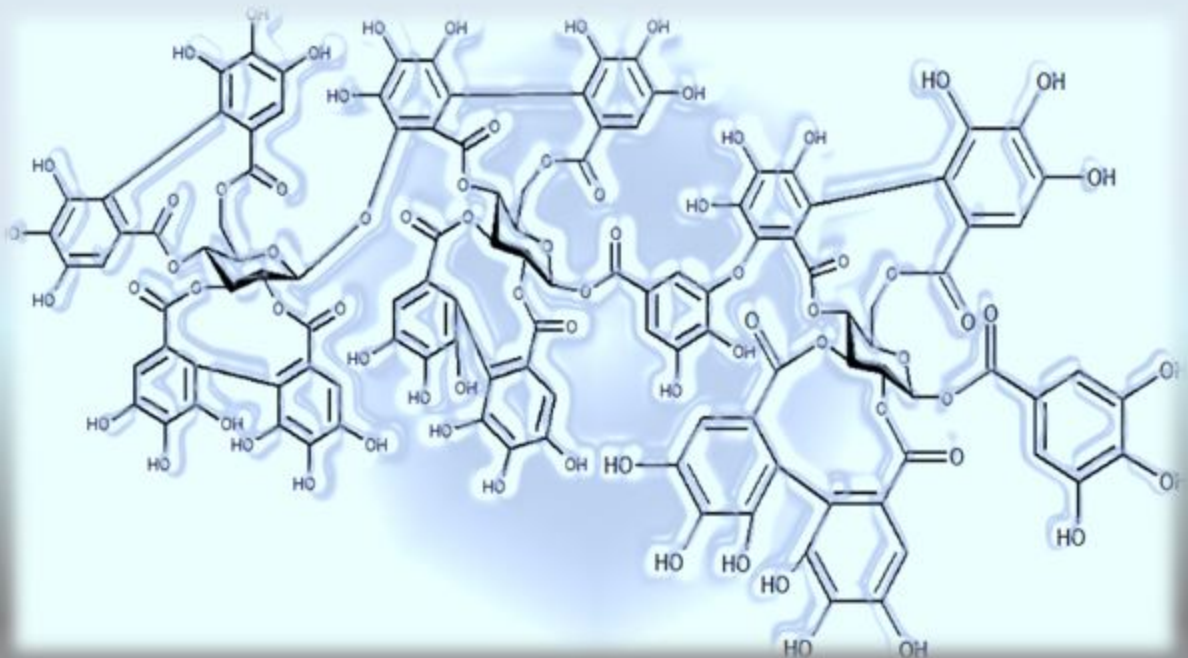
Departamento de Química e Ingeniería de Procesos y Recursos
E.T.S. de Ingenieros Industriales y de Telecomunicación
Universidad de Cantabria

MEJORAS EN UN PROCESO DE PASTEADO AL SULFITO PARA EL APROVECHAMIENTO DE CARBOHIDRATOS DENTRO DEL CONCEPTO DE BIORREFINERÍA

DEVELOPMENTS IN A SULPHITE PULPING PROCESS FOR THE VALORISATION OF ITS CARBOHYDRATE RESOURCES WITHIN THE BIOREFINERY CONCEPT



Memoria de Tesis Doctoral presentada para optar al título de Doctor por la Universidad de Cantabria

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Santander, Enero 2016

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Programa Oficial de Doctorado en Ingeniería Química y de Procesos (BOE núm. 36, de 10 de febrero de 2010. RUCT: 5311209) con Mención hacia la Excelencia (BOE núm. 253, de 20 de octubre de 2011. Referencia: MEE2011-0031)

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Imprime: Tratamiento Gráfico del Documento, S.L.

Avda. de los Castros s/n (Edificio Interfacultativo)

39005 Santander

Telf. 942201108

Email: tgd@edicionesTGD.com

ISBN: 978-84-608-5396-1

I would like to dedicate this thesis to my parents, the cornerstone of my life.

"One father is more than a hundred schoolmasters"
G. Herbert

ACKNOWLEDGEMENTS

This doctoral thesis wouldn't be possible without the financial support by the **EUCAFUEL 23.H031.64003** research project of the Spanish Ministry of Science and Innovation, together with the financial support of the European Comission by the **BRIGIT research project** under the seventh framework program.

In the first place, I would like to express all my gratitude to my supervisor, **Alberto Coz**, for his compression, support, and confidence in me, his advice and dedication. He provided me the oxygen to carry on by means of his innovative ideas and research experience. Thank you for listening to me, showing interest in what I was saying and proposing. Thank you for your sense of humour and humanity. You let me express myself naturally and comfortably from the beginning. Thank you for always being there.

Thanks to **Javier R. Viguri** and **Mari Cruz Payán** for bringing me the opportunity of working with them in their laboratory experiments as a Bachelor student of Chemical Engineering. They initiated me in the research field and aroused my interest for R&D.

I would like to express my gratitude to the hydrolysis laboratory team. My partners and colleagues: **Natalia Quijorna, Cristina Rueda and Ana I. Portilla**. I am lucky to have you as colleagues and I will always keep you in my heart. Thanks to **Javier Fernández**, who was helping me the last months with the HPLC.

I would like to make a special mention to the bachelor students who worked alongside me these years. I will quote them in chronologic order. Any other criteria would be difficult to me as I truly love all of them: **Alicia Pérez, Rafael Mendívil, Carlos Arce, Noelia García, Laura Ulloa, Patricia Velasco and Elisa Velasco**. I want

to tell you that this PhD has been possible thanks to your help and support, your hard-working spirit, good practices, teamwork abilities, technical guidance and goodness. You made the best for me guys and I would be silly if I didn't recognise it. Thank you so much. I wish you a shiny future. I am sure you will follow your dreams.

I want to express my gratitude to **Pedro A. Calvo** and **Beatriz Lastra** from Biopolymers laboratory in Sniace Company because they have been the best next-door colleagues that I'd wish for... Of course thanks to the Sniace Company and personnel, especially **Alain Blanco**, the technical office boss for all their knowledge and contributions. Alain, your enigmatic personality inspired me in many aspects. I hope to be a good professional and make you proud of me. Regarding Sniace's personnel I could fill pages naming people as they treated us very kindly, solving our doubts about the process and the analytical procedures. Thanks to **Angel Bercedo**, our brilliant chemist grandpa and of course thanks to **Carlos Tejedor**, **Antonio Carcedo, Estíbaliz Pacheco** and **Maite Turrado** from Sniace and **Daniel Gómez** from Lignotech Ibérica among others.

I couldn't forget the acknowledgements to the **GER research group** (www.geruc.es) of the Department of Chemistry and Process & Resource Engineering from the ETSIIT at the University of Cantabria. I would like to express acknowledgement to the professors and PhD. students who from the beginning made me feel part of their group. A special mention to **Ana Andrés** who has alwasys been there to bring me wise counsel. Thank you to people from **Room 20 of E.T.S. Navy** and our coffee break colleagues as well. I'm glad to share many good moments with you. **Elena Dosal**, after this time together, I can conclude that we've formed an incredible friendship. Thanks to you I finished my PhD on time... I really appreciate the time we spent together de-stressing and encouraging each other to finish our dissertations. You have always been there to lend me a hand, thanks. Thanks to **Juan**

Dacuba García who started being a good friend but transformed my entire world in a few months. You are an incredible person and I wish to spend a very very happy life next to you.
Finally, thanks a lot to the people I met at the Agriculture University of Athens during my internship. I was lucky to meet these marvellous, enthusiastic and hard-working people. A special mention to my mentor **Apostolis Koutinas** and **Mary Alexandri** a friend of mine that taught me, worked day by day next to me with patience in her best mood. Thank you because people like you made me feel at home.

What would I be without my friends? Thanks because this wouldn't be possible without you. Thank you **Esti Gómez**, **Pablo Sierra**, **Rafi Arriola**, **Laura Aranda**, **Pablo Saiz**, **Alejandra Pastor**, **Sandra Madrazo**, **Esther Barrio** and **Jose Palomares** because you have supported me during difficult moments. I would like to thank **Alberto Sierra**, my traveling companion, best friend and love for the last twelve years. I can't imagine this goal without you next to me. Best wishes for the best person.

The last paragraph belongs to my dearest family. Thanks **Fernando Llano Sierra** and **Mª Concención Astuy Torralbo** because I admire you day by day. They have shown me unconditional love, moral support, ethical values, honesty and professionalism among many other things. Thank you for inspiring me in all of my pursuits and encouraging me to follow my dreams.

"Knowledge is in the end based on acknowledgement"
L. Wittgenstein

AGRADECIMIENTOS

Esta tesis doctoral no habría sido posible sin el apoyo financiero del proyecto **EUCAFUEL 23.H031.64003** del Ministerio de Ciencia e Innovación del gobierno de España, junto con el apoyo financiero de la Comisión Europea a través del Proyecto BRIGIT del séptimo programa marco.

En primer lugar me gustaría expresar toda mi gratitud a mi director, **Alberto Coz**, por su comprensión y apoyo, la confianza depositada en mí, sus consejos y dedicación. Él me proporcionó el oxígeno necesario para continuar a través de sus ideas innovadoras y su experiencia investigadora. Gracias por escucharme mostrando en todo momento interés en lo que decía y proponía. Gracias por tu sentido del humor y humanidad. Desde el principio, me dejaste expresarme natural y cómodamente. Gracias por haber estado siempre ahí.

Gracias a **Javier R. Viguri** y **Mari Cruz Payán** por brindarme la oportunidad de trabajar con ellos en sus experimentos de laboratorio como proyectista de Ingeniería Química. Ellos me iniciaron en el campo de la investigación y suscitaron mi interés por el I+D+i.

Me gustaría expresar mi gratitud a todo el equipo del laboratorio de hidrólisis. Mis compañeros y colegas: **Natalia Quijorna, Cristina Rueda** y **Ana I. Portilla.** Tengo la suerte de haberlas tenido como compañeras y siempre os tendré en mi corazón. Gracias a **Javier Fernández** quien estuvo ayudándome los últimos meses con el HPLC.

Quisiera hacer una mención especial a las proyectistas que trabajaron junto a mí estos años. Las citaré en orden cronológico. Cualquier otro criterio sería difícil para

mí puesto que los quiero a todos ellos por igual: **Alicia Pérez, Rafael Mendívil, Carlos Arce, Noelia García, Laura Ulloa, Patricia Velasco y Eliza Velasco.** Quiero deciros que esta tesis ha sido posible gracias a vuestra ayuda y apoyo, vuestro espíritu de trabajo duro, buenas prácticas, habilidades de trabajo en equipo, vuestro asesoramiento técnico y bondad. Hicisteis lo mejor por mí y sería estúpida y no lo reconociera. Muchas gracias. Os deseo un brillante futuro. Estoy segura de que perseguiréis vuestros sueños.

Quiero darle las gracias a **Pedro A. Calvo** y **Beatriz Lastra** del laboratorio de biopolímeros de Sniace porque ellos han sido los mejores colegas del laboratorio de al lado que desearía tener… Por supuesto agradecer a la fábrica de Sniace y a su personal, en especial a **Alain Blanco** el jefe de la oficina técnica por su conocimiento y sus contribuciones. Alain, tu personalidad enigmática me ha inspirado en muchos aspectos. Espero ser una buena profesional y hacer que te sientas orgulloso de mí. Podría rellenar varias páginas citando al personal de Sniace dado que nos trataron muy amablemente y resolvieron nuestras dudas sobre el proceso y los procedimientos analíticos. Gracias a **Angel Bercedo,** nuestro brillante abuelo de la química y por su puesto gracias a **Carlos Tejedor, Antonio Carcedo, Estíbaliz Pacheco, Maite Turrado** de Sniace y **Daniel Gómez** de Lignotech Ibérica entre otros.

No podría olvidar en estos agradecimientos al **grupo GER de investigación** (www.geruc.es) del Departamento de Química e Ingeniería de Procesos y Recursos de la ETSIIT de la Universidad de Cantabria. Quisiera darles las gracias a los profesores y doctorandos quienes desde el principio me hicieron sentir como una más de su grupo. Y en especial, a **Ana Andrés** quien siempre estuvo ahí para darme sabios consejos. Gracias a la gente del **Aula 20 de la ETS de Náutica** y a mis colegas del café de la escuela de náutica. Me alegra haber compartido tantos buenos momentos con vosotros. **Elena Dosal,** después de este tiempo juntas puedo concluir que hemos formado una increíble amistad. Gracias a ti he acabado mi tesis a

tiempo… Aprecio mucho el tiempo que pasamos juntas relajándonos y al mismo tiempo animándonos para finalizar nuestras tesis. Siempre has estado ahí para echarme una mano, gracias. Gracias a **Juan Dacuba** quien empezó siendo un buen amigo y en unos pocos meses ha trastocado todo mi mundo. Eres una persona increíble junto a la cual deseo pasar una vida muy muy feliz.

Finalmente, muchas gracias a la gente que conocí en la Universidad de Agriculutura de Atenas durante mi estancia. Tuve suerte de conocer gente maravillosa, entusiasta y trabajadora. Especial mención a **Apostolis Koutinas** y **Mary Alexandri** amiga mía que me enseñó y trabajó día a día junto a mí con paciencia y la mejor de sus sonrisas. Gracias porque gente como tú hizo que me sintiera como en casa.

¿Qué sería de mí sin mis amigos? Gracias porque esto no habría sido posible sin vosotros. Gracias a **Esti Gómez, Pablo Sierra, Rafi Arriola, Laura Aranda, Pablo Saiz, Alejandra Pastor, Sandra Madrazo, Esther Barrio** y **Jose Palomares** por vuestro apoyo en los momentos difíciles. Me gustaría agradecer a **Alberto Sierra**, mi compañero de viaje durante los últimos 12 años. No concibo este objetivo sin haberte tenido a mi lado todo este tiempo. Mis mejores deseos a la mejor persona.
El último párrafo pertenece a mi querida familia. Gracias **Fernando Llano Sierra** y **Mª Concepción Astuy Torralbo** porque os admiro cada día. Me habéis mostrado amor incondicional, apoyo moral, valores éticos, honestidad y profesionalidad entre muchas otras cosas. Gracias por inspirarme en todos mis proyectos y animarme a perseguir mis sueños.

"El conocimiento es la base del reconocimiento"

L. Wittgenstein

PREFACE

Crude oil prices in the recent past have reached dizzy heights. This, joined to the fact of the energy dependence suffered in the European countries, has therefore revived a renewed interest in the research and development of renewable resources. In this sense, conversion of lignocellulosic biomass and residues is regarded as one of the most promising alternatives to fossil fuels. About 200 billion tons of biomass is produced annually of which less than 5 % is consumed by mankind. The effective utilisation of biomass to produce value-added chemicals, materials and fuels within the biorefinery concept may lay the foundation for a stepwise shift of the current global economy toward a sustainable bio-based economy.

Within this framework, pulping factories are potential candidates for biorefinery, accomplishing the current objectives and producing a wide variety of bio-based value-added products. In addition, the demand for high-purity cellulose pulps, also known as dissolving pulps, has substantially increased during the last few years. The upturn of dissolving pulps in the market may be attributed to a consistent growth of regenerated cellulose fibre production, largely initiated by an increasing demand in China and other Asian countries. Market studies indicate that this trend will prevail during the next decades. It is estimated that in 2050 the annual demand of textile fibre ranges between 120 and 130 million tons. Furthermore, environmental and agricultural restrictions for cotton production cannot keep the present share of 31 % from global fibre production. The growing demand is not only limited to textile applications, but also concerns the manufacture of cellulose acetate for films, plastics and coatings as well as cellulose mixed ethers for lacquers and printing, cellulose ethers and cellulose powder which have found important applications in food and pharmaceutical industries. Moreover, dissolving pulps seem to be the preferred substrate for the manufacture of nanofibrillated cellulose (NFC), a future precursor of advanced materials.

This dissertation is based on the results carried out in a R&D&I laboratory placed in a factory in the north of Spain and supervised by the University of Cantabria. The pulp mill produces

dissolving pulp as the main product; however, in addition to this product, other chemicals, materials and/or fuels can be obtained in order to be transformed into a biorefinery mill. Throughout the last five years of research, the obtained results can give future valorisation opportunities in the factory in order to be more competitive in the market.

PRÓLOGO

El precio del crudo ha alcanzado unas cuotas vertiginosas en los últimos años. Este hecho, unido al problema de dependencia energética sufrido en Europa, ha reavivado un interés renovado por la investigación y el desarrollo de recursos renovables. En este sentido, la conversión de biomasa vegetal y sus residuos está siendo considerada como una de las alternativas más prometedoras a los combustibles fósiles. Cerca de 200 billones de toneladas de biomasa se producen anualmente las cuales menos del 5 % son consumidas para fines humanos. El uso eficiente de la biomasa para producir químicos de alto valor añadido puede sentar las bases del cambio de economía global hacia una economía sostenible basada en una amplia plataforma de productos químicos, biopolímeros y biocombustibles como pilares fundamentales.

En este contexto, las fábricas de pasta son candidatos potenciales para su transformación en biorrefinerías, cumpliendo los objetivos actuales y produciendo una amplia variedad de productos de origen biológico de alto valor añadido. Además, la demanda de pasta de celulosa de alta pureza, también conocida como pasta dissolving, ha incrementado sustancialmente en los últimos años. Su repunte en el mercado puede atribuirse al crecimiento consistente de la producción de fibra regenerada, la cual ha sido motivada por el incremento de la demanda en China y otros países asiáticos. Estudios de mercado indican que esta tendencia prevalecerá durante las próximas décadas. Se estima que en 2050 la demanda de fibra textil se situará en el rango de 120 a 130 millones de toneladas. La demanda creciente no se limita únicamente a las aplicaciones textiles – las restricciones ambientales y agrícolas relativas a la producción de algodón no pueden mantener la actual cuota del 31 % de producción mundial de fibra- si no también el interés por la fabricación de acetato de celulosa para plásticos y revestimientos, así como éteres de celulosa para lacado e impresión, éteres y polvo de celulosa con importantes aplicaciones en la industria alimentaria y farmacéutica. Asimismo la pasta dissolving se utiliza en la fabricación de fibras de nanocelulosa, futuro precursor de materiales avanzados.

Esta tesis se basa en los resultados llevados a cabo en un laboratorio de I+D+i localizado en una fábrica del norte de España, y supervisado por la Universidad de Cantabria. La planta papelera produce pasta dissolving como producto principal; sin embargo, además de éste producto, otros químicos, materiales y/o biocombustibles pueden obtenerse para tranformar la actual planta en una biorrefinería. Mediante los últimos cinco años de investigación, los resultados obtenidos pueden dar lugar a futuras oportunidades de valorización en la fábrica para ser más competitiva en el mercado.

ABSTRACT

Dissolving cellulose is a low-yield chemical pulp (30-35 %) with a high alpha-cellulose content (91-93 %) and relatively low hemicellulose (1-10 %) and lignin (<0.05 %) content. It is traditionally manufactured by acid sulphite and nowadays by prehydrolysis-kraft pulping processes. Dissolving pulp is used in the production of cellophane, rayon and viscose fibres, cellulose esters, cellulose ethers, graft and cross-linked cellulose derivatives.

Sugar-rich residual streams generated during sulphite pulping for dissolving pulp manufacture convert such factories into potential lignocellulosic biorefineries by integrating several modifications into the current processes by means of chemical, thermo-chemical or biochemical pathways. Mixed five and six carbon sugar platforms produced from the hydrolysis of hemicelluloses serve as substrate for biological conversion providing fuels as hydrogen or ethanol, biopolymers, and chemicals as lactic, succinic, or levulinic acids, sorbitol, furfural or xylitol. Within this hypothesis, this dissertation evaluates the possibilities of producing fermentable substrate stream, transforming an acid sulphite pulping industry located in Cantabria (Spain) into a modern lignocellulosic biorefinery.

The main objective of this thesis is to study the acid sulphite process in a factory in the north of Spain and to identify the factory improvements within the biorefinery concept in order to not only obtain dissolving pulp but also to track down the residual sugar-rich streams.

In order to fulfil this objective, the first step of the work was the development of several physico-chemical characterisation methods of the feedstock (*Eucalyptus globulus)*, product (dissolving pulp) and waste streams (hydrolysates). In addition, four chromatographic methods for carbohydrate and derivative quantification of

woody samples were set up. The obtained results gave some recommendations to the analysis of these kinds of industrial samples.

Once the methods were obtained, the mass balance of the main components was carried out at industrial scale in order to give some conclusions about the fractionation processes and valorisation opportunities in the factory. From the study of the industrial digestion and bleaching processes, it can be concluded that there is a good cellulose separation during wood digestion (99.64 %) with the presence of 87.23 % hemicellulose and 98.47 % lignin in the spent liquor. As an example of valorisation option, 0.183 L of second-generation ethanol per kilogram of dry spent liquor can be obtained by the mill.

The following step was to study the sulphite digestion stage and the effect of the most important parameters on the final dissolved sugars. Cooking trials at laboratory scale in 1-L stainless steel reactors were carried out. The best results of the digestion step increased the amount of total monosaccharides of the spent liquor at 1.01T of dwell temperature, 0.196R and 6.20 % total SO_2. Experimental cooking results were modelled giving theoretical increases of 7.33 % of monosaccharides at 1.013T, 0.628R and 7.33 % total SO_2. In order to increase the total sugar content of the waste and maintaining the sulphite cooking conditions, the liquid samples were externally hydrolysed. Diluted-acid hydrolysis and concentrated-acid hydrolysis were chosen as the most adequate depolymerisation treatments. A factorial design of experiments was done to study the effect of temperature, acid concentration, acid-to-liquor ratio and time on hemicellulose depolymerisation. Diluted-acid hydrolysis showed the best results getting monosaccharide improvements of 7.9 % using 0.5 % w/w of H_2SO_4 at 80 ^{o}C for 60 minutes with acid-to-liquor ratio of 10 v/v. Finally, to separate sugars from the rest of fermenting inhibitors (lignin-derived and carbohydrates-derived constituents) several detoxification techniques were implemented. Overliming, adsorption, ion exchange and liquid-liquid extraction were studied. Treatment with anionic resins was proposed as the best solution with

maximum total inhibitor removals of 91.8 %. In addition, six regeneration cycles of resin were proposed for phenolics and lignosulphonate recovery. Adsorption was also adequate for lignosulphonate, phenolics and acetic acid removals reaching a maximum of 74.5 % of acetic acid removal using activated charcoal. However, adsorption processes gave high losses of sugar in the hydrolysate. Finally, overliming was proposed as the simplest way of fractionation giving mostly lignosulphonate removals (45.9 %). Maximum bioethanol potentials of 1.231, 1.818 and 1.799 L.EtOH/Kg.dry SSL were obtained after detoxification with overliming and liquid-liquid extraction with chloroform and diethyl ether. Due to the multiple fermenting scenarios applicable to the liquors, not only ethanol but also other byproducts working with different microorganisms were contemplated since the detoxification alternatives proposed separate the most important groups of inhibitors registered in this kind of lignocellulosic hydrolysates.

This research proposes some recommendations and improvements in the factory in order to valorise the residual streams, not only enhancing the separation of sugars from the rest of wood constituents for fermenting purposes, but also maintaining the quality properties of the main product, dissolving pulp. Based on the whole study, diluted-acid hydrolysis, detoxification, fermentation and purification steps should be incorporated to the current sulphite mill for carbohydrates valorisation within the biorefinery concept.

RESUMEN

La celulosa dissolving es una pasta química de bajo rendimiento (30-35 %) con un alto contenido en alfa celulosa (91-93 %) y bajo contenido en hemicelulosa (1-10 %) y lignina (<0.05 %). Tradicionalmente se produce por medio del proceso al sulfito y actualmente a través del proceso kraft con una etapa de hidrólisis previa. La pasta dissolving se utiliza en la producción de celofán, fibras de rayón y viscosa, ésteres de celulosa, éteres de celulosa y derivados de celulosa reticulados.

Las corrientes residuales ricas en azúcares generadas durante el proceso al sulfito para producción de pasta dissolving convierten dichas industrias en potenciales biorrefinerías lignocelulósicas mediante la integración de algunas modificaciones en los procesos actuales por medio de transformaciones químicas, termo-químicas o biológicas. Las plataformas de azúcares de cinco y seis carbonos procedentes de la hidrólisis de hemicelulosas sirven como sustrato para la conversión biológica dando lugar a combustibles tales como hidrógeno o etanol, biopolímeros y químicos como ácido láctico, succínico o levulínico, sorbitol, furfural o xilitol. Con esta hipótesis, esta tesis evalúa las posibilidades de producción de una corriente que sirva como sustrato fermentable, transformando una industrial de pasteado al sulfito ácido localizada en Cantabria (España) en una moderna biorrefinería lignocelulósica.

El objetivo principal de esta tesis es el estudio del proceso al sulfito ácido en una planta del norte de España e identificar las mejoras dentro de un concepto de biorrefinería para la no sólo obtener pasta dissolving si no también detectar las corrientes residuales ricas en azúcares.

Para cumplir este objetivo, el primer paso de este trabajo fue el desarrollo de varios métodos de caracterización físico-químicos de la materia prima (*Eucalyptus globulus)*, el producto (pasta dissolving) y las corrientes residuales (hidrolizados). Además se pusieron a punto cuatro métodos cromatográficos para la cuantificación de

carbohidratos y derivados en muestras de origen maderero. Los resultados obtenidos dieron algunas claves y recomendaciones para el análisis de este tipo de muestras industriales.

Una vez obtenidos los métodos, se llevó a cabo el balance de materia de los principales componentes a escala industrial lo cuales brindaron algunas conclusiones sobre los procesos de fraccionamiento y las oportunidades de valorización de la planta. Del estudio de los procesos industriales de digestión y blanqueo, puede concluirse que hay una buena separación de celulosa durante la digestión de madera (99.64 %) con la presencia de 87.23 % de hemicelulosa y 98.47 % de lignina en el licor gastado. Como ejemplo de opción de valorización, 0.183 L de etanol de segunda generación por kilogramo de licor gastado puede obtenerse en la planta.

El siguiente paso fue el estudio de la etapa de digestión al sulfito y el efecto de los parámetros más importantes que afecten a los azúcares disueltos. Se llevaron a cabo experimentos de cocción a escala de laboratorio en reactores de 1 L de acero inoxidable. Los mejores resultados de la etapa de digestión incrementaron la cantidad total de monosacáridos del licor gastado a una temperatura constante de 1.01T, rampa de cocción de 0.196R y 6.20 % de SO_2 total. Los resultados experimentales se modelaron dando lugar a incrementos de monosacáridos del 7.33 % a 1.013T, 0.628R y 7.33 % SO_2 total. Con el propósito de incrementar el contenido total de azúcares manteniendo las condiciones de cocción al sulfito, las muestras líquidas fueron hidrolizadas externamente. Hidrólisis ácida diluida e hidrólisis ácida concentrada se escogieron como los tratamientos de depolimerización más adecuados. Se hizo un diseño factorial de experimentos para estudiar el efecto de la temperatura, la concentración de ácido, el ratio ácido-licor y el tiempo total de depolimerización de hemicelulosas. La hidrólisis diluida mostró los mejores resultados dando mejoras del 7.9 % empleando 0.5 % w/w de H_2SO_4 a 80 ^oC durante 60 minutos con ratios ácido-licor de 10 v/v. Finalmente, para separar los azúcares

del resto de inhibidores de fermentación (constituyentes derivados de lignina y de carbohidratos) se implementaron varias técnicas de detoxificación. Se estudiaron las técnicas de overliming, adsorción, intercambio iónico y extracción líquido-líquido. Se propuso el tratamiento con resinas aniónicas como la mejor solución con una eliminación de inhibores totales del 91.8 %. Además se propusieron seis ciclos de regeneración de resina para una recuperación eficiente de compuestos fenólicos y lignosulfonatos. La adsorción con carbono activo fue también adecuada para la eliminación de lignosulfonatos, fenólicos y ácido acético, alcanzando un máximo de eliminación de ácido acético del 74.5 %. Sin embargo, los procesos de adsorción dieron como resultado altas pérdidas de azúcares en el hidrolizado. Finalmente, se propuso el overliming como la forma más simple de fraccionamiento causando principalmente pérdidas de lignosulfonatos (45.9 %). Los máximos potenciales de bioetanol (1.231, 1.818 y 1.799 L.EtOH/Kg.dry SSL) se obtuvieron después de la detoxificación del licor mediante overliming y extracción líquido-líquido con cloroformo y dietiléter. Debido a los múltiples escenarios de fermentación aplicables a este tipo de licores, no sólo etanol si no otros subproductos fueron contemplados, trabajando con diferentes microorganismos partiendo de la premisa de que las alternativas de detoxificación propuestas separan los gurpos de inhibidores más importantes registrados en este tipo de hidrolizados lignocelulósicos.

Esta investigación contribuye con algunas recomendaciones y mejoras en la fábrica para valorizar las corrientes residuales no sólo mejorando la separación de los azúcares del resto de constituyentes de la madera para su fermentación, si no manteniendo las propiedades de calidad del producto principal, la pasta dissolving. A partir del estudio completo, deberían incorporarse a la planta actual etapas de hidrólisis ácida diluida, detoxificación, fermentación y purificación para la valorización de los carbohidratos dentro del concepto de biorrefinería.

ABBREVIATIONS

List of Abbreviations

AA	Atomic absorption spectroscopy
AAI	Antioxidant activity
AC	Activated carbon
AFEX	Ammonia fibre Explosion
AR	Anionic resin
ASAM	Alkaline sulphite anthraquinone methanol pulping
ATR	Attenuated total reflectance
BC	Black carbon
BP	Biomass Pretreatment
C5	Pentoses
C6	Hexoses
CBP	Consolidated bioprocessing
CMP	Chemimechanical pulping
CR	Cationic resin
CTMP	Chemi-thermomechanical pulping
D	Digestion
DPPH	2,2-diphenyl-1-picrylhydrazyl
ECF	Elemental Chlorine Free
EOP	Alkaline extraction reinforced with oxygen and hydrogen peroxide
FTIR	Fourier transformed infrared (spectroscopy)
HMF	5-hydroxymethyl-2-furfural

HPLC/RID	High performance liquid chromatography/Refractive Index Detector
HPLC/DAD	High performance liquid chromatography/Diode Array Detector
IIR	Individual inhibitors removal
IPC	Individual total phenolics measured with HPLC/DAD; in g/L.TSSL
LCB	Lignocellulosic biomass
LCBR	Lignocellulosic biorefineries
LCH	Lignocellulosic hydrolysates
LS	Lignosulphonates
MEEP	Multiple effect evaporation plant
NSSC	Neutral sulphite semichemical pulping
OH-UNE	Phenolic hydroxyl groups by UNE EN 16109 Standard; in g/L.TSSL
OV	Overliming
P1	Crude pulp; after digestion stage
P2	Ozonated pulp; after ozonation stage
P3	Semi-bleached pulp; after alkaline extraction stage
P4	Total-bleached pulp; after peroxide bleaching stage
PGW	Pressurised groundwood pulping
PHAs	Polyhydroxyalkanoates
PO	Peroxide bleaching
P&P	Pulp and paper
RMP	Refiner mechanical pulping
SCP	Single cell protein
SEW	SO_2-ethanol-water pulping
SGW	Stone groundwood pulping
SHF	Simultaneous hydrolysis and fermentation

Soda-AQ	Soda-anthraquinone pulping
SPORL	Sulphite Pretreatment to Overcome Recalcitrance of Lignocellulose
SSCF	Simultaneous saccharification and co-current fermentation
SSF	Simultaneous saccharification and fermentation
SSL	Spent Sulphite Liquor
TCC	Total Carbohydrate Content
TCF	Total Chlorine Free
TIR	Total inhibitors removal
TMP	Thermo-mechanical pulping
TPC	Total phenolic content by Folin Ciocalteu method; in g.GAE/L.TSSL
TSL	Total sugar looses; in % with respect to the thick spent liquor
TSSL	Thick Spent Sulphite Liquor; concentrated liquor collected at the outlet of the evaporation plant
UV-Vis	Ultraviolet visible spectrophotometry
VFAs	Volatile fatty acids
WSSL	Weak Spent Sulphite Liquor; diluted liquor before evaporation stage
Z	Ozonation

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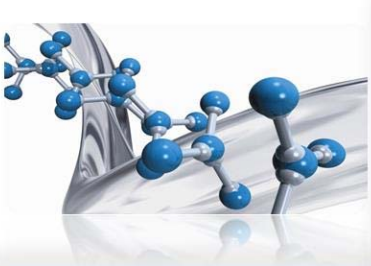

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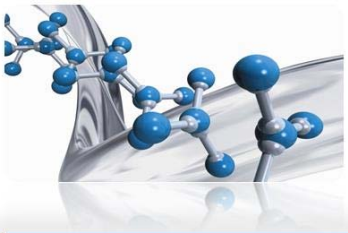

1. Introduction

1. INTRODUCTION

1.1 Overview of fossil fuels and new opportunities of lignocellulosic biomass

The 20th century has been dominated by petroleum providing a wide range of fuels and chemicals. A tremendous increase in energy demand is projected in the coming years and the preservation and management of natural resources is fundamental to foster sustainable development in the 21st century.

Currently fossil resources are not regarded as sustainable since they are questionable from the economic, ecological and environmental point of views (Naik et al., 2010). The burning of fossil fuels is a big contributor to increasing the level of CO_2 in the atmosphere which is directly associated with the global warming observed in recent years. The agreement to limit global warming to 2 ºC forces governments to focus on the energy sector which causes the highest greenhouse gas emission (Kircher, 2015).

About 90 % of fossil oil is used in the production of energy (power, heat and transport fuels) while 10 % is used in the production of chemicals and materials (Wertz and Bédué, 2013). Recent studies of Kircher (2015) reported that more than 95 % of fossil carbon sources (11 bn t of carbon annually from coal, gas, oil) are employed to generate energy for industrial, residential, commercial and transport applications whereas only 3.5 % gas, 0.1 % coal and about 8% of fossil oil is used in chemistry.

Figure 1.1 shows the total energy consumption of Europe in 2013. The energy consuption is dominated by the transport and industry sectors where renewable alternatives to the fossil fuels should be considered (EUROSTAT, 2015). Taking into account the global consumption, in 2010, 12.7 billion tons of oil equivalents were globally consumed, including, 32.4 % oil, 27.3 % coal and peat, and 21.4 % natural gas; however, biofuels and waste contributed with only 10.0 % (Lennartsson et al., 2014). These facts reveal the importance of biofuel development and how world biofuel production increased by 7.4 % in 2014 and global ethanol production increased by 6.0 % (BP, 2015).

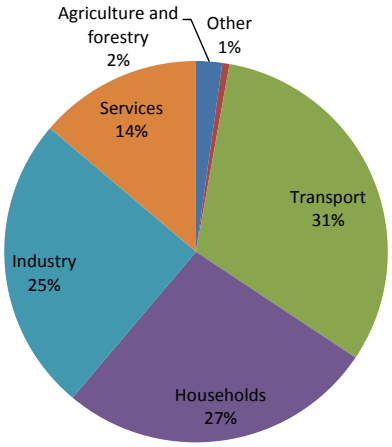

Fig. 1.1 *Final energy consumption EU-28 in 2013; tonnes of oil equivalent (EUROSTAT, 2015).*

Within the biofuels production, first generation biofuels come from sugar containing crops (i.e. sugar cane, wheat, beet, root, fruits or palm juice) or starch containing crops (i.e. wheat, barley rice, sweet sorgum, corn, potato or cassava). These kinds of biofuels can offer some CO_2 benefits; however they generate concerns about the sourcing of feedstocks including the impact it may have on biodiversity, land use and competition with food crops (Naik et al., 2010).

Second generation biofuels pursue the integral utilisation of biomass giving use to all parts of the plants such as leaves, bark, fruits and seeds. One renewable feedstock which is becoming popular nowadays for the production of second generation biofuels based on non-food feedstocks is the so-called lignocellulosic biomass (LCB) (Martins et al., 2011; Soccol et al., 2011).

LCB has emerged as a potentially attractive renewable resource providing not only second generation biofuels but also a wide variety of products. Among the main characteristics of LCB it is highlighted that they are cheap and abundant feedstocks with magnificent structural diversity and functional versatility (Dumitriu, 2005). LCB can be divided into seven categories such as crop residues, softwood, hardwood, cellulose wastes, herbaceous biomass and municipal solid waste (Cardona et al., 2010). The main drawback of the LCB is their complex structure which requires non-cost effective processes to overcome the recalcitrance of the LCB matrix. Nevertheless, a wide variety of value-added products well ranked in the bio-based and petro-chemical product markets can be obtained from LCB.

1.2 Lignocellulosic materials: fibre structure and chemistry

The LCB structure is formed by two types of cell wall that differ in function and composition (Complex Carbohydrate Research Center, 2015). Primary walls provide mechanical strength but must also expand to allow the cell to grow and divide. Secondary walls are thicker and stronger than primary walls and account for most of the carbohydrate in biomass. Primary and secondary walls contain cellulose and hemicellulose in different proportions. In Figure 1.2 the cell wall structure is shown. Such structure is composed of a network of cellulose microfibrils and cross-linking glycans embedded in a highly cross-linked matrix of polysaccharides surrounded by lignin polymers in the secondary wall (Alberts et al., 2002).

The three most important macrocomponents of LCB are cellulose, hemicelluloses and lignin (Ragauskas, 2015; Ochoa-Villareal et al., 2012). In addition, LCB contains a small quantity of extractives.

- **Cellulose** is a homogeneous linear polymer of 1,4 linked β-D-glucopyranose units with a degree of polymerisation (DP) ranging from 10000 to 15000. The basic repeating unit of cellulose is cellobiose. At ambient temperature, the rigid glucose rings are all found in their lowest energy, puckered chair conformation with all of the hydrogen-bonding hydroxyl substituents directed around the periphery of the ring.

- **Hemicelluloses** are amorphous heterogeneous polymers with a DP of 80-200 units. The monosaccharides comprising hemicellulose include the pentoses D-xylose and L-arabinose, as well as the hexoses D-glucose, D-galactose and D-mannose, and other related substances like acetyl groups, uronic acids or minor monosaccharides (rhamnose and fucose) embedded in the amorphous structure.

- **Lignin** is an aromatic polymer of phenylpropanoid precursors which surrounds and strengthens the cellulose-hemicellulose matrix. Softwoods generally contain more lignin than hardwoods. Lignin is mainly composed of coniferyl alcohol, sinapyl alcohol and hydroxycinnamyl alcohol which are polymerised in a random fashion.

- **Extractives** constitute the remaining low-molecular components extractable from the wood with water or organic solvents, excluding components which by definition belong to the hemicelluloses or lignin.

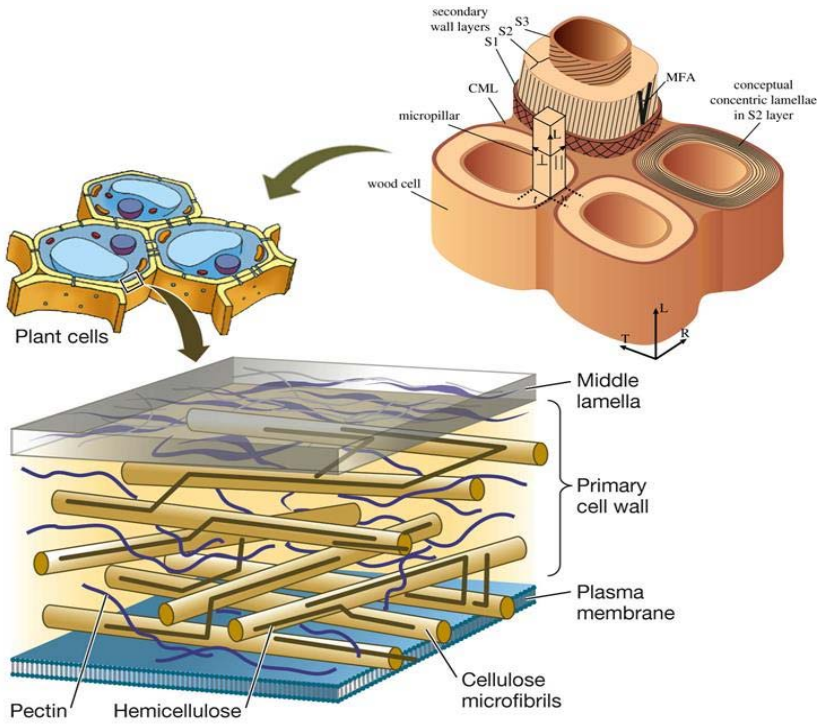

Fig. 1.2 *Cell wall structure (Biology forums, 2015).*

The composition of LCB depends strongly on the kind of specie. Different types of LCB species and their composition are displayed in Table 1.1. The variation ranges (Tolbert et al., 2014; Xu et al., 2014; Chen et al., 2010; Santana and Okino, 2007; Hayes et al., 2006) of the chemical composition of different LCB feedstocks are:

(i) 40-48 % of cellulose, 12-15 % of C6 sugars (hexoses), 7-10 % of C5 sugars (pentoses) and 26-31 % of lignin for softwood feedstocks;
(ii) 30-43 % cellulose, 2-5 % of C6, 17-25 % of C5, 20-25 % lingin for hardwood feedstocks;
(iii) 38-40 % cellulose, 2-5 % C6, 17-21 % C5 and 6-21 % lignin for cereal straw;
(iv) 35-41 % cellulose, 2 % C6, 15-28 % C5 and 10-17 % lignin for maize straw feedstocks.

Table 1.1 *Composition of lignocellulosic biomass feedstocks on % w/w dry weight.*

Biomass	Cellulose	Hemicellulose	Lignin
Switchgrass [a]	34	27	17
Monterery pine [a]	42	21	26
Eucalyptus saligna [a]	48	13	27
Corn stover [a]	37	24	18
Miscanthus [a]	37	36	25
Spruce [b]	43	26	29
Beech [b]	38	35	25
Ailanthus [b]	42	41	23
Hornbeam [b]	38	39	21
Alder [b]	42	29	26
Willow [b]	43	30	23
Oak [b]	35	32	26
Turkey oak [b]	38	31	28
Wheat straw [b]	32	37	18
Rice straw [b]	36.2	24.5 *	11.9
Barley straw [b]	33.8	24.7 *	14.5
Bagasse [b]	41.3	20.4	14.9
Poplar [c]	44.7	18.5	26.4
Pine [c]	43.3	20.5	28.3

*[a] Data taken from Hayes et al. (2006); [b] Data taken from Tolbert et al. (2014); [c] Data taken from Xu et al. (2014); * Only the pentosans were considered in the case of rice and barley straw.*

1.3 Lignocellulosic biorefinery

The energetic concerns previously overviewed and the characteristics inherent to LCB (abundance, versatility or low price) highlight the need to figure out new industrial models using these renewable feedstocks.

Within this framework, biorefinery is the sustainable processing of biomass into a spectrum of marketable products and energy (De Jong and Jungmeler, 2015). This term biorefinery arises to contribute towards reducing energy consumption and waste generation and establishes the utilisation of biomass for production of fuels and other bioproducts. The concept is analogous to a petroleum refinery where crude oil is converted into fuels and a wide range of valuable chemicals and materials. To achieve this, biorefineries embrace a wide variety of technologies able to separate biomass resources (wood, grasses, corn and so on) into their building blocks (carbohydrates, proteins, fats, etc.) which can be converted into value-added products, biofuels and chemicals (Wertz and Bédué, 2013; Pandey et al., 2011; De Jong et al., 2008). Such novel factories range from biomass production, biomass transformation/processing and end use (Naik et al., 2010).

According to De Jong and Jungmeler (2015), the biorefinery systems are classified by quoting the involved platforms (e.g. C5/C6 sugars, syngas and biogas), products (energy or chemicals), feedstocks (energy crops from agriculture or biomass residues from agriculture, forestry, trade and industry) and the conversion processes (thermochemical, biochemical, chemical and mechanical).

In this sense, lignocellulosic biorefineries (LCBR) comprise a wide variety of models and configurations as a function of the final target. In the case of sugar platforms, the main steps for LCB processing are shown in Figure 1.3. Biomass pretreatment followed by hydrolysis and detoxification prior to fermentation is usually required to get an efficient bioconversion into bio-based chemicals, biopolymers, biofuels and energy. Thus, a proper deconstruction of the lignin polymer from the plant cell wall (delignification); followed by an efficient carbohydrate depolymerisation (hydrolysis); and fractionation of undesirable compounds (detoxification) forming part of the lignocellulosic hydrolysates (LCH) should be carried out before fermentation. Optimisation of such stages permits the integral use of the LCB feedstock improving the economic margins of the mill.

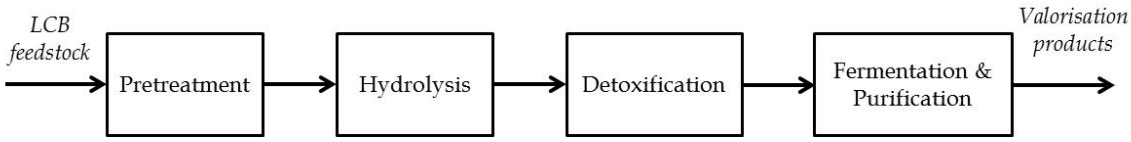

Fig. 1.3 *Main steps for LCB processing.*

The four steps of the process are detailed in the following sections, from 1.3.1 to 1.3.4. In addition, a 1.3.5 section about the valorisation options of lignocellulosic biomass has been added.

1.3.1 Pretreatment and delignification of lignocellulosic materials

Following the steps of Figure 1.3, the first step is the pretreatment of the LCB, breaking down the structure of the lignocellulosic matrix, rendering cellulose and hemicellulose accessible for subsequent steps. Biomass pretreatment (BP) which overcomes the biomass recalcitrance, is a process to ameliorate the accessibility for carbohydrates prior to the hydrolysis reactions. An easy way to see the effect of the BP on the complex LCB structure is shown in Figure 1.4.

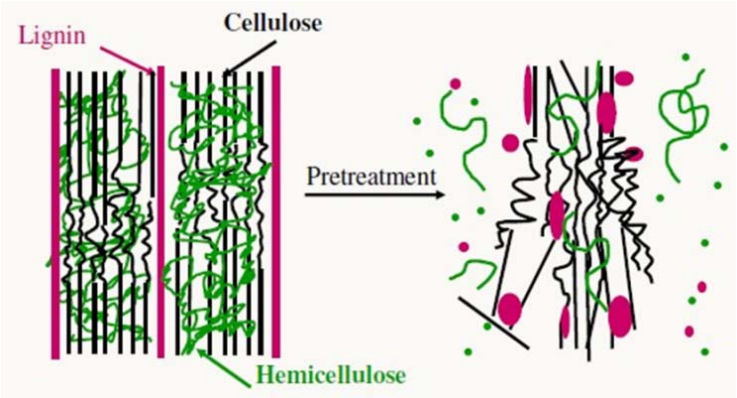

Fig. 1.4 *Effect of acid hydrolysis in the chemical structure of the LCB feedstocks (Liu and Fei, 2013).*

BP effects include: (i) an increase of the accessible surface area and porosity, (ii) cellulose decrystallisation, (iii) partial cellulose depolymerisation, (iv) hemicellulose solubilisation, and (v) delignification and modification of the lignin structure (Margeot et al., 2009). In addition, the process should meet the following

requirements: (i) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis; (ii) avoid the degradation or loss of carbohydrates; (iii) avoid formation of byproducts that are inhibitory to fermentation; and (iv) be cost-effective (Kumar et al., 2009). Selection of an appropriate BP depends upon the effective delignification or hemicellulose removal, minimum generation of inhibitors, low sugar loss, time saving, being economic and causing less environmental pollution (Canilha et al., 2012). Hemicelluloses should not be destroyed since hemicelluloses are the main source of sugars in the lignocellulosic residue, being potentially converted into marketable byproducts.

Several physico-chemical and/or biological BP can be used. A summary of the processes and conditions is listed in Table 2.2. Among the most popular pretreatment methods the following are highlighted (Hayes et al., 2006):

- **Physical pretreatment**: milling and grinding; high-pressure steaming and steam explosion; extrusion and expansion; high-energy radiation; pyrolysis.
- **Chemical methods**: alkali treatment (e.g. NH_3, NH_4SO_3, NaOH); acid treatment (e.g. H_2SO_4, HCl, H_3PO_4); gas treatment (e.g. ClO_2, NO_2, SO_2); oxidising agents (e.g. H_2O_2, O_3); cellulose solvents (e.g. Cadoxen, CMCS); solvent extraction of lignin (e.g. ethanol-water, benzene-ethanol, ethylene glycol, butanol-water); swelling agents.
- **Biological methods**: lignin-consuming microorganisms (fungi, e.g. *Phanerochaete chrysosporium* or bacteria e.g. *Nocardica sp.*); cellulose-attacking microorganisms (fungi, e.g. brown rot); lignin and cellulose-attacking microorganisms (white and red rot); lignin and/or cellulose attacking insects like termites.

Table 1.2 *Summary of pretreatments of LCB, their classifications, and processing conditions (Tolbert et al., 2014; Kumar et al., 2009; Carvalheiro et al., 2008).*

Pretreatment	Cathegory	Conditions
Organosolv	Chemical	180-195 °C, 30-90 min, 35-70 % ethanol
Steam Explosion	Physico-chemical	Saturated steam 160-260 °C and 0.69-4.83 MPa followed by a rapid decrease in pressure
Liquid Hot Water	Physico-chemical	Liquid water at 160-240 °C
Supercritical Water	Chemical	< 373 °C and < 22 MPa
White rot Fungi	Biological	Biomass containing 60-80 % moisture, pH 4-5, 15-35 °C, incubate for days, weeks or months
Alkaline treatment	Chemical	25-85 °C, 1-30 h
Dilute Acid	Chemical	0.3-2 wt% acid, 120-180 °C, 1min to 2 h
Ammonia Fibre Explosion (AFEX)	Physico-chemical	1-2 Kg liquid NH_3/kg.dry biomass, 60-100 °C, 10-60 min, > 3MPa
Wet Oxidation	Physico-chemical	1 L H_2O/ 6 g.biomass, 1.2 MPa, 195 °C, 10-20 min
Acid or SO_2 Catalysed Steam Explosion	Physico-chemical	190-220 °C, 1-10 min
Sulphite Pretreatment to Overcome Recalcitrance of Cellulose (SPORL)	Chemical	Sodium, calcium or magnesium bisulphite 160-190 °C, pH 2-5
Ionic Liquids	Chemical	20-150 °C, 1-48 h
Carbon Dioxide (CO_2) Explosion	Physico-chemical	Supercritical CO_2 at 6.8-27.6 Mpa, several minutes, < 200 °C

Pretreatment of LCB is one of the main challenges in biorefinery and regarded as the most expensive step where the cell wall complex structure should be broken down for the availability of free cellulose. Theoretically, less than 20 % cellulose hydrolysis is done in the absence of pretreatment while it is about 90 % when pretreatment is done (Srivastava et al., 2014).

1.3.2 Hydrolysis of lignocellulosic materials

After LCB pretreatment, the next step is the so-called hydrolysis which is the process converting the biomass carbohydrate polymers into fermentable sugars (see second step on Figure 1.3).

Cellulose and hemicellulose can be hydrolysed into sugars as is described in equations 1 and 2 respectively.

$$(C_6H_{10}O_5)_n + nH_2O \longrightarrow nC_6H_{12}O_6 \quad \text{(eq.1)}$$

$$(C_5H_8O_4)_n + nH_2O \longrightarrow nC_5H_{10}O_5 \quad \text{(eq.2)}$$

The primary challenge is the fact that the glucose in cellulose is joined by beta bonds in a crystalline structure, making it more difficult to be depolymerised than the alpha bonds in amorphous starch. The amorphous structure of hemicellulose makes it more easily hydrolysed than cellulose but there are also other components that can act as inhibitors in the fermentation step (Wyman et al., 2005):

$$\text{Cellulose} \longrightarrow \text{Glucose (C6)} \longrightarrow \text{HMF} \longrightarrow \text{Levulinic acid} \quad \text{(eq.3)}$$

$$\text{Hemicellulose} \longrightarrow \text{C5 sugars} \longrightarrow \text{Furfural} \longrightarrow \text{Formic acid} \quad \text{(eq.4)}$$

$$\text{Hemicellulose} \longrightarrow \text{C6 sugars} \longrightarrow \text{HMF} \longrightarrow \text{Levulinic acid} \quad \text{(eq.5)}$$

$$\text{Hemicellulose} \longrightarrow \text{acetyl} \longrightarrow \text{acetic acid} \quad \text{(eq.6)}$$

There are two major categories of lignocellulosic carbohydrate hydrolysis: the first and older method uses acids as catalysts (dilute acid hydrolysis or concentrated acid hydrolysis), while the second uses enzymes like cellulases (Verardi et al., 2012). In addition, there are other existing methods pursuing the same goal as alkaline hydrolysis and auto-hydrolysis. Acid hydrolysis aims at solubilisation of hemicellulose leaving the cellulose accessible for enzymatic saccharification. Most of the lignin remains in the insoluble cellulosic residue. Alkaline hydrolysis has been proven to solubilise lignin, while hemicellulose remains in the cellulosic residue. Alkali breaks ester-linkages and thereby increases the accessibility for enzymatic

hydrolysis (Murciano Martínez et al., 2015). Enzymatic hydrolysis is becoming popular for cellulose depolymerisation using cellulolytic enzymes (Cardona et al., 2010; Margeot et al., 2009). In the case of auto-hydrolysis, it is a hydrothermal process with no addition of reagents.

The hydrolysis mechanisms, operational conditions and the main characteristics of acid, alkaline, enzymatic and autohydrolysis are described below. In addition, a summary of the advantages and disadvantages of the main different kinds of hydrolysis is described in Table 1.3.

- **Acid hydrolysis** is a chemical process that uses an acid catalyst to break the polysaccharide chains into basic monomers. H_2SO_4 and HCl are the most commonly used. In contrast, H_3PO_4 can be more advantageous as it is less aggressive than other acids regarding the undesirable decomposition products formation (Lenihan et al., 2010). Depending on the acid concentration, there are two kinds of acid hydrolysis: (i) diluted-acid hydrolysis at high temperatures (80 °C < *T* < 200 °C) and an acid concentration between 0.5 % and 8% (w/w) used for hemicelluloses depolymerisation (Lenihan et al., 2010; Jiménez et al., 2007); and (ii) concentrated-acid hydrolysis at temperatures in the range of 20–50 °C and acid concentrations between 60 % and 84 % (w/w) used for higher recalcitrance cellulose depolymerisation (Gírio et al., 2010; Lenihan et al., 2010; Sánchez and Cardona 2008; Arni et al., 2007; Cardona and Sánchez 2007).

- **Alkaline hydrolysis** consists of the saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components like lignin and other cellulose. The effect of alkaline hydrolysis depends on the lignin content of the feedstocks. For instance, the digestibility of NaOH-treated hardwood increased from 14 to 55 % with a decrease in lignin content from 24-55 % to 20 % (Sun and Cheng, 2002). Low temperatures, long times under moderate base concentrations are used in this kind of process. Alkali such as sodium, potassium, calcium and ammonium hydroxides are the most employed reagents in the process (Toquero and Bolado, 2014; Chaturvedi and Verma, 2013).

- **Enzymatic hydrolysis** is naturally a very slow process compared to acid or alkaline hydrolysis due to the mild conditions (pH 4.8 and temperature in the range of 45 and 50 ºC) as well as the absence of corrosion problems (EC, 2015).

Enzymatic hydrolysis of plant carbohydrates has emerged as the most prominent technology for the conversion of biomass into monomer sugars for fermentation into second generation bioethanol (Pengilly et al., 2015; Van Dyk and Pletschke, 2012). Cellulases used for hydrolysis of LCB are produced from both bacteria and fungi which can be aerobic, anaerobic, mesophilic and termophilic. There are three main groups of cellulose-decomposition enzymes (cellulases): endoglucanases, exoglucanases and β-glucosidases (EC, 2015). The complete enzymatic hydrolysis of cellulose requires the synergistic effects of the three main cellulases (Jung et al., 2015). One major advantage of the enzymatic hydrolysis is that bioconversion and fermentation might take place in the same bioreactor by means of simultaneous saccharification and fermentation (SSF) process or consolidated bioprocessing (CBP) (Van Dyk and Pletschke, 2012).

- **Autohydrolysis** is a hydrothermal process promoting lower liberation of compounds derived from lignin and lower cellulose and hemicellulose degradation and consequently lower levels of microbial inhibitors are produced. This process does not require other reagents than water that selectively hydrolyse the hemicelluloses fraction (Silva-Fernandes et al., 2015). Nevertheless, enzymatic hydrolysis should be implemented thereafter to successfully convert the hydrolysis of cellulose into glucose (Silva-Fernandes et al., 2015-a; Silva-Fernandes et al., 2015-b).

Table 1.3 *Summary of hydrolysis conditions, advantages and disadvantages (Verardi et al.,2012; Binod et al., 2011; Sanchez and Cardona, 2008).*

Method	Advantages	Disadvantages
Dilute-acid hydrolysis	hydrolyse hemicellulose to xylose and other sugars; alters lignin structure	equipment corrosion, formation of toxic substances
Concentrated-acid hydrolysis	overcome the hydrolysis of crystalline cellulose which is very recalcitrant	equipment corrosion, high amount of toxic substances
Alkaline hydrolysis	removes hemicelluloses and lignin; increases accessible surface area	residual salts in biomass
Enzymatic hydrolysis	degrades lignin and hemicelluloses; low energy requirements	slow hydrolysis rates, very slow process

1.3.3 Detoxification of lignocellulosic hydrolysates

Detoxification is the next step proposed in LCB processing according to Figure 1.3. Detoxification treatments have the purpose of reducing toxic effects of inhibitory degradation compounds formed during the pretreatment and hydrolysis of LCB. Nowadays, most challenges in lignocellulosic detoxification consist of overcoming technical and economic barriers.

During the pretreatment or delignification and the hydrolysis process into C5 and C6 sugars from the hydrolysates and according to equations 3 to 6 in the previous section, different inhibitors of microbial metabolism are formed. A complex mixture of microbial toxins is generated including acetate from deacetylation of xylan, furan dehydration products, aliphatic acids and low-molecular weight phenolic compounds. Such decomposition products should be removed for an efficient sugar transformation (Cruz et al., 2005; Martínez et al., 2000). Figure 1.5 shows the macrocomponent structure, lignin precursors and sugar derivatives, formed as a consequence of LCB pretreatment and hydrolysis. Three main groups of inhibitors have been considered. Phenols (lignin-derived degradation compounds), furfurals (cellulose and hemicellulose-derived compounds) and weak acids (mostly hemicellulose-derived compounds):

(i) The first group of inhibitors corresponds to furfurals which inhibit the growth of yeast and decrease ethanol yield and productivity (Jönsson et al., 2013). Furfurals are formed from hexoses and pentoses at high temperatures and/or acid/alkaline conditions.

(ii) The second group of inhibitors is formed by the weak acids whose toxic effect strongly depends on the pH, due to partial dissociation of acetic acid, formic acid and levulinic acid. Weak acids inhibit cell growth and undissociated form of the acid because they become liposoluble and diffuse across the plasma membrane.

(iii) The third group of inhibitors is the low molecular weight phenolics from lignin which may act on biological membranes, causing loss of integrity, destroying the electrochemical gradient by transporting the protons back across the mitochondrial membranes (Almeida et al., 2007).

In addition to these inhibitors and depending on the pretreatment and hydrolysis process, lignosulphonates, insoluble lignin and metals can also act as inhibitors in the fermentation process (Alexandri et al., 2014).

Detoxification is based on separation technologies of these kinds of compounds which are toxic for the next fermentation step in the biorefinery process. Such techniques can be carried out by physico-chemical or biological treatments (Koivula et al., 2011). The most used processes include: neutralisation or overliming (Yu et al., 2012), liquid-liquid extraction (Alexandri et al., 2014), adsorption with activated charcoal (Lee et al., 2011; Canilha et al., 2004), ultrafiltration (Toledano et al., 2010), chromatography separation techniques (Ouyang et al., 2011), ionic exchange resins (Takasahi et al., 2013; Fernandes et al., 2012-b), treatment with reducing agents (Guo et al., 2013) or biological treatments e.g. *Laccase* and *Thricoderma reesei* (Jönsson et al., 2013). A summary of the main detoxification technologies of lignocellulosic hydrolysates is shown in Table 1.4. Unfortunately, the maximum allowable concentration of each inhibitor cannot be established with a general character, since it is strongly dependent on factors such as the microorganism utilised and its degree of adaptation; fermentation conditions; and the synergism effects caused by the simultaneous presence of several other inhibitors (Parajó et al., 1998). Therefore, a comprenhensive study of the feedstock and its processing behaviour should be done in order to determine the most suitable detoxification technique or combination of techniques adapting to a specific fermenting scenario.

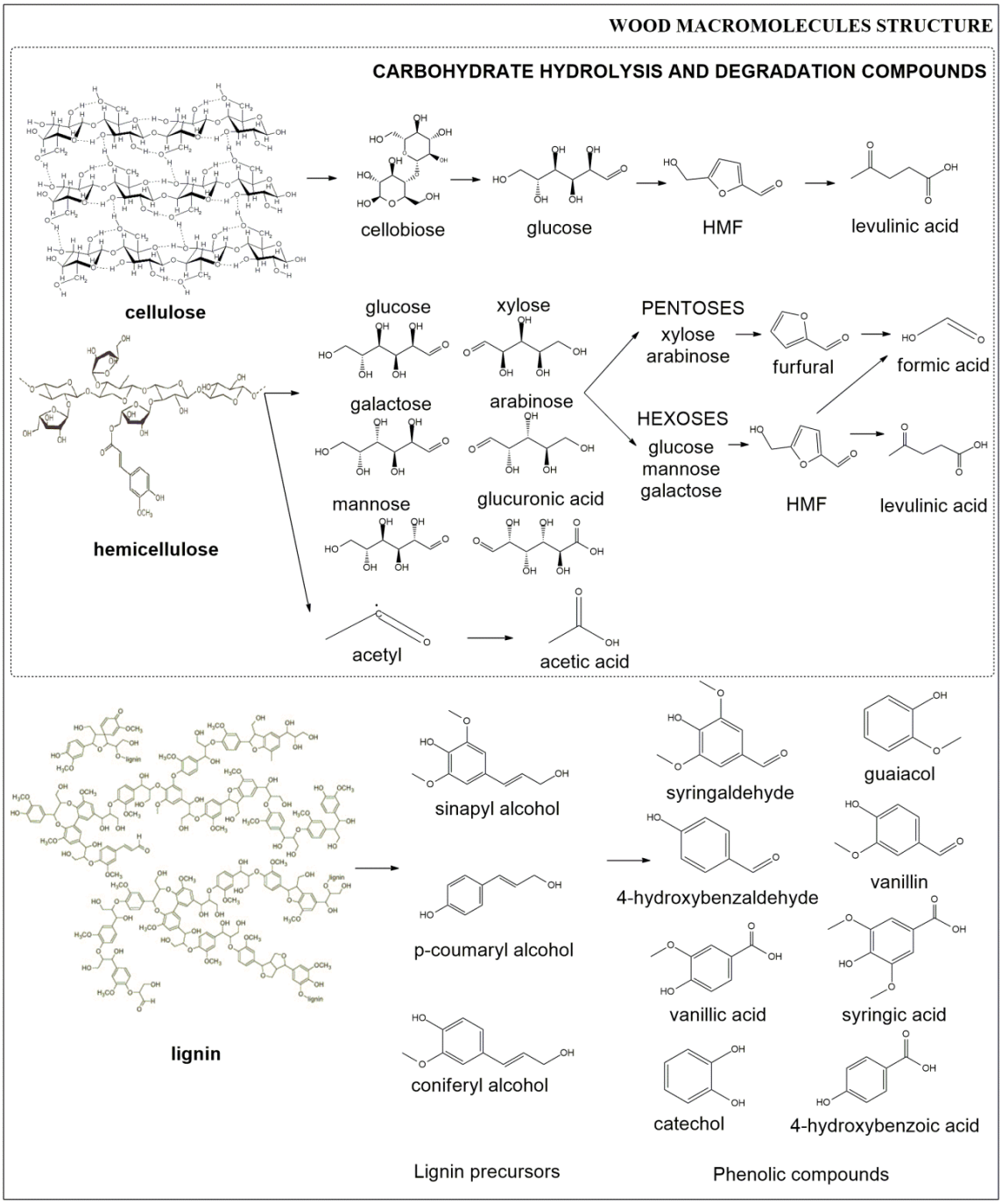

Fig. 1.5 *LCB constituents formed after pretreatment and hydrolysis.*

Table 1.4 ***S****trategies for lignocellulosic hydrolysates detoxification.*

Technique	Procedure	References
Chemical additives	Alkali such as $Ca(OH)_2$, NaOH, NH_4OH	Alriksson et al., 2006; Alriksson et al.,2005 Yu et al., 2012; Millati et al., 2002; Persson et al., 2002-a
	Reducing agents such as ammonium hydroxide, dithionite, dithiothreitol, sulphite	Alriksson et al., 2011; Lanka et al., 2011
Enzymatic treatment	Laccase	Ludwig et al., 2013; Jurado et al., 2009; Jönsson et al., 1998
	Peroxidase	Jönsson et al., 1998
Heating and vaporisation	Evaporation	Larsson et al., 1999
	Heat treatment	Ranatunga et al., 2000
Liquid-liquid extraction	Ethyl acetate	Alexandri et al., 2014; Faustino et al., 2010; Parajó et al., 2008; Cruz et al., 2005
	Supercritical fluid extraction such as supercritical CO_2	Persson et al., 2002-b
Adsorption	Activated carbon	Lee et al., 2011; Sainio et al., 2011; Canilha et al., 2004; Herbert Danner et al., 2002; Mussatto and Roberto, 2001; Parajó et al., 1996
	Ion exchange	Ludwig et al., 2013; Takahashi et al., 2013; Fernandes et al., 2012; Canilha et al., 2004; Mancilha and Karim, 2003; Nilvebrant et al., 2001
	Lignin	Björklund et al., 2002
Membrane separation	Ultrafiltration	Fernández-Rodríguez et al., 2015; Toledano et al., 2010; Restolho et al., 2009
Microbial treatment	*Coniochaeta ligniaria, Trichoderma reesei, Ureibacillus thermosphaericus*	Nichols et al., 2008; Okuda et al., 2008; López et al., 2004; Larsson et al., 1999

1.3.4 Fermentation of lignocellulosic hydrolysates

Fermentation is the following step of the LCB processing according to Figure 1.3. It is a process in which a microorganism (i.e. yeast, bacteria or fungi) causes an organic substance to break down into simpler substances like, for instance, the anaerobic break down of sugars into alcohol. The stoichiometric of hexoses and pentoses fermentation reaction into ethanol is shown in equations 7 and 8.

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \quad \text{(eq.7)}$$

$$3C_5H_{10}O_5 \longrightarrow 5C_2H_5OH + 5CO_2 \quad \text{(eq.8)}$$

Different fermentation strategies can be employed depending on the composition of the substrate, the organism used and the aim of the study. The basic fermentation modes regarding the addition of the substrate are: (i) batch fermentation where the cultivation media is added from the beginning; (ii) fed-batch fermentation where the substrate is fed into the system in a well-balanced mode in order to increase productivity of the organism due to low levels of inhibitors; and (iii) continuous fermentation which is constantly fed with substrate while an equal amount of spent substrate plus microorganisms is constantly withdrawn (Johansson, 2013).

The hydrolysis and fermentation processes are usually carried out by separate hydrolysis and fermentation (SHF). However, nowadays other modes of operation are being investigated: simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-current fermentation (SSCF), consolidated bioprocessing (CBP) (IEA Bioenergy, 2013; Van Dyk and Pletschke, 2012) or innovative simultaneous saccharification, filtration and fermentation (SSFF) (Ishola et al., 2013).

The microorganisms suitable for C5 and C6 sugar bioconversion, and therefore suitable for fermenting LCH, are summarised in Table 1.5. The most common fermenting scenarios are using naturally occurring bacteria (*Bacillus macerans* DMS 1574, *Bacteroides polypragmatus* NRCC 2288, *Clostridium saccharolyticum* ATCC 35040, *Erwinia chrysanthemi* B374 or *Thermoanaerobacter ethanolicus* ATCC 31938), recombinant bacteria (*Escherichia coli* B pLOI297, *Klebsiella oxytoca* M5A1, *Zymomonas mobilis* CP4), naturally occurring yeast (*Candida blankii* ATCC 18735, *Kluyveromyces cellobiovorus* KV 5199, *Pachysolen tannophilus* NRRL Y-2460, *Picchia stipites* CBS 5776), recombinant yeast (*Saccharomyces cerevisiae* TJ1, *Saccharomyces cerevisiae* H550, *Schizosaccharomyces pombe*) and fungi (*Aeurobasidium pullulans, Fusarium avenaceum* VTT-D-80146, *Monilia* sp., *Neurospora crassa* NCIM 870, *Paecilomyces* sp. NFI ATCC

20766) (Liang, 2012; Kim et al., 2010; Mussatto and Teixeira, 2010; Olsson and Hahn-Hägerdal, 1996).

Table 1.5 *Microorganisms suitable for C5 & C6 sugar bioconversion (Liang, 2012; Kim et al., 2010; Mussatto and Teixeira, 2010; Olsson and Hahn-Hägerdal, 1996).*

Feedstock	Microorganims
Cellulose bioconversion	*Saccharomyces cerevisiae*
	Lactobacillus species
	Acetobactor sp
	Penicillium luteum, P. citrinum, Aspergillus niger, A.wentii, A.clavatus, Mucor piriformis, Citromyces pfefferianus, Paecilomyces divaricatum, Trichoderma viride, Yarrowia lipolytica, Candida guilliermondii
	Manneheimia succiniproducens bacteria, *Actinobacillus succinogenes, Anaerobiospirillum succiniciproduens, Mannheimia succiniciproducens*
	Xanthophyllomyces dendrorhous
Hemicellulose bioconversion	*Pichia stipitis* (yeast) or *Escherichia coli, Klebsiella, Erwinia, Lactobacillus, Balcillus, Clostridia* (bacteria)
	Clostridium acetobutylicum, Clostridium beijerinckii
	Candida guilliermondii, Candida entomaea
	Pichia guilliermondii
	Bacillus polymyxa, Klebsiella pneumoniae, Bacillus subtilis, Seratia marcescens, Aerobacter hydrophia
	Lactobacillus pentosus, Bacillus coagulans JI12
	Aspergillus niger
	Clostridium tyrobutyricum

While decisions about the most adequate pretreatment and hydrolysis step depend on the feedstock, detoxification and fermentation of LCH change as a function of the product of interest; therefore, different detoxification-fermentation scenarios should be considered. For example, acetic acid inhibits the growth of yeasts in the production of either xylitol or ethanol (Parajó et al., 1998; Takahashi et al., 2013) whereas phenolics are problematic for growth of bacteria which produce succinic or PHA (Alexandri et al., 2014; Dietrich et al., 2013).

1.3.5 Valorisation options of lignocellulosic biomass in biorefinery

Throughout the steps described above (from section 1.3.1 *pretreatment* to section 1.3.4 *fermentation*) different hemicellulose and cellulose-derived products can be generated together with lignin-derived byproducts. A summary of by-products is shown in Figure 1.5. The main applications of byproducts derived from the three LCB macrocomponents are: (i) production of advanced materials, polymers and aromatic aldehydes in the case of lignin (Santos et al., 2011; Sanchez et al., 2008); fuels, oxygen barrier films, food additives, thickeners, emulsifiers and even medical uses in the case of hemicelluloses (Canilha et al., 2013); binders, glues, explosives, lacquers, cellophane films, sponge products, paper or textile fibres in the case of cellulose (Coz, 2014).

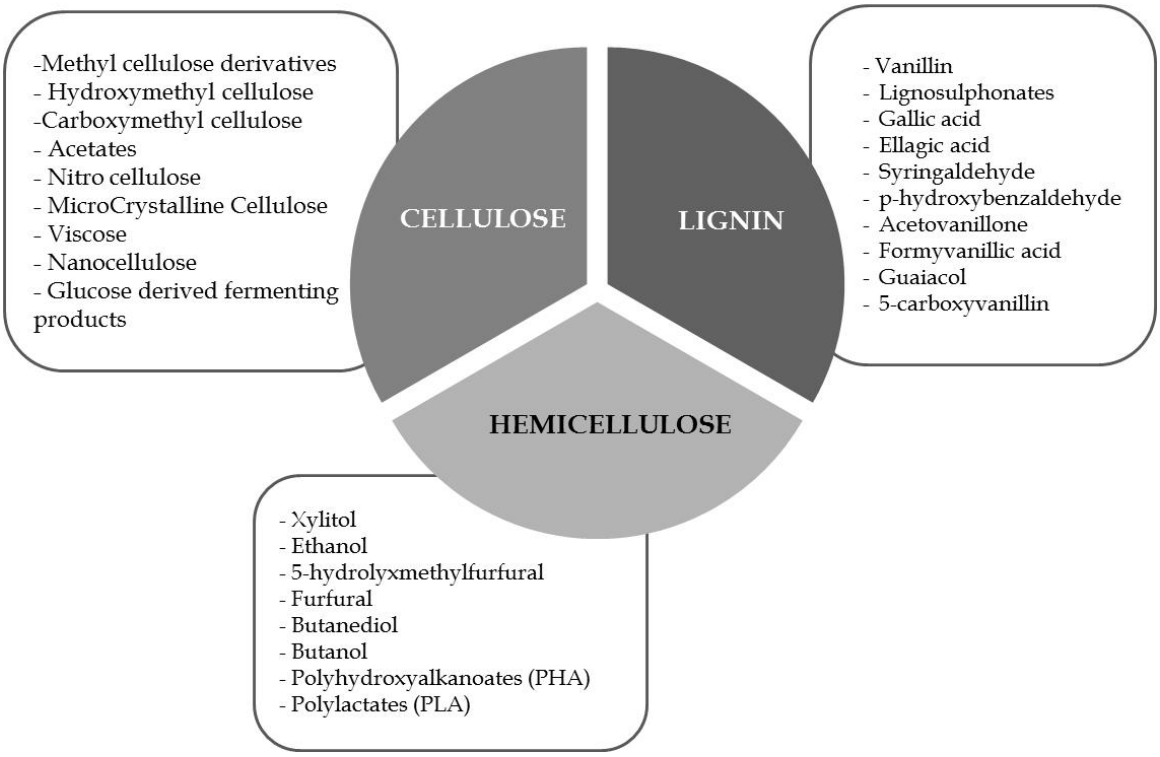

Fig 1.5 *Valorisation options from the three main LCB macrocomponents (Coz, 2014; Canilha et al., 2013; Bjørsvik and Liguori, 2002; Lai and Guo, 1991).*

Depending on the route used in the lignocellulosic biorefinery, different products can be obtained. Chemical based processes such as the hydrogenation of glucose to sorbitol, lignin depolymerisation by catalytic conversion producing vanillin, the

oxidation of glucose to gluconic then saccharic acid or acid dehydration of xylose to furfural can be used (Alonso et al., 2010). In addition, thermo-chemical processes including (i)-gasification where the material is gasified to yield a synthesis gas for energy production, methanol production, ammonia or methane; (ii)-pyrolysis giving activated carbon as a solid phase and pyrolytic oil used for fuel or for further chemical transformations; (iii)-liquefaction to obtain a liquid phase with a much higher calorific value than initial biomass; or (iv)-combustion which comprises the earliest use that has assigned the lignocellulosic material are also useful alternatives (Alonso et al., 2010; Goudriaan et al., 2000).

Finally, biochemical processes of sugar platforms (C5 and C6) presented in this dissertation are a good alternative in the case of transforming several industries into the biorefinery concept. The majority of uses of cellulose and hemicellulose products are derived from the sugar platform via microbial fermentation giving alcohols, organic acids, alkenes, lipids and other chemicals. Conversion can be achieved using bacteria, fungi or yeast, genetically modified or not in a wide variety of process conditions. There are many possibilities surrounding mixed-sugar fermentation such as ethanol, furfural, methanol, hydrogen, single cell protein (SCP), polyhydroxyalkanoates (PHAs), bacterial cellulose and volatile fatty acids (VFAs) like acetic acid, propionic and n-butiric acids can be obtained from the sugars presented in hardwood hydrolysates (Fernandes et al., 2012-a). Currently, ethanol production is one of the most studied and promising alternatives for LCB conversion, due to the large incentive that has been given to biofuel use in replacement of gasoline (Mussatto and Teixeira, 2010). Together with bioethanol, biohydrogen has shown potentialities for sustainable bioenergy production. In this case, not only hydrogen but also butyrate and acetate may be obtained as byproducts of the fermentation process. Biogas rich in H_2 and CH_4 may also be produced from glucose. Besides biofuels, several organic acids including lactic, citric and succinic may be produced. Production of carotenoids (natural pigments with a variety of applications in food technology) such as astaxanthin, has already been performed by fermentation of wood hydrolysates (Mussatto and Teixeira, 2010). Some of the most common examples of fuels, chemicals and polymers produced from sugar fermentation of LCH are shown in Figure 1.6.

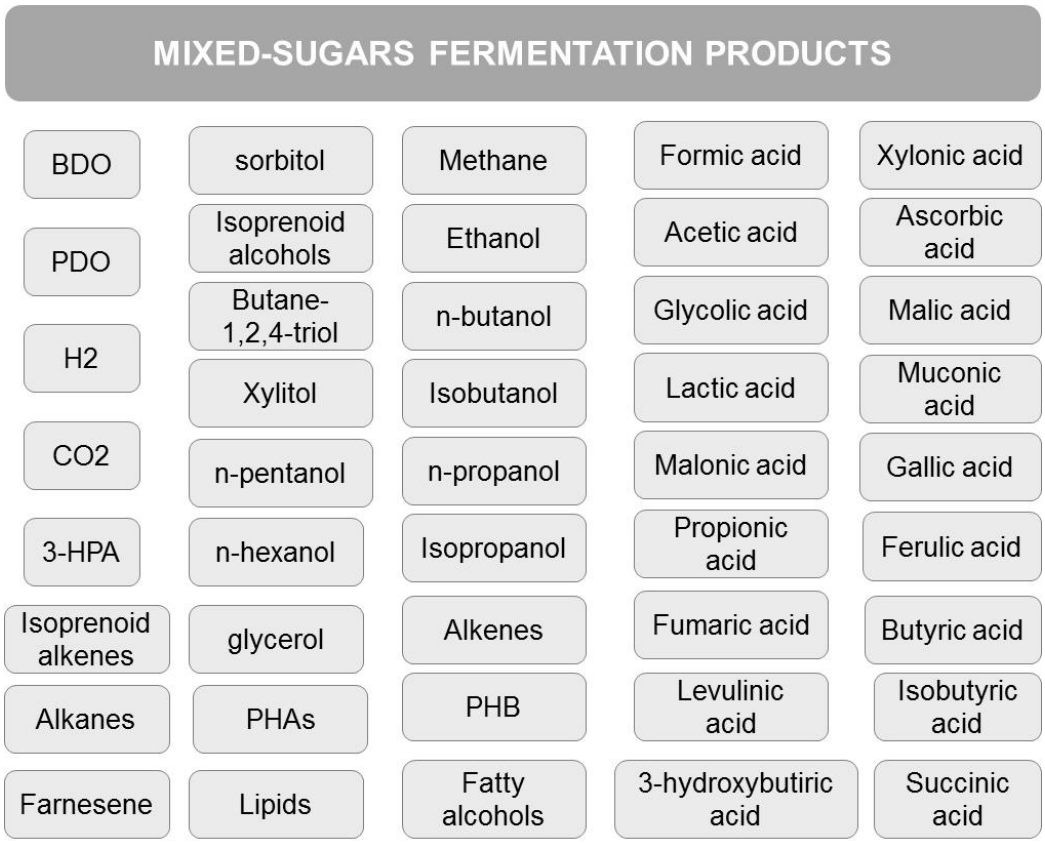

Fig 1.6 *Mixed sugars fermentation products: biofuels, biochemicals and biopolymers (EC, 2015). BDO: 1,4-butanediol; 3-HPA: 3-hydroxypropionic acid; PDO: propane-1,3-diol; PHAs: polyhydroxyalkanoates; PHB: polyhydroxybutyrate*

1.4. Pulp and Paper mills as a challenge for lignocellulosic biorefinery

The tendency for valorising residual sugar producing chemicals, biopolymers or biofuels is a motivation for the sustainable economy, the energy dependence issues and circular economy consciousness. Therefore, new industries or the integration of sugar-processing steps in existing industries should be done within the biorefinery concept in order to valorise all of the main fractions of the lignocellulosic biomass. One industrial sector that generates vast amounts of sugar-rich residues and which are consequently suitable to be transformed into LCBR is the pulping sector. Pulp and Paper (P&P) mills generate lignocellulosic residues (rich in lignin and hemicellulose) capable to be transformed into marketable bioproducts by partially changing their usual processes that are currently oriented in cellulose pulp production.

The world's annual wood pulp production in 2011 was at around $173 \cdot 10^6$ t and the world's top producers were USA and Canada ($67 \cdot 10^6$ t), Brazil ($15 \cdot 10^6$ t) and Sweden and Finland ($21 \cdot 10^6$ t) (Koutinas et al., 2014). The demand for high-purity cellulose pulps, the so-called dissolving pulps, has substaitially increased during the last few years and this trend will prevail during the next decades. It is estimated that in 2050 the annual demand of textile fibers will range between 120 and 130 million tons (Aalto University, 2015).

P&P mills comprise different produts (e.g. paper, paperboard or dissolving pulps) and pulping processes (e.g. kraft, sluphite, SEW or SPORL). The pulping processes can be classified into four broad categories: chemical, semi-mechanical, chemi-mechanical and mechanical. Chemical pulp accounts 75.6 % of the total produced pulp whereas mechanical pulp produced approximately 17 % followed by semi-chemical (4.9 %) and dissolving (2.5 %) (Koutinas et al., 2014). Chemical methods rely on the effect of chemicals to separate fibres, whereas mechanical pulping methods rely completely on physical action (Bierman, 1996). Among the **chemical processes**, kraft, also known as the sulphate process, is the most commonly used (Chakar and Ragauskas, 2004). However, other processes such as soda-anthraquinone (soda-AQ) (Wang et al., 2013); ethanol-water pulping also known as organosolv (Akgul et al., 2009; Pan et al., 2005); neutral, acid sulphite and bisulphite pulping (Shen and Chen, 2009; Gümüşkaya and Usta, 2006); or SO_2-Ethanol-Water process (SEW) can also be used (Sklavounos et al., 2013). **Mechanical pulps** are produced by grinding wood against a stone or between metal plates, thereby separating the wood into individual fibres (Teschke and Demers, 2011). Several

examples of mechanical pulps are stone groundwood pulping (SGW), refiner mechanical pulping (RMP) and thermo-mechanical pulping (TMP), a modification of RMP where chips are steamed before and during refining, usually under pressure (Teschke and Demers, 2011). On one hand, in the SGW process, debarked logs are forced against rotating stone grinding wheels that are constantly washed by a stream of water. On the other hand, in RMP and TMP the chips are ground by passing them through rapidly rotating disk grinders (OTA, 1989). **Chemi-mechanical pulps** combine treatment with chemical agents and mechanical treatments like Sulphite Pretreatment to Overcome Recalcitrance of Lignocellulose process (SPORL) (Tian et al., 2010; Zhu et al., 2009); The **chemi-thermo-mechanical** pulping process (CTMP), in which the wood is pre-softened with chemicals, is generally considered to be a mechanical pulping technique since the chemical principally soften the lignin prior to the mechanical stage rather than fully dissolve it out as in true chemical pulping processes (INERIS, 2015).

Regarding the pulp products, there are a wide variety of types of pulp based on their pulping process, raw material used or the type of bleaching. Some of the classifications employed worldwide are listed below:

- ✓ **Based on pulping process:** mechanical (GW, PGW, RMP), mechanical and thermal (TMP), semi-mechanical (CTMP), semi-chemical (NSSC, cold Soda), chemical (Sulphite, Sulphate)
- ✓ **Based on raw material:** wood (HW, SW), agricultural residue (straws, sugarcane bagasse), annual plants & grasses (hemp, jute, kenaf, bamboo), rags, recycled on secondary fibre, deinked
- ✓ **Based on bleaching:** total chlorine free (TCF), elemental chlorine free (ECF), chlorine bleaching (chlorine gass & hypochlorite), oxygen/ozone
- ✓ **Based on yield:** very high yield (>95 %) SGW, PGW; high yield (85 -95 %) RMP, TMP; medium high yield (65-85 %) CTMP, NSSC, CMP; medium yield (45-65 %) sulphite and sulphate pulping; medium low yield (35 -45 %) straw, grass, bagasse chemical pulp; low yield (<35 %) rayon, viscose
- ✓ **Based on fibre length:** long fibre pulp (>10 mm) cotton, hemp, flax, jute; medium fibre pulp (2-10 mm) northern SW, HW; short fibre pulp (<2 mm) tropical HW, straws, grasses.

A summary of the main pulping processes and conditions, pulp grades and end uses is shown in Table 1.6. In general terms, the main advantage of mechanical and thermo-mechanical pulps is the high yields. Looking at Table 1.6, yields are in the range of 85 % to 97 % for mechanical pulps and from 65 % to 94 % for chemi-

mechanical pulps. However, the main drawbacks of mechanical and thermo-mechanical pulps are their low strength and resistance (tendency to discolour). The addition of chemical fibers is required for producing printing papers. The main advantages of chemical pulps rely on the brightness which is higher in comparison with mechanical pulps, and kappa numbers which are lower than mechanical pulps. The kappa number estimates the amount of lignin of the pulp. In the case of kraft pulping there is no limitation on wood species and there is a good chemical recovery and kraft pulps have high strength. Nevertheless, kappa numbers in kraft pulping are higher than in sulphite pulps and brightnesses are lower than sulphite pulps. Sulphite pulping uses a wide variety of bases and delignification selectivity is better than kraft, soda-AQ or organosolv. The drawbacks of sulphite pulping are the vessel corrosion and cooking times which are longer than kraft pulping processes.

One of the main important issues of the final products is the grade of the pulp. In this case, three main products can be obtained: corrugated paper, pulp for paper grade and dissolving pulp (high purity cellulose pulp). Dissolving cellulose is a low-yield chemical pulp (30-35 %) with a high alpha-cellulose content (91-93 %) and relatively low hemicellulose (1-10 %) and lignin (<0.05 %) content and it can be produced by two main processes: kraft with a pre-hydrolysis process and sulphite pulping. However, other processes such as SEW or SPORL are being studied (Coz et al., 2015; Sixta et al., 2013). Nowadays, due to the fact that dissolving pulp can be a replacement of cotton, many paper-grade factories are trying to transform into dissolving factories for textile fibre production (Quijorna et al., 2011). The world demand for dissolving pulp is expected to grow from 4.1 million air dried tonnes per year in 2008 to 6.3 million ADt/year by 2025 (Flickinger et al., 2011). The trend consumption by region is reflected in Figure 1.7. Asia demanded 61 % of the total dissolving market in 2008 in comparison to Europe, the second demander that covered 25 %. Dissolving pulps prices on world markets per ton have skyrocketed from protracted lows of 300 $ to 500 $ per ton from the 1970s into the late 1990s to as high as 1,800 $ to 1,900 $ with spot prices up to 2,500 $ in 2011. The price still remains on the high side in the fourth quarter of 2013 (1,725 $/ton). The growing demand of dissolving pulp requires the development of novel processes within the sustainable biorefinery context, able to compete with the Asiatic markets.

Dissolving pulp is not only limited to textile applications, but it also concerns the manufacture of cellulose acetate for high value-added films, plastics and coatings among others (Sixta et al., 2013). The link between cotton and dissolving pulp prices has strengthened over the last several years. Small changes in cotton supply result

in large changes in dissolving pulp demand. The International Cotton Advisory Committee (ICAC) foresees the cotton acreage to be reduced from a historical trend of 33 million hectares to 32 million hectares by 2020 (Floe, 2011). The supply constraints of cotton are serious and the supply forecast for 2020 by ICAC would allow cotton to capture only a small portion of core demand growth leaving viscose fibres a significant market opportunity in substitution (Floe, 2011). Figure 1.7 shows the global demand of dissolving pulp distributed by region, common end uses and substituting products. Percentages of end-product and region graphs were calculated by Flickinger et al. (2011) considering the total market.

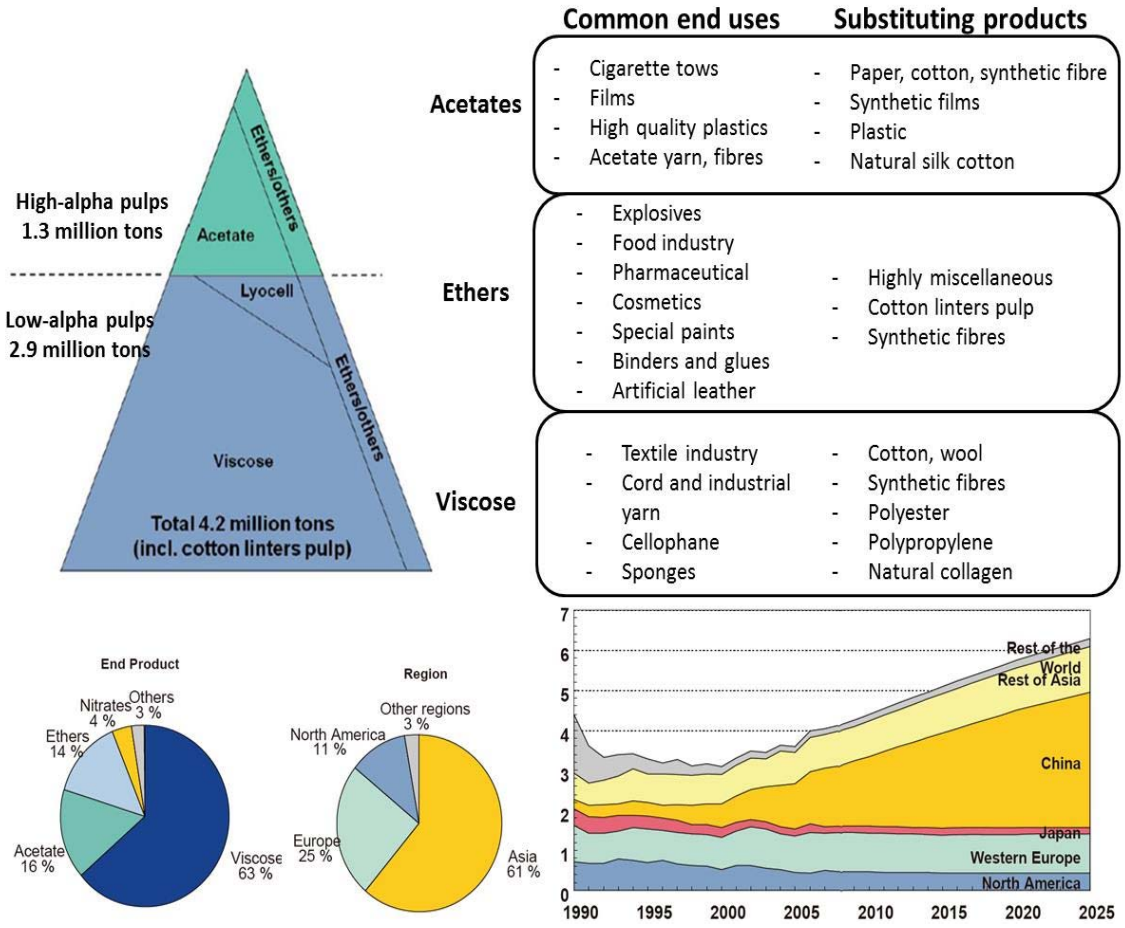

Fig. 1.7 *Dissolving pulp end use segments, demand by end product and region (Flickinger et al., 2011).*

Table 1.6 *Review of pulping processs, qualities and operational conditions (INERIS, 2015; Salmenoja, 2012; Flickinger et al., 2011; Floe, 2011; Sixta et al., 2011; Teschke et al., 2011; OTA, 1989).*

Pulping	Feedstock	Cooking Conditions	Yields (%)	Grade	Pulp properties
Organosolv (Chemical)	SW/HW	40-60 % ethylenglycol at 180-200 °C for 30 to 90 min	31.8-40.1	paper	kappa: 25.5-27.5; α-cellulose: 21.2-33.7 %
Soda-anthraquinone, Soda-AQ (Chemical)	SW/HW	5:1 or 4:1 liquor-to-wood ratio, 0.1-0.15 % AQ and 18 % NaOH; ramp 160-180 °C for 40 to 90 minutes; dwell time: 60-150 minutes	47.7-53.2	paper	kappa: 19.3-22.3 934-1000 mL/g; brightness 22.3 %
Alkaline sulphite anthraquinone methanol, ASAM (Chemical)	SW/HW	Heating ramp 135-150 min to 180 °C; using 22.5-25.0 % NaOH and Na_2SO_3/total alkali 0.7-0.8 ;AQ: 0.05-0.1 % w/w; EtOh of 10 % v/v ;3:1 to 5:1 liquor-to-wood	51.4-54.6	paper	brightness: 33.5-47.9 %; kappa 14.7-16.6; viscosity: 1285 mL/g
Kfrat (Chemical)	SW/HW	White liquor containing NaOH and Na_2S; pre-heating ramp: 85 min up to 80 °C; cooking temperature 165-170 °C; dwell time: 95-180 min; 4:1 liquor-to-wood ratio	47.0k-47.2	paper	brightness 28.9 %; kappa 26.8 1000-1100 mL/g
Prehydrolysis kraft (Chemical)	SW/HW	Cooking with steam prehydrolysis: prehydrolysis at 150-170 °C, 45-90 min; pH after hydrolysis 3.3-3.7; Total time: 300-350 min	35-39	dissolving	brightness 26.2-44.4 %; kappa 20.7-7.8; 1024-1211 mL/g
Acid sulphite (Chemical)	SW/HW	pH: 1.2-1.5, 80 % free SO_2-20 % bound SO_2, bases of Ca, Mg, Na or NH_3 can be used; pre-heated liquor: 70-80 °C; dwell temperature 130°C-145 °C; pressure: 5,5-7 bar; 7 hours; liquor-to-wood ratio 4:1	51.5 -35	dissolving	brightness 48.4-57.4 %; kappa: 5-7; 680-760 mL/g; alpha-cellulose > 88%
Bisulphite (Chemical)	SW/HW	pH:3-5; 50 % free SO_2-50 % bound SO_2; Na, Mg and NH_3 bases; dwell temperature 160 °C; 5-6 bar; 6-7 hours; liquor-to-wood ratio 4:1	50.1-45.3	paper	brightness 56.6 %
Alkaline sulphite (Chemical)	SW/HW	pH:12-14; 100 % of total SO_2 is bounded; sodium bases ($NaOH+Na_2SO_3$); dwell temperature: 175 °C; 5-6 bar; 6-7 hours; liquor-to-wood ratio 4:1	43.9-45.5	paper	brightness 33.0-37.9 % kappa 35.8-43.6
Neutral Sulphite NSSC (Chemi-mechanical)	HW	pretreatment with 4-12 % Na_2SO_3 and 3 % Na_2CO_3 at 154 to 186 °C from 10 to 30 minutes+ mechanical refining; pH:8-9; 100 % of total SO_2 is bounded; sodium, magnesium or ammonia bases; liquor-to-wood 4:1	79.9-93.9	corrugated paper	brightness: 44.0-55.9 %
Cold-caustic (Chemi-mechanical)	HW	NaOH bath; pH>12; pretreatment time: 120 min at 30 °C	65-85	corrugated paper	-
Bisulphite BCMP (Chemi-mechanical)	HW	Heating ramp 1-10 min to 110-140 °C; using 12-17 % Na_2SO_3 and 12-17 % $NaHSO_3$; pH 9-12; pretreatment time: 60 min at 130-170 °C	65-85	corrugated paper	-
Chemi-Thermo-Mechanical, CTMP (Chemi-mechanical)	SW	Heating ramp 1-10 min to 110-140 °C; using 2-5 % Na_2SO_3; pH 9-12; pretreatment time: 2-5 min at 120-130 °C	90-94	corrugated paper	-
Stone groundwood, SGWP (Mechanical)	SW	No cooking ramp, chemicals or pretreatment is needed	95-97	corrugated paper	-
Refiner mechanical, RMP(Mechanical)	SW	No cooking ramp, chemicals or pretreatment is needed	85-95	corrugated paper	-
Thermomechanical, TMP(Mechanical)	SW	Heating ramp 1-10 min to 110-140 °C; no chemicals or pretreatment is used	93-95	corrugated paper	-
Pressure Groundwood, PGWP (Mechanical)	SW	No cooking ramp, chemicals or pretreatment is needed	85-95	corrugated paper	-

1.5 Objectives and structure of the thesis

The research work presented in this thesis was conducted at the Chemistry and Process & Resource Engineering Department of the University of Cantabria within the Green Engineering & Resources group (GER) and at the Hydrolysis Laboratory of the Sniace, a company located in Cantabria. This doctoral dissertation is presented in the topic of *Valorisation of lignocellulosic waste in biofuels and bioproducts*. The thesis has been financially supported by the EUCAFUEL 23.H031.64003 research project under the framework of the Spanish Ministry of Science and Innovation and also by the KBBE-2012-6-311935 BRIGIT research project of the 7th Framework Programme of the European Union. In addition, three months were conducted at the Agricultural University of Athens, Laboratory of Engineering Food Science and Technology, under the Erasmus+ programme and the supervision of Dr. Apostolis Koutinas.

The working hypothesis is that pulp mills can be easily transformed into lignocellulosic biorefineries by changing operational variables at digestion and bleaching steps, and implementing fractionation steps, which permit the reuse of sugars for fermenting purposes.

The general objective of this thesis is to study the acid sulphite process in a factory in the north of Spain and to identify the improvements to the factory in order to not only obtain dissolving pulp but also to track down the residual sugar-rich streams within the biorefinery concept. The achievement of this general goal is accomplished by the following partial objectives:

- Study of the current acid sulphite process and the three wood macrocomponents throughout the whole process establishing the main variables affecting lignin and hemicellulose extraction at the industrial plant. In order to fulfil this sub-objective, all of the methods for carbohydrate quantification of *Eucalyptus globulus* hardwood (feedstock), cellulose dissolving pulp (product) and spent sulphite liquor (residue) were developed.
- Study of the digestion process and external hydrolysis processes of the spent sulphite liquor at laboratory scale in order to improve the sugar substrate and minimise the inhibitors for fermenting purposes.
- Study of different detoxification techniques at laboratory-scale, able to separate sugars from the rest of inhibitors for fermenting valorisation purposes.

According to the hypothesis, the main and specific objectives, and taking into consideration the requirements to the preparation of doctoral thesis the present dissertation is organised into the following chapters:

Chapter 1 entitled "Introduction" that includes the background regarding lignocellulosic feedstocks and valorisation options together with the use of pulp and paper mills in the transformation towards lignocellulosic biorefineries. The objectives, scope and thesis structure are outlined within this chapter.

Chapter 2 entitled "Methodology" containing the description of the analytical procedures for the physico-chemical characterisation of wood, pulp and hydrolysates, and the experimental assays in the processes performed in this research, digestion, external hydrolysis and detoxification.

Chapter 3 entitled "Results and discussion" contains the obtained results and discussion to fulfil the objectives of the dissertation. This chapter is subdivided into the following sections:

> **3.1 The acid sulphite pulping process:** describing the main steps of the current process taking place within the factory.
>
> **3.2 Towards transformation of the sulphite mill in a LCBR:** based on the current process, this section describes the possibilities and developments of the factory towards a lignocellulosic biorefinery according to the following steps: (i) firstly, the carbohydrate quantification was developed in order to characterise all of the fractions in the process. (ii) secondly, a mass balance through summative analysis of all of the main components in the industrial mill was studied; (iii) the third step was the study of the main pulping process in order to increase the sugar substrate for valorisation options; (iv) fourthly, an external hydrolysis was studied with the purpose of enhancing sugar content; and (v) as a sixth step, the study of different physico-chemical detoxification processes was carried out in order to increase the valorisation opportunities of the mill. In the last section, a discussion about the efficient transformation of the sulphite pulp mill into a lignocellulosic biorefinery is displayed.

2. Methodology

2. METHODOLOGY

This chapter is divided into the following sections: characterisation procedures of *wood (Eucalyptus globulus)*, dissolving pulp and hydrolysates; analysis of woody carbohydrates and lignin derivatives by HPLC; cooking trials for wood delignification at laboratyory scale; and finally the experimental procedures for hydrolysis and detoxification of the hydrolysates. A summary of the analytical procedures applied in this dissertation is shown below in Table 2.1 for wood, pulp and hydrolysate characterisation.

Table 2.1 *Analytical procedures used in this thesis for wood, pulp and hydrolysate characterisation.*

Parameter	Main Equipment	Standard/Source
(w,p) Samples conditioning	Mill and sieve for wood Disintegrator and Buchner filter for pulp	TAPPI T257 cm-02 (2002-a) for wood TAPPI T205 sp-02 (2002-b) for pulp
(w,p) Extractives	Soxhlet apparatus	ISO 14453 (2014), TAPPI T204 cm-97 (1997-a)
(w,p) Ash at 525 °C	Muffle furnace	TAPPI T211 om-02 (2002-c)
(w,p) Moisture	Oven	TAPPI T264cm-97 (1997-b)
(w) Cellulose	Analytical balance	Seifert method (Wright and Wallis, 1998)
(w) Holocellulose	Thermostatic bath	Wise method (Haykiri-Acma et al., 2014)
(w) Lignin	Spectrophotometer	TAPPI T222 om-02 (2002-d)
(p) Kappa	Titrator	TAPPI T236 om-99 (1999-a)
(p) Micro Kappa	Titrator	TAPPI UM 246 (1991)
(p) Lignin	-	Syverud et al. (1999)
(p) Intrinsic viscosity	Titration and capillary viscometer	ISO 5351:2010 (2010), TAPPI T230 om-99 (1999-b)
(p) α-cellulose	Titration and rotary shaker	TAPPI T203 cm-99 (1999-c)
(w,p,h) Glucan (w,p,h) Xylan (w,p,h) Arabinnan (w,p,h) Galactan (w,p,h) Mannan (w,p,h) Acetyl	[1] Thermostatic bath (hydrolysis) [2] Liquid chromatograph (sugars quantification) [3] Summative calculations (from sugars to homopolymers)	[1] TAPPI T249 cm-00 (2000-a) [2] Internal method developed in sections *2.5.1* and *2.5.2* [3] Internal method developed in section *2.5.3*
(h) Lignosulphonates	Spectrophotometer	UNE EN 16109 (AENOR, 2012)
(h) Total, free & combined SO_2	Titrator	TAPPI T 604 om-00 (2000-b)
(h) Weak acids and furfurals	Liquid chromatograph	Internal method developed in sections *2.5.1* and *2.5.2*
(h) C5 & C6 sugars	Liquid chromatograph	Internal method developed in sections *2.5.1* and *2.5.2*
(h) Total and reducing sugars	Spectrophotometer	Phenol- Sulphuric (Durmuş et al., 2002) and DNS (Wang et al., 2011-a) methods
(h) Metals	Spectrometer	Internal method developed in section
(h) Antioxidant Activity	Spectrophotometer	DPPH method (Scherer and Godoy, 2009)
(h) Phenolic hydroxyl groups	Spectrophotometer	UNE EN 16109 (AENOR, 2012)
(h) Total phenolic content *	Spectrophotometer	Folin Ciocalteu method (Faustino et al., 2010)
(h) Individual phenolics	Liquid chromatograph	External method (Proestos et al., 2006) see section *2.5.4*
(h) Functional groups	Spectrometer	Internal method see section *2.3.5*
(h) Density, (h) viscosity, (h) pH, (h) ash at 525 °C and 900 °C and (h) moisture	Picnometer, rotational viscometer, pHmeter, muffle	Internal methods (section *2.3.7*), TAPPI T211 om-02 (2002-c) TAPPI T413 om-02 (2002-e), TAPPI T264cm-07 (1997-b)

** Total phenolic content was determined over the liquid extracts*

(w,p,h) Wood, pulp and hydrolysate samples

2.1 Characterisation of *Eucalyptus globulus* labill hardwood

Figure 2.1 shows a summary of all of the characterisation procedures used in wood. As can be seen in the figure, there are two different pathways for determining the wood carbohydrates in cellulose and hemicellulose, one of them based on the total cellulose and hemicellulose content and the other one based on the sum of all of the individual carbohydrates by HPLC.

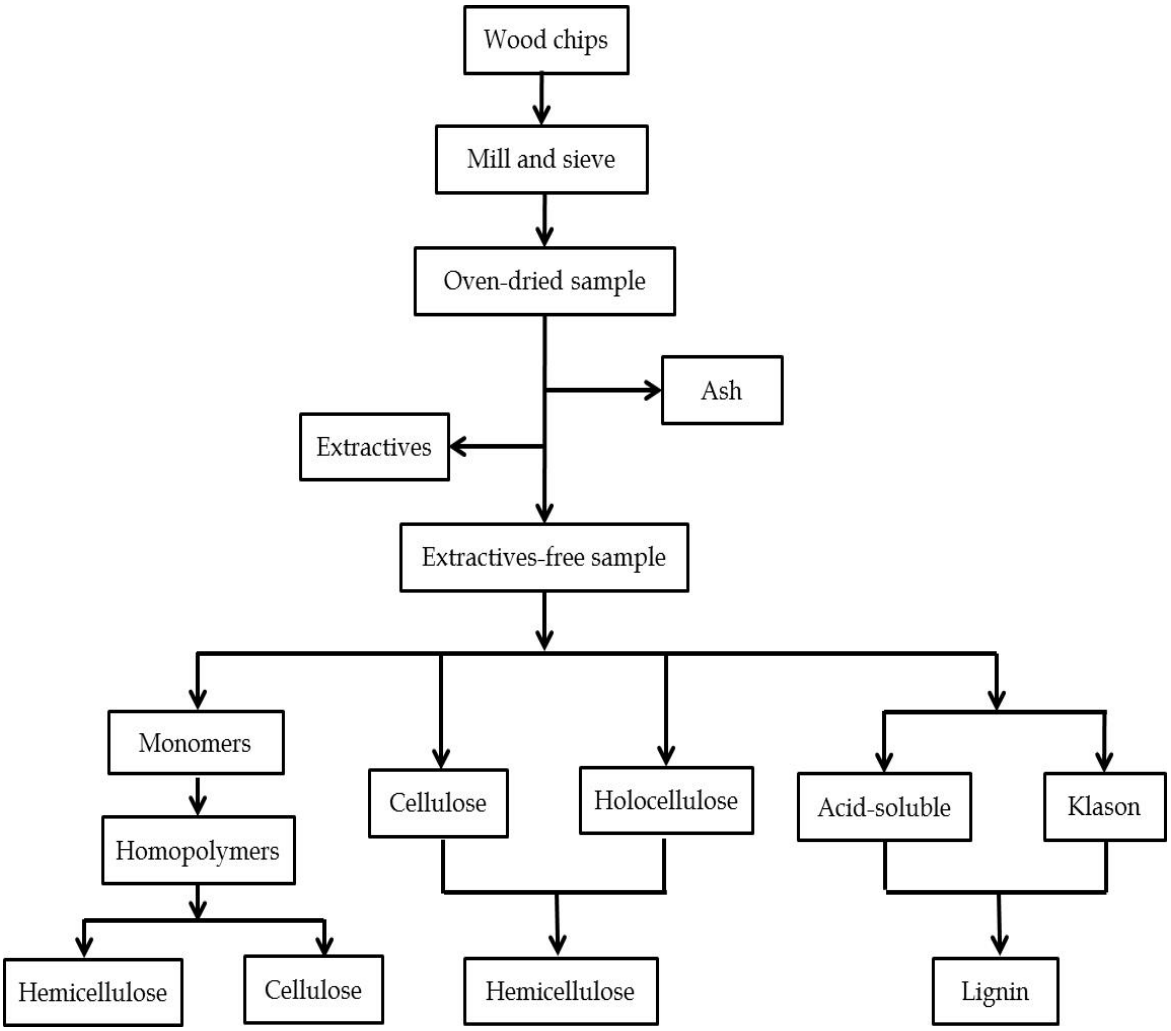

Fig. 2.1 *Chemical characterisation of Eucalyptus globulus hardwood.*

2.1.1 Wood conditioning and extractive removal

Eucalyptus globulus chips (15-30 mm length and 2-4 mm thickness) were supplied by the pulp mill and used afterwards for the analysis and the cooking trials. Sample conditioning includes mill, sieve, and dry before extractive removal. The whole description is included within TAPPI T257 cm-02 (TAPPI, 2002-a) standard. The samples were air-dried to constant moisture to the nearest 10 % w/w, milled and passed through 40 mesh sieve.

Wood was extracted with acetone in a Soxhlet apparatus according to the standard UNE EN ISO 14453 (ISO, 1999) for pulp adapted with the TAPPI T204 cm-97 (1997) standard for its use with wood. This permits the use of acetone instead of using hazardous solvents like dichloromethane or ethanol (1:3)-benzene (2:3). The aim of this analysis is to remove organic solvent-soluble extractives that might interfere in the subsequent analysis. 2±0.1 g of moisture-free wood was weighed in a tared extraction timble (10 g to the nearest 0.01 g in the case of pulp) and was placed it in the Soxhlet apparatus. Heaters should be adjusted to provide a boiling rate which will cycle the specimens for no less than 24 extractions over a 4-5 h period.

2.1.2 Holocellulose

Holocellulose, which represents the total carbohydrate content, sum of cellulose and hemicelluloses, was measured by means of the Wise chlorite method (Haykiri-Acma et al., 2014; Wahab et al., 2013). The holocellulose content was determined through treatment with an acidified sodium chlorite water solution in acidic medium (by adding several drops of acetic acid) at 70-80 ºC for 1 h. The process was repeated until the product became white. Working with *Eucalyptus globulus* samples are bleached after three times.

2.1.3 Cellulose

The cellulose content was determined using the Seifert procedure boiling a mixture of acetylacetone-dioxane-hydrochloric acid (Wright and Wallis, 1998). Two grams of free-extractive sawdust were mixed with 12 mL of acetylacetone, 4mL of 1,4-dioxane and 3mL of commercial HCl (37 % w/w). Then the mixture was heated and boiled for 35 minutes. Once the flask was warmed, the content was filtered using a

porous cresol, taking the whole flask content with methanol and then washing with distilled water.

Some authors (Jani and Rushdan, 2014; Rabemanolontsoa and Saka, 2012) used the TAPPI T203 cm-99 standard, which is only applicable for unbleached and semi-bleached pulps, on wood samples after making the holocellulose analysis to calculate afterwards the hemicellulose content by subtracting the weight of alpha-cellulose from that of holocellulose. However, the Seifert method is directly applicable to samples with high lignin content which is the case of *Eucalyptus globulus* studied in this dissertation.

2.1.4 Hemicellulose

Hemicellulose was calculated as the difference between holocellulose (Wise method) and Seifert cellulose. Another way to determine the hemicellulose content is the summative analysis taking into account all the monomers derived from hemicellulose, sugars and other decomposition products. In order to quantify all of the monomer, a standardised two-step acid hydrolysis method, T249 cm-00 (TAPPI, 2000-a) followed by chromatography analysis (see the internal procedure described in section *2.5.2*) and using stoichiometric factors calculations (see the internal procedure described in section *2.5.3*) was carried out.

2.1.5 Lignin

Acid-insoluble and soluble lignin (Klason lignin) were determined by using T222 om-02 method (TAPPI, 2002-d). Soluble-lignin and insoluble-lignin were extensively developed by Ehrman (1996) and Templeton and Ehrman (1995) respectively. The moisture content of the sample was determined for air-dried wood and used to weigh a known amount of wood. Concentrated sulphuric acid was utilised to hydrolyse and solubilise the carbohydrates in woody samples. The acid-insoluble lignin was filtered, dried and weighed. The acid-soluble lignin can be determined after filtering off the insoluble-lignin by a spectrophotometric method based on absorption of ultraviolet radiation at 205 nm.

2.1.6 Ash and moisture content

Ashes at 525 ºC were analysed by the T211 om-02 standard (TAPPI, 2002-c). The moisture content of pulpwood procedures was developed by means of the T264 cm-97 standard (TAPPI, 1997-b). Moisture was determined by the difference in weight as received and after drying 2 g to the nearest 0.001 g of specimen at 105 ± 3 ºC for 2 h in an oven. The specimen was cooled in a dessicator and placed in a climate chamber with a constant atmosphere in order to minimise changes in the moisture during subsequent handling and weighing operations.

2.1.7 Sugar and other decomposition products content

Individual carbohydrates, sugars and other decomposition products were determined by means of acid hydrolysis standardised method T249 cm-00 standard (TAPPI, 2000-a) and quantified by chromatographic internal procedures. The complete methodology is described in section *2.5.2*. Hemicellulose and cellulose from their individual monomers were calculated stoichiometrically (Alves et al., 2010; Santana et al., 2007; Kaar et al., 1991) as is described in section *2.5.3*.

2.2 Dissolving pulp analysis

A summary of the analytical procedures of dissolving pulp chemical characterisation is shown in Figure 2.2. As can be seen in the figure, there are two different pathways for determining the pulp carbohydrates in cellulose and hemicellulose, one of them based on the total cellulose and hemicellulose content and the other one based on the sum of all of the individual carbohydrates by HPLC.

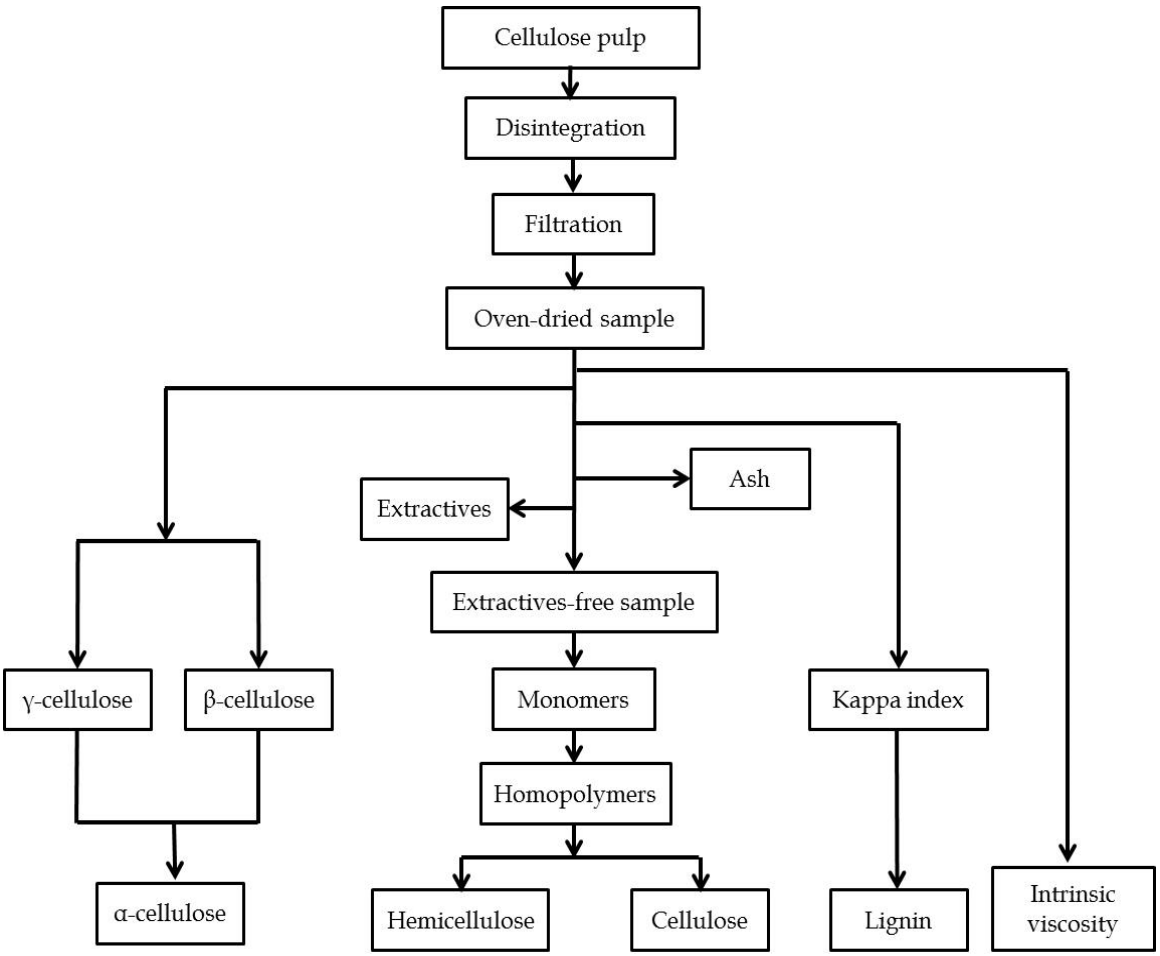

Fig. 2.2 *Chemical characterisation of dissolving cellulose pulp.*

Ash, extractives and moisture content were calculated in the same way as was explained before for wood samples. The only particularity relies on pulp sample preparation and conditioning.

2.2.1 Pulp conditioning for laboratory testing

Pulp handsheets, which are the beginning for all the subsequent analysis, are prepared as is contemplated in the standard T205 sp-02 (TAPPI, 2002-b). This step is necessary before making the physico-chemical tests. Cellulose pulp is disintegrated into a rotary stirrer to homogenise the sample, filtered and oven dried at 105 ºC. Pulp handsheets should be prepared with a Buchner filter. Handsheets were air dried until constant moisture. Afterwards, the sheets will be disintegrated for further chemical analysis.

2.2.2 Intrinsic Viscosity

The degree of polymerisation and consequently the degradation of carbohydrate chains were determined with the standard ISO 5351:2010 (ISO, 2010) also collected in the standard T230 om-99 (TAPPI, 1999-b). 0.25 g. oven-dried pulp is solvated with curpiethylendiamine solution and passed through Cannon-Fenske 150 viscometers at 25 ºC. The viscosity was converted to the degree of polymerisation (equation 9).

$$D.P. = -449.6+598.4 \ln[\eta]+118.02(\ln[\eta])^2 \quad \text{(eq.9)}$$

Where D.P. is the degree of polymerisation; η is the viscosity in cP measured according to TAPPI T230 om-89.

2.2.3 Alpha, beta and gamma cellulose

Cellulose was determined according to T203 cm-99 (TAPPI, 1999-c). An air-dried sample was weighed to an equal of 1.50 g of oven-dried sample to the nearest 0.10 mg. Pulp was consecutively extracted with 17.5 % and 9.45 % NaOH solutions at 25 ºC. The soluble fraction, consisting of β- cellulose and γ-cellulose, is determined volumetrically by oxidation with potassium dichromate. Furthermore, the α-cellulose is the insoluble fraction derived by difference.

2.2.4 Kappa Index

The kappa number estimates the amount of lignin by measuring the oxidant demand of the pulp. Kappa number methodology was performed on air-dried pulp samples, which were dried on a thermobalance to acquire their oven-dried weight.

Following the instructions dictated by T236 om-99 (TAPPI, 1999-a), the pulp was treated with $KMnO_4$ by titration. Air-dried pulp sheets were disintegrated in 500 mL distilled water. The disintegrated test specimen was transferred to a 200 mL reaction beaker and rinsed with distilled water to bring the total volume to 795 mL. The beaker was placed in a constant temperature bath so that the reaction temperature stayed at 25±0.2 ^{o}C. Continuously stir the suspension, pipet 100±0.1 mL of $5 \cdot 10^{-4}$ N $KMnO_4$ and thereafter add 100 mL of 4 N H_2SO_4. At the end of exactly 10 minutes the reaction was stopped by adding 20 mL of 1 N KI from a graduated cylinder. Immediately without filtering out the fibres, the free iodine with 0.2 N $Na_2S_2O_3$ was titrated, adding a few drops of the starch indicator toward the end of the reaction. The lignin content (% on weight) was calculated by multiplying the kappa number by 0.17 as was reported by Syverud et al. (1999).

2.2.5 Micro Kappa Index

Syverud et al. (1999) established a general rule in which the kappa analysis can be applied for crude pulps or those pulps with kappa numbers above 5. TAPPI UM 246:1991(TAPPI, 1991) method should be implemented in order to obtain the micro kappa number of high-purity pulps like for instance bleached pulps with high cellulose content and consequently low kappa numbers (below 5). This method uses 1/6 of the chemicals and sample volume versus the normal kappa method, using a water bath during the titration to allow for more accurate kappa number determination.

2.2.6 Sugar and other decomposition products content

The methodology is the same as described for wood samples in section *2.1.7*. Individual sugars and other decomposition products were determined by means of acid hydrolysis standardised method T249 cm-00 standard (TAPPI, 2000-a) and quantified by chromatographic internal procedures. The complete methodology is described in section *2.5.2*. Hemicellulose and cellulose from their individual monomers were calculated stoichiometrically (Alves et al., 2010; Santana et al., 2007; Kaar et al., 1991) as is described in section *2.5.3*.

2.3 Hydrolysate analysis

In this section the methodology of the physico-chemical characterisation of lignocellulosic hydrolysates will be explained. Lignosulphonates and hydroxyl groups (UV-Vis), cellulose and hemicellulose monomers (HPLC), metals (AA), total phenolic content, total and reducing sugars and antioxidant activity were analysed spectrophotometrically. In addition, FTIR spectroscopy was performed. Other properties such as suspended solids, pH, ashes at 525 and 900 ºC, density, dry matter, total, combined and free SO_2 were also determined.

2.3.1 Lignosulphonates and hydroxyl groups

Lignosulphonates (LS) and hydroxyl groups (OH) were determined in triplicate following the standard UNE EN 16109 (AENOR, 2012). The spectrophotometer UV-Vis (Lambda 25, Perkin Elmer) was used for LS and OH quantification.

The starting point calculating either LS or OH involves the preparation of a solution containing between 0.15 and 0.20 g dry hydrolysate matter and dilute to 250 mL with distilled water in order to have a clear solution with LS content between 0.6 and 0.8 g/L (*solution 1*).

Thereafter 3 mL of *solution 1* was placed into a 100mL beaker; approximately 60 mL of distilled water was added. The pH of the solution was adjusted between 4 and 5 by adding 0.125 M NaOH or 0.2 M HCl. 100mL volumetric flask was used and the volume was made up with distilled water (*solution 2*).

Afterwards sample and reference were filled in quartz cuvettes. The sample cell was filled with *solution 2* and the reference with distilled water. The absorbance value was recorded at 232.5 nm. Finally the LS content was calculated in equation 11:

$$LS(\%) = \frac{Abs(232.5nm)\cdot D}{f\cdot P(g)\cdot 10} = \frac{Abs(232.5nm)\cdot 100\cdot 250}{f\cdot P(g)\cdot 30} \qquad \text{(eq.11)}$$

Where f is a factor which is 35.2 for calcium LS; P is the sample weight in grams; D is the dilution factor in mL; Abs(273nm) is the absorbance of solution 2 at 232.5 nm.

To calculate the phenolic hydroxyl groups, 5 mL of *solution 1* were transferred to a 50 mL volume flask, the pH was adjusted above 11 using 10 mL of 5 M NaOH and

brought to volume with distilled water (*solution 3*). Besides, one more solution had to be prepared. It was named *solution 4* and was made by taking a part of *solution 1* and adjusted to pH 2-2.2 with a few drops of HCl 5 M. A 5mL aliquot was put into a 50mL volume flask and made up to the volume with distilled water.

The sample cell was filled with *solution 3* and the reference cell with *solution 4*. The last step was to scan the solution from 220 to 340 nm, and to record the absorbance at 255 nm and the minimum valley between 220 and 340 nm. Thus, the content of phenolic hydroxyl groups (g/100g dry sample) of the liquor was calculated according to equation 12.

$$OH_{phenolics}(\%) = 0.192 \cdot \frac{Abs(255nm) - Abs_{MIN}(340-220nm)}{Conc(g/L)} \qquad \text{(eq.12)}$$

Where Conc (g/L) is the value of dry lignosulphonates concentration in 50 mL flasks solutions, Abs(255nm) is the absorbance peak value of about 255 nm and Abs_{MIN}(340-220nm) the minimum valley to either side.

2.3.2 Folin Ciocalteau Method

The Folin-Ciocalteu method is widely used to measure the total phenolic content (TPC). The reaction is based on the reduction of the Folin-Ciocalteu reagent, which is a mixture of phosphotungstic acid, $H_3PW_{12}O_{40}$, and phosphomolybdic acid, $H_3PMo_{12}O_{40}$, producing a blue coloration with maximum absorbance at 765nm. The procedure is same as was proposed by Faustino et al. (2010).

TPC was determined spectrophotometrically according to the Folin-Ciocalteu reagent assay (Waterhouse, 2002), using gallic acid as standard. Briefly, 50 µL of diluted extracts or standard solutions of gallic acid were added to a test tube containing 450 µL distilled water. After the addition of 250 µL of 0.2 N Folin-Ciocalteu reagent, the mixture was stirred for 1 min and was allowed to stand for 8 min. Then, 2.0 mL of an aqueous solution of Na_2CO_3 (7.5 % w/v) were added and the mixture was incubated for 1.5 h at 30 °C. The absorbance relative to that of blank prepared using distilled water was measured at 765 nm using a double-beam UV-Vis spectrophotometer (Jasco V-530). The concentration of TPC in the methanolic extracts was determined as mg gallic acid equivalents/g dry weight by using the regression equation that was obtained from the standard curve of gallic acid plot. All determinations were performed four times.

The TPC method by means of Folin Ciocalteau is only applied to the liquid extracts. TPC of the hydrolysate could not be determined by means of Folin Ciocalteu (Waterhouse, 2002) because of the dark colour of the original sample. In this case, the content of phenolic hydroxyl groups was calculated following the UNE EN 1609 standard (AENOR, 2012).

Phenolic recovery was calculated according to equation 13, taking into account the concentration of the hydroxyl groups in the sample and the total phenolic content determined by Folin-Ciocalteu method of the extracts:

$$E._{phenolics}(\%) = \frac{\left[TPC\left(g.GAE/L\right)\right]_{extract}}{\left[OH_{phenolics}\left(g/L\right)\right]_{SSL}} \cdot 100 \qquad \text{(eq.13)}$$

Where TPC (g.GAE/L) represents the total phenolic concentration extracted per litre of sample and $OH_{phenolics}$ (g/L) corresponds to the total hydroxyl group concentration of the sample.

2.3.3 DPPH• radical scavenging method

Antioxidant activity can be attributed to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of hydrogen abstraction, and radical scavenging (Moure et al., 2005). For this reason, there are different analytical procedures to determine the antioxidant capacity in pure standards, plants or food extracts (Prior et al., 2005).

Among the techniques determining the antioxidant capacity of pure standards or extracts, in this thesis the antioxidant activity index was determined by means of DPPH• (2,2-diphenyl-1-picrylhydrazyl) scavenging radical method (Scherer and Godoy, 2009; Brand-Williams et al., 1995). This method is used in the quantification of free radical scavenging activity based on colour changes and it has been employed in many studies (Tirzitis and Bartosz, 2010). The odd electron of the nitrogen atom in DPPH• is reduced by receiving a hydrogen atom from antioxidant compounds and losing the characteristic deep purple colour. DPPH• is characterised as a stable free radical with a maximum absorption at about 517 nm. When a solution of DPPH• is mixed with an antioxidant this gives the reduced form with the loss of its colour.

A stock solution of DPPH• (31.6 µg/mL) was prepared in aqueous methanol (70:30 v/v). 0.1 mL of sample was added to 3.9 mL of the DPPH• solution. The mixture was then shaken vigorously and allowed to stand at room temperature in the dark. After 90 min, the decrease in absorbance at 517 nm was measured against a blank (aqueous methanol solution), by using a double–beam UV-Vis spectrophotometer (Jasco V-530, Tokyo, Japan). A mixture consisting of 0.1 mL of aqueous methanol (70:30 v/v) and 3.9 mL of DPPH• solution was used as a control. The radical stock solutions were freshly prepared every day, stored in a flask covered with aluminium foil and kept in the dark. The radical scavenging activities of the samples, expressed as percentage inhibition of DPPH•, were calculated according to equation 14.

$$Inhibition.(\%) = 100 \cdot \frac{A_B - A_A}{A_B} \qquad \text{(eq.14)}$$

The antioxidant activity expressed as the antioxidant activity index (AAI) is calculated according to equation 15.

$$AAI = \frac{\left[Final\_DPPH\left(\mu g / mL \right) \right]_{blank}}{IC_{50}\left(\mu g / mL \right)} \cdot 100 \qquad \text{(eq.15)}$$

A_B and A_A are the absorbance values of control and test samples. Inhibition (%) or IC_{50} corresponds to the minimum extract concentration required to decrease the initial absorbance of the DPPH• solution by 50 %.

Gallic acid was used as standard giving AAI of 26.69 ± 0.27. Similar values were found in literature in the range of 25.46 and 27.15 (Scherer and Godoy, 2009). Comparable results were exhibited by Faustino et al. (2010) giving AAI of 30.28.

2.3.4 Dissolved metals by Flame Atomic Absorption

Calcium, magnesium, sodium, potassium and iron were determined using flame atomic absorption spectroscopy AA-7000, Shimadzu (IZASA, Spain). Individual lamps (one for each metal) were run under the spectral bandwidth and wavelengths are displayed in Table 2.2. The fuel was a mixture of synthetic air and acetylene. Flow rate and the burner height were automatically adjusted in order to get the maximum absorbance signal. Calibration curves were prepared following Table 2.2 of metal standards preparation giving regression factors in the range of 0.9993 and 0.9998. Every standard is prepared in ultrapure water using 4 mL of commercial HCl

(Panreac, Spain) and 4 mL of a releasing agent which inhibits noise signals in the spectra. Releasing agents and standards were supplied by Panreac (Barcelona, Spain).

Table 2.2 *Preparation of the metal standards calibration curves.*

Metal	Wavelength	Slitwidth	Standards	Releasing agent	Acidic medium	Make up to volume
Ca	422.7 nm	0.5 nm	0.5; 1; 2, 5; 10; 50 ppm	4mL of 210 g/L $LaCl_3$	4mL of HCl 37 % w/w	ultrapure water
Mg	285.2 nm	0.5 nm	0.1; 0.2; 0.4; 0.8; 1 ppm	4mL of 99.77 g/L $SrCl_2$		
Na	589.0 nm	0.5 nm	0.1; 0.2; 0.4; 0.8; 1 ppm	4mL of 27.9 g/L KCl		
K	766.5 nm	1.0 nm	0.2; 0.4; 0.8; 1; 4 ppm	4mL of 63 g/L CsCl		
Fe	248.3 nm	0.2 nm	0.5; 1; 2; 10; 50 ppm	4mL of 55.01g/L $CaCl_2$		

Dilution of the hydrolysate depends on the stage of the process where it is collected. Different samples were collected from the pulp mill at the end of digestion stage or after passing through the evaporation plant. Such samples are named respectively weak spent sulphite liquor (WSSL) and thick spent sulphite liquor (TSSL). Synthetic liquors obtained in the laboratory were also measured. Dilutions also vary as a function of the metal which is of interest: (i) WSSL is 50 times diluted for Ca and Fe analysis, 100 times diluted for Na and K and 2000 times diluted for Mg analysis; (ii) TSSL is 200 times diluted for Ca and Fe, 500 times for Na and K and 10000for Mg. Sample solutions were prepared using 25 µL of releasing agent per 25 mL of sample and ultrapure water. As the pH was acid, addition of hydrochloric acid was not required. Dilution of detoxified samples is quite different. It depends on the experimental assay varying from 1:50 to 1:5 for Ca and from 1:25 to 1:1000 for Mg.

2.3.5 FTIR analysis for functional groups detection

Fourier transform infrared (FTIR) spectroscopy has been used as a simple technique for obtaining rapid information about the structure of constituents and chemical changes taking place in woody samples due to various treatments. Contrary to conventional chemical analysis, this technique requires small sample sizes and short analysis time (Chen et al., 2010). Infrared spectra were recorded on a Shimadzu

Fourier transform Infrared (FTIR) spectrophotometer FTIR-8400S equipped with attenuated total reflectance (ATR) made by Pike Technologies. Spectra were recorded in the range of 4000-400 cm^{-1}, and were measured with a resolution of $4cm^{-1}$, taking 32 scans per sample. Because of the ATR apparatus, samples were not conditioned e.g. KBr pellet or polyethylene card methods. Solid and liquid samples were placed in the ATR and directly the FTIR generated the spectrum under the conditions highlighted above.

2.3.6 Total and reducing sugars

Total sugars were determined by using the phenol-sulphuric method. Samples were treated with concentrated sulphuric acid and phenol, measuring intensity of orange at 490 nm to determine pentoses and hexoses according to Durmuş et al. (2002).

The measurement method of reducing sugars is based on the determination of the reducing groups of each monosaccharide and the reducing ends of the chains of oligo- and polysaccharides present in the liquor samples. Reaction between 3,5-dinitrosalicylic (DNS) acid and samples is quantified by measuring absorbance at 530 nm in accordance with the results of Wang et al. (2011-a).

2.3.7 Other physico-chemical parameters

The pH was measured by a pH meter Crison Kit 2012T with electrode 5012T.

Total suspended solids were determined gravimetrically, filtering 10 mL SSL aliquots using Millipore membrane filters of diameter 47 mm and pore size of 0.45 μm, dried at 105±3 ºC for 24 h (Clesceri et al., 1998).

Ash content was determined at 525 and 900 ºC by placing samples in a muffle furnace at 525±25 ºC for 30-60 minutes (TAPPI, 2002-c) and at 900±25 ºC for 30-60 minutes (TAPPI, 2002-e). After ignition, it was cooled slightly and then placed in a desiccator, containing indicating-grade anhydrous alumina. When cooled to room temperature, the ignited crucible was weighed on the analytical balance to the nearest 0.1 mg.

Density and viscosity at 20 ºC were determined using a glass picnometer and a rotational viscometer Fungilab Alpha L series.

Dry matter was measured by weighing approximately 2 g of the wet hydrolysate and then drying the samples at 105±3 ºC using a Selecta muffle. After drying, samples were carefully cooled in a desiccator. Moisture calculations and methodology were the same for wood and pulp samples according to the standard T264 cm-97 (TAPPI, 1997-b).

Free, total and combined SO_2 in the hydrolysates were determined by the iodine volumetric titration described in T604 om-00 (TAPPI, 2000-b). The first step was to prepare 100 mL of 1:10 sample solution using distilled water. Taking 10 mL of the aliquot and transfering into an erlenmeyer with a few drops of indicator (starch), and few drops of ethylic alcohol was the next step. Titration with 10 N iodine until dark blue colour was required. The following step was to add several drops of sodium thiosulphate till decolouration, and drops of methyl red indicator to titrate with NaOH 10 N. Thus, calculations of total, free and combined SO_2 are shown in equations 16 to 18:

$$SO_2(\%)tot = 0.32 \cdot V \quad \text{(eq.16)}$$

$$SO_2(\%)free = 0.32 \cdot (V_1 - V) \quad \text{(eq.17)}$$

$$SO_2\,(\%)comb = SO_2(\%)tot - SO_2(\%)free \quad \text{(eq.18)}$$

Where SO_2 (%) tot is the total SO_2 content of the cooking liquor as grams of SO_2 per 100 mL of solution and expressed as a percentage; SO_2 (%) free is the SO_2 in the form of sulphurous acid H_2SO_3 or $SO_2(H_2O)_x$, plus one-half the SO_2 combined as bisulphites, also referred to as available SO_2; SO_2 (%) comb is the SO_2 combined with the base determined by the difference between the total SO_2 and the free SO_2. The volume of iodine used is V whereas the volume of soda is V_1.

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2.4 Analysis of carbohydrates and lignin derivatives by HPLC

2.4.1 Carbohydrate quantification by HPLC/RID

Monosaccharides, aliphatic acids and furan aldehydes were determined by means of HPLC/RID technique. Four chromatographic methods were developed. The state of the art of the main chromatography techniques for LCB characterisation is described in the results chapter in section *3.2.1*. Details regarding operating conditions and column assayed and methods validation are also explained within section *3.2.1*.

The HPLC system used is a Shimadzu Prominence LGE-UV equipped with a control system CMB-20A, an inline degasser channel DGU-20-A5, an isocratic pump LC20AD, and auto sampler SIL-20AHT with thermostatic cooling (samples held at 4ºC), a column oven CTO-20ASVP and a refractive index detector RID-10A.

The methods developed were succesfuly applied within different LCB samples: wood, pulp and bleached pulps (Llano et al., 2015-a); detoxified liquors (Llano et al., 2015-b; Fernandez-Rodríguez et al., 2015), weak and thick industrial liquors (Rueda et al., 2015-a), synthetic sulphite spent liquors (Llano et al., 2013); paper and dissolving grade liquors (Llano et al., 2012; Quijorna et al., 2011); or hydrolysates from the kinetic study (Rueda et al., 2015-b).

The steps involved in pulp and wood samples depolymerisation, hydrolysis and sugar quantification are widely described in section *3.2.2*. Two-step acid hydrolysis in accordance with the T249 cm-00 standard (TAPPI, 2000-a) followed by HPLC analysis and stoichiometric calculations (Santana et al., 2007; Kaar et al., 1991) were carried out.

Briefly, the main steps regarding qualitative and quantitative total carbohydrate content (TCC) determination by HPLC techniques of solid biomass or liquid hydrolysates are shown in Figure 2.3.

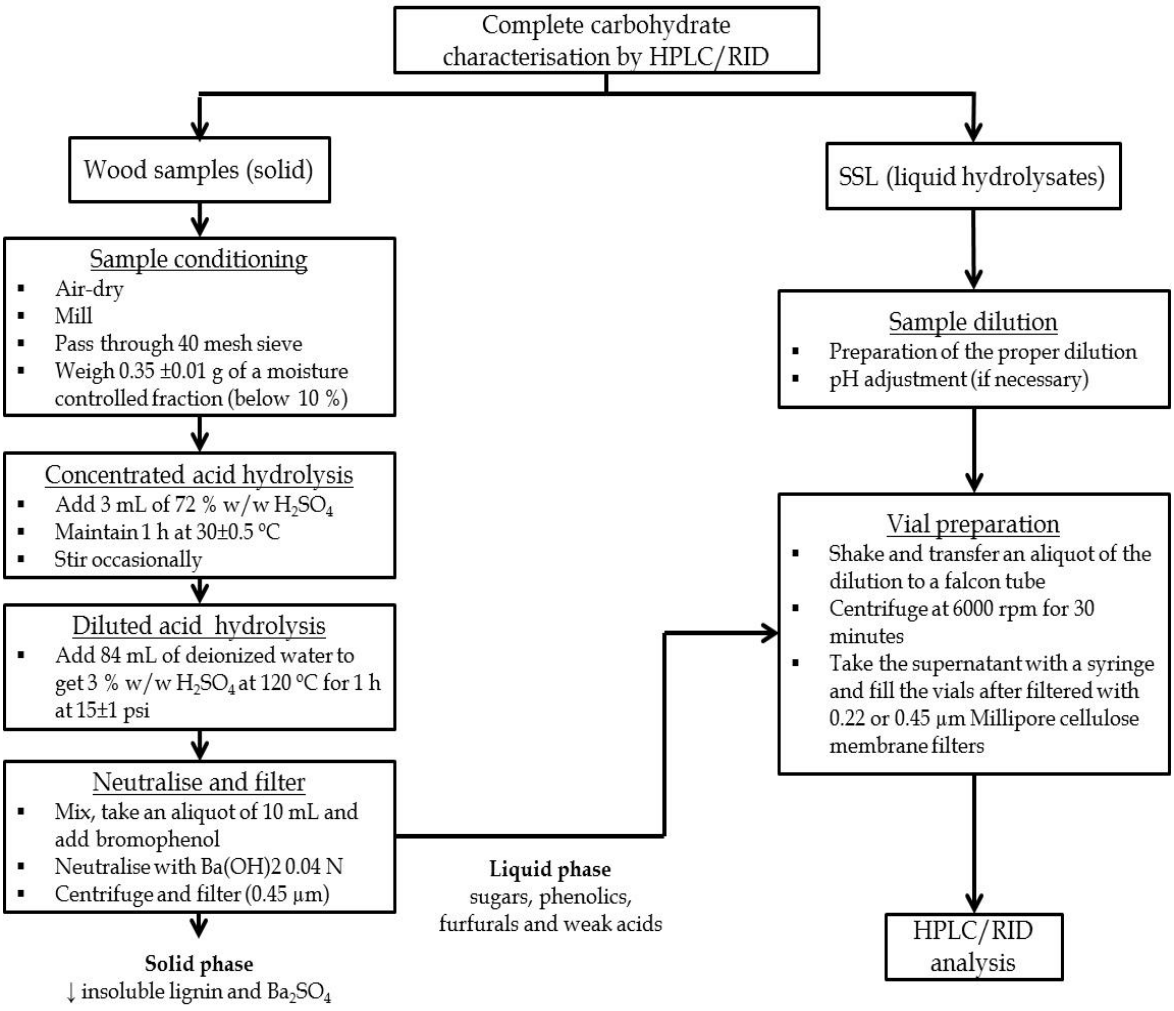


Fig. 2.3 *Stages that should be implemented working with solid biomass (left) or liquid hydroysates (right).*

2.4.2 Total carbohydrate content by means of summative analysis

Each monomer can be reported in the summative analysis as its pure theoretical homopolymer (Kaar et al., 1991). The weight of each constituent, determined quantitatively after the hydrolysis, has to be multiplied by a factor to calculate its contribution to the original wood component (as a theoretical homopolymer). Calculations were made by using the stoichiometric factors obtained in literature (Alves et al., 2010; Santana et al., 2007; Kaar et al., 1991). These factors consist of molecular mass of anhydrous unit divided by molecular mass of the isolated substance. Table 2.3 shows all of the conversion factors used in this work. It should

be noted that carbohydrate summative analysis calculations are based on models previously applied in wood samples (Santana et al., 2007; Kaar et al., 1991).

The percentage of each component in the extractives-free hydrolysates was obtained as follows in equations 19 and 20.

$$C^* = C_{HPLC} \cdot D_f = C_{HPLC} \cdot \frac{V_{Ba(OH)_2}+10}{10} \quad \text{(eq.19)}$$

$$C_w = \frac{C^* \cdot V_F}{P_S \cdot 1000} \cdot 100 \quad \text{(eq.20)}$$

C^* (g/L) = Real concentration of the component in the hydrolysate.
C_{HPLC} (g/L) = Concentration of the component giving by the HPLC peak integration.
$VBa(OH)_2$ (mL) = volume of barium hydroxide added into the hydrolysate.
Df = Dilution factor.
Cw (% w/w) = Concentration of the component referred to the dry weight of the woody hydrolysate.
VF (mL) = final volume of the hydrolysate.
PS (g) = dry weight of the initial woody sample, before hydrolysis.

To calculate the concentration of each homopolymer from their monomers according to Table 2.3, not only the monosaccharides but also the degraded-compounds derived from carbohydrates were considered; e.g. cellulose is the sum of cellobiose, glucose, HMF and levulinic acid multiplied by their stoichiometric factors.

The individual contribution of carbohydrate-derived compounds to the final cellulose or hemicellulose content depends on the chemical structure of the macromolecules forming the cell wall. In this work, all the glucose is assumed to generate from the cellulose (Kaar et al., 1991). Simultaneously, it was also assumed that formic acid is an inhibitor mostly produced from pentose sugars, the formation of formic from hexoses being a remainder against the levulinic acid conversion (Hayes et al., 2006). Acetic acid which is the major weak acid is not associated with pentoses or hexoses because it is considered a co-product (secondary reaction) from hydrolysis formed at the same time as the monosaccharides by the degradation of acetyl groups presented in polysaccharides (Rueda et al., 2015-b). Once the concentration of the main polymers is obtained, the macrocomponents and the total

mass balance of the sample were calculated considering the stoichiometric factors of Table 2.3.

Table 2.3 *Stoichiometric factors used to calculate the percentage of theoretical homopolymer.*

Hydrolysed constituents	Homo-polymer	Carbohydrate contribution	Conversion factor	Ethanol factor g.EtOH/ g.monomer
Glucose	glucan	cellulose	162/180	0.511
HMF*	glucan	cellulose	162/126	-
Levulinic acid	glucan	cellulose	162/116	-
Cellobiose	glucan	cellulose	324/342	-
Xylose	xylan	hemicellulose	132/150	0.511
Furfural	xylan	hemicellulose	132/96	-
Formic acid	xylan	hemicellulose	132/46	-
Arabinose	arabinan	hemicellulose	132/150	0.511
Galactose	galactan	hemicellulose	162/180	0.511
Mannose	mannan	hemicellulose	162/180	0.511
Acetic acid	acetyl	hemicellulose	43/60	-

2.4.3 Weak phenolics in the Spent Sulphite Liquor by HPLC/DAD

The individual phenolic method was developed by the Agricultural University of Athens and all the liquor samples and extracts were analysed within the laboratory of engineering food science and technology of this institution.

Purified phenolics are applied to an HPLC Jasco instrument utilising a reversed phase C18 column, Waters Nova-Pack C18 column (3.9 mm x 150 mm, 4 μm), multiwavelength detector (MD-910 Jasco), quaternary pump (PU-2089) and autosampler Jasco AS-1555.

Polar acidified organic solvents, in this case acetonitrile and methanol, are the dominant mobile phases. It is recommended to maintain the pH of the mobile phase in the range of 2-4 to avoid the ionisation of phenolics during the identification. The mobile phase consisted of water with 0.1 % v/v perchloric acid (solvent A), and pure methanol HPLC grade (solvent B). The gradient used was: 100 % A 0-5 min, 90 % A/ 10 % B 5-15 min, 82.5 % A/ 17.5 % B 15-25 min, 75 % A / 25 % B 25-45 min, 40 %A/ 60 % B 45-60 min, 100 % B 60-80 min, 100 % A 80-85 min. The flow rate was 1 mL/min and the injection volume was 20 μL. This method was previously reported

by Guendez et al. (2005) with modifications from methodology employed by Proestos et al. (2006). Two wavelengths, 280 and 320 nm were monitored. The identification of each compound was based on a combination of retention time and spectral matching, since polyphenols absorb in the ultraviolet (UV) region. Most benzoic acid derivatives show an absorption maximum at 246-262 nm with a shoulder at 290-315 nm, except gallic acid showing a maximum in the 240-285 nm range (Jönsson et al., 2013).

Standards in the range of 10 to 130 mg/L were prepared in methanol HPLC quality. Caffeic, syringic, gallic, vanillic, ellagic, rosmarinic and ferulic acids, rutin, syringaldehyde, lariciresinol, quercetin, 4-hydroxybenzaldehyde, 2-hydroxycinnamic acid, guaiacol, 1,2-dihydroxybenzene, naringenin, isorhamnetin, and luteolin were determined (see Figure 2.4).

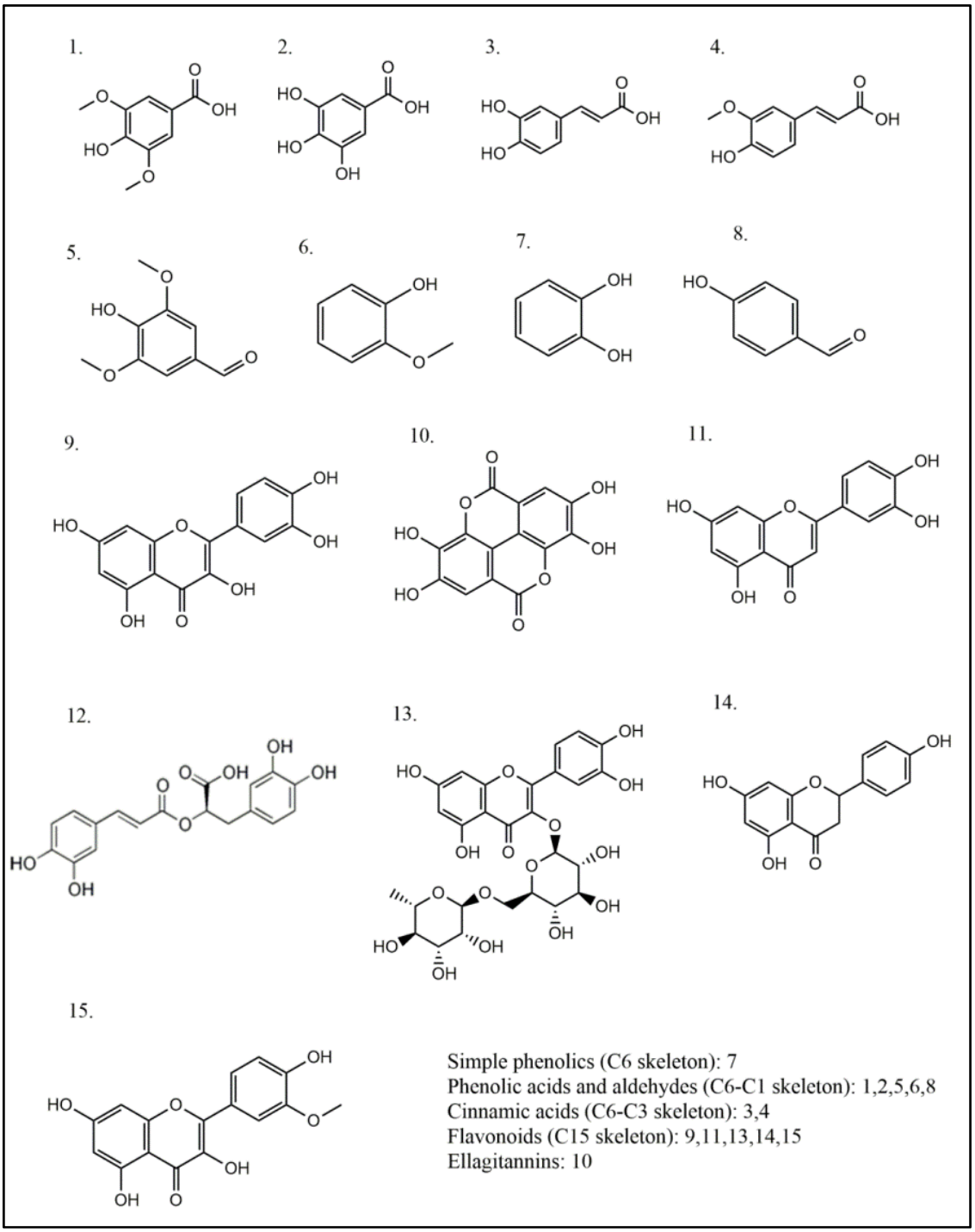


Fig. 2.4 *Structure of low molecular weight phenolics appeared in the extracts: [1]syringic acid, [2]gallic acid, [3]caffeic acid, [4]ferulic acid, [5]syringaldehyde, [6]guaiacol, [7]1,2-dihydroxybenzene, [8]4-hydroxybenzaldehyde, [9]quercetin, [10]ellagic acid, [11]luteolin, [12]rosmarinic acid, [13]rutin, [14]naringenin, [15]isorhamnetin.*

2.5 Cooking trials at laboratory scale

Some digestion trials at laboratory scale were done with the purpose of studying the operational variables affecting the final quality of pulp and the hydrolysate composition. Cooking tests were carried out in two 1-L stainless steel vessels with temperature and pressure control (see Figure 2.5). The control system of the laboratory digesters reproduces the industrial process ramps from acid pre-heating to degasification and cooling stages. The end point is crucial to obtain high quality pulp. For this reason, digesters are also equipped with a liquid sampler to extract liquor fractions to control the change of the colour to determine the end cooking point. This behaviour is due to the presence of furfural to predict the end of delignification (Sixta, 2006). After each experiment the hydrolysate and the cellulose pulp were analysed in terms of sugars, LS, furfurals, acids and kappa index, viscosity and alpha-cellulose content respectively.

Fig. 2.5 *Laboratory digesters used to make the cooking trials.*

The process is as follows: wood and fresh cooking liquor are loaded into the digesters using a liquid/solid ratio of 4:1 L/Kg. Then, the reactors are presurised using argon gas until 7 bar of pressure and the heating ramp until desired constant temperature is set (confidential data). Pressure is maintained constant along the process by an automatic system of pressure control.

Once the whole digestion cycle finished, heating is stopped and the pressure progressively releaved. Finally, the digesters are discharged at atmospheric pressure.

2.6 External Acid Hydrolysis of spent sulphite liquor

Variables affecting hydrolysis were studied, covering a wide range of process conditions. Based on the literature review, sulphite liquor was hydrolysed at different temperatures, residence times, acid concentrations and acid-to-liquid ratios. Concentrated-acid and diluted-acid hydrolysis were carried out. The study of an external hydrolysis stage in this dissertation accomplished a double objective: (i) to check that most of the carbohydrates were in the monomeric form; and (ii) to investigate the equilibrium displacement towards toxic fermentation inhibitors. Hydrolysis tests were carried out using the liquor collected after the evaporation plant of the factory. All experiments were done in triplicate.

2.6.1 Concentrated-acid hydrolysis

Concentrated acid hydrolysis was studied with temperatures in the range of 20 to 40 ºC; sulphuric acid concentration of 64, 72 and 80 % w/w. Ratios of 0.1 and 1 v/v were assayed at 1, 2.5 and 4 h considering previous studies (Girio et al., 2010; Roncero Vivero, 2001). A factorial design using Stat Graphics software with four experimental factors (A: temperature, °C; B: Acid/Sample ratio, v/v; C: acid concentration, g/L; and D: time, min) and three response variables (variable 1: sugars concentration, g/L; variable 2: inhibitors concentration, g/L; and variable 3: LS concentration, g/L) was made to determine hydrocarbon deconstruction capacity of the spent liquor. A total of 24 experiments and 3 central points were carried out. Table 2.4 shows the factorial design.

2.6.2 Diluted-acid hydrolysis

A factorial design was performed with 19 trials (24 experiments+3 central points) corresponding to four experimental factors and three response variables (the same as concentrated-acid hydrolysis). The experimental design was made with the Stat Graphics software. Table 2.5 shows the variables in the factorial design.

Table 2.4 *Factorial design of concentrated acid hydrolysis.*

Trial	A-factor Temperature	B-factor acid/SSL ratio	C-factor [H_2SO_4]	D-factor time
1	20	0.1	84	60
2	40	1.0	60	60
3	20	0.1	84	240
4	40	0.1	84	240
5	40	0.1	60	60
6	20	1.0	60	60
7	20	1.0	60	240
8	20	0.1	60	240
9	40	1.0	84	60
10	40	1.0	60	240
11	20	1.0	84	60
12	40	0.1	60	240
13	40	0.1	84	60
14	40	1.0	84	240
15	20	1.0	84	240
16	20	0.1	60	60
17-18-19	30	0.55	72	150

Table 2.5 *Factorial design of diluted acid hydrolysis.*

Trial	A-factor Temperature [°C]	B-factor acid/SSL ratio	C-factor [H_2SO_4]	D-factor time
1	80	10	9.5	240
2	80	10	0.5	240
3	80	0.1	0.5	240
4	80	0.1	9.5	240
5	80	10	9.5	60
6	80	0.1	0.5	60
7	80	10	0.5	60
8	80	0.1	9.5	60
9	160	10	0.5	60
10	160	0.1	9.5	240
11	160	10	0.5	240
12	160	0.1	9.5	60
13	160	10	9.5	240
14	160	0.1	0.5	240
15	160	0.1	0.5	60
16	160	10	9.5	60
17-18-19	120	5.05	5	150

2.7 Detoxification techniques of spent sulphite liquor

Different detoxification processes were carried out in this dissertation. The summary of techniques studied, the main purpose of each detoxication technique and parameters affecting such processes and studied in this dissertation are presented in Table 2.6.

Table 2.6 *Testing conditions and purpose of detoxification techniques assayed at laboratory scale.*

Technique	Aim	Tested conditions
Evaporation	Volatile compound removal	Same as industrial (confidential)
Overliming	Insoluble lignin separation	2 kinds of alkali, from 25 to 40ºC, pH from 5 to 10, 15 to 75 min
Anionic resins	LS, phenolic and furfural separation	Room T, from 5 to 120 min, 1.2 to 60 mL hydrolysate/g.wet resin
Cationic resins	Metal separation	Room T, from 5 to 120 min, 1.2 to 60 mL hydrolysate/g.wet resin
Adsorption	Phenolic and furfural separation	2 kinds of adsorbent, from 30 to 50 ºC, 1:5 and 1:10 w/v
L-L extraction	Phenolic removal	5 solvents; pH 1.5-6.5; 10-390 min; 1:1 to 1:5 (v/v); 1 to 3 stages

2.7.1 Evaporation

The industrial multiple effect evaporation plant (MEEP) was reproduced at laboratory scale in a rotary evaporator (Heidolph, LR-4000), a vacuum pump (Vacuubrand, MZ 2C NT) controlled by the vacuum controller CVC 3000. Pressure and temperature conditions, which are confidential data, were taken from the plant. Ten samples corresponding to five concentrates (bottom phase) and five condensates (light phase) of each effect were collected. Every sample was analysed in triplicate. Solid concentrations at the bottom phase were fixed according to the industrial plant; such concentrations were in every effect 10.0, 11.7, 14.4, 18.5, 27.6 and 55.3 % w/w respectively.

2.7.2 Selective precipitation by overliming

Precipitation of lignosulphonates (LS) from the hydrolysates can be achieved by overliming. The addition of an alkaline reagent was carried out in order to precipitate insoluble compounds from lignosulphonates and insoluble lignin.

The experiments were conducted in 250 mL erlenmeyer flasks. First, the hydrolysate was placed in 1-L beaker and neutralised with 2.5 M $Ca(OH)_2$ up to pH 7. Then the pH adjusted sample was distributed in the erlenmeyer flasks and maintained at a fixed temperature in the orbital incubator at 120 rpm for 30 minutes. Afterwards, samples were placed into falcon tubes and centrifuged. The supernatant was adjusted with commercial H_2SO_4 to pH 5.5. The influence of temperature, pH and the time were considered. Assays at pH 10 and 25, 30, 35 and 40 ^oC were done. Then, the pH (5, 6, 7, 8 and 9) at the best temperature (30 ^oC) was studied. Finally, a kinetic study was conducted at 15, 30, 45, 60 and 75 minutes.

2.7.3 Adsorption with black carbon and activated charcoal

Granulated activated charcoal (AC) and powered black carbon (BC) were used in this work as adsorbents. AC was provided from Panreac (Barcelona, Spain) with a particle size of 1.25-3.15 mm and a density of 1.8-2.1 g/cm^3 with a surface area of 500 m^2/g. The BC supplier was Columbian Carbon Spain S.L. BC N220 is cheaper than AC and it has a particle size between 0.044 and 0.5 mm.

Adsorption of most organic materials is higher at neutral conditions and consequently the pH of liquor samples was adjusted. SSL was neutralised at pH 7 with $Ca(OH)_2$ and adjusted to pH 5.5 with H_2SO_4. After neutralisation, batch experiments were conducted in 250 mL erlenmeyer flasks. Kinetic isotherms at 30 and 50 ^oC were constructed at adsorbent-to-SSL ratios of 1:10 and 1:5 w/v (grams of dry adsorbent per mL of neutralised-SSL). Equilibrium trials were also done varying the ratio from 1:50 to 1:5 w/v. In order to recover the adsorbent, spent carbon was washed and equilibrated sequentially with 0.4 N HCl or 0.05 M H_2SO_4, then washed with distilled water and dried at room temperature.

2.7.4 Ion-exchange resins

Detoxification of the hydrolysates with two exchange resins was conducted based on studies of Fernandes et al. (2012): cation exchange resin (Dowex 50WX2, mesh 100-200) in H^+ form and anion exchange resin (Amberlite IRA-96, mesh 20-50) in OH- form. In this sense, the cationic resin separates Ca^{+2} and Mg^{+2} while LS, phenolics and acetic acid can be mainly removed with the anionic resin.

The separation was carried out in falcon tubes (batch mode). Firstly, kinetic experiments were conducted at room temperature at 5, 15, 20, 40, 60 and 120 min. Then, equilibrium assays were run at different ratios varying from 1.2 to 60 mL hydrolysate/g.wet-resin. Resin activation was performed with 5 % w/w HCl for cation exchange resin, or with 4 % w/w NaOH for anion exchange resin. Afterwards, ultrapure water was used to wash the resin in order to get a neutral pH.

2.7.5 Liquid-Liquid extraction with organic solvents

In a separator funnel, 50 mL of each hydrolysate sample were vigorously mixed together with different volumes of solvent at room temperature (Faustino et al., 2010; Cruz et al., 2005). Then, the mixture was allowed to separate in two phases for the examined settling times. After the separation, the solvent was removed by vacuum evaporation and reutilised. The crude extracts were dried overnight in vacuum desiccators, weighed and re-dissolved in 5 mL methanol for their subsequent analysis. Once solvent extraction takes place, two different phases can be distinguished: the light phase represents the non-polar organic phase (phenolics-rich fraction) and the heavy phase in the bottom represents the aqueous phase (phenolics-free hydrolysate fraction). As a rule, this can be assumed knowing that in most cases solvent density is lower than water.

Several solvents have been used for antioxidant extraction. The activity of these compounds is closely dependent on the solvent used. Ethers and ketones are two of the most employed solvents for removing phenolics from water, whereas ethyl acetate and diethyl ether have been used for extracting low molecular weight phenolics from oak wood (Palmqvist et al., 2000). Five organic solvents were selected (i.e. chloroform, trichloroethylene, diethyl ether, hexane and benzene) based on literature (Parajó et al., 1998; Parajó et al., 1997). The main properties regarding the organic solvents checked are shown in Table 2.7. Low boiling points, non-miscibility

in water or low polarities (and consequently low dielectric constant) are some of the features for phenolic extraction purpose.

Table 2.7 *Physico-chemical properties of the selected solvents.*

Solvent	Boiling Point (°C)	Dielectric Constant (ε)	Density (g/cm³)	Solubility at 20 °C
Chloroform	61	4.8	1.48	8
Diethyl ether	34.6	4.3	0.71	69
Trichloroethylene	87.2	3.4	1.46	1.28
Benzene	80	2.3	0.88	1.8
Hexane	69	2	0.66	0.0095

After the determination of the two most efficient solvents, the effect of the initial pH values of the hydrolysate was studied. The pH values under investigation were: 1.5, 2, non-adjusted at 3.6, 4.5 and 6.5. In order to study the settling time on phenolics extraction, the resulting mixtures were left to separate at 10, 30, 150 and 390 minutes. The effect of hydrolysate-to-solvent ratio, for the two best solvents previously selected, was evaluated at four different ratios, i.e. 1:1, 1:2, 1:3 and 1:5 (v/v). The number of consecutive extraction stages was also studied.

Table 2.8 shows the studied parameters for the liquid-liquid extraction process.

Table 2.8 *Studied parameters for liquid-liquid extraction.*

Parameter	Tested conditions	Selected conditions
Solvent	Diethyl ether, chloroform, trichloroethylene, benzene and hexane	Diethyl ether
Initial pH	1.5, 2, 3.6, 4.5 and 6.5	3.6
Settling time	from 10 to 390 minutes	30 min
Sample-to-solvent ratio	from 1:1 to 1:5 (v/v)	1:3 (v/v)
Number of extraction stages	from 1 to 3	1

3. Results & Discussion

3. RESULTS & DISCUSSION

3.1 The acid sulphite pulping process

Figure 3.1 shows the pulping process studied in this dissertation. The main steps of the process are described below.

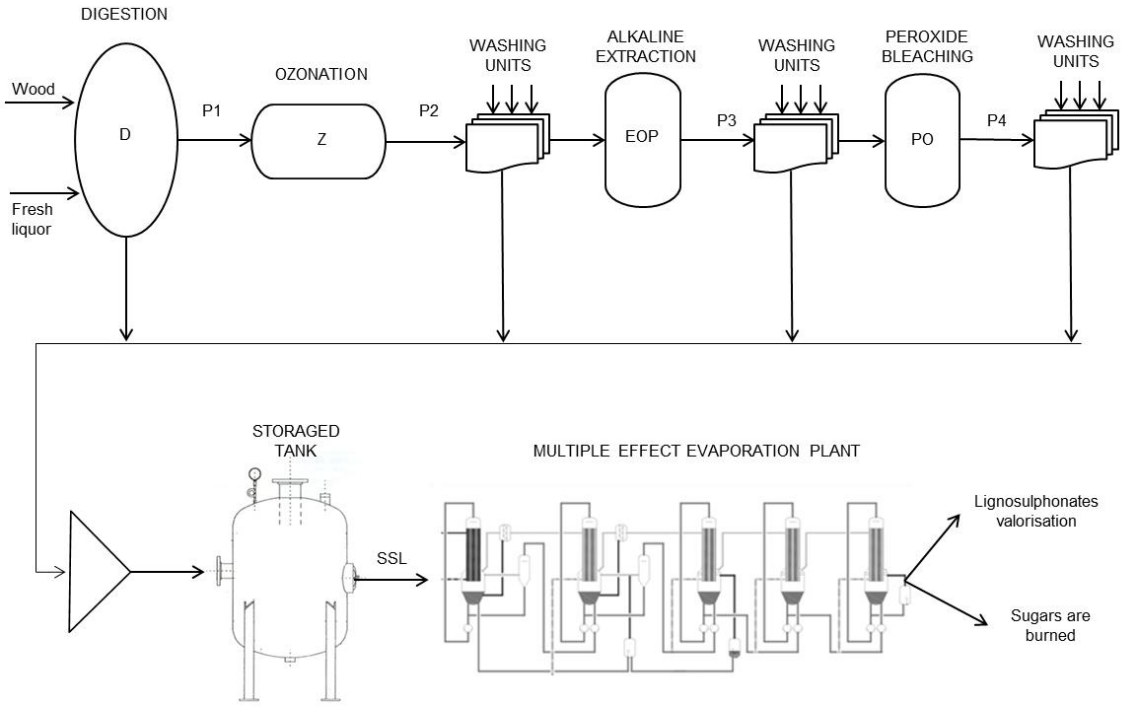


Fig. 3.1 *Acid sulphite pulping process.*

- ✓ **Raw material conditioning**

Eucalyptus globulus timber is used as a feedstock in the factory. Such hardwood specie belongs to the frondous family (together with poplar and birch) which is characterised by a short fibre structure 0.75-2 mm, high amounts of hemicelluloses - it can vary from 20 up to 43 %, slightly higher than coniferous timber from 25-30 % - and lower lignin contents 24 % than coniferous species 28 % (Pauly and Keegstra, 2008).

On one hand, wood chips (15-30 mm length and 2-4 mm thickness) are sent to the digester after being cut, debarked and sieved. The size distribution of the wood

chips is controlled during cutting and at least 75 % on weight should be between 8 and 21 mm length.

On the other hand, the fresh liquor, which is an acidic aqueous solution, is formed in absorption towers passing SO_2 in counter-current and dosing dolomite with 57 % of CaO and 37 % of MgO. Sulfur dioxide exists in different forms depending upon the pH. The chemical equilibrium is described by the following reactions (eq. 21):

$$SO_2+H_2O \leftrightarrow H_2SO_3\ (SO_2 \cdot H_2O) \leftrightarrow H^+ + HSO_3^- \leftrightarrow H^+ + SO_3^- \quad \text{(eq.21)}$$

- ✓ **Wood impregnation**

The first step in the digestion (see unit D in Figure 3.1) is the impregnation process which consists of the transportation of cooking liquor through the surface of the chip, followed by diffusion into the interior. As the chips heat up, lignin chemical reactions start to occur. When chips are being pulped at elevated temperatures, the rate of liquor diffusion into the wood is the rate-determining step (Santos et al., 2013). Efficient impregnation enables uniform pulping and reduced cooking time (Malkov et al., 2003). The non-uniformity of pulp has unfavourable effects on the cooking and bleaching. The cooking liquor must be equally distributed into the wood chips by getting a uniformly impregnated wood in the quickest possible time (Rueda et al., 2015-c).

Chemical transport into the chip voids is accomplished by two primary mechanisms: (i) liquor penetration into the air-filled voids of the wood chip under the pressure gradient; (ii) diffusion that refers to the movement of ions or other soluble matter through water under the influence of the concentration gradient (Malkov et al., 2003).

- ✓ **Wood cooking**

Once the wood feedstock has been impregnated, the next step occurring within the digestor (see unit D in Figure 3.1) is the so-called wood cooking. Such a step consists of complex physico-chemical reactions of the wood macrocomponents as it is heated above 100 ºC.

During the sulphite cooking, the composition of sulphite liquor varies very much since SO_2 is consumed by the sulphonation of lignin. The rate at which lignin is

sulphonated and hydrolysed depends strongly on the liquor composition. At 130 ºC, a maximum sulphonation reaction takes place (Shahzad, 2012). Hemicelluloses are affected first, followed by cellulose and lignin (Esteves et al., 2013). Lignin degrading reactions are characterised by sulphonation, hydrolysis between lignin and carbohydrates bonds, and condensation which is a detriment to lignin removal (Shahzad, 2012). Hydrolysis reactions consist of the cleavage of glycosidic bonds in the cellulose and hemicelluloses promoted by the acid conditions of the pulping process. Weaker glycosidic bonds, hemicelluloses and amorphous cellulose are depolimerised more easily than crystalline cellulose. Cellulose chains are also affected by acid hydrolysis during cooking but major cellulose depolymerisation does not happen until the end of delignification (Shahzad, 2012).

Figure 3.2 shows the temperature-pressure ramps regarding wood digestion including the impregnation and cooking stages previously explained. The specific temperature, pressure and time data are not displayed since they are confidential.

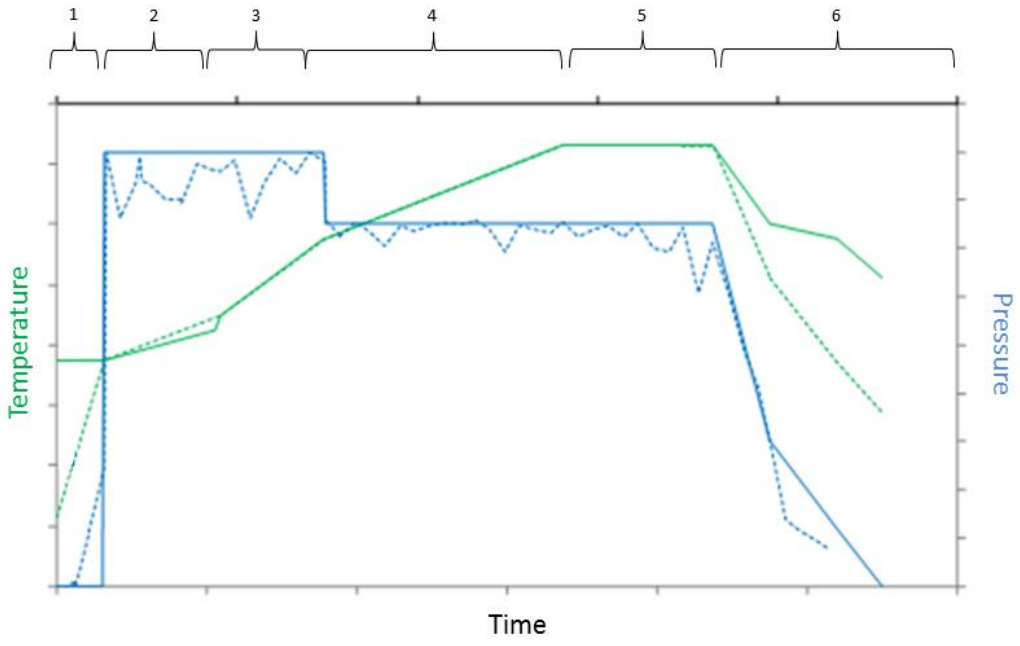


Fig. 3.2 *Typical T-P profile of acid sulphite pulping: (1) digester loading; (2) preheating step; (3) impregnation step; (4) cooking step; (5) dwell temperature step; (6) degasification and liquid purge.*

- ✓ **Total Chlorine Free bleaching**

After digestion, the next step of the pulp is bleaching to purify the cellulose pulp, removing little amounts of lignin and hemicellulose that still remain in the pulp.

The bleaching process taking place in the Sniace factory is total chlorine free (TCF) environmentally friendly process (Fardim, 2011; Sixta, 2006) formed by a combination of three selective oxidation stages: ozonation (Z), alkaline extraction (EOP) and peroxide bleaching extraction (PO). The complete bleaching sequence is shown in Figure 3.2. TCF processes solve the formation of toxic chemicals such as dioxins, furans, and adsorbable organic halides (AOX) in the wastewater avoiding the hazardousness to both humans and marine life (Bouiri and Amrani, 2010).

- ✓ **Evaporation**

The main residue formed at the end of the cooking step is the so-called SSL which is sent to the multiple effect evaporation plant (MEEP). The MEEP concentrates the SSL from 9 % up to 55 % w/w dry matter. This stage permits the recovery of the LS contained into the SSL for valorisation purposes. In addition, volatile compounds rich in SO_2 are recirculated to the accumulators for fresh liquor enrichment. Once the liquor has evaporated, it is sent to another factory in order to valorise the lignosulphonate content. However, the sugar content of the hydrolysate is destroyed.

3.2 Towards transformation of the sulphite mill in a lignocellulosic biorefinery

P&P industry is being reconsidered as an important source of hemicellulosic carbohydrates. Hence, there are currently many efforts from the P&P industries focused on sugar-rich resources valorisation into energy and a wide variety of products.

Several approaches tracing pathways and guidelines towards the conversion of pulping factories into LCBR can be found in the literature for kraft pulping (Saadatmand et al., 2013; Sixta et al., 2009; Towers et al., 2007), soda-AQ (Vena et al., 2013; García et al., 2011), organosolv (Shatalov et al., 2013; Bozell et al., 2011; Saake et al., 2011), or SEW process (Sklavounos et al., 2013; Sixta et al., 2013). The investment activity the field of P&P mills reveals the importance of transforming

traditional pulping factories into integrated LCBR, increasing the profit margin in the existing pulp mills.

The main advantage of sulphite pulping is the possibility to obtain a high separation of the main components, cellulose, hemicellulose and lignin, to give several applications within the biorefinery concept; nevertheless, only a few contributions have been studied in the case of sulphite pulping (Steindl et al., 2008; Steindl et al., 2003). Therefore, the study of this process for dissolving pulp manufacture and the conversion of hemicellulose-derived sugars and lignin-derivatives into fuels and other chemicals is a clear priority.

The general objective of this thesis is to identify the improvements to the acid sulphite mill described in the previous section in order to not only obtain dissolving pulp but also to track down the residual sugar-rich streams within the biorefinery concept. In order to fulfil this objective, and according to the main steps for lignocellulosic biomass processing shown in Figure 1.3 in the introduction section, the following steps have been carried out: (i) the carbohydrate quantification and mass balance of all of the main fractions in the lignocellulosic biomass of the factory; (ii) the study of the pretreatment process in the sulphite pulping in order to maximise the sugar content in the hydrolysate; (iii) the study of an external hydrolysis of the spent liquor in order to increase the sugar substrate of the liquor and decrease the inhibitor concentration; and (iv) the study of different detoxification processes of the hydrolysate in order to give a source of sugar substrate for fermentation and purification and/or separating the lignosulphonates and other possible by-products from the sugar substrate.

3.2.1 Carbohydrate quantification by HPLC

A preliminary stage towards the P&P mill transformation into a LCBR is to make an effective analysis of the lignocellulosic streams generated throughout the whole process. In a sulphite process, the main compounds in the streams are lignosulphonates (LS), phenolic hydroxyl groups (OH) and carbohydrates. The methodology for LS and OH has already been implemented (AENOR, 2012). However, in the case of carbohydrate and derivative quantification, there are too many alternatives.

In this sense, a literature review of the available techniques for carbohydrate analysis was done. Table 3.1 shows the literature review. Gas chromatography (GC) of alditol acetates constituted the standardised method for carbohydrate biomass feedstocks (Cao et al., 1997). The first application of GC to carbohydrates was reported in 1958 and it described the separation of fully methylated monosaccharides. GC of alditol acetates is widely used for determining the composition of monosaccharide mixtures, having better solutions than the other commonly used derivatives. In contrast, current methods for preparing alditol acetates involve relatively long acetylation times at elevated temperatures (Ciucanu and Caprita, 2007) using hazardous reagents. The GC-MS of carbohydrate derivatives has also been extensively used but there are some limitations generated by the low volatility of these derivatives (Blakeney et al., 1983). Paper chromatography (PC) has also been traditionally applied for carbohydrate quantification in wood samples (Irik et al., 1988; Puri and Anand, 1986). Nevertheless, either PC or GC has the disadvantage of extensive sample preparation, resulting in lengthy and tedious procedures. Nowadays, the analysis of monosaccharides and more complex carbohydrates is often performed by column liquid chromatography (LC) techniques. Normal-phase liquid chromatography (NPLC), Ligand-exchange chromatography (LEC), supercritical fluid chromatography (SFC) or capillary electrophoresis (CE) have been reported by Karlsson et al. (2005) who developed a method using hydrophilic interaction liquid chromatography with evaporative light scattering detection (HILIC-ELSD) to separate monosaccharides in glycoprotein. There are also chromatography techniques such as high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD); high performance size exclusion chromatography (HPSEC), high performance liquid chromatographic with atmospheric pressure chemical ionisation mass spectrometry (HPLC-APCI-MS) or reverse phase-high performance liquid chromatography colorimetric electrode array detection (RP-HPLC-CEAD). Techniques beforehand mentioned are used for sugars and sugar-derivatives qualitative and quantitative determination. Finally, there are semi-quantitative, qualitative or quantitative non-chromatographic techniques also summarised in Table 3.1 such as Fourier Transform Raman Spectroscopy (FT-Raman), Fourier Transform Infrared spectroscopy (FTIR) or Nuclear Magnetic Resonance (NMR) to figure out functional groups, empirical and structural formulas.

Among the processes, high performance liquid chromatographic, HPLC, methods using refraction index detector, RID, are the most promising, rapid and reliable analytical techniques for sugar quantification of lignocellulosic hydrolysates (Fernandes et al., 2012; Santana and Okino, 2007; Chávez-Servín et al., 2004; Kaar et al., 1991; Irick et al., 1988). However, the use of industrial waste streams can give several problems to the characterisation. In the case of sulphite pulping, the acidic and corrosive nature of the samples caused by the residual SO_2 content produces a reduction in the column lifetime; besides, high LS content causes fouling problems and columns must be subjected continuously to cleaning and regenerating cycles because of the high viscosity and sticky properties of the LS. Another issue inherent to carbohydrate characterisation is regarding the separation of sugars peaks. Wood monosaccharides are very similar and therefore a lot of effort needs to be made in order to achieve a correct separation of the five major monosaccharides dissolved in the lignocellulosic hydrolysates.

In this work, four methods of HPLC-RID have been developed by using four different chromatographic columns based on literature (Sluiter et al., 2006; Ruiz and Ehrman, 1996). Ligand exchange is the preferred method for the separation of these kinds of columns using deionised water as eluent (sugars separation) or diluted sulphuric acid (acids and furfurals). Other secondary mechanisms are also involved in the separation of carbohydrates including size exclusion and normal phase partitioning.

Table 3.1 *Review of analytical techniques for carbohydrate and degradation product determination in lignocellulosic feedstocks.*

Sample	Technique	Detector	References
Chromatographic techniques for sugar and derived products analysis			
Wood and pulp samples	GC Gas Chromatography	MS Mass spectrometry	(Santos et al., 2011-a; Syverud et al., 2003; Cao et al., 1997; Blakeney et al., 1983)
Lignocellulosic feedstocks	HPAEC High Performance Anion Exchange Chromatography	PAD Pulsed amperometric detector	(Kabel et al., 2002-a; Kabel et al., 2002-b; Persson et al., 2002-a; Davis, 1998; Wang et al., 1998)
Eucalyptus globulus, Corn cob, Brewery's spent grain	HPSEC High performance size exclusion chromatography	MS Mass spectrometry	(Kabel et al., 2002-a; Kabel et al., 2002-b)
Standard mixtures	HILIC Hydrophilic interaction liquid chromatography	ELSD Evaporative light scattering detector	(Karlsson et al., 2005)
Wood kraft black liquors	HPLC-APCI High Performance Liquid Chromatography with atmospheric pressure chemical ionization	MS Mass spectrometry	(Käköla et al., 2007)
Food plants	RP-HPLC Reverse phase high performance liquid chromatography	CEAD Colorimetric electrode array detector	(Schwartz and Sontag, 2006)
Lignocellulosic feedstocks	HPLC High Performance Liquid Chromatography	RID Refractive index detector	(Fernandes et al., 2012; Santana and Okino, 2007; Sluiter et al., 2006; Chávez-Servín et al., 2004; Ruiz and Ehrman 1996; Kaar et al., 1991; Irick et al., 1988)
SW, HW & kraft liquors	HPLC High Performance Liquid Chromatography	UV ultraviolet detector	(Rodrigues et al., 2011; Santos et al., 2011-b; Jönsson et al., 2008)
Eucalypt extracts, bagasse hydrolysates & orange juice	HPLC High Performance Liquid Chromatography	DAD diode array detector	(Kelebek et al., 2009; Persson et al., 2002-a; Nilvebrant et al., 2001)
Non-Chromatographic techniques for sugar and derived products analysis			
Eucalypt species	FT-Raman (Raman Spectroscopy)		(Ona, 2003)
SW &HW hydrolysates	FTIR (Fourier Transform Infrared Spectroscopy)		(Rodrigues et al., 2011; Tucker et al., 2000)
Spent liquors	NMR (Nuclear Magnetic Resonance)		(Evtugin et al., 2010)

Optimal conditions have been obtained in the four columns. Table 3.2 shows the obtained results. The adjustment of chromatographic conditions to separate sugars was tedious since the mobile phase flow or pH, injection sample volume or column oven temperature affect the solute elution. The mobile phase was fixed in ultrapure water (HPX-87P and CHO-782 columns) and diluted sulphuric acid (HPX-87H and SH1011 columns) from the literature review (Saari et al., 2010; Scarlata and Hyman, 2010; Kelebek et al., 2009). Therefore, the mobile phase flow, injection volume and column oven temperature were optimised. Such chromatographic parameters significantly affect residence times and peak resolution. Longitudinal diffusion of the solute in the mobile phase and a low mass transfer between the solute and the mobile phase might contribute to band broadening as Figure 3.3 shows. Finally, a compromise solution was found for each method giving good peak separation at acceptable retention times. Operating conditions were: (i) HPX-87P column, 0.3 mL/min ultrapure water, 79ºC, 20 µL, 940 psi; (ii) CHO-782 column, 0.3 mL/min ultrapure water, 68ºC, 20 µL, 453 psi; (iii) HPX-87H column, 0.5 mL/min H_2SO_4 0.005 M, 30ºC, 20 µL, 975 psi; (iv) 0.5 mL/min H_2SO_4 0.005 M, 60ºC, 20 µL, 198 psi.

Table 3.2 *Operating column guidelines, standards and method conditions.*

	HPX-87P	CHO-782Pb	HPX-87H	SH-1011
Resin ionic form	Lead	Lead	Hydrogen	Hydrogen
Support	Sulphonated divinyl benzene-styrene	Poly styrene-divinylbenzene	Sulphonated divinyl benzene-styrene	Poly styrene-divinylbenzene
Particle size	9 µm	7 µm	9 µm	6 µm
Dimensions	300 mm x 7.8 mm	300 mm x 7.8 mm	300 mm x 7.8 mm	300 mm x 8 mm
Max. Pressure	1500 psi	1100 psi	1500 psi	725 psi
Max. Flow	1 mL/min	0.7 mL/min	Unknown f(Pmax)	1.5 mL/min
Max Temp.	85 ºC	95 ºC	65 ºC	95 ºC
Mobile phase	ultrapure Water	Ultrapure Water	0.005M H_2SO_4	0.005M H_2SO_4
pH range	05-sep	ene-14	01-mar	0-14
Guard column	Micro-guard cartridge 125-0119	CARBOSep CHO-99-2354	Micro-guard cartridge 125-0129	SH-G SUGAR
Cleaning solvent (reverse column)	30 % CH_3CN in water, 4 h, 25 ºC, 0.2 mL/min	50 % CH_3CN in water 0.1 mL/min 65ºC	65 ºC, 0.2 mL/min 1) 4 h 5 % CH_3CN in 0.005M H_2SO_4 2) 12 h 30 % CH_3CN in 0.005M H_2SO_4	1 mL/min 0.005M H_2SO_4 in water, 15min
Standards (g/L)	0.5-20	0.5-20	0.5-20 (sugars) 0.1-10 (acids) 0.1-3 (furfurals)	0.5-20 (sugars) 0.1-10 (acids) 0.1-3 (methanol & furfurals)
Retention times (min)	25.01-33.07	22.07-35.70	9.21-13.66 (sugars) 17.48-21.64 (acids) 39.92-41.15 (furfurals)	13.33-18.04 (sugars) 21.03-24.11 (acids) 27.36-66.46 (furfurals)
R^2	0.99940-0.99993	0.99984-0.99999	0.99936-0.99988 (sugars) 0.99925-0.99998 (acids) 0.99950-0.99953 (furfurals)	0.99922-0.99992 (sugars) 0.99931-0.99998 (acids) 0.99980-0.99997 (methanol & furfurals)
Method conditions	0.3 mL/min ultrapure water, 79ºC, 20 µL, 940 psi	0.3 mL/min ultrapure water, 68ºC, 20 µL, 453 psi	0.5 mL/min H_2SO_4 0.005 M, 30ºC, 20 µL, 975 psi	0.5 mL/min H_2SO_4 0.005 M, 60ºC, 20 µL, 198 psi

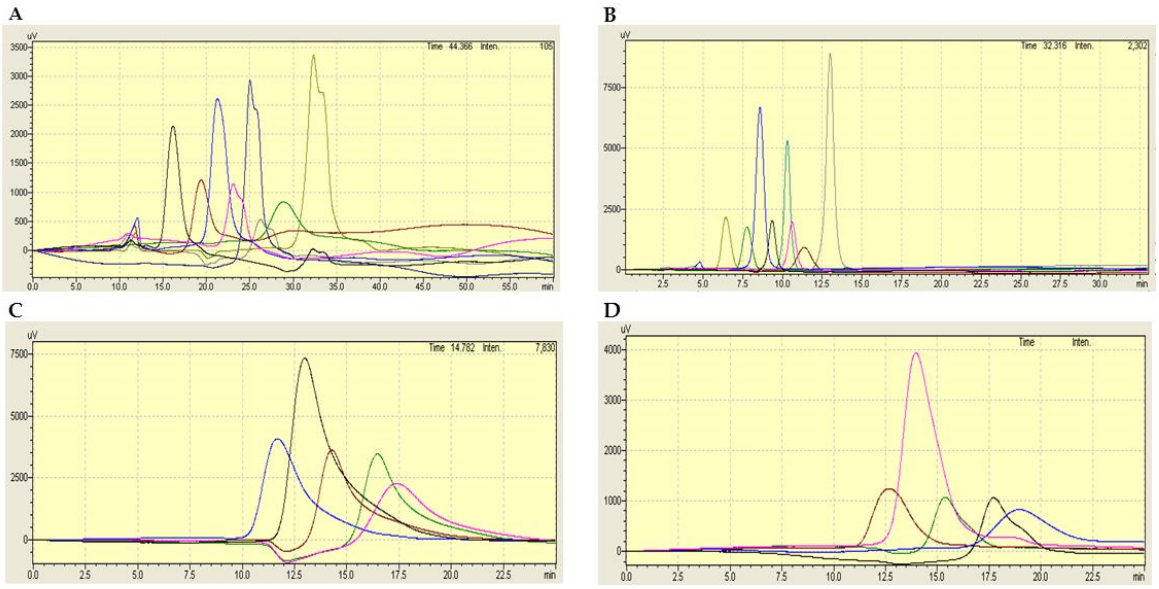


Fig. 3.3 *Ilustration of band broadening phenomenon occurring within HPX-87P column: (A) sugars peaks at 0.4 mL/min, 79 ºC and 5µL; (B) sugars peaks at 1mL/min, 79 ºC and 5µL; (C) sugars peaks at 0.6mL/min, 65 ºC and 25 µL; (D) sugars peaks at 0.6mL/min, 65 ºC and 5 µL.*

Calibration curves of the aforementioned methods are shown in Figure 3.4. An external standard as quantification method was used in all cases. Lineal adjustment force through zero with regression factors (R^2) up to 0.999 was obtained as can be observed in Figure 3.4 for each method developed. Standards were prepared in the range of 0.1 to 3 g/L for furfural, HMF and methanol; from 0.1 up to 10 g/L for acetic, levulinic and formic acids; and in the range of 0.5 to 20 g/L for sugars.

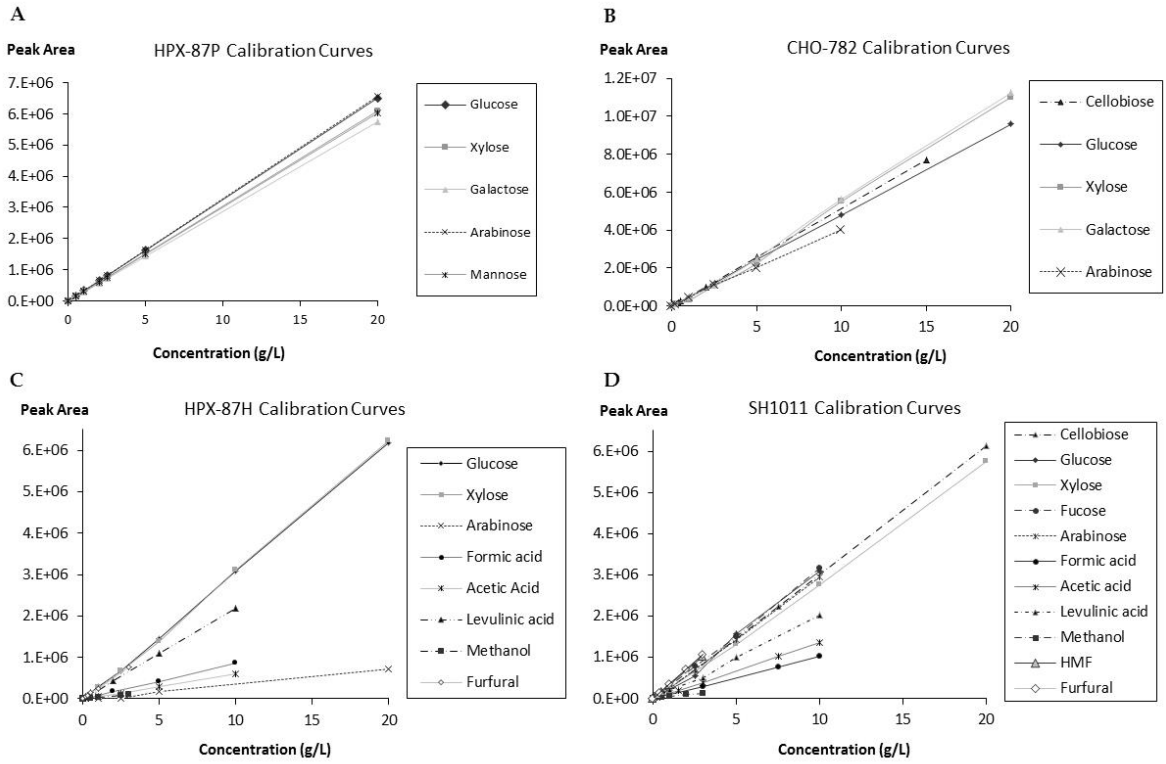


Fig. 3.4 *Calibration curves for sugars, acids, methanol and furfurals quantification of SSL.*

Final standards and sample chromatograms are represented in Figure 3.5. Peaks 1 to 13 correspond to: (1) cellobiose, (2) glucose, (3) xylose, (4) galactose, (5) fucose, (6) arabinose, (7) mannose, (8) formic acid, (9) acetic acid, (10) levulinic acid, (11) methanol, (12) HMF, (13) furfural. Biorad HPX-87P and Transgenomic CHO-782Pb columns were adequated to separate sugars. Major C6 sugars like glucose, galactose and mannose and major C5 sugars like xylose and arabinose could be integrated and separated in mixed standards and liquor samples. Biorad HPX-87H and Shodex SH-1011 columns are not adequate for sugar quantification since peaks of galactose, mannose, and xylose co-eluted and overlapped and it was only possible to assume that peak belonged to xylose, the major sugar of the SSL samples. Nevertheless, HPX-87H and SH-1011 separate furfurals and carboxylic acids. Furfural and HMF are separated mainly by SH1011 column because it has lower detection limits.

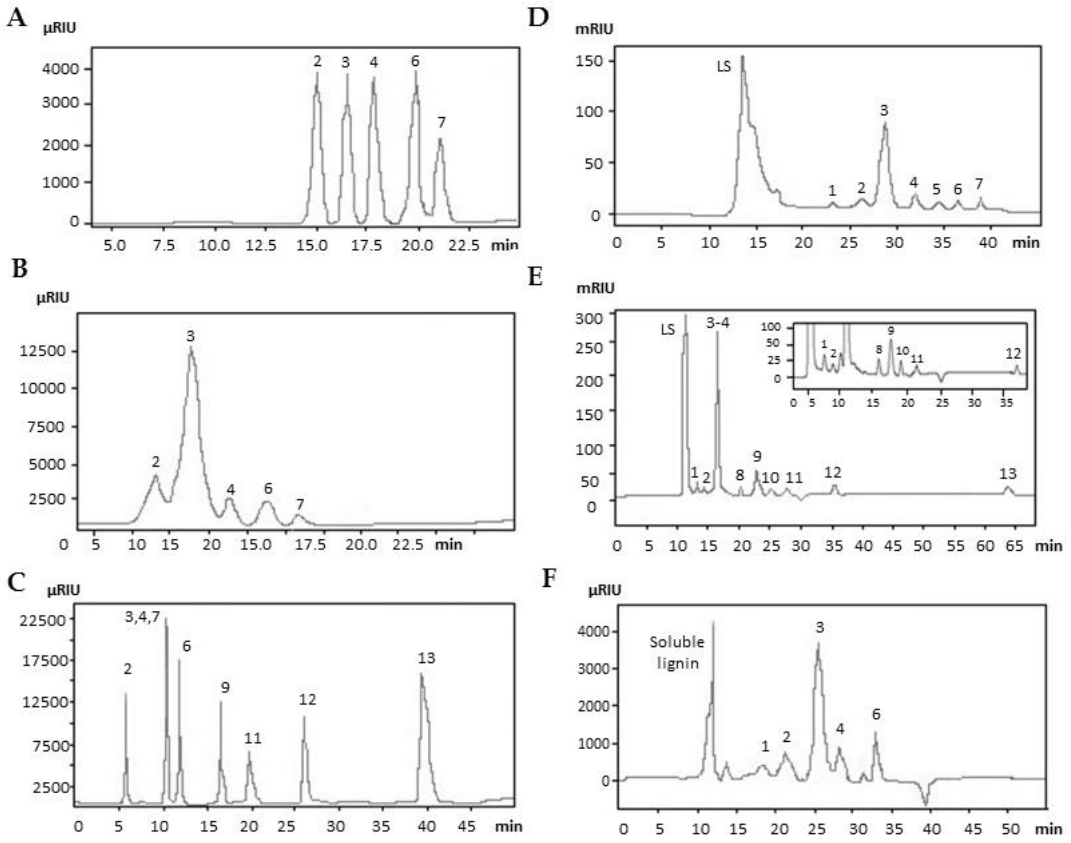


Fig. 3.5 *(A) Chromatogram of monosaccharides passed through the HPX-87P column; (B) chromatogram of the SSL using the HPX-87P column; (C) sugar, acid and furfural standards in the HPX-87H column; (D) chromatogram of the SSL using the CHO-782 column; (E) chromatogram of the SSL using the SH 1011 column; (F) chromatogram of wood and pulp hydrolyzates using the CHO-782 column.*

Regarding lead-based columns, either CHO-782Pb or HPX-87P columns are adequate for measuring monosaccharides. However, CHO-782Pb operates at a wider pH range in comparison to HPX-87P. Taking into account that liquor samples in sulphite pulping are acidic (pH=1-3), using the HPX-87P column would make it necessary to neutralise the samples. Regarding the hydrogen-based columns, SH-1011 has been chosen for this application because of the detection limits, the regression coefficients and the wider pH interval. This column can be used in all of the pH range and a correct separation of organic aliphatic acids, alcohols and furfurals is possible with this column. For all of these reasons both CHO-782Pb and SH-1011 columns are recommended as a solution for the separation of

monosaccharides and low-molecular organic derivatives in sulphite pulping samples.

Fouling of lead ionic columns (CHO-782Pb and HPX-87P) occurs frequently, increasing the pressure system, therefore it is necessary to clean and regenerate protocols to care for lifetime use. Operating column guidelines and characteristics, column cleaning protocols and details about the chromatographic methods developed are added in Table 3.2.

In order to validate the chromatographic analysis, twenty samples of weak spent sulphite liquor (WSSL) collected at the inlet of the evaportation units of the factory were analysed together with twenty samples of thick spent sulphite liquor (TSSL) collected at the outlet of the evaporation plant. The results are visualised in Table 3.3. The heterogeneity of the industrial liquor samples depends on many factors such as the wood used as raw material or the cooking conditions throughout the process. However, results do not depend strongly on the chromatographic method applied in every single case. Nevertheless, best standard deviations were obtained with the two proposed methods using CHO-782Pb and SH-1011 columns.

Comparing the results of Table 3.3 with other authors, similar results were obtained, and therefore the chromatographic methods checked are adequate for these kinds of samples. Total monosaccharides in the range of 29.1-43.2 g/L for WSSL and 75.6-145.2 g/L for TSSL; total acid content in the range of 8.2-10.3 g/L for WSSL and 4.2-12.6 g/L for TSSL; and total furfurals content in the range of 0.1-0.2 g/L in WSSL and lower than 0.06 g/L in TSSL were found in the literature (Nigam, 2011; Xavier et al., 2010; Chipeta et al., 2005). The industrial liquor samples analysed in Table 3.3 registered total monosaccharide contents in the range of 26.7-36.5 for WSSL and 185-214 g/L for TSSL; total acids in the range of 8.75-9.61 g/L for WSSL and in the range of 8.19-8.28 g/L for TSSL; and total furfurals between 0.43-0.52 g/L for WSSL and 0.20-0.27 g/L for TSSL.

In order to show all of the main compounds in spent sulphite liquors, Figures 3.6 and 3.7 visualise the chemical characterisation of both industrial weak liquor (WSSL) and thick liquor (TSSL) respectively. Graph bars showing the individual monosaccharide contribution to the total dry liquor content are also represented in Figures 3.6 and 3.7. In both cases, major compounds are LS and sugars. Monosaccharides, acids, furfurals and methanol data were taken from the CHO-782 and SH-1011 methods (Table 3.3). In addition to the HPLC results, lignosulphonates, phenolics (OH), SO2 and metals were added in the graph (Coz et al., 2015; Rueda et

al., 2015-a). WSSL contain 23.6 % of sugars and 41.8 % of LS whereas TSSL is composed by 27.8 % of sugars and 54.9 % of LS.

Not only monosaccharides (HPLC/RID method with the CHO-782 column) but also total sugars (phenol-sulphuric method) and reducing sugars (DNS method) were analysed in this work. Twelve industrial samples of WSSL and TSSL were taken for the colorimetric analysis. **Total monosaccharides** in WSSL and TSSL are respectively 26.7± 3.10 g/L and 214 ± 20.5g/L. **Reducing sugars** in WSSL and TSSL are respectively 29.6±6.43 and 188±14.0 g/L. **Total Sugars** in WSSL and TSSL are respectively 52.4±6.96 g/L and 212.5±13.5 g/L. In all cases, the resuls of total monosaccharides (HPLC/RID) give smaller concentrations than reducing sugars (DNS) and as it is expected, the concentration of reducing sugars is less than the concentration of total sugars. Consequently, there are some hemicellulosic sugars that still remain without depolymerisation within WSSL and TSSL waste streams. The presence of cellobiose (β-1,4-linked glucose dimer) is further evidence of the incomplete carbohydrate hydrolysis in WSSL and TSSL. Therefore, in order to increase the quantity of sugars, an optimisation of the pulping process or a post-hydrolysis step can be carried out.

Table 3.3 S*ugars and decomposition products of the spent liquors obtained by HPLC/RID.*

WSSL Weak liquors	Col. HPX-87P [a] Method	Col. CHO-782 [b] Method	Col. HPX-87H [c] Method	Col. SH-1011 [d] Method
Cellobiose (g/l)	-	2.24 ± 0.18	-	2.36 ± 0.90
Glucose (g/l)	4.53 ± 1.63	4.12 ± 0.72	1.67 ± 0.45	2.35 ± 0.72
Xylose (g/l)	23.6 ± 9.69	15.6 ± 2.05	26.2 ± 3.87	25.0 ± 6.23
Galactose (g/l)	3.70 ± 1.67	2.93 ± 0.09	-	-
Arabinose (g/l)	3.07 ± 1.88	1.53 ± 0.06	1.02 ± 0.89	1.67 ± 0.39
Mannose (g/l)	1.56 ± 1.66	1.45 ± 0.17	-	-
Fucose (g/l)	-	1.10 ± 0.09	-	0.63 ± 0.08
Formic acid (g/l)	-	-	0.032 ± 0.005	0.290 ± 0.002
Acetic acid (g/l)	-	-	9.56 ± 1.53	8.33 ± 1.87
Levulinic acid (g/l)	-	-	0.0154 ± 0.003	0.130 ± 0.001
Methanol (g/l)	-	-	1.03 ± 0.38	0.5542 ± 0.10
HMF (g/l)	-	-	-	0.170 ± 0.02
Furfural (g/l)	-	-	0.43 ± 0.014	0.35 ± 0.06
TSSL **Thick liquors**	**Col. HPX-87P** [a] Method	**Col. CHO-782** [b] Method	**Col. HPX-87H** [c] Method	**Col. SH-1011** [d] Method
Cellobiose (g/l)	-	5.22 ± 1.17	-	16.0 ± 3.04
Glucose (g/l)	27.6 ± 10.8	23.8 ± 3.29	9.36 ± 3.38	14.9 ± 2.21
Xylose (g/l)	114 ± 16.7	145 ±17.1	145 ± 13.7	164 ± 19.4
Galactose (g/l)	17.8 ± 3.94	22.8 ± 2.22	-	-
Arabinose (g/l)	17.5 ± 7.75	12.7 ± 1.20	1.98 ± 0.23	11.4 ± 1.22
Mannose (g/l)	9.05 ± 8.72	7.54 ± 1.45	-	-
Fucose (g/l)	-	2.6 ± 0.89	-	3.68 ± 0.40
Formic acid (g/l)	-	-	0.341 ± 0.071	0.228 ± 0.090
Acetic acid (g/l)	-	-	7.79 ± 1.27	7.85 ± 1.28
Levulinic acid (g/l)	-	-	0.151 ± 0.03	0.111 ± 0.02
Methanol (g/l)	-	-	0.63 ± 1.43	0.17 ± 0.06
HMF (g/l)	-	-	-	0.12 ± 0.05
Furfural (g/l)	-	-	0.20 ± 0.05	0.15 ± 0.03

[a] *Method : 0.3mL/min of ultrapure H_20 (isocratic flow), 79ºC, 20µl, 940psi*

[b] *Method : 0.3mL/min of ultrapure H_2O (isocratic flow), 68ºC, 20µl, 450psi*

[c] *Method : 0.5mL/min 0.05M H_2SO_4 (isocratic flow), 30ºC, 20µl, 975psi*

[d] *Method : 0.5mL/min 0.05M H_2SO_4 (isocratic flow), 60ºC, 20µl, 198psi*

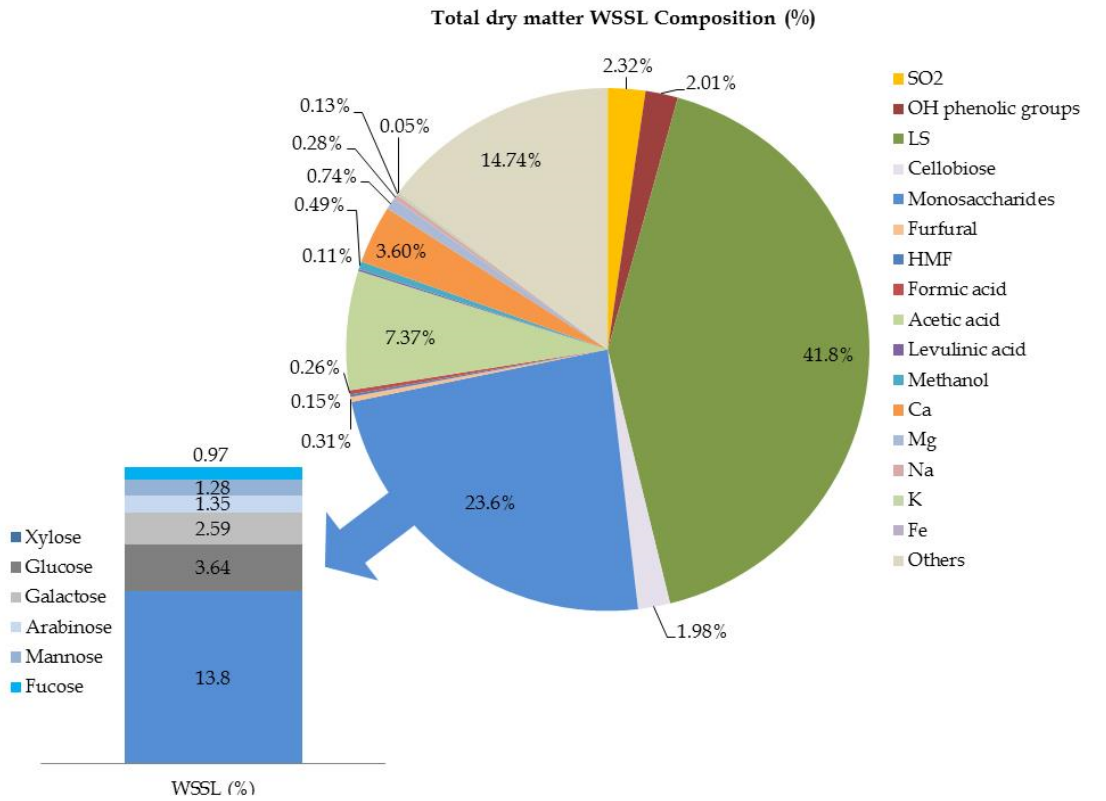


Fig. 3.6 *Characterisation of the weak spent liquor WSSL (results expressed in % w/w dry basis).*

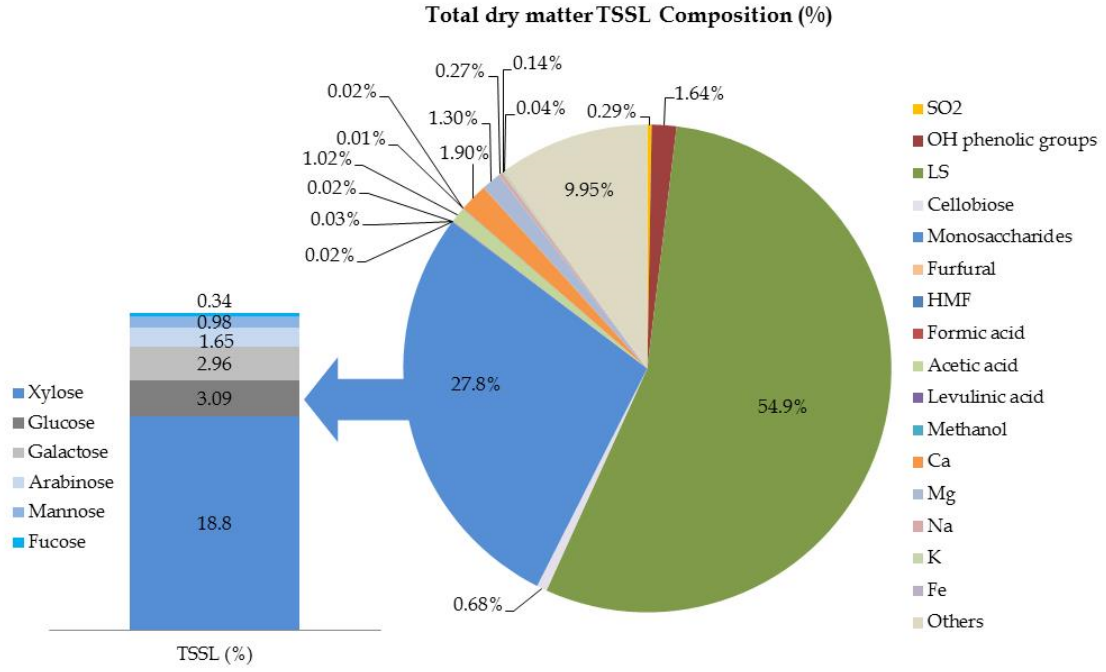


Fig. 3.7 *Characterisation of the thick spent liquor (results expressed in % w/w dry basis).*

3.2.2 Summative analysis and mass balance of the wood macrocomponents

Once the methodology for carbohydrate characterisation by means of HPLC was developed, the next step was to study the mass balances of the three main wood macrocomponents, lignin, cellulose and hemicellulose, in the whole industrial process (digestion and bleaching) and give some recommendations to the industrial plant to be converted into a lignocellulosic biorefinery.

✓ **Total composition of the lignocellulosic samples**

All of the samples, wood, pulp, and hydrolysates, were analysed in the main units of the industrial mill: digestion and bleaching steps (see Figure 3.1) in triplicate. The results of the total content per sample are shown in Table 3.4 including the major components, ash, and extractives. The results represent the total weight percentage content of the industrial samples collected in the pulp mill. The total mass closure was near 100 % in spite of the fact that some minority compounds were not analysed, such as low molecular phenolic compounds derived from lignin or aldonic and uronic acids derived from cellulose and hemicellulose. P1 to P4 are the pulp samples through the industrial process according to Figure 3.1, P1 being the pulp sample from the digestion step, P2 from the ozonation step, P3 from the alkaline extraction and P4 from the peroxide bleaching step.

The comparison of traditional characterisation using gravimetric and titration methods and the carbohydrate analysis derived from the summative analysis calculations is displayed in Table 3.4. Traditional cellulose methods include alpha-cellulose in pulp (TAPPI, 1999-c) and Seifert for cellulose in wood (Wright and Wallis, 1998). Traditional hemicellulose in wood is calculated as the difference between holocellulose (Haykiri-Acma et al., 2014) and Seifert cellulose (Wright and Wallis, 1998). Cellulose-HPLC and hemicellulose-HPLC of wood and pulp samples were obtained stoichiometrically, after acid hydrolysis of carbohydrates and HPLC sugar quantification. Otherwise, SSL sugars were measured directly in the HPLC avoiding the hydrolysis step.

Table 3.4 *Total weight content of the industrial samples.*

Total mass clossure	*Wood* (% w/w)	WSSL (% w/w)	P1 (% w/w)	P2 (% w/w)	P3 (% w/w)	P4 (% w/w)
Cellulose-HPLC	42.3	5.67	89.0	87.3	89.9	91.3
Cellulose	46.0	-	91.3	91.2	92.4	92.3
Hemicellulose-HPLC	24.9	30.4	6.2	5.1	2.2	2.1
Hemicellulose	31.6	-	-	-	-	-
Lignin	27.0	42.99*	0.8	0.4	0.4	0.1
Ash at 525 ºC	0.35	12.1	0.28	0.26	0.24	0.18
Extractives	1.50	-	0.3	0.2	0.2	0.2
TOTAL	**96.0**	**91.2**	**96.6**	**93.3**	**92.9**	**93.9**

**Lignin data in SSL is the lignosulphonate content, derived from lignin sulphonation.*

Cellulose obtained by traditional methods and cellulose-HPLC in *Eucalyptus globulus* samples present values of 42.3 % and 46.0 % respectively. The Seifert method entails higher experimental errors because of the wood digestion at high temperatures where some projections can be formed if the analysis is not carried out carefully. In pulp samples, the cellulose also showed higher values by means of the traditional method. Alpha-cellulose corresponds to the insoluble fraction produced after the digestion of pulp at 25 ºC using 17.5 % NaOH. Theoretically, beta and gamma-cellulose with a lower degree of polymerisation are excluded, but considering the results of Table 3.4, there are chains with similar molecular weights that were also quantified. Regarding the results of hemicellulose, the hemicellulose-HPLC is lower than hemicellulose calculated by traditional methods in wood 24.9 % and 31.6 % respectively. This behaviour could be explained by the assumption that glucose is only considered to form part of the cellulose fraction. In addition, gravimetric mistakes of Seifert and holocellulose methods are overlapping, giving more errors in comparison with the chromatographic method. An alternative to the study of hemicelluloses in pulp samples can be the pentosan determination with the T223 cm-01 procedure (TAPPI, 2001); however, pentosan analysis was not carried out since it only contemplates C5 sugars.

Table 3.5 *Total carbohydrate content and biofuel potentials of the woody hydrolysates.*

	Wood (% w/w)	SSL (% w/w)	Sulphite dissolving pulps (% w/w)			
			P1	P2	P3	P4
GLUCAN	**42.3**	**5.67**	**89**	**87.3**	**89.9**	**91.3**
Glucose	44.99 ± 2.13	4.12 ± 1.48	95.0 ± 3.81	93.7 ± 3.27	96.7 ± 2.42	98.1 ± 2.52
HMF	0.1 ± 0.03	0.02 ± 0.009	0.3 ± 0.001	0.3 ± 0.05	0.3 ± 0.002	0.3 ± 0.00
Levulinic	0.14 ± 0.03	0.01 ± 0.009	0.2 ± 0.09	0.2 ± 0.01	0.2 ± 0.02	0.4 ± 0.00
Cellobiose	1.51 ± 1.29	2.04 ± 0.16	3.1 ± 1.78	2.5 ± 1.95	2.4 ± 1.26	2.2 ± 1.84
XYLAN	**13.3**	**19.2**	**5.3**	**4.5**	**1.7**	**1.5**
Xylose	14.27 ± 0.47	21.43 ± 8.80	2.9 ± 0.67	2.7 ± 0.66	2.0 ± 0.42	1.7 ± 0.75
Furfural	0.2 ± 0.19	0.15 ± 0.055	0.4 ± 0.004	0.8 ± 0.02	0.1 ± 0.003	0.2 ± 0.001
Formic	0.15 ± 0.05	0.03 ± 0.028	0.3 ± 0.18	ND	ND	ND
ARABINAN	**0.52**	**2.46**	**0.3**	**0.3**	**0.2**	**0.2**
Arabinose	0.59 ± 0.36	2.79 ± 1.71	0.3 ± 0.13	0.4 ± 0.16	0.2 ± 0.09	0.3 ± 0.16
GALACTAN	**7.36**	**3.03**	**0.4**	**0.3**	**0.3**	**0.4**
Galactose	8.18 ± 1.53	3.36 ± 1.52	0.4 ± 0.09	0.4 ± 0.06	0.3 ± 0.09	0.4 ± 0.26
MANNAN	**1.00***	**1.28**	**ND**	**ND**	**ND**	**ND**
Mannose	0.011	1.42 ± 0.50	ND	ND	ND	ND
ACETYL	**2.78**	**4.51**	**0.2**	**ND**	**ND**	**ND**
Acetic	3.87 ± 0.32	6.30 ± 1.70	0.3 ± 0.24	ND	ND	ND
TCC (%)	**67.2**	**36.1**	**95.2**	**92.4**	**91.8**	**93.4**
[a] **EtOH** (L/Kg.dry sample)	0.467	0.227	0.683	0.664	0.662	0.672
[b] **EtOH** (L/Kg.dry sample)	0.441	0.215	0.639	0.630	0.642	0.651

[a] *EtOH (L/Kg.dry sample) calculated from the homopolymers using hydrolysis and fermentation factors*

[b] *EtOH (L/Kg.dry sample) calculated from the monomers using fermentation factors*

The results of the Total Carbohydrate Content (TCC) disclosure appear in Table 3.5. TCC of 67.2 % and 27.0 % of lignin was obtained in *Eucalyptus globulus* hardwood samples. Besides, the replicates checked showed average values of 42.3 % cellulose and 77.6 % holocellulose. Results of lignin varying from 23 % to 27 % and cellulose from 45 to 54 % of *Eucalyptus globulus* timber were found in literature (Cotterill and Macrae, 1997; Pereira, 1988). Such ranges are in accordance with the results obtained in this work.

The total content of xylan was 13.3 % in wood samples, representing more than 50 % of the total hemicellulose content. This is because hardwood -in contrast to

coniferous softwood with a higher portion of hexosans than pentosans-, is composed mainly of pentoses where xylose is the major monosaccharide (Saadatmand er al., 2013; Chirat et al., 2012; Irick et al., 1988; Pettersen et al., 1984). TCC is much higher in pulp samples in comparison to the *Eucalyptus globulus* samples. The difference is explained because little amounts of lignin and hemicellulose were found in pulp samples. Hemicellulose decreases from 6.2 % to 2.1 % and lignin from 0.8 % to 0.1 % (see Table 3.5). TCC in pulp samples decreases in the bleaching processes, as can be seen in Table 3.5, from values of 95.2 % to values of 91.8-93.4 %. This phenomenon can be explained by the fact that xylan drops from 5.3 % to 1.5 % despite cellulose increases from 89.0 % to 91.3 %.

Once the total carbohydrates were obtained, a theoretical quantity of bioethanol was calculated. Results varying from 0.215 L.EtOH/Kg.dry.biomass (WSSL) to 0.683 L.EtOH/Kg.dry.biomass (crude pulps named as P1) were obtained. One challenge would be to increase the bioethanol potential of the residual liquor stream. Despite the fact that pulps resulted in good ethanol potentials, it is not interesting to ferment the glucose contained in this samples since the P&P mill is focused on dissolving cellulose manufacture.

✓ **Dissolving pulp properties**

Pulp properties and their evolution within the sulphite process are represented in Figures 3.8A to D. Pulp transformation from crude pulp after digestion stage (P1) to final bleached pulp (P4) is graphed with error bars.

Pulp quality parameters are represented in Figures 3.8-A and 3.8-D. Glucan and alpha-cellulose have similar trends, especially in the alkaline extraction process; however, some differences can be found in the case of ozonation with a more noticeable decrease in glucan. Pulp impurities were plotted respectively in Figure 3.8-B and Figure 3.8-C. In this case, similar results were obtained. Lignin and hemicellulose content decreases as the process advances. These results showed that the most oxidative stage is the ozonation where the main losses of lignin are registered from 0.8 % to 0.4 %. Although delignification is the main function of this stage, there is also a depolymerisation of hemicelluloses from 6.2 % to 5.1 % because of the high oxidation produced by ozone. In spite of the recalcitrant nature of cellulose with no losses of alpha-cellulose, there is also a little decrease in glucan from 88.8 % up to 87.3 % probably due to the degradation of beta and gamma cellulose. Such behavior is also reflected in the viscosity falling from 706.4 mL/g to

568.2 mL/g. Figure 3.8-D shows the polymerisation degree of cellulose chains playing an important role in the quality of the final pulp. As was expected, the viscosity diminished stage by stage from 706.4 mL/g (P1) after digestion up to 492.5 mL/g (P4) after PO bleaching.

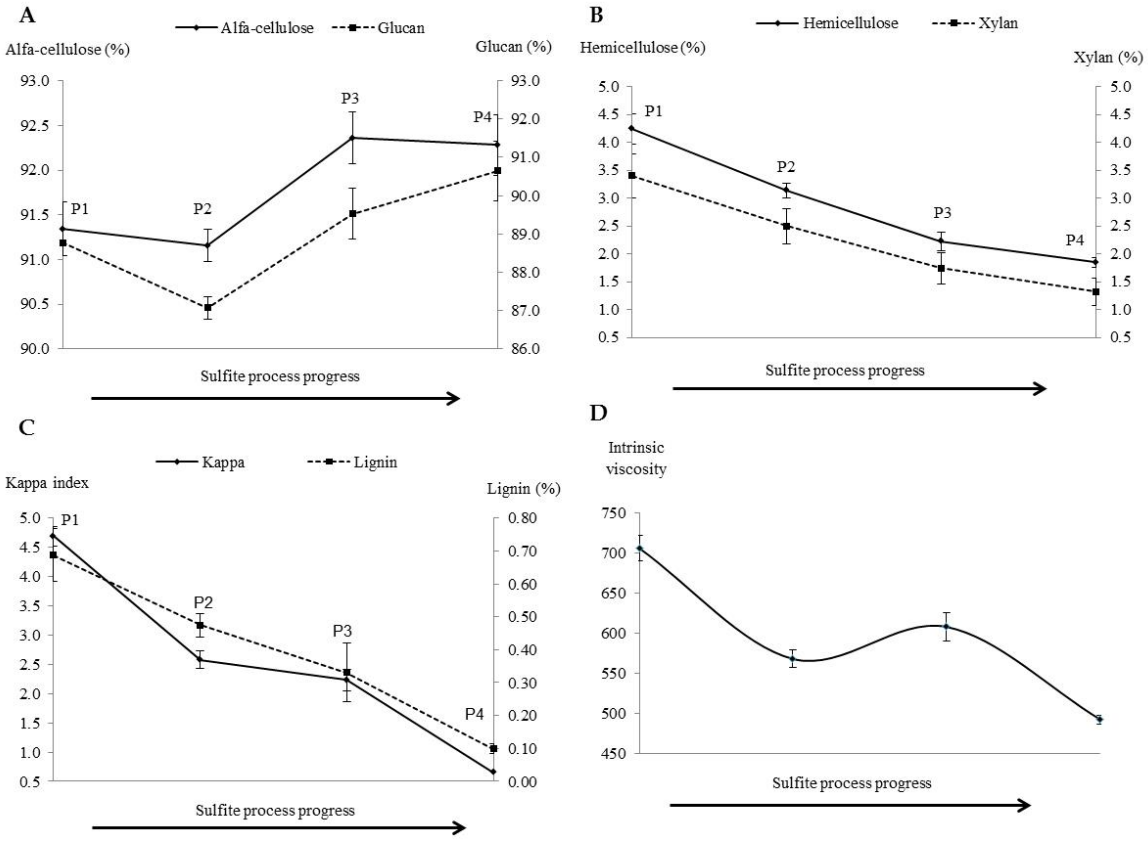


Fig. 3.8 *Evolution of wood macrocomponents in pulp throughout the sulphite mill: (A) glucan and alpha-cellulose; (B) hemicellulose and xylan; (C) Kappa index and lignin; (D) viscosity in pulp.*

Based on the experimental results shown in Figure 3.8 it can be concluded that: (i) the ozonation stage (Z) produces the destruction of mainly lignin and also carbohydrates. Z focuses on delignification and therefore kappa is notably reduced. Nevertheless, glucan is considerably diminished during Z whereas this does not affect the alpha-cellulose. This is due to the fact that the ozone is very aggressive as a bleaching agent (being less selective than chlorine derivatives) and attacks beta and gamma cellulose chains; (ii) the hot alkaline extraction stage (EOP), focuses on hemicellulose solubilisation, lowering hemicelluloses from 5.1 % to 2.2 % and

specifically xylan from 4.5 % to 1.7 %; (iii) the peroxide bleaching stage (PO) attacks the chromophore groups and the pulp is definitely purified by removing lignin traces from 0.4 % to 0.1 % and other groups responsible for the colour of the pulp; (iv) the selectivity in PO and Z stages should be improved in order to avoid the breakdown of the cellulose chains; (v) the results evidenced the importance of the waste water streams valorisation considering the high charge of organic compounds removed from the high-purity dissolving pulp along the sulphite process.

The obtained results were compared with other quality pulps as a function of the process (chemical or thermo-mechanical), the feedstock (softwood or hardwood), and the final application (paper-grade or dissolving grade) (Saadatmand et al., 2013; Gehmayr and Sixta, 2011; Alves et al., 2010; Sjöholm et al., 2000; Laver and Wilson, 1993). Figure 3.9-A shows the comparison data. HWDK, SWDK, TMP, HWPK correspond to hardwood dissolving grade from kraft process, softwood dissolving grade from kraft process, thermomechanical pulp (the worst quality) and hardwood paper grade from kraft process respectively. Results presenting major impurity removal are the more suitable for waste streams valorisation towards biofuels and other value-added products. The worst pulp quality is the thermo-mechanical (TMP) pulp with a total carbohydrate content of 64.4 % in comparison to chemical pulping processes with a total carbohydrate of 96.5 % (Ragauskas, 2013). The TMP constituted low-purity (regarding the lignin content) and high-yield pulp. The difference between paper-grade and dissolving-grade pulp resides in the total glucan content that is lower in case of paper grade, obtaining values of 74.7 % and 84.9 % for hardwood and softwood bleached pulps (Sjöholm et al., 2000) and 92.6 % in the case of dissolving-grade pulps (Laver and Wilson, 1993). Consequently, the hemicellulose content is higher in paper-grade pulps than in high purity dissolving-grade pulps. In this work, the total carbohydrate content in bleached pulp (P4) is 93.4 % where 91.3 % belongs to glucan with only 1.5 % of xylan.

Figure 3.9-B makes a comparison between different softwood (Kaar et al., 1991) and hardwood (Alves et al., 2010; Santana and Okino, 2007) species. SWA, HWE and HWP were softwood Aspen, hardwood Eucalypt and hardwood Parkia respectively. It should be noted that Figure 3.9-B does not reach content higher than 70 % w/w because lignin is not graphed. Only the carbohydrate fraction (hemicellulose and cellulose) was contemplated. The predominant homopolymer in all wood species is the glucan and consequently the cellulose fraction is always higher than hemicellulose.

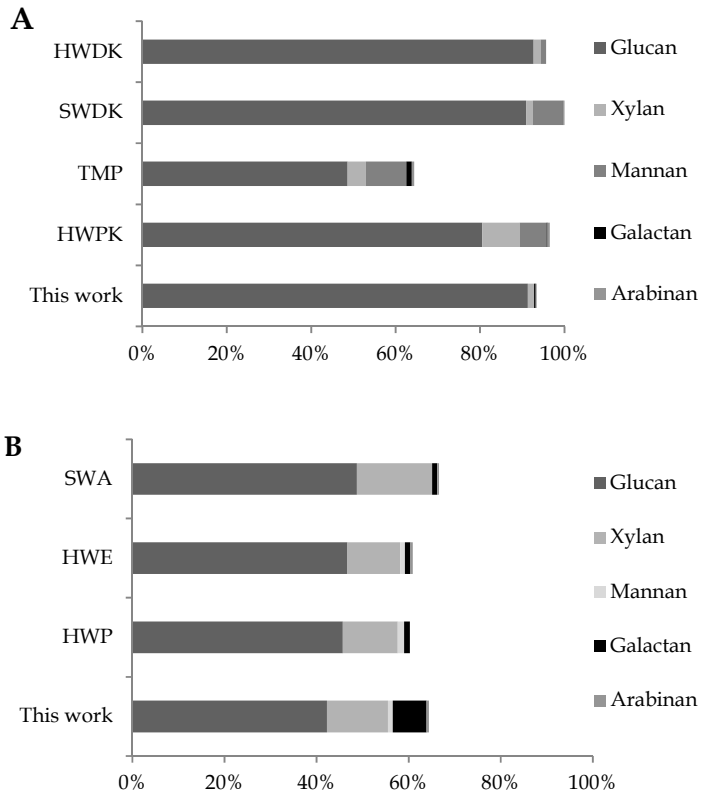


Fig. 3.9 *(A) Comparison of acid sulphite dissolving pulp with different quality pulps; (B) Comparison of Eucalyptus globulus feedstock with different wood species.*

✓ **Mass Balance of the Industrial Process**

The mass balance of the entire industrial process has been carried out taking into account the summative analysis. The complete characterisation of the feedstock (*Eucalyptus globulus* timber), the inlet-outlet pulps, and the main residual stream which is the SSL, were required. Data of the three macrocomponents throughout the process, flow rates, digestions per day, wood moisture or yields were considered. Some of the data are confidential to the factory and cannot be specifically displayed. Results appearing in Figure 3.10 have been correlated to the initial dry wood in terms of grams of cellulose, hemicellulose and lignin per grams of dry wood. The main discussion is described as follows:

(i) A total content of 99.6 % of cellulose provided from the feedstock goes to the main product, dissolving pulp, indicating the good performance of the digestion process. Only traces of wood cellulose are dissolved into the spent liquor. Thus, 0.032 g.hemicellulose/g.dry wood and $4.1 \cdot 10^{-3}$ g.lignin/g.dry

wood were detected in the crude pulp (P1) which will be removed throughout subsequent stages.

(ii) Based on the global mass balance some action lines can be made regarding Z and PO stages. A better use of the bleaching reagents and process conditions should be made in order to decrease the depolymerisation degree but not to the detriment of delignification.

(iii) The SSL generated after wood digestion is composed of 87.2 % of the total hemicellulose in wood (0.218 g.H/g.dw) and 98.5 % of the total lignin (0.266 g.L/g.dw). Hemicelluloses are hydrolysed and dissolved as monosaccharides and other derivatives. Likewise, lignin reacts with sulphite, bisulphite ions and sulphurous acid forming lignosulphonates. Based on these results SSL can be a perfect candidate for second-generation biofuel production.

The SSL is evaporated in the factory in order to reduce the water content. However, samples collected were collected before the evaporation plant, at the tank outlet (see WSSL in Figure 3.10). Tap water is used at the end of the digestion stage to stop hydrolysis and depolymerisation reactions. In addition, wastewater streams provided from pulp washing containing cellulose, hemicellulose and lignin are stored in the tank together with the SSL and sent to the evaporation plant as WSSL.

Theoretical bioethanol potential of the WSSL was calculated based on the carbohydrate content (0.031 g.C/g.dw and 0.205 g.H/g.dw). The hydrolysis stoichiometric factors for hexoses and pentoses were respectively 1.11 g.C6-sugars/g.cellulose and 1.136 g.C5-sugars/g.hemicellulose; the fermentation stoichiometric factor for ethanol production is 0.511 g.EtOH/g.monosaccharide. Assuming the complete conversion of C5 and C6 sugars, the second-generation bioethanol potential of the WSSL can be 0.183 L.EtOH/g.dw.

Based on the mass balance of the three wood macrocomponents throughout summative analysis procedures, **99.6 % of cellulose provided from the feedstock goes to the main product**, dissolving pulp, indicating the good performance of the digestion process. Only traces of wood cellulose are dissolved into the spent liquor.

The sulphite spent liquor generated after wood digestion is composed of **87.2 % of the total hemicellulose in wood** (0.218 g.H/g.dw) and 98.5 % of the total lignin (0.266 g.L/g.dw). Hemicelluloses are partially hydrolysed and dissolved as monosaccharides and other derivatives. Likewise, lignin reacts with sulphite, bisulphite ions and sulphurous acid forming lignosulphonates.

A better use of the bleaching reagents and process conditions should be made within the bleaching stage in order to decrease the depolymerisation degree but not to the detriment of delignification.

Spent sulphite liquor can be a perfect candidate for second-generation biofuel production since 0.183 L.EtOH/g.dw might be generated assuming the complete C5 & C6 conversion. Biofuel potential of this lignocellulosic residue will be further optimised.

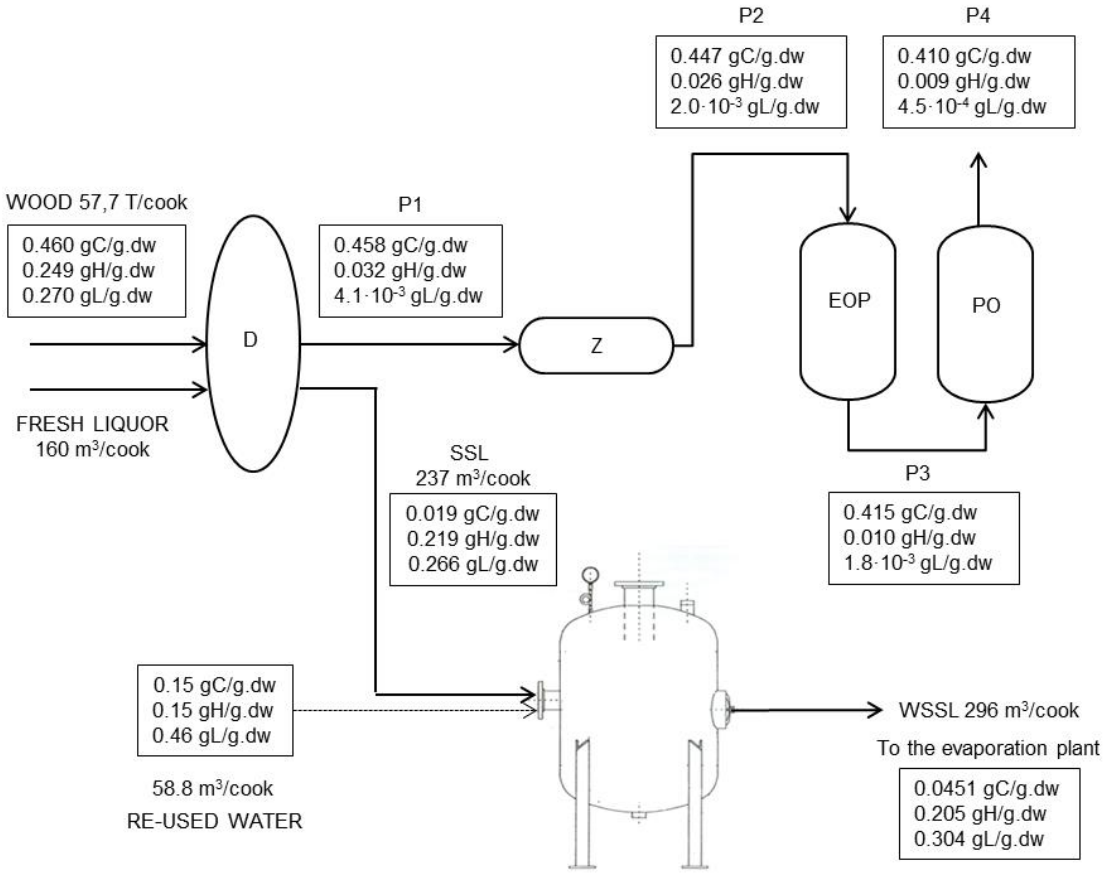


Fig. 3.10 *Mass balance results in the sulphite process. *Results expressed as g.C/g.dw, gH/g.dw and gL/g.dw correspond to grams of Cellulose, Hemicellulose and Lignin per grams of dry wood respectively.*

3.2.3 Optimisation of the cooking parameters

Taking into account the final percentage of the sugar in the industrial spent liquor coming from the hemicellulose of the wood and according to the results of the incomplete carbohydrate hydrolysis of the liquor, the following steps of this dissertation were the increase of sugar substrate by means of the study of the cooking process and by means of an external hydrolysis of the spent liquor. In both cases, not only an increase in the sugar content was studied but also a decrease in the inhibitors is taken into account with the same quality of dissolving pulp in the process. In this section, the optimisation of the cooking process is described.

✓ **Study of the main cooking parameters**

The main stage within the sulphite mill is the wood digestion which consists of an impregnation ramp, followed by a cooking ramp and a dwell time at constant and maximum temperature (Sixta, 2006; Casey, 1990; Gullichsen and Fogelholm, 1999). Cooking parameters were obtained from literature and the industrial Sniace process. Temperatures from 140 ºC to 150 ºC, liquor-to-wood ratio between 3:1 and 6:1 (m^3 fresh liquor/ton dry wood) and pH of the fresh liquor between 1.2 and 1.5 are necessary to extract the cellulose in pulping at about 7 bars of pressure (Marqués et al., 2009; Sixta, 2006; Casey, 1990). In this work, two cooking parameters, maximum temperature and heating rate, and total SO_2 content in the fresh liquor have been modified and analysed. A total of eight trials shown in Table 3.6 were performed. The exact values are confidential and therefore the cooking parameters (except total SO_2 that was modified from 6.20 % to 8.40 %) are expressed as T, R and t being respectively temperature, heating ramp and cooking time.

Table 3.6 *Cooking experiments at laboratory scale (parameters standardised preserving confidentiality).*

Trial	Temperature (°C)	Total SO_2 (%)	Temperature Ramp (°C/min)	cooking time (min)
C1	T	6.20	R	t
C2	1.03T	6.20	R	1.129t
C3	1.03T	6.20	0.334R	1.127t
C4	1.03T	8.40	0.501R	1.029t
C5	1.03T	6.20	0.501R	1.082t
C6	1.058T	6.20	0.334R	1.079t
C7	1.058T	6.20	0.334R	1.078t
C8	1.072T	6.20	0.285R	0.983t
Acid sulphite reference cook	1.01T	6.20	0.096R	-

Cooking time is standardised according to the equivalent energy employed to obtain a final kappa index of 6 and taking into account loads of wood in the reactor of 173.14 g. The equivalent energy concept refers to the energy employed during cooking step and an approximation can be calculated as the area under the curve of temperature versus time at constant temperature, excluding impregnation and cooking ramps. In addition, it is necessary to consider that kappa index decreases one unit every ten minutes according to the industrial data. Therefore, there are three possible situations depending on the final kappa index: (i) kappa equal to 6; (ii) kappa lower than 6; or (iii) kappa higher than 6. The following equations (22 to 25) were used for these situations:

For kappa = 6

$$tc' = tc \rightarrow E'eq = Eeq \rightarrow Esteq = Eeq\ (MSeq/MS) \rightarrow tst \rightarrow ttot = timp+tramp+tst \quad \text{(eq.22)}$$

For kappa < 6

$$tc'=tc-(6-kappa)\cdot 10 \rightarrow E'eq(tc') \rightarrow Esteq=E'eq\cdot(MSeq/MS) \rightarrow tst \rightarrow ttot=timp+tramp+tst \quad \text{(eq.23)}$$

For kappa > 6

$$tc'=tc+(kappa-6)\cdot 10 \rightarrow E'eq(tc') \rightarrow Esteq = E'eq\cdot(MSeq/MS) \rightarrow tst \rightarrow ttot=timp+tramp+tst \quad \text{(eq.24)}$$

Finally: t*= ttot/ttotref (eq.25)

Where: tc is cooking time; tc' is corrected cooking time to achieve kappa = 6; Eeq is the equivalent energy; E'eq is the corrected equivalent energy taking into account cooking times of tc'; Esteq is the standard equivalent energy, tst is the standard cooking time (taken from Esteq); MSeq is the equivalent dry mass of 173.14 g; MS is the real dry mass; ttot is total cooking time; timp is the impregnation time; tramp is the heating ramp time (after impregnation phase); t total standardised cooking time as a function of first trial (C1); ttotref is the total cooking non-standardised time in trial C1.*

Only for the experiment C8 at high temperature, low heating ratio and 6.20 % of total SO_2, it is possible to reduce the cooking time in comparison with the C1 trial. According to the cooking time, t*, an increase of the total SO_2 content in fresh liquor and higher temperatures contributes to a 1.7 % decrease of this value from 1.029t up to 1.082t when 6.20 % to 8.40 % of total SO_2 is used; and from 1.127t up to 1.078t when 1.03T to 1.058T is used.

The results of pulp and liquor obtained after each cooking trial are displayed in Table 3.7. The results of pulp and liquor related to the typical acid sulphite process held in the dissolving P&P mill are shown in the table as a reference.

Table 3.7 *Pulp and SSL results under conditions tested.*

Trial	C1	C2	C3	C4	C5	C6	C7	C8	Acid sulphite reference cook
Kappa	20.4	6.18	5.54	8.63	5.12	6.61	6.18	6.40	6.25±1.25
Viscosity (mL/g)	1285	539.7	841.2	1065	566.3	689.1	688.2	752.2	722.5±37.5
α-cellulose (%)	76.5	88.2	89.5	89.6	88.3	89.3	89.5	88.8	>88.0
LS (g/L)	63.4	86.4	77.1	81.9	96.6	87.1	86.1	84.8	47.3±4.51
Total sugars (g/L)	29.0	28.6	34.1	36.8	41.7	40.0	40.3	59.7	52.4±6.96
Reducing sugars (g/L)	23.9	23.2	31.3	30.6	32.4	26.3	27.5	32.9	29.6±6.43
Monosaccharides (g/L)	12.3	27.7	25.0	21.3	33.9	25.5	24.7	27.2	26.7±3.1
Acetic acid (g/L)	8.30	8.99	9.27	9.88	15.1	9.51	9.68	10.5	8.33±1.87
Metals (g/L)	8.65	4.17	4.34	5.78	4.54	4.64	4.99	4.59	5.12±0.32

Comparing the dissolving quality pulp requirements of the industrial digestion with the cooking trials assayed at laboratory scale, C6, C7 and C8 trials are

appropriate for dissolving pulp production. Furthermore, two of them, C6 and C7, with the same cooking conditions: 1.058T, 0.334R and 6.20 % total SO_2, were made to check the reproducibility of the reactors and the robustness of the obtained values. In both cases, very similar results on final pulp qualities, cooking time and physico-chemical properties of SSL were obtained.

The influence of the **maximum temperature** was studied by comparing C1 (T, R and 6.20 % total SO_2) and C2 (1.03T, R and 6.20 % total SO_2) trials. In this case, a higher temperature is necessary because kappa index of C1 is 20.4 typically from paper grade pulps. By increasing maximum temperature kappa and viscosity decrease considerably; alpha-cellulose jumps between C1 and C2 from 76.5 % up to 88.2 % by increasing temperature and cooking time but then, remains almost constant between 88.2 and 89.6 %. The results obtained in C1 are very different from the rest of the experiments not only from the standpoint of alpha-cellulose content but also the kappa index and viscosity of pulp.

In experiments C3 (1.03T, 0.334R and 6.20 % total SO_2) and C5 (1.03T, 0.501R and 6.20 % total SO_2) the effect of the **heating rate** was analysed. By increasing the heating rate kappa and alpha-cellulose slightly decrease from 5.54 to 5.12 and from 89.5 % to 88.3 %. However an increase in the cooking ramp significantly affected pulp viscosity causing depolymerisation of the cellulose chains. There is a viscosity falling from 841.2 mL/g up to 566.3 mL/g and therefore glucose contribution (derived from cellulose) to the total monosaccharides registered in the spent liquor of C8 is higher than the rest of cooking trials.

Finally, to study the effect of **total SO_2 content** in fresh liquor C4 (1.03T, 0.501R and 8.40 % total SO_2) and C5 (1.03T, 0.501R and 6.20 % total SO_2) trials were compared. By increasing the SO_2 content, both kappa index and viscosity rise from 5.12 up to 8.63 and 566 mL/g up to 1060 mL/g, respectively in contrast with the expected results because of different cooking times.

The end of the digestion process is given by the colour change of SSL and trial C4 (1.029t) was stopped before trial C5 (1.082t). If C4 and C5 had been stopped at the same time, a higher SO_2 content would have originated lower kappa index and viscosity because SO_2 affects the pH and consequently has repercussions in the polymerisation degree and the kappa index. This behaviour is an indication that maximum viscosity is reached and it is necessary to extend the constant temperature cooking time. Selectivity of delignification reaction and maximum viscosity grows

by increasing total SO_2 and consequently increasing bisulphite content, and heating rate.

Viscosity and kappa index are the main pulp properties regarding depolymerisation and delignification reactions. By means of the cooking results, it is observed that extending ramp (decrease heating rate) selectivity and consequently maximum intrinsic viscosity increased, because only lignin, hemicelluloses and short-chain polysaccharides are broken down. The process is selective to the delignification reaction and attacks less cellulose chains, therefore the maximum value of viscosity increases. For the same value of viscosity, the kappa index is higher in pulps obtained using shorter digestion ramps. At high heating rates, not only lignin but also hemicellulose and cellulose are degraded. It is also observed that maximum temperature affects decreasing dwell time but also decreases cellulose yield and selectivity which are not desirable (Sixta, 2006; Casey, 1990).

In addition to total sugars, reducing sugars and monosaccharides (by HPLC) were analysed and compared in all of the experiments. Figure 3.11 shows the obtained results. Total sugars are always higher than reducing sugars because this method measures not only the carbohydrate chain and monosaccharides but also crystalline-cellulose and other refractory hydrocarbons which have not been hydrolysed. In addition, reducing sugars are higher than monosaccharides because monomers as well as reducing extremes of non-hydrolysed polysaccharides are included.

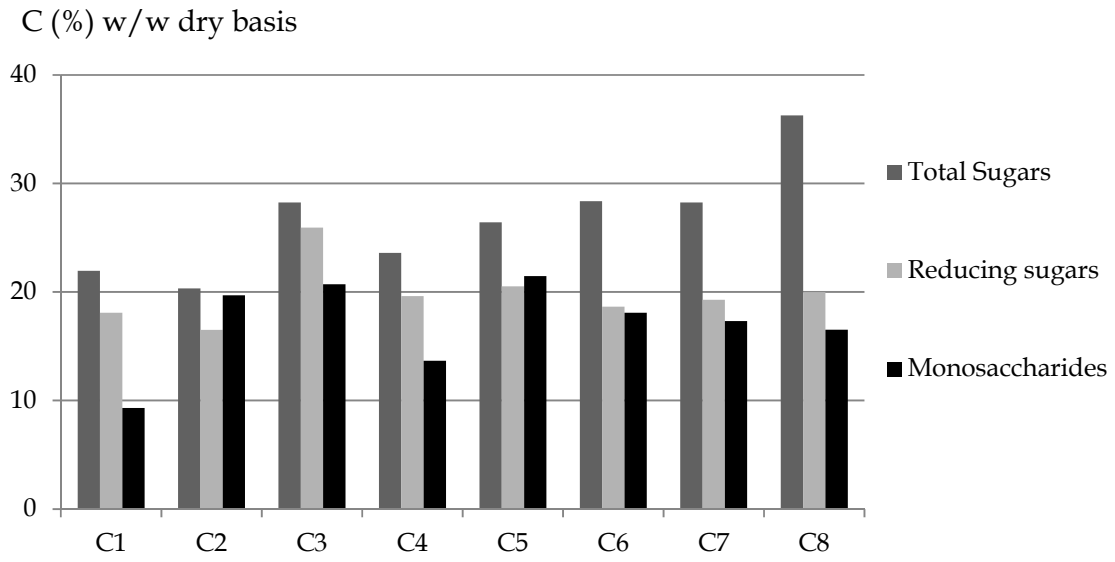


Fig. 3.11 *Total sugars, reducing sugars and monomers detected in the spent liquors under different cooking conditions.*

Taking into account total monosaccharides, there are improvements with respect to the reference acid sulphite cook (26.7 g/L of total monosaccharides) in trials C2, C5 and C8 giving concentrations of 27.7 g/L, 33.9 g/L and 27.2 g/L respectively. There was a considerably jump from C1 (12.33 g/L) to C2 (27.7 g/L) caused by the increase of maximum temperature in 4 ^{o}C, demonstrating that the effect of **maximum temperature affects the monosaccharides** which are hydrolysed and dissolved in the spent liquors. Another cooking parameter that also affects the amount of monosaccharides is the cooking ramp. Making the comparison between C2 and C5 which have the same maximum temperature (1.03T) but different heating rates (R and 0.501R) it is also demonstrated that **lowering heating rates promotes major release of sugars**. C8 (1.072T, 0.285R) is the only trial from the three selected (C2, C5 and C8) that fulfills the dissolving pulp quality properties. Making the comparison with the reference sulphite cook, total sugars increased from 52.4 g/L to 59.7 g/L, reducing sugars from 29.6 g/L to 32.9 g/L and monosaccharides from 26.7 g/L to 27.2 g/L. Considering total monosaccharides as the best sugar determining method, it can be concluded that increasing the maximum temperature 8 ^{o}C and 0.074 ^{o}C/min the cooking ramp, total monomers dissolved in the spent liquors rises by only 1.87 % which is not cost effective for the P&P mill.

Figure 3.12 shows the main components of SSL, ash, total sugars and LS, for the experimental trials. Similar results have been obtained in literature for this kind of liquor (Rueda et al., 2011; Xavier et al., 2010; Chipeta et al., 2005). The results of total sugar vary from 20.3 % to 36.8 %, ash at 900 ºC from 7.60 % to 9.73 % and lignosulphonates from 48 to 63.9 % w/w.

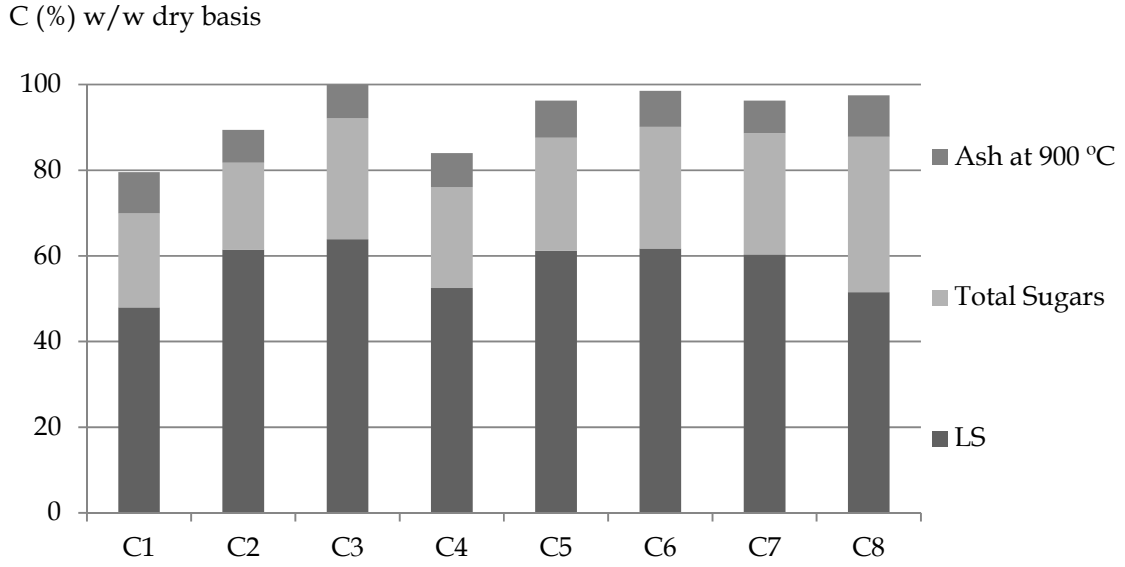


Fig. 3.12 *Results of the major compounds of the spent liquors under different cooking conditions.*

Regarding the amount of LS in the SSL for its valorisation options, the best result was registered in C5 (1.03T, 6.20 % SO_2, 0.501R and 1082t) with total LS concentration of 96.6 g/L produced by lignin sulphonation. Such delignification was pointed out in the kappa number. C5 registered a kappa number of 5.12 which is the lowest of the cooking trials assayed.

Depending on the operational cooking parameters, cellulose extraction could be more effective and consequently chemical composition of SSL could be different in each case. High contents of cellulose in pulp involve a good extraction of cellulose and consequently, high levels of sugars resulting from hemicellulose hydrolysis and LS resulting from lignin sulphonation in the spent liquors.

The relationships between the quality properties of pulp and composition of SSL are shown in Figure 3.13. On one hand, the monomers obtained by HPLC in SSL and viscosity of pulp for each cooking experiment are shown in Figure 3.13-A. On the

other hand, in Figure 3.13-B results of LS in SSL and kappa index of pulp for each cooking experiment are represented.

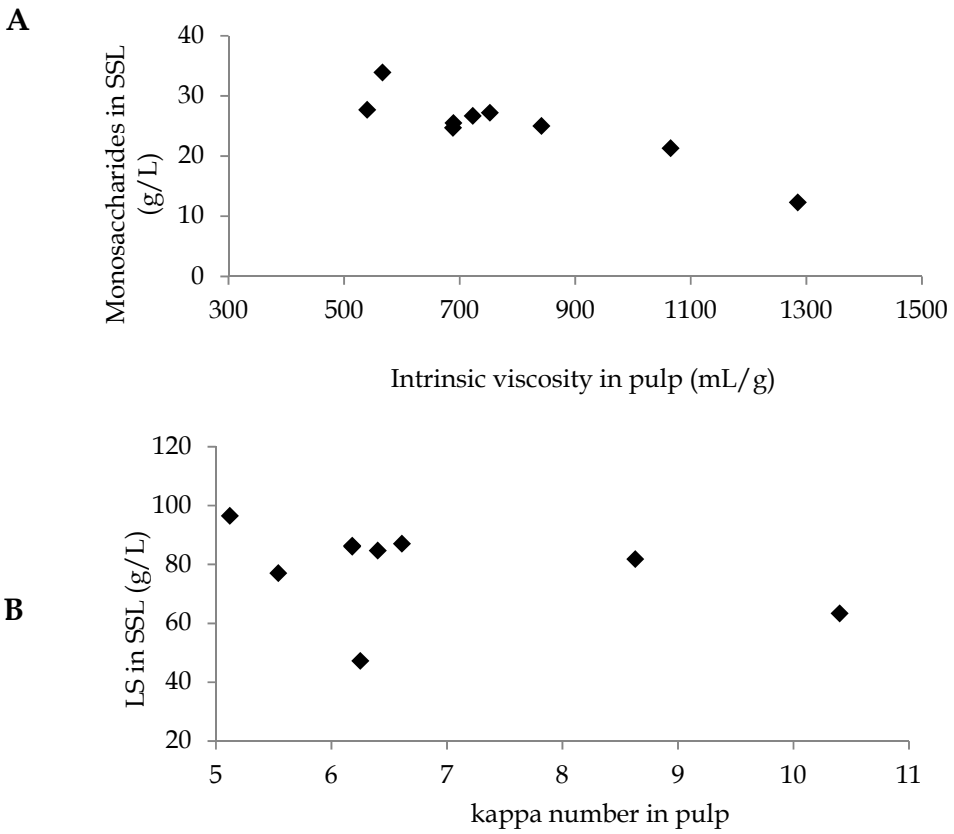


Fig. 3.13 *Trend curves of outlet SSL versus pulp quality: (A) Relationship between monosaccharides and intrinsic viscosity; (B) Relationship between LS and kappa index.*

The kappa number is related to the delignification degree and it is correlated with the lignin content. A high kappa number implies a low degree of delignification which gives a low LS content in the liquor. This is due to the fact that LS have not been dissolved in the liquor and therefore they remain in the pulp. The intrinsic viscosity is related to the polymerisation degree and high viscosity involves a lower hydrolysis grade which gives a low presence of monosaccharides in SSL. All of these conclusions are corroborated in Figure 3.13; however, no correlation has been obtained in the two cases.

✓ **Modelling of the main cooking parameters**

A mathematical model based on the previous set of laboratory cooking experiments has been conducted in order to predict the best cooking conditions to maximise LS and sugars, and simultaneously minimise acetic acid in the spent liquor. The quality parameters of pulp product have been monitored together with the chemical properties of the spent sulphite liquor, giving the best results of the process variables: maximum temperature, heating rate, SO_2 content and cooking time. After cooking, the unbleached dissolving pulp should have a kappa index between 5 and 7.5; an intrinsic viscosity between 685 mL/g and 760 mL/g; and alpha-cellulose content > 88% (Sixta, 2006; Casey, 1990). All of these paramters have been added in the optimisation model. According to the experimental results, only C6, C7 and C8 trials are appropriate for dissolving pulp production.

A model which predicts optimal conditions to produce dissolving-quality pulp with maximum concentrations of sugars and LS and minimum concentrations of acetic acid was carried out. In addition, the Stat Graphics Software which is specifically useful for statistical process control and design of experiments, allows us to make a multivariable linear regression. The following equations (26-31) were obtained by means of the multiple regression analysis:

$$LS = -1036+53.92 \cdot R+26.08 \cdot SO_2+4.909 \cdot T+0.7084 \cdot t \qquad R^2=93.50\ \% \qquad \text{(eq.26)}$$

$$Sugar = -450.7+33.45 \cdot R+3.816 \cdot SO_2+2.513 \cdot T+0.196 \cdot t \qquad R^2=95.10\ \% \qquad \text{(eq.27)}$$

$$Acetic = -113.7+15.36 \cdot R+0.7165 \cdot SO_2+0.5915 \cdot T+0.0736 \cdot t \qquad R^2=90.50\ \% \qquad \text{(eq.28)}$$

$$Kappa = 218.6+5.784 \cdot R-1.399 \cdot SO_2-0.9651 \cdot T-0.156 \cdot t \qquad R^2=85.50\ \% \qquad \text{(eq.29)}$$

$$Viscosity = 13330-501.2 \cdot R+37.15 \cdot SO_2-66.11 \cdot T-7.631 \cdot t \qquad R^2=79.90\ \% \qquad \text{(eq.30)}$$

$$\alpha\text{-cellulose} = -113.8-5.786 \cdot R+2.542 \cdot SO_2+0.9198 \cdot T+0.1316 \cdot t \qquad R^2=94.52\ \% \qquad \text{(eq.31)}$$

Modifications of the sulphite pulping process produce a higher purity of cellulose pulp. Due to the higher delignification and hydrolysis stages, it was possible to obtain SSL with higher contents of LS and reducing sugars. The higher proportions of hemicelluloses in spent liquor associated with a dissolving grade pulp allow the valorisation of this lignocellulosic waste material.

As can be seen from equations 26-31, an increase of all the compounds of the lignocellulosic waste, (LS, sugars and acetic acid) are obtained in relation to the heating rate (R), the maximum temperature (T), the SO_2 content in the fresh liquor and the cooking time (t). However, only LS and sugar content should be maximised in the process with a minimisation of the acetic acid because this acts as an inhibitor in the fermentation process. Furthermore, in all cases, good regression factors from 90.5 to 95.1 % have been obtained.

Regarding the results of kappa index, only the heating rate increases the value, however, the rest of the cooking variables decrease this property. Viscosity and alpha-cellulose are higher in the case of using more SO_2 content in the liquor; however ramp rate and cooking time diminish these parameters. The regression factors obtained in the case of final pulp properties were within 79.90 and 94.52 %.

Through equations (26-31) together with the objective function (equation 32), an optimisation model was built by means of the GAMS software. All of the Stat Graphics equations together with the upper and lower boundaries and variables initialisation were introduced in the GAMS Software. An arithmetic media method was applied in order to initialise all of the variables. Objective function (OF) was established as shows equation 32.

$$OF = LS + Sugars - Acetic \quad \text{(eq.32)}$$

After maximising equation 32, the best results obtained in GAMS were: 27.7 g/L of total monosaccharides, 108 g/L of LS and 12.4 g/L of acetic acid, and the best cooking conditions were: 0.628R ^{o}C/min, 1.013 ·T ^{o}C, 1.157t minutes and 7.33 % SO_2.

On the other hand, a minimum of 8.95 g/L of acetic acid was obtained at 0.285R, 1.068 ·T and 1.005t. However, it is preferable to make the detoxification prior to fermentation because it is not desirable to decrease sugar and LS concentration, 27.7 g/L and 108 g/L respectively.

Based on the study of the main cooking parameters affecting the sugar content in the spent liquor collected at the end of digestion stage, it can be concluded that maximum temperature is the most influencing parameter followed by heating ramp. Preserving dissolving pulp quality parameters, total monosaccharides rises from 26.7 g/L (1.01T, 0.196R, 6.20 % of total SO_2) up to 27.2 g/L (1.072T, 0.285R, 6.20 % of total SO_2).

Based on the optimisation results of the cooking trials and considering again dissolving pulp quality parameters, it is possible to achieve 27.7 g/L at 0.628R, 1.013T, 7.33 % of total SO_2.

Total monosaccharides in the spent liquors rises by 1.87 % in the first case (C8 trial) **and 3.75 % in the second case** (multivariate-linear modelling & optimisation). Such little improvements will take place by increasing maximum temperatures, heating rates and in the second case the total SO_2 content which is not cost effective for the P&P mill. The next section will test the external hydrolysis of the WSSL to improve the amount of monosaccharides from an economic viewpoint.

3.2.4 External acid hydrolysis

With the purpose of improving the fermentable sugars, a post-treatment was checked to achieve further depolymerisation of the polysaccharides into sugar monomers which could be fermented into several products (Lenihan et al., 2010; Jiménez et al., 2007). Acid hydrolysis experiments were carried out in the spent sulphite liquor obtained at the end of the evaporation plant (TSSL). Average concentrations of the TSSL were 14.2 g/L glucose, 131.7 g/L xylose, 8.6 g/L arabinose, 10.4 g/L acetic acid and 0.24 g/L furfural.

✓ **Concentrated acid hydrolysis**

Concentrated acid hydrolysis at temperatures in the range of 20-50 ^{o}C and concentrations between 60 and 84 % (w/w) is used to depolymerise cellulose chains (Gírio et al., 2010) which are more recalcitrant and difficult to break down in comparison to hemicellulosic carbohydrates. Sulphuric acid was chosen as the hydrolysing agent because it is the most common acid used for the hydrolysis of lignocellulosic wastes (Cardona et al., 2010; Gírio et al., 2010; Sun and Cheng 2002).

In this work, a factorial design of a total of 16 experiments and 3 central points has been carried out according to the variables shown in the methodology, section 2.6.1. Total sugars have been calculated as the sum of glucose, xylose, arabinose, mannose and galactose. Inhibitors are acetic acid and furfural.

Figure 3.14 illustrates the concentration of sugars, acetic acid and furfural in all of the hydrolysis experiments in relation to the hydrolysis time. Almost all the tests had a deconstruction of monosaccharides indicating that most hemicelluloses and amorphous-cellulose susceptible to hydrolysis had already been hydrolysed inside the digester.

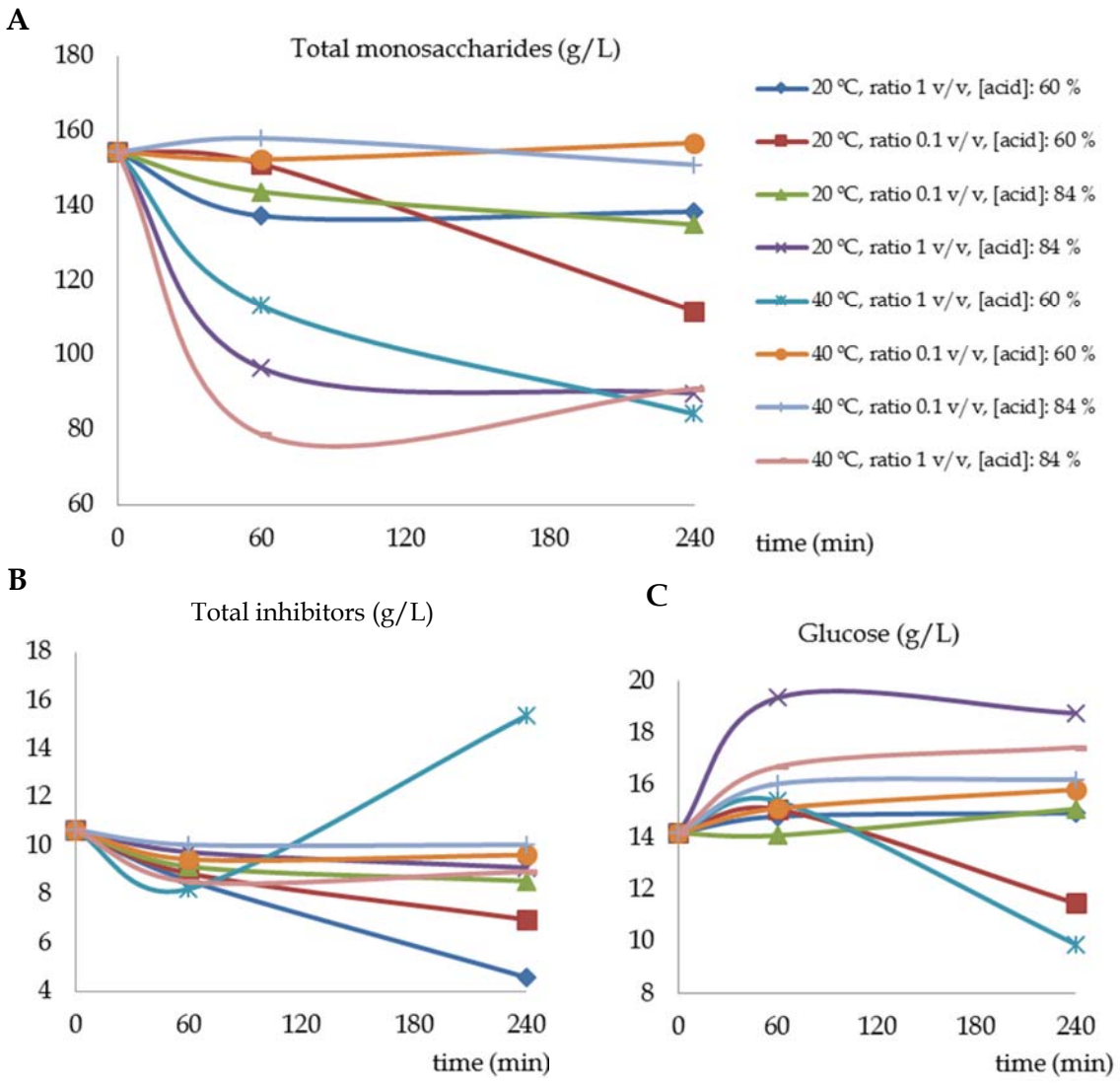


Fig. 3.14 *Curves of concentrated acid hydrolysis experiments under different conditions versus time: (A) Total monosaccharides content; (B) Total inhibitors; (C) Glucose.*

Figure 3.14 contemplates total monosaccharides (Figure 3.14-A) calculated as the sum of glucose, xylose and arabinose; total inhibitors (Figure 3.14-B) calculated as the sum of acetic acid and furfural; and glucose (Figure 3.14-C). Glucose was considered since concentrated hydrolysis is oriented to cellulose depolymerisation. Concentrated hydrolysis attacks high recalcitrant and crystalline polymers in comparison with diluted acid hydrolysis that mostly attacks hemicellulosic sugars. Glucose concentrations increase in the majority of cases. However, xylose and arabinose (C5 sugars) decrease and they contribute to a fall in monosaccharides.

Looking at Figure 3.14-A, total monosaccharides increase from 154.5 g/L up to 156.8 g/L at 40 ºC, acid-to-TSSL ratio of 0.1 v/v, 60 % w/w H_2SO_4, for 240 minutes. In this case, a hydrolysis reaction should be extent due to the fact that glucose and xylose concentrations are still increasing at 240 minutes. Total monosaccharides also increase from 154.5 g/L up to 158.1 g/L at 40 ºC, acid-to-TSSL ratio of 0.1 v/v, 84 % w/w H_2SO_4, for 60 minutes. At 240 minutes, within the same conditions, xylose starts to break down forming inhibitors.

Looking at Figure 3.14-B, equilibrium displacement towards the formation of inhibitors occurs at 40 ºC, acid-to-TSSL ratio of 1 v/v, 60 % w/w H_2SO_4, for 240 minutes were total inhibitors goes from 10.7 g/L to 15.38 g/L due to xylose degradation from 131.7 g/L to 70.1 g/L.

The inhibitor concentration is related to the sugar content with a regression factor of R^2=0.89. In this way, a compromise solution should be found. With the purpose of obtaining high monosaccharide while keeping the inhibitory compounds in their low concentrations, it is necessary to find a more selective hydrolysis method to increase sugar concentration with a reduction of the inhibitor concentration.

A multiple regression analysis was carried out to obtain the relation among all the variables and this is presented in equations 32, 33 and 34.

$$\text{Sugars (g/L)} = 26.5+0.017 \cdot A-13.5 \cdot B-0.0053 \cdot C-0.0029 \cdot D \quad \text{(eq. 32)}$$

$$\text{Acetic acid (g/L)} = 8.4+0.036 \cdot A-4.41 \cdot B+0.0057 \cdot C-0.0006 \cdot D \quad \text{(eq. 33)}$$

$$\text{Furfural (g/L)} = 0.415-0.000624 \cdot A-0.288 \cdot B-0.00105 \cdot C+0.000452 \cdot D \quad \text{(eq. 34)}$$

The regression coefficients were R^2= 92.50 %, 90.79 % and 82.26 %, respectively. Furthermore, the standard error of estimation in the three central experiments has been 1.62 for sugars, 0.59 for inhibitors, and 0.73 for lignosulphonates. Some variables can be removed from the model because they have little influence on the output variable. Therefore, new models are shown in equations 35, 36 and 37.

$$\text{Sugars (g/L)} = 26.2-13.5 \cdot B \qquad R^2 = 93.55\ \% \quad \text{(eq. 35)}$$

$$\text{Acetic acid (g/L)} = 8.82-4.41 \cdot B \qquad R^2 = 92.2\ \% \quad \text{(eq. 36)}$$

$$\text{Furfural (g/L)} = 0.31043-0.28 \cdot B+0.000525 \cdot D \qquad R^2 = 81.50\ \% \quad \text{(eq. 37)}$$

Equations determine that in all cases, total sugars and inhibitors strongly dependend on A/S ratio but not on the hydrolysis time, acid concentration or temperature.

✓ **Diluted acid hydrolysis**

Diluted acid hydrolysis at high temperatures between 80 ºC and 200 ºC and acid concentrations in the range of 0.5 and 8 % (w/w) is focused on hemicellulose depolymerisation (Lenihan et al., 2010; Jiménez et al., 2007). Diluted hydrolysis and steam explosion are the most widely studied methods. However, diluted acid hydrolysis is more advantageous because of the high recovery degree of hemicellulosic sugars, being for instance about 80 % for hardwood feedstocks (Sánchez and Cardona, 2008). An example of diluted acid hydrolysis is the named Saeman hydrolysis that uses acid in the range of 0.4-1.6 % (w/w), 170-190 ºC and 0-90 min (Wyman et al., 2013).

Diluted acid hydrolysis experiments were conducted at H_2SO_4 concentrations between 0.5 and 9.5 % w/w, temperatures in the range of 80 up to 160 ºC, residence times between 60 and 240 minutes and acid-to-TSSL ratios between 0.1 and 10 v/v. A factorial design of a total of 16 experiments and 3 central points has been carried out. All the experiments are shown in section 2.1.7.

The results of total monosaccharides, xylose and total inhibitors are displayed in Figure 3.15. Temperature is the most important variable affecting sugars and inhibitors and the ratio is significant for the total inhibitors. Maximum temperatures of 160 ºC destroyed the total sugar concentration significantly.

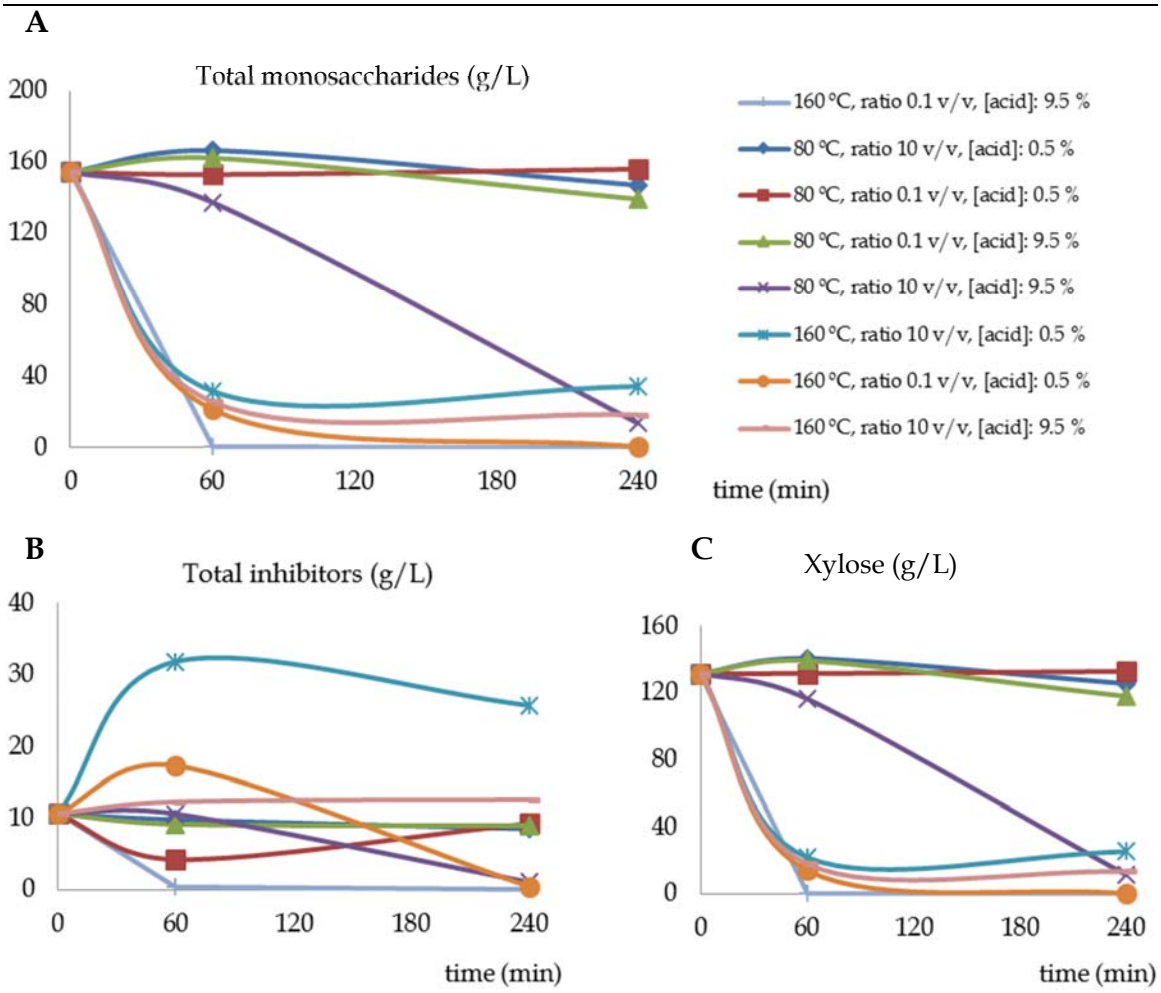


Fig. 3.15 *Curves of diluted acid hydrolysis experiments under different conditions versus time: (A) Total monosaccharides content; (B) Total inhibitors and (C) Xylose.*

Looking at Figure 3.15, experiments performed at 80 ºC obtained increases in total monosaccharide (Figure 3.15-A) and xylose (Figure 3.15-C) contents. The best results increasing the total monosaccharides from 154.5 g/L to 166.7 g/L producing extra xylose (from 131.7 to 140.8 g/L) and glucose (from 14.2 g/L to 17.1 g/L) contents were obtained at 80 ºC, acid-to-TSSL ratio of 10 v/v, 0.5 % w/w H_2SO_4, for 60 minutes.

Regarding the experiments at 160 ºC there is no sugar improvement. In fact, some of the experiments totally destroyed the sugars presented in the TSSL samples. Looking at Figure 3.15-B, the experiment run at 160 ºC, acid-to-TSSL ratio of 10 v/v, 0.5 % w/w H_2SO_4, for 60 minutes registered total inhibitors of 31.9 g/L due to xylose displacement towards furfural. In this case, xylose decreased from 131.7 g/L to 22.0 g/L and furfural increased from 0.25 g/L to 24.78 g/L.

A multiple regression lineal analysis has been done using the Stat graphics software making the adjustment with Cochrane-Orcutt optimisation model, since least-square adjustment had lower regression coefficients than Cochrane Orcutt adjustment.

The relation among all the variables is presented in equations 37 to 39 and simplified in equations 40 to 42 with a confidence interval of 95 %.

$$\text{Sugars (g/L)} = 685.0 - 3.79 \cdot A + 1.74 \cdot B - 4.87 \cdot C - 0.191 \cdot D \quad \text{(eq. 37)}$$

$$\text{Acetic acid (g/L)} = 45.6 - 0.248 \cdot A + 0.754 \cdot B - 0.310 \cdot C + 0.02737 \cdot D \quad \text{(eq. 38)}$$

$$\text{Furfural (g/L)} = 7.55 + 0.046 \cdot A + 0.252 \cdot B - 0.86 \cdot C - 0.0135 \cdot D \quad \text{(eq. 39)}$$

The regression coefficients have been R^2= 52.84 %, 54.73 % and 29.66 %, respectively. Furthermore, the standard error of estimation in the three central experiments has been 1.62 for sugars, 0.59 for inhibitors, and 0.73 for lignosulphonates. Some variables can be removed from the model because they have little influence on the output variable (se new models in equations 40, 41 and 42).

$$\text{Sugars (g/L)} = 605.9 - 3.26 \cdot A \qquad R^2 = 38.29\ \% \quad \text{(eq. 40)}$$

$$\text{Inhibitors (g/L)} = 43.77 - 0.228 \cdot A + 0.0769 \cdot B \qquad R^2 = 41.08\ \% \quad \text{(eq. 41)}$$

$$\text{Furfural (g/L)} = 12.89 - 0.922 \cdot C \qquad R^2 = 24.56\ \% \quad \text{(eq. 42)}$$

Based on the results of the mass balance of the three wood macrocomponents, 87.2 % of hemicellulose provided from the *Eucalyptus globulus* feedstock is in the spent liquor. **External concentrated and diluted acid hydrolysis** was studied in order to increase the total monosaccharides presented in such liquors. Acid concentration, temperature and acid-to-sample ratio were modified in both concentrated and diluted acid hydrolysis using factorial design analysis. The best results were obtained using 0.5 % w/w diluted sulphuric acid at 80 ºC, acid-to-TSSL ratio of 10 v/v, for 60 minutes. Under these conditions, **total monosaccharides increased by 7.9** % (from 154.5 g/L to 166.7 g/L) caused mainly by xylose growth. Concentrated acid hydrolysis only increases 2.3 % of total monosaccharides in the TSSL and therefore is not worthwile.

3.2.5 Detoxification techniques for inhibitors removal

Detoxification is the following step in the LCB processing. It is required for sugar bioconversion and hydrolysate valorisation within the biorefinery concept. In this section, detoxification of the hydrolysate by means of evaporation and other phydrico-chemical processes was studied. Overliming (OV), cation exchange resins (CR), anion exchange resins (AR), adsorption with activated charcoal (AC), adsorption with black carbon (BC) and liquid-liquid (L-L) organic solvent extraction were studied to separate the sugars from the rest of by-products and fermenting inhibitors.

✓ Pretreatment of the hydrolysate by evaporation

Concentration by evaporation is considered as a previous detoxification stage, removing most volatile compounds. In the factory, there are five effects working in series in order to concentrate the SSL. Two kinds of streams are generated at the end of every effect: condensates and concentrates. Condensates (light phase) are the fraction rich in volatile compounds. Such condensates are currently being reintroduced into the accumulators for fresh liquor SO_2 enrichment in the factory. At the end of the process, the last concentrate stream is directly sent to another enterprise where the LS are reused and the sugars are burned.

The industrial plant was reproduced at laboratory scale in a rotatory evaporator according to the experiments described in section 2.7.1. WSSL from the factory was used as raw material. Average concentrations of the industrial WSSL samples taken for the evaporation experiments were 47.3 g/L of LS, 26.7 g/L of total monosaccharides, 5.43 g/L of metals, 8.33 g/L of acetic acid, 2.27 g/L of phenolic hydroxyl groups, 0.17 g/L of HMF and 0.35 g/L of furfural.

Results of the evaporation at laboratory scale were similar to the industrial plant. Sugars increase from 28.9 g/L to 61.0 g/L; LS also increase from 72.3 g/L to 412 g/L in the last effect. The pH increases throughout the evaporation process from 1.8 to 2.3 due to combined SO_2, water, methanol and acetic acid were partially removed. Results of the inhibitors appear in Table 3.8. They are expressed as percentage removals being HAcR, MetOHR, coSO_2R and TIR, acetic acid, methanol, combined SO_2 and total inhibitor removals respectively. Acetic removals reached 53 % in the last effect whereas 95.3 % MetOHR and 28.6 % coSO_2R were the maximum removals of methanol and combined SO_2 respectively.

Table 3.8 *Removals and concentrations of SSL within five evaporation effects.*

Removals in %	1st effect	2nd effect	3th effect	4th effect	5th effect
HAcR	6.3	8.9	12.7	19.6	53.0
MetOHR	61.6	23.8	95.3	30.8	14.6
coSO_2R	28.6	7.1	11.4	20.0	6.3
TIR	4.4	4.0	4.0	4.3	8.6

Analysis of furfurals, formic and levulinic acids was undertaken but instead of removals, such woody constituents were concentrated throughout the evaporation effects giving increases of concentration of 37.2 % (furfurals), 26.0 % (formic acid) and 53.1 % (levulinic acid) at the outlet of the fifth effect. Consequently, evaporation gets to remove some critical inhibitors but there are other woody constituents that still remain in the spent liquor and have to be removed before fermentation.

Based on Table 3.8, it can be said that evaporation fulfilled a double objective. Evaporation can be considered as a preliminary detoxification stage with total inhibitor removals of 8.61 % at the last effect. Thus, evaporation is pretreatment where not only LS and sugars increased but also several volatile toxic inhibitors i.e. SO_2, methanol or acetic acid are totally or partially separated. For these reasons, the concentration stream of the fifth effect (TSSL) constitutes the most suitable fraction for the hydrolysate detoxification and valorisation.

✓ **Detoxification by overliming**

Once the evaporation has been carried out, other separation techniques were studied. In all cases and due to the conclusion in the previous section, TSSL from the last evaporation effect of the industrial process has been detoxified. Average concentrations of the industrial TSSL samples taken for the rest of detoxification experiments were 422 g/L of LS, 214 g/L of total monosaccharides, 28.16 g/L of metals, 7.85 g/L of acetic acid, 12.6 g/L of phenolic hydroxyl groups, 0.12 g/L of HMF and 0.17 g/L of furfural.

Precipitation of LS from SSL can be achieved by addition of lime or other alkali, forming the insoluble calcium lignosulphonate compounds according to the following reaction:

$$(\text{Lignin-SO}_3)_2\text{Ca}+\text{Ca(OH)}_2 \rightarrow (\text{Lignin-SO}_3)_2\text{Ca}\cdot\text{Ca(OH)}_2\downarrow \quad \text{(eq.38)}$$

Furthermore, the insoluble lignin is also contained on the precipitate. Alkali treatment has been reported to be one of the best and most common detoxification methods because of its low cost and high efficiency (Millati et al., 2002; Persson et al., 2002-a). Unlike neutralisation, which consists of working within a pH range of 5 and 7, overliming can raise the pH from 10 to 12. In addition to the removal of LS, this effect promotes the precipitation of low molecular weight phenolics and furfurals. Besides, some carboxylic acids with pKa 3-4 are converted to salts, which decrease their ability to form hydrogen bonds with the lignin network (Koivula et al., 2011).

The effect of the kind of alkali was studied firstly. On one hand, $Ca(OH)_2$ was employed as a neutralizing agent which is the most commonly used (Millati et al., 2002; Persson et al., 2002-a). On the other hand, NH_4OH was selected according to Xavier et al. (2010). According to literature (Xavier et al., 2010; Millati et al., 2002; Persson et al., 2002-a), all of the experiments were carried out at 25 ºC, pH of 10 and 30 minutes. Results of the effect of the kinds of alkali showed that LS removals were higher using $Ca(OH)_2$ in comparison to NH_4OH giving values of 36.5 % and 13.3 % respectively. Nevertheless, sugar losses were slightly higher in the case of $Ca(OH)_2$ giving values of 11.1 % instead of 5.5 % using NH_4OH due to the link between sugars and calcium lignosulphonate.

Overliming has a drawback in terms of sugar losses. The main variables that can drastically contribute to sugar losses are high values of pH, temperatures or residence times. Based on literature, moderate temperatures below 60ºC and pH under 12 are recommended in order to avoid high sugar losses (Millati et al., 2002). In this work, the influence of all of these parameters was studied. In all cases, the same alkali, 0.5 M $Ca(OH)_2$, was used due to previous results and availability.

The effect of time and temperature is plotted in Figures 3.16-A and Figure 3.16-B. Error bars are represented in the figures. These experiments were performed holding the pH at 10 which is the most commonly used in overliming (Millati et al., 2002). Due to the fact that overliming mainly affects the LS removal, only the concentration of these compounds and sugars has been represented. According to the figure and as was expected, both LS and sugars decrease by increasing temperature, reaching a minimum at 5 ºC with 218.7 g/L of LS and 174.2 g/L of sugars. Furthermore, the rest of inhibitors had a similar behaviour, phenolics

decreased from 12.60 g/L to average values of 7.57 g/L regardless of the temperature checked, and only a small decrease in the concentration of acetic acid, from 7.85 to 7.80 g/L, and furfurals, from 0.27 to 0.20 g/L, was obtained.

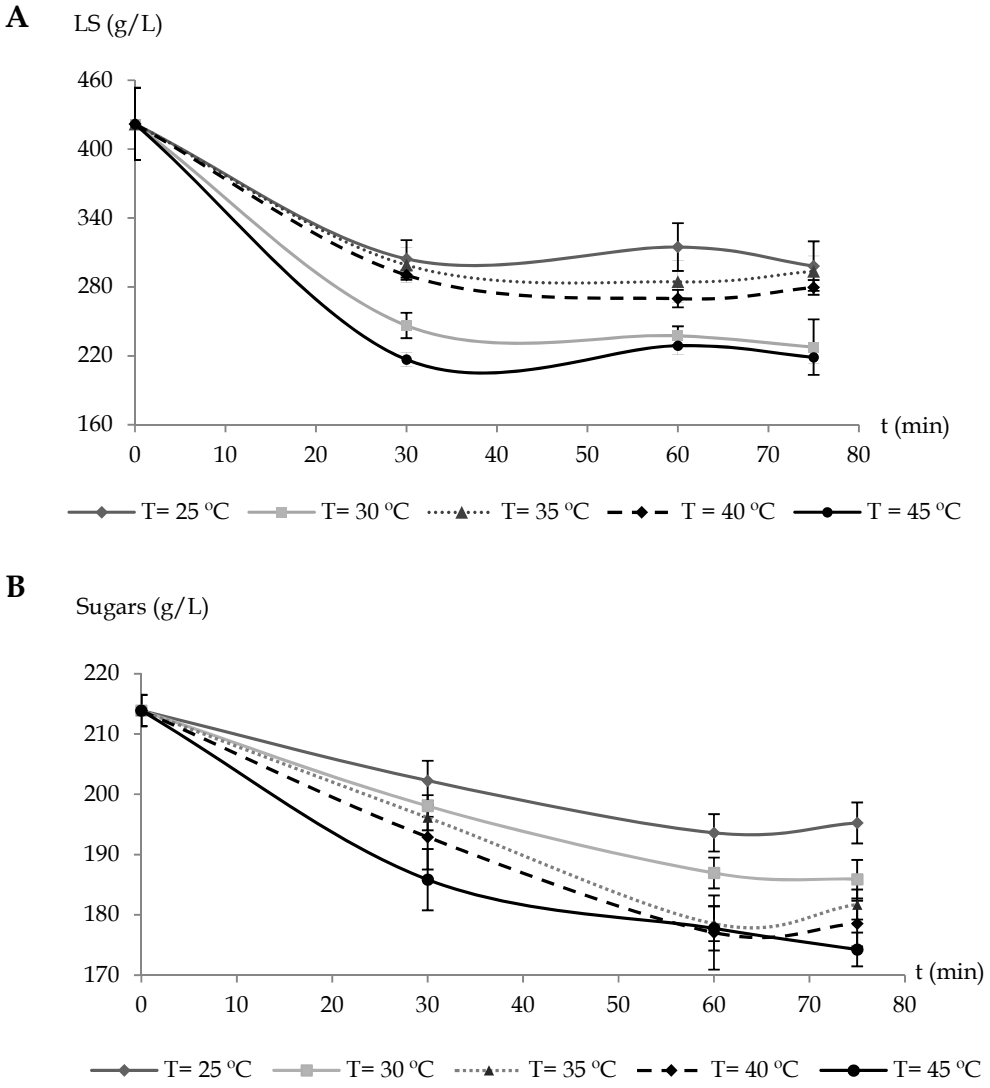


Fig. 3.16 *(A) Variation in time and temperature over LS removals; (B) Variation in time and temperature over sugars.*

Once the alkali and temperature were considered, the next step was to study the initial pH value. Experiments under equilibrium at 25 ºC were plotted in Figure 3.17-A and Figure 3.17-B. An increase in pH enhances detoxification and therefore

increases the fermentability of the hydrolysates. Maximum LS precipitation occurs at pH 10 where the LS decrease from 421.9 to 260.4 g/L, phenolics from 12.60 to 8.0 g/L and furfurals from 0.27 to 0.076 g/L. However, acetic acid maintained its concentration at 7.80 g/L. The effect of pH is not as drastic as the effect of temperature. The treatment at 25 ^{o}C and pH 10 resulted in a better fermentability regarding sugar concentration and LS precipitation.

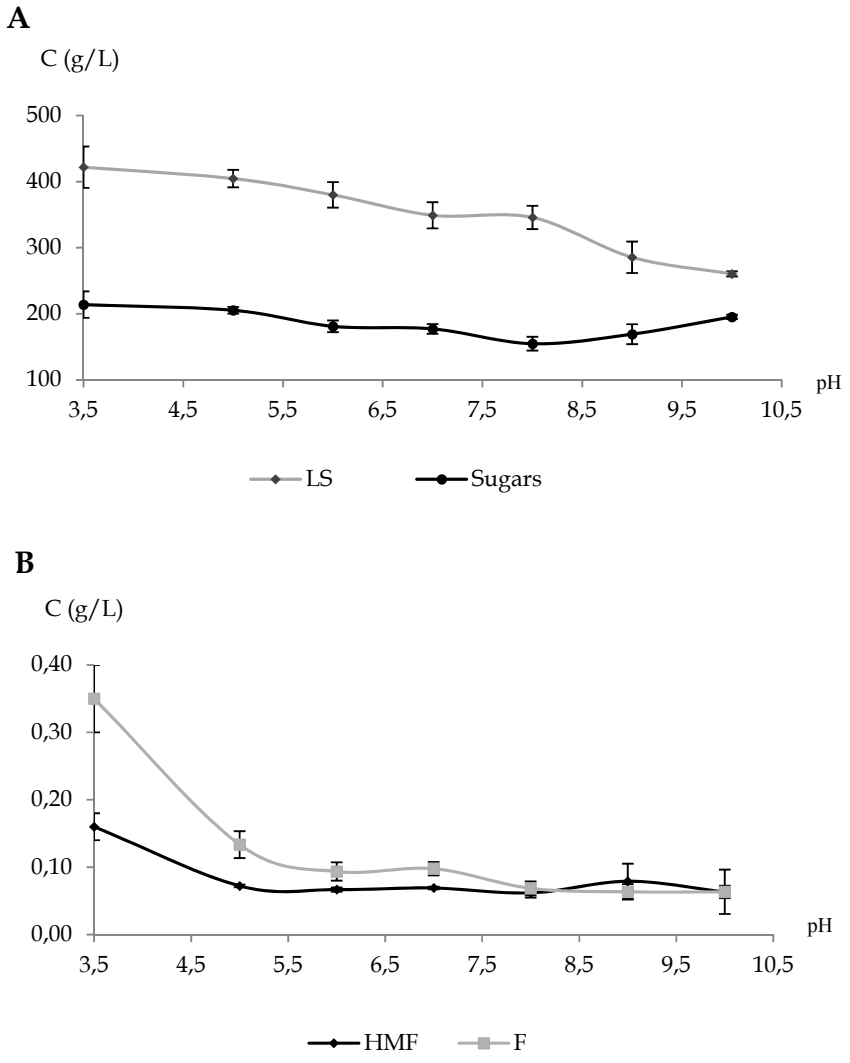


Fig. 3.17 (A) *Effect of pH over LS and sugar; (D) Effect of pH over furfural concentration.*

✓ **Detoxification by adsorption**

Adsorption enables the separation of selected organic compounds from aqueous streams. Among the advantages, its design simplicity, operation and scale up, high capacity and favourable rate, insensitivity to toxic substances, easy regeneration and low cost can all be highlighted. Additionally, adsorption avoids using toxic solvents and minimises degradation (Soto et al., 2011).

In this work, activated charcoal (AC) and black carbon (BC) were used as adsorbents. In both cases, the effect of the time was studied firstly. Because the shape of curves is quite similar in all cases studied, Figure 3.18 shows only the experiments using BC at 50 ºC, 1:5 w/v. Error bars are also represented in both figures. Furfurals were not plotted because separation took place almost immediately. Selectivity of AC and BC towards the components analysed is, in descending order: furfurals, acetic acid, phenolics, and LS. In all cases, saturation times were reached so fast (before 20 minutes) regardless of the adsorbent, temperature or ratio employed.

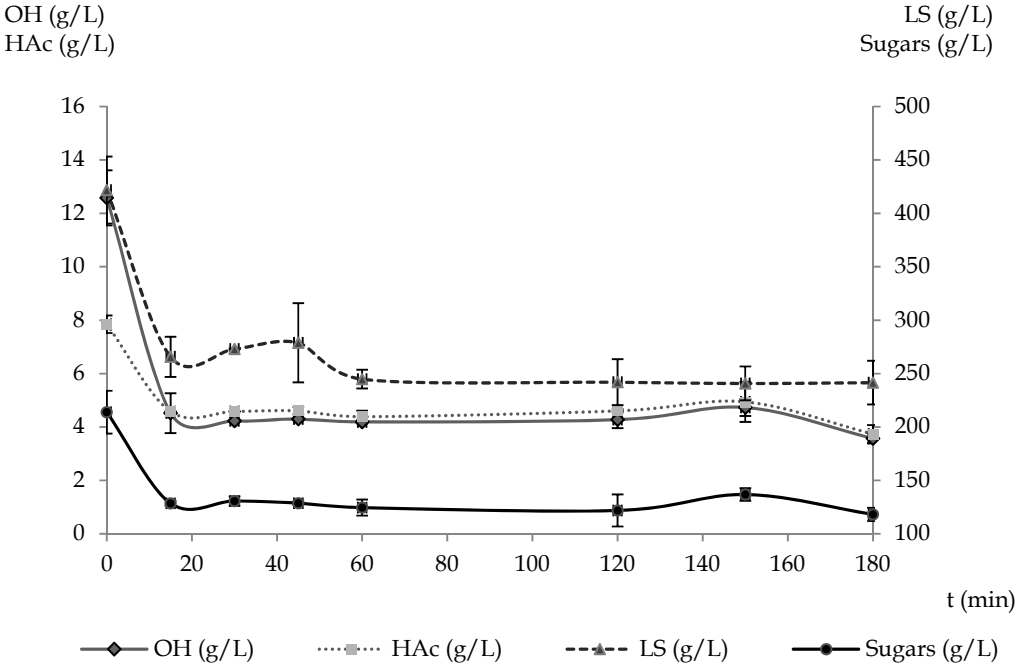


Fig.3.18 *Concentration of sugars and inhibitors over time, using BC at 50ºC and 1:10 w/v ratio.*

Once the equilibrium was reached, final concentrations and consequently maximum removals are summarised in Table 3.9 at different temperatures, adsorbents and sample-to-adsorbent ratios. The two adsorbents (AC and BC) at 30 and 50ºC and ratios of 1:10 and 1:5 grams of adsorbent per mL of SSL were considered based on literature (Lanka et al., 2011; Lee et al., 2011).

Among the two adsorbents, BC seems to be the most suitable in terms of low molecular phenolic and LS removals. The affinity of BC towards toxic substances contemplated in this research is higher than using AC, and therefore the adsorption efficiency is better using BC. Another advantage of BC with respect to AC is related to the sugar losses. Despite the fact that sugar losses are inherent to any detoxification technique as they are chemically bonded to the LS structure, sugar losses were lower using BC in comparison to AC under the same conditions. The only toxic substance, which is better removed by AC, is the acetic acid working at 1:5 w/v either at 30 or 50 ºC.

Considering the results appearing in Table 3.9, maximum LS, phenolic, acetic and furfural removals of 76.2 %, 71.4 %, 77.1 % and 100 % were achieved under conditions checked. These results give better results of furfurals and weak acids in comparison to Guo et al. (2013) and Lee et al. (2011) who removed 94-96 % of furfurals, 14-28 % of acetic acid or 88 % of total phenolics.

Table 3.9 *Detoxification by means of adsorption: influence of temperature, adsorbent and ratio.*

	LS (g/L)	OH (g/L)	HAc (g/L)	Furfurals (g/L)	Sugars (g/L)
TSSL	**422±31.4**	**12.6±1.03**	**7.85±0.33**	**0.27±0.19**	**214±20.1**
BC 30ºC 1:10 w/v	231±4.59	5.24±0.96	3.57±1.32	<DL [a]	119±22.7
BC 50ºC 1:10 w/v	218±54.6	3.69±0.12	2.93±0.08	<DL	77.5±0.59
BC 30ºC 1:5 w/v	112±0.6	3.63±0.13	4.14±0.04	<DL	99.7±1.35
BC 50ºC 1:5 w/v	100±9.80	4.19±0.37	4.49±0.37	<DL	125±9.03
AC 30ºC 1:10 w/v	271±52.1	7.70±1.25	5.03±1.35	<DL	117±25.3
AC 50ºC 1:10 w/v	228±25.4	6.31±0.07	3.27±0.50	<DL	104±28.4
AC 30ºC 1:5 w/v	193±16.1	4.90±0.06	1.96±0.09	<DL	94.5±15.1
AC 50ºC 1:5 w/v	193±15.14	5.21±0.20	1.76±0.11	<DL	86.3±5.09

✓ **Detoxification by ion exchange resins**

According to the results of Fernandes et al. (2012), two resins, Dowex 50WX2 cationic resin (CR) and Amberlite IRA-96 anionic resin (AR), were used. CR separates mainly Ca^{+2} and Mg^{+2} while AR removes mainly LS, phenolics and acetic acid. 18 experiments were carried out working with only one single resin (AR & CR) and working in series. Table 3.10 presents the main results under the conditions tested once the equilibrium was given. The first 7 experiments were developed with AR, using 1.2 mL/g to 60 mL/g of SSL/AR ratio. In the case of CR, 7 experiments from 0.67 mL/g to 60 mL/g were used. Moreover, four sets of experiments were done working in series: Set I consists of CR at 3mL SSL/g.wet resin followed by AR treatment at 2mL SSL/g wet resin; set II was run with CR at 3 mL/g and AR at 6 mL/g; Set III using ratios of 5 mL/g CR and 6 mL/g AR; and finally, Set IV at 5 mL/g CR and 8 mL/g AR.

CR working at 0.67 mL/g exhibited metal removals of Ca^{+2} 98.8 % and 99.7 % of Mg^{+2} in one single fractionation step. Similar results were obtained by Fernandes et al. (2012) after two-step fractionation working in series. AR has an amine group that can selectively adsorb anions with the following selective order: $OH^- < CH_3COO^- < Cl^- < HSO_3^- < HSO_4^-$. This fact reveals that major inhibitor losses were produced in the case of LS and phenolics. Major removals were registered using AR at 1.5mL/g: 96.1 % of LS, 98.1 % of OH, 61.3 % of acetic acid, 65.0 % metals and 100 % of furfurals were trapped by AR.

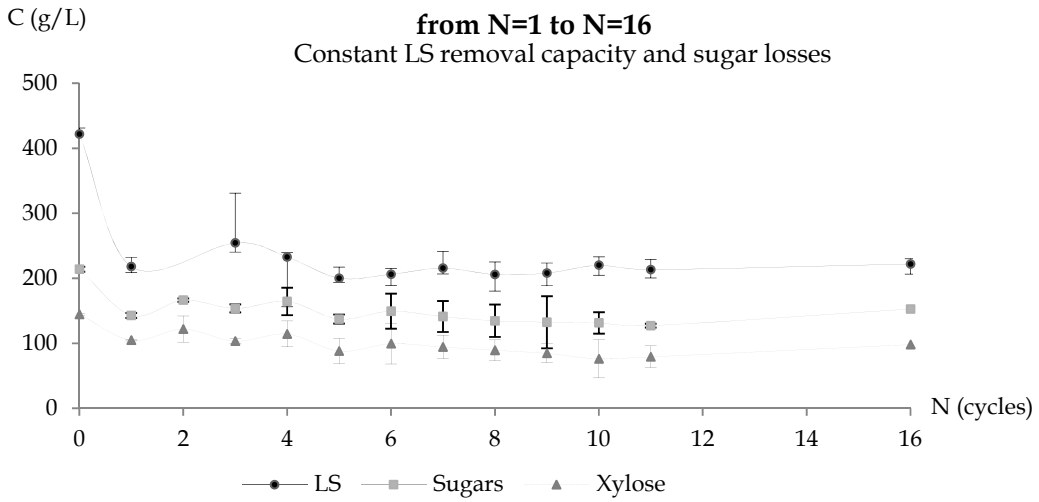
The results of ion exchange were better than the rest of detoxification processes, especially considering the high initial amounts of LS in the SSL. Takahashi et al. (2013) observed that the presence of LS hinders the ability of acetic acid removals by strong base resins. 120 g/L of LS decreased the efficiency of acetic removals from 98 % to only 30 % (Takahashi et al., 2013). Looking at phenolic removals, the results decrease from 12.6±1.03 g/L towards 0.26±0.05 g/L. by Nilvebrant et al. (2001) and exhibited phenolic losses of 21 % from 3.7 g/L to 0.77 g/L.

Table 3.10 *Ion exchange resin experiments at laboratory scale.*

	Ca (g/L)	Mg (g/L)	LS (g/L)	OH (g/L)	HAc (g/L)	Furfurals (g/L)	Sugars (g/L)
TSSL	**14.6±0.74**	**10.0±0.79**	**422±31.4**	**12.6±1.03**	**7.85±0.33**	**0.27±0.19**	**214±20.1**
AR 1.2 mL/g	6.18±0.08	4.77±0.70	13.7±1.12	0.16±0.03	2.72±0.13	<DL	71.9±1.20
AR1.5 mL/g	4.51±1.28	4.1±0.12	15.9±3.3	0.26±0.05	3.04±0.47	<DL	78.4±13.3
AR 2 mL/g	1.59±0.22	7.51±0.96	57.1±15.4	1.29±0.33	4.60±0.27	<DL	128±7.60
AR 3 mL/g	2.22±0.31	8.27±1.42	95.0±5.29	2.84±0.37	4.60±0.33	<DL	128±11.1
AR 6 mL/g	3.96±0.55	9.74±0.10	159.9±5.4	5.69±0.06	5.87±0.34	<DL	180±4.14
AR 30 mL/g	12.55±1.30	11.9±0.36	326±18.1	7.99±3.26	7.14±1.41	<DL	170±41.0
AR 60 mL/g	14.07±2.15	10.9±0.20	341±9.77	9.90±0.60	7.44±0.48	<DL	181±3.46
CR 0.67 mL/g	0.18±0.01	0.028±0.01	99.5±9.00	5.69±0.50	1.11±0.14	<DL	31.3±2.33
CR 1.33 mL/g	0.32±0.02	0.20±0.01	209±19.7	5.84±0.66	3.15±0.24	<DL	83.7±5.92
CR 2 mL/g	0.55±0.02	0.33±0.01	233±1.96	6.02±0.02	3.49±0.17	<DL	91.7±7.24
CR 3 mL/g	1.28±0.10	0.41±0.09	272±6.49	9.28±0.50	5.16±0.07	<DL	134±2.75
CR 4 mL/g	1.32±0.08	0.94±0.19	281±12.7	11.7±1.3	5.34±0.21	<DL	143±9.18
CR 30 mL/g	12±0.33	7.93±1.05	354±0.05	12.5±2.40	7.67±0.74	<DL	189±23.9
CR 60 mL/g	11.48±3.10	9.6±1.73	367±0.06	10.84±1.76	7.65±0.72	<DL	189±24.3
Series-I	0.34±0.05	0.53±0.02	4.3±1.30	<DL	0.63±0.05	<DL	80.5±8.77
Series-II	1.04±0.03	0.97±0.06	90.8±4.40	1.80±0.06	0.81±0.37	<DL	131±16.7
Series-III	0.81±0.01	1.69±0.03	99.5±3.96	2.03±0.03	1.31±0.02	<DL	157±5.56
Series-IV	0.85±0.05	1.67±0.05	121±15.9	1.91±0.59	1.4±0.09	<DL	162±29.5

One of the advantages of ion exchange resins is their ability to be regenerated, recovering the LS and phenolics. Because of the conclusions of the results, the ability of the AR to be reused after cleaning and regenerating was determined. Figures 3.19-A and 3.19-B show the obtained results by subjecting the AR to 16 successive cycles. Regarding recovery efficiency of LS, acetic acid and furfurals (Figure 3.19-A), 16 cycles do not affect the selectivity of the AR towards LS, acetic acid and furfurals. However, low molecular phenolic efficiency decreases after seven cycles (Figure 3.19-B). Due to the fact that these compounds can be recovered from the regenerated solution for valorisation purposes because of their strong antioxidant capacities (Alexandri et al., 2014), six cycles of regeneration are recommended in this work.

A



B

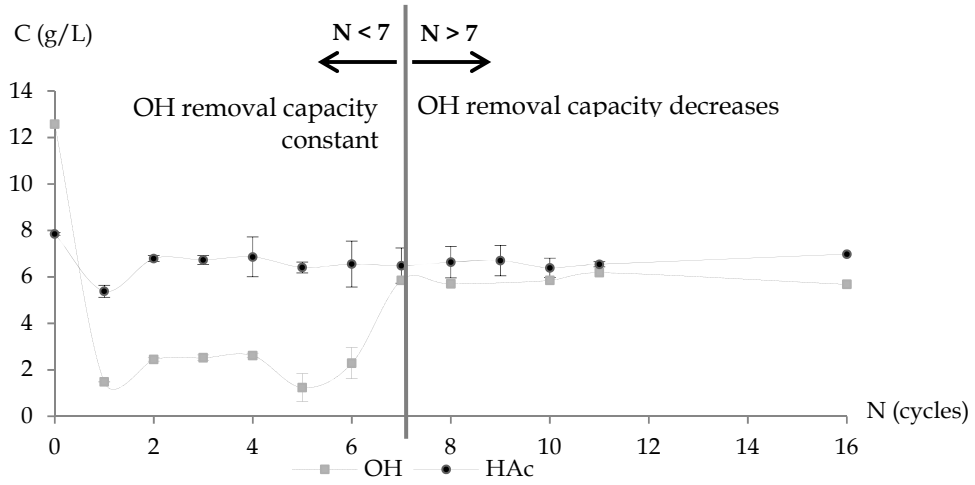


Fig.3.19 *Sequential use of anionic resins: (A) Evolution of LS and sugars; (B) Evolution of phenolics and acetic acid.*

✓ **Liquid-liquid extraction for phenolics removal**

The main target of this work was to investigate solvent extraction parameters leading to the highest phenolic extraction from the SSL. The removal of phenolics from the SSL enhances the microbial fermentation of the hemicellulosic hydrolysate (Alexandri et al., 2014; Palmqvist and Hahn-Hägerdal, 2000; Parajó et al., 1998), being a useful alternative to convert the traditional pulping manufacture concept into forestry biorefineries.

Different solvents and extraction parameters (number of extraction stages, settling time, initial pH, and sample-to-solvent ratios) were evaluated based on the total phenolic content (TPC), antioxidant activity (AAI) and individual phenolic content (IPC) results.

The obtained results of TPC (%) and AAI are shown in Figure 3.20. These results indicate that the recovery of total phenolics is better in all cases when no acidification is carried out at pH of 3.6. Similar results were found in literature using ethyl acetate in the extraction of olive tree hydrolysates. Castro et al. (2008) achieved phenolic extraction from 42 up to 57 %. In this research, maximum phenolic recovery of 50.89 % has been attained using diethyl ether. Not only the amount of phenolics recovered in every extraction (Figure 3.20-A), but also the quality of the extract based on their AAI (Figure 3.20-B) was evaluated. AAI values ranged from 1.44 to 4.64 and from 0.55 to 5.37 at pH 2 and 3.6 respectively, showing strong antioxidant activities in all cases at pH 3.6, apart from benzene extracts.

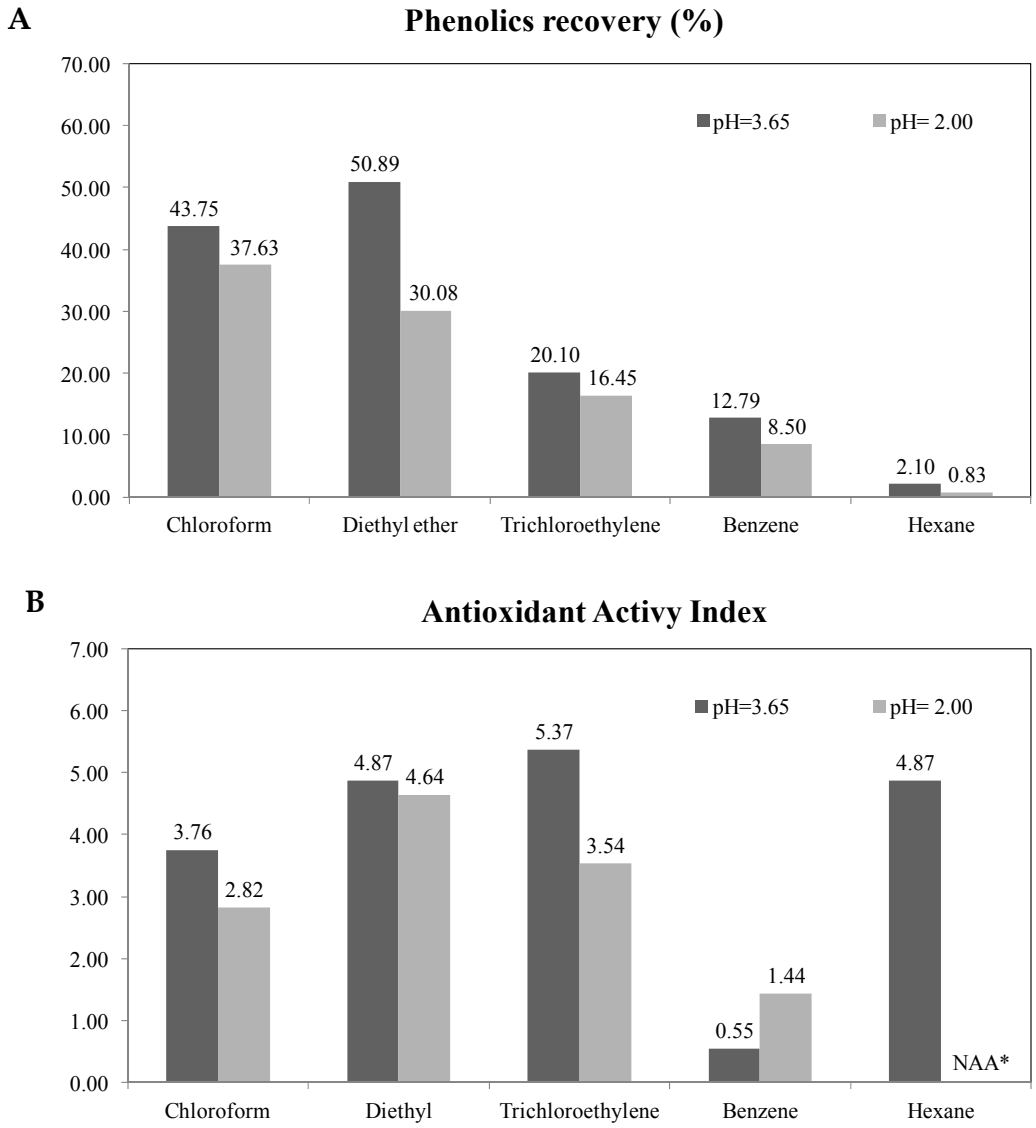


Fig. 3.20 *Results at two different pH values (A) Effect of solvents on phenolic recovery; (B)Effect of solvents on antioxidant activity.*NAA = not antioxidant activity registered (under the detection limits).*

Cruz et al. (1999) concluded that phenolics derived from *Eucalyptus globulus* present high antioxidant and antimicrobial activities. Faustino et al. (2010) determined the radical scavenging capacity of kraft and sulphite liquors extracted with ethyl acetate at 1:3 v/v sample-to-solvent ratio giving AAI of 3.29 ± 0.35 at pH 2 for sulphite liquors and 3.41 ± 0.33 at pH 6 for black liquors. Looking at Figure 3.14-B, the best

results were obtained at the original liquor pH (3.6) giving higher antioxidant activities with a maximum in trichloroethylene extracts (AAI = 5.37).

According to the criteria established by Scherer and Godoy (2009) with values of AAI lower than 0.5, the extract has poor antioxidant activity, increasing to moderate when AAI is between 0.5 and 1, strong activity with 1 <AAI< 2, and very strong when AAI is higher than 2. Based on this, the extracts show strong antioxidant activity and can be further valorised. The only exception appeared with benzene at two pH assays, presenting poor (0.56) and moderate-strong (1.45) antioxidant activities.

The results of TPC and AAI demonstrate that chloroform and diethyl ether are the most efficient solvents among those that were tested. As Figure 3.21 indicates, there is a liner regression between the TPC and AAI as well as TPC and IPC expressed as the sum of individual phenolics obtained by HPLC/DAD. According to this, the effect of settling time, initial pH and sample-to-solvent ratio have been studied measuring the TPC in the extracts (see Table 3.10).

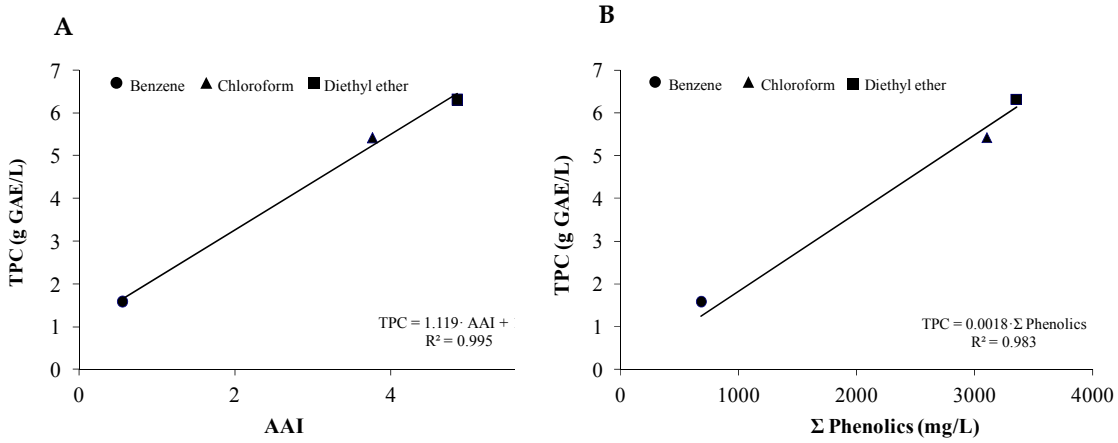


Fig. 3.21 *Linear correlations using benzene, chloroform and diethyl ether (A) TPC and AAI correlation; (B) TPC and ITP correlation.*

Settling time was also studied and the results highlighted that equilibrium was reached in a few minutes regardless of the sample-to-solvent ratio. Thirty minutes was more than enough to reach the equilibrium. Similar results were also found in literature. Thirty minutes were considered sufficient for the whole extraction of eucalypt hydrolysates (Faustino et al., 2010; González et al., 2004; Cruz et al., 1999).

In Table 3.11, the initial pH ranging from strong acidic (1.0) to semi-neutral (6.5) was evaluated together with the ratio for the best two solvents.

Table 3.11 *Effect of L-L solvent extraction variables in the two best solvents on the TPC results.*

Experiments at room T, 1:3 v/v ratio, 30 min settling time						
Input variable	pH	1.05	2.00	3.65	4.5	6.5
TPC (g GAE/L.TSSL)	chloroform	3.2	4.67	5.42	5.25	0.65
TPC (g GAE/L.TSSL)	diethyl ether	3.37	3.73	6.31	2.08	2.02
Experiments at room T, pH 3.65, 30 min settling time						
Input variable	ratio v/v	1:1	1:2	1:3	1:5	Economically not worth continuing
TPC (g GAE/L.TSSL)	chloroform	4.63	4.62	5.42	5.59	
TPC (g GAE/L.TSSL)	diethyl ether	3.72	2.94	6.31	6.23	

Regarding sample-to-solvent ratio, the experimental assays indicate that the ratio 1:3 (v/v) extracts the available phenolic fraction as was corroborated by Cruz et al. (2005). Such results were compared to other authors that also observed this behaviour (Faustino et al., 2010; González et al., 2004; Cruz et al., 1999).

Finally, the optimum number L-L extraction of stages was also contemplated at the best conditions found in this work. The flowsheet of the number stage study is represented in Figure 3.22, adding a graph bar with the TPC recovery after sequential extraction. From the results given, it is not worth extending the number of stages. The cost surrounding the equipment, the energy employed and the residence time increases whereas separation effectiveness decrease from 84 % to 52.61 % in one step and up to 8.64 and 8.66 % for steps two and three respectively. Cruz et al. (2005) obtained TPC recoveries of 75.4 % with small improvements (8.7 %) by coupling an additional extraction step.

L-L extraction with diethyl ether (one single step, 1:3 v/v, without pH adjustment at room temperature) led to sufficient extraction of the phenolic compounds from the SSL. The TPC in the extract recovered was of 6.31 g.GAE/L.SSLwith strong AAI of 4.87. Moreover, 18.46 % HMF, 12.5 % furfural and 41.7 % of acetic acid were also removed from the aqueous phase with minor sugar losses (0.72 %). As the crude

extract obtained presented very strong antioxidant activity, it is worthwhile evaluating it further as a value-added co-product.

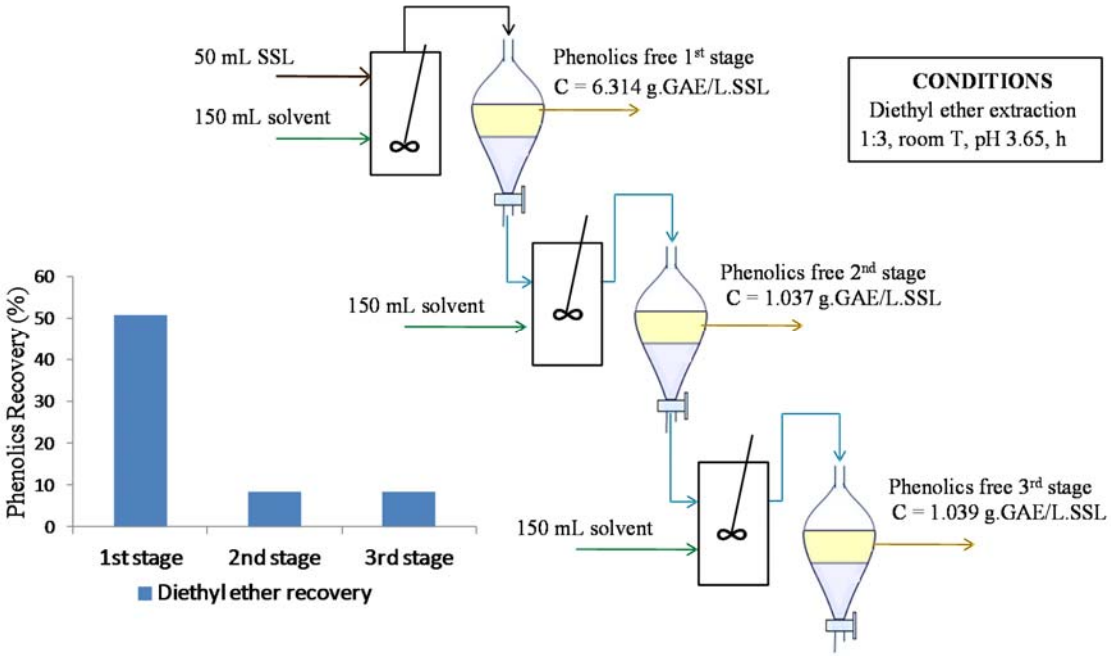


Fig. 3.22 *Sequential L-L extraction and effects of the stage number on the TPC.*

The comprehension and discussion of the HPLC-DAD results have a special interest considering that phenolic hydroxyl groups vary significantly among hardwood species (Lai and Guo, 1991). Similar phenolics from literature were found with the presence of low molecular weight phenolic compounds like acids (gallic, vanillic and ellagic), aldehydes (syringaldehyde and sinapaldehyde) and flavonols (naringenin and quercetin) as well as ferulic acid, homovanillyl alcohol and syringic acid recently detected in *Eucalyptus globulus* species (Marqués et al., 2009; González et al., 2004). These kind of phenolic compounds are reported to have higher anti-oxidant and anti-microbial activity than others (Cruz et al., 2005). In addition, the major contribution of gallic acid and syringic acid in sulphite liquors was confirmed by Marqués et al. (2009).

Among the phenolics found in lignocellulosic hydrolysates from either pine, oak, willow, wheat straw, bagasse, poplar, corn stover or switch grass were typically 4-hydroxybenzoic acid, vanillin, dihydroconiferyl alcohol, coniferyl aldehyde, syringaldehyde, and syringic acid (Klinke et al., 2004). Typically, 4-hydroxybenzoic

acid, ferulic acid and guaiacol are the most common phenolic compounds of lignocellulosic hydrolysates (Chandel et al., 2013). Besides, p-hydroxybenzoic acid, m-hydroxybenzoic acid, vanillic acid, syringic acid, p-hydroxybenzaldehyde, vanillin and cinnamic acid were detected in steam-exploded poplar (Parajó et al., 1998). The specific individual phenolics found in chloroform and diethylene extracts are shown is Figure 3.23.

HPLC-DAD results complete the information about the presence of strong antioxidants in the extracts. The structure of phenolic compounds is a key factor of their radical scavenging activity and this is referred to as structure-activity relationships (Scherer and Godoy, 2009). For instance, the AAI of phenolic acids increases with increasing degree of hydroxylation. It was also found that substitution of the hydroxyl group by methoxyl groups reduces the activity. In this study, the main individual phenolics found in the extracts have a high degree of hydroxylation. The structure-activity relationships of flavonoids such as quercetin, luteolin, naringenin, rutin or isoharmnetin are more complicated. In this case, the degree of hydroxylation as well as the position of the hydroxyl groups increase the radical scavenging capacity of flavonoids (Scherer and Godoy, 2009).

Regarding their toxicity towards microorganisms, their inhibition is thought to be proportional to their molecular weights. The lower molecular weight compounds (in this case simple phenolics, acids and cinnamic acids) are generally more inhibitory to the microorganisms than higher molecular weight compounds (i.e. flavonoids like quercetin, luteolin, rutin, naringenin or isorhamnetin) (Chandel et al., 2013). According to the phenolic analysis by HPLC-DAD in conjunction with TPC and AAI, diethyl ether and chloroform extract the highest amounts of phenolic compounds.

The phenolic compounds identified have a high antioxidant activity; therefore, they can be used in the manufacture of adhesives, composites, polymer synthesis or protectors in wood surfaces. A comprehensive study of Bjørsvik et al. (2002) presents some first-, second- and third generation fine chemicals produced via lignin oxidation, playing an important role in the synthesis of a variety of pharmaceutical chemicals i.e. cyclovalone, etamivan and levodopa. SSL oxidation affords a large variety of compounds like vanillin, vanillic acid, acetovanillone, p-hydroxybenzaldehyde, syringaldehyde, syringic acid, 5-formyvanillin, 5-formylvanillic acid, 5-carboxyvanillin, dehydrodivanillin, dehydrodivanillic acid and guaiacol that could be used as substrates for new drugs and other fine chemicals.

Phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents and range from simple molecules to highly polymerised compounds. Among the phenolic classifications appearing in literature, the Vermerris and Nicholson (2008) criteria used is based on the number of carbons in the molecule. Phenolics derived from *Eucalyptus globulus* hydrolysates and dissolved into the SSL can be divided into: (i) simple phenolics like 1,2-dihydroxybenzene; (ii) phenolic acids such as gallic acid, p-hydroxybenzoic acid or vanillic acid and phenolic aldehydes with the presence of an aldehyde group substituted on a phenol like 4-hydroxybenzaldehyde and syringaldehyde; (iii) cinnamic acids like caffeic acid and ferulic acid; (iv) hydrolysed tannins like ellagic acid; (v) flavonoids with two benzene rings linked by a group of three carbons like quercetin, luteolin, naringenin, rutin or isoharmnetin.

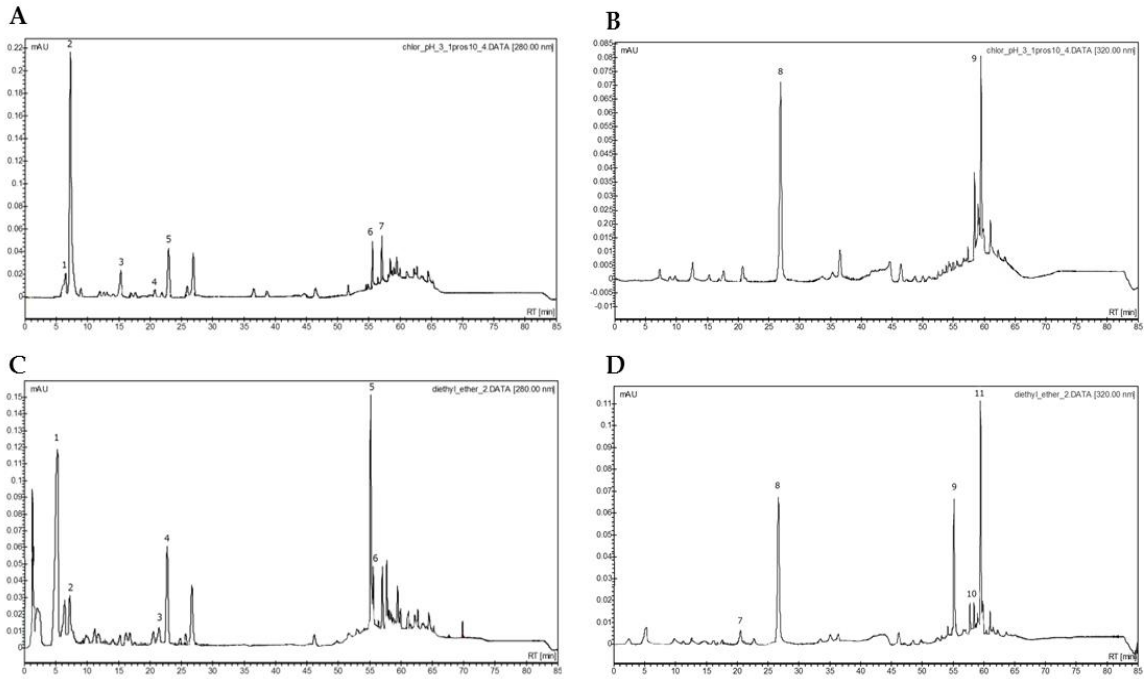


Fig. 3.23 *(A) Chromatogram at 280 nm of SSL-extract using chloroform as solvent: (1) 1,2-dihydroxybenzene (2) 4-hydroxybenzaldehyde (3) guaiacol (4) syringic acid (5) ellagic acid (6) rosmarinic acid (7) naringenin; (B) Chromatogram at 320 nm of SSL-extract using chloroform as solvent: (8) syringaldehyde; (9) luteolin; (C) Chromatogram at 280 nm of SSL-extract using diethyl ether as solvent: (1) gallic acid; (2) 1,2-dihydroxybenzene; (3) guaiacol; (4) syringic acid; (5) ellagic acid; (6) rosmarinic acid; (D) Chromatogram at 320 nm of SSL-extract using diethyl ether as organic solvent: (7) caffeic acid (8) syringaldehyde (9) rutin (10) quercetin (11) luteolin.*

The complete list of individual phenolics detected by HPLC/DAD within five SSL extracts studied in the Agriculture University of Athens (AUA) is presented in Table 3.12.

Table 3.12 *Individual phenolic compounds found in the extracts.*

Concentration (mg/L)	Hexane	Benzene	Trichloro-ethylene	Chloroform	Diethyl ether
syringaldehyde	24.5	192.6	204.8	261.9	232.6
syringic acid	<DL[1]	<DL	<DL	213.2	<DL
1,2dihydroxy-benzene	<DL	191.2	ND	2426.1	373.3
4-hydroxy-benzaldehyde	<DL	<DL	9.5	43.76	<DL
caffeic acid	4.38	<DL	<DL	<DL	22.08
ferulic acid	1.77	<DL	<DL	<DL	<DL
gallic acid	<DL	<DL	15.83	<DL	848.4
ellagic acid	5.73	56.54	42.47	48.79	856.2
guaiacol	<DL	139.7	72.18	85.81	240.9
naringenin	<DL	23.08	27.58	24.22	<DL
quercetin	<DL	77.7	83.37	<DL	107
isorhamnetin	2.9	<DL	<DL	<DL	<DL
rosmarinic acid	<DL	<DL	<DL	<DL	132.8
luteolin	0.0015	<DL	0.0062	0.016	0.019
rutin	<DL	<DL	<DL	<DL	539.37
Σ Phenolics	39.28	680.8	455.7	3103.8	3352.7

[1] *Concentrations < DL means below the detection limit*

✓ **Result comparison of the detoxification alternatives**

The best results of detoxification techniques studied in this work are summarised in Table 3.13. Technique effectiveness under equilibrium was calculated in terms of Individual Inhibitor Removal (IIR). In addition, Total Inhibitor Removal (TIR) and Total Sugar Losses (TSL) have been calculated.

IIR is calculated as the difference between the initial and final concentration of the inhibitor before and after detoxification divided by the initial inhibitor concentration and multiplied by 100, being MetalR, LSR, OHR, HAcR and FuR the IIR value of

metals, LS, phenolics, acetic acid and furfurals, respectively. TIR is calculated as the difference between 442.6 and the total inhibitors removed divided by 442.6 and multiplied by 100, where 442.6 g/L is the total amount of inhibitors in the TSSL. Finally, TSL is calculated as the difference between 214 and the sum of total sugars presented after detoxification, divided by 214 and multiplied by 100. The concentration 214 g/L corresponds to the total amount of sugars in the TSSL.

In addition, a new ratio was made in order to establish the selectivity of the fractionation alternative towards inhibitor removals and thereby sugar-to-inhibitor removal ratio (S/I) was calculated as the TSL divided by TIR. Among all the experiments checked, only the optimum ones regarding maximum TIR and IIR within the minimal TSL were selected and displayed in Table 3.13.

Table 3.13 Summary of the best *detoxification techniques.*

	MetalR (%)	LSR (%)	OHR (%)	HAcR (%)	FuR (%)	TSL (%)	TIR (%)	S/I Coef.	EtOH[c] L/Kg.dry.SSL
AR 1.5mL/g	65.0	96.2	98.1	61.3	100	63.3	91.8	0.69	0.509
AR 6mL/g	44.3	62.1	54.8	25.2	100	15.8	55.1	0.29	1.167
CR 1.3 mL/g	97.9	50.5	53.7	59.9	100	60.9	50.5	1.21	0.542
CR-AR Series I	96.5	99.0	100	92.0	100	62.4	98.5	0.63	0.521
CR-AR Series IV	89.8	71.3	84.8	82.2	100	24.2	70.8	0.34	1.051
BC 50°C 1:5	NM [a]	76.2	66.7	42.7	100	42.6	47.6	0.89	0.796
AC 30°C 1:5	NM	54.3	61.1	74.5	100.0	56.1	33.6	1.7	0.608
OV [b] 30°C pH10	NM	45.9	39.6	31.3	50.0	11.2	44.9	0.25	1.231
L-L ext. Chloroform	NM	<DL	43.1	45.0	26.2	0.23	2.36	0.10	1.818
L-L ext. Diethylether	NM	<DL	50.1	73.6	73.4	1.31	2.89	0.45	1.799

[a] NM not measured; [b] OV overliming; [c] EtOH calculated from the monomers using the theoretical stoichiometric factors reported by Thomsen et al. (2014)

Ethanol potentials after detoxification at optimal conditions under equilibrium are displayed in Table 3.13. Such potentials were calculated considering the sugar losses resulted from each detoxification technique. From the mass balances of the whole process, untreated liquors resulted in 0.215 L.EtOH/Kg.dry.SSL calculated from the monomers (see Table 3.5). Looking at Table 3.13, ethanol potentials after detoxification were considerably ameliorated. The best results were 1.231, 1.818 and 1.799 L.EtOH/Kg.dry.SSL after detoxification with OV and L-L extraction with chloroform and diethyl ether. Such values are significantly higher than values of the

West African agricultural residues previously reported by Thomsen et al. (2014). Nevertheless these factors do not take into account the inhibition of fermentation caused, for instance, by the presence of acetic acid. Olsson and Hahn-Hägerdal (1996) reported that acetic acid concentrations above 4.3 g/L inhibit fermentation 50 % with *S.cerevisiae* at pH of 5.5. In the case of *P.stipitis* concentrations above 8 g/L inhibit 98 % or 25 % at pH of 5.1 or 6.5 respectively. Considering the acetic removals (HAcR) appearing in Table 3.13, OV and L-L extraction are not the best options for SSL fermentation with *S.cerevisiae* in order to produce EtOH. Within this framework, resin in series (Series I and IV) or AC at 30ºC and 1:5 w/v would be suitable for the biological conversion of sugars into ethanol with low inhibition grade towards cell growth of *S.cerevisiae* or *P.stipitis*.

Regarding IIR and TIR, detoxification with resins is better than detoxification by means of adsorption. CR-AR Series I of resins has the best results with a total toxic separation of 98.5 %. The coefficient S/I is also low (0.63) demonstrating that this method has a good affinity for inhibitors with respect to sugars. A slightly worse result was obtained with one-single resin, AR 1.5 mL/g, presenting very good TIR of 91.8 %, S/I of 0.69 but a high TSL value of 63.3 %. Series IV preserves sugars, giving acceptable TIR of 70.8 % with TSL of only 24.2 %.

Regarding the adsorption methods, the adsorption with BC is a better alternative in comparison to AC because it eliminates more inhibitors with low sugar losses. However, the S/I coefficient is worse in the case of BC experiments. Sugar losses (42.6 %) and inhibitor removals (47.6 %) are almost in the same proportion and consequently S/I is close to 1. Nevertheless, it is a good solution in those cases working with microorganisms affected in a moderated way by phenolics, LS and acetic acid. AC adsorption seems to be the most adequate adsorption option in the case of acetic acid removal, presenting losses from 74.5 to 92 %. Using this adsorbent, high losses of sugar are obtained.

Overliming should not be considered as a unique detoxification alternative, but it might be used in combination with adsorption or ion exchange resins. In any case, a pH adjustment of the lignocellulosic hydrolysate has to be done in order to create a medium where yeasts can be grown and survive.

Considering the results of Table 3.13, treatments with ion exchange resins are the most suitable techniques for SSL detoxification prior to sugar fermentation, the best method being the utilisation of CR at 3 mL SSL/g.wet resin followed by AR treatment at 2 mL. The maximum separation degree of the toxic substances plays an

important role in terms of yield, productivity or rate of fermentation working with sensitive microorganisms under adverse conditions. Nevertheless, a drawback of resins is their cost in comparison to overliming, organic solvent extraction, or adsorption.

Figure 3.24-A and Figure 3.24-B show the HPLC chromatogram and the FTIR spectrum of the untreated SSL and their comparison to detoxified-SSL which corresponds to this experiment. FTIR is used as a simple technique for obtaining information about the structure of wood constituents in short analysis time. The main bands are the following ones: (i)- the band between 3700 and 3000 cm^{-1} is assigned to O-H groups (typically presented in cellulose and hemicellulose); (ii)- bands from 2200 to 2050 cm^{-1} are associated to alkynes groups C≡C; (iii)- other peaks are assigned to characteristic stretching vibrations of C=O (ketones belonging mainly to the hemicelluloses) around 1725-1650 cm^{-1}, (iv)- aromatic rings at 1510 cm^{-1} from lignin; and (v)- carbonyl groups (C-O) at 1030 cm^{-1} from hemicelluloses. Bands centered at 1463, 1425, 1375, 1160, 1111 and 1030 cm^{-1} are assigned to characteristic bending or stretching vibrations of lignin and cellulose groups. Besides, the band intensity at 1739 cm^{-1} is attributed to the stretching of the free carbonyl groups mainly contained in the hemicellulose branched component. Based on this spectral information, the main differences between the original and detoxified SSL are (i)-the low intensity of O-H or C≡C bands caused by phenolics and lignin losses after detoxification; (ii)- at 1725 cm^{-1} the ketone C=O group appears in the detoxified-SSL spectra because of the higher concentration of hemicelluloses; (iii)- and the presence at 1030 cm^{-1} of a notorious peak in the case of detoxified-SSL sample, caused by carbonyl groups C-O derived from hemicelluloses.

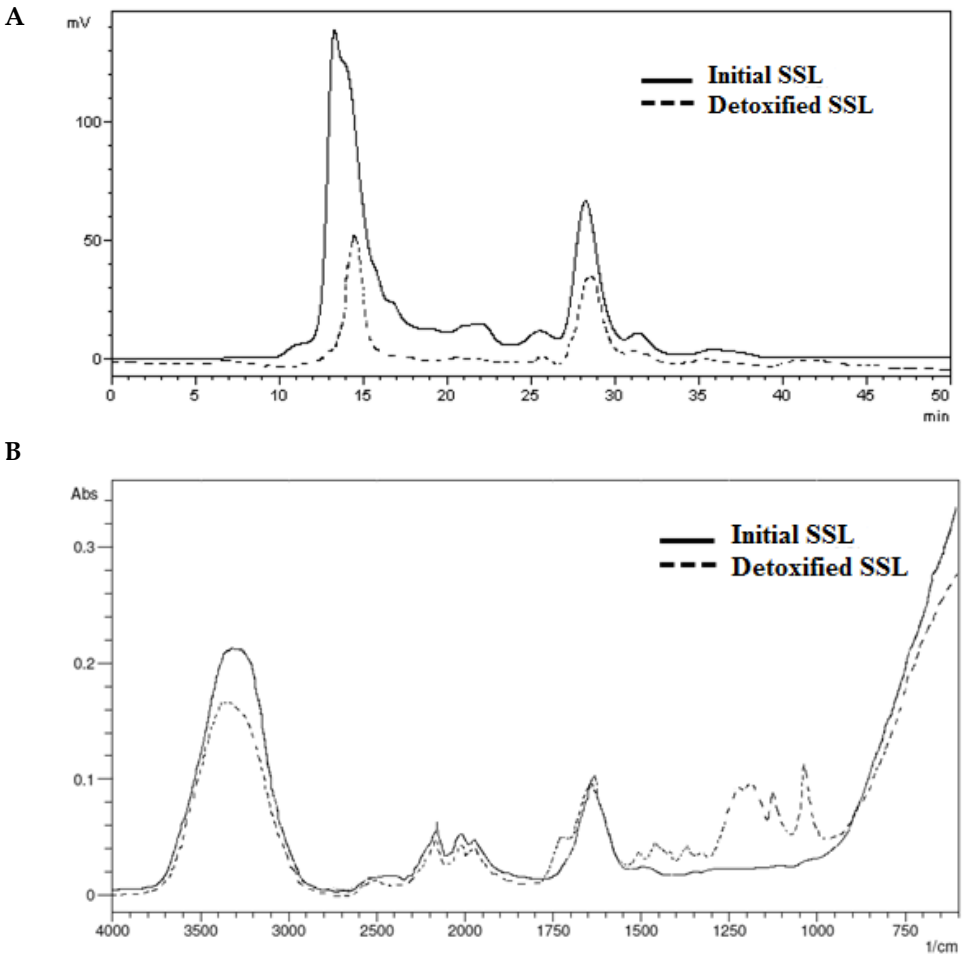


Fig. 3.24 *(A) HPLC chromatogram of initial and detoxified SSL; (B) FTIR spectrum of initial and detoxified SSL.*

The results of the HPLC in Figure 3.24-B also show the difference between the detoxification of SSL with resin in series and the concentrated SSL. This chromatogram belongs to the sugar and LS analysis at the CHO-782 column. The first peak at 13.5-16 min corresponds to LS whereas peaks at 25.8, 28.5, 31.4, 35.6 and 38.1 min belong to glucose, xylose, galactose, arabinose and mannose. It can be seen how either LS or sugars give lower signals in the detoxified-SSL than in the original-SSL, caused by the simultaneous removal of LS and sugars. The most suitable detoxification treatment would be one that maximizes TIR while minimizing TSL.

Detoxification of lignocellulosic residues is an attractive way to transform pulp mills into biorefineries in order to valorise all of the main components. Spent sulphite liquor has been treated by evaporation, overliming, adsorption and ion exchange in order to separate the sugar substract from the rest of by-products or inhibitors. In this case, there are many aspects to be considered from the technical standpoint. Regarding selectivity, individual and total inhibitor removals, experiments conducted with ion exchange resins in series (CR-AR Series I) have the best results with a total toxic separation of 98.5 %, followed by detoxification with anionic resins (AR at 1.5 mL/g) with TIR of 91.8 %. Regarding acetic acid removals, adsorption with AC seems to be the most adequate presenting losses ranging from 74.5 to 92 %. Nevertheless, the selectivity coefficient is too high with a Sugar/Inhibitor ratio iqual to 1.7. Adsorption with BC is a good solution in those cases working with microorganisms affected in a moderate way by phenolics, LS and acetic acid. Nevertheles, the potential concentration of the final product obtained by the process should be taken into account. In this case, bioethanol potentials have been calculated, the most adecuate detoxification techniques being L-L extraction and overliming followed by anionic resin.

3.2.6 Adapting detoxification technologies for fermenting purposes

The results of detoxification processes depend strongly on the application of the following step in biorefinery because the microorganisms are more or less sensitive in function of the type of inhibitor and its concentration. Substrate quality, inhibitor tolerance or the product of interest are some of parameters affecting fermentation and therefore, the detoxification step. Because of this, one of the objectives pursued in this work was to establish the most suitable technique or combination of techniques for the most common fermentating scenarios.

Figure 3.25 highlights the importance of a convenient detoxification technique for an integral use of the SSL. In order to study some biorefinery options of the pulp mill, not only dissolving pulp, but also other products such as weak acids, phenolics, lignosulphonates and sugars can be recovered. To achieve this objective, some detoxification/separation methods must be carried out. The experimental results obtained in this paper together with the fermentation requirements of a specific microorganism allow us to establish the detoxification possibilities that might be implemented in different case studies. Figure 3.23 shows a summary of the biorefinery process. From the cooking (delignification) and hydrolysis steps of the factory, dissolving pulp is produced and spent liquor is formed. In order to separate the different inhibitors or by-products of the liquor and obtain a sugar rich hydrolysate, a detoxification step is necessary. However, it depends on the fermentation scenario and the obtained product. It should be noted that the study cases represented in this section are based on other similar lignocellulosic biomass to the industrial liquors used in this work such as hardwood hydrolysates (Lee et al., 2011; Canilha et al., 2004), softwood hydrolysates (Guo et al., 2013; Millati et al., 2002) and spent sulphite liquors (Alexandri et al., 2014; Queirós et al., 2014; Takasahi et al., 2013; Xavier et al., 2010).

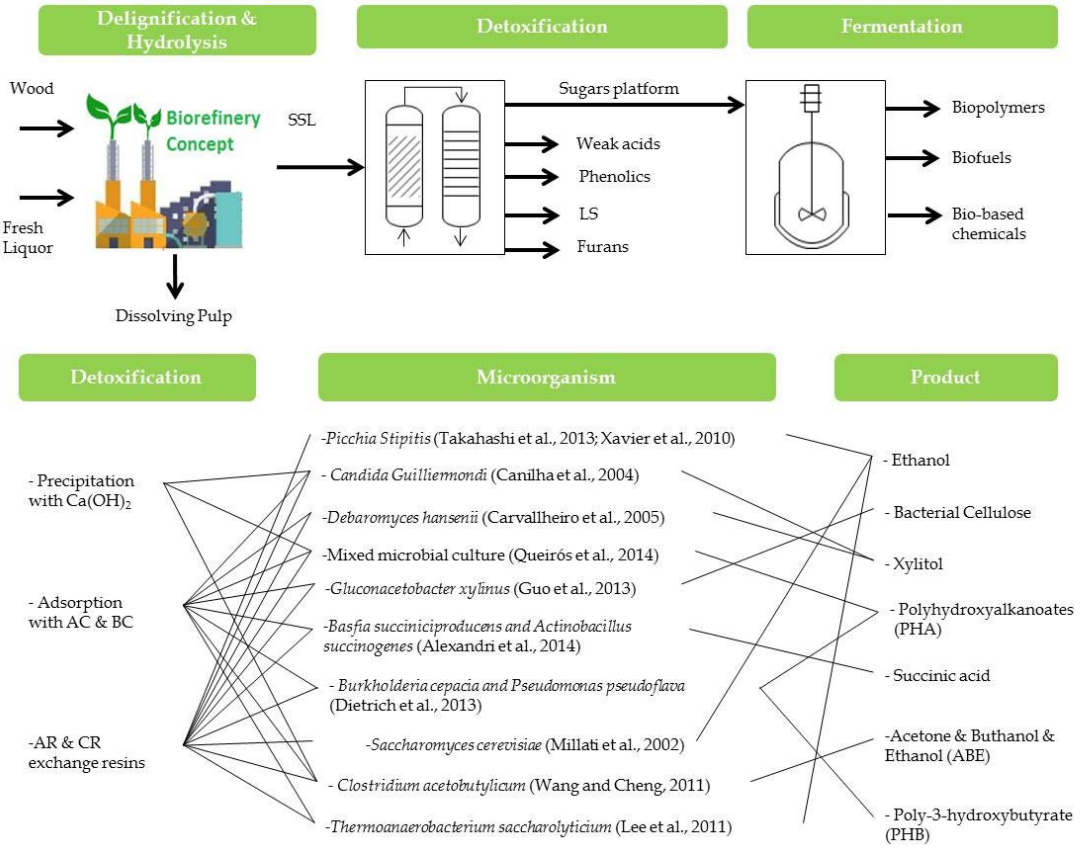


Fig. 3.25 *Detoxification pathways of lignocellulosic hydrolysates within the most common LCH fermentation scenarios.*

According to literature (see 1.3.3 section), SSL inhibitors can be classified into furan derivatives, weak acids and phenolics among others (Jönsson et al., 2013; Almeida et al., 2007). Furthermore, the separation of lignosulphonates, the major compound of the SSL, is also important since LS hinders the ability to separate other toxic compounds (Takasahi et al., 2013).

The first group of inhibitors corresponds to furfurals which inhibit the growth of yeast and decrease ethanol yield and productivity (Jönsson et al., 2013). This group is not problematic working with concentrated SSL since concentration of F and HMF correspond to 0.20 and 0.07 g/L respectively. More concentration of these inhibitors in necessary according to literature. There are studies demonstrating that furfural concentrations above 2 g/L stopped the cell growth of *Pichia stipitis* almost completely (Sainio et al., 2011), a concentration of 1.5 g/L gives a reduction of the 90 % of ethanol yield and 85 % of the productivity, whereas only 9.9 % of reduction is obtained in the case of using a concentration of furfural of 0.27 g/L (Nigam et al.,

2001). Although HMF is not as serious an inhibitor as furfural, it was also reported by Sainio et al. (2011) that the concentrations in the range of 1 to 5 g/L reduce ethanol production by 71-96 %.

The second group of inhibitors is formed by the weak acids whose toxic effect strongly depends on the pH, due to partial dissociation of acetic formic and levulinic acid. Weak acids inhibit cell growth and undissociated form of the acid because they become liposoluble and diffuse across the plasma membrane. Levulinic acid and formic acid do not have any toxic effect in the concentrations of this work, the final concentration of the hydrolysate in our case being 0.11 and 0.23 g/L, respectively. However, the acetic acid can constitute a problem in the concentration of this work: 7.85 g/L. Bibliography results suggest that the acetic acid concentration should be reduced to less than 1 g/L to effectively produce ethanol from hardwood SSL using *P.stitipis* (Takahashi et al., 2013). *S. cerevisiae* can only survive at an external pH higher than 4.5 and maximum concentration of undissociated acetic acid of less than 5 g/L (Purwadi et al., 2004). Concentrations higher than 1.45 g/L completely inhibit the growth of the *Pachysolen tannophilus* yeast, concentrations above 1.2 g/L inhibit the growth of *Candida utilis* and concentrations higher than 2.7 g/L reduce the pentose utilisation by *P. stipitis* (Parajó et al., 1998). The negative effect of acetic acid on yeasts is almost the same producing either xylitol or ethanol.

The third group of inhibitors is the low molecular weight phenolics which may act on biological membranes, causing loss of integrity, destroying the electrochemical gradient by transporting the protons back across the mitochondrial membranes (Almeida et al., 2007). Phenolic compounds are considered the major inhibitors in lignocellulosic hydrolyzates, even more than weak acids and furfurals (Wang and Chen, 2011). This behavior becomes significant working with bacteria strains for succinic acid production (Alexandri et al., 2014), PHA production (Dietrich et al., 2013), ABE fermentation (Wang and Chen, 2011) or bacterial cellulose (Guo et al., 2013). Alexandri et al. (2014) observed an increase of 62 % on succinic acid production fermenting with two wild-type bacteria strains after SSL organic solvents detoxification which partially removed phenolic compounds. Therefore, detoxification methods that efficiently remove phenolics benefit from bacterial growth. An exception to this is observed working with mixed cultures consuming acetic acid, a part of phenolics and a low amount of sugars for PHA production (Queirós et al., 2014). Afterwards, remaining sugars can be used for other bioprocesses like bioethanol production.

Figure 3.25 at the bottom shows the recommended detoxification step in the most common scenarios. The detoxification technique was selected in every case considering the inhibitor tolerance previously analysed towards furfurals, weak acids and phenolics. Discussion of Figure 3.25 was possible assuming the individual inhibitor results obtained in this work for overliming, exchange resins and adsorption. In the case of liquid-liquid extraction, this is only recommended in the case of valorising the phenolic contents of the hydrolysate. However, the combination of this process with other detoxification techniques will be necessary for separating lignosulphonates.

Based on the results of the previous section and the information collected from literature it can be concluded that: (i) bioprocess working with bacteria strains requires detoxification processes like anionic exchange resins or overliming coupled with adsorption, removing LS and phenolics in large amounts; (ii) however, bioprocess working with yeasts mostly requires acetic acid and furan losses or a convenient pH adjustment, and therefore only detoxification by adsorption coupled with overliming could be enough.

The versatility of the sugar platform offers different business opportunities for the sulphite pulp biorefinery. As detoxification is the main challenge for the pulp and paper mill, this work recommends a future study by means of multi-criteria decision-making tools, including not only the technical but also economical and environmental aspects.

3.2.7 Developments of the sulphite mill towards the lignocellulosic biorefinery

The stages studied in this dissertation regarding industrial sulphite wood processing are represented in Figure 3.26. Currently, the enterprise Lignotech Ibérica reuses the LS from the SSL which constitutes 65 % (w/w on dry weight) of the spent liquors (Coz et al., 2015). Nevertheless, hemicellulosic sugars and other woody constituents are not being valorised.

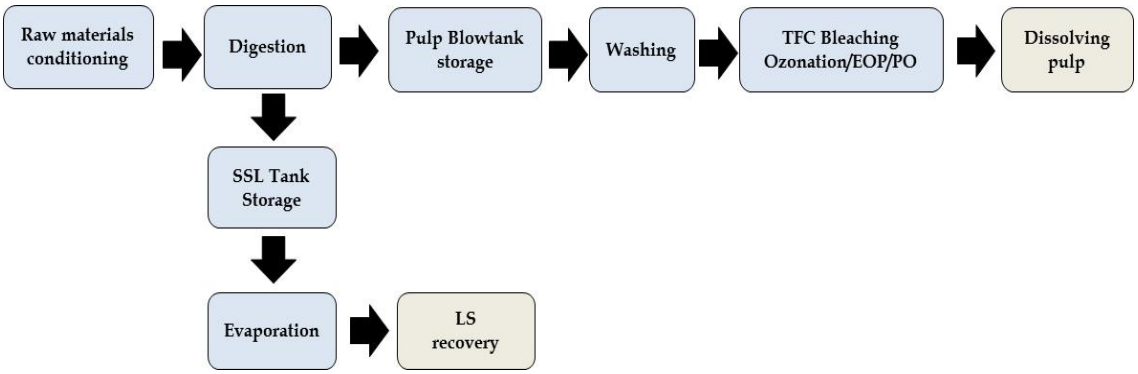


Fig. 3.26 *Block diagram with main steps of the sulphite P&P mill.*

SSL sugars are not being utilised at most of the sulphite P&P mills since they are destroyed during the LS recovery (Koutinas et al., 2014). Sugar valorisation is very important for an efficient use of the natural resources within the biorefinery concept. From the technical standpoint, the experimental results obtained evidenced the possibility of carbohydrate valorisation within the Sniace factory. This is possible by means of the new stages proposed in Figure 3.27. The model of Figure 3.27 is based on SSL material valorisation instead of energetic valorisation due to the low calorific value of the SSL (Petersen et al., 2014).

In this dissertation, some developments and modifications are recommended in order to transform the existing facility into a modern biorefinery. On the one hand, an optimisation of the existing processes (digestion, ozonation and peroxide bleaching) is recommended in order to increase the sugar content of the hemicellulose and to avoid the degradation of the cellulose. On the other hand, the modifications shown in Figure 3.27 (in green) are recommended to obtain not only dissolving pulp and lignosulphonates but also other products from the sugar content with or without the combination of the production of phenolics, weak acids and/or furfurals (in red). In this case, the stages proposed in Figure 3.27 consist of

an acid hydrolysis process before liquor concentration followed by evaporation and some detoxification stages, separating LS and other minor inhibitors from the sugar-rich substrate and adjusting the pH to mild conditions. Finally, fermentation and purification of the sugar-substrate should be implemented.

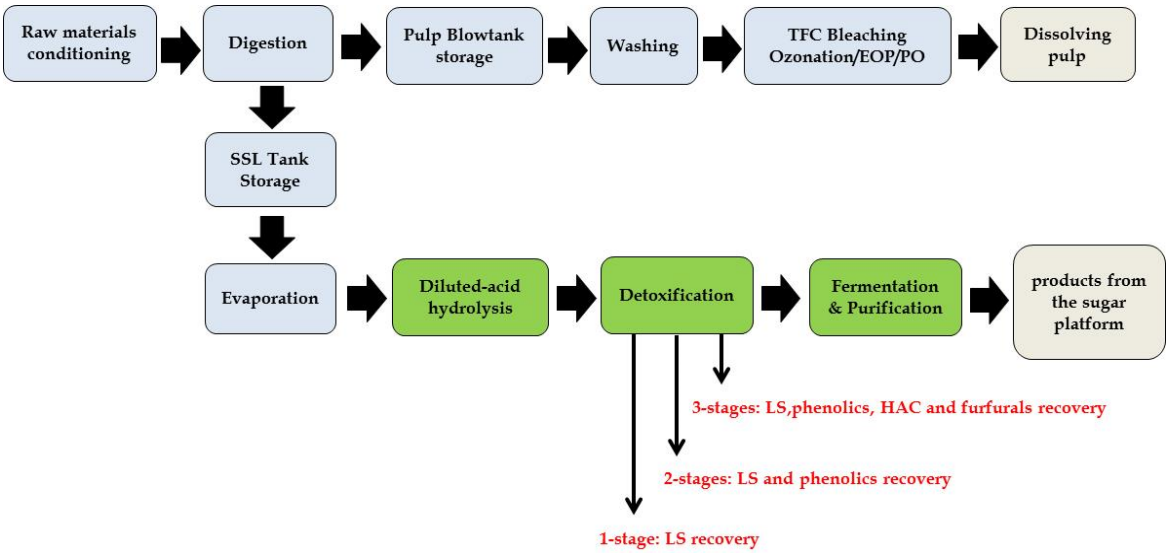
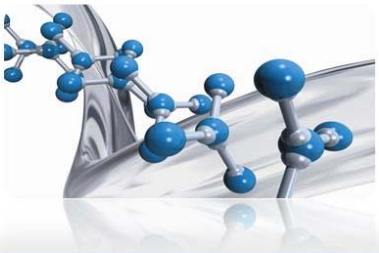


Fig. 3.27 *Stages to be integrated in the current process for the complete SSL valorisation within the sulphite pulp mill.*

A diagram about the improvements and modifications of the sulphite pulping mill has been developed. Different models can be assayed and simulated, working with detoxification stages in series producing different qualities of sugar substrates able to adapt to market value-added product fluctuations. The complexity of such models depends not only on the substrate quality but also on the variety of wood compounds separated from the indutrial waste stream. In order to simplify the models, a diluted acid hydrolysis stage to improve hemicellulosic sugar depolymerisation and pH adjustment with a moderated neutralisation stage up to pH of 5.5 before sugars fermentation are recommended. In addition to the valorisation of the sugar substrate and depending on the main by-products (phenolics, acetic acid and lignosulphonates), different detoxification steps can be used: liquid-liquid extraction in the case of phenolics, overliming and/or anionic exchange resin when lignosulphonates are valorised and the use of adsorption with activated charcoal or anionic exchange resin in the case of acetic acid.



4. Conclusions

CONCLUSIONS

Based on the hypothesis of this dissertation supporting that it is able to transform pulp and paper mills into a modern biorefineries, the aim of this thesis was to find out ways of transforming an existing sulphite pulping mill into a lignocellulosic biorefinery where cellulose, hemicellulose and lignin fractions provided from *the Eucalyptus globulus* feedstock were valorised.

The main steps of the dissertation included the characterisation of the main fractions of the lignocellulosic biomass in the whole process, the optimisation of the pulping process and the study of external hydrolysis and detoxification procedures with the purpose of enhancing the content of sugar substrate and the separation of the inhibitors or other by-products in the hydrolysate of the mill.

Taking into account these results the main conclusions of this doctoral thesis can be summarised in the following points:

- Four **chromatographic methods** were developed for sugar, acid and furfural quantification. Shodex SH-1011 at 0.5 mL/min of H_2SO_4 0.005 M, 60 ^oC and 20 µL of injection sample and Transgenomic CHO-782 at 0.3 mL/min of ultrapure water, 68 ^oC and 20 µL of injection sample were the most suitable methods for sugar and inhibitor analysis in wood, pulp and hydrolysates streams generated in the pulp and paper mill.

- The **digestion stage** was studied to obtain not only dissolving pulp but also fermentable sugars as much as possible at different cooking conditions. Total SO_2 content of the fresh liquor, heating rate and maximum temperature were studied. Heating rate affects the depolymerisation grade and the sugar content in the SSL whereas total SO_2 and maximum temperature affects the delignification grade and consequently the concentration of lignosulphonates. Regarding total monosaccharides, the best results increased the amount of total monosaccharides of the spent liquor at 1.01T of dwell temperature, 0.196R and 6.20 % total SO_2. Experimental cooking results were modelled giving theorethical increases of 7.33 % of sugars at 1.013T, 0.628R and 7.33 % total SO_2.

- **Mass balances of the three wood macrocomponents (**lignin, hemicellulose and cellulose) were done throughout the whole process (digestion and bleaching stages). The characterisation of the woody materials has been developed comparing traditional methods to more novel methods based on the hydrolysis

and individual characterisation of the monomers. Acid hydrolysis is a useful method for the analysis of carbohydrate composition of wood and pulp samples. Using the TAPPI T249 cm-00 standard in combination with HPLC-RID technique can give complete information of the main components for valorisation options in pulping processes. The results of the mass balance in the mill reported the presence of 87.2 % and 98.5 % of hemicellulose and lignin in the spent liquor. Bioethanol potentials of this waste were determined giving 0.183 L of second-generation ethanol per Kg of dry liquor.

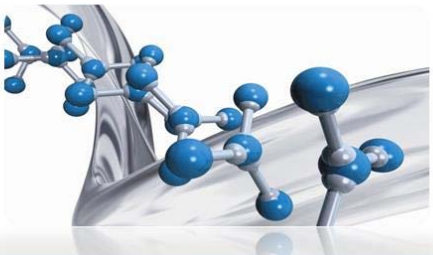
- **External concentrated and diluted acid hydrolysis** was done in order to increase the total monosaccharides present in the spent liquors. Acid concentration, temperature and acid-to-sample ratio were modified in both concentrated and diluted acid hydrolysis using factorial design analysis. The best results were obtained using 0.5 % w/w diluted sulphuric acid at 80 ºC, acid-to-TSSL ratio of 10 v/v, for 60 minutes. Under these conditions the total monosaccharides increased 7.9 % (from 154.5 g/L to 166.7 g/L) caused mainly by xylose growth.

- With the purpose of determining the most suitable fraction to be further detoxified prior to fermentation, **evaporation (pre-detoxification step) of the liquor from the digestion step** was done by reproducing the fifth effects of the industrial evaporation plant. Results showed that the last concentrated sample (the fith effect) is the perfect candidate for subsequent detoxification because it presents the highest inhibitor removals (8.6 %), especially acetic acid removals (53 %) and an increase in the sugar concentrations and lignosulphonates.

- **Different detoxification techniques** were assayed for sugars purification and microbial inhibitor separation: ionic exchange resins, adsorption with activated charcoal and black carbon, overliming and liquid-liquid extraction. Results showed that liquid-liquid **extraction** with diethylether is a good solution for phenolic extraction, which are very easy to recover in order to use them as strong antioxidants in a wide variety of applications. Separation by **ionic exchange resins** was proposed as the best solution with maximum total inhibitor removals of 91.8 %. In addition, six regeneration cycles were proposed for phenolics and LS recovery. **Adsorption** with BC and AC was also adequate for LS, phenolics and acetic acid removals reaching a maximum of 74.5 % of acetic acid removal using activated charcoal. However, adsorption processes gave high losses of sugar in the hydrolysate. **Overliming** was proposed as the simplest way of fractionation giving mostly LS removal (45.9 %).

- Considering all of the detoxification results, different options were recommended depending on the fermentation process. In general, a **bioprocess working with bacteria** strains e.g. *Gluconacetobacter xylinus, Basfia succiniproducens*, *Burkholderia ceppacia*, *Pseudomonas pseudoflava* or *Clostridium acetobutylicum* requires detoxification processes like anionic exchange resins or overliming coupled with adsorption, removing lignosulphonates and phenolics in large amounts. However, a **bioprocess working with yeasts** e.g. *Picchia Stipitis*, *Candida Guilliermondi*, *Debaromyces hansenii* or *Saccharomyces cerevisiae* mostly requires acetic acid and furan losses and a convenient pH adjustment. In this case, detoxification by adsorption coupled with overliming could be enough.

- Based on the whole study, the following developments to be incorporated into the current process are recommended: (i) the digestion conditions should be optimised in order to increase the depolymerisation of hemicelluloses in the spent liquor; (ii) ozonation and peroxide bleaching extraction processes should also be improved, avoiding the degradation and destruction of the cellulose chains and obtaining similar values of impurities; (iii) the spent liquor should be conveniently hydrolysed and detoxified, separating sugars from the rest of microbial inhibitors for microbial fermentation. In order to transform the sulphite mill into a modern lignocellulosic biorefinery, the following steps should be incorporated: hydrolysis, detoxification, and fermentation/purification stages.



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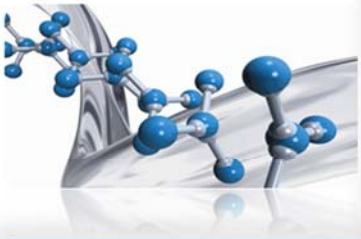

Dissemination of results

DISSEMINATION OF RESULTS

References corresponding to scientific publications directly related to this doctoral thesis are listed below:

1. Llano, T., Rueda, C., Quijorna, N., Blanco, A., Coz, A. 2012. *Study of the delignification of hardwood chips in a pulping process for sugar production. Journal of Biotechnology 162(4), 422-429.*

2. Llano, T., García-Quevedo, N., Quijorna, N., Viguri, J., Coz, A. 2015. *Evolution of lignocellulosic macrocomponents in the wastewater streams of a sulphite pulp mill: A preliminary biorefining approach. Journal of Chemistry. DOI: http://dx.doi.org/10.1155/2015/102534*

3. Llano, T., Alexandri, M., Koutinas, A., Gardeli, CHR., Papapostolou, H., Coz, A., Quijorna, N., Andres, A., Komaitis, M. 2015. *Liquid-liquid extraction of phenolic compounds from spent sulphite liquor. Waste and Biomass Valorisation 6(6), 1149-1159.*

4. Llano, T., Ulloa, L., Quijorna, N., Coz, A. 2015. *Detoxification of a lignocellulosic residue for biorefining purposes. [In development]*

5. Llano, T., Quijorna, N., Coz, A. 2015. *Sugars, aliphatic acids and furfurals quantification of a sulphite pulp mill by HPLC/RID. [In development]*

Next oral communications and posters that were sent to relevant International Congresses in the research field are also listed below:

1. Llano, T., Rueda, C., Blanco, A., Andrés, A., Coz, A. *Design of a laboratory for the study of the digestión of hardwood chips to obtain pulp and sugars for bioethanol production. 3th International Symposium on Energy from Biomass and Waste. Oral Communication. Venice, November 2010.*

2. Llano, T., Quijorna, N., Rueda, C., Mendívil, R., Galán, B., Coz A. *Analysis of the sugars and inhibitors obtained by the digestion of hardwood chips for bioethanol production. 8th European Congress of Chemical Engineering. Oral Communication. Berlin, September 2011.*

3. Llano, T., Rueda, C., Quijorna, N., Galán, B., Blanco, A., Coz, A. *Influence of the digestion variables on a pulping process to maximise all of the lingocellulosic waste options. 4th International Conference on Engineering for Waste and Biomass Valorisation. Oral Communication. Porto, September 2012.*

4. Llano, T., Quijorna, N., Portilla, A.I., Andrés, A., Coz, A. *Analysis of sugars, intermediates and inhibitors in sulphite pulping by HPLC/RID. XXII TECNICELPA- International Forest, Pulp and Paper Conference. Poster. Tomar, October 2013.*

5. Llano, T., García-Quevedo, N., Quijorna, N., Portilla, A.I., Viguri, J., Coz, A. *Analysis of e.globulus wood, spent liquor, acid sulphite crude and bleached pulp hydrolysates from dissolving processing. EPNOE International Polysaccharide Conference. Oral Communication. Nice, October, 2013.*

6. Llano, T., Alexandri, M., Koutinas, A., Gardeli, CHR., Papapostolou, H., Komaitis, M., Andres, A., Coz, A. *Solvent extraction of phenolics from lignocellulosic waste. WASTEEng 5th International Conference on Engineering for Waste and Biomass Valorisation. Poster. August 2014.*

7. Llano, T., Ulloa, A., Quijorna, N., Coz, A. *Fractionation of a lignocellulosic residue towards its valorisation into biopolymers and construction additives. WASCON 9th International Conference on the Environmental and Technical Implications of Construction with Alternative Materials. Santander, June 2015.*

8. Llano, T., Dosal, E., Coz, A. *Multi-criteria decision making tools for assessing spent liquor detoxification alternatives. European Cooperation in Science and Technology. FP1306 Cost Action. WG1 & WG3 Meeting. Litvínov, October 2015.*

The following scientific papers were published as co-author:

1. *Rueda, C., Fernández-Rodriguez, J., Ruiz G., Llano, T., Coz, A. 2015. Monosaccharide production in an acid sulphite process: Kinetic modeling. Carbohydrate Polymers. 116, 18-25.*
2. *Coz, A., Llano, T., Rueda, C., Quijorna, N., Maican, E. 2015. Hydrolysis and separation of lignocellulosic biomass in a sulphite process to valorise the main fractions within the biorefinery concept. INMATEH-Agricultural Engineering, ISSN 2344 – 4118, 223-228.*

In addition, other side works related to this dissertation were sent to international congresses:

1. *Rueda, C., López-Álvarez, J., Llano, T., Quijorna, N., Blanco, A., Viguri, J., Coz, A. Physico-chemical characterisation of a spent sulphite liquor oriented to its valorisation options. 16th International Symposium on Wood, Fibre and Pulping Chemistry (ISWFPC). Oral Communication. Tianjin, June 2011.*

2. *Quijorna, N., Llano, T., Portilla, A.I., Rueda, C., Blanco, A., Andrés, A., Coz, A. Comparison of sugar content of two pulping processes in order to produce bioethanol of second generation. 1st International Conferences WASTES: Solutions, Treatments, Opportunities. Oral Communication. Guimarães, September 2011.*
3. *Rueda, C., Llano, T., Quijorna, N., Ruiz, G., Coz, A. Study of acid hydrolysis conditions of a lignocellulosic industrial waste on the sugar content for bioethanol production. 12th Mediterranean Congress of Chemical Engineering. Poster. Barcelona, November 2011.*
4. *Rueda, C., Llano, T., Tejedor, C., Quijorna, N., Andrés, A., Coz, A. Characterisation of species of eucalyptus and comparison of its behaviour as a lignocellulosic biomass in a pulp process. 4th International Conference on Engineering for Waste and Biomass Valorisation. Poster. Porto, September 2012.*
5. *Coz, A., Quijorna, N., Rueda, C., Llano, T., Portilla, A.I., Blanco, A., Andrés, A., Galán, B., Ruiz, G., Viguri, J. Valorisation of lignocellulosic waste from a sulphite pulp mill in bioproducts. 3rd International Forest Biorefinery Symposium, Paper Week Canada. February 2013.*
6. *Alexandri, M., Llano, T., Gardeli, C., Papapostolou, H., Komaitis, M., Papanikolaou, S., Coz, A., Koutinas, A. Solvent extraction and analysis of phenolic compounds derived from spent sulphite liquor for enhanced succinic acid fermentation. 36th Symposium on Biotechnology for Fuels and Chemicals. Florida, April 2014.*
7. *Alexandri, M., Papapostolou, H., Gardeli, C., Llano, T., Komaitis, M., Papanikolaou, S., Coz, A., Koutinas, A.: Detoxification of spent sulphite liquor for succinic acid production. 10th European Symposium on Biochemical Engineering Sciences and 6th International Forum on Industrial Bioprocesses. Lille, September 2014.*

Dissolving pulp mills are perfect candidates for the bio-based sustainable economy.
High-purity cellulose pulp is manufactured by means of pretreatment and hydrolysis of lignocellulosic feedstocks giving sugar-rich effluent streams that might be transformed into biofuels by biochemical pathways. The major challenge in sulphite pulp factories is to conveniently fractionate the waste streams. The integral use of the renewable resources will control the global market for cellulosic pulp. Nowadays, China leads demand growth of pulp. Such trend might change by taking the advantage of residues valorisation within the biorefinery framework.

Las plantas de pasta dissolving son las candidatas perfectas para la bioeconomía sostenible.
La celulosa de alta pureza se produce mediante la hidrólisis y el pretratamiento de materia prima lignocelulósica, dando lugar a efluentes ricos en azúcares que podrían ser transformados en biocombustibles por vías bioquímicas. El mayor reto en las fábricas de pasta al sulfito es el de fraccionar convenientemente las corrientes residuales. El uso integral de estos recursos renovables controlará el mercado global de pasta celulósica. Actualmente China lidera el crecimiento de la demanda de pasta. Dicha tendencia podría cambiar teniendo en cuenta las ventajas que ofrece la valorización de los residuos en el marco de una biorrefinería.

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