extract with 10 kDa PS hollow fiber membrane. At the maximum TMP tested (2.8 bar), the critical flux had not yet been reached, and a permeate flux of 16 L/h/m<sup>2</sup> was achieved at 20 °C.

Filtration in concentration mode of the sugars and lignin oligomers on this membrane should lead to high recoveries of these compounds and to the removal of inorganic salts, acetic acid, phenolic monomers and ASL to a lesser extent. The concentration of the retained compounds in the retentate would probably lead to higher rates of retention rates due to the build-up of a cake and/or the formation of a polarization layer at the surface of the membrane. Membrane filtration presents the advantage of not only separating molecules, but also concentrating the retentate flow which is particularly useful if the next step in the purification process requires a concentrated feed, as is the cases for chromatography or precipitation. By performing ultrafiltration before selective precipitation, it would be possible to obtain pure hemicelluloses or lignin fractions (free of salts, phenolic monomers, acetic acid), a key advantage given that the main concern raised about selective precipitation on lignocellulosic alkaline extracts is the low purity of the supernatant and the precipitate. Diafiltration could be investigated as a way of eliminating additional salts, acetic acid and phenolic monomers, if a higher purity of retained compounds is required.

### Acknowledgments

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### 3.3. Membrane filtration in concentration and diafiltration mode

#### 3.3.1. Introduction

The membrane screening and the study of the effect of the filtration parameters showed that the 10 kDa polysulfone hollow fiber membrane had the highest retention for hemicelluloses and acid insoluble lignin, above 90% and 85%, respectively, independently from shear rate and with limited transmembrane pressure influence. The retention of salts, acetic acid and phenolic monomers was about 10%. The permeate flux reached  $16 \text{ L h}^{-1} \text{ m}^{-2}$  at 2.8 bar and the critical flux was not yet reached.

This membrane was selected to run filtration tests in concentration and diafiltration modes.

#### 3.3.2. Materials and methods

This section is similar to the one described previously (3.2.2 Materials and methods), except that the membrane filtration part evolved with the addition of concentration and diafiltration modes, and the membrane cleaning procedures.

SCB mild alkaline extract was ultrafiltered in concentration mode on the 10 kDa PS hollow fiber membrane. Compared to the full-recycling mode, the concentration mode is expected to increase the retention of molecules and decrease the flux so the temperature was set at  $40 \text{ }^\circ\text{C}$ , due to the increase of the polarization layer and of the viscosity. To minimize the polarization layer, the shear rate was also set at the maximum value tested previously:  $10,187 \text{ s}^{-1}$ . The TMP was set at 2.4 bar to optimize the flux. Concentration was run in semi-batch conditions, meaning that the permeate was collected and the retentate was recycled. Concentration was expressed as volume reduction factor (*VRF*), calculated following Eq. (8):

$$VRF = \frac{V_{initial}}{V_{final}} \quad (8)$$

Where  $V_{initial}$  is the volume of the feed at the beginning of the concentration experiment and  $V_{final}$  is the volume of the feed at the end of the concentration experiment, i.e., the final retentate.

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SCB mild alkaline extract was also ultrafiltered in diafiltration mode with water until 4.3 diavolumes were reached. A peristaltic pump (Cole Parmer Masterflex) was used to add distilled water at 40°C in the feed tank at the same flow as the permeate flow (constant volume in the feed tank). Constant volume diafiltration (i.e., continuous diafiltration) is more efficient than volume reduction diafiltration, for instance on molecules with 0% retention, 3 diavolumes addition gives 88% recovery in the permeate with volume reduction by a 2-fold factor whereas 95% recovery is reached with continuous diafiltration (Schwartz, 2003).

When the observed retention ( $R$ ) is constant during concentration, the yield of a compound is given by Eq. (9):

$$Yield = VRF^{R-1} \quad (9)$$

For concentration and diafiltration experiments, yield ( $Y$ ), also named as recovery rate, of a given compound was determined following Eq. (10) and (11):

$$Y_{retentate} = \frac{C_{final\ retentate} V_{final\ retentate}}{C_{feed} V_{feed}} \quad (10)$$

$$Y_{permeate} = \frac{C_{global\ permeate} V_{global\ permeate}}{C_{feed} V_{feed}} \quad (11)$$

Where  $C$  is the concentration of the compound in the feed, the final retentate or the global permeate and  $V$  the volume of feed, final retentate or global permeate.

The permeate flux can be expressed according to Eq. (12):

$$J_{40} = \frac{\Delta P - \Delta \Pi}{R_t \cdot \mu_{40}} \quad (12)$$

Where  $J_{40}$  is the permeate flux at 40 °C ( $L \cdot h^{-1} \cdot m^{-2}$  or  $m^3 \cdot s^{-1} \cdot m^{-2}$ ),  $\Delta P$  is the applied pressure on the membrane what can be called TMP (bar or Pa or  $kg \cdot m^{-1} \cdot s^{-2}$ ),  $\Delta \Pi$  is the osmotic pressure (bar or Pa or  $kg \cdot m^{-1} \cdot s^{-2}$ ),  $R_t$  is the total resistance ( $m^{-1}$ ) and  $\mu_{40}$  the dynamic viscosity of the solution (Pa.s or  $kg \cdot m^{-1} \cdot s^{-1}$ ). Osmotic pressure is usually negligible for ultrafiltration. Previous tests have shown that retention rate of the small molecules is close to 0% so no osmotic pressure will be induced by these molecules.



Concerning bigger molecules, they do not contribute to significant osmotic pressure so only  $\Delta P$  was considered in the rest of this chapter (Ladisich, 2001).

The resistance-in-series model commonly admitted can be described by Eq. (13):

$$R_t = R_m + R_a + R_{pl} + R_{pb} + R_d \quad (13)$$

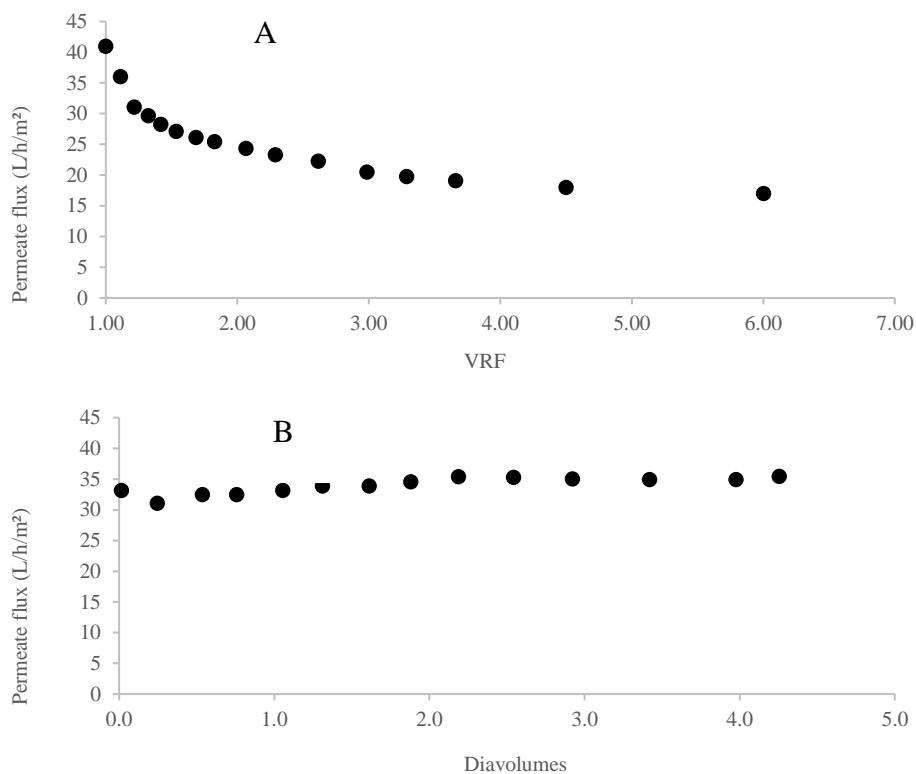
Where  $R_m$  is the resistance of the membrane,  $R_a$  the resistance due to the adsorption,  $R_{pl}$  the resistance due to the polarization layer,  $R_{pb}$  the resistance due to the pore blocking and  $R_d$  the resistance due to the deposit (i.e., cake formation or gel layer).

### 3.3.3. Results and discussion

#### 3.3.3.1. Concentration mode

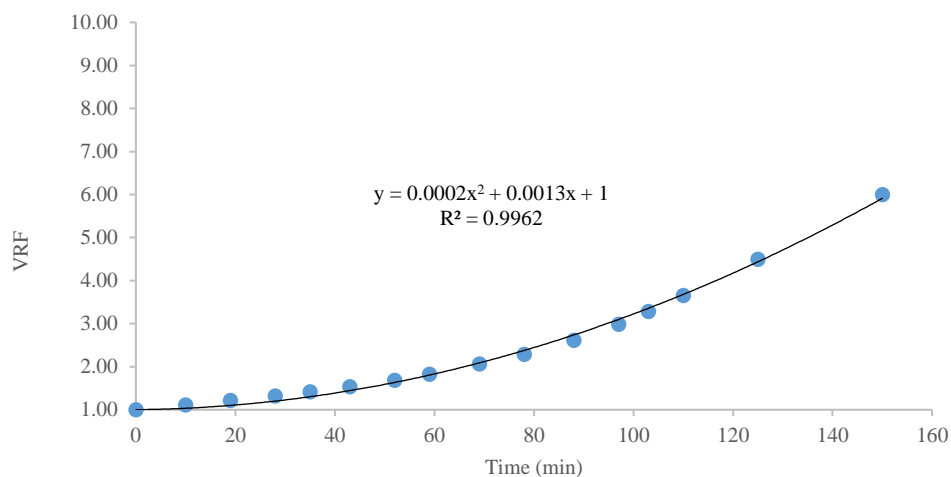
The permeate flux presented a logarithmic decrease during the concentration of the SCB alkaline extract. At the beginning of the concentration, the flux was 41 L/h/m<sup>2</sup>, and rapidly dropped to eventually reach 17 L/h/m<sup>2</sup> at a VRF of 6.0 (**Fig. 3.11A**). The flux decrease was faster at the beginning of the concentration because of the apparition of the concentration polarization layer adding a resistance ( $R_{pl}$ ) to the membrane resistance ( $R_m$ ). After the stabilization of the layer, the flux decrease was regular due to the increase of the viscosity of the solution and the increase of the osmotic pressure.

### Chapter 3: PURIFICATION BY MEMBRANE FILTRATION



**Fig. 3.11** Evolution of the permeate flux during (A) the concentration and (B) the diafiltration.

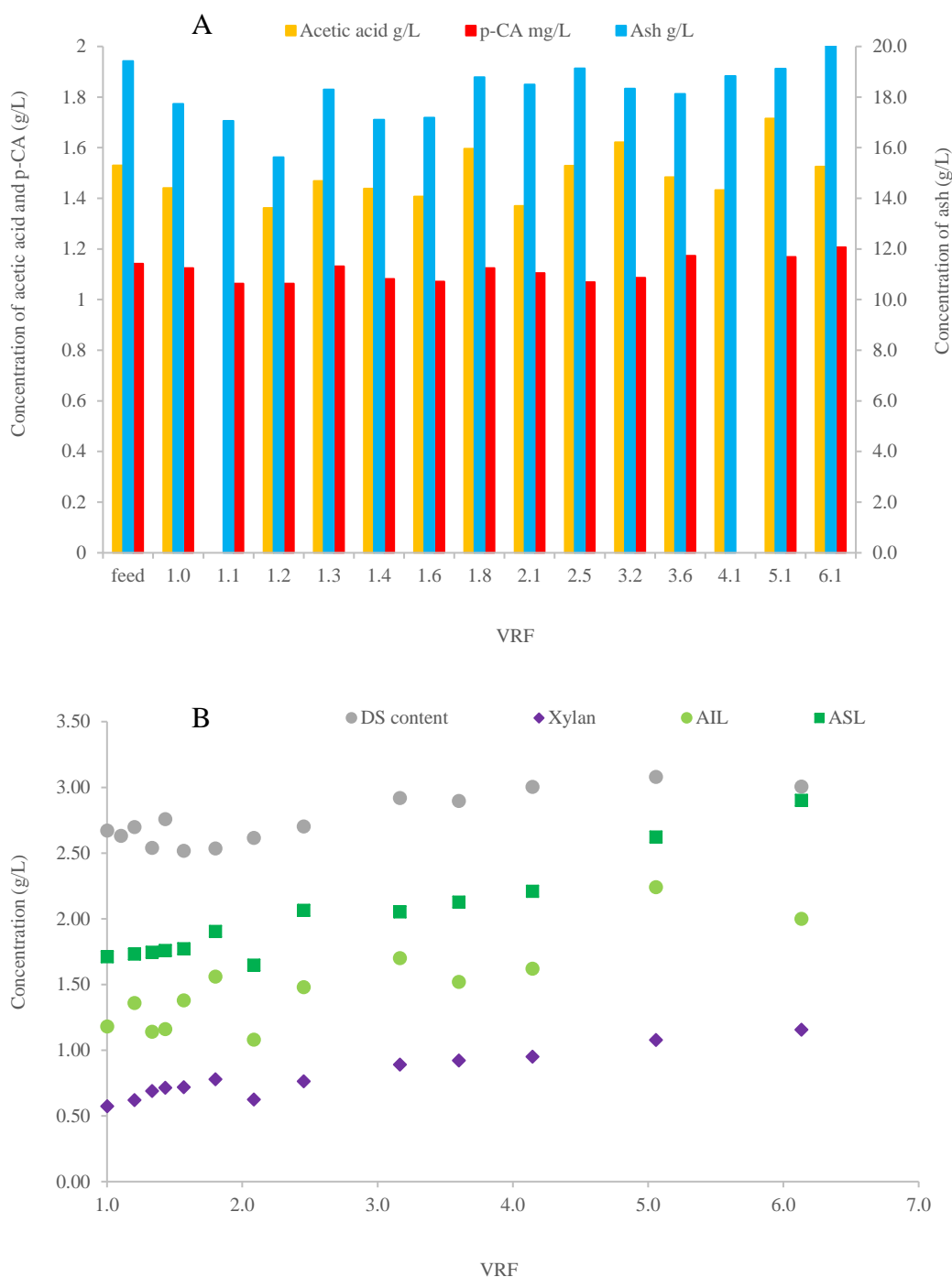
At constant TMP, the VRF was found to vary following a second order polynomial equation with the time (**Fig. 3.12**). A VRF of 2 was obtained after 67 min and the final VRF of 6.0 was reached after 150 min.



**Fig. 3.12** Evolution of the volume reduction factor (VRF) with time.

During the concentration experiment, the molecules with retention rates close to 0%, i.e., the small molecules (inorganic salts or ash, acetic acid and the five phenolic monomers) had a stable concentration in the permeate (**Fig. 3.13A**). The concentration of the molecules mainly retained by the membrane (AIL, ASL and xylan) increased in the permeate with increasing VRF (**Fig. 3.13B**). An increase in concentration of the retained molecules in the retentate side led to an increase in the concentration gradient with the permeate side, inducing higher dragging force by diffusion (Pontalier et al., 1997).

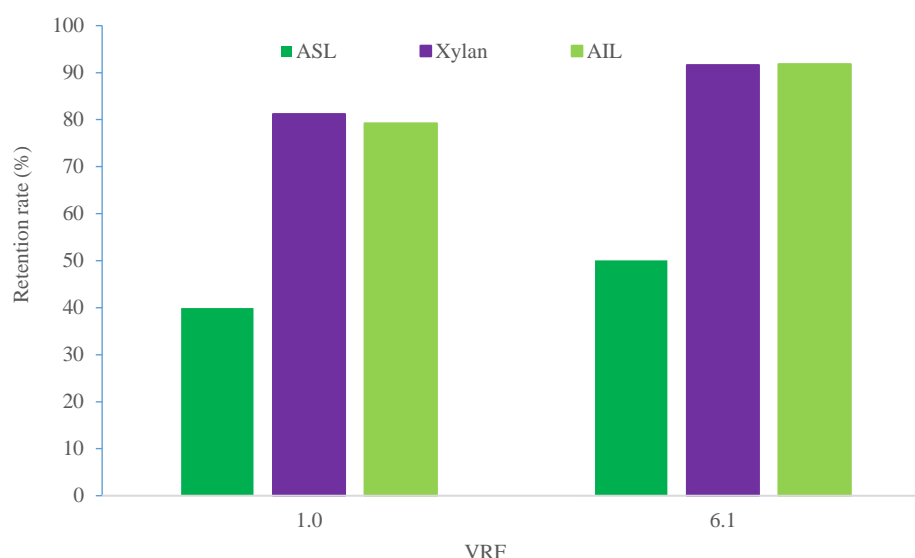
### Chapter 3: PURIFICATION BY MEMBRANE FILTRATION



**Fig. 3.13** (A) Concentration of acetic acid, *p*-CA and ash in the feed and in the permeates collected during the concentration of the SCB mild alkaline extract. Same trend was observed with vanillic acid, 4HBA, vanillin and FA but as their concentration were much lower they were not presented in this graph. (B) Evolution of the concentration of AIL, ASL and xylan in the permeate.

Nevertheless, the retention rate of AIL and xylan both increased by about 10% to reach 92% at a VRF of 6 (Fig. 3.14). This result differs from a study by Wallberg et al. (2003), where lignin retention was constant during concentration experiments on black liquor on an 8 kDa PS tubular membrane as its measured concentration fitted the concentration predicted by Eq. (9).

The retention rates of inorganic salts, acetic acid and *p*-CA remained stable as their concentration did not significantly evolve from the beginning to the end of the experiment in the permeate and the retentate. Increasing the concentration of the retentate is interesting to increase the recovery of AIL and xylan in the retentate side while still allowing the smaller molecules to pass through the membrane.



**Fig. 3.14** Retention rate of SCB mild alkaline extract components at the beginning of the concentration (VRF = 1) and at the end of the concentration (VRF = 6.1).

Composition of global permeate and final retentate, and the yield of the different components of the feed in these two fractions are given in Table 3.5. With a VRF of 6.1, the salts (78%), acetic acid (81%) and phenolic monomers, i.e., *p*-CA (84%) were mostly recovered in the permeate. On the contrary, glucan (55%), xylan (82%), arabinan (74%) and AIL (72%) were mostly recovered in the retentate. Therefore, the purity of glucan, xylan, arabinan and AIL was increased in the retentate compare to the feed from 1.4 to

### Chapter 3: PURIFICATION BY MEMBRANE FILTRATION

2.0%, 8.8 to 18.2%, 2.2 to 4.0% and 17.9 to 32.4%, respectively. The inorganic salts content in the retentate was drastically decreased but they were still accounting for 27.8% of the DS content of the final retentate. After the filtration of SCB mild alkaline extract in concentration mode by a VRF of 6.1, the concentrations of glucan, xylan, arabinan and AIL were multiplied by 3.0, 4.5, 4.0 and 4.0, respectively.

**Table 3.5**

Feed, global permeate, final retentate composition and yields of the various components after concentration by a VRF of 6.1.

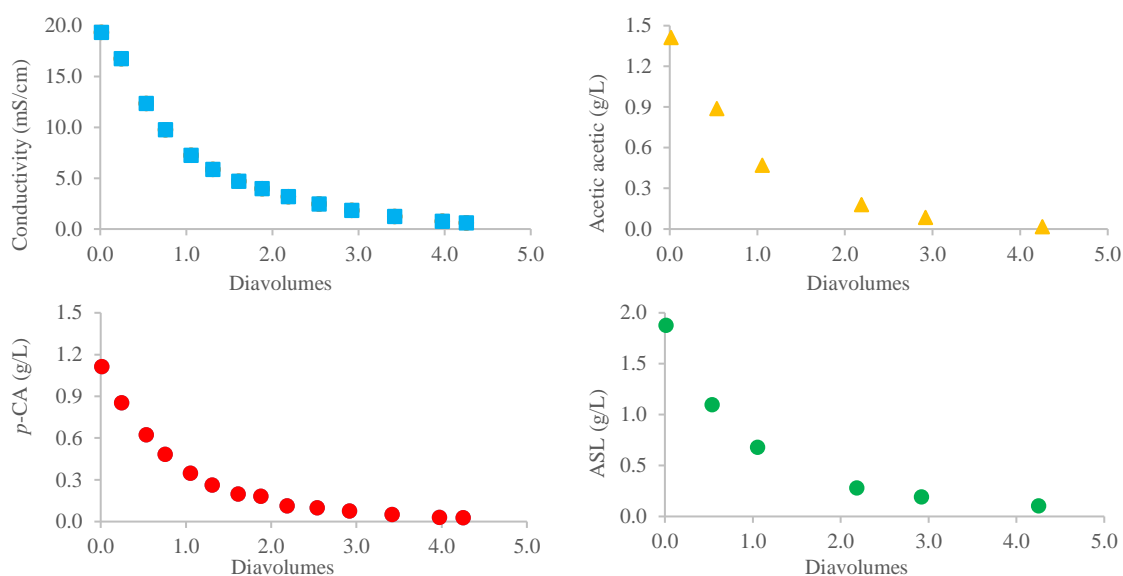
Components	<b>Feed</b>	<b>Global permeate</b>		<b>Final retentate</b>		Yield closure
	Content	Content	Yield	Content	Yield	
DS	3.4	2.5	61	7.8	39	100
Ash	56.0	71.5	78	27.8	27	105
Glucan	1.4	NA	NA	2.0	55	NA
Xylan	8.8	3.0	21	18.2	82	102
Arabinan	2.2	NA	NA	4.0	74	NA
Acetic acid	4.4	5.9	81	2.1	19	100
AIL	16.6	6.0	21	32.4	72	92
ASL	8.2	8.1	55	7.7	34	89
VA	NA	NA	NA	NA	NA	NA
4HBA	0.1	0.1	86	0.0	14	101
Vanillin	0.1	0.1	88	0.0	16	104
<i>p</i> -CA	3.3	4.5	84	1.3	16	100
FA	0.4	0.5	77	0.1	15	92
Mass closure	101.5	99.7		95.7		

All the values are calculated based on the percentage of dry solid.

The concentration step enabled to concentrate the retained species and also to purify them by removing a fraction of the small molecules from the SCB alkaline extract. However, as the inorganic salts content was initially high in the alkaline extract, they were still accounting for 27.8% of the DS content of the retentate. A diafiltration step could be run afterward on the retentate to further remove the salts.

## 3.3.3.2. Diafiltration mode

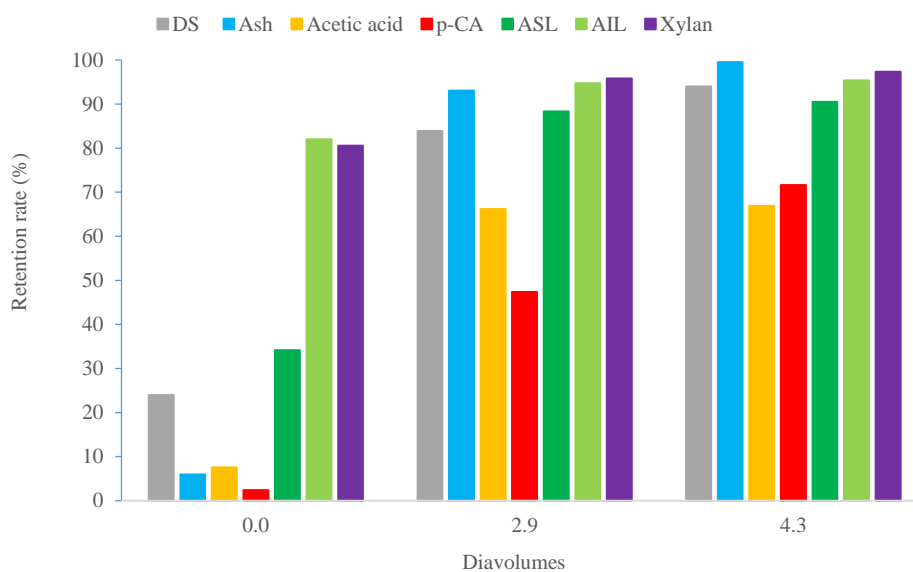
Diafiltration was directly run on another batch of SCB alkaline extract (5 L) and not on the concentrated SCB alkaline extract in order to study the effect of running it potentially before the concentration mode. After a small decrease down to 31 L/h/m<sup>2</sup> at the beginning of the diafiltration experiment, the permeate flux slightly increased during the diafiltration, from 33 to 35 L/h/m<sup>2</sup> after 4.25 diavolumes of water added (**Fig. 3.11B**). On average, diafiltration was run at higher flux than concentration. As the concentration of the small molecules, (inorganic salts, acetic acid and phenolic monomers) decreased in the retentate, the osmotic pressure decreased as well, leading to a higher effective TMP and a higher flux, compensating the drop of flux usually observed overtime during filtration experiments. The small molecules concentration rapidly decreased in the permeate during the diafiltration (**Fig. 3.15**).



**Fig. 3.15** Evolution of the conductivity (reflecting the ash concentration) and the concentrations of acetic acid, *p*-CA and ASL in the permeate during the continuous diafiltration in function of the diavolumes added.

As for the concentration mode, the retention rates of large molecules increased with the diavolumes (**Fig. 3.16**). After 2.9 diavolumes, the rejection rates of AIL and xylan reached 95% and 96%, respectively. But unlike the concentration mode experiment, the rejection rates of the small molecules did not remain stable, they drastically increased. Ash rejection was over 90% after 2.9 diavolumes and reached a quasi-total rejection (over

99%) after 4.3 diavolumes. At the end of the diafiltration, the retention rates of *p*-CA and acetic acid increased to a lower extent, by 47% and 60%, respectively. The purification was slowed down by the rise in the retention rates of the small molecules. However, at 2.9 diavolumes, the concentrations of the small molecules in the retentate was already drastically reduced compared to their initial concentration, for instance for acetic acid and *p*-CA, from 1.4 to 0.1 g/L and 1.1 to 0.1 g/L, respectively (**Fig. 3.15**). Therefore, in an integrated process, diafiltration should be stopped at 3 diavolumes or before, to optimize the water consumption.



**Fig. 3.16** Retention rates of the SCB alkaline extract components during the ultrafiltration in diafiltration mode.

Inorganic salts constituted the main impurities with 25.4% of the retentate DS content. Their yield in the retentate is surprisingly high (17%) given that a molecule with 25% retention should be eliminated in the permeate at 95% with 4 diavolumes if the retention remains stable during the filtration. Salts retention rate actually increased drastically from 6% at the beginning of the diafiltration to 93% after 2.9 diavolumes, thus limiting salts elimination in the permeate (**Fig. 3.16**). Acetic acid (0.4% of the DS) and phenolic monomers (1.3% of the DS) were almost totally removed from the retentate (**Table 3.6**), as their rejection did not increase to the same extent as the salt rejection rate (**Fig. 3.16**). The high rejection rate of the salts could be due to the formation of bound between the salts and the large retained molecules. In diafiltration mode the increase in purity for



lignin and xylan was limited compared to concentration mode mainly because the inorganic salts were poorly eliminated with diafiltration.

**Table 3.6**

Feed, global permeate, final retentate composition and components yield after diafiltration with 4.3 diavolumes

Components	Feed	Global permeate		Final retentate		Yield closure
	Content	Content	Yield	Content	Yield	
DS	3.4	0.6	77	1.0	29	105
Ash	56.0	61.0	84	25.2	13	97
Glucan	1.4	NA	NA	2.3	46	NA
Xylan	8.8	2.3	20	20.2	66	86
Arabinan	2.2	NA	NA	4.7	64	NA
Acetic acid	4.4	3.3	89	0.5	3	93
AIL	16.6	7.7	36	42.3	75	111
ASL	8.2	8.0	74	11.4	40	114
VA	NA	NA	NA	NA	NA	NA
4HBA	0.1	0.1	92	0.0	3	95
Vanillin	0.1	0.1	100	0.0	5	105
p-CA	3.3	4.0	92	1.0	9	101
FA	0.4	0.4	86	0.3	8	94
Mass closure	101.5	86.8		107.9		

All the values are calculated based on the percentage of dry solid.

Diafiltration with 4.3 diavolumes of water led to higher purity for xylan (20.2%) and AIL (42.3%) than concentration with a VRF of 6.1. Surprisingly, the retention of xylan and AIL differed from concentration to diafiltration mode with xylan being more retained than AIL in concentration mode, 82% and 72%, respectively; and AIL being more retained than xylan in concentration mode, 75% and 66%, respectively.

For both the concentration and diafiltration experiments, the recovery rates of xylan and arabinan in the retentate are close whereas the recovery of glucan is about 20% lower. The significant difference in recovery for both concentration and diafiltration experiments between glucan on one side, and xylan and arabinan on the other side suggests that glucose is not part of the same oligomers as xylan and arabinan, and glucan probably present a lower molecular weight.

### 3.3.4. Conclusion

Ultrafiltration can be used for the purification of SCB alkaline extract by separating sugar and lignin oligomers from inorganic salts, phenolic monomers and acetic acid. Membrane filtration also enabled to concentrate xylan and AIL in the retentate until a VRF of 6.1 with recovery yields of 82% and 72%, respectively. However, high content of inorganic salts remained in the retentate impacting the purity of hemicelluloses and lignin. Diafiltration was tested to remove the inorganic salts to a larger extent, but was found poorly efficient since the retention of salts increased with the diavolumes added. Diafiltration allows to work with higher permeate flux, leads to a similar recovery yield and with a higher purity than concentration mode. Nevertheless, it produces a retentate with a lower concentration.

The choice between concentration and diafiltration of SCB alkaline extract has to be determined according to the objectives of the separation and the cost of the process. Concentration will lead to better recovery of the sugar oligomers and lignin in the retentate but a lower purity of these molecules. Diafiltration will lead to lower recovery but higher purity of these molecules with a higher consumption of water and possibly energy as evaporation or reverse osmosis will be required to concentrate the retentate to further process it. Our suggestion for the following integrated process was to use concentration mode in a first time then diafiltration in a second time.

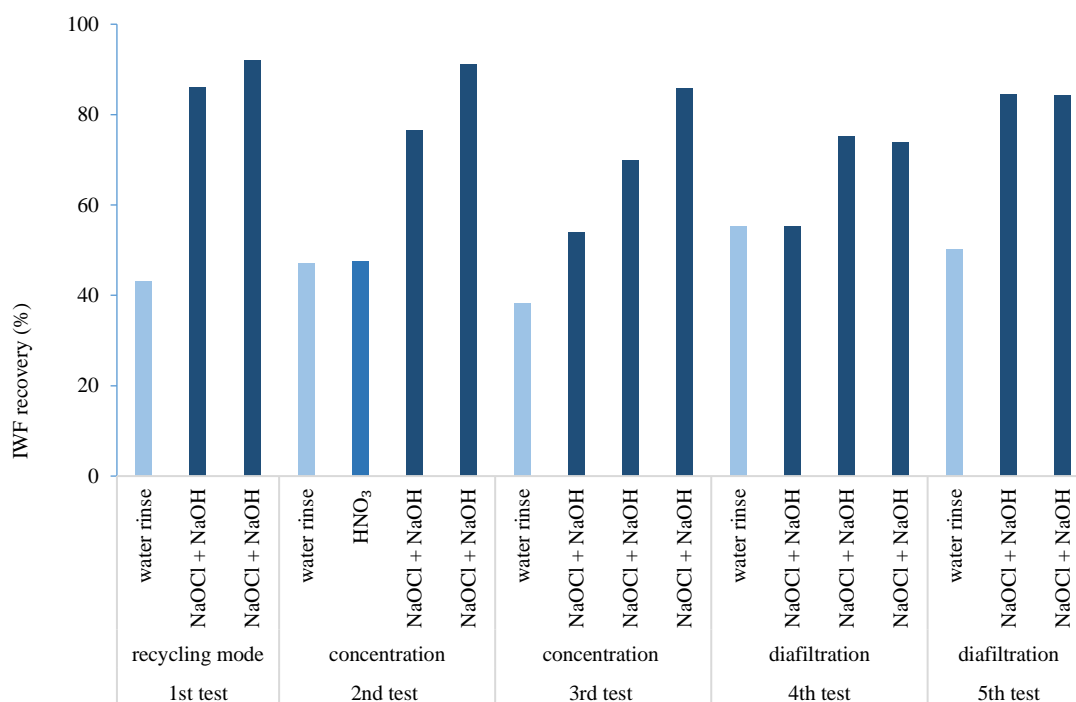
### 3.4. Membrane cleaning

To check the efficiency of a cleaning method, the flux obtained after the filtration then the rinsing/cleaning step must be close to the initial water flux (IWF) of the new membrane.

Cleaning procedure was studied on the 10 kDa PS hollow fiber membrane as this membrane presented the best results for the separation of SCB alkaline extract components and was used for several tests in concentration and diafiltration modes.

After SCB alkaline extract filtration and several water rinses on the new 10 kDa PS hollow fiber membrane, only 43% of the initial water flux was recovered after the first filtration in recycling mode (**Fig. 3.17**). A simple rinse on the membrane was not sufficient to restore its IWF, therefore a chemical cleaning was tested. A solution of NaOCl at 100 ppm and pH adjusted to 11 with NaOH was recirculated for 30 min at 45 °C, then the installation was rinsed with water. The IWF was recovered at 86% which can be considered as an efficient cleaning. A second cleaning with the same solution at 50 °C and for 1 h followed by several rinses led to another increase in the IWF with a recovery of 92%. The membrane was then used for the first filtration test in concentration mode. After the test and an extensive rinse, a solution of HNO<sub>3</sub> at pH 4 was recirculated at 150 L/h, a PTM of 0.8 bar, at 50 °C for 60 minutes, then the installation was rinsed with water. This cleaning procedure had no influence on the water flux, showing that salts precipitation was not responsible for the membrane pollution. The procedure with a solution of NaOCl at 100 ppm and pH adjusted to 11 with NaOH recirculated for 30 min at 50 °C led again to a substantial IWF recovery (71%). It was repeated and 91% flux recovery was reached. Several cycles of i) SCB alkaline extract filtration, ii) water rinse, iii) cleaning with sodium hypochlorite at alkaline pH and iv) water rinse showed that the IWF was recovered efficiently and the performance of the membrane, its selectivity and its flux, was kept constant.

### Chapter 3: PURIFICATION BY MEMBRANE FILTRATION



**Fig. 3.17** Initial water flux (IWF) recovery after each test and the different cleaning procedures on 10 kDa polysulfone hollow fiber membrane.

### 3.5. Conclusion

Membrane filtration has been reported in many studies dealing with lignocellulosic alkaline extracts obtained in severe conditions (pulp and paper industry) to purify lignin or hemicelluloses from the other compounds of the extracts. In this part, we studied the separation of the compounds from a lignocellulosic mild alkaline extract by membrane cross-flow filtration. A membrane screening was run showing that the best selectivity was reached on a 10 kDa polysulfone hollow fiber membrane. In recycling mode, up to 90% of the acid insoluble lignin and xylan could be retained by this membrane whereas inorganic salts, acetic acid and phenolic monomers almost totally passed through the membrane. The flux varied linearly with the TMP and the shear rate did not influence the flux within the range of values tested. At 20 °C and 2.8 bar, the flux was 16 L/h/m<sup>2</sup>.

In concentration and diafiltration conditions, it was observed that the retention of xylan and lignin increased. The retention of the small molecules increased only in diafiltration mode, therefore it limited the interest of this technique. After concentration with a VRF of 6.1 no flux limitation was achieved and the purities of xylan and AIL increased from 8.8% to 18.2% and from 16.6% to 32.4%, respectively. The recovery rates of xylan (82%) was slightly higher than the recovery of lignin (72%). After diafiltration with 4.3 diavolumes of distilled water, the flux slightly increased and the purities of xylan (20.2%) and AIL (42.3%) increased to a larger extent than in diafiltration mode. With this filtration mode, the yield of xylan (66%) was lower than the yield of AIL (75%).

Cleaning of the 10 kDa PS hollow fiber membrane was efficient after several filtration experiments in different modes on SCB mild alkaline extract. Initial water flux recovery above 80% was achieved with a cleaning procedure implying the use of NaOCl at 100 ppm at pH 10-11.

## Chapter 4:

# PURIFICATION BY CHROMATOGRAPHY

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### 4.1. Resin process introduction

#### 4.1.1. Resin properties

Resins are organic polymers with bead shapes obtained by batch polymerization, leading to some heterogeneity in the bead size or by extrusion resulting in higher conformity coefficient (bead shape and size homogeneity) with higher production cost.

Resins have several characteristics like their type, their structure, their porosity and their size distribution. Among the different types of resins, there are weak acid cation, strong acid cation, weak base anion, strong base anion, adsorbent or chelating resins. Ion exchange resins are able to adsorb positively or negatively charged ions or molecules from an electrolyte solution and release an equivalent amount of their original counter ion of equal charge to the solution.

The structure of the resins is mainly styrenic, acrylic or phenolic based ; polystyrene divinylbenzene (DVB) resins being the most common due to their their high resistance to base and acid. During the resin production, DVB is added to strengthen the structure of the resin, particularly when large porogen are used like for macroporous-type resin. Higher DVB content in the resin reduces the swelling of the resin and increases its mechanical resistance. However, increasing the DVB content reduces the humidity of the resin (i.e., the water solvating the counter ion and the water contained in the pores), and high humidity leads to faster diffusion of molecules inside the pores.

Two main porosities can be distinguished, gel-type resin having small pores (1-5 nm) and macroporous-type resin having wider pores (up to 50 nm), the latter being also qualified as highly reticulated (due to their high level of DVB). The manufacture of ion exchange resins involves first the preparation of a cross-linked bead copolymer (styrene-DVB for example) followed by the activation of the resin, for instance sulfonation in the case of strong acid cation resins, or chloromethylation and amination of the copolymer for anion resins.

Their size distribution ranges from polydispersed resins with pores of about 0.3 mm to 1.2 mm, cheaper and mainly used for adsorption processes (for instance ion exchange), to uniformed particle sized (UPS) resins with all beads presenting a narrow particle size



range, more expensive to manufacture, but required for chromatographic processes were preferential pathways must be absolutely avoided. Smaller beads are interesting for chromatographic processes as they increase the resolution but they lead to increased pressure drop.

### 4.1.2. Adsorption/desorption process

Adsorption occurs when the affinity of a compound is higher for the stationary phase (here the resin) than for the mobile phase. Various bonds are responsible for the adsorption of a compound on the resin including covalent, ionic, van der Waals and H bonds. The desorption of the compound from the sorbent is triggered by the use of another eluent for which the compound has an increased affinity or by increasing the osmotic pressure with the use of saline solution.

The process implies several steps – loading (i.e., injection of the solution to purify), rinsing, desorption, regeneration, equilibration – leading to high solvent and/or chemical consumptions. Compared to activated charcoal, the use of polymeric resin presents the advantage of better performances preservation over several cycles of adsorption-desorption and of the use of lower temperatures.

### 4.1.3. Chromatographic process

The separation is mainly based on ionic exclusion, size exclusion and affinity (e.g., formation of a complex between the counter ion of the resin and the hydroxyl group of the sugars varying in stability with the position of the hydroxyl groups of the sugars). The efficiency of the separation depends on these three parameters and on the column length. However, increase in column length leads to an increase of pressure drop: industrially the column length is generally limited to 2 m. There is no need for regeneration of the resin, the process has a simple set-up: loading, elution.

### 4.2. Batch column chromatography on a synthetic monomeric sugars solution

#### 4.2.1. Introduction

Lignocellulosic acid hydrolysates contain monomeric sugars and mineral acid that are usually separated by chromatography using a gel-type strong acid cationic (SAC) exchange resin with  $H^+$  as counter ion and water as eluent. This first chromatographic step has been largely studied in the literature (see 1.3.3.5.), whereas less experimental data exist on the second chromatographic step in batch column mode, where the sugars are separated by the same kind of resin except that  $Ca^{2+}$  is the first choice as counter ion.

Adsorption of the monomeric sugar on the resin is occurring through the formation of a complex between the oxygens of hydroxyl groups of adjacent carbons on sugar and the cation linked to the resin. The complex formation is affected on one side by the acidity of the cation (alkaline earth metal are strong acid while lead is a soft acid for instance), his radius and his charge, and on the other side by the steric hindrance and configuration ( $\alpha$ - and  $\beta$ -pyranose/furanose form and open-chain form equilibrium) of sugars (Caruel et al., 1991). Once an aqueous solution of sugar is injected on a column filled with cationic exchange resin and flushed with water as eluent, successive adsorption-desorption occurs along the column.

The resin, XA2004-30Na<sup>+</sup>, provided by Novasep was converted to Ca<sup>2+</sup> form and tested for this separation on a synthetic glucose, xylose and arabinose mixture at concentrations close to what is commonly found in SCB acid hydrolysates.

#### 4.2.2. Materials and methods

##### 4.2.2.1. Chemicals

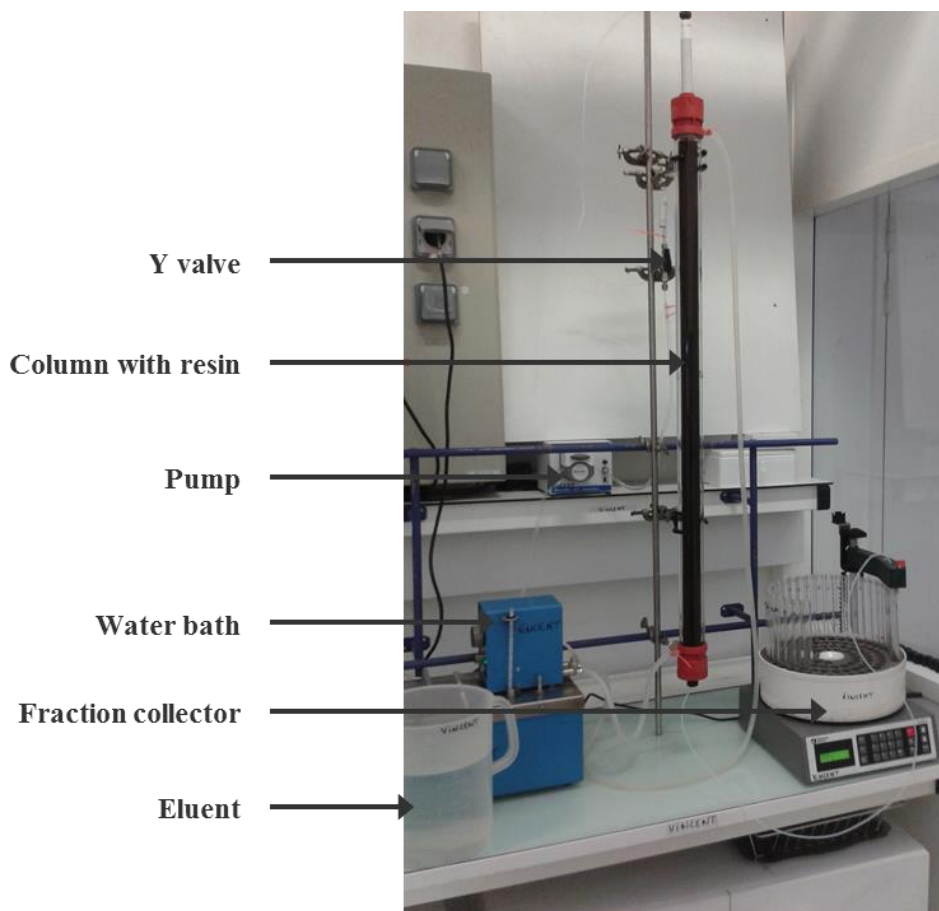
Sulfuric acid 95% to prepare HPLC eluent was purchased from VWR. D-(+)-glucose ( $\geq 99.5\%$ ), L-(+)-arabinose (99%), D-(+)-xylose (99%), for the preparation of the synthetic sugar solution and HPLC standards, were all purchased from Sigma Aldrich. Gel-type SAC exchange resin, XA2004-30Na<sup>+</sup> was provided by Novasep Process,

France. Calcium chloride ( $\geq 99\%$ ) for the resin conversion was purchased from Sigma Aldrich.

### 4.2.2.2. Pulse test

The pulse test was run on 500 mL resin packed in a 1 m high and 26 mm diameter jacketed glass column. The resin was mixed with water at 60 °C for degassing and packed from the top of the column. The upper piston was brought as close as possible to the top of resin to minimize the dead volume. A Y-valve successively enabled the injection of 5 mL feed (synthetic solution) or eluent (distilled water) on top of the column. The eluent was circulated from the top to the bottom of the column thanks to a peristaltic pump and its volume was accounted as resin Bed Volume (BV). The temperature of the column was maintained at 40 °C thanks to a water bath. At the outlet of the column, a fraction collector (GradiFrac, from Pharmacia Biotech) was set to collect 20 mL fractions representing 0.04 BV. The collected samples were analyzed after the run was completed. **Fig. 4.1** displays the set-up of the batch column chromatography.

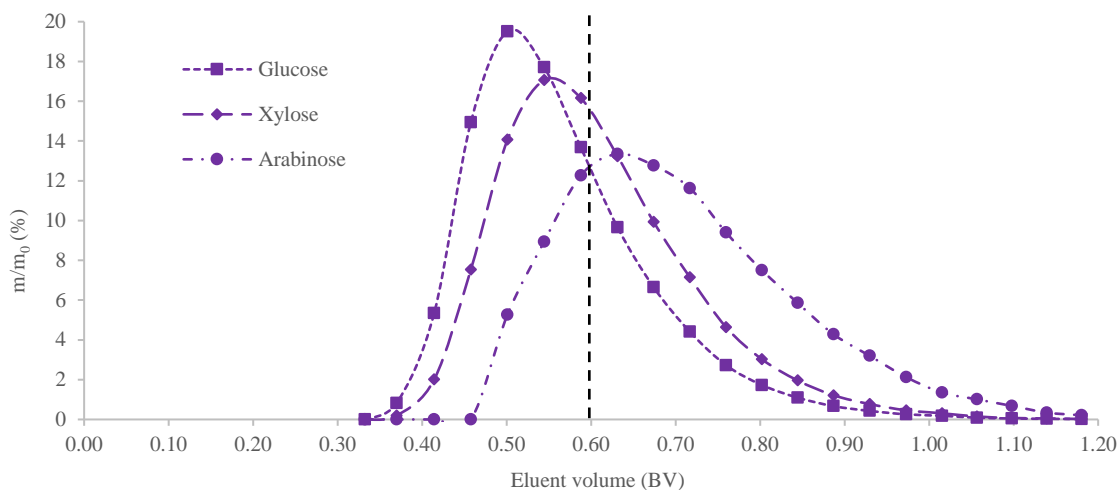
Before the pulse test, the XA2004-30 resin provided under  $\text{Na}^+$  form was converted into  $\text{Ca}^{2+}$  form with the elution of 1 L of 8%  $\text{CaCl}_2$  (w/v) at 2 BV/h.



**Fig. 4.1** Set-up of the batch column chromatography

#### 4.2.3. Results and discussion

Glucose can be separated from arabinose whereas xylose was not separated (**Fig. 4.2**). Glucose started to elute after 0.37 BV and reached a peak at 0.50 BV. 73% of the glucose was recovered in the fraction before 0.60 BV, whereas 24% of the arabinose was recovered in this fraction.



**Fig. 4.2** Synthetic solution of glucose xylose and arabinose, pulse test on XA2004-30Ca<sup>2+</sup> resin, injection of 0.01 BV, distilled water as eluent at a velocity of 2.26 m/h and temperature of 40 °C, with  $m$  the mass of a given compound in the fraction and  $m_0$  its mass in the feed. Experiment was run in triplicate showing similar peaks shape and BV. Lines are presented to guide the eyes.

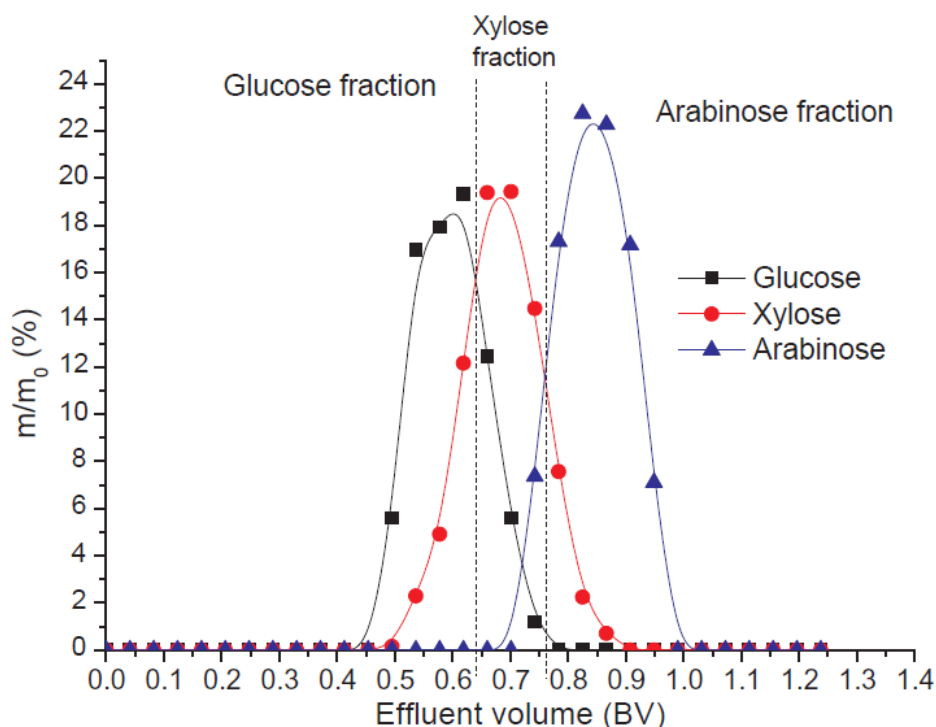
Chen et al. (2018) used a pine branches hydrothermal hydrolysate to study the separation of monomeric sugars (glucose, xylose and arabinose) by elution chromatography on Amberlite IRP69Ca<sup>2+</sup> resin with water as eluent. The comparison of their results with our results showed that the same elution order was observed but sugars were more retained on this resin: glucose started to elute at 0.48 BV and reached a peak at 0.63 BV, the peaks of xylose and arabinose were obtained at 0.65 BV and 0.85 BV, respectively (**Fig. 4.3**). The separation performance can be assessed by the resolution ( $R_s$ ) given by Eq. (14) (Kromidas and Kuss, 2009):

$$R_s = 1.18 \frac{(t_{R2} - t_{R1})}{(W_{h1} + W_{h2})} \quad (14)$$

With  $t_{R1}$  and  $t_{R2}$  retention time of compounds 1 and 2,  $W_{h1}$  and  $W_{h2}$  the width of the peaks at half height.

The resolution obtained by Chen et al. (2018) was 0.065 glucose/arabinose separation, whereas it was 0.031 from our experiment. The conditions used were very different since they used a slow eluent velocity (0.15 m/h, fifteen times less than we did) that is unlikely to be used industrially since the productivity would be certainly too low. They noticed on synthetic sugars solution that decreasing the velocity from 0.30 m/h to 0.15 m/h increased

the resolution by 50%. Despite the significantly shorter resin bed they used (25 cm), their experiment resulted in higher resolution.



**Fig. 4.3** Pine branches hydrothermal hydrolysate pulse test on Amberlite IRP69Ca<sup>2+</sup> resin with a column height of 25 cm, injection of 0.05 BV, with distilled water as eluent at a velocity of 0.15 m/h and a temperature of 30 °C, with  $m$  the mass of a given compound in the fraction and  $m_0$  its mass in the feed (Chen et al., 2018).

#### 4.2.4. Conclusion

Efficient glucose and arabinose separation on SAC resin with Ca<sup>2+</sup> as counter ion and with water as eluent has been reached. The process could be optimized, since lower resolution with the conditions employed in this work were obtained whereas the conditions from the study in the literature gave better resolution but is unlikely to be used industrially due to low productivity. Xylose separation from glucose and arabinose by chromatography still need investigation.

### 4.3. Batch column chromatography on SCB mild alkaline extract

Vincent Oriez<sup>a,\*</sup>, Marlène Beyerle<sup>b</sup>, Pierre-Yves Pontalier<sup>a,\*</sup> Jérôme Peydecastaing<sup>a</sup>

<sup>a</sup> Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, 4 allée Emile Monso, 31030 Toulouse, France

<sup>b</sup> Novasep Process, 5 chemin du Pilon, 01708 St-Maurice de Beynost, Miribel, France

\*Corresponding authors at: Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France

*E-mail addresses:* [vincentoriez@yahoo.fr](mailto:vincentoriez@yahoo.fr), [vincent.oriez@ensiacet.fr](mailto:vincent.oriez@ensiacet.fr) (V. Oriez), [pierreyves.pontalier@ensiacet.fr](mailto:pierreyves.pontalier@ensiacet.fr) (P.Y. Pontalier)

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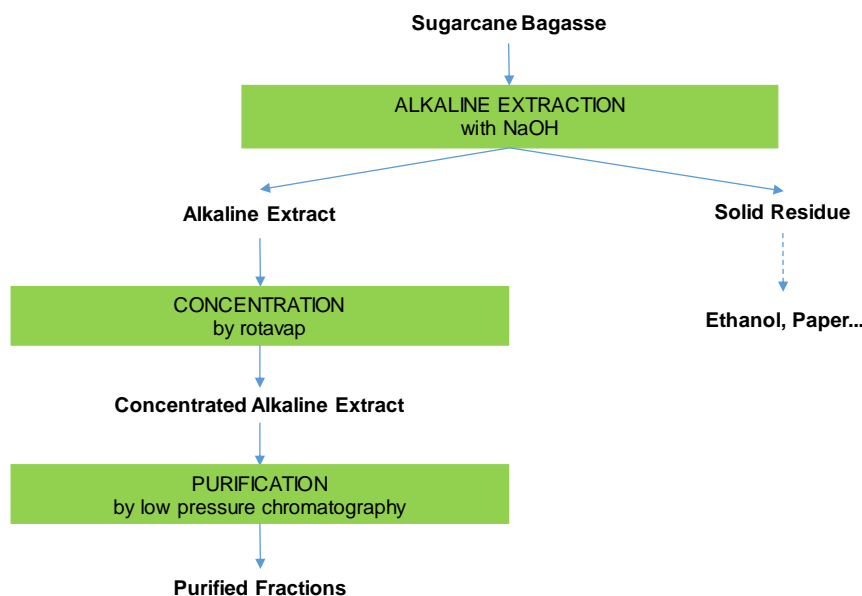
#### Abstract

Sugarcane bagasse (SCB) was treated under mild alkaline conditions (solid:liquid ratio of 1:20 (w/v), 1.5% NaOH (w/v), 60 °C, 6h) to fractionate the lignocellulose in order to produce a typical mild alkaline extract from a lignocellulosic biomass. The solid residue was enriched in cellulose, while the SCB alkaline extract contained lignin and hemicelluloses, but also inorganic salts, five phenolic monomers and acetic acid. After concentration of the alkaline extract by evaporation, low pressure chromatography with water as eluent was performed to produce purified fractions. Two different strong acid cation (SAC) exchange resins were tested: one gel-type resin and one macroporous-type resin. The lignin and hemicelluloses were separated from the inorganic salts by the gel-type SAC exchange resin. On this resin, the phenolic monomers were partitioned regarding the presence or absence in their structure of a carboxyl group. On the macroporous-type SAC exchange resin, the largest sugar oligomers and lignin oligomers were obtained in a fraction free of inorganic salts, phenolic monomers and acetic acid.

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Keywords: *Sugarcane bagasse fractionation; alkaline pretreatment; lignin; hemicelluloses; pulse chromatography*

Graphical abstract



### 4.3.1. Introduction

Sugarcane was the most produced crop in the world in 2013 with 1.9 billion tons (FAO, 2015). Sugarcane bagasse (SCB) is a lignocellulosic by-product of the sugar and alcohol industry from sugarcane, and is nowadays mainly burnt to produce electricity. However, in the last decade, SCB has been widely studied as a substrate to produce ethanol by fermentation of the glucose coming from the cellulose, or of the other C6 and C5 sugars coming from the hemicelluloses (Cardona et al., 2010). The pretreatment of the incoming lignocellulosic material into the second generation ethanol biorefinery, consisting in the separation of the three main components, cellulose, hemicelluloses and lignin, is a key step for economic viability and environmental efficiency in the overall process (Mosier et al., 2005; Yang and Wyman, 2008). Acidic conditions for the pretreatment were extensively studied and have been applied industrially for twenty years, and present the advantage of obtaining monomeric sugars in a single step process (Farone and Cuzens, 1997; Mosier et al., 2005).

Chromatography was investigated to purify the monomeric sugars resulting from lignocellulosic biomass treatment by concentrated acid (usually  $\text{H}_2\text{SO}_4$  at 70-75%), using



gel-type SAC exchange resin under  $H^+$  form and water as eluent (Neuman et al., 1987; Hester et al., 1995). Monomeric sugars can be separated from sulfuric acid and other impurities such as acetic acid, furfural and hydroxymethylfurfural (HMF) (Heinonen and Sainio, 2010). Once the acid and other impurities are removed, the mixture of sugars can be purified by another chromatographic step with water as eluent. When the monomeric sugars are a mixture of glucose, xylose and arabinose, gel-type SAC resin with  $Ca^{2+}$  as counter ion was found to be the most efficient resin for their separation (Caruel et al., 1991; Lei et al., 2010; Chen et al., 2018). However, prior to this second chromatographic step, the extract had to undergo decationization through ion exchange and neutralization with the addition of NaOH inducing extra economic and environmental cost to the process (Lodi et al., 2017).

Inspired from pulp and paper processes, alkaline pretreatment is gaining importance in the second generation ethanol biorefinery (Hayes, 2009) due to improved overall ethanol yields (Saha and Cotta, 2007; Kim et al., 2016), mild reaction conditions and possible valorization of the solubilized lignin and hemicelluloses (Cardona et al., 2010; Kim et al., 2016). Among the different alkaline pretreatments mentioned in the literature, mild sodium hydroxide conditions appear to lead to the highest lignin and hemicelluloses extraction yields at reasonable costs (Peng et al., 2012; Kim et al., 2016). These conditions also induce the hydrolysis of ester bonds – between hemicelluloses and lignin, phenolic monomers and lignin, acetate groups from hemicelluloses (Xiao et al., 2001; Chen et al., 2012). Purifying the alkaline extract components to enable their further valorization is of major importance to give value to the whole process of lignocellulosic ethanol production after alkaline pretreatment (Ragauskas et al., 2014).

Recovery of lignin or hemicelluloses from lignocellulosic alkaline extracts (black liquors in the pulp and paper industry) have been investigated by acid precipitation (Uloth and Wearing, 1989; Sun and Tomkinson, 2001), ethanol precipitation (Peng et al., 2009; Bian et al., 2012) and membrane filtration (Uloth and Wearing, 1989; Wallberg et al., 2003). However, purification through precipitation led to high chemical consumption, while membrane filtration generated fractions of mediocre purity due to difficult salt removal. Resin adsorption process has also been investigated either for the production of pure phenolic compounds such as *p*-coumaric acid (*p*-CA) (Ou et al., 2009) and ferulic acid (FA) (Ou et al., 2007), or for hemicelluloses purification (Zeitoun et al., 2010). These

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operations can lead to high economic and environmental costs through a significant consumption of chemicals and numerous process steps - loading, rinsing, desorption, regeneration, equilibration - and so far, no industrial development has been reported.

Chromatography is an interesting alternative purification technique, implying both size exclusion and ionic repulsion phenomena. It presents the advantage of using only one eluent and an easier process set-up - loading, elution - both for batch (pulse chromatography) and continuous process (Sequential Moving Bed). However, unlike for lignocellulosic acid extracts, very few studies can be found on chromatography to purify lignocellulosic alkaline extracts. In the case of liquors from soda-anthraquinone pulping process, separation was not performed directly on the alkaline extract. The media was first treated with acid until pH 1.2 to precipitate the lignin, then by chromatography on SAC exchange resin with water as eluent at 65 °C to specifically separate aliphatic carboxylic acids from sodium sulfate (Alén et al., 1991). More recently, chromatography was tested on a corn stover alkaline extract, but mesoporous silica materials were used as stationary phase, acidic water or organic solvent as mobile phase and the goal was to specifically separate monomeric C5 sugars from monomeric C6 sugars (Modenbach, 2013).

This paper focuses on the purification of raw SCB extract, obtained under mild alkaline conditions, to give a higher value to the overall biorefinery scheme. Pulse chromatography, using water as mobile phase and SAC exchange resins as adsorbents was studied in order to produce purified fractions from the SCB alkaline extract, composed mainly of lignin oligomers, hemicelluloses, acetic acid, phenolic monomers and inorganic salts.

### 4.3.2. Materials and methods

#### 4.3.2.1. Chemicals

Sodium hydroxide ( $\geq 98.5\%$ ), sulfuric acid 72% for analytical hydrolysis, sulfuric acid 95% and acetonitrile ( $\geq 99.9\%$ ) to prepare HPLC eluents, and methanol ( $\geq 99.8\%$ ) used as a tracer for column void volume, were purchased from VWR. Calcium carbonate ( $\geq 98.5\%$ ) was purchased from Merck. HPLC standards: D-(+)-cellobiose ( $\geq 98\%$ ), D-(+)-

glucose ( $\geq 99.5\%$ ), D-(+)-galactose, L-(+)-arabinose (99%), D-(+)-xylose (99%), D-(+)-mannose ( $\geq 99\%$ ), fructose ( $\geq 99\%$ ), acetic acid ( $\geq 99\%$ ), furfural (99%), 5-hydroxymethyl-2-furfuraldehyde (99%), gallic acid (97%), 4-hydroxybenzoic acid ( $\geq 99\%$ ), caffeic acid ( $\geq 98\%$ ), vanillic acid (97%), syringic acid ( $\geq 95\%$ ), 4-hydroxybenzaldehyde (98%), vanillin (99%), *p*-coumaric acid ( $\geq 98\%$ ), syringaldehyde (99%), *trans*-ferulic acid ( $\geq 99\%$ ), sinapic acid ( $\geq 98\%$ ), *trans*-3-hydroxycinnamic acid (99%), were all purchased from Sigma Aldrich. Blue Dextran, 2,000,000 Da molecular weight, came from Sigma Aldrich too. Both SAC exchange resins, XA2004-30Na<sup>+</sup> and XA2054Na<sup>+</sup> (**Table 3.3**) were provided by Novasep Process, France.

**Table 4.1**

Characteristics of the resins

	XA2004-30Na <sup>+</sup>	XA2054Na <sup>+</sup>
Nature	SAC	SAC
Matrix	Styrene + DVB (6%)	Styrene + DVB
Type	Gel (pore size: 3 nm)	Macroporous (max pore size: 20-50 nm)
Active site	-SO <sub>3</sub> <sup>-</sup>	-SO <sub>3</sub> <sup>-</sup>
Capacity	1.4 Eq/L	1.1 Eq/L

#### 4.3.2.2. Alkaline extraction

Dry SCB was provided by eRcane (La Réunion, France) and ground on a 2 mm mesh by a knife mill (Mill F6 N V, Electra). The alkaline extraction conditions are based on Sun *et al.* study (1995) to optimize the extraction yield of lignin and hemicelluloses (Sun *et al.*, 1995). The conditions were the following: 150 g of SCB in 3 L of sodium hydroxide solution at 1.5% (w/v) in a 4 L jacketed glass reactor, leading to a solid:liquid ratio of 1:20 (w/v) and a NaOH:SCB ratio of 0.3:1 (w/w), under continuous stirring (200 rpm) for 6 h at 60 °C. The SCB solid residue was removed from the alkaline extract on Whatman filters grade 3 (150 mm diameter) on a Büchner filtration device, then dried at 50 °C for 48 h and finally ground by a microfine grinder (IKA MF 10 basic) on a 1 mm sieve prior to analysis. The filtrated alkaline extract was concentrated by Rotavap at 55 °C under 100 mbar. A dry solid content of at least 20% is generally required to reach a good

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productivity on the chromatographic purification step and make it economically viable at industrial scale.

### 4.3.2.3. Pulse tests

Pulse tests were run on 500 mL resin packed in a 1 m high and 26 mm diameter jacketed glass column. Two resins were tested, their characteristics are indicated in **Table 3.3**. Both resins are under Na<sup>+</sup> form as the main cation in the alkaline extract is Na<sup>+</sup> due to the sodium hydroxide introduced during the extraction step. The resin was mixed with water at 60 °C for degassing and packed from the top of the column. The upper piston was brought as close as possible to the top of resin to minimize the dead volume. A Y-valve successively enabled the injection of 5 mL feed (Blue Dextran, methanol, synthetic solutions or concentrated alkaline extract) or eluent (distilled water) on top of the column. The eluent was circulated from the top to the bottom of the column thanks to a peristaltic pump and its volume was accounted as resin Bed Volume (BV). The temperature of the column was maintained at 40 °C thanks to a water bath. At the outlet of the column, a fraction collector (GradiFrac, from Pharmacia Biotech) was set to collect 15 mL fractions representing 0.03 BV. The collected samples were analyzed after the run was completed. Blue Dextran (Sigma Aldrich, France) at 0.1% (w/v), was used to determine the void volume of the resin bed (i.e., inter-particles porosity), as it cannot enter the pores of the resins (2,000 kDa molecular weight) or interact with the resin matrix (Ladisch, 2001). A pulse test was also run with methanol at 5% (v/v) in order to determine the total void volume of the resin bed (i.e., inter- and intra-particles porosity), since methanol, a small uncharged molecule, can penetrate all the pores of the resins without adsorbing on the styrene-DVB matrix of the resins thanks to its polarity (Lodi et al., 2017).

### 4.3.2.4. Analytical methods

#### a) Dry solid and ash

Dry solid (DS) content was gravimetrically determined at 103 °C for 12 h and ash content at 500 °C for 12 h. The conductivity (mS/cm) was measured for every fractions

of the pulse tests and converted into ash concentration (g/L) from a linear relationship with a coefficient of 0.443.

### b) Carbohydrates and lignin

Based on Laboratory Analytical Procedure of the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008), Acid Insoluble Lignin (AIL) was gravimetrically quantified and Acid Soluble Lignin (ASL) was determined at 240 nm using an absorptivity constant of 25 L/g/cm. High Performance Liquid Chromatography (HPLC) on a Rezex RPM-Monosaccharide  $Pb^{+2}$  300 x 7.8 mm column (Phenomenex) in conjunction with a Rezex RPM-Monosaccharide  $Pb^{+2}$  50 x 7.8 mm guard column (Phenomenex) was used to quantify cellobiose, glucose, xylose, galactose, arabinose, mannose and fructose coming from the acidic hydrolysis of cellulose (yields glucose and potentially cellobiose if the hydrolysis is not complete), hemicelluloses (yields all C5 and C6 sugars) or residual sucrose. Prior to the injection, the samples were filtered on SPE cartridge ABW (Phenomenex) to remove the salts and avoid interference with the sugar peaks. Isocratic conditions were used with Milli-Q water at 0.6 mL/min, the injection volume was 20  $\mu$ L, the column was maintained at 80 °C and the RI detector at 50 °C. For alkaline extract and purified samples, HPLC on a Rezex RHM-Monosaccharide  $H^+$  300 x 7.8 mm column (Phenomenex) in conjunction with a Rezex RHM-Monosaccharide  $H^+$  50 x 7.8 mm guard column (Phenomenex) was used to quantify glucose, xylose, arabinose, acetic acid, furfural and HMF (Sluiter et al., 2006). Unlike with the RPM column, salts did not interfere with sugar peaks on the RHM column, therefore no desalting of the samples was required prior to the injection. Isocratic conditions were applied with 5 mmol/L  $H_2SO_4$  at 0.6 mL/min, the injection volume was 50  $\mu$ L, the column was maintained at 65 °C and the RI detector at 50 °C. Since solid residue, alkaline extract and purified fractions were very alkaline, the NREL protocol was adapted to ensure total hydrolysis of the sugar oligomers under acidic conditions. 150 mg of solid residue were analyzed instead of 300 mg (Sluiter et al., 2008) and liquid samples (Sluiter et al., 2006) were diluted by 4 with distilled water before acid hydrolysis.

### c) Monomeric sugars and hemicelluloses acetyl groups

Sulfuric acid was added to the alkaline extract to adjust its pH to 2, corresponding to the pH of HPLC eluent with RHM column, then the extract was analyzed on RHM column without running NREL protocol (Sluiter et al., 2006). pH adjusted samples directly injected on HPLC enabled the quantification of monomeric sugars and free acetic acid, whereas samples analyzed through NREL protocol gave the total amount of sugars (monomeric and oligomeric forms) and acetic acid (free and bound to hemicelluloses).

### d) Phenolic monomers

Quantification of twelve phenolic monomeric compounds potentially present in SCB alkaline extract (Xu et al., 2005; Capriotti et al., 2015) - gallic acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid (VA), syringic acid, 4-hydroxybenzaldehyde (4HBA), vanillin, *p*-CA, syringaldehyde, FA, sinapic acid and hydroxycinnamic acid - was studied by HPLC on an OmniSpher 3 C18 100 x 4.6 column (Agilent Technologies). The gradient was as follow: 91% acidified water (1% acetic acid (v/v)) and 9% acetonitrile for 25 min, from 9 to 90% acetonitrile in 5 min, kept constant for 5 min, then decreased back to 91% acidified water in 5 min and the column was equilibrated for 7 min between runs. The flow rate was 0.5 mL/min, the injection volume was 10  $\mu$ L and the column temperature was maintained at 25 °C. The UV detector was set at 280 nm. Concentrations for the calibration curves ranged between 0 and 200 mg/L. Standard and process samples were diluted in acetonitrile:water at a ratio of 50:50 (v/v) prior to injection.

### e) Pulse chromatography tracers

The Blue Dextran concentration was monitored by UV-Vis at 620 nm. The methanol concentration was followed by HPLC on Rezex RHM column under isocratic conditions with 5 mmol/L H<sub>2</sub>SO<sub>4</sub> at 0.6 mL/min, the injection volume was 50  $\mu$ L, the column was maintained at 60 °C and the RI detector at 50 °C.

### 4.3.3. Results and discussion

#### 4.3.3.1. Alkaline extraction

HPLC analysis of raw SCB on the RPM Rezex column revealed the presence of three main sugars: glucose, xylose and arabinose. Traces of galactose and mannose were detected but their concentrations were too low to enable a reliable follow-up at the extraction and purification stages. In other studies on SCB from China, galactose and mannose have been quantified in hemicelluloses at levels of about 2 to 3% and traces to 1%, respectively (Sun et al., 2004; Cheng et al., 2008). Fructose was not found in the raw SCB. The retention time and response factor of all the detected components on these two HPLC columns are presented in **Table 4.2**.

For every compound, the mass balance between the inlet (SCB) and the outlet (solid residue and alkaline extract) was close to 100% (**Table 4.3**). Glucan was almost fully recovered in the solid residue (95%) and its purity increased from 35.9 to 43.6%. Under the alkaline extraction conditions employed in this work, 22% of the xylan, 50% of the arabinan and 46% of the lignin were recovered in the alkaline extract. SCB hemicelluloses are composed of a xylan backbone on which arabinosyl substituents are bonded (Sun et al., 2004), the difference between xylan and arabinan yields found in our work suggested that the branched portions of hemicelluloses were preferably extracted compared to the xylose backbone. Besides, no significant quantity of furfural and HMF were detected in the alkaline extract, confirming that the alkaline extraction conditions did not produce these sugar degradation products. Indeed, furfural and HMF come from C5 and C6 sugar degradation, respectively, mainly in acidic conditions (Mosier et al., 2005). Most importantly, unlike in lignocellulosic acid extracts, no sugar monomers were found in the alkaline extract. In this extract, glucose, xylose and arabinose were under oligomeric form: glucan, xylan and arabinan, respectively. The same amount of free acetic acid and total acetic acid was found in the extract, indicating that all the acetate groups have been released from the solubilized hemicelluloses. Once released from the hemicelluloses in the alkaline extract, acetic acid concentration (8.1 g/L) was about 40% of the xylan concentration (19.4 g/L) and higher than the concentration of glucan (3.2 g/L) and arabinan (5.3 g/L). We could also consider that with these alkaline conditions, ester bonds linking *p*-CA and FA to hemicelluloses were all broken (Sun et al., 2004).

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**Table 4.2**

Retention time and response factor of identified components in the sugarcane bagasse on Rezex-RPM column with RI detector

Component	Retention time (min)	Response factor
Glucose	13.5	4.339
Xylose	14.4	4.086
Arabinose	16.4	4.128

Retention time and response factor of identified components in the sugarcane bagasse alkaline extract on Rezex-RHM column with RI detector

Component	Retention time (min)	Response factor
Glucose	10.8	11.357
Xylose	11.5	11.027
Arabinose	12.4	11.749
Acetic acid	16.5	8.023
HMF	30.6	13.422
Furfural	42.8	13.101

Retention time and response factor of identified components in the sugarcane bagasse alkaline extract on Omnispher 3 C18 column with UV detector at 280 nm

Component	Retention time (min)	Response factor
VA	6.8	0.515
4HBA	8.2	2.187
Vanillin	11.2	1.228
<i>p</i> -CA	13.7	1.445
FA	19.2	0.852

Out of the twelve phenolic monomers tested, five of them (VA, 4HBA, vanillin, *p*-CA, FA) were present in detectable quantities in the alkaline extract. The retention time and



response factor of the detected phenolic monomers are presented in **Table 4.2**. They accounted for 4.1% of the DS of the alkaline extract, *p*-CA being the main compound with 3.6% of the DS (**Table 4.3**).

**Table 4.3**

SCB, solid residue, alkaline extract and concentrated alkaline extract composition and extraction yields

Components	SCB	Solid Residue		Alkaline Extract		Concentrate
	Content	Content	Yield	Content	Yield	Composition
DS	92.5	96.4	59	2.6	35	19.2
Ash	9.9	19.7	36	56.1	61	53.6
Glucan	35.9	43.6	95	1.5	2	1.5
Xylan	19.4	16.6	67	9.4	22	9.0
Arabinan	2.3	1.6	53	2.5	50	2.5
AIL	21.6	13.8	50	21.1	45	18.0
ASL	5.5	3.8	54	6.2	52	5.2
Total Lignin	27.1	17.7	51	27.3	46	23.2
VA				traces		traces
4HBA				0.1		0.1
Vanillin				0.1		0.1
<i>p</i> -CA				3.6		3.4
FA				0.4		0.4
Total phenolic monomers				4.1		4.0
Mass closure	95.2	99.1		98.9		92.4

All the values are calculated based on the percentage of dry solid. All the analyses were run in duplicate, standard deviation was at most 1%

After concentration of the alkaline extract on Rotavap from 2.6% to 19.2% DS at 55 °C, no significant difference was observed in the composition of the alkaline extract and the concentrate (**Table 4.3**) and no degradation products were observed in the HPLC chromatograms. A progressive increase of pH by 0.75, at 55 °C did not hydrolyze sugars and phenolic oligomers nor degrade phenolic monomers. Concentrated SCB alkaline extract was composed of five major pools of molecules: 92.4 g/L salts, 38.5 g/L AIL, 27.9 g/L oligomeric sugars (19.4 g/L xylan, 5.3 g/L arabinan, 3.2 g/L glucan), 8.6 g/L phenolic monomers and 8.1 g/L acetic acid. Different concentration of these five same

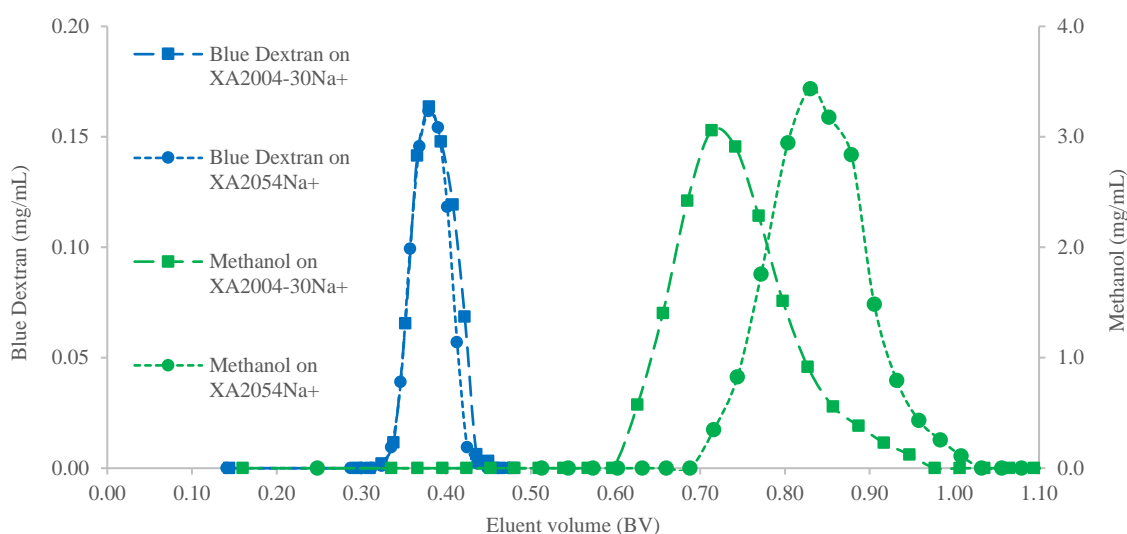
groups of molecules is unlikely to change their separation behavior by chromatography as demonstrated with pulse test run on SAC resin with lignocellulosic acid hydrolysates (Chen et al., 2018).

Hence, this SCB alkaline extract constituted a model lignocellulosic alkaline extract in order to study the separation of the solubilized molecules by low pressure chromatography.

### 4.3.3.2. Pulse tests

The mass balance was established between the inlet of the column (feed) and the outlet (the sum of the purified fractions) for every compound of every pulse test. All mass balances ranged between 90 and 110% indicating that there is no significant adsorption of any compound on the resins. ASL mass balance was largely over 100%, because the polystyrene-divinylbenzene backbone of the resins released compounds absorbing at 240 nm, so ASL was not presented in the results.

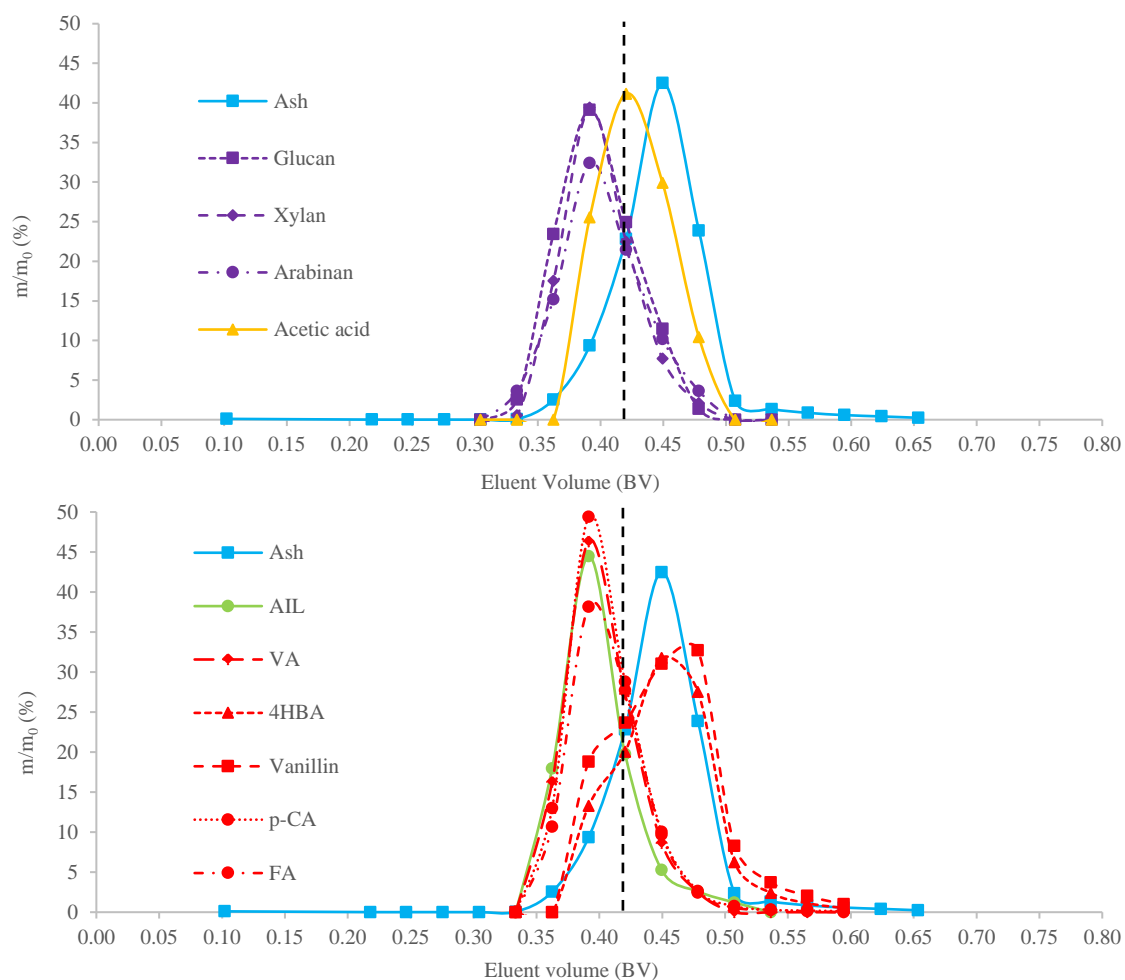
Blue Dextran pulse test showed a column void volume or inter-particle porosity of about 0.38 BV for both resins, with symmetric peaks, the front of the peak appearing at 0.33 BV due to diffusion phenomena (**Fig. 4.4**). All the molecules exiting the column at this BV were excluded from the resin pores either due to size exclusion or ionic repulsion. The pulse tests with methanol showed that the total void volume (i.e., inter- and intra-particle porosity) was 0.71 and 0.83 BV for the gel-type resin and the macroporous-type resin, respectively. The molecules eluting from 0.38 to 0.71 BV and from 0.38 to 0.83 BV for the gel-type and the macroporous type resins, respectively, can penetrate from a partial to a full extent into the resin pores.



**Fig. 4.4** Blue Dextran and methanol pulse tests at 2.26 m/h on the gel-type (XA2004-30Na<sup>+</sup>) and the macroporous-type (XA2054Na<sup>+</sup>) SAC exchange resins. Lines are presented to guide the eyes.

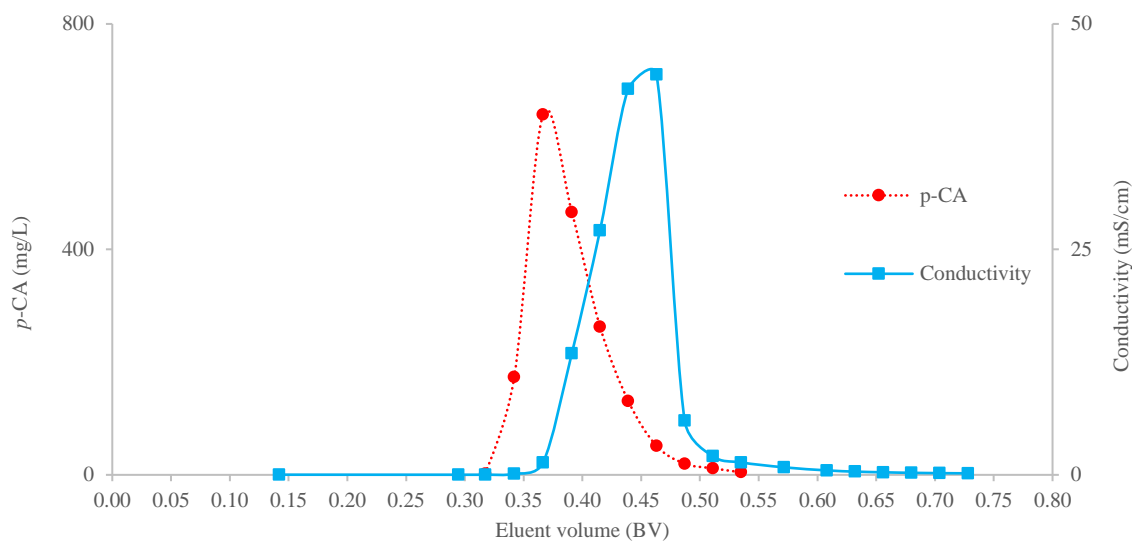
a) Molecules separation

Sugar oligomers, AIL and phenolic monomers with a carboxyl group (VA, *p*-CA and FA) were eluted at 0.38 BV and can be separated from salts, acetic acid and phenolic monomers with no carboxyl group (vanillin and 4HBA), on the XA2004-30Na<sup>+</sup> resin with an eluent velocity of 2.26 m/h (**Fig. 4.5**).



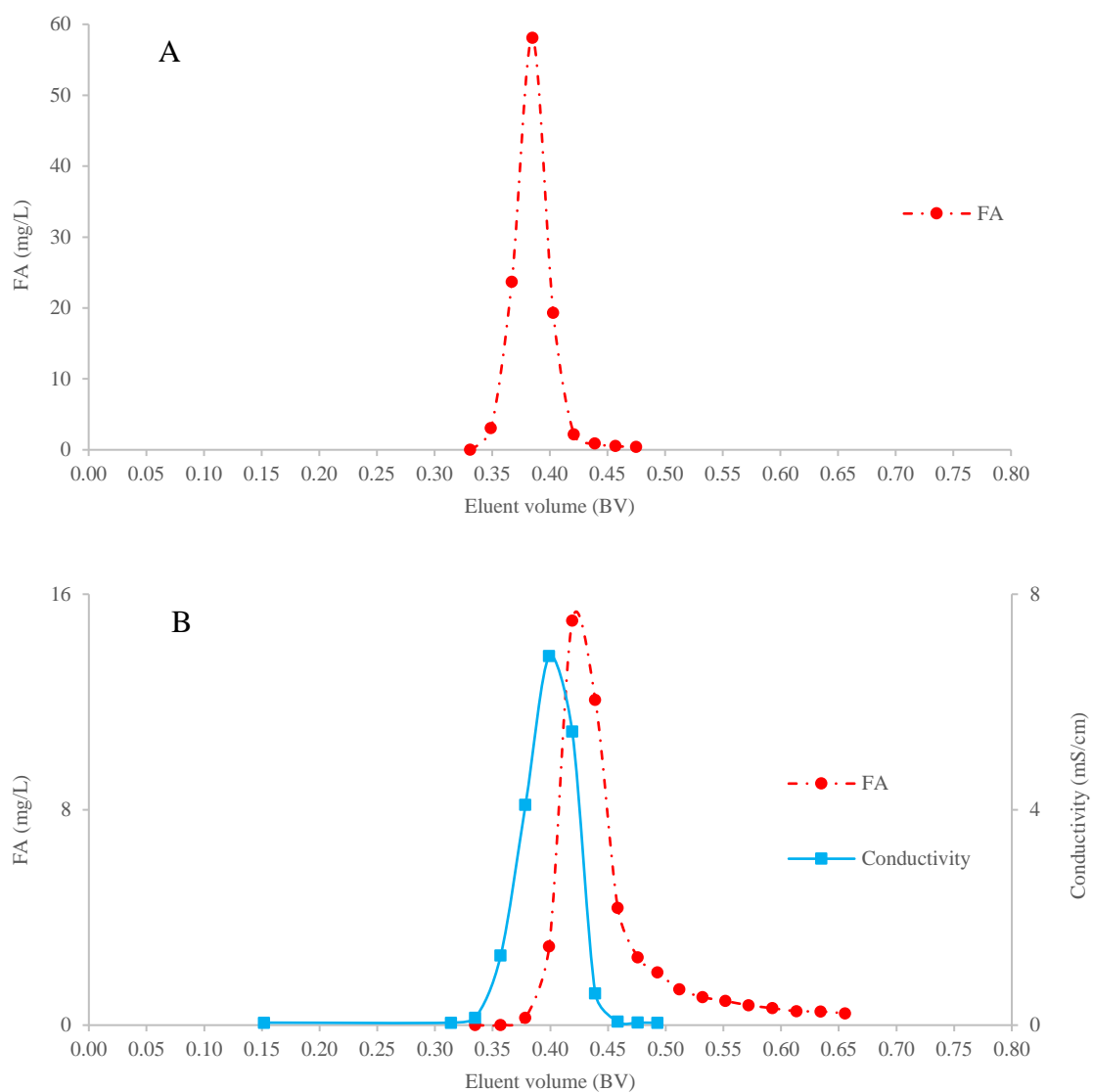
**Fig. 4.5** Concentrated alkaline extract pulse test on XA2004-30Na<sup>+</sup> with distilled water as eluent at 2.26 m/h, with  $m$  the mass of a given compound in the fraction and  $m_0$  its mass in the feed. Experiment was run in duplicate showing similar peaks shape and BV. Lines are presented to guide the eyes.

In order to confirm the behavior of the phenolic monomers, several pulse tests were run with synthetic solutions. With a solution of *p*-CA at 4.79 g/L and NaOH added to adjust the pH to 13.7 (pH of the concentrated alkaline extract), both compounds had the same retention volume and peak shape as in the alkaline extract (**Fig. 4.6**). This result confirmed that the behavior of *p*-CA was not due to interactions with other compounds of the concentrated alkaline extract. To study the influence of the charge of the phenolic monomers with a carboxyl group, pulse tests were run at lower pH.



**Fig. 4.6** *p*-CA synthetic solution at 4.79 g/L at pH=13.7 adjusted with NaOH, pulse test on XA2004-30Na<sup>+</sup> with distilled water as eluent at 2.26 m/h. Lines are presented to guide the eyes.

Due to the very limited solubility of *p*-CA in acid conditions, the next pulse tests were run with FA. A synthetic solution of FA at 200 mg/L (**Fig. 4.7A**) was eluted on the column. The fractions exiting the column had a very low conductivity (below 10  $\mu$ S/cm), leading to an unreliable pH measurement. FA peak exited the column at 0.38 BV indicating that FA was excluded from the resin pores, it must have been negatively charged. Therefore, a synthetic solution was prepared with FA at 200 mg/L, NaCl at 21.9 g/L in order to have a Na<sup>+</sup> concentration close to its concentration in the concentrated alkaline extract, and HCl to adjust the pH to 2.0 (**Fig. 4.7B**). At a pH more than 2 points below its pKa, FA was protonated and was eluted at about 0.42 BV. This retardation was caused by the ability of uncharged FA to penetrate partially into the resin pores. These pulse tests confirmed that despite having approximately the same size and structure, VA, 4HBA, vanillin, *p*-CA and FA did not present the same behavior on gel-type SAC exchange resin under alkaline conditions. Phenolic monomers with a carboxyl group can be negatively charged when the pH is over their pKa and ionic repulsion prevented them from diffusing inside the pores of the gel-type resin. Unexpectedly, conductivity, mainly due to NaCl (**Fig. 4.7B**), exited the column at 0.38 BV, showing that NaCl was totally rejected by ionic repulsion from the resin pores, whereas NaOH was eluted at 0.45 BV. Different salts were not excluded to the same extent of the resin pores.



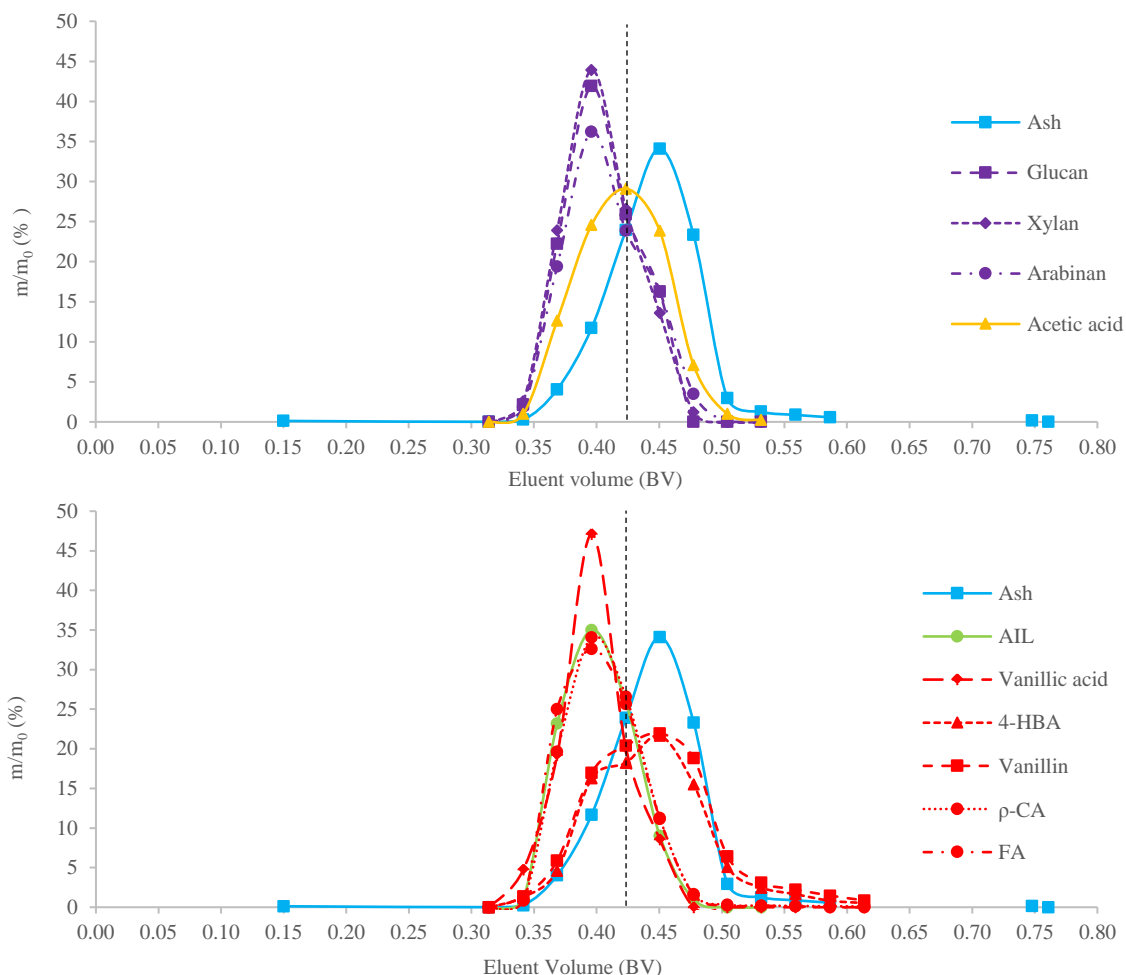
**Fig. 4.7** FA synthetic solutions pulse tests on XA2004-30Na<sup>+</sup> with distilled water as eluent at 2.26 m/h. (A) FA at 200 mg/L at pH=3.73. (B) FA at 113 mg/L, NaCl at 22.3 g/L and pH adjusted to 2.0 with HCl. Lines are presented to guide the eyes.

#### b) Flow rate influence

The flow rate was increased from 20 mL/min to 40 mL/min in order to define the influence of the hydrodynamic flow on the separation. On the XA2004-30Na<sup>+</sup> resin with an eluent velocity of 4.52 m/h, the BVs of the different compounds were the same as at 2.26 m/h, but the separation was less efficient, due to the broadening of the peaks (**Fig. 4.8**). This can be quantified by the resolution ( $R_s$ ) given by Eq. (14) (Kromidas and Kuss, 2009):

$$R_s = 1.18 \frac{(t_{R2} - t_{R1})}{(W_{h1} + W_{h2})} \quad (14)$$

With  $t_{R1}$  and  $t_{R2}$  retention time of compounds 1 and 2,  $W_{h1}$  and  $W_{h1}$  the width of the peaks at half height.



**Fig. 4.8** Concentrated alkaline extract pulse test on XA2004-30Na<sup>+</sup> with distilled water as eluent at 4.52 m/h, with  $m$  the mass of a given compound in the fraction and  $m_0$  its mass in the feed. Experiment was run in duplicate showing similar peaks shape and BV. Lines are presented to guide the eyes.

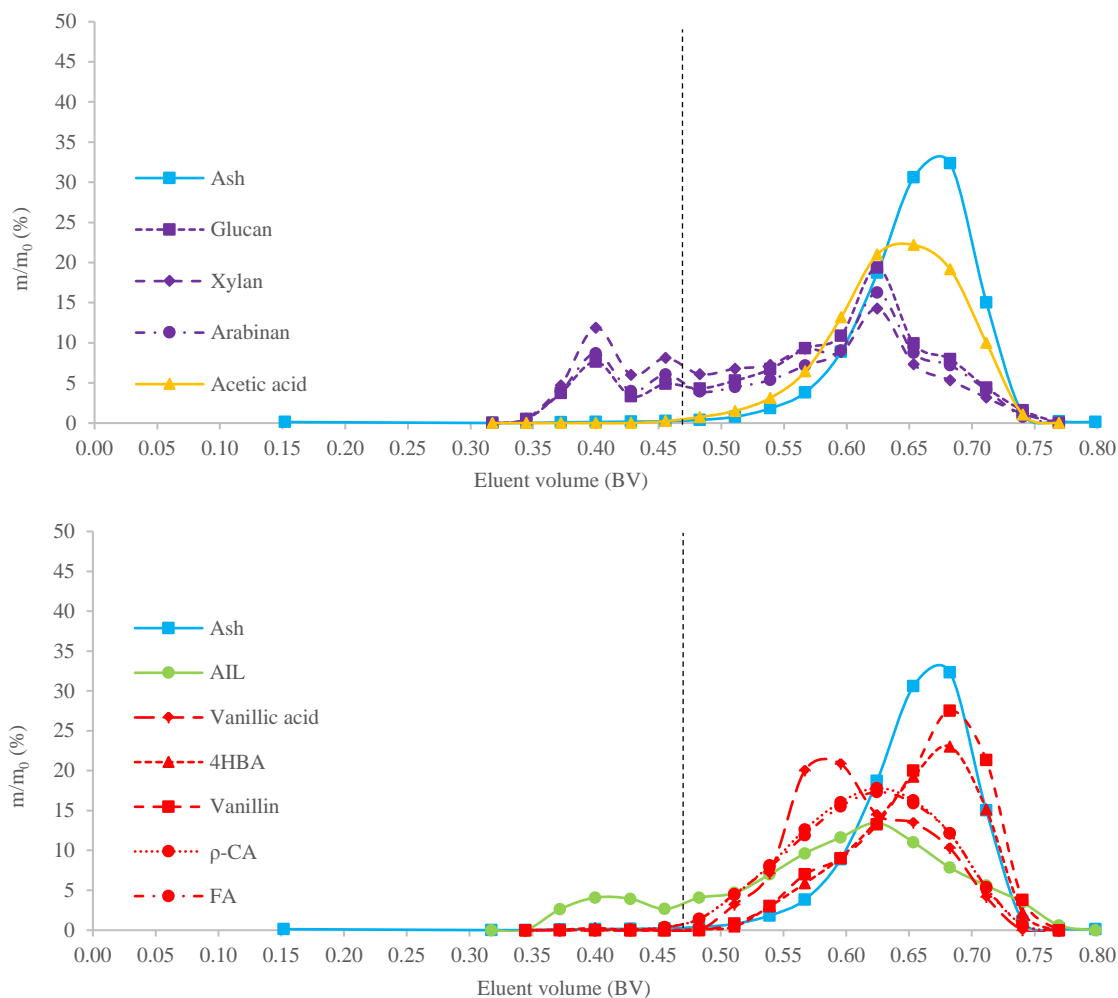
For instance, at 20 mL/min the resolution for the xylose/ash separation was 0.024, whereas at 40 mL/min the resolution decreased to 0.016. Increasing the eluent velocity led to a lower peak resolution, showing that the optimal velocity for this system is below 4.52 m/h according to the Van Deemter equation (Ladisch, 2001). Increasing the flow

rate induced an increase of the dispersion due to hydrodynamic hindering and thus the enlargement of the peaks, but did not reduce the influence of the electrostatic forces on the rejection of the small ionized molecules. However, the loss of resolution is counter balanced by the gain in productivity due to a higher eluent flow rate.

### c) Pore size influence

The influence of the size of the resin pores on the separation of the different compounds of the alkaline extract was studied by running another pulse test on the macroporous-type XA2054-Na<sup>+</sup> resin. This resin presents wider pores - 20 to 50 nm – and a more heterogeneous pore size distribution, but otherwise it has similar properties as the gel-type XA2004-30Na<sup>+</sup> resin (**Table 3.3**). The SCB alkaline extract elution on the XA2054-Na<sup>+</sup> resin showed that a pool of molecules constituted of sugar and AIL oligomers eluted at 0.40 BV (**Fig. 4.9**). Different sugar peaks were observed between 0.40 and 0.77 BV probably representing different size groups of sugar oligomers well separated from each other. On the gel-type and macroporous-type resins, xylan, arabinan and glucan profiles exhibited the same tendency, suggesting that xylose, arabinose and glucose are linked and part of the same oligomer structure. AIL presented a single massive peak from 0.45 to 0.77 BV suggesting a more homogeneous size dispersion of AIL oligomers compared to sugar oligomers. We can consider the lignin fragments as dissociated from each other and their rejection representative of their size and not of their aggregation. Indeed, the pH of the collected samples after 0.33 BV ranged between 10.8 and 12.9, so acidic groups in lignin are dissociated yielding polyelectrolytes formation and preventing the aggregation of lignin molecules that is observed at pH below 10.5 (Wong and de Jong, 1996). Phenolic monomers with a carboxyl group (VA, *p*-CA and FA) were recovered after 0.47 BV like the uncharged phenolic monomers (vanillin and 4HBA). Acetic acid and salts were also eluted later than on the gel-type resin. This was due to a larger intra-particle volume on the macroporous-type resin. The resin pores were too wide to prevent charged molecules from penetrating into the pores because of ionic repulsion. It confirmed the observation made with acetic acid and salts on XA2004-30Na<sup>+</sup> gel-type resin.





**Fig. 4.9** Concentrated alkaline extract pulse test on XA2054- $\text{Na}^+$  with distilled water as eluent at 2.26 m/h, with  $m$  the mass of a given compound in the fraction and  $m_0$  its mass in the feed. Experiment was run in duplicate showing similar peaks shape and BV. Lines are presented to guide the eyes.

If the limit between the two fractions was set at 0.47 BV (**Fig. 4.9**), recoveries in the fraction before 0.47 BV for xylan, arabinan, glucan and AIL were 32, 26, 20 and 14%, respectively. They can be completely separated from smaller sugar oligomers, phenolic oligomers, all phenolic monomers, acetic acid and ash. Recoveries of all phenolic monomers, acetic acid and ash were all over 99% in the fraction after 0.47 BV, leading to a conductivity in the fraction before 0.47 BV below  $100 \mu\text{S}/\text{cm}$ . The use of macroporous-type SAC resin enabled to produce a pure polymer fraction containing only the largest oligomers of lignin and hemicelluloses.

## Chapter 4: PURIFICATION BY CHROMATOGRAPHY

Soft alkaline extraction conditions helped producing large oligomers (Sun et al., 2004; El Mansouri and Salvadó, 2006) that can be better purified in a single economical step by low pressure chromatography on macroporous-type SAC cation resin. These polymers could be interesting for some applications where long chain of lignin or hemicelluloses are looked for, as non-exhaustively in coatings, surfactants, adhesives and cosmetics applications. (Werpy et al., 2004; Holladay et al., 2007).

### 4.3.4. Conclusion

Alkaline pretreatment of lignocellulosic biomass produced original pools of molecules – oligomeric lignin, oligomeric hemicelluloses, phenolic monomers, acetic acid and inorganic salts – compared to the acid pretreatment.

For the first time, purification of a lignocellulosic alkaline extract was studied by chromatography on SAC exchange resins with water as eluent, without chemical addition. SCB alkaline extract elution on a gel-type SAC exchange resin enabled the separation of sugar oligomers, AIL and phenolic monomers with a carboxyl group from ash and neutral phenolic monomers. However, acetic acid was partitioned between these two pools of molecules. This procedure could be used for the demineralization of the sugars and lignin or for the separation between phenolic monomers with a carboxyl group from the other phenolic monomers or salts.

Pure fraction of the biggest AIL and sugar oligomers was obtained using macroporous-type SAC resin, while all the other components of the SCB alkaline extract were recovered in another fraction. Playing on the porosity of the SAC resin can help to adjust the fractionation of the different pools of molecules.

This purification technique could be part of an integrated process, with other purification techniques such as precipitation, membrane filtration or crystallization, whose goal would be to obtain pure molecules from a lignocellulosic alkaline extract. Lignin and hemicelluloses obtained on macroporous-type resin could be separated by precipitation with alcohol for instance as mentioned previously, to keep the high purity of both lignin and hemicelluloses.

The transfer from pulse chromatography to simulated moving bed (SMB) would improve the selectivity of the process. These pulse tests gave enough information to

consider that an efficient separation will be performed with a SMB, but yield, purity, productivity and utilities consumption at pilot scale are to be investigated.

### Acknowledgements

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### 4.4. Conclusion

Chromatography as a purification technique on lignocellulosic acid extracts has been reported in many studies to separate inorganic acid and monomeric sugars, whereas a few recent studies referred to the separation of monomeric sugars from one another. We confirmed this second point on a synthetic solution of sugars using a gel-type strong acid cation exchange resin with  $\text{Ca}^{2+}$  as counter ion for the stationary phase and water for the mobile phase. The same resin with  $\text{Na}^+$  as counter ion tested on the purification of SCB mild alkaline extract showed that its components were separated in two pools of molecules: one fraction containing lignin and sugar oligomers and phenolic monomers with a carboxyl group, the other fraction containing inorganic salts and phenolic monomers without carboxyl group. The use of a similar resin differing only by its larger pore size (macroporous-type) under the same conditions led to the separation of a very pure fraction containing about 30% of the sugars and 15% of the lignin without inorganic salts, acetic acid and phenolic monomers. This pure fraction is composed by the largest sugar and lignin oligomers. Preliminary analyses by Size Exclusion Chromatography (SEC) with LS, UV and RI detectors, from the team of Professor José Kovensky working in the Laboratoire de Glycochimie des Antimicrobiens et des Agroressources (LG2A), Amiens, France, confirmed this point with oligomer size above 300 kDa.

## Chapter 5:

# INTEGRATED PURIFICATION PROCESS

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**Integrated process combining membrane filtration, pulse chromatography and precipitation to obtain purified components from a sugarcane bagasse mild alkaline extract**

Vincent Oriez\*, Jérôme Peydecastaing, Pierre-Yves Pontalier\*

Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, 4 allée Emile Monso, 31030 Toulouse, France

\*Corresponding authors at: Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France

*E-mail addresses:* [vincentoriez@yahoo.fr](mailto:vincentoriez@yahoo.fr), [vincent.oriez@ensiacet.fr](mailto:vincent.oriez@ensiacet.fr) (V. Oriez), [pierreyves.pontalier@ensiacet.fr](mailto:pierreyves.pontalier@ensiacet.fr) (P.Y. Pontalier)

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This paper will be submitted soon

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Abstract

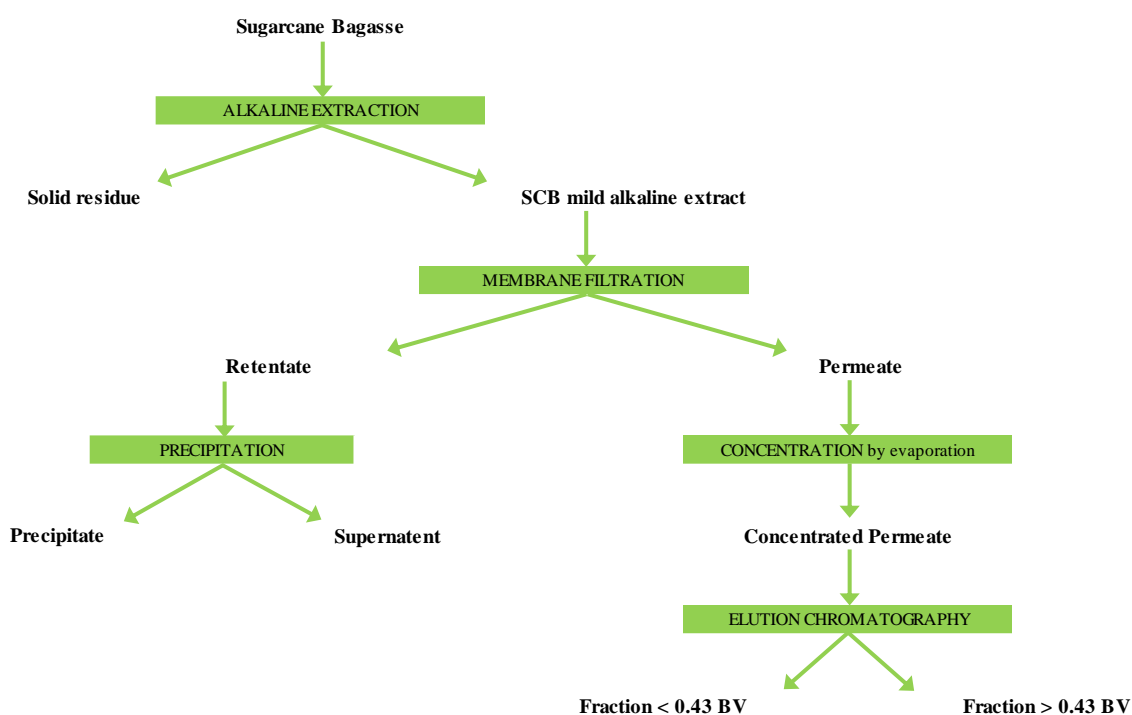
In the frame of the ethanol lignocellulosic biorefinery concept, pretreatment of sugarcane bagasse was made in mild alkaline condition (1.5% NaOH (w/v), solid:liquid ratio of 1:20 (w/v), 60 °C, 6h). Recovery of 95% of the initial cellulose in the solid residue combined with the solubilization of 64% of the lignin and 40% of the hemicelluloses in the extract were obtained. The alkaline extract, usually considered a by-product, contained lignin oligomers, hemicelluloses oligomers, acetic acid (from acetate groups on the hemicelluloses), phenolic monomers and inorganic salts. Downstream processing was run at low temperature (40-70 °C) to separate the major components of the alkaline extract. First, ultrafiltration (UF) on 10 kDa polysulfone hollow fiber membrane was used to separate large molecules (retentate stream) –lignin and hemicelluloses oligomers – from smaller molecules (permeate stream) – inorganic salts, acetic acid, phenolic monomers. UF was run in concentration mode until fouling limitation of the membrane was reached, then diafiltration mode was tested to further purify the retentate and increase the recovery of small molecules in the permeate. Permeate was concentrated by evaporation to make the following chromatographic step economically viable. Chromatography on strong acid cation exchange resin using water as eluent was run on the UF permeate and a fraction enriched in vanillic acid (VA), *p*-coumaric acid (*p*-CA)

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and ferulic acid (FA) was obtained, whereas the other fraction contained other phenolic monomers without carboxylic function and inorganic salts. From the UF retentate, precipitation of the lignin by acid addition and precipitation of the hemicelluloses by ethanol addition were tested. For the whole process, a particular attention was put on limiting chemical, water and energy consumption to make the whole process environmentally friendly and economically sustainable.

Keywords: *Sugarcane bagasse fractionation, hemicelluloses, lignin, ultrafiltration, low pressure chromatography, acid precipitation*

### Graphical abstract





## 5.1. Introduction

Second generation biorefineries aim at producing fuels, chemicals and materials from lignocellulosic materials. The first step consists in the fractionation of lignocellulose into cellulose, hemicelluloses and lignin to make further valorization of these pools of molecules possible. Various techniques exist for this fractionation step such as biological, physical, chemical and physico-chemical treatments (Alvira et al., 2010). Nowadays, chemical treatments with concentrated acid, dilute acid or both sequentially applied are mostly used because of their higher efficiency and lower cost, and have been already applied at industrial scale. However, another chemical treatment with mild alkaline conditions has been shown in various studies to be more efficient than acid treatments for the production of ethanol from the fractionated cellulose (Hernández-Salas et al., 2009; Wu et al., 2011b). The major hurdle for the implementation of this treatment at larger scale is its higher cost compare to acid treatments (Sánchez and Cardona, 2008). Lignocellulosic mild alkaline extracts usually considered by-products, contain hemicelluloses oligomers, lignin oligomers, phenolic monomers, acetic acid and a high salt content. Their purification, i.e., separation of the various pools of molecules, is necessary to enable their further valorization of the molecules and make the biorefineries using alkaline treatment economically viable.

Hemicelluloses can be valorized in their oligomeric form for instance as polymeric blend films (Ruiz et al., 2013) or as sugar monomers for further fermentation into ethanol (biofuels) or reduction into lactic acid (chemical intermediates) for example (Werpy et al., 2004). Likewise, lignin in their oligomeric form present potential valorization for the replacement of phenol-formaldehyde foam or the generation of epoxy polymers (Holladay et al., 2007) and monomeric phenol, e.g., *p*-CA or FA can be used as functional ingredients for example (Zhao et al., 2011). The main components of alkaline extracts in terms of dry solid content are the inorganic salts introduced at the pretreatment step. Their recovery during downstream processing to recycle them at the pretreatment step is of major importance for the economic and environmental sustainability of the process.

The existing studies on the purification of lignocellulosic mild alkaline extract focused on one given component, combining various techniques to reach good purity. For instance, the combination of ultrafiltration (UF) on a 30 kDa membrane retaining

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hemicelluloses and removing salts and color, adsorption on anion exchange resin to remove more color compounds and ethanol precipitation to reach a purity of 95% for hemicelluloses from a wheat bran mild alkaline extract (Zeitoun et al., 2010). Another process yielding 95.2% purity for *p*-coumaric acid from sugarcane bagasse (SCB) mild alkaline extract, combined UF on a 3 kDa membrane to remove hemicelluloses and lignin in the retentate, adsorption with activated charcoal (AC) of phenolic compounds, adsorption with anion exchange resin then desorption with a water:ethanol:HCl solution with a ratio of 36:60:4 (v/v/v) and crystallization of *p*-CA by removing ethanol (Zhao et al., 2011). Integrated process combining several purification techniques to obtain various pool of purified molecules has not been studied yet.

Based on previous chromatographic (Oriez et al., 2018) and membrane filtration (Oriez et al., 2019) experiments, size exclusion cannot be used as a separation method between lignin and hemicelluloses. Acid addition to strong alkaline extract (black liquors) is the traditional technique used by the pulp and paper industry to precipitate lignin and separate them from hemicelluloses (Uloth and Wearing, 1989). Acid precipitation of lignin presented higher yield at temperatures higher than 50 °C (Minu et al., 2012), large flocks facilitating precipitate/supernatant separation occurred at temperatures higher than 70 °C (Glasser and Wright, 1998), however temperature above 85 °C produced flocks too big that hinder the mixing of the solution (Uloth and Wearing, 1989). More recently, ethanol addition has been applied to lignocellulosic mild alkaline extracts to precipitate and purify hemicelluloses (Peng et al., 2010; Zeitoun et al., 2010). High hemicelluloses precipitation yields (70-95%) are obtained with high ethanol:alkaline extract ratio (4:1 to 9:1 (v/v)), and temperature are usually kept between 5 and 25 °C (Brillouet et al., 1982; Bian et al., 2010; Peng et al., 2011; Xu et al., 2014). No comparison in terms of efficiency (recovery and purity) has been established yet between the two precipitation processes.

The goal of this work was to study an original integrated process combining membrane filtration with chromatography of the permeate and precipitation by either acid or ethanol addition of the retentate to produce purified pools of molecules from a model lignocellulosic biomass. Purities and recoveries (also referred as composition of a given stream and yield of a component) were thoroughly followed on the various streams generated by the mild alkaline fractionation of sugarcane bagasse (SCB) and by the next purification steps applied on the mild alkaline extract.

## 5.2. Materials and methods

### 5.2.1. Chemicals

Sodium hydroxide ( $\geq 98.5\%$  purity) for the alkaline extraction, sulfuric acid (72% (w/w)) for NREL hydrolysis and precipitation test, sulfuric acid (95% (w/w)) and acetonitrile ( $\geq 99.9\%$ ) for HPLC eluents, ethanol (96% (v/v)) for precipitation tests were purchased from VWR, and calcium carbonate (NREL protocol) was obtained from Merck. The following HPLC standards were purchased from Sigma Aldrich: D-(+)-cellobiose ( $\geq 98\%$ ), D-(+)-glucose ( $\geq 99.5\%$ ), D-(+)-galactose, L-(+)-arabinose (99%), D-(+)-xylose (99%), acetic acid ( $\geq 99\%$ ), furfural (99%), 5-hydroxymethyl-2-furfuraldehyde (99%), gallic acid (97%), 4-hydroxybenzoic acid ( $\geq 99\%$ ), caffeic acid ( $\geq 98\%$ ), vanillic acid (97%), syringic acid ( $\geq 95\%$ ), 4-hydroxybenzaldehyde (98%), vanillin (99%), *p*-coumaric acid ( $\geq 98\%$ ), syringaldehyde (99%), *trans*-ferulic acid ( $\geq 99\%$ ), sinapic acid ( $\geq 98\%$ ), *trans*-3-hydroxycinnamic acid (99%).

### 5.2.2. Alkaline extraction

Dry SCB was provided by eRcane (La Réunion, France) and ground on a 2 mm mesh by a knife mill (Mill F6 N V, Electra). The mild alkaline extraction was carried out on 3 kg of SCB and 60 L of 1.5% NaOH (w/v) in a stainless steel-lined vessel (De Dietrich), resulting in S:L ratio of 1:20 (w/v) and NaOH/SCB ratio of 1:3 (w/w), with continuous mechanical stirring (200 rpm), at 60 °C for 6 h. These are optimized conditions reported by Sun *et al.* (1995), for maximizing the recovery of hemicelluloses and lignin by the mild alkaline pretreatment of wheat straw (Sun *et al.*, 1995). The SCB solid residue was removed from the alkaline extract using a top-discharge vertical basket centrifuge (RC 50 PX R, Rousselet) equipped with a polypropylene bag with 5  $\mu\text{m}$  pores. This residue was rinsed with distilled water, dried at 50 °C for 48 h and ground using a microfine grinder (IKA MF 10 basic) on a 1 mm sieve before analysis. The filtered SCB alkaline extract and the filtered solution employed to rinse the solid residue were mixed, analyzed and used as the feed for the membrane filtration experiments (mixture referred to hereafter as the SCB alkaline extract).

### 5.2.3. Membrane filtration

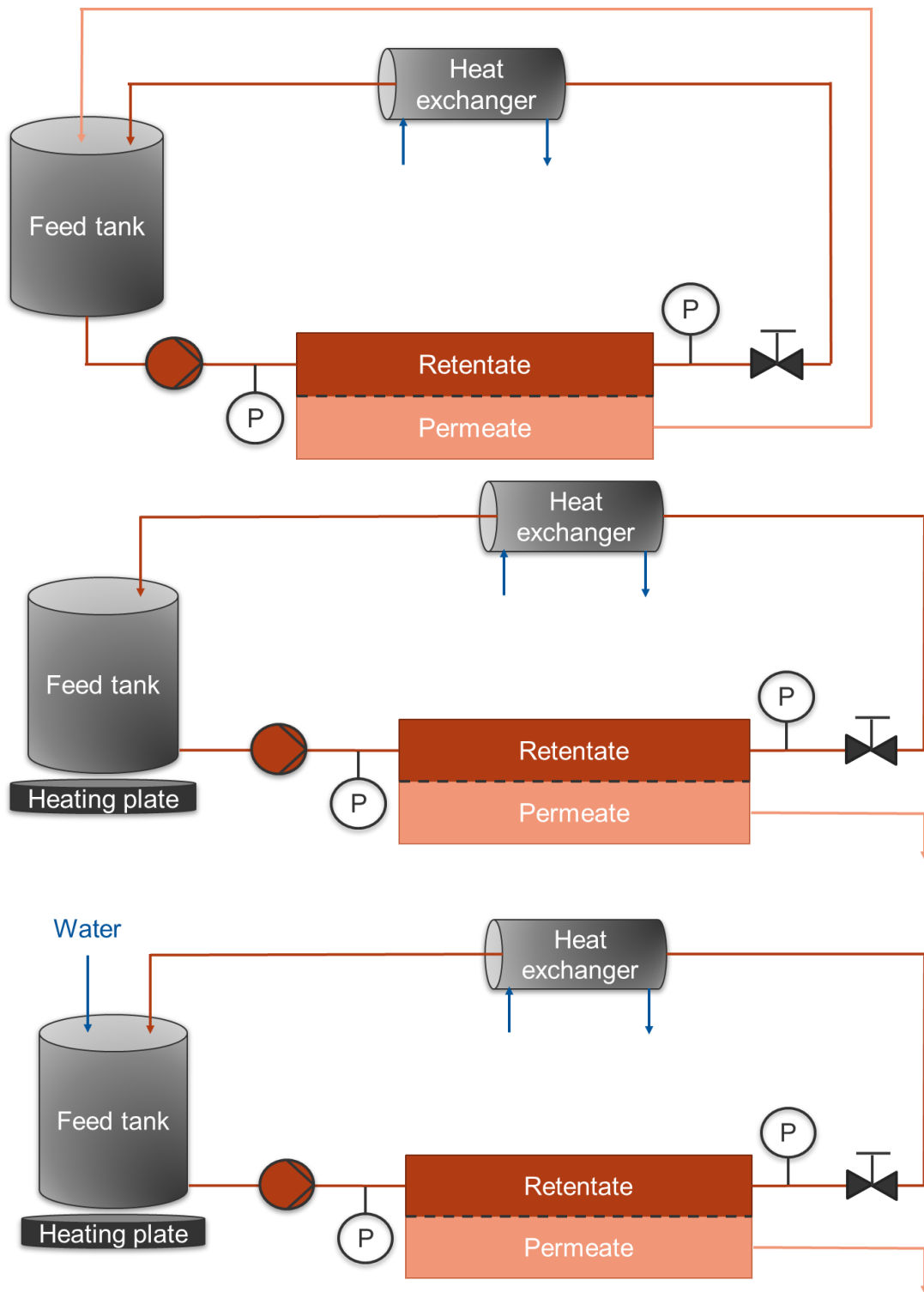
Membrane filtration was carried out on the filtered SCB alkaline extract. The feed tank contained 5 L of water or filtered SCB alkaline extract. The feed was circulated in the membrane with a gear pump (Johnson Pump, model 10/0005). Feed flow was measured with a flowmeter (Rosemount, Mexico). Permeate flux was assessed by collecting permeate over a given time period and weighing the sample collected. TMP was set with a valve on the retentate stream and checked with two manometers (Tecsis), one on either side of the membrane. The temperature was maintained at 20 °C during the membrane screening experiments, with a monotube heat exchanger located in the retentate flow. The temperature was maintained at 40 °C using a hot plate (Heidolph) under the feed tank, during the membrane filtration experiments in concentration and diafiltration modes (**Fig. 5.1**).

First, five new PS hollow fiber membranes (GE Healthcare) and two Kerasep ceramic tubular membranes (Novasep Process) with various MWCO were tested (**Table 3.3**) to determine which membrane presented the best separation potential based on the rates of rejection ( $R$ ) of the components of the SCB alkaline extract. Experiments were run in recycling mode, with both the retentate and the permeate recirculated to the feed tank (**Fig. 5.1**).

The rejection or retention rate  $R$  is given by Eq. (7):

$$R=1-\frac{C_P}{C_R} \quad (7)$$

where  $C_P$  and  $C_R$  are the solute concentrations (g/L) in the permeate and the retentate streams, respectively.



**Fig. 5.1** Set-up of the filtration systems for the filtration of sugarcane bagasse mild alkaline extract (A) recycling mode for the membrane screening, (B) concentration mode, (C) diafiltration mode.

**Table 5.1**

Characteristics of the membranes used for the filtration of sugarcane bagasse mild alkaline extract

	<b>UFP-1-C- 4X2M</b>	<b>UFP-5-E- 4X2MA</b>	<b>UFP-10-E- 4X2MA</b>	<b>UFP-30-E- 4X2MA</b>	<b>UFP-50-E- 4X2MA</b>	<b>K1335</b>	<b>K927</b>
Module configuration	Hollow fiber	Hollow fiber	Hollow fiber	Hollow fiber	Hollow fiber	Tubular	Tubular
Membrane material	Polysulfone	Polysulfone	Polysulfone	Polysulfone	Polysulfone	ZrO <sub>2</sub> /TiO <sub>2</sub>	ZrO <sub>2</sub> /TiO <sub>2</sub>
Membrane area (cm <sup>2</sup> )	1400	850	850	850	850	75	75
Number of fibers/channels	140	50	50	50	50	1	1
Channel inner diameter (mm)	0.5	1	1	1	1	6	6
Molecular weight cut-off (kDa)	1	5	10	30	50	5	15
Range of feed pH	2-13	2-13	2-13	2-13	2-13	0-14	0-14
Range of feed temperature (°C)	0-80	0-80	0-80	0-80	0-80	0-80	0-80
Range of feed pressure (bar)	0-3.1	0-3.1	0-3.1	0-3.1	0-3.1	0-6	0-6
Initial water flux (L/h/m <sup>2</sup> /bar) at 20 °C	21.3	40.7	47.5	76.3	113.2	38.1	57.0

Before each experiment, the new membrane (PS hollow fiber membranes were stored in glycerol), was washed several times with an ethanol/water solution (1:1, v/v), rinsed with water and the initial water flux (IWF) was measured at 20 °C and various TMP values (**Table 3.3**). Water was drained from the installation and the SCB alkaline extract was loaded into the feed tank and recirculated at a TMP of 0.8 bar until the flux was stable over time (about 15 min) and a quasi-stationary state was reached. Permeate flux was measured at different TMP values, from 0.8 to 2.8 bar, at shear rates of 3408 s<sup>-1</sup> for ceramic tubular membranes (corresponding to a cross-flow velocity of 2.6 m/s) and 3396 and 10,187 s<sup>-1</sup> for PS hollow fiber membranes (corresponding to a cross-flow velocity of 0.4 and 1.3 m/s, respectively). Three permeate samples were collected for analysis at three different TMP values (0.8, 1.6 and 2.4 bar). The feed volume was large enough relative to the total volume of permeates collected for analysis to assume that the composition of the SCB alkaline extract remained constant throughout each experiment. The final retentate was collected for analysis, to confirm that there had been no change in the composition of the SCB alkaline extract during the filtration process. At the end of the experiment, the SCB alkaline extract was drained and the membrane was washed several times with water.

In a second time, SCB mild alkaline extract was ultrafiltered in concentration mode on the 10 kDa PS hollow fiber membrane, which was the best membrane selected after the membrane screening. Concentration was run in semi-batch conditions, meaning that permeate was collected and retentate was recycled (**Fig. 5.1**). Concentration was expressed as volume reduction factor (*VRF*), calculated as follow, Eq. (8):

$$VRF = \frac{V_{initial}}{V_{final}} \quad (8)$$

Where  $V_{initial}$  is the volume of the feed at the beginning of the concentration experiment and  $V_{final}$  is the volume of the feed at the end of the concentration experiment, i.e., the final retentate.

Then, the retentate was continuously diafiltrated with water until 3.0 diavolumes were reached. A peristaltic pump (Cole Parmer Masterflex) was used to add distilled water at 40 °C in the feed tank at the same flow as the permeate flow (constant volume in the feed tank) (**Fig. 5.1**). Constant volume diafiltration (i.e., continuous diafiltration) is more

efficient than volume reduction diafiltration, for instance on molecules with 0% retention, 3 diavolumes addition gives 88% recovery in the permeate with volume reduction by a 2-fold factor whereas 95% recovery is reached with continuous diafiltration (Schwartz, 2003)

When the observed retention is constant during concentration, the yield of a compound is given by Eq. (9):

$$Yield = VRF^{R-1} \quad (9)$$

### 5.2.4. Chromatography

The permeate from the concentration and diafiltration of the SCB alkaline extract was concentrated by an 8.2-fold factor in order to reach about 20% DS to feed the chromatography. Concentration was made by evaporation on a Rotavap (brand) at 45 °C and 70 mbar. About 8 h were necessary to concentrate 1000 mL of permeate.

Batch-column chromatography was run following the conditions described in our previous work with a gel-type strong acid cation exchange resin (XA2004-30Na<sup>+</sup>, Novasep) (Oriez et al., 2018)

The resolution  $R_s$  of the separation between two components by chromatography is given by Eq. (14):

$$R_s = 1.18 \frac{(t_{R2} - t_{R1})}{(W_{h1} + W_{h2})} \quad (14)$$

With  $t_{R1}$  and  $t_{R2}$  the retention time of compounds 1 and 2,  $W_{h1}$  and  $W_{h2}$  the width of the peaks at half height.

### 5.2.5. Precipitation

The retentate from the UF in concentration mode then diafiltration mode was precipitated in order to separate lignin oligomers (AIL) from hemicelluloses oligomers (xylan and arabinan) by the addition of sulfuric acid or ethanol.



### 5.2.5.1. Precipitation by acid addition

Sulfuric acid at 72% (w/w) was added to 100 mL of UF retentate kept at 70 °C by a hot plate (Heidolph) with continuous magnetic stirring (600 rpm), until pH 1.9 was reached. When the temperature of the resulting mixture reached room temperature, the precipitate was separated from the supernatant by centrifugation at 10,000 g for 5 min, dried at 50 °C for 48 h and dry ground in a mortar and pestle before analysis.

### 5.2.5.2. Precipitation by ethanol addition

Ethanol at 96% (v/v) was added to 100 mL of retentate at room temperature with continuous magnetic stirring (200 rpm) at two ethanol:retentate ratio: 1:4 (v/v) and 1:9 (v/v). The resulting mixtures were left overnight at 5 °C and the precipitates were separated from the supernatants by centrifugation at 10,000 g for 5 min, dried at 50 °C for 48 h and dry ground in a mortar and pestle before analysis.

### 5.2.6. Analytical methods

The following analytical methods were applied to the initial SCB, the mild alkaline extract, the various retentates and permeates obtained by the UF treatment of the mild alkaline extract, the concentrated permeate and the resulting precipitate, the fractions obtained at the outlet of the pulse chromatography run on the concentrated UF permeate, and on the precipitates produced by the precipitation tests on the UF retentate.

#### 5.2.6.1. Dry solid and ash

Dry solid (DS) content was determined gravimetrically by heating at 103 °C for 12 h and ash content was determined at 500 °C for 12 h: 1 g was used for solid samples, 1 mL was used for alkaline extract and UF retentate samples, and 5 mL was used for UF permeate samples and fractions from the pulse chromatography.

#### 5.2.6.2. Carbohydrates and lignin

Based on Laboratory Analytical Procedure of the National Renewable Energy Laboratory (Sluiter et al., 2008), acid-insoluble lignin (AIL) was quantified gravimetrically and acid-soluble lignin (ASL) was determined spectrophotometrically, at

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a wavelength of 240 nm using an absorptivity constant of 25 L/g/cm. High-performance liquid chromatography (HPLC) on a Rezex RPM-Monosaccharide  $\text{Pb}^{+2}$  300 x 7.8 mm column (Phenomenex), used in conjunction with a Rezex RPM-Monosaccharide  $\text{Pb}^{+2}$  50 x 7.8 mm guard column (Phenomenex) was performed to quantify the cellobiose, glucose, xylose, galactose, arabinose, mannose and fructose released by the acidic hydrolysis of cellulose, hemicelluloses or residual sucrose. Before injection, the samples were filtered on an ABW solid phase extraction (SPE) cartridge (Phenomenex) to remove salts and prevent interference with the sugar peaks. Isocratic conditions were used with Milli-Q water at a flow rate of 0.6 mL/min; the injection volume was 20  $\mu\text{L}$ , the column was maintained at 80 °C and the RI detector was maintained at 50 °C. For the alkaline extract and purified samples, HPLC on a Rezex RHM-Monosaccharide  $\text{H}^{+}$  300 x 7.8 mm column (Phenomenex), used in conjunction with a Rezex RHM-Monosaccharide  $\text{H}^{+}$  50 x 7.8 mm guard column (Phenomenex) was performed to quantify glucose, xylose, arabinose, acetic acid, furfural and hydroxymethylfurfural (HMF) (Sluiter et al., 2006). The salts did not interfere with the sugar peaks on the RHM column, so, by contrast to the RPM column, no desalting of the samples was required before their injection. Isocratic conditions were applied, with 5 mmol/L  $\text{H}_2\text{SO}_4$  at a flow rate of 0.6 mL/min; the injection volume was 50  $\mu\text{L}$ , the column was maintained at 65 °C and the RI detector at 50 °C. The SCB alkaline extract and the UF retentates and permeates collected were diluted by four-fold with distilled water before the NREL protocol.

### 5.2.6.3. Monomeric sugars and hemicelluloses acetyl groups

Sulfuric acid was added to the alkaline extract to adjust its pH to 2, corresponding to the pH of the RHM column HPLC eluent. The extract was then analyzed on the RHM column without running the NREL protocol. The direct injection of pH-adjusted samples onto the HPLC column made it possible to quantify monomeric sugars and free acetic acid, whereas the analysis of samples with the NREL protocol provided data for total sugars (monomeric and oligomeric forms) and acetic acid (free and bound to hemicelluloses).

### 5.2.6.4. Phenolic monomers

Twelve phenolic monomeric compounds potentially present in SCB alkaline extract (Xu et al., 2005; Capriotti et al., 2015) – gallic acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid (VA), syringic acid, 4-hydroxybenzaldehyde (4HBA), vanillin, *p*-CA, syringaldehyde, FA, sinapic acid and hydroxycinnamic acid – were quantified by HPLC on an OmniSpher 3 C18 100 x 4.6 column (Agilent Technologies). The gradient was as follows: 91% acidified water (1% acetic acid (v/v)) and 9% acetonitrile for 25 min, acetonitrile concentration increasing from 9 to 90% over 5 min, then kept constant for 5 min, before decreasing back to 91% acidified water over 5 min, with column equilibration for 7 min between runs. The flow rate was 0.5 mL/min, the injection volume was 10  $\mu$ L and the column temperature was maintained at 25 °C. The UV detector was set at 280 nm. The concentrations used to plot the calibration curves ranged from 0 to 200 mg/L. Standards and process samples were diluted in acetonitrile:water at a ratio of 50:50 (v/v) before injection.

### 5.3. Results and discussion

#### 5.3.1. Alkaline extraction

Glucan, xylan and arabinan were the only sugars detected in significant amounts in the SCB raw material. The SCB alkaline extract contained no monomeric sugars (glucose, xylose, arabinose); all the extracted sugars were under oligomeric form. No sugar degradation products (furfural and HMF) were detected and the acetate groups bound to hemicelluloses were completely hydrolyzed in the alkaline extract. Five of the 12 phenolic monomers tested, (VA, 4HBA, vanillin, *p*-CA, FA) were present in detectable amounts in the alkaline extract. Chromatograms of these analyses are available as supplementary materials in a previous study (Oriez et al., 2018).

The mass balance between the inlet (SCB) and the outlet (solid residue and alkaline extract) for every compound, represented by the sum of the yield for solid residue and alkaline extract is close to 100% (**Table 5.2**), except for ASL with 140%. The yield of AIL is 93%, the alkaline conditions may have hydrolyzed some AIL from the raw SCB that were accounted as ASL in the alkaline extract after NREL analysis. Based on the results of the extraction on a 3 L reactor (Oriez et al., 2018), changing the scale (better agitation and temperature control) as well as rinsing solid residue considerably increased the extraction yield of most of the compounds (**Table 5.3**). For instance, xylan, arabinan, AIL and ASL extraction yield increased from 22%, 50%, 45% and 52%, to 29%, 59%, 49% and 96%, respectively.

**Table 5.2**

SCB, solid residue, alkaline extract and concentrated alkaline extract composition and extraction yields

Parameters	SCB	Solid residue		Alkaline extract	
	Content	Content	Yield	Content	Yield
DS	92.5	97.5	48	3.4	49
Ash	9.9	17.1	26	56.0	85
Glucan	35.9	48.2	86	1.4	3
Xylan	19.4	18.1	60	8.8	29
Arabinan	2.3	2.0	55	2.2	59
Hemicelluloses	21.8	20.1	59	13.3	40
AIL	21.6	14.9	44	16.6	49
ASL	5.5	3.8	44	8.2	96
Total Lignin	27.1	18.7	44	24.6	58
VA				0.0	
4HBA				0.1	
vanillin				0.1	
p-CA				3.3	
FA				0.4	
Total phenolic monomers				3.9	
Mass Closure	97.0	104.2		97.0	

All the values are calculated based on the percentage of dry solid. All the analyses were run in duplicate, standard deviation was at most 1%.

**Table 5.3**

Comparison of the alkaline extraction composition and yields at different scale

Components	SCB	3 L alkaline extract		60 L alkaline extract	
	Content	Content	Yield	Content	Yield
DS	92.5	2.6	35	3.4	49
Ash	9.9	56.1	61	56.0	85
Glucan	35.9	1.5	2	1.4	3
Xylan	19.4	9.4	22	8.8	29
Arabinan	2.3	2.5	50	2.2	59
Hemicelluloses	21.8	11.9	25	13.3	40
AIL	21.6	21.1	45	16.6	49
ASL	5.5	6.2	52	8.2	96
Total Lignin	27.1	27.3	46	24.6	58
VA		0.0		0.0	
4HBA		0.1		0.1	
vanillin		0.1		0.1	
p-CA		3.6		3.3	
FA		0.4		0.4	
Total phenolic monomers		4.1		3.9	
Mass Closure	95.2	98.9		97.0	

All the values are calculated based on the percentage of dry solid. All the analyses were run in duplicate.

The SCB mild alkaline extract (i.e., the feed of the following UF step) was composed of six major pools of molecules: 19.4 g/L inorganic salts, 6.2 g/L AIL, 3.1 g/L ASL, 5.3 g/L oligomeric sugars (3.8 g/L xylan, 0.9 g/L arabinan, 0.6 g/L glucan), 1.5 g/L acetic acid and 1.3 g/L phenolic monomers.

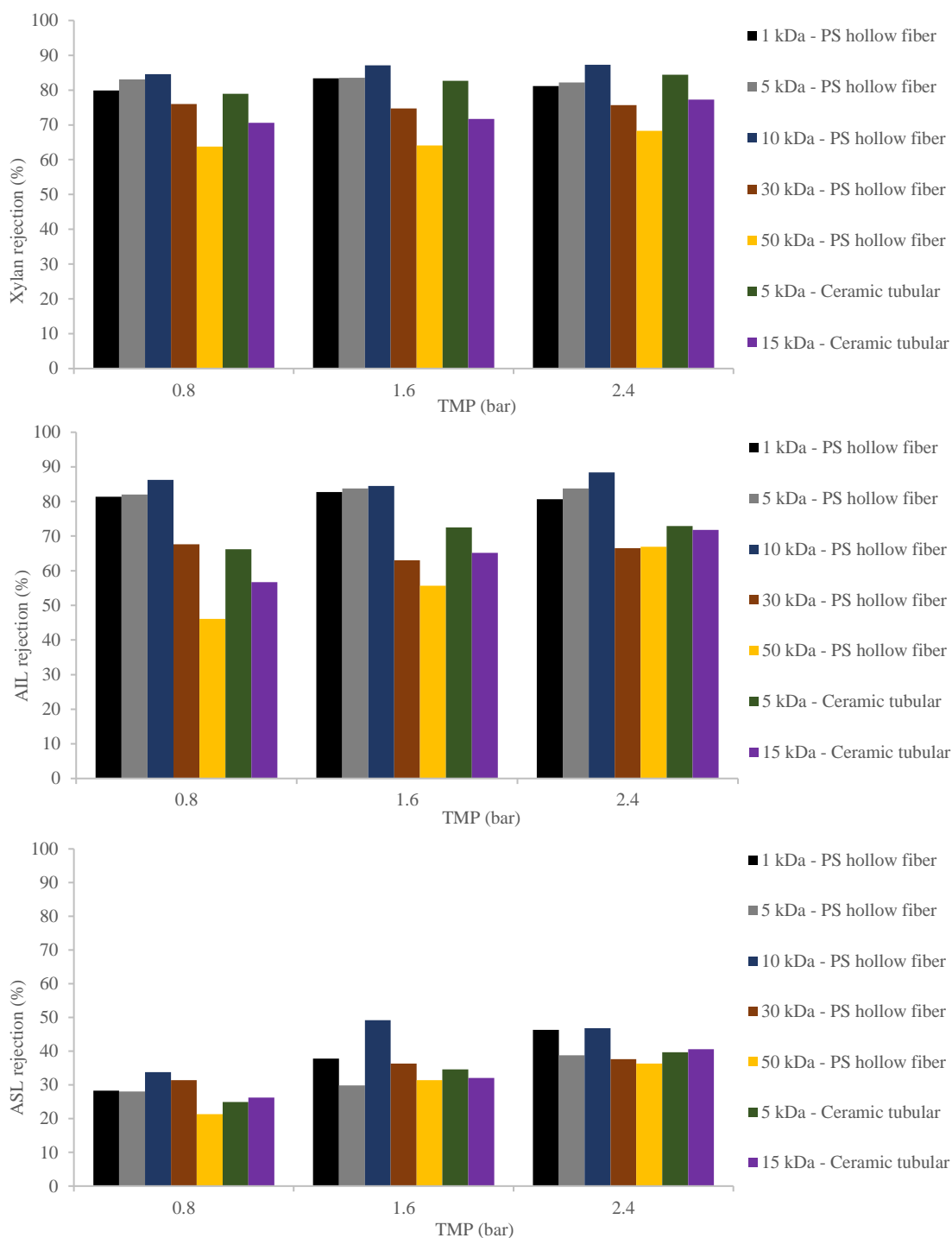
### 5.3.2. Ultrafiltration

#### 5.3.2.1. Membrane screening in full recycling mode

In most of the permeates analyzed, glucan and arabinan were barely detectable by HPLC. Xylan was, therefore, the only sugar oligomer displayed in the results for the permeate composition.

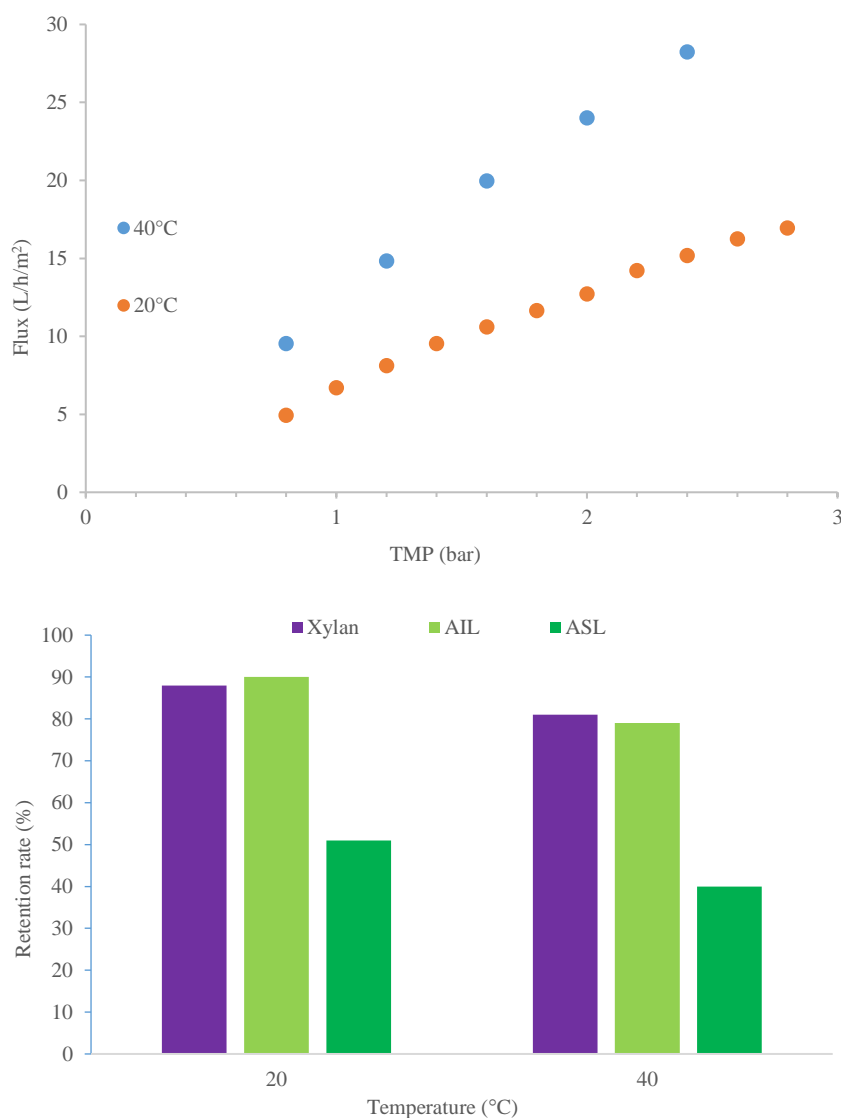
The membrane screening showed that the 10 kDa membrane presented the best rates of rejection for xylan (85-87%), AIL (85-88%) and ASL (34-49%) (**Fig. 5.2**). On all the membranes, the rejection rates for salts, phenolic monomers and acetic acid was below 10-15%. Besides, the 10 kDa and the 1 kDa PS hollow fiber membranes were the only ones presenting a linear evolution of flux within the TMP range tested, meaning that the critical flux was not yet reached at 2.8 bar and thus minimizing the fouling of the membrane. The flux was 5 L/h/m<sup>2</sup> at 0.8 bar and reached 17 L/h/m<sup>2</sup> at 2.8 bar, 20 °C and a shear rate of 10,187 s<sup>-1</sup> (**Fig. 5.3A**). Based on the high selectivity and the flux behavior obtained, the 10 kDa PS hollow fiber membrane was selected to run further experiments on the SCB mild alkaline extract.

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**Fig. 5.2** Effect, during the filtration of sugarcane bagasse mild alkaline extract, of the molecular weight cut-off, the transmembrane pressure (TMP) and the nature of the membrane on the rejection rates of (A) xylans, (B) acid insoluble lignin (AIL) and (C) acid soluble lignin (ASL). Shear rate of  $3396 \text{ s}^{-1}$  for PS hollow fiber membranes and  $3408 \text{ s}^{-1}$  for ceramic tubular membranes.





**Fig. 5.3** (A) Permeate flux evolution with transmembrane pressure (TMP) and (B) Evolution in xylan, AIL and ASL retention rates, during the filtration of sugarcane bagasse mild alkaline extract on 10 kDa polysulfone hollow fiber membrane, with a TMP of 2.4 bar, a shear rate of  $10,187 \text{ s}^{-1}$ , and temperatures of 20 °C and 40 °C.

A full recycling experiment on the 10 kDa PS hollow fiber membrane at 40 °C produced higher flux by about a two-fold factor compared to 20 °C (e.g., 15 L/h/m<sup>2</sup> at 20 °C and 28 L/h/m<sup>2</sup> at 40 °C, at 2.4 bar), but lower rates of retention for xylan, AIL and ASL with decrease of 7%, 11% and 11%, respectively (**Fig. 5.3**).

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### 5.3.2.2. Successive concentration and diafiltration mode

The mass balance between the inlet (feed) and the outlet (global permeate and final retentate) for every compound, represented by the sum of their yield for global permeate and final retentate is close to 100% (**Table 5.4**), no compounds were lost during the UF in concentration mode.

**Table 5.4**

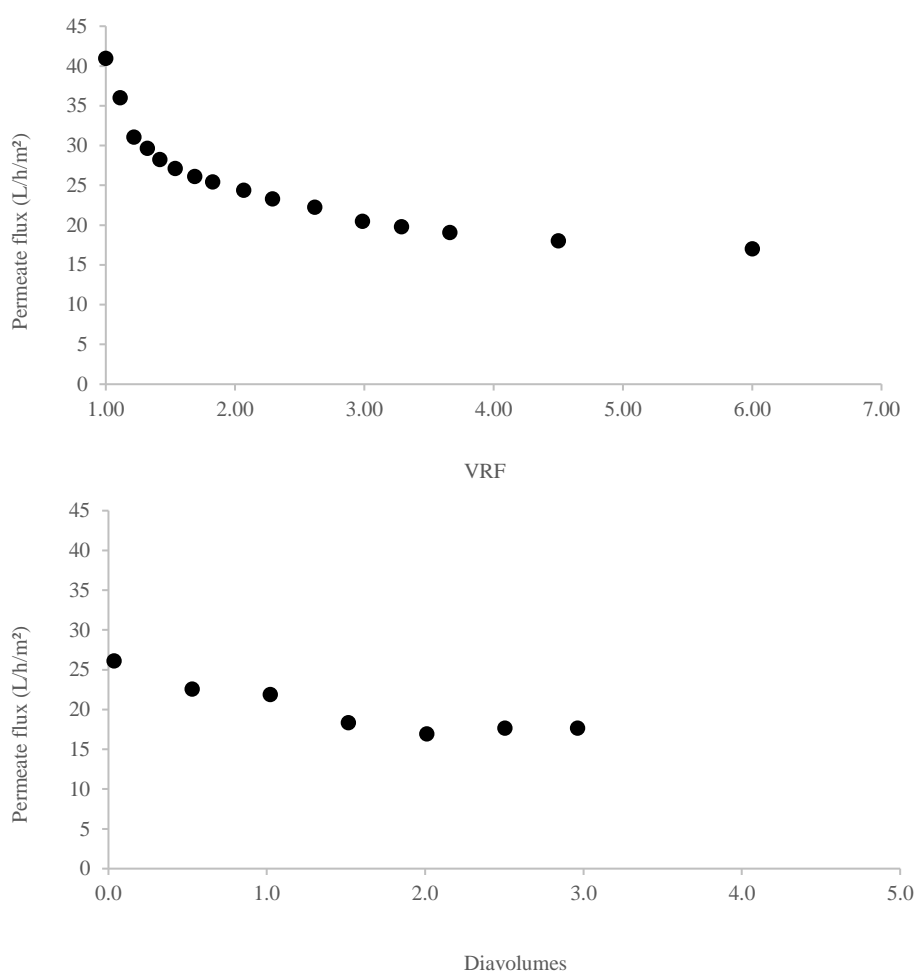
Composition and components yield in global permeate and final retentate after ultrafiltration in concentration mode (volume reduction factor of 6.1) of the SCB mild alkaline on the 10 kDa PS hollow fiber membrane with a transmembrane pressure of 2.4 bar, a shear rate of 10,187 s<sup>-1</sup> and a temperature of 40 °C

Compounds	<b>Feed</b>	<b>Global permeate</b>		<b>Final retentate</b>	
	Content	Content	Yield	Content	Yield
DS	3.4	2.5	61	7.8	39
Ash	56.0	71.5	78	27.8	20
Glucan	1.4	NA	NA	2.0	55
Xylan	8.8	3.0	21	18.2	82
Arabinan	2.2	NA	NA	4.0	74
Acetic acid	4.4	5.9	81	2.1	19
AIL	17.9	6.0	22	32.4	78
ASL	9.0	8.1	60	7.7	37
VA	NA	NA	NA	NA	NA
4HBA	0.1	0.1	86	0.0	14
Vanillin	0.1	0.1	88	0.0	16
<i>p</i> -CA	3.3	4.5	84	1.3	16
FA	0.4	0.5	77	0.1	15
Mass closure	103.5	99.7		95.7	

All the values are calculated based on the percentage of dry solid. All the analyses were run in duplicate.

Based on Eq. (9) where a VRF of 6.1 should produce yields of 71% for xylan and 68% for AIL, the yields for xylan and AIL in the final retentate were higher after UF in concentration mode with 82% and 78%, respectively (**Table 5.4**). It showed that the rates

of rejection increased during the concentration experiment. The phenomenon could be due to the raise in concentration in the polarization layer and to the potential cake formation at the surface of the membrane (Bacchin et al., 2006). High yield was also observed for arabinan with 74%, whereas glucans were less retained with 55% yield in the retentate, suggesting that glucans are smaller oligomers than xylans or arabinans. In parallel, the recoveries of small molecules in the permeate were high: 78% for inorganic salts, 81% for acetic acid, 86% for 4HBA, 88% for vanillin, 84% for *p*-CA and 77% for FA. The composition of the final retentate was: 22.0 g/L inorganic salts, 24.5 g/L AIL, 5.8 g/L ASL, 18.3 g/L oligomeric sugars (13.8 g/L xylan, 3.0 g/L arabinan, 1.5 g/L glucan), 1.6 g/L acetic acid and 1.2 g/L phenolic monomers. The concentration mode enabled the concentration of retained molecules (e.g., by 3.6 for xylan and by 4.0 for AIL), while the concentration of unretained molecules stayed stable, which also contributed to the increase in purity for the retained molecules, for instance from 8.8% to 18.2% for xylan and from 17.9% to 32.4% for AIL. During the concentration by a VRF of 6.1, the flux decreased following a logarithmic pattern from 41 L/h/m<sup>2</sup> to 17 L/h/m<sup>2</sup> (**Fig. 5.4**).



**Fig. 5.4** Evolution of the flux during sugarcane bagasse mild alkaline extract filtration on 10 kDa polysulfone hollow fiber membrane (A) in concentration mode, then (B) in diafiltration mode.

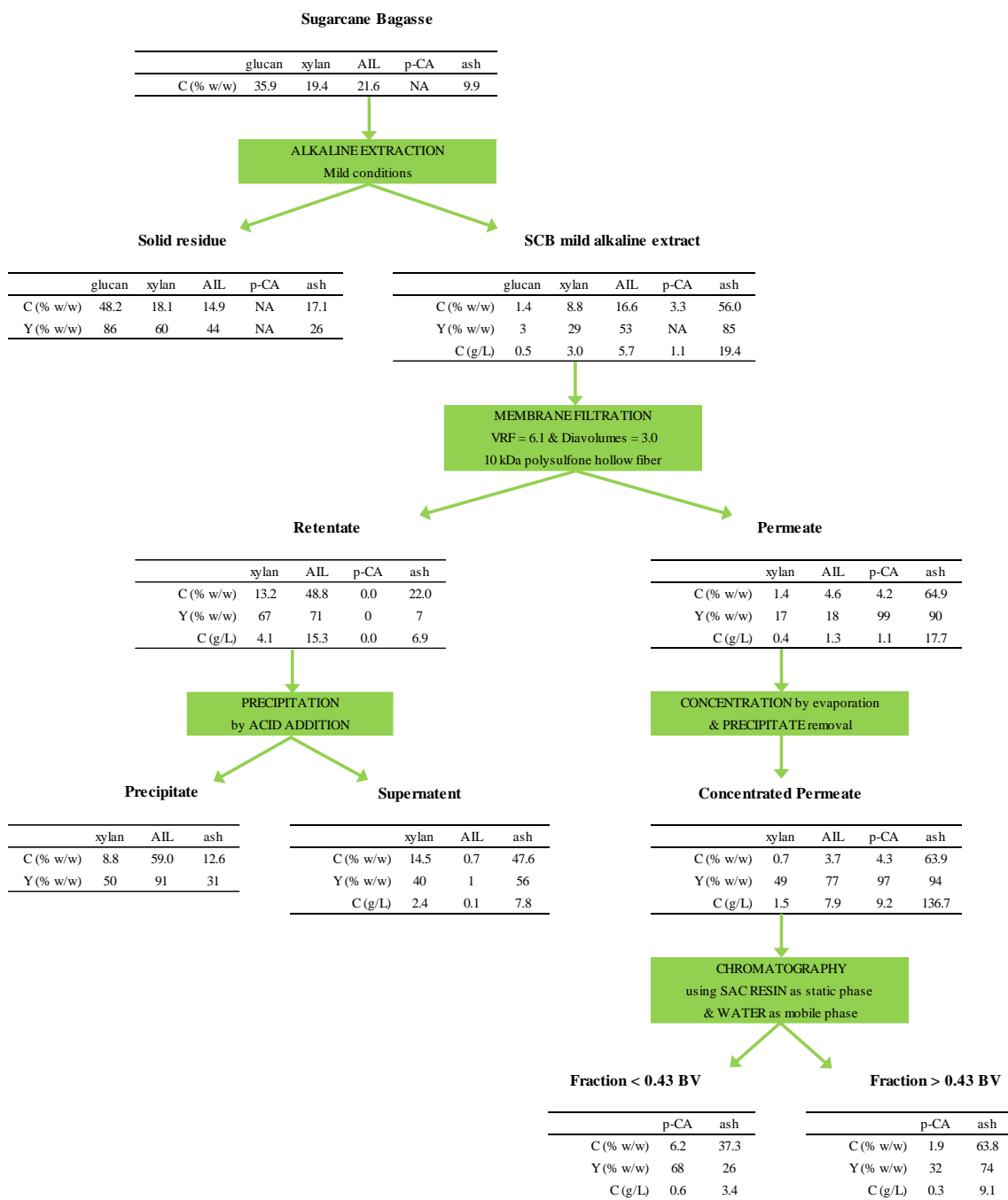
In order to increase the purity of the retained molecules and increase the yield of the molecules passing through the membrane, diafiltration was tested with 3.0 diavolumes of distilled water at 40 °C. The membrane was cleaned between the concentration mode experiment and the diafiltration mode experiment. At the beginning of the diafiltration, the flux was higher than at the end of the concentration experiment with a value of 26 L/h/m<sup>2</sup> (**Fig. 5.4**). The flux slowly decreased until 1.5 diavolumes of water was added, then remained stable at about 18 L/h/m<sup>2</sup>.

The diafiltration mode increased the purity of retained molecules by removing the small ones, which also contributed to the increase in purity for the retained molecules, for instance from 32.4% to 48.5% for AIL. However, the salts were not fully recovered, since

as for the concentration mode, the retention of the molecules increased during the process, making additional diavolumes inefficient to increase the xylan and AIL purity. Only 7% of the salts were recovered in the retentate after the concentration and diafiltration modes (33% and 20% recovery for these steps, respectively) (**Fig. 5.5**), but due to their initial content in the SCB alkaline extract the salts still constitute 22.0% of the retentate (27.8% after the concentration mode). After both filtration modes, the recovery rates of AIL and xylan reached 71% and 67% in the retentate, with AIL being more recovered during the concentration mode (91%) than xylan (82%) but the reverse occurring during the diafiltration mode with a recovery of AIL of 78% whereas xylan was recovered at 82%.

Overall, after concentration by a VRF of 6.1 and diafiltration with 3.0 diavolumes of distilled water, the retentate composition was 6.7 g/L inorganic salts, 15.3 g/L AIL, 3.0 g/L ASL, 5.7 g/L oligomeric sugars (4.3 g/L xylan, 1.0 g/L arabinan, 0.4 g/L glucan), 0.3 g/L acetic acid and phenolic monomers were not detected in quantifiable concentration.

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**Fig. 5.5** Integrated process scheme for the fractionation of sugarcane bagasse (SCB) by alkaline pretreatment and for the separation of the components of the SCB alkaline extract. Content (C) and yield (Y) at the various process steps are displayed for all the fractions and for the major components.

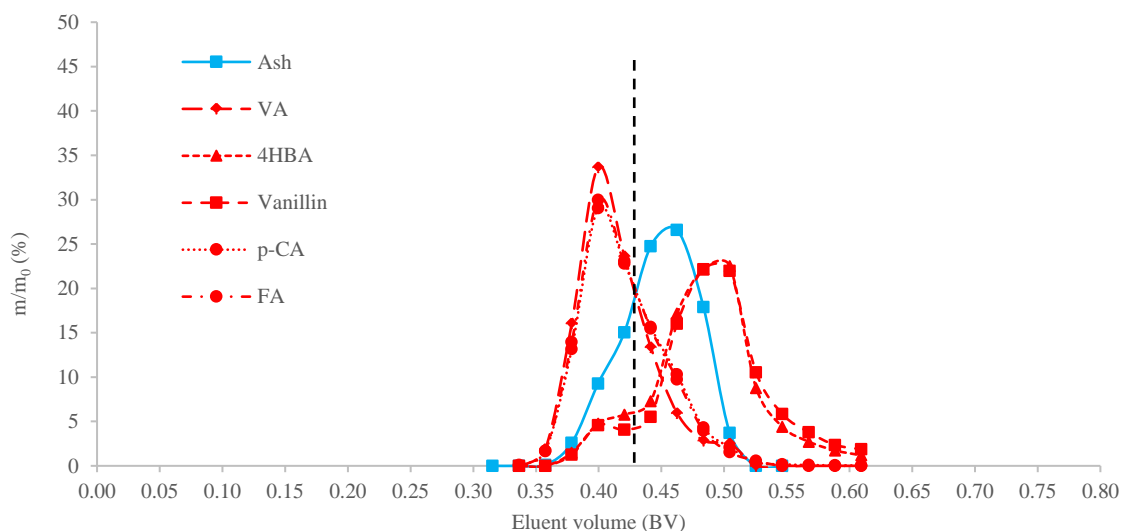
### 5.3.3. Chromatography on ultrafiltration permeate

The permeate from the UF concentration of the SCB mild alkaline extract had the following composition: 18.1 g/L inorganic salts, 1.5 g/L AIL, 2 g/L ASL, 0.8 g/L xylan, 1.5 g/L acetic acid and 1.3 g/L phenolic monomers (*p*-CA being the most important with 1.1 g/L), corresponding to purity of 71.5%, 6.0%, 8.1%, 3.0%, 5.9% and 5.2% (4.5% for *p*-CA), respectively. A chromatographic separation developed by Oriez et al. (2018), based on the use of a gel-type strong acid cation exchange resin packed in a column with water as eluent was tested on the permeate in order to separate the salts and the phenolic monomers without a carboxyl function from the salts and the phenolic monomers with a carboxyl function.

Before the chromatographic pulse test, the DS content of the permeate was increased from 2.5% to 19.0% by evaporation. It induced the formation of a precipitate that was removed by centrifugation at 10,000 g for 5 min, it accounted for 13.9% of the DS of the permeate. Minor loss of ash (5%), ASL (3%) and phenolic monomers (1, 9, 3, 3, 5 and 3% for VA, 4HBA, vanillin, *p*-CA, FA and ASL occurred in the concentrated UF permeate, whereas AIL and xylan were lost in significant proportion, 23% and 51%, respectively, meaning that the precipitate contained mainly these 2 compounds. As a comparison, the concentration of the SCB mild alkaline extract to 20% DS did not lead to a precipitate (Oriez et al., 2018). The higher salt content in the UF permeate (71.5%) compared to the mild alkaline extract (56.0%) may have led to a salting out phenomenon. The water molecules may have solvated preferably the salts due to their charge, and interactions between xylan chains probably occurred, resulting in their precipitation. The same phenomenon probably occurred with lignin oligomers to a lower extent, since at alkaline pH their negatively charged phenolate and carboxyl groups may have limited interactions between each other. After concentration by evaporation of the permeate, the content of AIL and xylan in the permeate dropped from 6.0% and 3.0% to 3.7% and 0.7%, respectively.

The pulse test run on the centrifuged concentrated UF permeate (**Fig. 5.6**) resulted in an unchanged separation resolution, given by Eq. (14), between phenolic monomers with a carboxyl group and phenolic monomers without carboxyl group (about 0.030 for *p*-CA and vanillin for instance) from the pulse test directly performed on the concentrated SCB

alkaline extract run in a previous work (Oriez et al., 2018). The quasi-complete removal of lignin and hemicelluloses oligomers had no impact on the separation of the other compounds. The shoulder in front of the peaks of 4HBA and vanillin is unexplained on both the SCB alkaline extract and the centrifuged concentrated UF permeate. VA, *p*-CA and FA had high recovery rate in the fraction before 0.43 BV with 75, 70 and 68%, respectively. In the fraction after 0.43 BV, inorganic salt 73%, 4HBA 88% and vanillin 90% were mainly recovered. Batch-column chromatography showed great separation performance between phenolic monomers, and even higher yield, purity and productivity are expected on continuous chromatography, with the use of Simulated Moving Bed (SMB) (Nicoud, 2000).



**Fig. 5.6** Elution test of centrifuged concentrated permeate from concentration mode on 10 kDa membrane on XA2004-30Na at 1.24 m/h, with  $m$  the mass of a given compound in the fraction and  $m_0$  its mass in the feed. Lines are presented to guide the eyes.

### 5.3.4. Precipitation on ultrafiltration retentate

#### 5.3.4.1. Precipitation by acid addition

1.10 g of  $H_2SO_4$  at 72% (w/w) was added to 100 g of UF retentate to adjust its pH to 1.9. High recoveries of AIL (91%) and ASL (79%) were obtained in the precipitate, however a significant amount of xylan (53%), arabinan (45%), glucan (46%) and salts (43%) were also recovered in the precipitate (**Table 5.5**). Therefore, the purity of AIL moderately increased from 48.9% to 59.0%. In parallel, the purity of xylan, arabinan and



glucan remained stable in the supernatant as salts were mainly recovered in this fraction. In a study of Alekhina et al. (2015) on softwood black liquor acid precipitation, high recovery of lignin coupled with low purity was also achieved at low pH (2.5). In order to increase the purity of the precipitate, several wash with acid could have been interesting to remove some of the sugars. However, Alekhina et al. (2015) reported that even extensive wash of the lignin precipitate with acidified water and water was not efficient to remove the co-precipitated sugars (mainly xylan chains), since the polymeric form of xylan and its linear structure may prevent its solubilization in water (Alekhina et al., 2015).

**Table 5.5**

Precipitations, by acid addition, by ethanol addition with an ethanol:retentate ratio of 4:1 and 9:1 (v/v), on the retentate from the ultrafiltration on the 10 kDa polysulfone membrane in concentration mode and diafiltration mode of the sugarcane bagasse alkaline extract. Composition of the precipitates and yields of the various components in the precipitates.

Compounds	Retentate Content	Acid addition until pH 1.9		Ethanol:retentate 4:1 (v/v)		Ethanol:retentate 9:1 (v/v)	
		Content	Yield	Content	Yield	Content	Yield
DS	3.1	92.5	75	93.3	43	92.9	56
Ash	21.3	12.6	43	23.6	47	19.3	49
Glucan	1.4	0.8	44	2.0	64	1.8	75
Xylan	13.7	8.8	50	19.3	63	17.5	75
Arabinan	3.2	1.6	42	3.7	55	3.4	66
Acetic acid	0.9	0.0	0	0.0	0	0.0	0
AIL	48.5	59.0	91	32.1	29	40.6	47
ASL	9.6	10.9	79	11.6	48	13.6	73
Mass closure	98.6	93.6		92.3		96.3	

All the values are calculated based on the percentage of dry solid. All the analyses were run in duplicate.

### 5.3.4.2. Precipitation by ethanol addition

63% of the xylan, 55% of the arabinan and 64% of the glucan were recovered in the precipitate with an ethanol:retentate ratio of 4:1, whereas 30% of AIL was recovered (**Table 5.5**). The purity of the sugars increased from 13.7% to 19.3%, 3.2% to 3.7% and 1.4% to 2% for xylan, arabinan and glucan, respectively.

75% of the xylan, 66% of the arabinan and 75% of the glucan were recovered in the precipitate with an ethanol:retentate ratio of 9:1, but 47% of AIL was also recovered in the precipitate. Other studies reported higher precipitation yields for hemicelluloses, e.g., about 70-80% with an ethanol concentration at 70% or above, and 80 to 95% with concentration of ethanol at 80% or above, respectively, depending on the initial biomass and the hemicelluloses extraction conditions (Brillouet et al., 1982; Bian et al., 2010; Peng et al., 2011; Xu et al., 2014). The recovery of the sugars increased with increasing ethanol:retentate ratio but their purities decreased since more lignins (AIL and ASL) were co-precipitated. Sun et al. 1998 also observed high recovery of lignin in the precipitate (38%) after ethanol precipitation (ethanol:extract ratio of 4:1 (v/v)) of a wheat straw mild alkaline extract (Sun et al., 1998)

Precipitates from ethanol addition contained higher salts content than acid precipitate despite the addition of salt constituted by the addition of sulfuric acid. With the conditions used in this work, acid precipitation was more efficient than ethanol precipitation for the separation of hemicelluloses and lignin. Acid precipitation of lignin followed by ethanol precipitation of hemicelluloses could be investigated.

#### 5.4. Conclusion

Temperature was kept low for the mild alkaline fractionation of SCB and the purification of the resulting extract, at 60 °C and 40-70 °C, respectively. The process consumed only 0.3 g NaOH per gram of SCB treated at the alkaline fractionation step and the equivalent of 2.2 mg of H<sub>2</sub>SO<sub>4</sub> per gram of SCB at the precipitation step, since acid precipitation was preferred to ethanol precipitation.

Membrane screening showed that the best separation between hemicelluloses and lignin oligomers on one side and salts, acetic acid and phenolic monomers on the other side, was achieved with the 10 kDa PS hollow fiber membrane. Concentration experiment on this UF membrane increased significantly the concentration and purity of sugar and lignin oligomers in the retentate stream. A following diafiltration step showed limited improvements in the purity of the oligomers. Concentration by evaporation of the UF permeate by evaporation before batch-column elution chromatography increased the purity of the phenolic monomers by the precipitation of residual hemicelluloses and lignin. Pulse test on a gel-type strong acid cation exchange resin confirmed the separation of phenolic monomers with a carboxyl group from salts and phenolic monomers without carboxyl group in their structure. In parallel, precipitation by the addition of acid on the UF retentate was more efficient than precipitation by the addition of ethanol for the separation of lignin and hemicelluloses oligomers. However both precipitation processes resulted in moderate purification performance.

Further optimization of the process could include an increase in the S:L ratio (w/v) at the fractionation step to reduce the water and sodium hydroxide consumptions and the study of its impact on the extraction yields. The concentration by evaporation of the UF permeate could be increased (over 20% DS) to observe the impact on the precipitation of the residual lignin and hemicelluloses in the UF permeate, it would also increase the productivity of the chromatographic step. Continuous chromatography (e.g., SMB) could be tested to check if productivity, yield and purity could be increased from the batch-column elution chromatography tests. The separation of lignin and hemicelluloses oligomers, by sequential precipitation with acid addition then ethanol addition could be assessed. Lignin precipitate by acid addition could be washed with an acidic solution to remove the co-precipitated sugars and increase lignin purity. Adsorption could be

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investigated as well for the separation of lignin and hemicelluloses oligomers. With the use of an adsorbent resin that could retain lignin, higher purity than with precipitation processes could be reached but at a higher economic and environmental cost. If the valorization of hemicellulosic sugars under their monomeric form is targeted, then enzymatic hydrolysis of hemicelluloses then UF to retain lignin and recover the sugars in the permeate could also be conceivable.

### Acknowledgements

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## Chapter 6:

# ANALYTICAL METHODS

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### 6.1. Dry solid content and ash

Dry solid (DS) content was gravimetrically determined at 103 °C for 12 h and ash content at 500 °C for 12 h. Additional methodology was provided in the concerned parts of the manuscript when necessary.

The precise composition of ash was not especially followed in this work. For some lignocellulosic biomass, like rice hull, silica is the major ash component at levels up to 20% of the DS (Kamath and Proctor, 1998). Its follow-up by specific measurement did not appear of major interest in our case, since first we worked on SCB that has a lower ash content and secondly because silica was indirectly measured. Alkaline conditions solubilize silica (Kamath and Proctor, 1998), the undissolved fraction is retained with the solid residue, the dissolved fraction  $\text{Na}_2\text{SiO}_3$  which is issued from the reaction of  $\text{SiO}_2$  with  $\text{NaOH}$  ( $\text{SiO}_2 + 2 \text{NaOH} \rightarrow \text{Na}_2\text{SiO}_3 + \text{H}_2\text{O}$ ) is recovered in the alkaline extract.

## 6.2. Extractives

Extractives were determined following NREL methodology (Sluiter et al., 2005). The method consists in submitting biomass to a first Soxhlet extraction with distilled water to remove hydrophilic compounds (e.g., non-structural sugars, proteins, minerals) and a second Soxhlet extraction with ethanol 95% (v/v) to remove hydrophobic compounds (e.g., lipids, wax, pigments) that could interfere with the further analyses of the biomass to determine its lignocellulosic composition.

This analysis was carried out only on the raw SCB and raw SuOC (Chapter 2) to provide an exhaustive characterization of these lignocellulosic materials. 5 g of dry biomass was wrapped in tared cellulose paper and submitted to reflux in a Soxhlet apparatus with 200 mL distilled water for 9 h. At the end of the extraction, the solid residue was dried at 50 °C for 36 h and two times 200 mg were used for dry solid and ash analyses. The water extract was also analyzed for dry solid and ash contents determination. The solid residue was packed in a new tared cellulose paper and submitted to reflux in a Soxhlet apparatus with 200 mL ethanol 95% (v/v). At the end of this second extraction, the solid residue was dried at 50 °C for 36 h and two times 200 mg were used for dry solid and ash analyses. The ethanol extract was also analyzed for dry solid and ash contents determination. Reflux extractions were run in triplicate and analyses were run in duplicate.

### 6.3. Carbohydrates and lignin determination

Three main methods were developed during the 20<sup>th</sup> century to quantify cellulose, hemicelluloses and lignin in lignocellulosic biomass: ADF-NDF methodology, the method developed by The Technical Association of Pulp and Paper Industry (TAPPI) and more recently the method from the National Renewable Energy Laboratory (NREL). The method from TAPPI is not tackled here since, NREL used the work of TAPPI to produce an improved version of the procedure.

#### 6.3.1. ADF-NDF

The acid detergent fiber (ADF) and neutral detergent fiber (NDF) method to determine the contents of cellulose, hemicelluloses and lignin originally in forage was developed in the 60s with improvements in the following decades (Van Soest, 1963; Van Soest and Wine, 1967, 1968; Goering, 1970; Van Soest et al., 1991). Later, it was used in studies on lignocellulosic biomass in the frame of the biorefinery concept (Okano et al., 2006; Chen et al., 2011), so this method was tested in this work (**Fig. 6.1**).

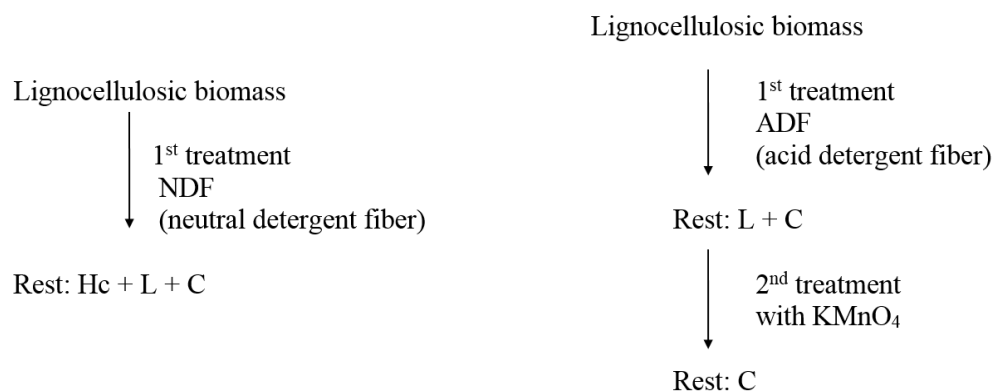
This is a gravimetric method based on the difference in solubility of the compounds in various detergents:

- A neutral detergent, NDF (Neutral Detergent Fiber), which solubilize all the non-lignocellulosic compounds (e.g., proteins, pectins). The solid residue (N) is composed of cellulose (C), hemicelluloses (Hc) and lignin (L).
- An acid detergent, ADF (Acid Detergent Fiber), which solubilize all the non-lignocellulosic compounds and hemicelluloses (Hc). The solid residue (A) is composed of cellulose (C) and lignin (L).
- Lignin (L) are solubilized from (A) by a powerful oxidant solution, based on potassium permanganate, producing a solid residue containing cellulose (C) only.

After drying of the fractions, mass content of the compounds are given by:

- % Cellulose = C
- % Lignin = A-C
- % Hemicelluloses = N-A





**Fig. 6.1** Principle of ADF-NDF methodology with C: cellulose, Hc: hemicelluloses and L: lignin.

NDF solution was made from 1 L of distilled water in which 30 g of sodium lauryl sulfate, 18.61 g of sodium tetra acetate ethylene diamine, 4.56 g of disodium phosphate, 6.81 g of sodium borate and 10 mL of ethylene glycol monoethyl ether were added. ADF solution was made from 1 L of distilled water in which 20 g of cetyltrimethylammonium bromide and 26.8 mL of sulfuric acid 96% (w/w) were added. The solution for the solubilization of lignin (i.e., 2<sup>nd</sup> treatment after ADF treatment) was made of 2 volumes of saturated potassium permanganate solution (50 g/L  $\text{KMnO}_4$ ) and 1 volume of buffer solution (6 g of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  + 0,15g of  $\text{AgNO}_3$  in 100 mL distilled water, 500 mL of pure acetic acid + 5 g of potassium acetate, 400 mL of ter-butanol). A demineralizing solution was made of 50 g of dehydrated oxalic acid in 700 mL of ethanol 95% (v/v), 50 mL of HCl 35% (w/w) and 250 mL of distilled water).

ADF and NDF extractions were run on a Fibertec 1020 Hot extractor (Foss) with 1 g of raw material (>85% DS, ground on 2 mm mesh) put in 250 mL flasks, where 100 mL of the corresponding reagent was added (ADF or NDF) as well as 2 drops of octanol (anti-foaming agent). The flasks were put under reflux condenser and heated to reflux (about 100 °C) during 1 h. Then, after the flasks have been cooled down, the mixtures were filtrated on a glass filter (Foss 2) under vacuum and the solid residues were extensively washed with hot distilled water. Solid residues on glass filter were dried at 105 °C for 12 h and the dry weight measured. For NDF solid residue only, the ash content was assessed.

## Chapter 6: ANALYTICAL METHODS

The solubilization of lignin on ADF solid residue was run on a Fibertec 1021 Cold extractor (Foss) where 25 mL of saturated potassium permanganate and buffer solution was added in the glass filter. The reaction was made at room temperature during 1h30 with regular manual agitation (PTFE stirring rod). The solution was filtrated under vacuum, then washed 3 times with demineralizing solution, twice with ethanol 80% (v/v) and twice with acetone. DS and ash content were eventually measured.

### 6.3.2. NREL

Compared to ADF-NDF methodology, the procedure developed by the National Renewable Energy Laboratory (NREL), fund by the US Department of Energy and based in Golden, Colorado, has become the reference procedure for studies dealing with the characterization, fractionation and valorization of lignocellulosic biomass. The two methodologies were compared for the characterization of SCB in Chapter 2.

NREL developed two methods for the determination of carbohydrates and lignin content, one for solid materials (biomass or solid fractions) (Sluiter et al., 2008) and one for liquid fractions issued from processed samples of biomass (Sluiter et al., 2006).

#### 6.3.2.1. Protocol adapted by IATE

From the original procedure for solid material (Sluiter et al., 2008), IATE adapted slightly the protocol. An oil bath instead of an autoclave was used for the dilute acid hydrolysis at 121 °C, and the solid residue from this step, was filtrated using a GF/A filter (Whatmann), particle filtration size of 1.6 µm, instead of ceramic filtering crucible Coors #60531, with a 15 µm porosity.

#### 6.3.2.2. Protocol adapted by LCA

LCA also adapted slightly the procedures from NREL. Instead of ceramic filtering crucible Coors #60531, with a 15 µm porosity, LCA used glass filtering crucible (Robu porosity 4, 10-16 µm). HPLC columns were Rezex RPM and Rezex RHM (Phenomenex), the measured retention times of various standard compounds on this column according to the HPLC procedure developed by NREL are presented in **Table 6.1** and **Table 6.2**. Besides, chromatograms obtained on Rezex RPM column from the analysis of SCB are

reported in **Fig. 6.2** and **Fig. 6.3**, where salt peak interference with the peaks of sugars can be noticed. NREL advises the use of ionic form  $H^+/CO_3^-$  deashing guard column to avoid this interference. However, LCA tried to remove the salts from the samples for HPLC analyses before the injection with different means, using filtration on SPE cartridges (Strata XL-C and Starta ABW from Phenomenex) and or contact in a beaker with the resin XA7111 MB (Novasep). The use of Strata XL-C (Polymeric strong cation exchange sorbent) was inefficient to remove the salts (**Fig. 6.4**). Strata ABW (mixed bed – strong cation and strong anion exchange sorbent) was efficient to remove salts without altering the peaks of sugars (**Fig. 6.5**). XA7111 MB resin induced removal of salts and sugars, making it unsuitable for sample deashing (**Fig. 6.6**). Chromatograms obtained on Rezex RHM corresponding to the analysis of the SCB and its alkaline extract are presented in **Fig. 6.7** and **Fig. 6.8**. No sugar monomers were found in the SCB mild alkaline extract, and all galacturonic acid and acetic acid were under their free form (i.e., not bound to hemicelluloses) (**Fig. 6.8**).

Sugars can also be analyzed using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD) which allow the detection of smaller concentration of sugars (down to 1 mg/L) compare to HPLC as described above (down to 100 mg/L). However, many limitation have been reported with HPAEC/PAD, such as the lengthy chromatographic running time due to column conditioning and re-equilibration steps, the difficulty of separating arabinose, galactose, and rhamnose, while maintaining resolution of the xylose-mannose pair, and the poor quantitation of low amounts of mannose, because of the common tendency of late eluting peaks to tail excessively (Davis, 1998).

Sugar recovery standards (SRS) were run in triplicate and the following values were found and used in all the calculations in this work: 93.6% for glucose, 90.5% for xylose, 93.1% for arabinose and 94.5% for galactose. These values correspond to what was reported in previous studies, where xylose was noticed to be more degraded than the other sugars (Templeton et al., 2010).

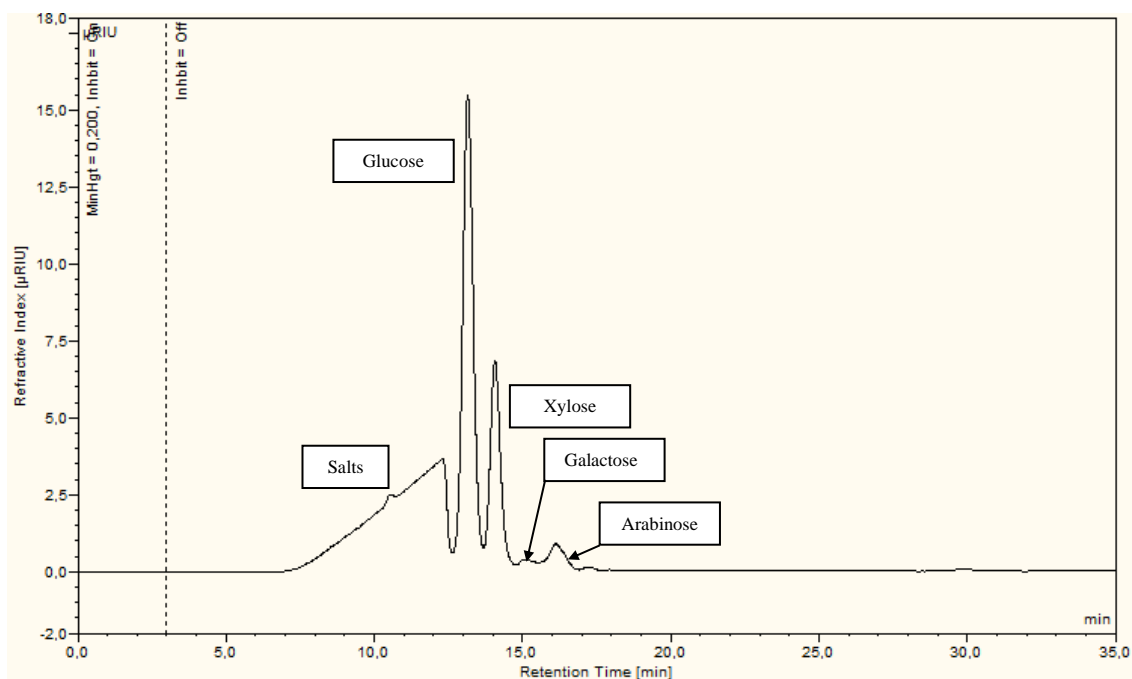
Acid soluble lignin (ASL) was calculated using the adsorptivity constant of 25 L/g/cm given for SCB in the procedure from NREL (Sluiter et al., 2008). A method was developed by NREL to precisely quantify ASL (Hyman et al., 2008) in lignocellulosic

biomass, however the same adsorptivity constant was used for SuOC characterization in order to simplify analysis.

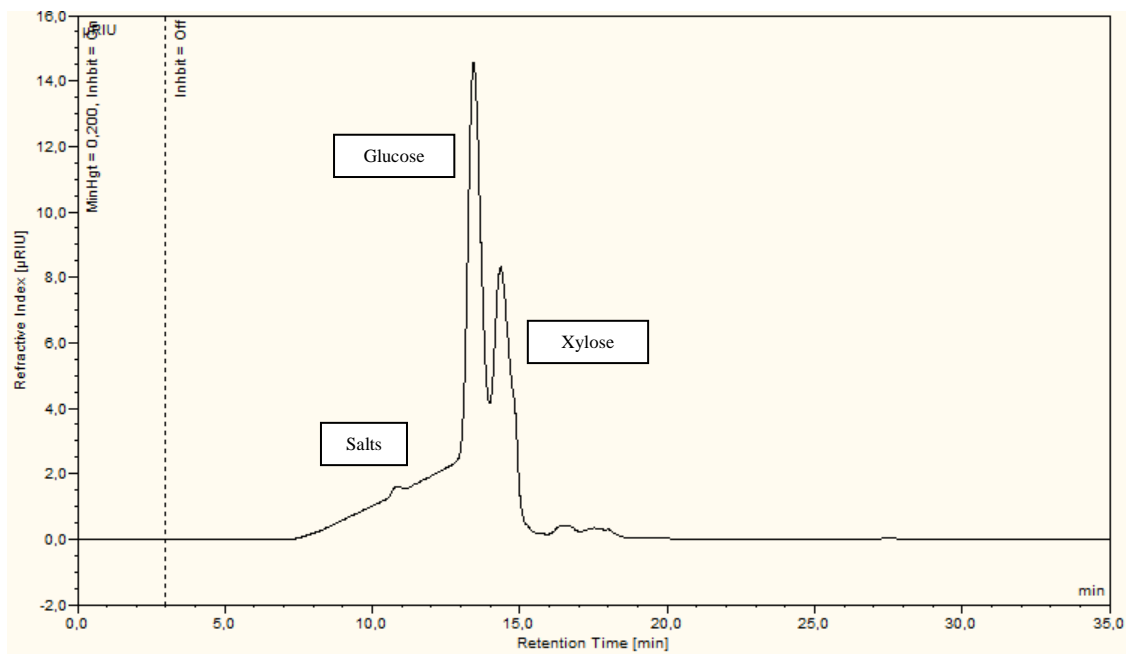
**Table 6.1**

Retention time of standard compounds on Rezex-RPM column with RI detector

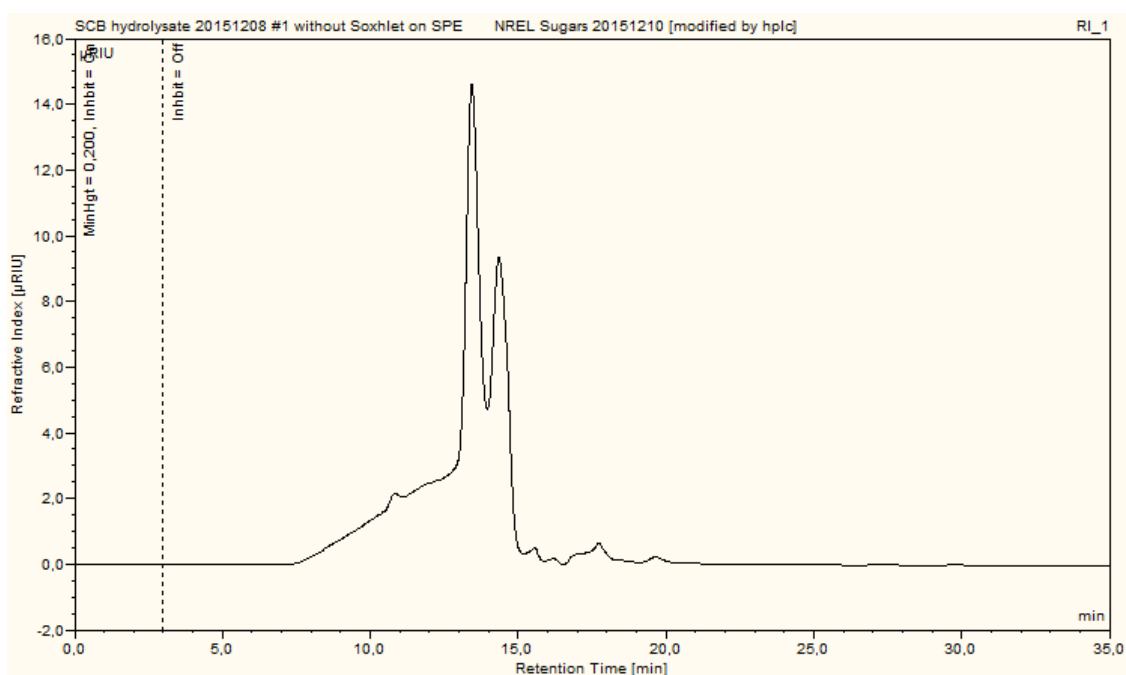
Compound	Retention time (min)
Cellobiose	11.51
Glucose	13.45
Xylose	14.36
Galactose	15.27
Arabinose	16.37
Rhamnose	16.43
Mannose	16.79
Fructose	17.29



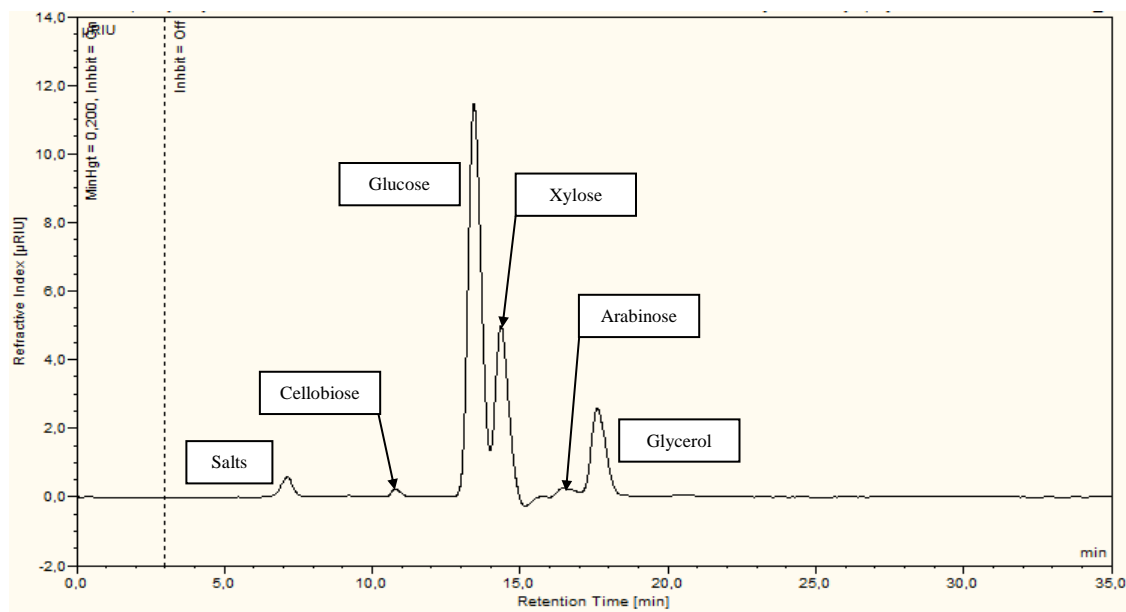
**Fig. 6.2** HPLC chromatogram on Rezex RPM column of SCB raw material analyzed following NREL protocol (including Soxhlet extractions with water and ethanol).



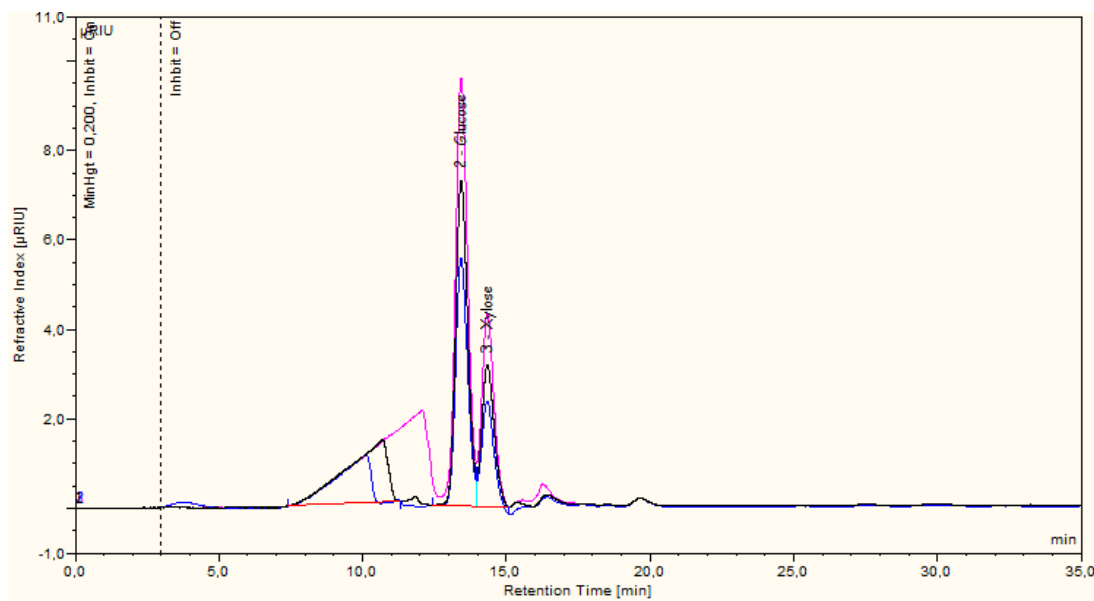
**Fig. 6.3** HPLC chromatogram on Rezex RPM column of SCB raw material analyzed by NREL protocol (without Soxhlet extractions). Salt peak interfered with the peaks of sugars.



**Fig. 6.4** HPLC chromatogram on Rezex RPM column of SCB raw material analyzed following NREL protocol (without Soxhlet extractions), samples filtrated on SPE cartridge Strata XL-C.



**Fig. 6.5** HPLC chromatogram on Rezex RPM column of SCB raw material, analyzed following NREL protocol (without Soxhlet extractions), samples filtrated on SPE cartridge Strata ABW. Glycerol appeared as a preservative solution of the cartridge.

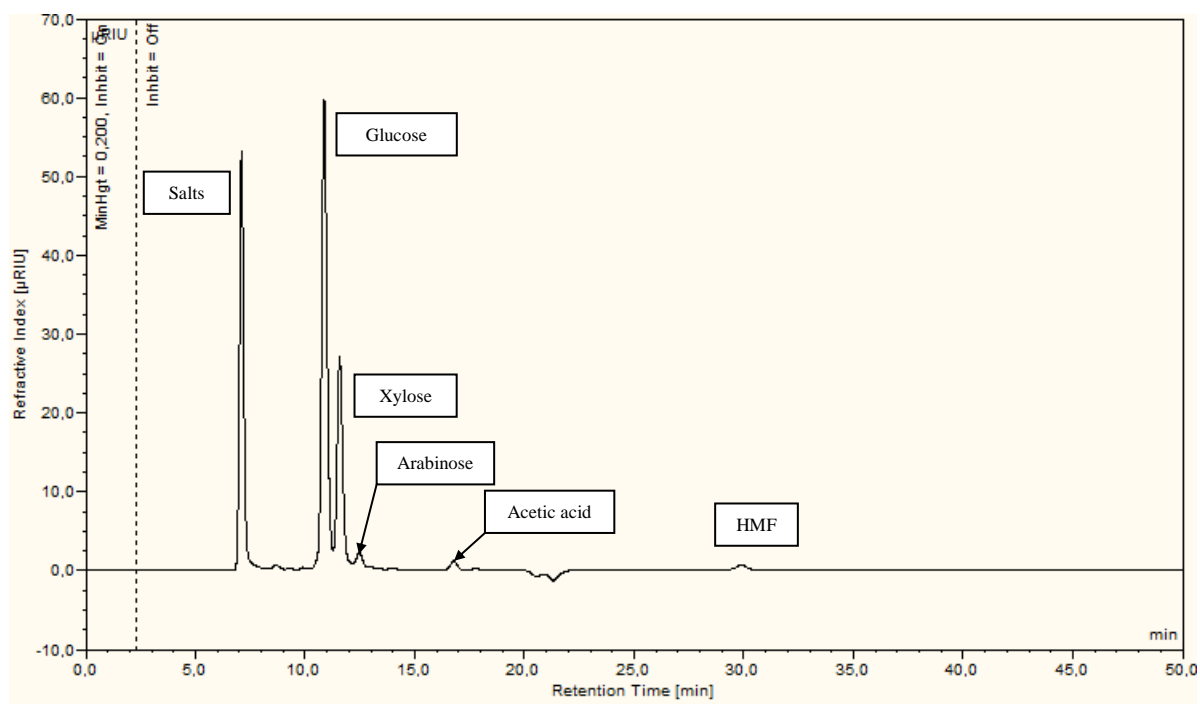


**Fig. 6.6** HPLC chromatogram on Rezex RPM column of SCB raw material analyzed following NREL protocol (without Soxhlet extractions), samples to analyze were mixed with XA7111 MB resin prior to injection. Three injections are presented corresponding to increasing quantity of resin in the similar samples to analyze: pink, black, blue lines – showing decreasing peaks of salt and sugars.

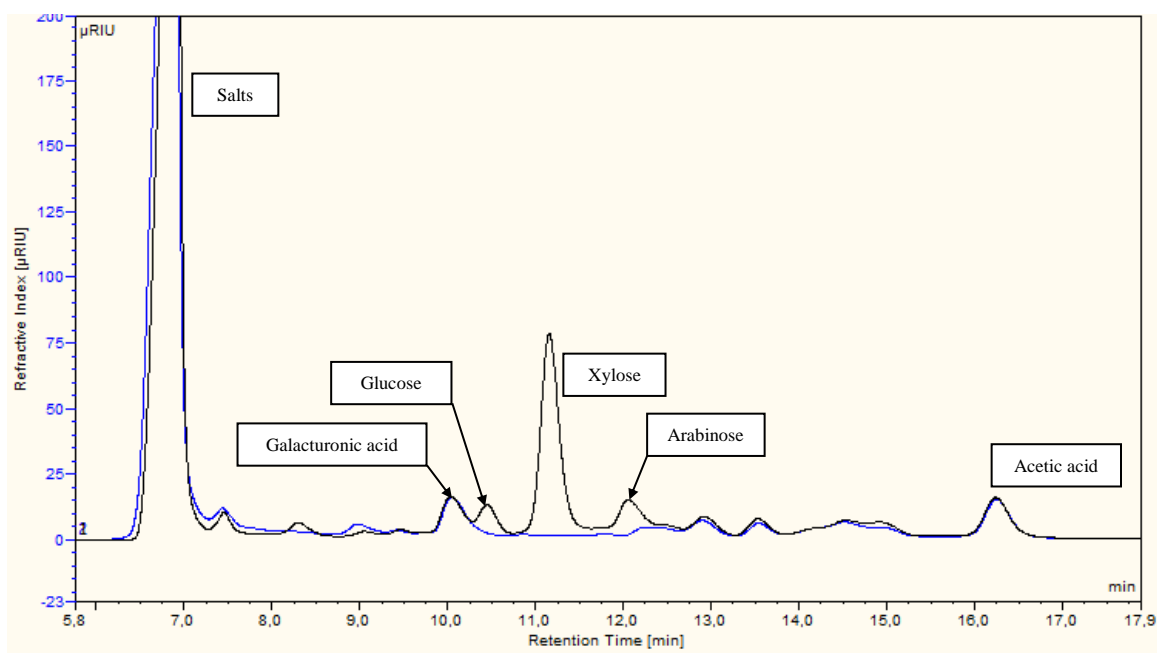
**Table 6.2**

Retention time of standards on Rezex-RHM column with RI detector

<b>Compound</b>	<b>Retention time (min)</b>
Cellobiose	8.85
Glucuronic acid	9.49
Galacturonic acid	10.08
Glucose	10.79
Galactose	11.43
Xylose	11.49
Arabinose	12.38
Xylitol	12.98
Succinic acid	13.28
Acetic acid	16.51
Ethanol	23.02
HMF	30.62
Furfural	42.76



**Fig. 6.7** HPLC chromatogram on Rezex RHM column of SCB raw material analyzed following NREL protocol (without Soxhlet extractions).



**Fig. 6.8** HPLC chromatograms on Rezex RHM column of SCB concentrated mild alkaline extracts, analyzed after only a pH adjustment to 2 (blue line) and analyzed by NREL protocol on liquid fractions (black line).



#### 6.4. Proteins

Protein content was determined in solid fractions (initial SCB and SuOC, and solid residue after alkaline extraction on SuOC) following Kjeldahl official method AOAC 984.13-1994. Samples of 1.2 g of SCB, 400 mg of SuOC (F0), 200 mg of solid residue after alkaline extraction of SuOC (F0), 800 mg of SuOC (F2A-) and its solid residue after alkaline extraction were mineralized using a solution of 12.5 mL sulfuric acid at 96% (w/w) with 2 tablets of catalyst (Kjeltabs: 5.0 g  $K_2SO_4$ , 0.15 g  $CuSO_4$ , 0.15 g  $TiO_2$ ) in each sample for 1 h at 400 °C in tubes of Tecator 2020 digester (Foss). After cooling, the tubes were transferred to auto sampler Kjeltac 8420 (Foss) and titration of mineralized nitrogen was carried out by full automated Kjeltac analyzer 8400 (Foss). First, 80 mL of distilled water then 50 mL of sodium hydroxide at 40% (w/v) were introduced into the tubes, then their contents were distilled and evaporated ammonia was recovered in 30 mL of indicator solution containing boric acid at 1%, bromocresol green and methyl red. The solution was titrated with hydrochloric acid at 0.1 mol/L. Total nitrogen content measured was multiplied by a factor of 6.25 (average content of nitrogen in proteins of 16%) to determine the protein content of the samples.

### 6.5. Phenolic monomers

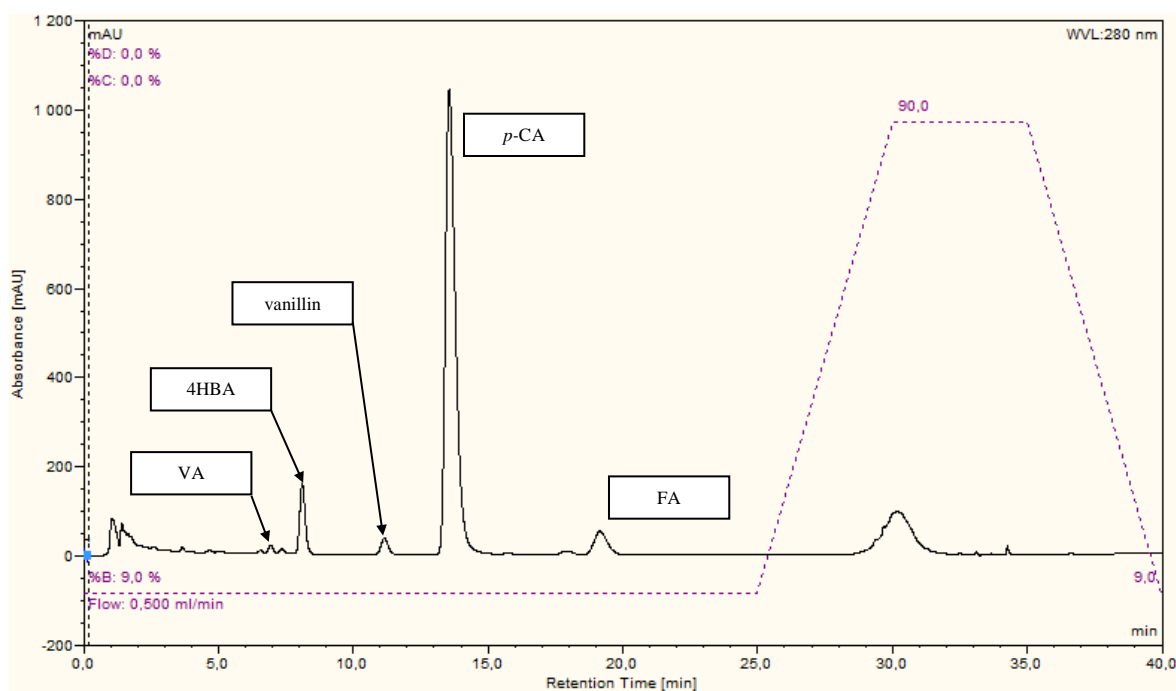
A HPLC method was developed based on other methods from the literature to quantify the highest number of phenolic monomers commonly contained in lignocellulosic biomass (Xu et al., 2005; Capriotti et al., 2015). Gallic acid, 4-hydroxybenzoic acid, caffeic acid, syringic acid, vanillic acid (VA), 4-hydroxybenzaldehyde (4HBA), vanillin, *p*-coumaric acid (*p*-CA), syringaldehyde, ferulic acid (FA), sinapic acid and hydroxycinnamic acid were quantified by HPLC on an OmniSpher 3 C18 100 x 4.6 column (Agilent Technologies). The gradient was as follow: 91% acidified water (1% acetic acid (v/v)) and 9% acetonitrile for 25 min, from 9 to 90% acetonitrile in 5 min, kept constant for 5 min, then decreased back to 91% acidified water in 5 min. The column was equilibrated for 7 min between runs. The flow rate was 0.5 mL/min, the injection volume was 10  $\mu$ L and the column temperature was maintained at 25 °C. The UV detector was set at 280 nm. Concentrations for the calibration curves ranged between 0 and 200 mg/L. Standard and process samples were diluted in acetonitrile:water at a ratio of 50:50 (v/v) prior to injection.

Several temperatures (20 to 40 °C) and solvent ratio (5% to 30% acetonitrile) were tested and the best separation between phenolic monomers while minimizing the analyses duration was obtained with the conditions detailed above. The retention time of the different phenolic monomers are given in **Table 6.3**.

**Table 6.3**

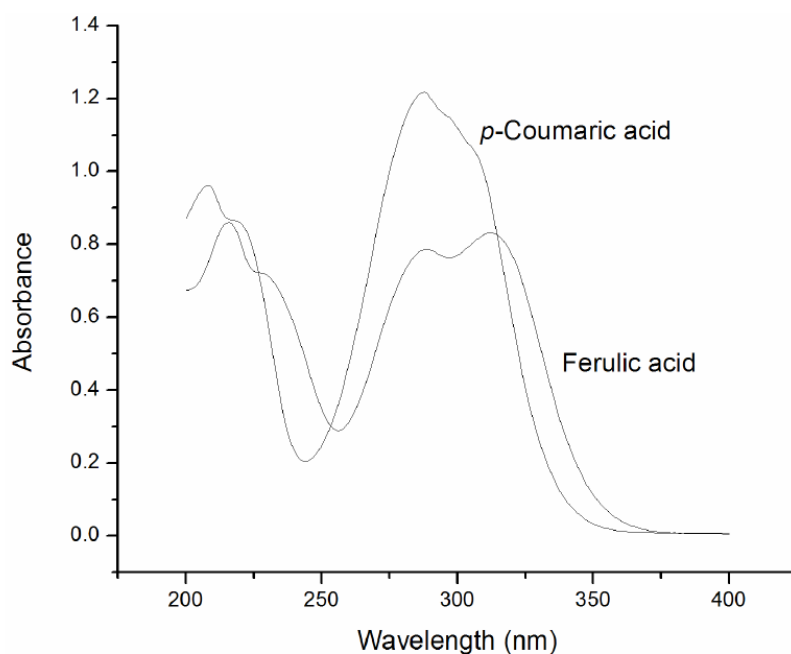
Retention time and response factor of identified components in the sugarcane bagasse alkaline extract on Omnispher 3 C18 column with UV detector at 280 nm

Compound	Retention time (min)
Gallic acid	1.5
HMF	2.4
Furfural	3.8
4-hydroxybenzoic acid	4.5
Caffeic acid	6.0
Syringic acid	6.4
VA	6.8
4HBA	8.2
Vanillin	11.2
Syringaldehyde	13.0
<i>p</i> -CA	13.7
FA	19.2
Sinapic acid	21.4
Hydroxycinnamic acid	23.8



**Fig. 6.9** HPLC chromatogram on OmniSpher 3 C18 column of SCB concentrated mild alkaline.

Phenolic monomers measured by this method could be accounted twice in numerous mass closures provided in this work since they can be accounted in the ASL as well. However, we considered that it could lead to minor discrepancies in the mass closure because they absorb less at 240 nm (NREL analysis for ASL) than at 280 nm as measured by this method. For instance, the two main phenolic monomers detected in SCB mild alkaline extract, *p*-coumaric acid and ferulic acid present a maximal absorbance at a wavelength of 280 nm, whereas their absorbance at 240 nm is close to a minimum (**Fig. 6.10**) (Holser, 2014).



**Fig. 6.10** Ultraviolet spectra of *p*-coumaric acid and ferulic acid (Holser, 2014).

## GENERAL CONCLUSIONS

To answer the post-petroleum issue, lignocellulosic material valorization into liquid fuels and more importantly into molecules (synthons and biomaterials) in biorefineries represents the only available option nowadays. Lignocellulosic biomass is essentially made of cellulose, hemicelluloses and lignin. Fractionation and purification of these three compounds are necessary for their valorization as substitutes to fossil hydrocarbons. Collaboration between Novasep, the joint research unit Agropolymer Engineering and Emerging Technologies, and the Laboratory of Agro-industrial Chemistry within LigNov project was created to explore promising fractionation and purification pathways on lignocellulosic biomass.

Organosolv process has already been claimed as the only process able to separate the three fractions of lignocellulose but industrial development has not been achieved yet. Acid fractionation process has been extensively studied, it is currently the most used in the industry. Purification pathways of the generated acid hydrolysate containing monomeric sugars rely on the use of polymeric resin (adsorption and chromatographic processes). However the valorization of the residue, mainly made of lignin, is rarely tackled. In the last decade, alkaline fractionation process, inspired from the pulp and paper industry, but using milder conditions has received more interest. Indeed, higher monomeric glucose yield can be achieved on the cellulose fraction after enzymatic saccharification. Moreover, the hemicelluloses and lignin are solubilized which favors their potential separation and valorization. For these reasons, the experimental work mainly focused on mild alkaline extract purification. In this work, the alkaline fractionation was studied on two lignocellulosic biomasses, sugarcane bagasse and sunflower oil cake, which presents different compositions. Sugarcane bagasse was selected as a model lignocellulosic material for the purification experiments because of its high content in lignocellulose and its interest in the scientific and industrial communities.

The thorough characterization of the initial sugarcane bagasse (SCB) and sunflower oil cake (SuOC) and their respective alkaline extracts showed that:

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- Electrostatic fractionation is promising for the separation of proteins and lignocellulose from SuOC. However, the protein left in the fraction enriched in lignocellulose still hindered the extraction of hemicelluloses and lignin. Mild alkaline extraction should be carried out on lignocellulosic biomass presenting low level of proteins.
- Lignocellulosic mild alkaline extracts are composed with up to 50% of potentially high added value molecules: lignin and hemicelluloses oligomers, phenolic monomers and acetic acid. The rest is inorganic salts from the initial biomass and mainly from the added alkaline salts that needs to be removed.
- Solubilized hemicelluloses are exclusively under oligomeric form; acetate groups and galacturonic acid (pectin) are fully released from the dissolved hemicelluloses.

Membrane filtration was studied in detailed showing that the choice of a membrane in terms of nature, structure and MWCO has to be based on experimental assays since performance cannot be extrapolated from one to another. Performance of the membranes (rejection rates of the molecules and flux) was dependent on some of the operating conditions such as transmembrane pressure and temperature but not significantly on cross-flow velocity in the range of values studied. On the membrane exhibiting the best performance in recycling mode, the 10 kDa polysulfone hollow fiber, up to 90% of the lignin and hemicelluloses oligomers were retained whereas inorganic salts, phenolic monomers and acetic acid were almost fully non-retained. Filtration in concentration and diafiltration modes confirmed the separation of these two pools of molecules with recoveries of about 70-80% for hemicelluloses and lignin and an increase in purity by a two-fold factor in concentration mode. However, during these filtration modes and particularly in diafiltration mode, the rejection rates of small molecules increased progressively, reducing the separation efficiency of the process.

Batch column elution chromatography on strong acid cation exchange resins, with water as eluent revealed that on a gel-type resin, phenolic monomers with a carboxyl group were separated from phenolic monomers without a carboxyl group and inorganic salts. This discovery was further used in the integrated process that we developed. On a

macroporous-type resin, a very pure fraction of the biggest hemicelluloses and lignin oligomers was obtained.

An integrated purification process was designed to produce purified fractions from the sugarcane mild alkaline extract. First, ultrafiltration in concentration then diafiltration mode was applied to the extract; 70% of the lignin and hemicelluloses oligomers were recovered in the retentate whereas 90% of the inorganic salts, and close to 100% of the phenolic monomers and acetic acid were recovered in the permeate. Separation of the lignin and sugar oligomers and was not achieved by membrane filtration nor elution chromatography, therefore, acid addition and ethanol addition processes were tested on the ultrafiltration retentate. Acid addition resulted in the precipitation of 91% of the lignin oligomers but sugar oligomers co-precipitation occurred. The ultrafiltration permeate was concentrated, and surprisingly 50% of the few hemicellulosic sugars contained in the permeate were precipitated as well as some lignin. The concentrated permeate underwent batch column chromatography on gel-type strong acid cation exchange resin and phenolic monomers with carboxyl group were recovered in a pool of molecules eluted fast whereas inorganic salts and phenolic monomers without a carboxyl group were eluted in a later pool.

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Many investigations have been carried out during this research work. However, some scientific aspects of the characterization, the fractionation and the separation could be further tackled:

- Extensive analysis of the lignin and hemicelluloses oligomers by Size-Exclusion Chromatography (SEC) could be of interest to confirm the potential different pools of sugars based on their size and the more homogeneous size distribution of lignin oligomers observed during the batch column elution chromatography experiments. It could also bring information about the mild alkaline extraction effect on the hemicelluloses and lignin fractionation and more understanding of the retention of these oligomers by membrane filtration.

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- Still on an analytical point of view, the follow-up of uronic acids (mainly galacturonic and glucuronic acids) was possible by HPLC on H<sup>+</sup> column within the NREL protocol. Galacturonic acid was detected during the characterization of the initial SCB and its resulting alkaline extract. However, the peak was not discernible after ultrafiltration and elution chromatography, the causes could be investigated.
- A precise composition of the mineral content of SCB could be investigated for instance by ionic HPLC or inductively coupled plasma atomic emission spectroscopy (ICP-AES) . The precise composition of the salts of the SCB and the resulting alkaline extract is a prerequisite for an efficient sodium hydroxide recycling.
- The rates of rejection of small molecules evolved during ultrafiltration in concentration and diafiltration modes. One explanation could be that inorganic salts are trapped or complexed within the retained lignin or sugar oligomers, but this should be demonstrated.
- To enrich the discussion on the fouling and the resistance-in-series model for the membrane filtration, the resistance linked to the adsorption R<sub>a</sub> could be assessed by circulating the alkaline extract without TMP and then measure the loss in permeate flux with distilled water.
- Understanding of the mechanism of the retention of salts during chromatographic experiments could have been carried out, since different salts presented different elution time (NaOH vs. NaCl and HCl). This would have permit to answer questions such as: during pulse test run with synthetic feed with a pH of 2, is there some conversion of the cation exchange resin from Na<sup>+</sup> to H<sup>+</sup> despite the excess of Na<sup>+</sup>? What is the role of the counter anion (OH<sup>-</sup> or Cl<sup>-</sup>)?
- The reasons of the formation of a precipitate after the concentration of the ultrafiltration permeate but not after the concentration of the alkaline extract could be investigated. Salting out phenomena was suggested as an explanation but this needs to be confirmed. Understanding how the concentration step impacts the precipitate formation and thus the removal of xylan and lignin would also be interesting; especially since we dealt with 20% DS content in this work, but increase up to 50% DS could be considered.



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- Similarly, chromatographic separation between phenolic monomers with carboxyl group and salts exhibited slightly lower resolution when performed on the ultrafiltration permeate rather than on the alkaline extract directly. The influence of xylan and lignin on the separation have to be examined.
- With both SCB alkaline extract and UF permeate as feed for the chromatographic pulse tests, a shoulder was observed in front of the main peaks for phenolic monomers without a carboxyl group (4-hydroxybenzaldehyde and vanillin). Explanation of this shoulder have not been provided, phenolate form (pKa of 9.95) of these compounds due to alkaline pH could be a reason for their partial rejection of the resin pores. Further pulse tests with synthetic solutions of various pH could be run.

It would also be of interest to explore further some technical points:

- Successive extractions with first mild acid to remove the hemicelluloses then mild alkaline to remove the lignin could be investigated as a fractionation scheme, hence reducing the purification requirements of the two extracts obtained. The composition of the fractions and the recovery rates of the different compounds could be compared to those observed in this work. Life Cycle Analysis (LCA) would then be of interest to compare this fractionation scheme to the one developed in this work and more generally to assess the environmental impact of the different purification steps.
- Alkaline extract from SuOC had a different composition in phenolic monomers (caffeic acid and 4-hydroxybenzaldehyde being the main ones) than SCB alkaline extract. Their separation on gel-type strong acid cation exchange resin depending on the presence or not of a carboxyl group in their structure could be checked.
- During the ultrafiltration experiment the volume reduction factor (VRF) was stopped due to the dead volume of the equipment. Larger volume of feed should be tested to push the VRF to a maximum were the performance would be too degraded (flux decrease or rejection rate increase).
- An intermediate step between ultrafiltration and precipitation on the retentate could be added for a complete demineralization of the UF retentate by ion

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exchange to assess how it affects the separation of xylan and AIL during the precipitation step.

- For the separation of lignin and sugar oligomers contained in the UF retentate, investigations on adsorption on non-functionalized resins (since the retentate has neutral pH) could be run. For instance, on resin with hydrophobic structure (e.g., styrenic resin), lignin could be fixed whereas xylan would be eluted. Then, study on the desorption of lignin with different solutions (e.g., alkaline solution or ethanol) would be required. Fractions with higher purities are expected but also a higher process costs than with precipitation processes as presented in this work. LCA could be performed to compare the different processes for the separation of xylan and AIL.
- This work showed the interest of chromatographic process to separate the compounds of a lignocellulosic mild alkaline extract. Optimization of the process conditions such as the temperature, the eluent velocity, the feed loading and the feed concentration have to be explored to guarantee good resolution and productivity at industrial scale.
- The chromatographic separations at larger scale in continuous system (e.g., simulated moving bed) should be performed to check that the yields and purities are improved, and to assess the productivity of the system to consider industrialization of the proposed process. The selection of the valuable compounds should be carried out, for instance *p*-CA, to set the parameters of the separation (BVs) to favor the recovery or the purity of this compound. Before running a SSMB experiment at pilot scale, a feed overload elution test could be run in batch column chromatography until breakthrough curves are achieved.
- To complete the purification process proposed, a demineralization step on the fraction eluted before 0.43 BV containing phenolic monomers with carboxyl groups could be carried out, then crystallization or preparative HPLC to obtain pure molecules.

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## **RESUME GENERAL EN FRANCAIS**

### INTRODUCTION

Les travaux présentés dans ce manuscrit ont été financés par l'ANR dans le cadre du projet LigNov (ANR-14-CE06-0025-01), et ils ont été réalisés au Laboratoire de Chimie Agro-industrielle (LCA) à Toulouse. Deux partenaires ont été impliqués dans le cadre de ces travaux, l'unité de recherche Ingénierie des Agropolymères et Technologies Emergentes (IATE) à Montpellier et Novasep Process, une société basée à Saint-Maurice-de-Beynost, spécialisée dans la purification de molécules notamment au travers des procédés à résines et à membranes.

La raréfaction du pétrole entraîne la recherche de nouvelles sources d'énergie et de molécules pour diverses applications de la chimie. Les biomasses lignocellulosiques, résidus forestiers et agricoles, constituent de par leur quantité et leur structure un potentiel unique pour la production d'énergie et de molécules d'origine renouvelable. En effet, en 2016, 4 milliards de tonnes de pétrole ont été consommé par l'humanité, en parallèle en 2008, les résidus de culture de seulement 6 espèces végétales cultivées par l'Homme (le blé, la canne à sucre, le riz, l'orge, le soja et le maïs) ont représenté 3,7 milliard de tonnes. La transformation de ces matières végétales pour la production d'énergie, de molécules et de matériaux a donné lieu au concept de bioraffinerie, inspiré de la raffinerie du pétrole.

## Chapitre 1 : LA BIORAFFINERIE LIGNOCELLULOSIQUE

Sur la base d'une étude de la littérature, ces travaux se sont tout d'abord attachés à la description et la compréhension des fractionnements chimiques acides et alcalins de la lignocellulose et aux voies de purification qui leur sont actuellement associées. Les biomasses lignocellulosiques sont constituées de cellulose, hémicelluloses et lignines. Le fractionnement et la purification de ces trois constituants est nécessaire à leur valorisation comme produits de substitution du pétrole. Au sein des bioraffineries, de nombreux procédés ont été étudiés pour l'étape de fractionnement : procédés biologiques (utilisation de champignons, de bactéries, d'enzymes), physiques (explosion à la vapeur, liquéfaction), chimiques (Organosolv, acide, alcalin) entre autres. Les fractionnements chimiques en milieu acide et alcalin semblent être les plus efficaces et les plus employés, respectivement pour la production d'éthanol cellulosique et de papier.

Le fractionnement en milieu acide a pour effet d'hydrolyser les liaisons glycosidiques entre les sucres constitutifs de la cellulose (le glucose) et des hémicelluloses (sucres en C5 et C6 tels que le xylose, l'arabinose, le glucose, le galactose, le mannose). L'hydrolyse est influencée par quatre paramètres principaux que sont la concentration en acide, le ratio solide/liquide (autrement dit matière première/solution acide), la température et la durée de la réaction. D'autres paramètres tels que la taille des particules solides ou l'agitation influencent l'hydrolyse mais la plus part des études ne les évoquent pas. La nature de l'acide inorganique a également une influence sur l'efficacité du fractionnement et l'acide sulfurique s'est montré le plus intéressant. Les polysaccharides pariétaux sont donc solubilisés dans le milieu acide, hydrolysés en monomères puis les monomères peuvent être eux même dégradés en dérivés furaniques, furfural pour les sucres en C5 et hydroxyméthylfurfural pour les sucres en C6, enfin ceux-ci peuvent à leur tour être dégradés en acide lévulinique, acide formique et autres produits de dégradation. Le changement de valeur d'un des paramètres de la réaction peut être compensé par celui d'un autre paramètre, par exemple, pour un même rendement en monomères de sucre à partir de lignocellulose, une augmentation de température peut être compensée par la diminution du temps de réaction. Deux principaux procédés de fractionnement en milieu acide sont employés, l'acide dilué couplé à des hautes températures, généralement des

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concentrations de 1 à 4% (m/v) et des températures entre 120 et 180°C sont utilisées, et l'acide concentré couplé à des températures plus basses, où des concentrations de l'ordre de 70% (m/v) et des températures entre 30 et 60°C sont utilisées. De nombreux chercheurs et industriels ont finalement retenus l'association des deux procédés, ainsi dans un premier temps la lignocellulose est soumise à de l'acide concentré à basse température puis l'acide est dilué par ajout d'eau et la température d'hydrolyse est élevée. Ce procédé a d'ailleurs fait l'objet d'une technique analytique développée par le Laboratoire National des Energies Renouvelables (National Renewable Energy Laboratory – NREL) aux Etats-Unis d'Amérique entre les années 2000 et 2010. Cette technique est aujourd'hui utilisée par l'ensemble de la communauté scientifique qui travaille sur la thématique de la bioraffinerie. Elle repose donc sur une double hydrolyse acide de la lignocellulose qui a pour effet de convertir totalement les polysaccharides pariétaux en monomères de sucres, qui sont ensuite quantifiés par HPLC (Chromatographie en phase Liquide à Haute Performance). Une petite fraction de la lignine est solubilisée dans l'hydrolysate acide (Acid Soluble Lignin – ASL) et quantifiée par spectrophotométrie, tandis que la majeure partie de la lignine est récupérée dans le résidu solide de la double hydrolyse acide (Acid Insoluble Lignin – AIL) et quantifiée par gravimétrie. Cette méthode analytique a été utilisée tout au long des travaux expérimentaux ici présentés.

Les hydrolysats lignocellulosiques acides sont donc constitués de monomères de sucres, de produits de dégradation des sucres (dérivés furaniques), de dérivés phénoliques (ASL), d'acide acétique (dû à l'hydrolyse des groupements acétates fixés sur les hémicelluloses). La production d'éthanol à partir des monomères de sucres, qui peuvent être également valorisés sous d'autres formes (d'autres alcools, entre autres le sorbitol, le xylitol ou l'arabinol ; des acides organiques, entre autres l'acide citrique, succinique ou lactique), nécessite une étape de fermentation via l'utilisation d'enzymes, de levures ou de bactéries. Au préalable à cette étape, le retrait des dérivés furaniques, phénoliques et de l'acide acétique est nécessaire puisque ceux-ci ont une action inhibitrice sur la fermentation. Leur retrait par purification des hydrolysats acides peut d'ailleurs conduire à leur future valorisation, puisque le furfural ou l'hydrométhylfurfural ont par exemple une valeur intrinsèque élevée. Parmi les procédés de purification des hydrolysats acides un des plus simples à mettre en œuvre consiste à alcaliniser le milieu par ajout de bases ce qui entraîne la précipitation des inhibiteurs de fermentation. L'utilisation d'hydroxyde



de calcium s'avère plus efficace que l'utilisation de soude au regard des rendements en éthanol à l'étape suivante de fermentation, mais ce procédé induit une consommation importante de produits chimiques puisque le pH doit être augmenté d'environ 1 jusqu'à 9-10 puis diminuer aux alentours de 5,5 qui est le pH auquel les réactions de fermentation ont généralement lieu. L'ammoniac est une base dont le prix est élevé, mais son utilisation pour l'augmentation du pH des hydrolysats acides présente l'intérêt de ne nécessiter une augmentation de pH que jusqu'à 5,5 pour une efficacité équivalente à la soude ou l'hydroxyde de calcium, de réduire les pertes en sucre puisqu'il n'y a pas de précipitation et de réduire également l'apport nécessaire en substrat azoté au cours de l'étape suivante de fermentation. L'évaporation des hydrolysats acides est également une technique simple à mettre en œuvre et entraîne le retrait des dérivés furaniques et de l'acide acétique sans perte de sucres, en revanche les dérivés phénoliques restent présents. L'extraction liquide/liquide, notamment avec l'utilisation d'acétate d'éthyle s'est avérée plus efficace que l'évaporation pour le retrait des inhibiteurs de fermentation et pour les rendements en éthanol, cependant, cette technique entraîne une consommation importante de solvant organique et nécessite son recyclage. Les procédés d'absorption sur charbon actif ou sur résines s'avèrent être les plus efficaces en terme d'élimination d'inhibiteurs de fermentation et de préservation des sucres conduisant ainsi aux plus forts rendements en éthanol. L'adsorption d'inhibiteurs est plus importante sur le charbon actif que sur les résines mais la désorption est compliquée et pénalise donc le recyclage du charbon actif. L'utilisation de résines anioniques couplées à une alcalinisation des hydrolysats acides permet d'atteindre de haut niveau d'adsorption pour les inhibiteurs, notamment en les chargeant négativement (acétate pour l'acide acétique, phénolate pour les dérivés phénoliques). Cependant, afin d'éviter une consommation importante de produits chimiques une adsorption sans ajustement de pH peut être préférable. Le mécanisme de fixation des inhibiteurs est alors essentiellement par liaisons hydrophobes, ce qui entraîne une faible fixation de l'acide acétique ; de plus une concentration en acide importante a pour effet d'augmenter l'adsorption des inhibiteurs sur la résine par relargage, les molécules d'eau solvatant préférentiellement les sels acides. A pH acide, la capacité d'absorption des résines cationiques est plus faible que celles des résines non-ioniques ou du charbon actif, néanmoins l'étape de désorption à l'avantage de pouvoir se faire qu'avec de l'eau comme éluant ceci étant dû à la disparition du phénomène de relargage.

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Cependant, toutes les techniques de purification d'hydrolysats lignocellulosiques acides ne semblent pas être privilégiées au niveau industriel. En effet, l'utilisation de résines cationiques en mode chromatographique pour la purification d'hydrolysats lignocellulosiques acides semble favorisée. La chromatographie continue sur résines cationiques avec  $H^+$  comme contre-ion et de l'eau comme éluant permet la séparation des monomères de sucre et de l'acide. Une deuxième étape de chromatographie, toujours avec de l'eau comme éluant et sur résines cationiques mais avec  $Ca^{2+}$  comme contre-ion permet la séparation des monomères de sucres entre eux (glucose, xylose, arabinose par exemple). La filtration membranaire est un procédé qui a été peu étudié pour la purification d'hydrolysats acides, puisque la différence de taille entre les monomères de sucre et les impuretés (acide sulfurique, inhibiteurs de fermentation) est minime. L'électrodialyse, un autre procédé à membrane, s'est avérée plus intéressante pour la séparation de l'acide sulfurique et des sucres.

Le fractionnement en milieu alcalin est l'autre type de fractionnement chimique auquel nous nous sommes intéressés. Il trouve son origine dans l'industrie papetière où la récupération de cellulose à haut niveau de pureté est obtenue par la solubilisation des hémicelluloses et de la lignine en milieu alcalin dans des conditions drastiques. Il a été repris dans le concept de bioraffinerie lignocellulosique pour la production d'éthanol notamment, mais avec des conditions plus douces, entraînant un retrait partiel des hémicelluloses et des lignines, qui est suffisamment efficace pour obtenir de meilleurs rendements en éthanol sur le résidu solide cellulosique après saccharification enzymatique et fermentation du glucose, comparé aux fractionnements acides suivis de fermentation. Les conditions alcalines ont pour effet de cliver les liaisons esters entre les groupements acétates et les hémicelluloses, entre les monomères phénoliques (acide coumarique et acide férulique essentiellement) et la lignine ou les hémicelluloses. Ainsi, les hydrolysats alcalins contiennent des sels inorganiques (base), des oligomères de lignine et d'hémicelluloses, des monomères phénoliques et de l'acide acétique (sous forme acétate). De même que pour le traitement acide, quatre paramètres principaux influencent la solubilisation des hémicelluloses et des composés phénoliques : la concentration en base, le ratio solide/liquide (autrement dit matière première/solution alcaline), la température et la durée de la réaction. La nature de la base a également une grande importance et la soude s'avère être la plus efficace en terme de solubilisation

d'hémicelluloses et de lignines et ainsi de conversion de la cellulose en éthanol par saccharification enzymatique puis par fermentation du glucose. Des concentrations en soude de l'ordre de 1-2% (m/v), des ratios solide/liquide de 1/10-1/20 (m/v), des températures de 60-80 °C et des durées de réactions de quelques heures ont donné des taux élevés de solubilisation d'hémicelluloses et de lignine, et donc de forts rendements de production d'éthanol. Le fractionnement en milieu alcalin nécessite des conditions plus douces que celles employées dans le fractionnement en milieu acide et donc entraîne une réduction des coûts liés aux équipements du procédé, aux produits chimiques et aux utilités, mais une réaction supplémentaire d'hydrolyse enzymatique des polymères de sucres est à mettre en œuvre.

Bien que les principaux produits valorisés après traitements alcalins soient les résidus solides (cellulose), la valorisation des composés des hydrolysats alcalins, présente un grand intérêt pour augmenter l'efficacité économique des bioraffineries employant ces traitements. La séparation des oligomères d'hémicelluloses, de lignines et les monomères phénoliques est un prérequis pour leur valorisation dans un large spectre d'applications tels que les tensioactifs, les adhésifs, les résines ou la production de synthons pour l'industrie chimique. Pour les hydrolysats alcalins obtenus par utilisation de soude, la lignine peut être purifiée par floculation par ajout de chlorure de calcium, le calcium créant des liaisons entre les groupements chargés négativement des composés phénoliques (groupement phénolates et carboxyliques) et entraînant ainsi leur floculation. La précipitation de la lignine par ajout d'acide inorganique dans les hydrolysats alcalins papetiers a été très utilisée. Plus récemment, le procédé a été transposé aux hydrolysats alcalins obtenus en conditions plus douces. L'addition d'éthanol a également été testée et mène à la précipitation des hémicelluloses. Les procédés de précipitation entraînent généralement de forts taux de récupération, mais les puretés obtenues sont impactées par des co-précipités de sucres dans le cas d'ajout d'acide, et de lignine dans le cas d'ajout d'éthanol, de plus ces procédés nécessitent la consommation de grandes quantités d'acide ou de solvant. L'utilisation de charbon actif sur les hydrolysats alcalins permet la fixation de composés phénoliques, ainsi il peut permettre de fixer des molécules considérées comme des impuretés lorsque les oligomères de sucres sont les molécules ciblées lors de la purification ou bien les molécules fixées constituent la fraction à valoriser, leur désorption est alors nécessaire et l'utilisation de soude s'est avérée efficace. Parmi les

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différents types de résine, les résines anioniques présentent les meilleurs taux d'adsorption de composés phénoliques et d'acétate à partir d'hydrolysats alcalins. Des solutions eau/éthanol/acide chlorhydrique et d'acide sulfurique ont été utilisées respectivement pour la désorption de monomères phénoliques et d'acide acétique. Tout comme les procédés de précipitation, la filtration membranaire est employée de longue date pour la purification d'extraits alcalins papetiers et récemment sur les extraits alcalins obtenus en conditions douces. L'utilisation de membranes avec des seuils de coupure de l'ordre de 1 à 30 kDa permettent de retenir les hémicelluloses alors que les autres composés des extraits alcalins obtenus en conditions drastiques sont récupérés dans le perméat. L'électrodialyse, en amont de la méthode de précipitation des lignines par acidification évoquée précédemment, a permis de réduire la consommation d'acide et en parallèle de recycler la soude.

Après le fractionnement en milieu acide ou en milieu basique, les différentes techniques de purification décrites ci-dessus ont également été utilisées en combinaison afin d'augmenter le niveau de pureté des molécules ciblées. Les fractionnements acide et alcalin ont aussi été étudiés de manière séquencée afin dans un premier temps d'hydrolyser les hémicelluloses dans des conditions acides puis dans un deuxième temps de solubiliser les composés phénoliques. Cette méthode d'extraction séquencée a pour avantage de réduire les étapes de purification.

## Chapitre 2 : FRACTIONNEMENT CHIMIQUE

Les travaux expérimentaux ont été réalisés à partir de deux matières premières : la bagasse de canne à sucre et le tourteau de tournesol. La deuxième partie de ce manuscrit a porté sur la caractérisation de ses biomasses, puis des fractions obtenues après prétraitement électrostatique, après extraction en condition alcaline douce et après extraction en condition acide concentré. La bagasse constitue une biomasse lignocellulosique modèle car elle est très étudiée dans la littérature, en effet elle est constituée quasiment exclusivement de lignocellulose, elle contient très peu d'autres éléments comme les protéines, les lipides ou les minéraux et la canne à sucre est l'espèce végétale la plus cultivée par l'Homme au niveau mondial (2 milliards de tonnes/an). La bagasse est actuellement brûlée dans les sucreries pour produire de l'électricité, il serait intéressant de lui trouver des débouchés à plus forte valeur ajoutée comme la production de polymères ou synthons pour l'industrie chimique. Le tournesol est une plante typique du Sud-Ouest de la France (où les travaux ont été réalisés) et le tourteau généré après l'extraction de l'huile à partir des graines est actuellement essentiellement utilisé en alimentation animale du fait de sa forte teneur en protéine. Cependant, le tourteau de tournesol contient aussi de la lignocellulose qui constitue un frein à la digestion des protéines par les animaux. Une étape de fractionnement en milieu sec, par tri-électrostatique a été réalisée par IATE, un des partenaires du projet LigNov avec l'objectif de produire une fraction de tourteau enrichie en protéines et donc destinée à l'alimentation animale et une fraction enrichie en lignocellulose qui pourrait être valorisée pour sa teneur en cellulose, hémicelluloses et lignine. Deux passes de tri-électrostatique ont conduit à la production d'une fraction enrichie en lignine (33% contre 20% initialement) et appauvrie en protéines (13% contre 31% initialement) avec un rendement massique de 21%.

Une caractérisation fine de ces matières premières ainsi que des extraits acides et alcalins obtenus à partir de ces matières a été réalisée. La méthodologie développée par NREL (Colorado, Etats-Unis d'Amérique) pour le dosage de la cellulose, des hémicelluloses et des lignines a été utilisée pour ces travaux, puisqu'elle s'est avérée plus fiable et plus facilement applicable, notamment pour les échantillons liquides, que la méthode ADF-NDF développée par Van Soest. Les monomères phénoliques ont été dosés

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par HPLC sur colonne C18, les protéines par la méthode Kjeldahl, les sels inorganiques par calcination à haute température (500 °C) et parfois suivis par conductivité, et enfin l'acide acétique, les dérivés furaniques et les acides uroniques par HPLC sur colonne H<sup>+</sup>.

Nos travaux n'ont pas eu pour but d'optimiser l'étape de fractionnement en conditions alcalins douces. Les conditions utilisées pour cette étape se sont basées sur une étude de Sun et al. (1995) sur l'extraction des hémicelluloses et de la lignine à partir de paille de blé. Les conditions optimales déterminées dans cette étude et reprises dans nos travaux ont été les suivantes : solution de soude à 1,5% (m/v), ratio solide/liquide de 1/20 (m/v), température de 60 °C et durée de 6 h avec agitation. Le but de nos travaux expérimentaux était de caractériser finement l'hydrolysat lignocellulosique alcalin ainsi produit afin d'étudier des voies de purification des différents composés le constituant.

L'extraction alcaline sur la bagasse de canne à sucre a été réalisée à trois échelles différentes, d'abord sur 10 g de bagasse en vue de réaliser une étude préliminaire, puis sur 150 g afin de produire un extrait pour tester sa purification par chromatographie et enfin sur 3 kg pour produire un lot d'extrait unique suffisant pour tester les différentes membranes d'ultrafiltration (UF) et éviter les biais liés à un produit à filtrer non uniforme d'un essai à l'autre. La composition du résidu solide et de l'extrait alcalin a peu évolué en fonction de l'échelle à laquelle a été réalisée l'extraction. Cependant les rendements d'extraction des hémicelluloses et de la lignine se sont avérés plus élevés à la plus grande échelle probablement grâce à une technique de séparation solide/liquide plus efficace (filtration centrifuge à la plus grande échelle contre filtration sur Büchner pour les autres échelles) et au rinçage du résidu solide durant la séparation solide/liquide à la plus grande échelle. Le résidu solide a été enrichi en cellulose et appauvri en hémicelluloses et lignines. L'extrait alcalin contient principalement des sels (plus de la moitié de sa matière sèche), de la lignine (environ 25%), des hémicelluloses sous forme d'oligomères (environ 10%), des monomères phénoliques (l'acide coumarique étant le principal, puis l'acide férulique, le 4-hydroxybenzaldéhyde, la vanilline et l'acide vanillique) et de l'acide acétique.

L'extraction alcaline a été aussi réalisée sur le tourteau de tournesol initial (F0) et sur le tourteau de tournesol obtenu après deux passes de fractionnement électrostatique (F2A-). Pour ces deux matières, les protéines ont été extraites à 80%, pénalisant ainsi la

séparation résidus solides/extraits par filtration sur Büchner et de futures étapes de purification, où une étape de retrait des protéines sera nécessaire, par centrifugation par exemple. En parallèle, la lignine a été faiblement extraite (9% pour F0 et 7% pour F2A-), bien moins que pour la bagasse. En revanche, le fractionnement électrostatique a eu une influence positive sur la quantité de monomères phénoliques extraits, leur part dans la composition de l'extrait passant de 10% pour F0 à 20% pour F2A-. La composition en monomères phénoliques des extraits alcalins de tourteaux est très différente de celle des extraits alcalins de bagasse puisque l'acide caféique est de loin celui retrouvé en plus grande quantité devant le 4-hydroxybenzaldéhyde, puis la vanilline, l'acide férulique, l'acide coumarique et l'acide sinapique.

L'extraction en conditions acide concentré a été testée sur la bagasse avec les conditions suivantes : solution d'acide sulfurique à 72% (m/m), ratio solide/liquide de 1/20 (m/m), température de 50 °C et durée de 1 h avec agitation. L'extrait obtenu contenait toute la cellulose initiale dont 45% sous forme de monomère de glucose, 73% du xylan initial dont 43% sous forme de monomère de xylose, 67% de l'arabinan initial dont 49% sous forme de monomère d'arabinose, une partie du xylose et de l'arabinose ayant été en partie dégradée en furfural, tandis que très peu de lignine et de monomères phénoliques ont été solubilisés.

### Chapitre 3 : PURIFICATION PAR FILTRATION MEMBRANAIRE

Les étapes de purification se sont focalisées sur l'extrait alcalin de bagasse. En effet, la purification d'extraits alcalins obtenus en conditions douces, a été peu étudiée malgré l'intérêt de ce procédé de fractionnement. La filtration membranaire et la chromatographie sur résine échangeuse de cation ont été étudiées séparément puis en association, afin de séparer les cinq grandes familles de molécules constitutives de l'extrait : des oligomères de lignines, des oligomères de sucres, des monomères phénoliques, de l'acide acétique et des sels inorganiques.

La troisième partie aborde la purification par filtration membranaire. Tout d'abord, un screening de membrane ainsi qu'une étude sur l'influence des paramètres de filtration en recyclage total (rétentat et perméat) ont permis de déterminer que les oligomères de lignine et de sucres, récupérés dans le rétentat, sont séparés des monomères phénoliques, de l'acide acétique et des sels inorganiques, récupérés dans le perméat. Le taux de rétention de toutes ces molécules et le flux ont été évalués sur cinq membranes fibres creuses en polysulfone ayant des seuils de coupure de 1 à 50 kDa et deux membranes tubulaires céramiques à des pressions transmembranaires (PTM) de 0,8 à 2,8 bar et trois taux de cisaillement différents. La membrane en fibres creuses de 10 kDa en polysulfone a présenté les meilleures performances de séparation, avec des taux de rétention en lignine (AIL) et en hémicelluloses atteignant 90% tout en retenant très peu les autres molécules, par ailleurs le flux critique n'a pas été atteint sur la gamme de pression testée. Le flux critique est observé par le suivi de l'évolution du flux en fonction de la PTM. L'évolution est tout d'abord linéaire, puis à partir d'une pression un point d'inflexion est observé, correspondant au flux critique, avant l'obtention d'un flux limite lorsque le flux atteint un plateau malgré une augmentation de la PTM. Le flux critique marque en général la transition entre un colmatage réversible de la membrane et un colmatage irréversible, et il est donc conseillé d'utiliser une PTM sous la PTM du flux critique. Durant le screening de membrane, il a été observé qu'une augmentation de la PTM augmentait le taux de rétention des oligomères de lignines (AIL) et de sucres (xylans et arabinans). Le taux de cisaillement n'a pas eu d'influence notable ni sur le taux de rétention des molécules ou ni sur le flux. L'influence d'une augmentation de température de 20 °C à 40 °C a également



été étudiée sur cette membrane. Il en a résulté une diminution de 10% taux de rétention des oligomères de lignine et de xylane mais une augmentation du flux d'un facteur 2. Cette membrane a été retenue pour les essais suivant en mode concentration et diafiltration. Etant attendu que la filtration en mode concentration provoque une augmentation de la rétention des molécules et une diminution du flux par augmentation de la couche de polarisation et de la viscosité, une température de 40 °C et un taux de cisaillement élevé ( $10\ 187\ s^{-1}$ ) ont été sélectionnés pour réaliser cet essai. Le mode concentration présente l'intérêt d'augmenter la concentration des hémicelluloses et des lignines dans le rétentat tout en les purifiant par le retrait des molécules plus petites dans le perméat. Ainsi un facteur de réduction volumique (FRV) de 6 a permis de concentrer les lignines (AIL) et les hémicelluloses (xylans et arabinans) d'un facteur supérieur à 4, tout en augmentant leur pureté d'un facteur 2.

### Chapitre 4 : PURIFICATION PAR CHROMATOGRAPHIE

Dans la quatrième partie, des essais de chromatographie d'élution en batch sur colonne effectué à 40°C avec de l'eau pour éluant en testant plusieurs résines acides cationiques fortes sont présentés. Des essais d'élution ont été effectués sur une résine cationique avec  $\text{Ca}^{2+}$  comme contre-ion de type gel, à partir d'une solution synthétique de glucose, xylose et arabinose, afin de valider la dernière étape de chromatographie présentée dans littérature comme étant capable de séparer ces monomères de sucres. La résolution de la séparation de ces sucres s'est avérée plus faible que celle présentée dans la littérature mais ce résultat est cependant à relativiser car une vitesse d'élution quinze fois plus élevée a été utilisée pour les tests ici présentés.

Deux résines acides cationiques fortes avec  $\text{Na}^+$  comme contre-ion, l'une de type gel, l'autre de type macroporeuse, ont été utilisées pour des tests d'élution sur l'extrait alcalin de bagasse décrit dans le deuxième chapitre. Les essais sur la résine de type gel ont montré le potentiel de séparation des composés constituant l'extrait. Ainsi une fraction éluee avant 0.42 volume d'éluant a été enrichie en oligomères de lignine (80% de récupération) et d'hémicelluloses (78% de récupération pour le xylan et 72% pour l'arabanan) et en monomères phénoliques possédant une fonction carboxyle (taux de récupération de 76%, 73%, 71% respectivement pour l'acide vanillique, l'acide coumarique et l'acide férulique) tandis que l'autre fraction éluee après 0.42 BV a été enrichie en sels inorganiques (79% de récupération) et en monomères phénoliques ne possédant pas de fonction carboxyle (taux de récupération de 78% pour le 4HBA et 74% pour la vanilline). L'acide acétique, sous la forme d'acétate, a été partagé entre ces deux fractions. L'élution de solutions synthétiques d'acide coumarique ou d'acide férulique en présence de base ou d'acide a permis de montrer que la charge négative de ces molécules à pH basique ( $\text{pH} \gg \text{pKa}$ ), due à la déprotonation de leur groupe carboxyle, est responsable du rejet de ces molécules hors des pores de la résine et de leur élution avant 0.42 BV. L'acide acétique, sous forme acétate étant plus petit il n'a été que partiellement rejeté des pores de la résine et donc élue à 0.42 BV.

Les essais sur la résine de type macroporeuse ont montré qu'une fraction très pure d'oligomères de lignines et de sucres peut être obtenue. Ainsi la fraction éluee avant 0,47

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volume d'éluant contient 20 à 30% des plus oligomères de sucre et 15% des plus oligomères de lignine. Des essais préalables de chromatographie d'exclusion stérique réalisés au Laboratoire de Glycochimie, des Antimicrobiens et des Agroressources sous la supervision du professeur José Kovensky ont montré que les plus gros oligomères de sucres atteignaient 300 kDa. La fraction éluée au-delà de 0,47 volume d'éluant contient plus de 99% des sels inorganiques, des monomères phénoliques et de l'acide acétique.

### Chapitre 5 : PROCEDE DE PURIFICATION INTEGRE

L'ultrafiltration et la chromatographie d'élution ont été combiné afin d'obtenir des fractions purifiées à partir de l'extrait alcalin de bagasse obtenu en conditions douces. L'ultrafiltration constitue la première étape, elle a été menée sur la membrane 10 kDa sélectionnée après le screening de membrane. Les essais ont été réalisés en mode concentration avec un FRV de 6 comme décrit dans le chapitre 3 puis en mode diafiltration avec l'ajout de 3 diavolumes d'eau distillée en continu. Comme observé précédemment le taux de rétention des petites molécules a fortement augmenté durant l'étape de concentration et surtout durant l'étape de diafiltration. 93% des sels inorganiques ont été récupérés dans le perméat, contre plus de 99% attendu. Du fait de leur teneur initiale dans l'extrait alcalin de bagasse, ils représentaient toujours 22% de la composition du rétentat. A la fin de la diafiltration, 71% de la lignine et 67% des xylanes ont été récupérés dans le rétentat. Ainsi, la composition finale du rétentat fût la suivante : 15.3 g/L d'AIL, 6.7 g/L de sels inorganiques, 5.7 g/L d'oligomères de sucre (4.3 g/L de xylanes, 1.0 g/L d'arabinanes, 0.4 g/L glucanes), 3.0 g/L d'ASL, 0.3 g/L d'acide acétique et les monomères phénoliques n'ont pas été détecté en concentration quantifiable.

La composition du perméat d'ultrafiltration fût la suivante : 18.1 g/L de sels inorganiques, 2.0 g/L d'ASL, 1.5 g/L d'AIL, 1.5 g/L d'acide acétique, 1.3 g/L de monomères phénoliques (l'acide coumarique étant le plus important avec 1.1 g/L) et 0.8 g/L xylan, correspondant à des puretés respectives de 71.5%, 8.1%, 6.0%, 5.9%, 5.2% (4.5% pour l'acide coumarique) et 3.0%. Le perméat a été concentré par évaporateur rotatif jusqu'à 19% de matière sèche (MS) afin d'augmenter la productivité de l'étape suivante à savoir de la chromatographie d'élution. La concentration du perméat de 2,5 à 19% de MS a entraîné la formation d'un précipité. Ce précipité a été séparé du perméat concentré par centrifugation. Il représente 14% de la MS du perméat correspondant à 51% des xylanes et 23% des lignines initialement présents dans le perméat. Cette étape de concentration a donc constitué une étape de purification également, faisant chuter la teneur en lignine de 6,0 à 3,0% et celle des xylanes de 3,7 à 0,7% dans le perméat. La chromatographie d'élution à partir du perméat d'UF concentré sur la résine cationique fortement acide de type gel a confirmé la récupération des monomères phénoliques

possédant une fonction carboxyle dans une fraction éluée avant 0,43 volume d'éluant et la récupération dans la fraction suivante des sels inorganiques et des monomères phénoliques sans fonction carboxyle.

Le rétentat d'UF a subi des essais de précipitation afin de séparer les lignines des xylanes. L'ajout de 1,1 g d'acide sulfurique à 72% (m/m) à 100 g de rétentat jusqu'à atteindre un pH de 1,9, a mené à la précipitation de 91% de la lignine insoluble dans l'acide et 79% de la lignine soluble dans l'acide. Cependant, des quantités importantes de xylanes (53%), d'arabinanes (45%), de glucanes (46%) et de sels inorganiques (43%) ont également été récupérées dans le précipité. La pureté de la lignine insoluble dans l'acide (AIL) a donc peu augmenté : de 49 à 59%.

La précipitation des hémicelluloses par ajout d'éthanol a également été testée. Les résultats se sont montrés moins bon que ceux obtenus par ajout d'acide puisque 63% des xylanes, 55% des arabinanes et 64% des glucanes ont été récupérés dans le précipité mais aussi 30% de l'AIL.

A partir d'extrait alcalin de bagasse, l'association de la filtration membranaire puis de la chromatographie sur le perméat et de la précipitation par ajout d'acide sur le rétentat a mené à l'obtention de quatre fractions purifiées : (i) les oligomères de lignine, (ii) les oligomères de sucres, (iii) les monomères phénoliques avec fonction carboxyle, (iv) les sels inorganiques et les monomères phénoliques sans fonction carboxyle.

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### Chapitre 6 : ANALYTICAL METHODS

Les sous-parties *Matériels et méthodes* de chaque chapitre attendant aux procédés de purification contient tous les éléments nécessaires à la compréhension et la reproduction des expériences réalisées. Cependant, un dernier chapitre a été ajouté pour récapituler toutes les analyses effectuées ainsi que le développement de celles-ci lorsque cela a été nécessaire. Les chromatogrammes lié aux analyses effectuées par la méthode NREL sont présentés, car trop difficilement disponibles dans la littérature. Une méthode pour le dosage des 12 monomères phénoliques suivis dans ce travail a été développée à partir de plusieurs méthodes existantes dans la littérature pour le dosage de composés phénoliques.

## CONCLUSIONS GENERALES

Afin de trouver une solution à l'après-pétrole, la valorisation de la biomasse lignocellulosique en carburant liquide et de manière plus nécessaire encore en molécules (intermédiaires chimiques et matériaux) au sein de bioraffineries constitue la seule option envisageable aujourd'hui. La biomasse lignocellulosique est essentiellement composée de cellulose, d'hémicelluloses et de lignine. Leur fractionnement et la purification des molécules ainsi obtenues est un prérequis pour leur valorisation en tant que substituts des hydrocarbures fossiles. La collaboration entre la société Novasep, l'unité mixte de recherche Ingénierie des Agro-polymères et Technologies Emergentes, et le Laboratoire de Chimie Agro-industrielle dans le cadre du projet LigNov a été mise en place afin d'explorer des voies de fractionnement au potentiel de développement intéressant afin de leur y associer des voies de purification efficaces.

Le procédé Organosolv a été présenté dans certains travaux comme étant le seul à séparer les trois fractions lignocellulosiques mais son développement industriel n'est toujours pas abouti. Le fractionnement en voie acide a été l'objet de nombreuses études et est actuellement le plus utilisé par les bioraffineries (industrie papeterie mise à part). La purification de l'hydrolysat acide ainsi généré contenant les sucres sous leur forme monomérique, repose sur l'utilisation de résines polymériques par des procédés d'adsorption ou chromatographique. Cependant, la valorisation du résidu solide contenant la lignine est rarement abordée. Au cours de la dernière décennie, le fractionnement en voie alcaline, inspiré des procédés papetiers, mais en conditions plus douces, a connu un intérêt croissant. En effet, des rendements plus élevés en monomères de glucose peuvent être obtenus par ce fractionnement suivi d'une hydrolyse enzymatique du résidu solide de cellulose, que par le fractionnement en voie acide. De plus, les hémicelluloses et la lignine sont solubilisés ce qui favorise leur séparation puis leur valorisation. Pour ces raisons, le travail expérimental s'est focalisé sur la purification d'extrait alcalin obtenu en conditions douces. La bagasse de canne à sucre a été sélectionnée pour générer un extrait lignocellulosique alcalin modèle pour étudier différentes voies de purification. Sa caractérisation minutieuse a montré qu'il était composé de plus de 50% de sels inorganiques, d'oligomères de lignine et

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d'hémicelluloses, de monomères phénoliques et d'acide acétique. Les sucres hémicellulosiques extraits sont exclusivement sous forme oligomérique. Les groupements acétates et pectiniques sont totalement clivés après l'extraction alcaline.

La filtration membranaire de l'extrait alcalin de bagasse a été étudiée en détails et il a été montré que le choix d'une membrane (nature, configuration, seuil de coupure) doit être basé sur des essais expérimentaux dans la mesure où les performances d'une membrane ne peuvent pas être extrapolées à une autre membrane. Les performances des membranes, en terme de rétention des molécules et de flux, ont été dépendantes des conditions opératoires comme la pression transmembranaire (PTM) ou la température mais faiblement dépendantes du taux de cisaillement sur la gamme de valeurs testées. Sur la membrane présentant les meilleures performances, la 10 kDa fibres creuses en polysulfone, jusqu'à 90% des oligomères de lignine et d'hémicelluloses ont été retenus tandis que les sels inorganiques, les monomères phénoliques et l'acide acétique ont eu des taux de rétention avoisinant 0%. La filtration en mode concentration et diafiltration a confirmé la séparation de ces deux groupes de molécules avec des taux de récupération de 70-80% pour les hémicelluloses et la lignine et une augmentation de leur pureté d'un facteur 2 en mode concentration. Cependant, avec ces deux modes de filtration et particulièrement en diafiltration, la rétention des petites molécules a augmenté de manière significative, entraînant ainsi une diminution de l'efficacité de la séparation.

Les essais de chromatographie d'élution ont été réalisés sur des résines cationiques fortement acides, avec de l'eau distillée pour éluant. L'utilisation d'une résine de type gel (taille des pores d'environ 3 nm) a montré que les monomères phénoliques avec une fonction carboxyle (acide coumarique, acide férulique et acide vanillique) étaient récupérés dans une fraction éluee avant 0,42 volume d'éluant (75-80% de récupération) alors que les monomères phénoliques sans fonction carboxyle (4-hydroxybenzaldéhyde et vanillin) et les sels inorganiques étaient éluées après 0,42 volume d'éluant à 75-80%. L'utilisation d'une résine de type macroporeuse (taille des pores de 20 à 50 nm) a conduit à l'obtention d'une fraction éluee avant 0,47 volume d'éluant contenant uniquement les plus gros oligomères de sucres (20-30% d'entre eux) et de lignine (15% de la lignine totale). Les autres oligomères, les sels inorganiques, les monomères phénoliques et l'acide acétique ont été élués après 0,47 volume d'éluant.



Un procédé intégré de purification a été conçu afin de produire des fractions purifiées à partir de l'extrait alcalin de bagasse. Dans un premier temps, une étape d'ultrafiltration (UF) en mode concentration puis en mode diafiltration a permis la récupération de 70% des oligomères de lignine et d'hémicelluloses dans le rétentat tandis que 90% des sels inorganiques et près de 100% des monomères phénoliques et de l'acide acétique ont été récupérés dans le perméat. Puisque la séparation des oligomères de lignine et de sucres n'a pu être effectuée ni par filtration membranaire ni par chromatographie d'élution, des procédés de purification par précipitation par ajout d'acide ou d'éthanol ont été testés sur le rétentat d'UF. L'ajout d'acide a conduit à la précipitation de 91% de la lignine mais des sucres ont également été co-précipités en partie. Le perméat d'UF a été concentré et étonnamment 51% des quelques oligomères d'hémicellulose de petite taille ayant pu passer dans le perméat ont été précipités ainsi que 23% de la lignine résiduelle. Pour finir, le perméat concentré a subi une étape de chromatographie d'élution sur résine cationique fortement acide de type gel avec  $\text{Na}^+$  comme contre-ion ce qui a permis de confirmer la séparation des monomères phénoliques présentant une fonction carboxyle des sels inorganiques et des monomères ne présentant pas de fonction carboxyle.

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De nombreux essais ont été réalisés durant ce travail de recherche. Cependant, certains aspects scientifiques concernant la caractérisation, le fractionnement ou la séparation des molécules restent à traiter :

- Des analyses par chromatographie d'exclusion stérique pourraient être intéressantes pour confirmer la distribution hétérogène des oligomères de sucres par rapport à la distribution plus homogène des oligomères de lignine observées durant les essais de chromatographie d'élution. Elles pourraient également apporter des informations concernant l'action du fractionnement alcalin en conditions douces sur l'extraction des hémicelluloses et de la lignine, et sur leurs rétentions par filtration membranaire.
- Toujours d'un point de vue analytique, le suivi des pectines via les acides uroniques (acides galacturonique et glucuronique) est possible par HPLC sur

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colonne  $H^+$  dans les conditions décrites par le protocole NREL. L'acide galacturonique a été détecté à partir de la bagasse initiale et dans l'extrait alcalin correspondant. Néanmoins, le pic d'acide galacturonique n'a plus été distingué après les essais de filtration membranaire et de chromatographie d'élution, les causes de ce phénomène pourraient être étudiées.

- Une détermination précise de la composition en minéraux de la bagasse pourrait être réalisée par exemple par HPLC ionique ou bien par spectrométrie à plasma à couplage inductif. La composition précise en sels inorganiques de la bagasse et donc de l'extrait alcalin qui en issue est un prérequis pour un recyclage efficace de la soude.
- Les taux de rétention des petites molécules ont évolué durant l'UF en mode concentration et diafiltration. Une explication pourrait être que les sels inorganiques sont piégés ou complexes dans les oligomères de lignines et de sucres retenus par la membrane, mais il faudrait le démontrer.
- Afin d'enrichir la discussion sur le colmatage des membranes et le modèle des résistances en série, la résistance liée à l'adsorption  $R_a$  pourrait être évalué en recirculant de l'extrait alcalin sans pression et ensuite mesurer la perte de flux à l'eau.
- Les mécanismes de rétention des sels durant les essais de chromatographie d'élution seraient également à élucider puisque des sels différents (NaOH vs. NaCl et HCl) n'ont pas été élués à la même vitesse. Cela pourrait permettre de répondre à des questions concernant l'essai avec une solution d'acide férulique à pH 2 : telles que : y-a-t'il eu conversion de la résine de la forme  $Na^+$  à la forme  $H^+$  malgré l'excès de  $Na^+$  en solution ? Quel est l'impact du contre-ion ( $OH^-$  ou  $Cl^-$ ) durant l'élution ?
- Les raisons de la formation d'un précipité après la concentration du perméat d'UF mais l'absence de précipité lorsque l'extrait alcalin de bagasse est directement concentré pourraient être investiguées. Comment l'étape de concentration influence-t-elle le retrait de xylanes et de lignine par précipitation ? La concentration a été augmentée jusqu'à 20% de matière sèche (MS), mais une augmentation jusqu'à au moins 50% de MS pourrait être envisagée.

- De même, la chromatographie d'élution du perméat concentré a montré un décalage entre la sortie des sels inorganiques et la sortie des monomères phénoliques sans fonction carboxyle par rapport à l'élution de l'extrait alcalin concentré. L'influence des oligomères de lignine et d'hémicelluloses pourrait être étudiée.
- Durant les essais d'élution, aussi bien avec l'extrait alcalin de bagasse qu'avec le perméat d'UF, un épaulement devant les pics principaux des monomères phénoliques sans fonction carboxyle a été observé. Une explication pourrait être la présence de la forme phénolate (pKa de 9,95) pour justifier de leur exclusion partielle des pores de la résine. Des tests d'élution avec des solutions synthétiques de différents pH pourraient être menés.

Il serait également intéressant d'explorer les points techniques suivant :

- Des extractions successives en conditions acides douces pour récupérer les hémicelluloses puis en conditions alcalines douces pour récupérer la lignine pourraient être étudiées comme schéma de fractionnement, afin de réduire la nécessité de purifier les deux extraits obtenus. La composition des extraits obtenus ainsi que le rendement des différents composés pourraient être comparés à ceux obtenus dans ces travaux. Une analyse de cycle de vie (ACV) permettrait ensuite de comparer ce schéma de fractionnement à celui développé dans ces travaux, et plus généralement d'évaluer l'impact environnemental des différentes étapes de purification.
- L'extraction alcaline de tourteau de tournesol a conduit à une composition en monomères phénoliques différente (acide cafféique et 4-hydroxybenzaldéhyde étant les principaux) de celle de la bagasse de canne à sucre. Leur élution dans les mêmes conditions que celles de ces travaux permettrait de vérifier leur séparation en fonction de la présence ou non d'un groupe carboxyle dans leur structure.
- Durant les essais d'UF en mode concentration, le facteur de réduction volumique (FRV) a été arrêté à 6 dû au volume mort de l'installation. Un volume d'extrait plus important devrait être testé pour pousser le FRV jusqu'à atteindre un

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maximum où les performances seraient trop dégradées (chute brutale du flux par exemple).

- Dans le procédé intégré, une étape intermédiaire de déminéralisation complète par échange d'ions entre les étapes d'UF et de précipitation du rétentat pourrait permettre d'étudier l'influence de la présence de sels dans la séparation des oligomères de sucre et de lignine par précipitation. La précipitation à l'éthanol pourrait alors s'avérer intéressante afin de garder des fractions ne contenant pas de sels.
- Concernant la séparation des oligomères de lignine et de sucre contenu dans le rétentat d'UF, des essais d'adsorption sur résines non-fonctionnalisées (puisque le rétentat à un pH neutre) pourraient être menés. Sur une résine avec une structure hydrophobe (par exemple un squelette styrénique), les lignines pourraient être fixées tandis que les oligomères de sucre seraient élués. Ensuite, la désorption de la lignine en utilisant différents éluants (par exemple une solution alcaline ou de l'éthanol) pourrait être examinée. Des fractions d'une plus grande pureté mais aussi un coût de procédé plus important sont attendus par rapport au procédé de précipitation proposé dans ces travaux. Une ACV s'avèrerait pertinente pour comparer ces différents procédés de purification.
- Ces travaux ont montré l'intérêt d'un procédé chromatographique pour séparer les composés d'un extrait alcalin lignocellulosique obtenu en conditions douces. L'optimisation des conditions du procédé telles que la température, la vitesse de l'éluant, le chargement en alimentation, la concentration de l'alimentation doivent être explorées afin d'obtenir la meilleure résolution possible et la productivité la plus élevée possible à l'échelle industrielle.
- Les étapes de séparation chromatographique devraient être ensuite testées sur des systèmes continus (par exemple le lit de résine mobile simulé) pour vérifier que la résolution est augmentée et donc avec elle les rendements et les puretés, et pour estimer la productivité du système en vue de l'industrialiser. Par ailleurs, la fraction à plus forte valeur ajoutée devrait être sélectionnée, par exemple l'acide coumarique, afin de fixer les paramètres de séparation (volume d'éluant) pour favoriser la récupération ou la pureté de la molécule visée. Au préalable à un essai de chromatographie en système continu, un essai de surcharge de

l'alimentation devrait être mené jusqu'à ce que les courbes de percement de chaque composé soient atteintes.

- Enfin, pour compléter le procédé de purification propose, une étape de déminéralisation pourrait être effectuée sur la fraction éluée avant 0,43 volume d'éluant et contenant les monomères phénoliques avec un groupe carboxyle. Puis une étape de cristallisation ou de chromatographie préparative devrait permettre l'obtention de molécules pures.