Åbo Akademi

Topochemistry of physical and chemical pretreatments of biomass as investigated by FE-SEM, XPS and ToF-SIMS

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Turku/Åbo 2014

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Academic Dissertation

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Åbo, Finland, 2014

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ISBN 978-952-12-3061-5
Painosalama Oy – Turku, Finland 2014

博学之，审问之，慎思之，明辨之，笃行之。

《礼记.中庸》

Learn extensively, inquire thoroughly, ponder prudently,
discriminate clearly and practice devotedly.

《The Book of Rites. The Doctrine of Mean》

Preface

This work was conducted in the Lab. of Fibre and Cellulose Technology (FCT) from 2010 to 2013 under the financial support of the Graduate School for Biomass Refining (BIOREGS).

However, I am deeply grateful to my supervisor, Professor Pedro Fardim for providing me guidance during my academic research, and for his encouragement and strong support even when I felt disappointed with my work. Also, I would like to thank Professor Huaiyu Zhan and Professor Menghua Qin for their guidance during my study in China and establishing my scientific perspective. And thanks Professor Kristiina Kruus and Professor Maija Tenkanan for their kindly support to my study.

I acknowledge all my colleagues of FCT for their kind support to my work. I am grateful to Dr. Elina Heikkilä for the encouragement and useful suggestion with regarding to this work. My appreciation goes to Olga Grigoray and Konstantin Gabov for the sharing of chocolate and their nice conversations which gave me strength in solving the problems encountered during this work. Special thanks go to Agneta Hermansson for taking care of many economic and practical issues and to Jan Gustafsson for his usual support and good suggestions during this work. Special thanks to Top Analytica Ltd for supplying the instruments for the surface analysis which was carried out during this work.

Furthermore, I would like to thank all my Chinese friends at Åbo Akademi University before and after I came to Finland. Thus, the happy times we spend together will be unforgettable. I also give my best wishes to the lovely kids of all the families wishing them good and sound health. And, I am grateful to Dr. Bin Li for his kind and attentive care.

Lastly, I would like to thank my family for their endless support. I appreciate my parents, my brother and sisters for their care, encouragement and firm support all the time, otherwise I would not be able to complete my study. I love you!

Turku, March 2014

Hong Yan Mou 牟洪燕

Abstract

Surface chemistry is of great importance in plant biomass engineering and applications. The surface chemical composition of biomass which includes lignin, carbohydrates and extractives influences its interactions with chemical agents, such as pulp processing/papermaking chemicals, or enzymes for different purposes.

In this thesis, the changes in the surface chemical composition of lignocellulosic biomass after physical modification for the improvement of resulting paper properties and chemical treatment for the enhancement of enzymatic hydrolysis were investigated.

Low consistency (LC) refining was used as physical treatment of bleached softwood and hardwood pulp samples, and the surface chemistry of refined samples was investigated. The refined pulp was analysed as whole pulp while the fines-free fibre samples were characterized separately. The fines produced in LC-refining contributed to an enlarged surface specific area as well as the change of surface coverage by lignin and extractives, as investigated by X-ray photoelectron spectroscopy (XPS). The surface coverage by lignin of the whole pulp decreased after refining while the surface coverage by extractives increased both for pine and eucalyptus. In the case of pine, the removal of fines resulted in reduction of the surface coverage by extractives, while the surface coverage by lignin increased on fibre sample (without fines). In the case of eucalyptus, the surface coverage by lignin of fibre samples decreased after the removal of fines. In addition, the surface distribution of carbohydrates, lignin and extractives of pine and eucalyptus samples was determined by Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS). LC-refining increased the amounts of pentose, hexose and extractives on the surface of pine samples. ToF-SIMS also gave clear evidence about xylan deposition and reduction of surface lignin distribution on the fibre of eucalyptus. However, the changes in the surface chemical composition during the physical treatment has led to an increase in the adsorption of fluorescent whitening agents (FWAs) on fibres due to a combination of electro-static forces, specific surface area of fibres and hydrophobic interactions.

Various physicochemical pretreatments were conducted on wood and non-wood biomass for enhancing enzymatic hydrolysis of polysaccharides, and the surface

chemistry of the pretreated and enzymatically hydrolysed samples was investigated by field emission scanning electron microscopy (FE-SEM), XPS and ToF-SIMS. A hydrotrope was used as a relatively novel pretreatment technology both in the case of wood and non-wood biomass. For comparison, ionic liquid and hydrothermal pretreatments were applied on softwood and hardwood as well. Thus, XPS analysis showed that the surface lignin was more efficiently removed by hydrotropic pretreatment compared to ionic liquid or hydrothermal pretreatments. SEM analysis also found that already at room temperature the ionic liquid pretreatments were more effective in swelling the fibres compared with hydrotropic pretreatment at elevated temperatures. The enzymatic hydrolysis yield of hardwood was enhanced due to the decrease in surface coverage of lignin, which was induced by hydrotropic treatment. However, hydrotropic pretreatment was not appropriate for softwood because of the predominance of guaiacyl lignin structure in this material. In addition, the reduction of surface lignin and xylan during pretreatment and subsequent increase in cellulose hydrolysis by enzyme could be observed from ToF-SIMS results. The characterisation of the non-wood biomass (e.g. sugarcane bagasse and common reed) treated by hydrotropic method, alkaline and alkaline hydrogen peroxide pretreatments were carried out by XPS and ToF-SIMS. According to the results, the action for the removal of the surface lignin of non-wood biomass by hydrotropic pretreatment was more significant compared to alkaline and alkaline hydrogen peroxide pretreatments, although a higher total amount of lignin could be removed by alkaline and alkaline hydrogen peroxide pretreatment. Furthermore, xylan could be remarkably more efficiently removed by hydrotropic method. Therefore, the glucan yield achieved from hydrotropic treated sample was higher than that from samples treated with alkaline or alkaline hydrogen peroxide. Through the use of ToF-SIMS, the distribution and localization of lignin and carbohydrates on the surface of lignocelluloses during pretreatment and enzymatic hydrolysis could be detected, and xylan degradation during enzymatic hydrolysis could also be assessed. Thus, based on the results from XPS and ToF-SIMS, the mechanism of the hydrotropic pretreatment in improving the accessibility of enzymes to fibre and further ameliorating of the enzymatic saccharification could be better elucidated.

Referat

Ytkemi är av stor betydelse för processeringar och applikationer av biomassa. Den ytkemiska sammansättningen av biomassa, vilket inkluderar lignin, kolhydrater och extraktivämnen, påverkar biomassans växelverkan med olika kemiska substanser, så som enzymer för olika ändamål samt massa- och papperskemikalier.

I denna avhandling har förändringar i den ytkemiska sammansättningen av biomassa från lignocellulosa undersökts efter både fysiska modifikationer och kemiska behandlingar. Biomassan modifierades fysiskt för att förbättra pappersegenskaper medan de kemiska behandlingarna förbättrade den enzymatiska hydrolysen av biomassa.

Lågkonsistensmalning (eng. LC-refining) användes som fysisk modifiering av blekta barr- och lövvedsmassor, varefter de malda provens ytkemi analyserades. Den malda massan studerades som odelad massa, medan de fiberprov där finmaterialet fraktionerats bort karakteriserades separat. Finmaterialet som tillkom under malningen bidrog både till en ökad specifik ytarea och till en förändring i ytans täckningsgrad av lignin och extraktivämnen, vilka undersöktes med röntgenfotoelektronspektroskopi (XPS/ESCA).

Ytan täckningsgrad av lignin för den fullständiga massan minskade efter malning medan ytans täckningsgrad av extraktivämnen ökade för massaprov av både tall och eukalyptus. För tall resulterade en avlägsning av finmaterial i en minskning av extraktivämnen på ytan medan täckningsgraden av lignin på ytan ökade för fiberprov som inte innehöll finmaterial. För eukalyptusproven minskade ytans täckningsgrad av lignin för fiberprov efter att finmaterialet hade avlägsnats. Därtill fastställdes fördelningen av kolhydrater, lignin och extraktivämnen för tall och eukalyptusprov med hjälp av flygtidsseparerad sekundär-jon massaspektrometri (ToF-SIMS). Lågkonsistensmalningen ökade mängden av pentoser, hexoser och extraktivämnen på ytan av tallprov. ToF-SIMS gav också klara bevis för en utfällning av xylan och en minskning av andelen lignin på eukalyptusfibrernas yta.

Förändringen i den ytkemiska sammansättningen som en följd av den fysiska modifieringen ledde till en ökad adsorption av fluorescerande vitmedel (eng.

FWA) till fiberytan tack vare en kombination av elektrostatiska krafter, hydrofobisk växelverkan och en ökad specifik ytarea.

Olika fysikalisk-kemiska förbehandlingar gjordes för biomassa från ved och växter för att öka den enzymatiska hydrolysen av polysackarider. Ytkemin av de förbehandlade och enzymatiskt hydrolyserade proven studerades med fältemmissions-svepelektronmikroskop (FE-SEM) och ToF-SIMS. En hydrotrop användes som en relativt ny teknik för att förbehandla biomassa från ved och från växter. Därtill utnyttjades joniska lösningar och hydrotermala förbehandlingar av barr- och lövvedsprov som jämförelse. XPS analyser visade att förbehandling med hydrotrop avlägsnade lignin mera effektivt än joniska lösningar och hydrotermala förbehandlingar. Istället indikerade SEM analyserna att en förbehandling med joniska lösningar svällde fibrerna mera effektivt redan vid rumstemperatur än en förbehandling med hydrotrop vid förhöjd temperatur. Utbytet efter enzymhydrolys av lövved ökade när ytans täckningsgrad av lignin minskade som en följd av hydrotropbehandling. Däremot var förbehandling med hydrotrop inte lämplig för barrved på grund av den övervägande mängden guaiacylgrupper i barrvedsligninet. Därutöver kunde minskningen av ytans lignin-och xylanhalt som en följd av förbehandlingen och den påföljande ökningen av enzymatisk hydrolys av cellulosa observeras från ToF-SIMS resultaten.

Karakteriseringen med XPS och ToF-SIMS gjordes för biomassa från växter, dvs. sockerrör och vass, vilka behandlats med alkali, alkalisk väteperoxid samt med hydrotrop. Enligt resultaten är funktionen för avlägsnande av ytlignin från växtbiomassa mera markant för de hydrotropbehandlade proven än för prov vilka förbehandlats med alkali eller alkalisk väteperoxid, trots den större totala mängden lignin som kunde avlägsnas genom förbehandling med alkali eller alkalisk väteperoxid. Dessutom kunde xylan bli anmärkningsvärt effektivare avlägsnat med hjälp av den hydrotropiska metoden. Följaktligen var glukanernas utbytet större från hydrotropbehandlade prov är från prov behandlade med alkali eller alkalisk väteperoxid. Ligninets och kolhydraternas lokalisering och distribution på ytan av lignocelluloser efter förbehandling och enzymatisk hydrolys kunde detekteras med ToF-SIMS. Samtidigt kunde den enzymatiska hydrolysens inverkan på xylanens nedbrytning också bestämmas. Sammanfattningsvis, baserat på resultaten från XPS och ToF-SIMS kunde mekanismen för hydrotropisk förbehandling för att förbättra enzymernas

åtkomlighet till fibrer och en följande förbättring av enzymatisk sackarifiering tydligare klargöras.

List of publications

I. **Hongyan Mou**, Eduardo Iamazaki, Huaiyu Zhan, Elina Orblin, Pedro Fardim. Advanced studies on the topochemistry of softwood fibers in Low-Consistency refining as analyzed by FE-SEM, XPS, and ToF-SIMS. *BioResources*, 2013, 8 (2), 2325-2336.

II. **Hongyan Mou**, Bin Li, Elina Heikkilä, Eduardo Iamazaki, Huai-yu Zhan, Pedro Fardim. Low consistency refining of eucalyptus pulp: Effects on surface chemistry and interaction with FWAs. *BioResources*, 2013, 8(4), 5995-6013.

III. **Hongyan Mou**, Elina orblin, Kristiina Kruus, Pedro Fardim. Topochemical pretreatment of wood biomass to enhance enzymatic hydrolysis of polysaccharides to sugars. *Bioresource Technology*, 2013, 142, 540-545.

IV. **Hongyan Mou**, Elina Heikkilä, Pedro Fardim. Topochemistry of alkaline, alkaline-peroxide and hydrotropic pretreatments of common reed to enhance enzymatic hydrolysis efficiency. *Bioresource Technology*. 2013, 150, 36-41.

V. **Hongyan Mou**, Elina Heikkilä, Pedro Fardim. Topochemistry of enviornmental-friendly pretreatment to enhance sugarcane bagasse enzymatic hydrolysis polysaccharides to fermentable sugar. *Journal of Agricultural and Food Chemistry*. 2014, 62 (16), 3619-3625.

Contributions of author

Paper I-II: All experiments work, interpretation of the results, and the writing of the manuscripts as the first author.

Paper III: All the experimental work, HPLC, SEM and part of XPS measurement, interpretation of the results, and the writing of the manuscripts as the first author.

Paper IV-V: All the experimental work, HPLC, SEM and ATR-IR measurement, interpretation of the results, and the writing of the manuscripts as the first author.

Other publications

The publications below are related to this thesis but are not included in it.

Hongyan Mou, Bin Li, Pedro Fardim. Pretreatment of corn stover with the modified hydrotropic method to enhance enzymatic hydrolysis of polysaccharides. 2014, *Energy & Fuels* (DOI: 10.1021/ef5001634).

Hongyan Mou, Pedro Fardim. Surface-chemistry of pretreatment birch and pine to enhancement enzyme hydrolysis 245th ACS National Meeting & Exposition. **Abstract**, April 7-11, 2013. New Orleans, Louisiana, USA.

Hongyan Mou, Elina Orblin, Kristiina Kruus, Pedro Fardim. Topochemistry of pretreatment wood biomass to enhance enzymatic hydrolysis. EPNOE 2013 International Polysaccharide Conference. **Abstract**, Epnoe 2013, Oct. 21-24, 2013, Nice, France.

Hongyan Mou, Huaiyu Zhan, Eduardo Iamazaki, Pedro Fardim. Effects of LC-refining on fibre surface chemistry of eucalyptus. Proceeding, Nov. 8th, 2010, 4th ISETPP, Guangzhou, China.

Hongyan Mou, Imazaki Eduardo, Pedro Fardim. Effects of Low-consistency refining (LCR) on fibre surface chemistry and nanomorphology of eucalyptus Pulp. **Abstract**, SIMS Europe. Münster, Germany, Sep.19-21, 2010, p68.

Abbreviations

AIL……..Acid insoluble lignin
ASL……Acid soluble lignin
ATR-IR…….Attenuated total reflectance infrared spectroscopy
B……...Bagasse
BI……..Birch
BH……Alkaline peroxide pretreated bagasse sample
BN……Alkaline pretreated bagasse sample
B_{SXS}……..Hydrotropic pretreated sample
C_1, C_2, C_3, C_4……Assignment in the XPS C1s peak as the bond C-H or C-C, C-O, C=O or O-C-O and O-C=O respectively
ECF……..Elemental chlorine free
F……...Fibre sheet
FWA……...Fluorescent whitening agents
G……...Guaiacyl lignin
HPLC……High performance liquid chromatography
k……Light absorbency coefficiency
L……Lignin
LC……..Low consistency
P……Pulp sheet
PI……Pine
PS……Polysaccharides
R……Common reed
RH……...Alkaline peroxide pretreated reed sample
RN……..Alkaline pretreated reed sample
R_{SXS}…….. Hydrotropic pretreated reed sample
s……Light scattering coefficiency
S……Syringyl lignin
S_{car}……Surface coverage by carbohydrates
S_{ext}……..Surface coverage by extractives
S_{lig}……...Surface coverage by lignin
SEC……Specific energy consumption
FE-SEM……....Field emission scanning electron microscopy
SXS……Sodium xylenesulfonate
ToF-SIMS……Time-of-flight secondary ion mass spectrometry

XPS……X-ray photoelectron spectroscopy

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1. Introduction

Lignocellulosic biomass has been the major sources of raw materials for pulp and paper production. The threats related to oil shortage and global warming also made lignocellulosic biomass to be a sustainable and renewable material for feedstock, for biofuel, biochemical and biomaterial production in the recent years (Figure 1). The renewable plant biomass materials which generally include wood, agricultural residues, energy crops and grasses are promising for biofuel production (e.g. bioethanol) to reduce the pressure of energy crisis. Approximately 200 billion tons (annually) of lignocellulosic biomass which includes wood, grass, and agricultural and forest residues was produced in the world (Zhang, 2008). Global annual production of ethanol has now surpassed 536 million barrels per year according to the data reported by Global Renewable Fuels Alliance (GRFA). In Europe, 20% of conventional fossil fuels in transportation will be replaced by biofuels by 2030 (Himmel et al., 2007). Hence, the study and development of technologies (e.g. suitable pretreatment and enzymatic hydrolysis) for the conversion of lignocellulosic biomass to biofuels, biochemical, or biomaterials (Figure 1) is of significant importance, particularly for large commercial production.

Cellulose, hemicelluloses and lignin are the three main components of lignocellulosic biomass. The various pretreatment technologies change the biomass properties to be more feasible for the downstream processes; and the chemical composition of biomass depends on the type of biomass such as wood or non-wood raw material. Further, modification of the components during different types of treatments will ultimately impact the end product properties. Recently, the surface chemical composition of biomass was found to play an important role for the biomass modification and bioconversion process (Jung et al., 2010; Goacher et al., 2012). Therefore, the study of the topochemistry of lignocellulosic biomass after physical or chemical treatment with the use of state-of-art surface analysis technologies is crucial. In this work, lignocellulosic biomass treatment with mechanical or chemical methods was characterized using X-ray photoelectron spectroscopy (XPS, also named as electron spectroscopy for chemical analysis (ESCA)), time-of-flight secondary ion mass spectrometry (ToF-SIMS) and other imaging and spectrometric methods. Finally, the impact of

the treatments on the topochemical changes of biomass were correlated with process ability in selected cases.

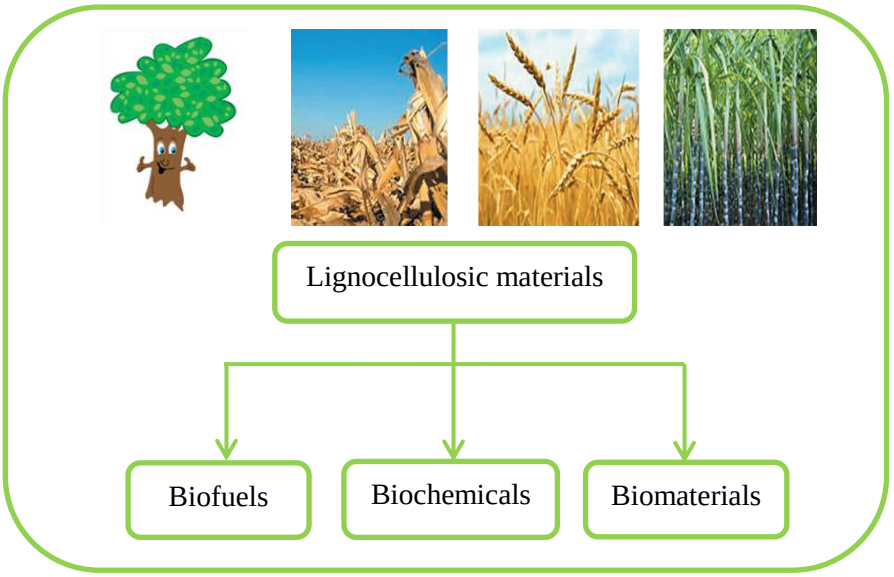


Figure 1. *Utilization scheme of lignocellulosic biomass.*

1.1. Chemical components of biomass

Lignocellulosic biomass has been developed as renewable alternative sources to supply energy, chemicals and materials. The chemical compositions could impact the pretreatment technology and the product properties. Cellulose, hemicelluloses and lignin are the main compositions of lignocellulosic biomass and the main objectives that should be considered in biorefinery process (da Costa Lopes et al., 2013). The chemical composition is different depending on the plant species, age and growth conditions (Jørgensen et al., 2007). Different types of biomass contain different ratio of components. Generally, wood biomass contains relatively higher quantity of lignin (16-31%) and cellulose (40-48%) than non-wood biomass (10-20% and 30-40%, respectively). Non-wood biomass has relatively higher ash (3-11%) and extractives contents (3-18%) than wood biomass (0.3-2% and 1-8%, respectively).

Cellulose is a linear homopolymer composed of D-anhydroglucan units linked by β-(1, 4)-glycosidic bonds (Klemm et al., 1998). Thus, it is made up of crystalline and amorphous regions. Crystallinity is an important factor for improving enzyme hydrolysis rate (Hall et al., 2010). Hemicelluloses are a kind of

heterogeneous polysaccharides with various side groups, and their structures are different according to the species of raw materials. In grass materials, hemicellulose is mainly composed of glucuronoarabinoxylans (Carpita et al., 1996); however, glactoglucomannans and 4-O-methyl-glucuronoxylans are abundant in softwood and hardwood, respectively (Jørgensen et al., 2007). Cellulose and hemicellulose can provide valuable possibilities for sustainable materials development and production of high added value chemicals. After hydrolysis, the monomer sugar from cellulose and hemicellulose can be converted into biofuels.

Lignin is a cross-linked polymer that chemical monomers differ depending on the lignocellulosic biomass sources. The polymer is composed of three basic units which are: p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S). Guaiacyl type lignin is the main constituent in softwood lignin, and guaiacyl and syringyl are commonly found in hardwood, while grass plants have the lowest content lignin with form S, G and H (Jørgensen et al., 2007; Zhao et al., 2012).

In the chemical pulping industry, the aim is to selectively remove lignin (and in some cases also hemicellulose) by chemical treatment. Pulp fibre can be developed by physical treatment to obtain different qualities of end product for different purposes. Biomass pretreatment is the basis of the biorefinery concept with the main goal of overcoming the natural recalcitrance of lignocelluloses in order to improve the enzymatic saccharification by increasing the accessibility of enzymes to fibres. An effective pretreatment is in demand to remove lignin/hemicellulose and sufficiently expose cellulose to achieve more fermentable sugars during enzymatic hydrolysis process (Ding et al., 2012; Mosier et al., 2005). In general, non-wood biomass (like sugarcane bagasse, reed, and corn stover) is easier to be pretreated than wood biomass because of the lower lignin content and density. Therefore, non-wood biomass (i.e. agricultural wastes and herbaceous biomass) will play an important role in biorefinery production, without any competition with food. However, regardless of the pulping or biorefinery process being traditional or state-of-art, the pretreatment technologies are generally classified as physical, chemical, or physical-chemical treatments before further utilization. Therefore, how the different treatments impact the chemical composition of the biomass will be of critical importance in obtaining good quality of end products or studying the downstream process.

1.2. Physical pretreatment of biomass

Physical pretreatment is one of the ways of modifying the fibre properties which has become an easier treatment method for further utilization and manufacture processing. Hence, this can be achieved through the application of physical pretreatment such as reduction in the size of the materials, and beating or refining of the fibres to improve the fibre accessibility in biorefinery process. In the pulping mill, the pulp fibres are commonly modified using some physical treatment for improving fibre strength properties.

Low-consistency (LC) refining is a mechanical treatment process commonly used to improve the strength and properties of fibre in papermaking process; however, the pulp consistency in the refiner is usually between 2% and 6% (Bajpai, 2005). During refining under mechanical and hydraulic forces, both the morphological structure of fibres and the macroscopic properties of the paper are changed such as fibre fracture and shape flattening (Touzinsky et al., 1977), fibre curling (Fardim et al., 2005), fibrillation (Buchanan and Washburn, 1962), delamination of the secondary cell wall S_2 (Molin and Daniel, 2004), and added swelling (Enomae and Lepoutre, 1998) have been reported. External fibrillation causes delamination of the surface layers, which contributes to the fibre-to-fibre bonding potential and also to the retention of filler, pigments, and colloidal particles in papermaking. The fibre shortening also produces fines that fill up the empty spaces in the sheet network and contribute to its formation and bonding (Clark, 1957). Thus, most of the strength properties of paper are increased by pulp refining (Miles and May, 1990; Stationwala et al., 1991). The most significant effects of low consistency refining treatment can be favourable or detrimental. In general, most of the strength properties of paper are consequently increased by refining, but the optical properties are commonly decreased because the paper becomes denser which is undesired. The drawback of refining is the deteriorated dewatering properties, as measured by Schopper-Riegler drainability (SR), freeness and water retention value, which may cause unwanted issues like increased sheet shrinkage due to the effects of fibre swelling (Berthold and Salmén, 1997). Bonding and surface specific area could be increased by refining (Baker, 2003). Therefore, other effects such as the releasing of colloidal and dissolved substances into the liquid media which forms a gelatinous layer have also been proposed (Laine et al., 2004).

Usually, it has been considered that refining has no effect on fibre chemistry. However, changes in the topochemistry of refined pulp were found by Fardim and Durán (2003) that surface lignin was reduced and carbohydrates were exposed on the fibre surface with refining. In addition, several studies have also reported that the surface chemical composition and morphology of the pulp was changed by refining (Fardim and Durán, 2000, 2003, 2005; Kleen, 2000; Fardim et al., 2005). The advanced surface sensitive technique, XPS has been established in pulp and paper research as a tool for detecting the elemental composition and the carbon boundary state in the surface within a range of 3 to 10 nm deep (Brinen, 1993). Also, applications of ToF-SIMS have been reported for paper and pulp (Fardim and Durán, 2000, 2003, 2005; Kleen, 2000; Fardim et al., 2005; Orblin and Fardim, 2010). Our hypothesis is that during refining, a multitude of surface chemical interactions takes place which possibly influence the chemical agent performance such as fluorescent whitening agents (FWAs) which was used in improving the whiteness and brightness of pulp fibre in the papermaking process.

1.3. Chemical pretreatments of biomass for bioconversion

In the biorefinery process, it is usually an integration of physical and chemical treatment together. Enzymatic hydrolysis of lignocellulosic materials after pretreatment is an important environment-friendly process used for the production of fermentable sugars which was further conversed into biofuels and biobased chemicals. However, enzymatic hydrolysis efficiency is limited by the resistant structure of plant walls with the presence of lignin, crystalline regions of cellulose, and covalent cross-linkages between lignin and hemicelluloses in the cell wall (Blanch and Wilke, 1982). Lignin content and its distribution in the plant cell wall play an important role in the process of enzymatic hydrolysis (Mooney et al., 1998). However, it has been reported that the location and the chemical structure of lignin have a more significant effect on the enzymatic hydrolysis than the absolute amount of lignin (Rahikainen et al., 2011). It has also been indicated that an effective lignocellulose treatment process should remove all of the acetyl groups (mainly from xylan) and reduce the lignin content to about 10% in the treated biomass (Kim et al., 2006). Deacetylation could improve the enzymatic hydrolysis because the steric hindrance for the hydrolytic enzymes is reduced, thus increasing the sugar yield (Kong et al., 1992). Crystallinity was believed to have a more significant effect on the initial

hydrolysis rate than on the ultimate sugar yields (Chang et al., 2000). Consequently, both delignification and deacetylation could remove parallel barriers for enzymatic hydrolysis. Thus, pretreatment of biomass is a prerequisite to remove the hindrances to enzymatic hydrolysis.

So far, pretreatment (e.g. physical, chemical, physical-chemical, and biological methods) is required to break down lignin that binds cellulose, and to destroy the crystalline structure of cellulose and increase its specific surface area so that the fragments become accessible to the enzyme active sites (Balat, 2009; Kumar et al., 2012). Especially hydrothermal pretreatment, alkaline pretreatment, steam explosion and ionic liquid are popular and have been widely studied. Pretreatment is the first-step in the conversion of lignocellulosic biomass to biofuels because it significantly improves the processing and reduces the operating costs of the downstream units (Tian et al., 2012). The ideal pretreatment should provide maximum removal of lignin yet minimizing the polysaccharides modification (Ding et al., 2012). Up till now, the work of exploring and improving novel pretreatment was being carried out; but the mechanism of pretreatments for improving the enzymatic hydrolysis is not completely understood. In this thesis, the unexplored potential of hydrotropic pretreatment was investigated from surface chemistry perspective and compared with the alkaline; thus alkaline-hydrogen peroxide, ionic liquids, and hydrothermal pretreatments were also studied, which are briefly reviewed below.

1.3.1. Hydrothermal pretreatment

Hydrothermal pretreatment, also called liquid hot water pretreatment, autohydrolysis, and hot-water compression pretreatment is one of the most common pretreatment methods. One important advantage of hydrothermal pretreatment is that there are no chemicals added and only liquid hot water is used in the process, usually with the temperature between 150 ℃ and 230 ℃ (Garrote et al., 1999).

Hydrothermal pretreatment is efficient hydrolysing hemicelluloses to soluble oligomers and it enhances the accessibility and susceptibility of the surface of cellulose to improve enzymatic digestibility (Zeng et al., 2007). However, it has also been reported that xylan oligomers from hydrothermal pretreatment can be adsorbed onto cellulose, thus hindering the hydrolysis of cellulose by cellulase (Kabel et al., 2007). Hydrothermal pretreatment can remove hemicelluloses, and

result in lignin re-deposition on the wood biomass under hot water condition above 140 ℃ (Xiao et al., 2011). Therefore, adding acid could enforce the hydrolysis in pretreatment but could also cause the so-called pseudo-lignin formation via dehydration of carbohydrates and lignin degradation, and those by-products have been found to decrease the downstream enzymatic hydrolysis. (Pu et al., 2013; Sannigrahi et al., 2011; Hu et al., 2012).

1.3.2. Alkaline and alkaline-hydrogen peroxide pretreatment

Alkaline pretreatment has been presented to cause lignin solubilisation with less sugar degradation and was indicated to be efficient on agricultural residues such as corn stover, straw and sugarcane bagasse (Carvalheiro et al., 2008; Kumar and Wyman, 2009; Bjerre et al., 1996). For hardwood, diluted alkaline pretreatment has been shown to perform well, but it was not found excellent for softwood treatment (Millet et al., 1976). This method could be carried out at high temperature in seconds as well as at low temperature in a few days. Many of the caustic salts can be recovered and/or regenerated. Among the alkaline pretreatment agents, sodium hydroxide has been most studied (Kumar et al., 2009). In addition to the partial removal of lignin and hemicelluloses, alkaline pretreatment can result in swelling of cellulose and decrease in the crystallinity of cellulose, thus improving the enzymatic hydrolysis yield (Li et al., 2012). Alkaline-hydrogen peroxide is a typical environment-friendly agent used for delignification in wood pulping process (Sixta, 2006). Thus, the addition of hydrogen peroxide in alkaline pretreatment could facilitate lignin removal by detaching and loosening the lignocellulosic matrix (Banerjee et al., 2011; Carvalheiro et al., 2008). It has been indicated as an environment-friendly treatment method for removing lignin by HOO· free radical that is formed during the pretreatment process (Costa et al., 2013; Mishima et al., 2006). Also, no detectable and harmful by-products such as furfural for the fermentable stage were generated (Rabelo et al., 2011).

1.3.3. Ionic liquid pretreatment

Ionic liquids are kinds of organic salt solvents composed of ions (cations and anions) with features such as non-flammability, recyclability, good thermal stability and low melting point (below 100 ^{o}C) (da Costa Lopes et al., 2013; Sun et al., 2009). Ionic liquid basically has a capability to dissolve biomass completely. After having been treated by ionic liquid, cellulose can be

precipitated by the addition of anti-solvent for further glucose hydrolysis (Shill et al., 2011). Ionic liquids may be recovered by using an anti-solvent such as water, ethanol, acetone, or even supercritical CO_2 (Blanchard and Brennecke, 2001; Zhu et al., 2006). Ionic liquids have already been demonstrated as efficient solvents for wood biomass and can be used for improving the enzyme hydrolysis of that (Dadi et al., 2006; Lee et al., 2009). However, with heating, ionic liquids can dissolve biomass materials, while at room temperature, ionic liquids such as 1-ethyl-3-methylimidazolium acetate (EmimAC) act as swelling agent of biomass instead of dissolution (Lucas et al., 2010; Marsh et al., 2004). With ionic liquid pretreatment, the biomass particle size is decreased and the crystallinity index of cellulose is lowered, which leads to higher sugar conversion (Dadi et al., 2006; Weerachanchai et al., 2012). Ionic liquid has been reported to be suitable for non-wood biomass treatment such as agriculture residues and sugarcane bagasse as well (da Costa Lopes et al., 2013; Singh et al., 2009; Sarath et al., 2008). However, there is no knowledge of whether ionic liquid has selectivity on lignin, cellulose and hemicelluloses. Also, the recovery and purification of ionic liquid is still expected to ameliorate.

1.3.4. Hydrotropic pretreatment

Hydrotropic pretreatment is the process of extracting lignin from lignocellulosic biomass using aqueous solutions of hydrotropes (Andelin, 1989). Hydrotrope is a compound containing both hydrophilic and hydrophobic functional groups that solubilise hydrophobic compounds in aqueous solutions. Therefore, the mechanism of hydrotropic pretreatment for lignin removal is based on this chemical property of hydrotrope agents. The most generally used hydrotrope agent is sodium xylenesulphonate (SXS). McKee was the first patented hydrotropic method for pulping process in 1943. Through McKee's work, few outstanding advantages of hydrotropic method were presented as higher α-cellulose content in pulp compared with kraft pulp, spent solution which is easily recycled and also easy recovery of lignin. After recovery of the lignin, the spent solution from the previous cooking can be reused 5 to 6 times until it is saturated with dissolved lignin (about 350 g/L), thus saving the energy cost of pulping process (McKee 1954; Gabov et al., 2013).

To meet the demand of improving the solubility of lignin by SXS, the minimum SXS concentration required is at least 0.38-0.40 M (Varade and Bahadur, 2004). It has been reported that the efficiency for sugarcane bagasse pulping is improved

at lower pH, yet more furfural was formed as by product (Hinrichs et al., 1957). Hydrotropic method was used for recovering lignin and cellulose in the experiment by Korpinen and Fardim (2009). The spruce and birch were extracted with 30% SXS at 150 ℃, and after 12 hours of extraction, the yield of lignin with this method varied from 20% to 70% depending on the type of wood material. Lignin extraction by SXS for hardwood is easier than that of softwood due to the presence of guaiacyl units in the softwood lignin chemical structure that leads to condensation reactions (Andelin, 1989; Procter, 1971). Lignin is easily precipitated and the solvent can be recovered (Gabov et al., 2013; Korpinen and Fardim, 2009). Normally, the hydrotropic treatment can be carried out at 150 ℃-170 ℃ with 30-40% SXS relying on the feedstock materials and expected properties of the product (Procter, 1971). Treatment time can be reduced at higher temperature. Take birch as an example: when treatment was carried out using 36% SXS at 170 ℃ for 2 hours with the addition of H_2O_2 or acid as catalysts, the delignification efficiency could be improved dramatically, and the resident lignin of birch was only 1.4% (Gabov et al., 2013). Hydrotropic method deserves to be explored as an environment-friendly method for producing biomaterial and high value-add chemicals.

1.4. Surface chemistry analysis of biomass

The term “topochemistry” has been interpreted as the specific character of reactions proceeding in the solid phase connected with its development in space coordinates (Boldyrev, 1990). It involved a chemistry field concerned with chemical reactions occurring on surfaces or in the interiors of solids where the reactants locally bound (Jakubke and Jeschkeit, 1993). In this work, topochemistry was defined as the surface chemical composition or reactions taking place at specific surface depth of biomass materials in nano-scale. This study principally focuses on the surface chemical composition and component distribution of lignocellulosic biomass which were mainly characterized by XPS and ToF-SIMS. The investigated biomass samples were analysed by XPS and ToF-SIMS after mechanical and chemical treatments for better understanding the change of topochemistry influence on the properties in the following interactions of the material.

XPS is a surface chemical analysis instrument which is able to measure elemental compositions, empirical formula, chemical state and electronic state of the elements that exist in a material. The detecting surface depth is about 1-10 nm.

XPS detects the elements except for H and He. XPS has been used for the analysis of wood surface (Brinen et al., 1991), and it has been applied for investigating the surface coverage by lignin, extractives and carbohydrates of various papermaking pulp fibres: chemical and mechanical, hardwood and softwood (Fardim and Durán, 2003; Koljonen et al., 2003), including enzyme treated spruce pulp (Kangas et al., 2007).

ToF-SIMS is another surface sensitive technique. ToF-SIMS uses a pulsed primary ion beam to detach and ionize species from a sample surface, therefore the masses of which are then identified by measuring their time-of-flight from the sample surface to the detector. Both mass spectra and secondary ion images can be supplied by ToF-SIMS to determine both the composition and the distribution of sample surface constituents.

ToF-SIMS had been applied to chemical characterization of wood surfaces at 1 nm analysis depth (Kangas et al., 2007). Characteristic secondary ions emitted from the wood surface can be used to identify lignin, carbohydrates (polysaccharides), extractives and inorganic ions. It can provide information on the spatial distribution of these compounds in wood tissues and cell walls (Fukushima et al., 2001; Tokareva et al., 2007a). ToF-SIMS has been used for the characterization of the compositions of pulp fibres and paper surfaces (Fardim and Durán, 2003; Fardim and Holmbom, 2005). Furthermore, it has been applied to the analysis of extractives in wood tissues (Imai et al., 2005). Kleen has studied pulp fibre by using ToF-SIMS to supply both spectral information and distribution images simultaneously. Also, applications within wood surface analysis have been reported in recent years (Jung et al., 2010); thus ToF-SIMS was applied in the field of biotechnology recently. Kangas studied the surface chemistry of TMP pulp treated with different enzymes (Kangas et al., 2007). ToF-SIMS has also been used for the direct measurement of enzyme activity on milled spruce and aspen by Goacher et al. (2012).

The sample preparation for ToF-SIMS measurement should be done carefully. The preparation of samples and extra contamination may affect the chemical composition of the surface and consequently affect the results gotten from ToF-SIMS. Therefore, particular care to sample preparation prior to ToF-SIMS analysis is of utmost importance (Tokareva et al., 2007b).

However, the application of advanced surface techniques to biomass characterization in different industrial purpose could give a new view of chemical analysis method.

2. Experimental (Materials and Methods)

2.1. Materials

2.1.1. Lignocellulosic biomass

Paper I-II: ECF (elemental chlorine free) bleached pine and eucalyptus pulp board was obtained from a Finnish pulp mill.

Paper III: Birch (*Bentula pendula*) and pine (*Pinus sylvestris*) chips obtained from a Finnish pulp mill were milled into coarse powder of the particle size of approximately 2 mm in diameter. The carbohydrates composition of milled birch and pine is shown in Table 1.

Table 1. *The carbohydrates composition (per 100 mg) of reference milled birch and pine wood.*

Materials	Glucose	Xylose	Mannose	Arabinose	Galactose
Birch	40.4 (2)	14.1 (0.03)	0.9 (0.3)	0.1 (0.1)	0.4 (0.1)
Pine	38.5 (2)	3.6 (0.6)	7.3 (1.6)	1.7 (0.1)	1.5 (0.2)

Values in brackets are standard deviation.

Paper IV: Sugarcane bagasse was obtained from Centro de Tecnologia Canavieira, Brazil. Bagasse was washed with tap water to remove sucrose until it was colourless and thereafter stored in freeze room.

Paper V: Common reed (*Phragmites australis*) was sourced in summer from native plant in Finland. The whole herbage reed without root and head was first milled and screened to 1mm.

2.1.2. Chemicals

Paper II: Both di-sulphonic DS (with the formula of $C_{40}H_{42}N_{12}Na_2O_{12}S_2$) and tetra sulphonic P01 (with the formula of $C_{40}H_{40}N_{12}Na_4O_{16}S_4$) are stilbene derivatives. $MgSO_4$ was purchased from Fluka and $CaSO_4$ was supplied from Kemira.

Paper III: EmimAC ($C_8H_{14}N_2O_2$) and 1-butyl-3-methylimidazolium chloride (BmimCl, $C_8H_{15}ClN_2$).was obtained from Sigma Aldrich and Fluka respectively.

Paper IV-V: Sodium hydroxide (NaOH) and 30% purity hydrogen peroxide (H_2O_2) was achieved from Fluka.

Paper III-V: Sodium xylene sulfonate (SXS) were purchased from Fluka. Commercial enzymes cellulase (Celluclast 1.5L) and β-glycosidase (Novozyme 188) used in the experiments were kindly supplied by VTT Technical Research Centre of Finland. The activities of the cellulase and the β-glycosidase were 75 FPU/ml and 5900 nkat/ml, respectively.

All chemicals and enzymes were used as received without further purification.

2.2. Methods

2.2.1. LC-Refining (Paper I-II)

LC-Refining was carried out in a ProLab™ laboratory station (Metso Paper, see Figure 2). Technical details of the refining station can be found in Lundin et al., 2008.

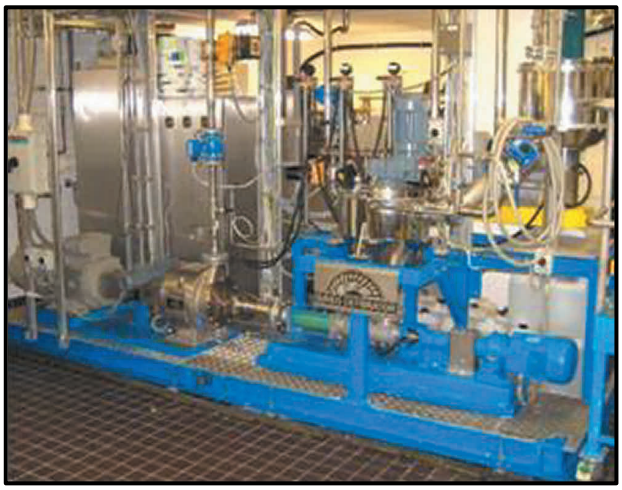


Figure 2. *ProLab™ refiner.*

Paper I: The pine pulp was refined at a specific edge load of 2 J/m and a consistency of 5%. The specific energy consumption (SEC) levels were 0, 75, 150, and 250 kWh/t respectively, with a long medium conical filling.

Paper II: The eucalyptus pulp was refined at a specific edge load of 0.5 J/m and a consistency of 5% with a SF-(short fibre) filling (Lundin et al., 2008). The specific energy consumption (SEC) levels were 0, 50, 100, and 150 kWh/t respectively, and Low SEC levels under 20, 30, 40, 60 kWh/t were studied for FWAs adsorption on Eucalyptus with addition of $CaSO_4$ experiment.

2.2.2. Fines removal and optical properties of paper sheets (Paper I-II)

Two series of samples were made: refined whole pulps which include pulp fines, and fibre fraction samples where the pulp fines were removed after refining. The separation was done with a Dynamic Drainage Jar (DDJ), equipped with a 200-mesh wire and propeller stirring (TAPPI T261cm00). Pulp fines mean the fraction passing a 200-mesh screen. Schopper-Riegler (SR) for statement drainability of pulp suspension was measured after each refining level (ISO5267-1 1999). Fibre length and fines content were measured by Kajaani fibre lab. Handsheets were prepared in a Rapid Köthen apparatus using deionised water (ISO5269-2 1988), which is used for measuring optical properties (L&W Elrepho Spectrophotometer Routine SE070R according to SCAN-CM 27:00).

2.2.3. FWAs adsorption (Paper II)

The anionic FWAs (DS and P01) and calcium sulphate were supplied from Kemira Company. Both di-sulphonic DS (with the formula of $C_{40}H_{42}N_{12}Na_2O_{12}S_2$) and tetra sulphonic P01 (with the formula of $C_{40}H_{40}N_{12}Na_4O_{16}S_4$) are stilbene derivatives.

0.3 g $MgSO_4$ was added into 1% concentration of pulp and then reacted with FWAs for 15 min at pH 6 with magnetic stirring. Paper sheet was formed with a glass funnel. Filtrated solution was determined by fluorescence spectroscopy using a calibration curve (Yamaki et al., 2005). Milli-Q purified water was used throughout during paper sheets preparation. FWAs sheets were used in optical properties measurement as mentioned in the section 2.1.

Fluorescence spectroscopy using steady-state fluorescence spectra of FWAs in solutions were recorded from λem=370 nm to λem=600 nm with λexc= 348 nm using an UV-Vis spectrofluoremeter Lambda 40. However, all samples were examined two or three times at room temperature and their average was reported.

2.2.4. Chemical pretreatment of biomass (Paper III-V)

Different pretreatments were carried out and compared with hydrotropic pretreatment in this work.

Hydrotropic pretreatment

Wood and non-wood biomass which was both treated by hydrotropic pretreatment at different conditions was studied.

Wood biomass (birch and pine) was treated with SXS at the concentration of 30% as an aqueous extraction solvent. The extraction temperature was 150 ℃ during 30 min or 2 h, respectively, and the liquor/wood ratio (w/w) was 8:1. After extraction, the samples were washed by 15% (w/w) SXS solution, followed by hot water to prevent the precipitation of lignin. Subsequently, the washed samples were centrifuged, and the substrates were kept frozen for the next step.

Non-wood biomass (sugar cane bagasse) was pretreated by 30% (w/v) SXS and 0.17% (w/v) formic acid in a digester under the controlled temperature of 160 ℃ for 40 min (Sugarcane bagasse) and 60 min (Common reed), hence temperature segment was 3 ℃/min, the liquor/wood ratio (w/w) was 10:1, and the pH was 3.5±0.05. After pretreatment, the substrates were disintegrated and washed by 5% (w/w) NaOH solution and was followed with tap water until it became colourless. Subsequently, the washed samples (denoted Rsxs) were centrifuged and stored in freeze room.

Ionic liquid pretreatment

Ionic liquid pretreatment was conducted at room temperature with the stirring speed of 500 rpm in a glass bottle with cap. The dosage of EmimAC or BmimCl was 20 mmol/g or 50 mmol/g (dry matter). Ethanol was used as anti-solvent to stop the reaction. After a 1 h or 3 h treatment, the reacted samples were filtrated and washed with deionized water 10 times until no colour of the filtrated liquid was observed.

Hydrothermal pretreatment

The hydrothermal pretreatment was used as static mode pressurized hot water extraction. It was controlled at a desired temperature of 165 ℃ with a revolving digester. The treatment time was 30 min or 120 min and the liquor-to-wood ratio (w/w) was 5:1.

Alkaline pretreatment

Reed or bagasse was mixed with 0.2 g NaOH /g-substrate and 0.25 g H_2O_2 /g-substrate in a flask covered with foil under stirring at 150 rpm. Liquor/wood ratio (w/w) was 10:1.The reaction was running for 24 h in dark place at room temperature. Subsequently, the substrates (named as RH) was washed with distilled water to neutralize pH and stored in freezer.

Alkaline-hydrogen peroxide pretreatment

Reed or bagasse was pretreated by 0.2 g NaOH /g-substrate at 60 ℃ for 2 h with the liquid/solid ratio (w/w) of 10:1. After pretreatment, the solid residue was washed by distilled water until neutral pH. Afterwards, the wet samples were stored in freezer.

2.2.5. Enzymatic hydrolysis (Paper III-V)

The pretreated wood samples were hydrolyzed by the commercial cellulase mixture Celluclast 1.5 at dosage of 10 FPU/g (dry matter), 20 FPU/g and 50 FPU/g fortified with Novozym 188, 300 nkat/g (dry matter) β-glucosidase at 50 ℃ in pH 5.0, for 2, 6, 24, and 48 h, respectively.

Pretreated non-wood biomass was hydrolyzed by cellulase at dosage of 20 FPU/g-substrate together with 300 nkat β-glycosidase /g-substrate at 50 ℃ in pH 5.0, for 2, 6 , 24, 48 and 72 h, respectively.

Enzyme hydrolysis consistency was 20 mg/ml (w/v) in 5 mL sodium acetate/acetic acid buffer, pH 5.0. Upon completion, the enzymatic hydrolysis was ended by boiling the hydrolyzate for 10 min. After cooling with cold water, the hydrolyzate was centrifuged at 1740 *g* for 20 min. The supernatant was used for glucose monomers determination.

The supernatants were analysed by High Performance Liquid Chromatography (HPLC), and the glucan and the xylan yield after enzymatic hydrolysis were calculated based on the glucan and xylan contents of raw material.

2.2.6. Topochemical characterization (Paper I-V)

X-ray spectroscopy (XPS) analysis

XPS spectra were obtained with a Physical Electronics PHI Quantum 2000 ESCA instrument equipped with a monochromatic Al Kα X-ray source. Low

resolution spectra were measured in 3.5 min with the pass energy 187 eV, and high resolution C1s scanning was done in 9 min using the pass energy 23 eV. At least, four different spots were measured on each sample. The oxygen-to-carbon (O/C) ratios were calculated from the low resolution XPS spectra. Both acetone-extracted samples (Soxhlet, acetone-water mixture 9:1 (v:v), overnight) and unextracted samples were analysed. The surface coverage by lignin (S_{lig}), carbohydrates (S_{car}) and extractives (S_{ext}) were calculated according to the following equations using the average O/C ratio value (Ström and Carlsson, 1992; Laine et al., 1994):

$$S_{ext} = (O/C_{extracted} - O/C_{before\ extraction}) / (O/C_{extracted} - O/C_{extractives}) * 100 \quad (1)$$

$$S_{lig} = (O/C_{extracted} - O/C_{carbohydrate}) / (O/C_{lignin} - O/C_{carbohydrate}) * 100 \quad (2)$$

$$S_{car} = 100 - S_{lig}\ \% \quad (3)$$

$O/C_{carbohydrate} = 0.83$, $O/C_{lignin} = 0.33$, $O/C_{extractives} = 0.09$

ToF-SIMS characterization

ToF-SIMS analysis was performed with a Physical Electronics ToF-SIMS TRIFT II spectrometer. The instrument was equipped with a primary ion beam of $69Ga^+$ liquid metal ion source and an electron flood gun for charge compensation. Positive secondary ions were detected using 25 kV acceleration voltage in 8 min. At least, three measurements were done on each sample.

Different with paper I-III, for paper IV-V, acetone-extracted samples of the pretreated and enzymatically hydrolyzed materials were prepared for the ToF-SIMS analysis. Sugar monomers from hexosans (cellulose, mannan, galactan) were identified by ToF-SIMS spectrum at m/z 127 and 145, and from pentosans at m/z 115 and 133 (Fardim and Durán, 2003). Lignin gives the characteristic mass fragments by ToF-SIMS: m/z 107 and 121 from p-hydroxyphenyl (H) unit, m/z 137 and 151 from guaiacyl (G) unit, m/z 167 and 181 from syringyl (S) unit (Saito et al., 2005), and m/z 77 and 91 from general aromatic ring (Koljonen et al., 2003; Goacher et al., 2012).

2.2.7. Determination of chemical composition (Paper III-V)

Carbohydrates determination

HPLC was applied for carbohydrates determination. The milled reference sample and the pretreated samples screened through 60-80 mesh were extracted by acetone for 5 h. The extractive-free samples were used for characterization of the chemical composition. The glucan, xylan, and lignin contents of the untreated and the pretreated biomass were determined by following the NREL (National Renewable Energy Laboratory) procedure for the determination of structural carbohydrates and lignin in biomass. Dissolved lignin was measured by UV spectrometer at 205 nm. Also, carbohydrates were measured by HPLC.

For paper III: The glucose monomers were measured by High Performance Liquid Chromatography (HPLC) Dionex ICS-5000 with PA20CarboPac anion-exchange resin column (0.4×150 mm) in the temperature 30 ℃, 1.0 mM KOH as eluent from 0 to 12 min following with 10 mM in the rest 8 min with the flow rate 0.008 ml/min. Sample injection volume was 20 μL.

Paper IV-V: Both the enzyme and sulphuric acid hydrolyzates of the pretreated samples were detected by HPLC system (Model 1200, Agilent, USA) equipped with a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm) and refractive index detector. The column was operated at 55 ℃ with 0.005 mol/L H_2SO_4 solution as the mobile phase at a flow rate of 0.5 mL/min, and the quantitative analysis was performed using calibration with external standards of known concentration. Sample injection volume was 20 μL.

ATR-FTIR measurement (Paper IV-V)

Attenuated Total Reflectance (ATR)-Fourier Transform Infrared (FTIR) spectroscopy was used to characterize the samples after pretreatment. Small amount of freeze dried samples after pretreatments were placed on the diamond probe of Nicolet IS50 FTIR (software is OMNIC 7.3, wave numbers in cm^{-1}). 36 scans were acquired for each sample and the results were recorded from 4000 cm^{-1} to 400 cm^{-1}.

2.2.8. Morphology of physically and chemically treated samples (Paper I-V)

Field Emission Scanning Electron Microscope (FE-SEM) was applied to supply the morphology information of treatment samples.

Paper I-II: SEM images were obtained using a JEOL JSMT 300 microscope, operated in secondary electron mode at a beam current of 100 μA and

accelerating voltage of 20 kV. Samples were previously coated with Pt for 20 s with an Agar scientific sputter coating system equipped with a rotating base. Images were obtained at magnifications of 500×, 2000×, and 20,000× for paper I. Images for paper II were at magnifications of 5000×.

Paper III-V: SEM LEO 1530 Gemini equipment. The freeze-dried samples were previously coated with carbon using a coating system equipped with a rotating base. Images were obtained at the 500× and 15,000×magnifications.

3. Results and discussion

3.1. Topochemistry of softwood and hardwood fibres in Low-consistency refining (Paper I-II).

The optical properties, morphology and surface chemical composition of the pulp fraction with fines and fibre fraction without fines after LC-refining were studied in this part. The representative results were shown below.

3.1.1. Effects of refining on pulp and fibre properties

For both pine and eucalyptus, the effect of refining on the optical properties of the pulp and the fibre samples was investigated. The whiteness, the brightness and the light scattering coefficient (*s*) of pine and eucalyptus in relation to SEC were all decreased in addition to the light absorption coefficient (*k*).

Table 2. *Effects of LC-refining on the whole pulp (P) and fibre fraction (F) properties of pine sample.*

SEC	**0 kwh/t**		**75 kwh/t**		**150 kwh/t**		**250 kwh/t**	
	P	**F**	**P**	**F**	**P**	**F**	**P**	**F**
SR	13.0	ND	19.3	ND	37.4	ND	72.0	ND
Fibre length (mm)	2.4	ND	2.2	ND	1.9	ND	1.5	ND
Fines wt %	2.1	ND	2.4	ND	3.5	ND	4.4	ND
CIE Whiteness %	71.4	69.6	68.0	66.7	63.6	64.6	55.4	57.3
Brightness % ISO	85.1	85.8	82.9	83.5	80.8	82.4	75.3	77.2
Opacity	78.5	88.6	74.0	77.9	71.2	73.4	58.7	60.2
s m^2/g	34.9	35.2	27.3	25.6	22.3	22.4	12.6	12.9
k m^2/g	0.18	0.11	0.21	0.15	0.22	0.15	0.25	0.20

ND: not determined. *s*: light scattering coefficient; *k*: light scattering coefficient. All optical properties data reported were the mean of ten readings for each test.

For both the whole pulp samples and the fibre fraction samples, the pulp drainage ability was reduced by refining due to the creation of fines, fibre fibrillation, and delamination, as shown by a fibre length reduction and a pulp fines weight percentage increase in Table 2 and 3 (Li et al., 2011b). The optical properties of the whole pulp sheets did not obviously differ from those of the fibre fraction

sheets at the same SEC level. The role of the fine material in the optical properties of paper is known to be related to the high specific surface area as well as the eventual lignin content (Asikainen et al., 2010), whereas the fibres' contribution can be speculated to be mainly due to the properties of the fibre cell wall, that is, the light reflecting surfaces of the external cell wall layer. Therefore, the impacts tendency of refining on optical properties was similar between eucalyptus and pine.

Table 3. *Effects of LC-refining on P and F properties of eucalyptus sample.*

	0 kWh/t		**50 kWh/t**		**100 kWh/t**		**150 kWh/t**	
	P	**F**	**P**	**F**	**P**	**F**	**P**	**F**
SR (^{o})	22.2	ND	37.2	ND	53.4	ND	76	ND
Fines wt %	1.7	ND	2.6	ND	2.7	ND	3.0	ND
Fibre length (mm)	0.79	ND	0.77	ND	0.75	ND	0.73	ND
Brightness % ISO	87.7	87.4	87.0	86.8	86.4	85.9	85.0	84.2
CIE Whiteness %	77.2	76.4	75.2	74.8	73.9	73.5	71.4	70.3
Opacity %	82.8	82.8	81.9	81.9	79.9	79.5	73.7	76.4
s m^2/g	48.5	40.1	42.5	38.2	40.1	32.3	28.3	28.3
k m^2/g	0.16	0.15	0.16	0.15	0.17	0.15	0.17	0.15

3.1.2. Effects of refining on the surface morphology

The sheets made from the whole pulp and the fibre fraction samples of pine and eucalyptus refined with different SEC levels were analysed by SEM. The morphology of refined samples of pine and eucalyptus influenced by refining can be seen in Figure 3-7. During refining, the fibre shape of pine sample shown in Figure 3 was changed from tubular to flat. It is reasonable to suppose that higher contact areas between the fibres are achieved with flat shapes than with tubular-shaped fibres. The refining process initially caused a shearing in the fibre wall, followed by an increase in the surface roughness and release of superficial layers. The increased surface fibrillation of the fibres can be distinguished in Figure 3b and 6b, compared to Figure 3a and 6a.

Comparing the fibre fraction and the whole pulp sheets of pine, it can be noted that the fibre fraction sheet structure in Figure 3 resembled that of the whole pulp sample in Figure 3a, and after refining (Figure 3b), the sheet structure was denser in a similar manner as in Figure 3b.

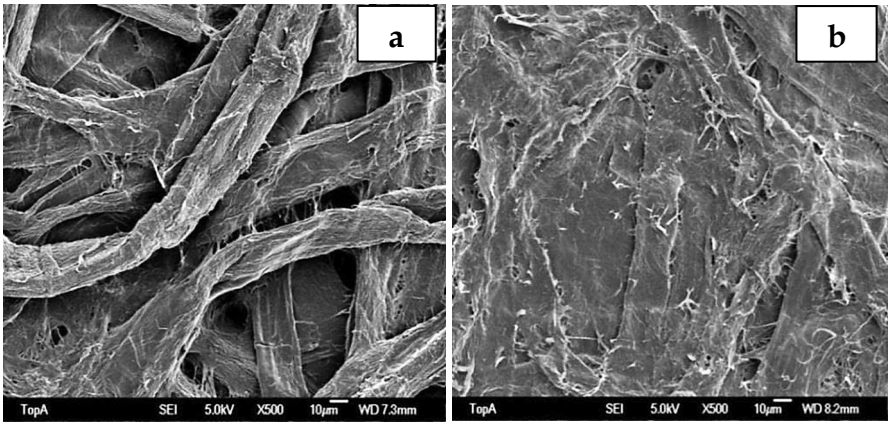


Figure 3. *FE-SEM images of the whole pulp sheets of pine with SEC of 0 kWh/t (a) and 150 kWh/t -P (b) at 500× magnification.*

In addition to the fibre flattening, voids between fibres of pine were filled (Figure 3b and 4b), supposedly due to external fibrillation and additional fines created, and a film-like connection between fibres was formed which is obviously found on pine samples (marked by white cycles in Figure 5).

The film-like connections were even more prominent in the fibre samples (Figure 4). Perhaps the film was more easily seen because of the lower amount of fine material that would cover it. Another possibility is that in the fibre fraction, more hydrophilic compounds from the cell walls were leaked out during the pulp fines separation stage, thereby contributing to the formation of gel-like material, which after drying appears as film. The film was presumably formed with extracellular material including polysaccharides and extractives, as well as very fine cellulosic fibrils produced after high-energy refining. Thus, the pulp fines and fibrils were probably generated from the primary wall and outer secondary wall (Li et al., 2011a).

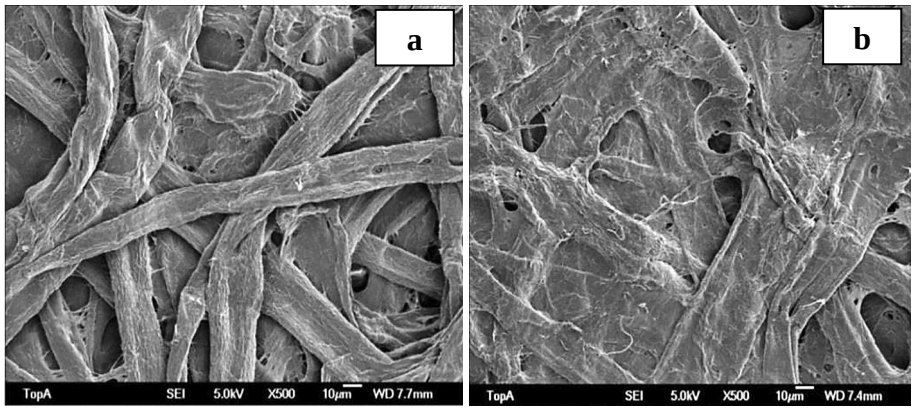


Figure 4. *FE-SEM images of the fibre fraction sheets of pine with SEC of 0 kWh/t -F (a) and 150 kWh/t -F (b) at 500× magnification.*

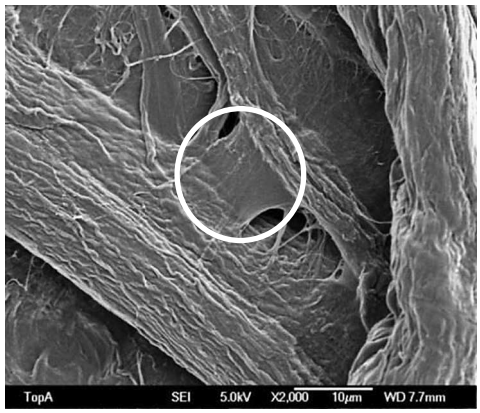


Figure 5. *FE-SEM image of the whole pulp with SEC of 150 kWh/t -P at 2,000x. The parts marked with white circles are gel-like materials.*

The fibrillation of eucalyptus fibre after refining was observed also in Figure 6. Severe shrinkage after fibre swelling was detected on P samples and F samples of pine and eucalyptus by FE-SEM. After removing fines and fibrils, the compactly shrinkage was exposed on F samples in Figure 7b.

The increased pulp fines and fibrils can lead to the increase in the specific surface area of pulp fibres (Li et al., 2011b), thus increasing the bonding potential (i.e. the strength properties of the whole pulp sheets) and affecting the optical properties of the sheet (Fardim and Durán 2003). This is also in good agreement with the increased SR and WRV values (Table 2-3) after refining.

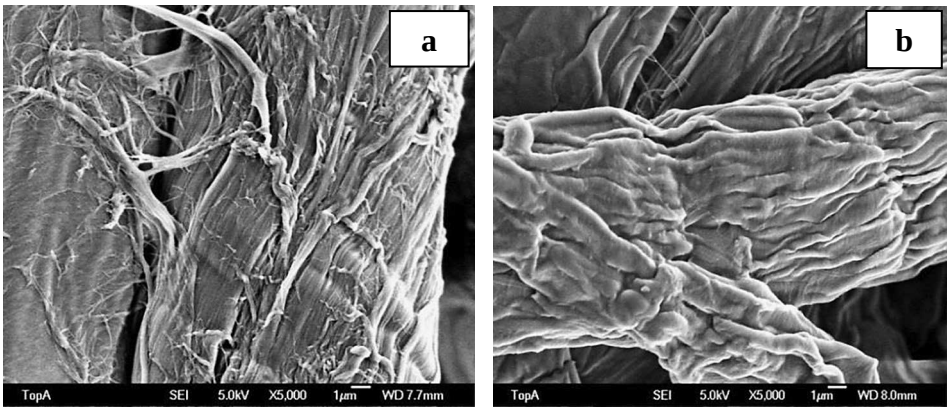


Figure 6. *Pulp (P) samples with FE-SEM magnification of 5,000×, at 0 kWh/t (a) and 100 kWh/t (b).*

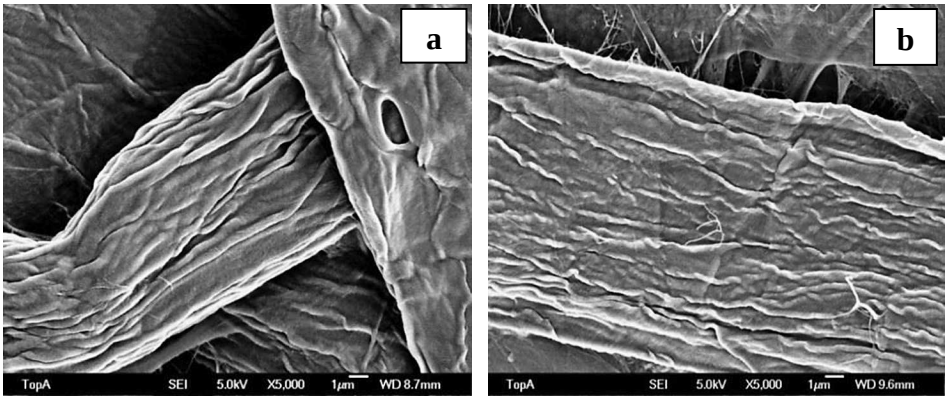


Figure 7. *Fibre fraction (F) samples with FE-SEM magnification of 5,000×, at 0 kWh/t (a) and 100 kWh/t (b).*

3.1.3. Effects of refining on surface chemical composition

Techniques with different surface sensitivity and specificity were used to study the surface chemical composition before and after refining. Surface chemical composition of pine and eucalyptus samples after refining detected by XPS was shown in Table 4-5.

In Table 4-5, the carbon boundary state (C_1 to C_4 was originated as the bond C-H or C-C, C-O, C=O or O-C-O and O-C=O respectively) and the O/C ratios of the samples observed from the XPS spectra are listed, and the surface coverage by lignin (S_{lig}) and by extractives (S_{ext}) were presented.

Table 4. *Effects of LC-refining on surface chemical composition of whole pulp (P) and fibre fraction samples (F) of pine as analysed by XPS.*

SR-Sample	C_1%	C_2%	C_3%	C_4%	O/C	S_{lig}%	S_{ext}%
13-F	18.3	60.4	19.8	1.5	69.6	26.3	2.3
19-F	21.3	58.1	18.1	2.4	67.3	27.4	2.8
37-F	19.8	60.1	18.2	1.9	67.9	28.6	3.3
72-F	19.9	59.8	18.5	1.8	66.1	33.4	5.2
13-P	20.2	66.6	11.3	2.1	67.3	31.1	2.4
19-P	22.4	63.6	11.4	2.7	63.8	27.3	9.2
37-P	20.9	62.4	13.6	3.1	64.1	25.3	10.3
72-P	22.9	62.4	12.2	2.5	65.8	23.5	10.5

13-F, 19-F, 37-F, 72-F of fibre fraction samples, and 13-P, 19-P, 37-P, 72-P of whole pulp samples were with the SEC of 0, 75, 150, and 250 kWh/t, respectively.

In Table 4, the whole pulp samples (with pulp fines) of pine had higher C_1 content and lower O/C ratios than the fibre fraction samples (without pulp fines) at the same SEC level. In Table 4, under the SEC level of 0, the fibre fraction samples of pine (13-F) had lower S_{lig} (26.3%) and S_{ext} (2.3%), compared to the whole pulp samples (13-P) with S_{lig} of 31.1% and S_{ext} of 2.4%. Removal of fines could obviously result in the S_{ext} value of the fibre fraction sample to be lower than that of the whole pulp samples. This is probably due to the pulp fines, which typically in chemical pulps contain more extractives and lignin than in the long fibres (Bäckström et al., 2008; El-Sharkawy et al., 2008). Thus, a higher O/C ratio of the fibre fraction samples is probably due to the removal of pulp fines.

Table 4 also shows that S_{lig} of the fibre fraction samples increased with the increase of SEC level, whereas the S_{lig} of the whole pulp samples decreased when the SEC level was increased. A decrease in S_{lig} was also previously reported for eucalyptus kraft pulp during refining (Fardim and Durán, 2003). The opposite phenomenon observed in the fibre fraction of pine could be explained by the creation of fibrils, which involves the outer layers of cell walls. Even if the lignin-rich middle lamellae are assumed to be practically all dissolved in a bleached chemical pulp, removal of deposited hemicelluloses in refining and subsequent fractionation stage could reveal residual lignin. Another possibility is that the detected carbon content perceived as lignin with this technique comprises

of fatty acid salts that were not removed in acetone extraction and therefore appeared as lignin (Fardim and Durán 2003).

In Table 5, in the results obtained from eucalyptus sample after refining, it is shownthat P samples have higher lignin and extractive content than F samples at the same SEC level due to the fact that fines (typically in chemical pulps) contains more extractives and lignin (Orblin and Fardim, 2010; El-Sharkawy et al., 2008; Bäckström et al., 2008). The surface coverage by extractives of eucalyptus was increased, oppositely to the lignin decrease with refining power increasing. Between P and F samples of eucalyptus, the surface coverage by lignin was decreased and carbohydrates were increased along with the added refining energy. It has been suggested that hemicelluloses, particularly xylan, arabinan, and galactan, are deposited on fibre surfaces during kraft pulping, as they have been detected and was clearly enriched in the primary wall of pine kraft pulp, compared to the outer secondary wall (Kibblewhite and Brookes, 1976). The pulp fines generated from primary wall with the increase of SEC level presumably contain deposited hemicelluloses. Deposition of dissolved hemicelluloses (xylan) is also plausible. The decrease in surface coverage by lignin refers to external fibrillation where cellulosic fibrils are shredded, opened up and thereby spread more on the surface.

Whether pine or eucalyptus, with the increasing of the SEC level, the S_{ext} increased for both of fibre fraction and whole pulp samples. Probably this is due to the fact that hydrophobic compounds that are released from the fibre wall during refining become distributed and re-deposit on the cellulosic surfaces. More extractives may re-deposit on fines, as fines have larger specific surface area. Thus, S_{ext} of pulp samples increased obviously with increasing SEC, as presented in Table 4-5. However, the S_{ext} increases observed in the case of the fibre fraction samples is possibly due to the deposition of extractives on the surface of fibrils, as pulp fines were already removed after refining.

The content of aliphatic carbon (C_1) in high-resolution XPS was higher in the refined samples than in the unrefined samples. This change is clearer seen in eucalyptus samples than in pine samples. It refers to re-localization of components in the fibre cell wall during refining. In Table 5, extraction naturally decreased the C_1 and increased the C_2 as the carbon-rich extractives were removed. The extraction (the proportional decrease in C_1) was more efficient in the refined P samples compared to the unrefined sample (22-P), also confirming

that components from the cell wall pores were probably redistributed and spread on the fibre surfaces during the process, and were thereafter easily washed away. The C_4 content of P and F samples of eucalyptus was raised typically by refining performance. After removing fines, the C_4 proportion in F samples was higher than that in P samples. The carbon bond C_4, that is, the amount of carboxyl groups raised by refining. It was assumed that carboxyl group from fatty acids or xylan was exposed on the surface of fibre after refining and thus can potentially affect the retention of chemical additives. This effect on C_4 by refining seems not to be appropriate for pine. The difference expression on the change of surface chemical composition on pine and eucalyptus was also due to the chemical compositions content between softwood and hardwood.

Table 5. *Effects of LC-refining on surface chemical compositions of extracted (E) and the un-extracted whole pulp (P) and fibre fraction samples (F) of eucalyptus.*

Samples	**C_1%**	**C_2%**	**C_3%**	**C_4%**	**O/C**	**S_{lig}%**	**S_{car} %**	**S_{ext}%**
22-P	19.9	47.2	32.2	0.66	0.69	23.1	76.9	4.7
22-E-P	14.9	61.7	22.5	1.01	0.71			
37-P	26.9	50.0	17.4	5.70	0.60	21.7	78.3	18.3
37-E-P	12.1	66.0	22.4	1.01	0.72			
53-P	26.9	50.6	18.9	3.73	0.60	19.2	80.8	20.7
53-E-P	16.2	62.9	17.6	4.25	0.73			
76-P	24.0	52.6	19.6	3.76	0.61	15.7	84.3	21.0
76-E-P	11.9	66.8	18.5	2.87	0.75			
22-F	26.8	45.9	24.7	2.62	0.66	19.0	81	11.6
22-E-F	10.8	68.6	17.6	2.99	0.73			
37-F	28.1	46.3	20.7	4.90	0.66	18.3	81.7	13.4
37-E-F	12.3	66.4	19.7	1.61	0.74			
53-F	25.7	47.8	22.0	4.54	0.63	16.8	83.2	17.4
53-E-F	11.7	65.0	21.8	1.56	0.75			
76-F	24.9	51.5	17.0	6.71	0.62	16.4	83.6	19.9
76-E-F	13.0	63.3	22.4	1.29	0.75			

22-P, 37-P, 53-P, 76-P of whole pulp samples, and 22-F, 37-F, 53-F, 76-F of fibre fraction samples without fines were with the SEC of 0, 50, 100, and 150 kWh/t, respectively.

The ToF-SIMS spectra can give more detailed information on the chemical composition despite the limitations in quantitative application of the technique.

In order to compare the relative contents of components on the surfaces, the relative peak intensities were considered. Ratios between the normalized peaks of the whole pulp and the fibre fraction samples with different refining energy were worked out. Thus, the characteristic peaks of carbohydrates, lignin, and extractives were selected (Table 6-7). Any value differing from 1 basically signals a change in the surface chemical composition in the outmost layer.

For pine samples in Table 6, the refining effect on the surface of the fibre fraction (column 37-F/13-F) was evident. Carbohydrate signals were increased, and most of the extractives were decreased. However, the decrease in the lignin signals was not noticeable, which supports the possibility interference of the ToF-SIMS results from fatty acid salts (Goacher et al., 2013). In general, the peak ratios were in agreement with the XPS results. For the whole pulp (column 37 P/13 P), the changes after refining as detected by XPS were in good correspondence with ToF-SIMS peak ratios. Mechanical action during refining clearly contributed to the release of components entrapped in fibre wall pores to the external liquid phase, as previously suggested (Fardim and Durán, 2003). Xylan (pentosan) was likely re-adsorbed rather than exposed on the surface during refining, based on the difference between the surface carbohydrate contents evaluated with ToF-SIMS. It seems reasonable to suppose that cellulose (hexosan) was exposed by a peeling action on the fibre surfaces and that part of the xylan was adsorbed after being released from the cell wall pores in a similar way with the fatty acids. Hydrogen or dispersion bonds are possibly the driving forces for the adhesion of xylan and extractive aggregates onto external fibre surfaces. Fibre chemistry has been shown to affect paper strength properties (Fardim et al., 2005; Sundberg et al., 2000), and thus, the chemical changes along with the morphological modifications caused by refining are assumed to be of importance in papermaking.

Table 6. *The secondary ions in the positive ToF-SIMS spectra, the relative intensities of which were observed for the whole pulp (P) and fibre fraction (F) of pine.*

Components	**Peaks (m/z)**	**Ratio of peak intensity (normalized)**			
		13-P/13-F	**37-P/37-F**	**37-F/13-F**	**37-P/13-P**
Carbohydrates					
Pentosans	115+	2.0	1.7	2.1	2.0
	133+	5.5	1.7	2.0	1.4
Hexosans	127+	2.4	1.7	2.3	1.3
	145+	3.1	1.7	2.6	1.4
Lignin	137+	1.7	0.7	1.2	0.7
	151+	1.2	1.1	1.0	0.8
Extractives					
Resin acid	300+	1.2	1.3	0.9	1.2
	301+	3.7	0.9	1.1	1.0
	302+	1.5	0.9	0.6	0.8
	303+	3.5	0.5	0.8	0.7
Fatty acid					
Palmitic acid	239+	1.8	0.6	0.7	0.9
	257+	0.3	2.4	1.0	1.0
Stearic acid	267+	3.8	1.7	2.8	1.0
	285+	1.1	0.9	0.8	1.5
Oleic acid	265+	3.7	1.9	3.3	1.0
	283+	0.5	2.2	3.8	1.2
Linoleic acid	263+	1.8	1.4	1.9	1.2
	281+	6.6	2.6	4.5	0.9
Linolenic acid	261+	2.4	0.7	0.8	1.3
	279+	5.0	2.6	4.7	1.1
Arachidic acid	295+	1.9	1.6	1.9	0.7
	313+	1.1	0.8	0.8	1.2
Behenic acid	323+	3.3	1.9	2.5	0.9
	341+	2.1	2.4	2.4	0.9
Tetracosaboic acid	351+	1.4	1.5	1.3	1.2
	369+	1.6	0.9	2.2	1.0

On the other hand in Table 7, the surface compositions of P and F samples of eucalyptus at different power levels were also investigated by ToF-SIMS spectrometry. Characteristic peaks of cellulose, xylan, lignin and extractives are shown in Table 7. Higher ratios for carbohydrate peaks may be an indication of cellulose and xylan enrichment after refining. The xylan ratio value of P samples was higher than that of F samples with increasing refining power. According to the ToF-SIMS peak ratio, the reason for the increase of surface amount of extractives on sample 37-P was due to a release of myristeric, pentadecan, and oleic acids, as well as Ca myristate, pentadecanoate, and palmitate during refining (Fardim and Durán, 2000). It was assumed that because of the internal fibrillation of fibre cell wall as a result of the mechanical refining performance, carbohydrates and extractives were exposed. Evidence of xylan deposition or cellulose exposure could be observed in the ToF-SIMS peak ratios. Deposition of lignin did not seem to be the case for eucalyptus, despite being reported for pine (Laine et al., 1996).

For both pine and eucalyptus, the results obtained by the peak intensity ratios of ToF-SIMS spectra had a good agreement with surface coverage results by XPS. The increase in S_{ext} and the decrease in S_{lig} during refining as estimated by XPS (Table 4-5) were also observed by the ToF-SIMS peak ratios (Table 6-7). The different extractives component was charaterized by ToF-SIMS.The increase in the S_{ext} of pine detected by XPS was due to free fatty acids, mainly oleic, linoleic, and arachidic acid. However, the remarkable increase of S_{ext} detected by XPS was not accompanied by any clear increase in the extractive peak intensities. The detection depth of ToF-SIMS is restricted on the outmost monolayer, whereas XPS collects information slightly deeper, and a difference in the component distribution is plausible. Furthermore, the signal intensities in ToF-SIMS depend not only on the component content of interest, but also on the various electronic, physical, and chemical states during the measurement.

Combination of the XPS and ToF-SIMS results was shown, typically for eucalyptus sample, and the peak ratio of xylan was obviously increased by refining treatment. C_4 (O-C=O) and carbohydrates increase was observed in XPS results. Carbohydrates were known to be containing carboxylic acid side groups, and therefore its exposure probably affects the adsorption of cationic chemical agents. Moreover, extractives on fibre surfaces during refining, particularly for fatty acid salts, may enhance the hydrophobic interactions with molecules

containing hydrophobic portions in chemical structure. This will have the unexpected influence in the downstream papermaking process. Therefore, in this work, taking eucalyptus as example, effects of LC-refining on chemical agent FWAs adsorption were studied.

Table 7. *The secondary ions in the positive ToF-SIMS spectra, the relative intensities of which were observed for eucalyptus refined pulp and fibre samples.*

Component	**Peaks (m/z)**	**Ratio of peak intensity (normalized)**				
		22P/22F	**37P/22P**	**37P/37F**	**53P/37P**	**53P/53F**
Carbohydrates						
Cellulose	127+	1.0	1.6	1.1	1.6	1.4
	145+	0.5	0.8	1.3	1.8	1.4
Xylan	115+	2.1	1.4	1.0	1.3	1.2
	133+	1.4	1.5	1.1	1.6	1.4
Lignin	137+	1.2	1.7	0. 9	0.9	0.7
	151+	1.0	0.8	0.9	0.9	0.6
	167+	1.0	0.6	0.7	0.8	0.7
	181+	0.6	0.9	0.7	1.1	1.2
Extractives						
Myristeric	211+	0.7	3.7	0.6	2.1	2.0
	229+	0.9	4.3	0.1	1.3	2.6
Pentadecanoic	225+	2.3	4.4	1.9	2.1	3.6
	243+	0.8	1.5	0.6	1.4	1.9
Palmitic	239+	1.2	1.3	1.2	0.6	1.6
	257+	1.1	7.3	1.3	1.1	2.8
Oleic	283+	1.5	3.2	2.9	0.3	1.9
	265+	1.2	1.9	0.5	1.0	3.1
Steric	285+	1.0	1.2	0.4	1.2	6.6
	267+	1.5	2.4	1.4	0.9	5.4
Lignoceric	369+	0	0	0.4	1.8	4.7
	351+	0	0	0	0.6	2.0
	339+	2.1	0.3	0.8	1.5	0.7
Pentadecosanic	383+	1.0	0.1	0.8	0	0.9
	365+	0.6	0.2	0.3	1.6	3.3

	355+	0	0.2	0.5	0.5	0.4
Calcium	268+	0.8	0.3	0	1.0	1.4
Myristate	495+	0.5	2.2	0.5	3.8	0.4
Pentadecanate	281+	1.2	0.4	0.2	0.2	5.6
	523+	0	0	0.7	6.3	3.9
Palmitate	296+	0.3	0.2	0.6	2.6	2.9
	551+	0	0	0.6	0	1.5
Stearate	323+	1.2	0.2	0.7	0.3	2.6
	324+	0	0.2	1.4	1.4	3.5
Sodium						
Oleate	304+	0	0	1.1	3.1	1.3
	305+	0.5	0.3	0.8	0.4	0.9
Eicosenoate	332+	0	0	1.3	4.2	0.7
	333+	0.2	0.4	0.5	0	1.5
Lignocerate	391+	0	0	1.1	0.9	2.6
	413+	1.0	0.2	0.6	1.0	10.4
Hexadecanoate	441+	1.0	0.1	1.0	1.3	1.3
Sterols						
Sitosterol	415+	0	0	0.8	0	1.0
	414+	0	0	1.0	2.1	1.3
	397+	0.5	0.2	1.1	2.5	6.5
Sitostanol	416+	0	0	3.9	2.1	1.3
	398+	0.7	0.1	0.5	1.6	0.5
oxo-sitosterol	429+	0.5	0.1	0.2	2.1	1.5
	411+	1.5	0.1	0.3	2.5	0.5

3.1.4. Effects of LC-refining on FWAs adsorption

FWAs adsorption on eucalyptus after refining

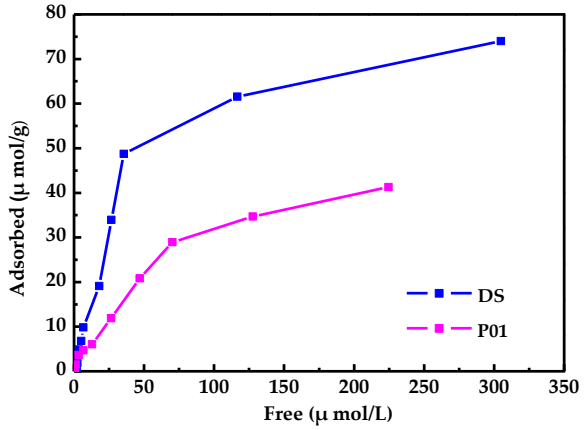


Figure 8. *Adsorption isotherm of FWAs onto reference Eucalyptus pulp (DS: di-sulphonic FWA (with the formula of $C_{40}H_{42}N_{12}Na_2O_{12}S_2$); P01: tetra-sulphonic FWA (with the formula of $C_{40}H_{40}N_{12}Na_4O_{16}S_4$)).*

The anionic FWAs (DS and P01) adsorption isotherms on eucalyptus pulp without refining are shown in Figure 8, which presents that DS was more easily adsorbed on eucalyptus than P01. This is possibly because of the different structures of DS and P01. Both molecules have hydrophobic groups that can interact with fibres and promote sorption of FWAs. However, there are two sulphonic acid groups in the DS molecule, while there are four sulphonic acid groups present in the molecule of P01. More sulphonic acid groups may generate stronger repulsion forces between the negatively charged fibres and P01 in comparison with DS.

After being treated at different refining power, pulp and fibre samples were treated with fluorescence DS and P01 with dosage 1 kg/ton, 2 kg/ton, 5 kg/ton, 7 kg/ton, 10 kg/ton respectively, and the adsorbed amounts are shown in Figure 9. As can be seen from Figure 9a, at the same refining power, DS adsorbed more on the P samples compared to the F samples. This is probably due to the large specific surface area of fines in P samples. High specific surface area benefits the adsorption of FWAs (Shi et al. 2012). However, for P01, the opposite phenomena were observed in Figure 9b, i.e. more P01 could be adsorbed on F samples at the same refining power. It was speculated that more binding sites (more surface

carbohydrates amount on F samples than P samples in Table 4) of F samples after removing fines could improve the FWAs retention on fibres (Alén, 2007). On the other hand, this may be also related to the different adsorption behaviour of DS and P01 as mentioned previously (Figure 8). The sulphonic acid groups of P01 are doubled compared to DS. C_4 values were relatively lower in P samples than in F samples. As a result, less P01 was adsorbed on P samples despite P samples include many fines which have large specific surface area. Overall, the adsorption performance of both DS and P01 was improved by refining, as exhibited in Figure 9. Our hypothesis is that hydrophobic extractives trapped in the fibre wall are released to fibre surfaces and favours hydrophobic interactions with FWAs. This hypothesis is supported by adsorption isotherms and XPS and ToF-SIMS results. Positive effects of cationic surfactants on the sorption of FWAs were reported before (Iamazaki and Atvars, 2007).

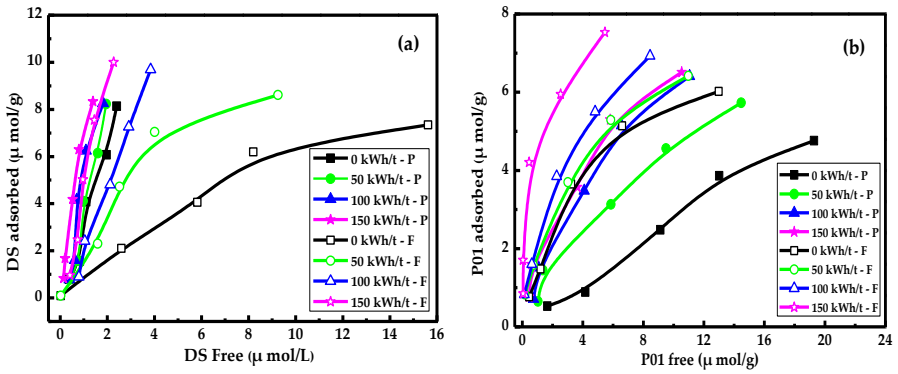


Figure 9. *The adsorption of DS (a) and P01 (b) FWAs on refined P and F samples with different SEC levels (DS: di-sulphonic FWA (with the formula of $C_{40}H_{42}N_{12}Na_2O_{12}S_2$); P01: tetra-sulphonic FWA (with the formula of $C_{40}H_{40}N_{12}Na_4O_{16}S_4$)).*

Effects of adding FWAs before LC-refining

The addition of DS and P01 before LC-refining was also investigated. The FWAs went through the refining process together with fibres, and the adsorption results are shown in Tables 8 and 9. Therefore, by increasing the amount of DS and P01, the adsorbed amount on fibre increased significantly. However, the adsorption amount of DS (Table 8) and P01 (Table 9) did not change with the increasing of SEC. It is obvious that the mechanical hydraulic force generated by the high refining power could disturb the FWAs retention.

Table 8. *DS adsorption on pulp before refining.*

Refining power (kWh/t)	**2 g/ton**		**7 kg/ton**	
	µmol Free/L	**µmol adsorbed/g**	**µmol Free/L**	**µmol adsorbed /g**
0	3.8	1.7	3.0	4.9
50	5.1	1.6	2.9	4.9
100	5.0	1.6	2.3	5.0
150	6.8	1.4	2.9	5.0

Table 9. *P01 adsorption on pulp before refining.*

Refining power (kWh/t)	**2 kg/ton**		**7 kg/ton**	
	µmol Free/L	**µmol adsorbed/g**	**µmol Free/L**	**µmol adsorbed/g**
0	0.9	1.5	6.9	4.7
50	1.2	1.5	7.8	4.5
100	0.7	1.6	7.8	4.5
150	0.9	1.5	6.3	4.8

Comparing the adsorption amount in Figure 9, and the dosage being 2 kg/ton and 7 kg/ton, DS was adsorbed less on pulp when added before refining compared to its addition after refining. However for P01, a different tendency was seen. At lower refining power, P01 added before refining reacted better than that added after refining. Yet with an increasing refining power, the adsorbed amount of P01 added before refining was less compared to its addition after refining. The mechanical hydraulic force generated by the high refining power that disturbed the FWAs retention behaviour could not be neglected. Therefore, it could be concluded that the refining performance could help FWAs adsorption because of the change of morphology and surface chemical composition and component distribution. But if FWAs were added before refining at lower power, refining action would be beneficial for its adsorption on eucalyptus fibre even better than if it was added after refining.

3.1.5. The effect of LC-refining on the optical properties of pulp sample with the addition of FWAs

Optical properties of P samples with FWAs addition after LC-refining

LC-refining had a negative impact on the optical properties of eucalyptus pulp (Table 3). To overcome/minimize the loss of optical properties, different dosage of FWAs was added and the optical properties were determined. The results are shown in Figure 10.

As can be seen, both brightness and whiteness increased dramatically with the increase of FWAs dosage. For instance, the brightness was increased by about 4%-units when DS dosage increased from 1 to 5 kg/ton.

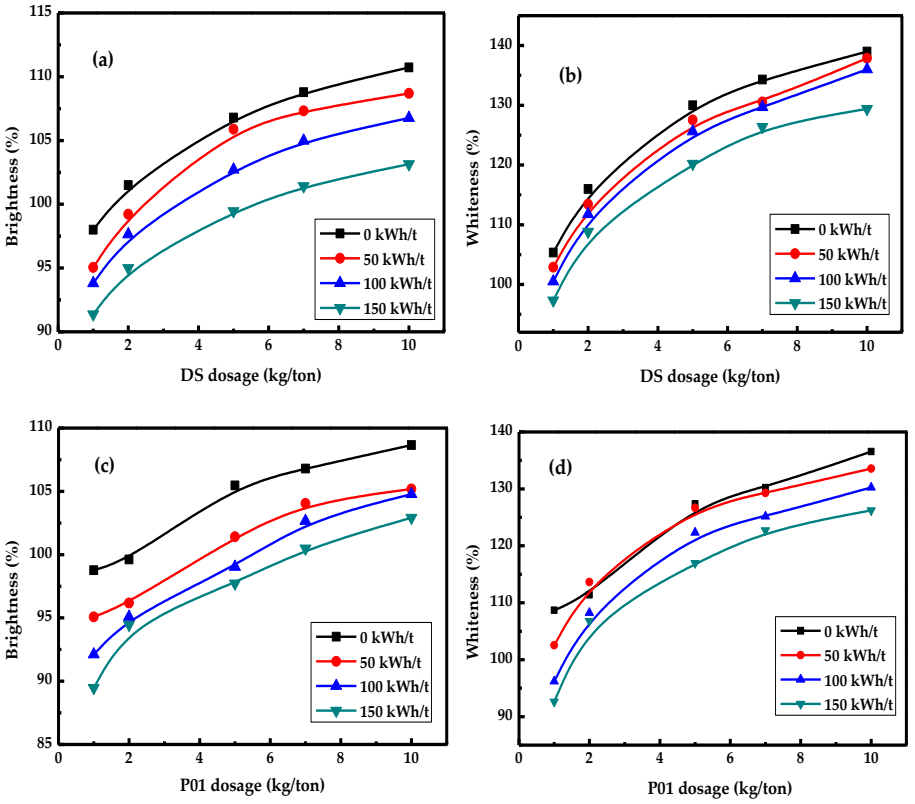


Figure 10. *Effect of DS (a, b) and P01 (c, d) on brightness and whiteness of P samples.*

The enhancement of the brightness and whiteness obtained from FWAs can compensate well the loss of optical properties resulted from LC-refining. In addition, Figure 10 also shows that DS worked better on the improvement of

brightness and whiteness, and this may be because more DS can be adsorbed onto the fibres than P01. However, there is a clear trend indicating that to obtain the best performance of FWAs, the pulp needs to be refined with as low SEC levels as possible.

3.1.6. Optical properties of eucalyptus with FWAs addition before LC-refining

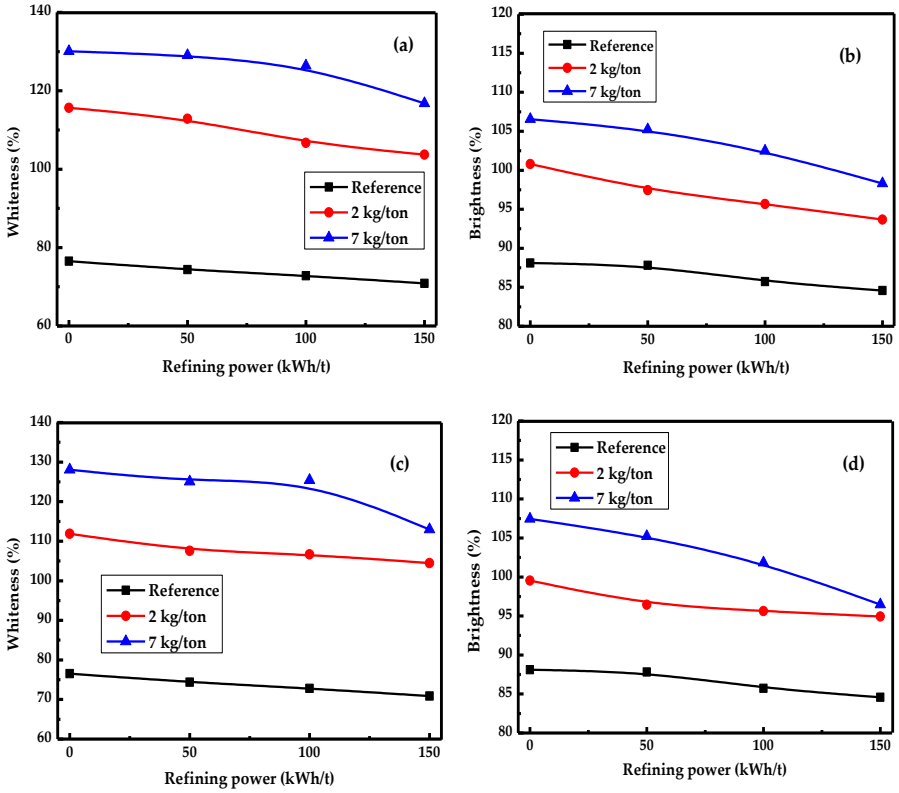


Figure 11. *The effect of DS ((a), (b)) and P01((c),(d)) addition before refining on whiteness and brightness of P samples.*

The addition of DS and P01 before LC-refining could significantly improve the brightness and whiteness of P samples as well, as shown in Figure 11. However, at the same refining power, both whiteness and brightness with the addition of DS and P01 before refining (Figure 11) were slightly lower than that with their addition after refining (Figure10). For example, under the refining power of 50kWh/t with the DS dosage of 2 kg/ton, the brightness and whiteness of P samples where DS was added after refining, are 99 and 114 respectively, while

the corresponding brightness and whiteness for the samples where DS was added before refining are 97 and 113. This may be due to the fact that more FWAs can be adsorbed on fibres after refining, as mentioned previously (Figure 9, Table 8 and 9). With an increasing refining energy, the whiteness and brightness were deteriorated by refining (Figure 11) while FWAs adsorption amount was not promoted either.

3.1.7. FWAs adsorption on eucalyptus with addition of CaSO4 before and after refining at lower refining power

Calcium sulphate is one kind of filler used in paper coating or filling (Alén, 2007) to increase the sheet smoothness, brightness, and printing performance of paper. The impact of calcium sulphate at two dosage points on FWAs adsorption under refining performance was investigated here and it was shown in Figure 12 and Figure 13.

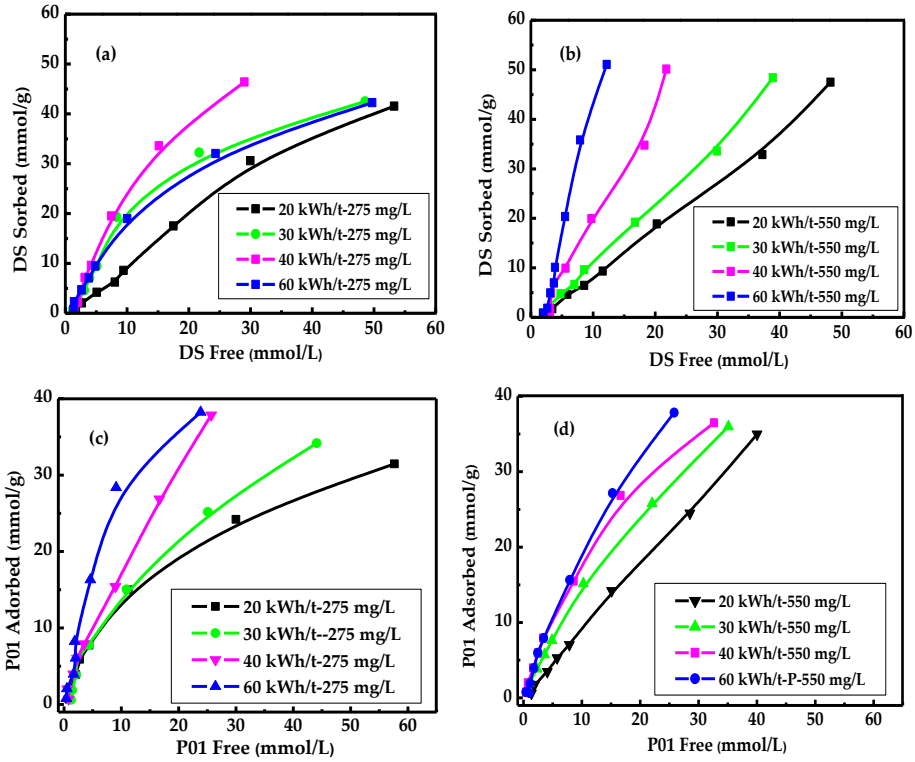


Figure 12. *The adsorption of DS (a, b) and P01 (c, d) on P samples with $CaSO_4$ addition after refining.*

Low power refining was found to be favourable for FWAs adsorption on fibres. After refining, both DS and P01 sorption amount was increased under the power level from 20 to 60 kWh/t with 275 mg/L calcium sulphate addition to the pulp, especially in the case of P01 which was shown in Figure 12. However, the P01 sorption on pulp was not changed when increasing the calcium sulphate dosage to 550 mg/L, which did increase the adsorption of DS a little. It was assumed that the calcium ions retains on fibres and supply more cationic bond sites for anionic FWAs, thus improving the FWAs adsorption on fibre. Yet this improvement ability was limited by the amount of calcium sulphate staying on fibre. Furthermore, native chemical features of DS and P01 are certainly important factors too.

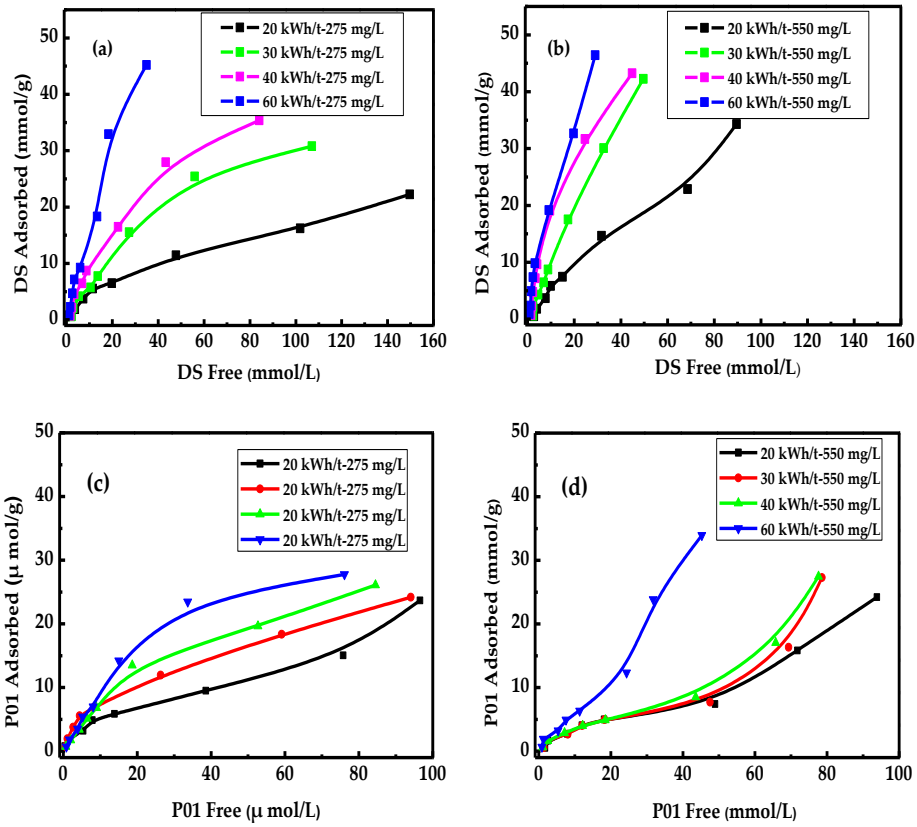


Figure 13. *DS/P01 absorption on eucalyptus pulp with Ca addition before refining process.*

In the cases presented in Figure 13, calcium sulphate was added into pulp solution before refining. DS and P01 were reacted with refined calcium sulphate

fibre afterwards. For DS, the adsorption value was similar as the value given in Figure 12. P01 adsorption seemed to be on a lower level with $CaSO_4$ addition before refining than it was added to fibre after refining. High calcium amount was unfavourable for the P01 adsorption. It was probably because the refining forces affect the calcium sulphate attachment and distribution on fibre. The adsorption improvement of DS and P01 by calcium sulphate is limited. Hence, this will be studied in the future work.

3.2. Topochemical pretreatment of wood biomass to enhance enzymatic hydrolysis of polysaccharides to sugars (Paper III)

3.2.1. Surface coverage by lignin before and after pretreatments

The data obtained by XPS consists of the O/C ratios and elemental surface composition. The elements detected by XPS were O and C and minor amounts of N and S from natural wood. The O/C ratios were used for the estimation of the surface coverage by lignin. The O/C ratio of the wood samples before and after acetone extraction and the surface coverage by lignin of birch and pine wood from hydrothermal, ionic liquid and hydrotropic pretreatment are summarized in Table 10.

The O/C ratio of all the extracted samples was higher than that in the samples before extraction because of the removal of carbon-rich wood extractives. The difference in the O/C ratios was remarkable in the reference wood. During the pretreatments, particularly for hydrothermal and ionic liquid treatments, some of the extractives were already removed before exposure to acetone extraction, which can be roughly estimated on the O/C ratios. Hydrotropic treatment again did not increase the O/C as such, but prepared the samples to a significant carbon removal during the following acetone extraction.

Table 10. *The O/C ratios, surface coverage by lignin (S_{lig}) by XPS, and total lignin of birch (BI) and pine (PI) with different pretreatment. Standard deviations are in parentheses.*

Samples	**O/C**	**O/C extracted**	**S_{lig} %**	**Total lignin%**
BI_{ref}	34 (7)	54 (1)	59	23.3 (0.5)
PI_{ref}	31 (2)	47 (2)	72	27.9 (3)
Hydrothermal pretreatment[a]				
BI 30 min	41 (1)	44 (7)	78	23.8 (1)
BI 120 min	40 (0)	44 (1)	78	22.8 (2)
PI 30 min	46 (1)	54 (2)	59	22.8 (1)
PI 120 min	42 (1)	59 (6)	48	21.8 (1)
Hydrotropic pretreatment[b]				
BI 30 min	34 (5)	66 (1)	34	16.6 (0.8)
BI 120 min	40 (5)	65 (1)	36	13.9 (1)
PI 30 min	27 (5)	71 (11)	25	27.7 (2)
PI 120 min	26 (5)	38 (3)	41	25.5 (2)
Ionic liquid pretreatment				
BI 20 mmol, 1 h[c]	46 (2)	47 (3)	43	24.6 (1)
BI 20 mmol, 3 h[c]	39 (6)	55 (1)	53	27.6 (1)
BI 50 mmol, 3 h[c]	37 (6)	56 (6)	45	24.5 (0)
PI 20 mmol, 1 h[c]	46 (4)	57 (6)	52	24.9 (2)
PI 20 mmol, 3 h[c]	39 (4)	64 (1)	39	27.2 (1)
PI 50 mmol, 3 h[c]	46 (1)	60 (3)	46	27.1 (2)
BI 20 mmol, 1 h[d]	44 (0)	49 (3)	69	23.5 (1)
BI 20 mmol, 3 h[d]	35 (5)	55 (2)	57	24.1 (3)
BI 50 mmol, 3 h[d]	38 (6)	56 (5)	55	24.0 (2)
PI 20 mmol, 1 h[d]	48 (2)	59 (7)	49	28.9 (1)
PI 20 mmol, 3 h[d]	47 (4)	61 (1)	45	21.0 (3)
PI 50 mmol, 3 h[d]	46 (4)	60 (6)	46	27.0 (1)

a 165 ℃, liquor-to-wood ratio (w/w) 5:1; b 30% SXS, 150 ℃, liquor-to-wood ratio (w/w) 8:1.
c EmimAC pretreatment at room temperature; d BmimCl pretreatment at room temperature.

After the hydrothermal treatment for birch, the surface coverage by lignin clearly increased already during the first 30 min of the process. Hot water could remove

hemicelluloses and extractives from hardwood with cellulose and lignin retention, as also reported by Liu et al. (2010). Thus, the surface lignin ratio was increased due to the removal of the other main components. The surface lignin of pine underwent a comparative decrease after hydrothermal pretreatment. Yet, the large deviation in the surface coverage by lignin for pine after 120 min treatment refers to the uneven removal of surface lignin. Total lignin of pine (Table 10) was reduced from 27.9% to 21.8% during the hydrothermal pretreatment while the lignin content in birch did not obviously change. Softwood lignophenol decomposition under hydrothermal conditions at high temperature has been reported before (Nonaka and Funaoka, 2011). Hydrotropic extraction seemed to be an efficient method to remove lignin from lignocellulosic biomass materials, as seen in Table 10. For birch, lignin was reduced to 16.6% after 30 min and to 13.9% after 120 min pretreatment. On the other hand, the surface coverage by lignin was reduced fast in the first 30 min, and after 120 min pretreatment, there was an additional minor decrease. For pine, the reduction in the surface coverage by lignin was not that evident compared to the pine reference, as after 120 min treatment time, the measured surface coverage was bigger than after 30 min. The total content of lignin in pine was lightly decreased during hydrotropic pretreatment. Pine has more chemical resistant guaiacyl lignin compared to birch, which is the probable explanation for the lesser lignin removal from pine compared to birch wood (Andelin, 1989). Moreover, it was assumed that SXS was not able to extract lignin from pine as well as from birch, and with longer treatment time, lignin redeposit on the surface of pine.

Further in Table 10, the effect of the ionic liquid pretreatment on the surface coverage by lignin on birch and pine is presented. Two different ionic liquids, two different dosages of them, as well as two different treatment times are considered. After the ionic liquid pretreatment, both birch and pine had a reduction in surface lignin but in a different extent. However, reduction of total lignin was not found, compared to Table 10. Surface coverage by lignin in both pine and birch was typically reduced by EmimAC, whereas the surface lignin degradation efficiency by BmimCl was lower to birch than to pine. At the same dosage and reaction time, EmimAC worked better than BmimCl to remove surface lignin, both on birch and pine. It has been reported that ionic liquid could dissolve lignin and hemicelluloses of pine, thus improving the following enzymatic digestion (Cox and Ekerdt, 2013). It was also verified by Varanasi et al. (2013) that ionic liquid had an ability to degrade lignin at certain extent at

high temperature, and lignin was broken down by ionic liquid into syringol and allyl guaiacol from pine. At room temperature, lignin was reduced on the surface without significant change in the total lignin content.

To our knowledge, there are no previous publications considering the effects of pretreatments on the wood surface coverage by lignin. However, lignocellulosic pulp fibres have been studied, and handlings such as ozone treatment (Koljonen et al., 2003), peracetic acid treatment (Wang et al., 2010), and lipase treatment (Kangas et al., 2007), are known to reduce the surface lignin through degradation, and mechanical refining of fibres through exposure of carbohydrates (Fardim and Durán, 2003).

3.2.2. Morphology of birch and pine wood after different pretreatments

In all of the presented FE-SEM images (Figure 14-16), the wood fibre cells are in upright position. The wood morphology before treatments appeared dense with some longitudinal fractures between the fibres; and on the wood particle surfaces, there were half-loosened fibre cell wall flakes, probably created from the primary walls during the milling (PIREF and BIREF in Figure 14). In birch, having shorter fibre cells (compared to the tracheids in pine) also, horizontal fractures were detected on the surface, but the structure was otherwise dense.

The morphology of pine and birch wood after hydrothermal pretreatment lasting 30 min or 120 min was investigated, but no significant changes were detected in the treated samples. Even with the longer pretreatment time, hydrothermal pretreatment did not visibly influence the surface morphology (images not shown). However, going to larger magnifications, features resembling deposits of so called pseudo-lignin were detected in pine after 30 min hydrolysis time (marked with arrows in Figure 14/PI 1) and in birch both after 30 min (Figure 14/BI 1) and after 120 min (Figure 14/BI 2). The droplets were larger after 120 min. Pseudo-lignin has been reported to be formed via acid catalysed dehydration of carbohydrates during dilute acid pretreatment, and deposited as spherical droplets of the diameter up to about 1 μm on the biomass surface (Pu et al., 2013; Sannighari et al., 2011; Hu et al., 2012). The conditions in hydrothermal pretreatment are milder than in dilute acid hydrolysis, but the reactions are similar even if in lesser extent. Therefore, formation and deposits of pseudo-lignin are plausible. Possibly the process was slower in birch because the droplets

clearly increased after 120 min pretreatment time. Contradictory with the pine sample, the droplets were not detected anymore after the longer pretreatment time (Figure 14/PI 2). Pseudo-lignin, consisting of carbohydrates and lignin degradation products, could be part of the surface coverage by lignin which in pine was higher after 30 min pretreatment time than after 120 min, as shown in Table 10. However, the reason for this is unknown.

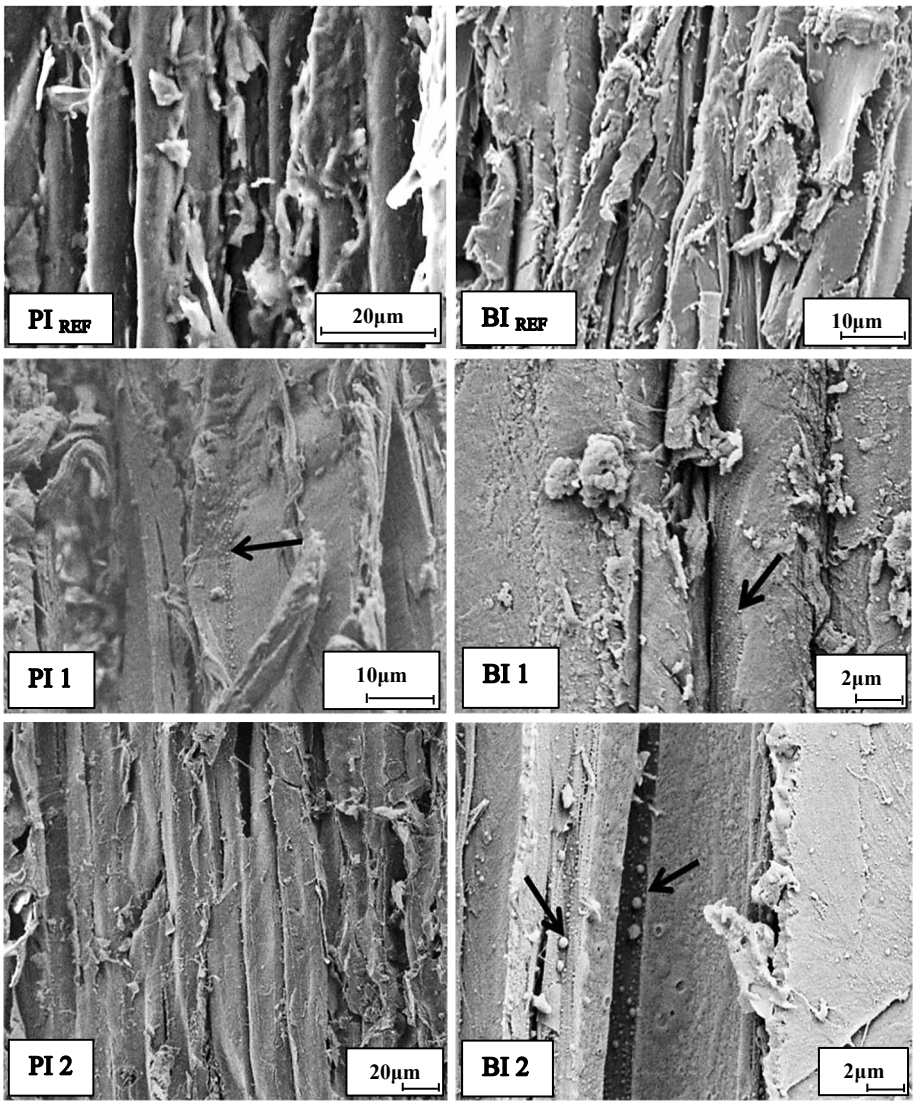


Figure 14. *SEM images of wood before and after hydrothermal pretreatment: (PI_{REF}) pine before treatment, (BI_{REF}) birch before treatment, (PI 1) pine treated 30 min, (BI 1) birch treated 30 min (PI 2) pine treated 120 min, (BI 2) birch treated 120 min.*

Images of pine and birch wood after hydrotropic pretreatment are presented in Figure 15. Surface degradation was not detected in any of the wood samples, neither swelling. In the pretreated pine (PI), fractures presumably following the direction of the fibril aggregates of the secondary cell wall were observed. The reason for the fractures was assumed to be changed lignin distribution in the cell wall after 9 hours pretreatment with SXS, because the surface coverage by lignin was lower than that in the reference pine in Table 10.

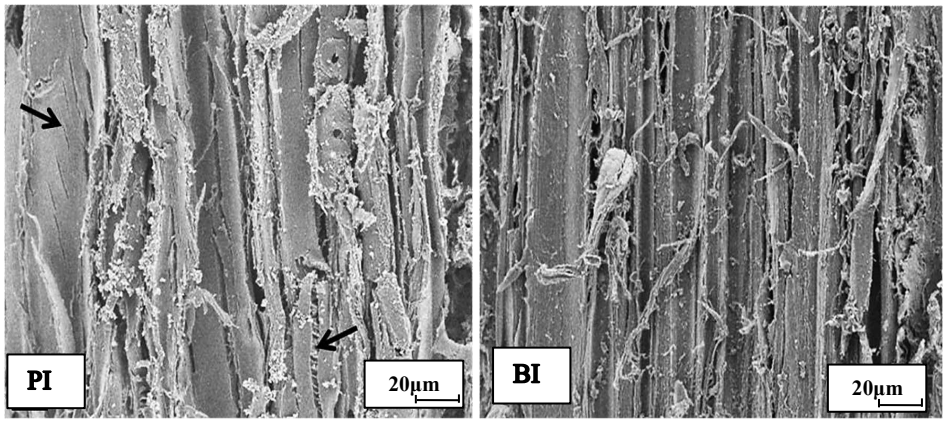


Figure 15. *SEM images of wood after hydrotropic pretreatment at 150 ℃ in 2 h: (PI) pine, (BI) birch.*

The morphology of pine and birch wood after ionic liquid pretreatment is shown in Figure 16. Both with EmimAC and BmimCl, the woody structure was more open and more swollen in the horizontal direction, compared to the untreated wood (Figure 14). Swelling of the fibres by ionic liquid is in agreement with the results published by Lucas et al. (2010). In addition, the surface was degraded and most of the cell wall flakes had disappeared, as if the outer cell wall layer had been exfoliated. This effect of ionic liquid was more obvious on pine than on birch. In pine, the ring pores were more exposed compared to pine without treatment, due to the surface degradation and opening up. However in birch as well, surface degradation was observed (BI b in Figure 16) and part of the outer cell walls was broken into shreds (BI a in Figure 16). The reason for this was demonstrated before, when smaller wood chips were treated long time and the contact between the solvent and the fibres during ionic liquid pretreatment was probably promoted (Lennartsson et al., 2011).

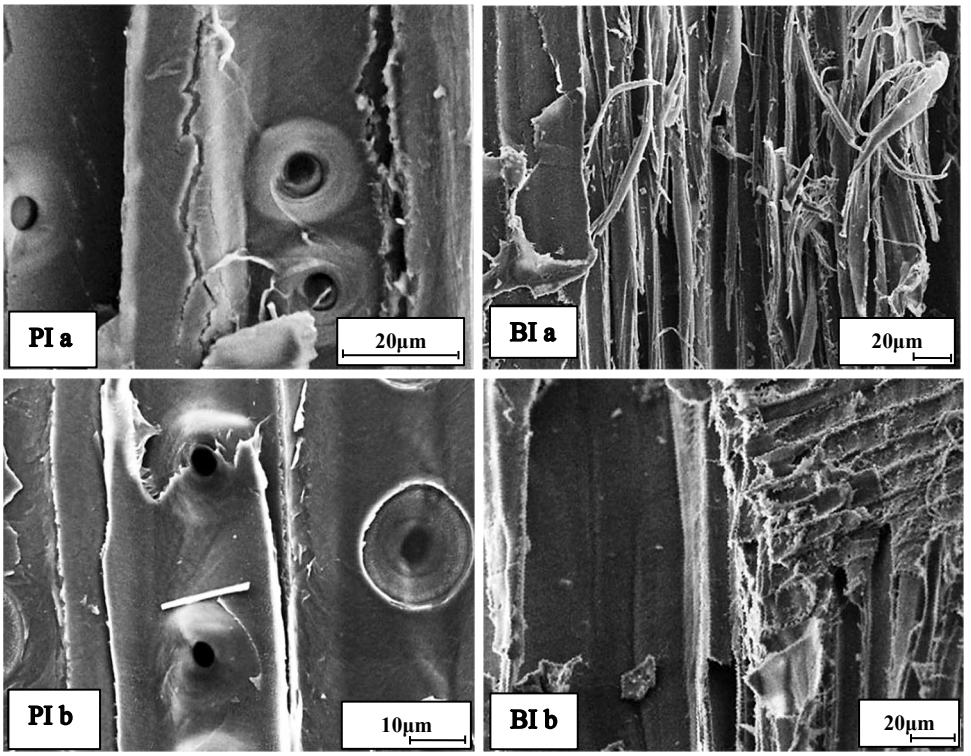


Figure 16. *SEM images of wood after pretreatment with 50 mmol ionic liquids in 3 h: (PI a) pine treated with EmimAC, (PI b) pine treated with BmimCl, (BI a) birch treated with EmimAC, (BI b) birch treated with BmimCl.*

3.2.3. Effect of hydrotropic pretreatment on enzymatic hydrolysis

Comparing those three pretreatment methods, hydrotropic extraction has been proven to be the most efficient way of removing surface lignin. The samples were introduced to enzymatic hydrolysis, where they were hydrolysed using different hydrolysis time and different amounts of commercial enzyme mixture.

Glucose yield of birch and pine wood by enzymatic hydrolysis

The glucose yield after the enzymatic hydrolysis is given in Figure 17 and 18. Figure 17 shows that birch after 120 min hydrotropic pretreatment was easier to hydrolyse than with 30 min pretreatment, expectedly, and that the hydrolysis efficiency was obviously higher on both of the pretreated samples than on birch without pretreatment. With an increasing dosage of enzyme and prolonging time, the glucose yield in the liquid increased. During the first 6 hours, the enzyme hydrolysis speed was fast, but became slower after that. Birch wood pretreated with hydrotrope for 120 min had a maximum glucose conversion of 83.9 %, after

48 hours with 50 FPU/g d.m. cellulase hydrolysis (Figure 17). This is close to the result from steam explosions in mild conditions (Vivekanand et al., 2013). Harsh steam explosion can increase the glucose yield clearly more than when the wood structure is destroyed (Grethlein and Converse, 1991; Horn and Eijsink, 2010). However, intensifying the hydrotropic pretreatment such as increase temperature and prolonging the treatment time is probably helpful to boost the enzyme hydrolysis. Severe conditions of sulphite pretreatment on pine wood has been demonstrated to be able to improve the glucose saccharification, while a certain of amount of inhibitors furfural and hydroxymethylfurfural were produced (Lan et al., 2013).

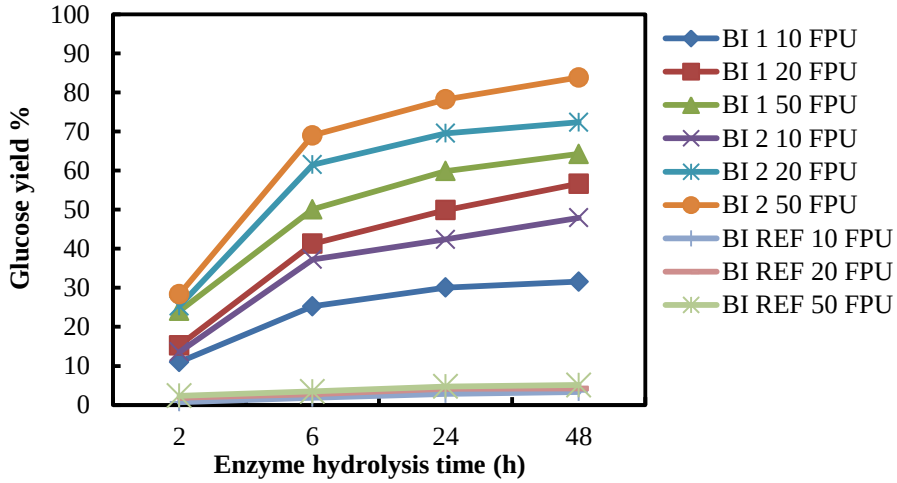


Figure 17. *Glucose yield of birch (BI) after enzyme hydrolysis, with the hydrotropic pretreatment time 30 min (1) or 120 min (2), or no pretreatment (REF).*

Figure 18 shows the glucose concentration released from pine before and after hydrotropic pretreatment, hydrolysed by the enzyme mixture. Also with pine, the hydrotropic treatment improved the enzymatic hydrolysis. Pine treated 120 min resulted in better hydrolysis than those detected from pine treated for 30 min at different dosages of cellulase mixture. However, the hydrolysis level with pretreated pine remained much lower as compared with birch. Even after 24 hours hydrolysis time, for pine with 120 min pretreatment, the glucose yield was only 15.5%.

In Figure 18, it can be seen that the hydrolysis is still proceeding, but not levelling. The enzyme hydrolysis efficiency was improved with hydrotropic pretreatment, however the glucose yield remained rather modest, and more glucose monomers were hydrolysed from birch, than from pine at the same conditions.

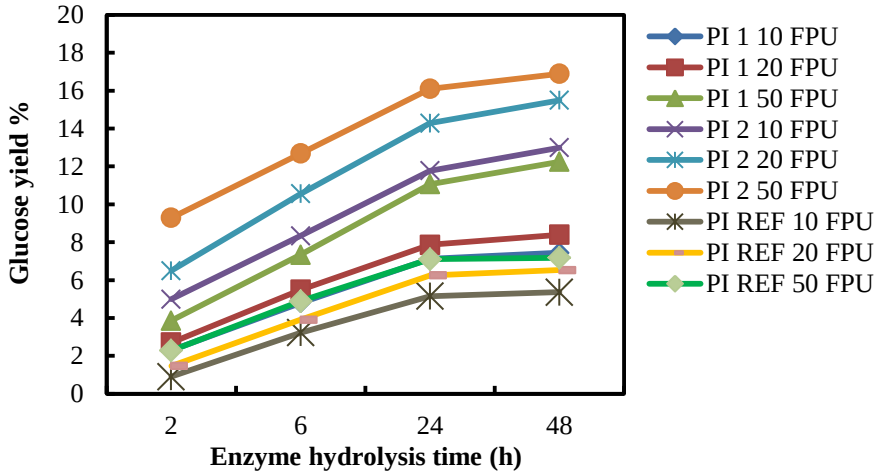


Figure 18. *Glucose yield of pine (PI) after enzyme hydrolysis, with the hydrotropic pretreatment time 30 min (1) or 120 min (2), or no pretreatment (REF).*

Surface chemical composition of birch and pine wood after enzymatic hydrolysis

The surface chemical composition after enzymatic hydrolysis is reported for the samples that provided the most evident contrast to reference, that is, the ones with the highest enzyme dosage and longest time. The samples were denoted as: BI (untreated birch reference), BI 1 (birch after 30 min hydrotropic treatment), BI 2 (birch after 120 min hydrotropic treatment), EBI 1 (B1 after enzyme hydrolysis), EBI 2 (BI 2 after enzyme hydrolysis), and for pine samples (PI) in analogy.

Surface coverage by lignin in pretreated birch and pine after enzymatic hydrolysis were studied by XPS. Thus, the results are presented in Table 11. In birch, the O/C ratio was not changed after enzyme hydrolysis, comparing BI to EBI, but the difference was visible only after acetone extraction, where the removal of extractives (mainly carbon-rich material) was clearly more intense in the enzyme hydrolysed samples. In pine again, the ratio was increased which is

already going from PI to EPI. Before the enzyme hydrolysis, birch had a smaller surface coverage by lignin than that of pine. After enzyme hydrolysis, the surface coverage by lignin had increased for both birch and pine. The observed increase of surface lignin is presumably due to degradation of carbohydrates by the enzyme. Hence, the hydrolysis made carbohydrates to dissolve to constituent sugars together with extractive reduction leading to the relative enrichment of surface lignin.

Table 11. *The O/C ratio and surface coverage by lignin (S_{lig}) by XPS before and after enzymatic hydrolysis (E) of hydrotropic pretreated birch (BI) and pine (PI) where the pretreatment time was 30 min (1) or 120 min (2).*

Samples	**O/C**	**O/C extracted**	**S_{lig} %**
BI 1	34(5)	66(1)	34
EBI 1[a]	37(6)	45(1)	75
BI 2	40(5)	65(1)	36
EBI 2[a]	39(1)	43(3)	79
PI 1	27(5)	71(11)	25
EPI 1[a]	41(2)	63(1)	89
PI 2	26(5)	38 (3)	41
EPI 2[a]	42(4)	46(2)	73

[a] Enzyme hydrolysis conditions: 50 FPU/ g (dry matter) cellulase and β-glucosidase 300 nkat/g (dry matter) at 50 ℃, pH 5.0, 48 hours.

The effect of the hydrotropic treatment and enzyme hydrolysis on the wood powder outmost surfaces was further investigated by comparing the ToF-SIMS spectra of the treated samples to that of the untreated samples. It is important to note that the peak intensities in ToF-SIMS are not directly quantitatively relative to the surface content of the component in question but the secondary ion formation is ruled by a multitude of chemical and physical interactions. However, certain commensurateness is valid, particularly in comparing similar types of samples to each other. The peak intensities of the characteristic lignin (Saito et al., 2005), pentose (xylan), and cellulose peaks (Fardim and Durán, 2003) were normalized against the total counts, and in addition, the ratios between the lower mass region counts from polysaccharides and from lignin accordingly to Goacher et al. (2012) were compared, and the ratios of guaiacyl and syringyl units were also studied. Because of lacking consensus on the origin of the mass peak 115 Da,

it was not used in the peak ratios (Goacher et al., 2012). These values are shown in Table 12.

Table 12. *Surface chemical composition of birch (BI) and pine (PI) by ToF-SIMS after hydrotropic pretreatment in 30 min (1) or 120 min (2) and enzyme hydrolysis (E). REF stands for untreated wood. PS=polysaccharides, L=lignin, S=syringyl, G=guaiacyl. Standard deviations in parentheses.*

Samples	PS/(PS+L)[a]	S/G	Cellulose[b]/ Total	Pentoses[b]/ Total	Lignin[c]/ Total
BI 1	0.29 (0.01)	0.83 (0.06)	3.7 (0.1)	3.2 (0.6)	5.7 (1.2)
EBI 1[d]	0.29 (0.00)	0.80 (0.06)	2.3 (0.2)	1.7 (0.2)	3.2 (0.3)
BI 2	0.30 (0.09)	0.77 (0.02)	3.3 (1.3)	2.2 (0.3)	4.7 (1.7)
EBI 2[d]	0.31 (0.03)	0.83 (0.14)	2.1 (0.2)	1.6 (0.1)	3.0 (0.7)
BI $_{REF}$	0.34 (0.03)	0.79 (0.46)	3.9 (0.6)	3.3 (0.5)	3.9 (0.9)
PI 1	0.26 (0.02)	0.82 (0.22)	4.3 (0.1)	3.2 (0.2)	2.1 (0.1)
EPI 1[d]	0.21 (0.00)	1.13 (0.09)	3.1 (0.3)	2.6 (0.1)	1.3 (0.1)
PI 2	0.26 (0.01)	0.40 (0.02)	3.8 (0.3)	3.1 (0.2)	4.1 (0.5)
EPI 2[d]	0.21 (0.01)	0.84 (0.11)	3.1 (0.5)	2.4 (0.4)	1.8 (0.4)
PI $_{REF}$	0.30 (0.02)	0.80 (0.20)	4.8 (0.4)	3.9 (0.4)	3.4 (0.2)

[a]Goacher et al. 2012; [b]Fardim and Durán, 2003; [c]Saito et al. 2005.;
[d] Enzyme hydrolysis conditions: cellulase 50 FPU/g (dry matter) and 300 nkat/ml β-glycosidase at 50 ℃, pH 5.0, 48 h.

During hydrotropic extraction for birch, the lignin ratio was first increased and then decreased (BI $_{REF}$ compared to BI 1 and to BI 2). Furthermore, the proportion of polysaccharide peaks of the sum of the polysaccharide peaks and lignin peaks (PS/ (PS+L)) was not changed, and the treatment time did not affect this. The result was suggesting that hydrotropic method was not only removing the lignin but also resulted in polysaccharide loss, probably from low mass hemicelluloses, and it mainly happened during the first half hour of the pretreatment process. The pentose ratio from fibre surface was reduced with long pretreatment, but the cellulose was well retained. Therefore, this proved that SXS which had good selectivity for lignin extraction rely on wood species with lower cellulose removal.

It can be estimated that with a prolonged hydrotropic treatment, xylan started to degrade, which can be seen also in Table 12. Furthermore, after the enzyme hydrolysis, the polysaccharide ratio (PS/(PS+L)) was clearly increased. EBI 1

had a higher ratio value than EBI 2, which indirectly means that the relative distribution of polysaccharides on the surface of EBI 1 was denser than on EBI 2. This is probably due to more lignin on the outmost surface of the material after 30 min pretreatment and the incomplete break down of the bond between lignin and carbohydrates, resulting to the fact that the enzyme was not able to hydrolyse the carbohydrates into monomer sugars, and more sugar fragments stayed on the surface. In accordance with the decreased lignin ratio in B1during enzyme hydrolysis, enzyme was capable to break down the bond between lignin and polysaccharides.

For pine, some more evident affects were observed in the surface lignin, compared to those in birch. Hydrotropic treatment seemed to first decrease and then to increase the proportional surface lignin, which probably is a sign of polysaccharide degradation in a longer treatment time. The S/G peak ratios were in general higher than what is known about the bulk proportion of the lignin units in pine. The fragmentation inclination of the two in ToF-SIMS could be different. Hydrotropic treatment degraded syringyl groups, and enzymatic hydrolysis affected the guaiacyl groups in lignin, thereby clearly decreasing the lignin peak intensities. The polysaccharide ratio, as well as the cellulose ratio, was also decreasing during hydrotropic treatment and further during enzymatic hydrolysis.

The trends of the lignin peak ratios are contradictory with the XPS results on the surface coverage by lignin, which was increasing after enzymatic hydrolysis for both birch and pine. The peak intensities cannot be straight forward compared to a surface content. It is possible that the effects of pretreatment and enzyme hydrolysis on the lignin-carbohydrate-complexes (Lawoko et al., 2006) are influencing the fragmentation of each of the components in ToF-SIMS. Also, the detection depth is different for XPS and ToF-SIMS. It was shown that the chemical composition of wood material surface was different under different pretreatment conditions. Two hours hydrotropic pretreatment removed the lignin hindrance and improved the enzyme hydrolysis. Enzyme hydrolysis decreased both lignin and polysaccharides on the surface, simultaneously with breaking down polysaccharides deeper in the tissue as detected by the high glucose yield.

Hydrothermal hot water pretreatment, ionic liquid and hydrotropic pretreatment worked differently on surface lignin on birch and pine chopped wood. Therefore, hydrothermal treatment did not directly remove lignin; and ionic liquid could swell the fibre to increase the surface area without the removal of lignin.

Hydrotropic pretreatment was an efficient way to improve enzyme accessibility through lignin removal, especially syringyl from birch. Hydrotropic pretreatment also preserved carbohydrates. Therefore, changes in the surface chemical composition could be evaluated by XPS and ToF-SIMS.

3.3. Topochemistry of alkaline, alkaline-hydrogen peroxide and hydrotropic pretreatment of sugarcane bagasse and common reed to enhance enzymatic hydrolysis efficiency (Paper IV-V)

Sugarcane bagasse and common reed pretreated by alkaline, alkaline-hydrogen peroxide and hydrotropic methods were studied in this part.

3.3.1. Chemical compositions of bagasse and reed with dilution alkaline, alkaline-hydrogen peroxide and hydrotropic pretreatment

Pretreatment technology applied before enzyme hydrolysis process is aimed at overcoming the limit factors for enzyme access to cellulose. With diluted alkaline, alkaline-hydrogen peroxide and hydrotropic pretreatment, lignin amount of bagasse was reduced from 17.7% to 6.4%, 8.4% and 6.4%. For reed, the total lignin was decreased to 7.3%, 7.9%, 12.0% as it is in the reference reed which was 19.7% as shown in Table 13-14. The lignin was removed at different extent by three different pretreatment methods as expected.

When the carbohydrates of bagasse and reed after the pretreatments in Table 14 are compared to the reference materials in Table 13, it can be seen that the hemicelluloses were degraded little after the diluted alkaline and the alkaline-hydrogen peroxide pretreatments. But typically for bagasse, the hydrotropic pretreatment seems not only to have removed lignin but also hemicelluloses. Arabinan was almost totally degraded, but cellulose has remained well in the residue substrates, and thus the hydrotropic pretreatment loading yield is lower than that of the other pretreatments. For reed, diluted alkaline and alkaline-hydrogen peroxide reduced the total lignin amount slightly less than the hydrotropic treatment, as is shown in Table 14. Hemicelluloses were degraded heavily both in reed and in bagasse during the hydrotropic pretreatment. However, there was no obvious change in the xylan content after alkaline and alkaline-hydrogen peroxide pretreatments. Alkaline and alkaline-hydrogen

peroxide pretreatments could degrade lignin into smaller fragments through cleavage of the β-ether bond that contributed to the lignin removal (Banerjee et al., 2011; Cao et al., 2012). The mechanism of hydrotropic pretreatment improves the lignin solubility to the solvent (Korpinen and Fardim, 2009). The total lignin value of Rsxs is lightly higher than RN and RH. But for bagasse, hydrotropic pretreatment was the most efficient method to eliminate the main cause of lignocellulosic biomass recalcitrance and appeared promising to improve enzymatic hydrolysis.

Table 13. *Chemical compositions of original bagasse (Bref) and reed (Rref), standard deviation was given also.*

	Glucan %	**Xylan %**	**Arabinan %**	**AIL %**	**ASL %**	**Extrac-tives %**	**Ash %**
Bref	40.9 (0.2)	23.9 (0)	1.5 (0)	16.7 (0.2)	1.1 (0)	19.0 (0.5)	1.2 (0.6)
Rref	37.7 (0.3)	22.2 (0.1)	2.2 (0.1)	18.5 (0)	1.2 (0)	11.4 (0.6)	1.9 (0.3)

AIL: Acid insoluble lignin; ASL*:* Acid soluble lignin.

Table 14. *Carbohydrates and lignin of bagasse (B) and reed (R) after various pretreatments: sodium hydroxide pretreatment (N), sodium hydroxide and hydrogen peroxide pretreatment (H) and hydrotropic pretreatment (SXS). Standard deviations are in parentheses.*

	Glucan %	**Xylan %**	**Arabinan%**	**Total lignin %**	**Loading Yield %**
Bref	40.9 (0.2)	23.9 (0)	1.5 (0)	17.7 (3)	-
BN	56.1 (0.3)	22.4 (0.1)	1.7 (0)	6.4 (0.1)	60.8 (2)
BH	54.9 (0)	24.1 (0.1)	1.7 (0)	8.4 (0.3)	65.5 (1)
Bsxs	77.3 (1)	7.0 (0)	0.1 (0)	6.3 (1.1)	43.0 (2)
Rref	37.7 (1.1)	22.2 (0.1)	2.2 (0.1)	19.7 (0.1)	-
RN	49.8 (0)	23.8 (0.1)	3.3 (0.1)	7.3 (0)	62.2 (1)
RH	50.7 (0.4)	24.8 (0.1)	2.8 (0)	7.9 (0.5)	63.8 (2)
Rsxs	70.9 (0.1)	9.2 (0.2)	-	12.0 (0.6)	48.7 (3)

3.3.2. Surface composition of pretreated bagasse and reed by XPS

So far, there is no ideally fractionation method of separating cellulose, hemicelluloses and lignin. Except for the barriers to enzyme accessibility caused by lignin and acetyl group of hemicellulose, according to literatures, the distribution of xylan and lignin on the surface of biomass materials could directly influence the enzyme hydrolysis efficiency also (Blanch and wilke, 1982, Moony et al., 1998). The elemental composition of the pretreated sample surfaces was investigated by XPS. The main constituents were carbon and oxygen. In the reference bagasse, additionally, small amounts of N, Al, Si, were detected in the XPS low resolution spectra. In the reference reed, Si and Cl traces were revealed by the XPS low resolution spectra.

In Table 15, the surface lignin, carbohydrates and extractives of bagasse and reed after different pretreatment methods were listed. Also the O/C ratios of the acetone-extracted and not extracted samples were given in Table 15. As can be seen, the removal of carbon-rich material from the bagasse surface during pretreatments, and on the other hand during the acetone extraction, is demonstrated by the increase of the O/C ratio.

Table 15. *Surface coverage by lignin (S_{lig}), carbohydrates (S_{carb}) and extractives (S_{exts}) of bagasse and reed.*

Samples	**O/C$_{ext}$**	**O/C**	**S$_{exts\ \%}$**	**S$_{lig\ \%}$**	**S$_{carb\ \%}$**
Bref	0.68 (0.11)	0.45 (0.03)	38.1	31.0	69.0
BN	0.77 (0.06)	0.69 (0.03)	12.6	11.8	89.2
BH	0.84 (0.11)	0.63 (0.03)	28.6	0	100
Bsxs	0.77 (0.04)	0.61 (0.02)	24.7	11.3	89.7
Rref	0.32 (0.11)	0.14 (0.06)	80.5	~100	~0
RN	0.30 (0.11)	0.33 (0.14)	~0	~100	~0
RH	0.37 (0.09)	0.41 (0.15)	~0	91.5	8.5
Rsxs	0.75 (0.03)	0.43 (0.19)	48.7	16.5	83.5

Further, the surface coverage by lignin, carbohydrates and extractives of bagasse after different pretreatment methods were gained from the XPS results (Table 15). After the pretreatments were applied, the surface of bagasse samples contained more carbohydrates and less lignin than the reference sample. The values of

surface coverage by lignin value of BN and Bsxs were on the same level. However, during alkaline pretreatment, together with lignin removal, a notable amount of extractives was reduced from the fibre surface as well. Table 15 also shows that, the O/C value of BH after extraction was close to that of pure cellulose. This high O/C ratio was probably caused by the oxidation of surface lignin and the insertion of oxygen atoms to form carbonyl and carboxyl groups. According to the results shown in Table 14, the residual total lignin of BH was 8.4%, which was higher than that of BN and Bsxs, but the surface coverage by lignin for BN and Bsxs was more than 11% while the BH had 0%. This was a limitation of XPS method to assess detailed chemical changes in surface lignin. ATR-FTIR and ToF-SIMS spectrometry was applied here to complement XPS data.

As to the XPS results of reed in Table 15, the O/C ratio value between extracted and unextracted sample was close to the theoretical O/C ratio of 0.33 in lignin. The surface coverage by lignin of RN was approximately 100% while it in RH was 95.6%. It seems that the fibre surface was mainly covered by lignin after sodium alkaline and alkaline-hydrogen peroxide pretreatments, even if the total lignin amount had a clear reduction. During alkaline delignification processes, lignin is easily precipitated on fibre (Sixta, 2006). The high surface coverage by lignin was probably caused by the lignin redeposition during pretreatment and washing processes. However, in the difference with RN and RH, the surface coverage by lignin of Rsxs was dramatically reduced from 31% to 16.5%, resulting to as high as 83.5% carbohydrates exposure on the surface.

3.3.3. Chemical structure measurement of bagasse and reed after pretreatments

The samples after pretreatments were measured by ATR-FTIR. The analysis depth of ATR-FTIR was from <200 nm to >1 μm) which was deeper than XPS (5-10 nm). The spectra are shown in Figure 19-20. The broad band from 3340 cm^{-1} has been reported as the O-H bond which can be found in cellulose and lignin. For agriculture materials, it also has a possible origin in the aliphatic fraction of waxes (Sun et al., 1995, Shen et al., 2011). With the different pretreatments, this group did not obviously change in Figure 19 and 20.

In Figure 19, 2896 cm^{-1} is the C-H bond in polysaccharides which is stronger in the pretreated bagasse samples. 1733 cm^{-1} is the C=O in the acetyl groups in

hemicelluloses and is shown clearly in untreated bagasse but not in BN, BH or Bsxs samples because of hemicellulose degradation during the pretreatment (Pereira et al., 2011). 1605 cm^{-1} and 1510 cm^{-1} is the sign of lignin and 1240 cm^{-1} is the aryl C-O bond from guaiacyl lignin which was only existing in untreated bagasse. It is a clear indication of lignin dissolution during pretreatment (Sun et al., 1995; Derkacheva and Sukhov, 2008). 904 cm^{-1} was assigned as β-glucosidic bond which was stronger in the pretreated samples because of more carbohydrate located on fibre surface (Sun et al., 1995), in agreement with the results by XPS in Table 15. 835 cm^{-1} has been reported as ester carbonyl signal of p-coumaric ester groups (Fan et al., 1987). It was only detected in the untreated sample.

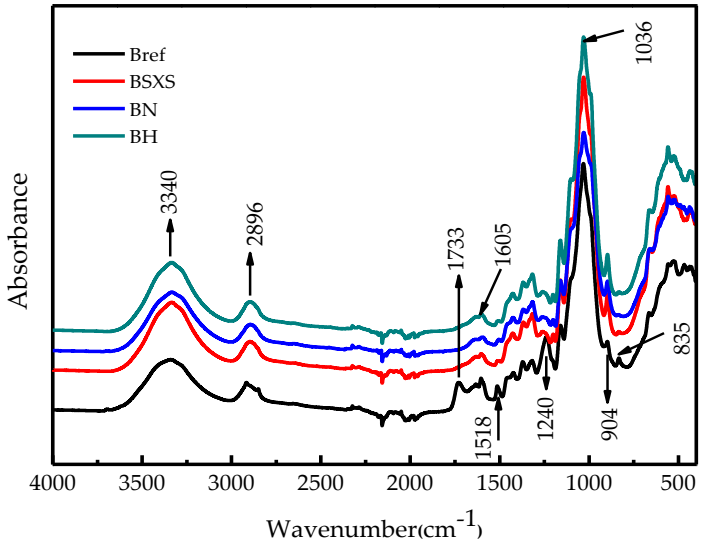


Figure 19. *ATR-FTIR of bagasse after pretreatment.*

In untreated and treated reed (Figure 20), the band at 2926 cm^{-1} is the C-H stretching of CH_3 and CH_2, respectively which has been reported to originate from cellulose, hemicelluloses and lignin (Corrales et al., 2012; Guo et al., 2011; Sun et al., 1995). The peak at 2847 cm^{-1} is assigned to -OCH_3 which is commonly present in lignin (Corrales et al., 2012). The intensities of both of the two peaks were lowest in Rsxs and highest in RN. It is because of the more efficient hemicellulose and lignin removal after the hydrotropic pretreatment, while the increasing intensities of CH_2, CH_3 and -OCH_3 groups could be speculated to represent the breaking of the aryl-ether bonds, especially the β-O-4 bonds in the alkaline pretreatment. Stretching at 1735 cm^{-1} is assigned to the ester bond from

hemicelluloses, and the intensity order is Rref >RN>RH>Rsxs. At 1640 cm^{-1}, the C=C bond of lignin aromatic ring is not detected in Rsxs samples. The peak at 1460 cm^{-1} has usually been seen as the sign of cellulose and lignin (Sun et al., 1995). It is plausibly a reflectance of cellulose increasing in the pretreated samples, according to the results in Tables 14.

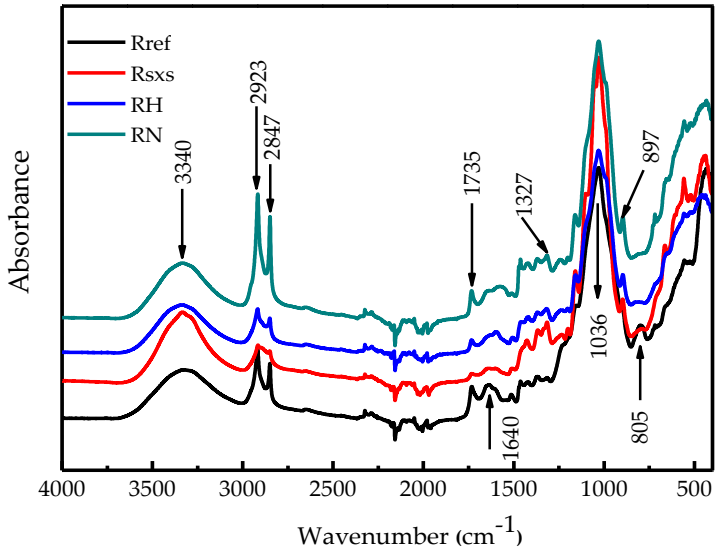


Figure 20. *ATR-FTIR of reed after pretreatment.*

The peaks between 1700-900 cm^{-1} are located in the characteristic region especially for the guaiacyl (G) and syringyl (S) units (Todorciuct et al., 2009), but the spectra for all reed samples are similar in this area. Peaks at 1357 cm^{-1} for syringyl with C-O stretching were not clear, but peaks at 1430 cm^{-1} and 1323 cm^{-1} in Rsxs have been reported as the assignment of guaiacyl lignin (Todorciuct et al., 2009) that was relatively more intense than that in Rref. In addition, the peak at 1036 cm^{-1} was attributed to C-O-C bond from polysaccharides. Peak at 897 cm^{-1} was related with the β-glucosidic bond of polysaccharides (Corrales et al., 2012), while peak at 805 cm^{-1} is speculated to be C-H out of plane binding, which originates from P-substituted benzenes that are only existing in untreated reed. As detected by ATR-FTIR, there is no S bond in Rsxs.

3.3.4. Morphology of bagasse and reed after pretreatments

The surface morphology of bagasse and reed samples after the pretreatment was investigated by SEM.

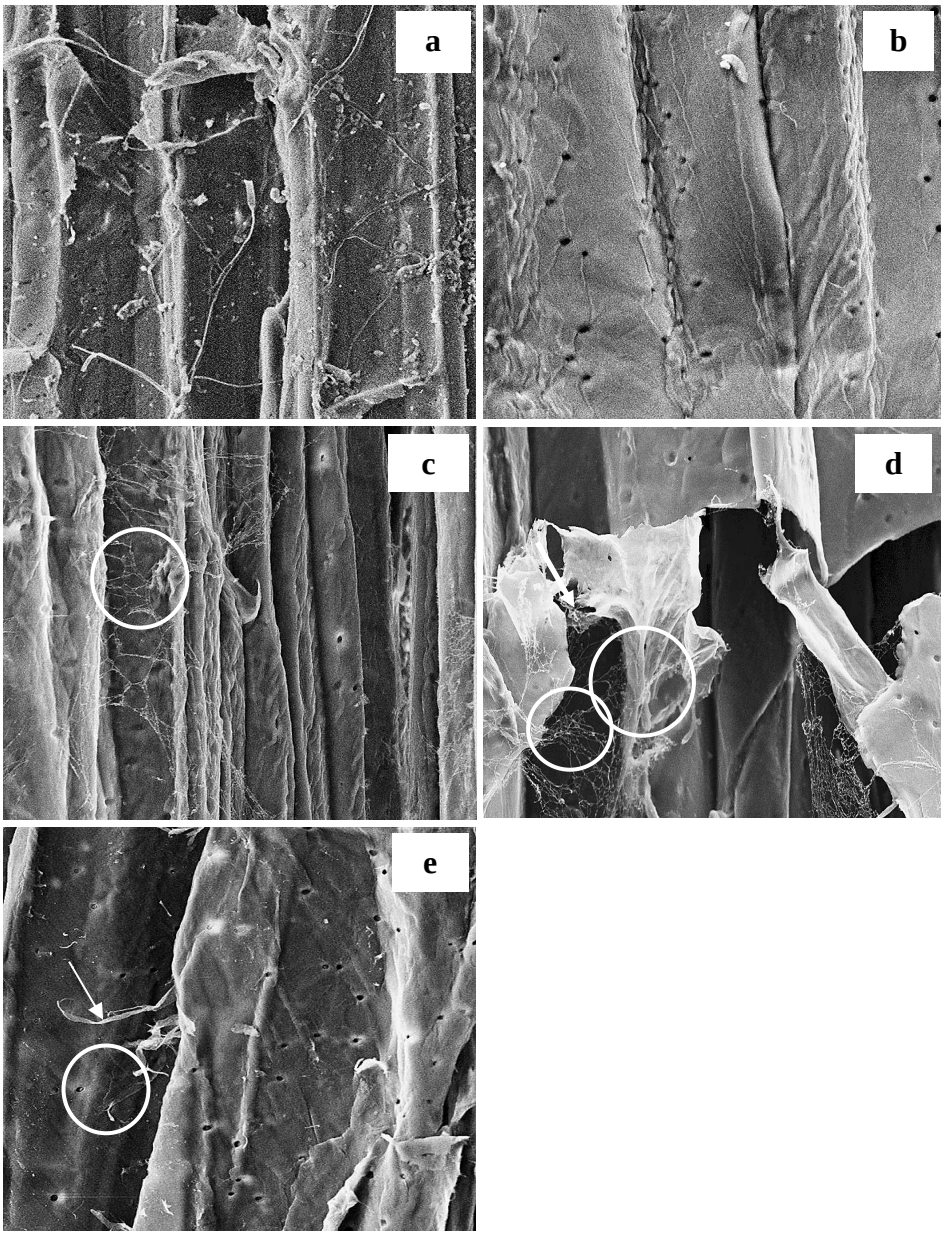


Figure.21. *Morphology of bagasse after pretreatment (reference (a), BN (b), BH(c-d), Bsxs(e))*

In Figure 21a, the reference bagasse fibre was covered by some fragments, probably fibrils created by mechanical forces during the sugarcane squeeze stage in the sugar mill. The pits in cell wall were shown in all samples at such high magnification 15,000 ×. Shrinkage trace was present on alkaline treated fibre that proved the fibre swelling caused by alkaline pretreatment in Figure 21b. Alkaline

treatment could swell wheat straw fibre leading to the increase of the internal surface (Jackson, 1977), and the same action to sugarcane bagasse was found. After alkaline-hydrogen peroxide pretreatment, some substances formed spider web-like structures covering the fibre surface in Figure 21c. Similar phenomenon was found out on softwood fibre surface after mechanical refining treatment because of xylan released from fibre wall re-adsorbed on the surface (Fardim and Durán, 2003). It was probably due to the high surface carbohydrates covering bagasse fibre, as detected by XPS (Table 15). These substances were probably released from the collapsed inner fibre wall which is shown in Figure 21d. After hydrotropic pretreatment, the fibrils could be seen in Figure 21e. It seems that the surface cell wall was ripped off. Fibres were expanded and more pores exposed. The performance of the fibre digestibility improved by pretreatment cannot be sufficiently explained by the change of chemical composition only. The fibre structure features and cell wall damage created by the pretreatment procedure could assist the enhancement of the fibre accessibility for enzyme as well (Donohoe et al., 2011). The factors affecting on the fibre accessibility are complementing each other.

In Figure 22, after alkaline-hydrogen peroxide pretreatment, the broken outer layer was peeled off from reed surface compared with the coarse surface of reference that had more broken outer layer due to grinding process. Apparently, the layer of lignin deposited was detected on alkaline-hydrogen peroxide pretreated sample and fibre walls were with sparse broken cell wall residues on surface (arrow in Figure 22b). The fibre appearance became smoother than the reference reed and long cells were clearly visible. For the samples RN (alkaline pretreated), platode mastoid epidermal were found by SEM, the exterior surface was dense and the cell wall showed delamination tendency (arrow in Figure 22c). Similar findings have also been reported previously (Corrales et al., 2012). Same platode mastoid epidermal located along vertical fibre direction were detected on the hydrotropic treated reed (arrow in Figure 22d). Some of them were broken to pores on fibre leading to the exposure of internal layer of fibres. The generation of broken pores is usually favourable for accelerating enzymatic hydrolysis efficiency. The performance of the fibre digestibility which is improving by pretreatment cannot be sufficiently explained by the change of chemical composition only. The fibre structure features and cell wall damage created by the pretreatment procedure could assist the enhancement of the fibre accessibility

for enzyme as well (Donohoe et al., 2011). Thus, the factors affecting the fibre accessibility are complementing each other.

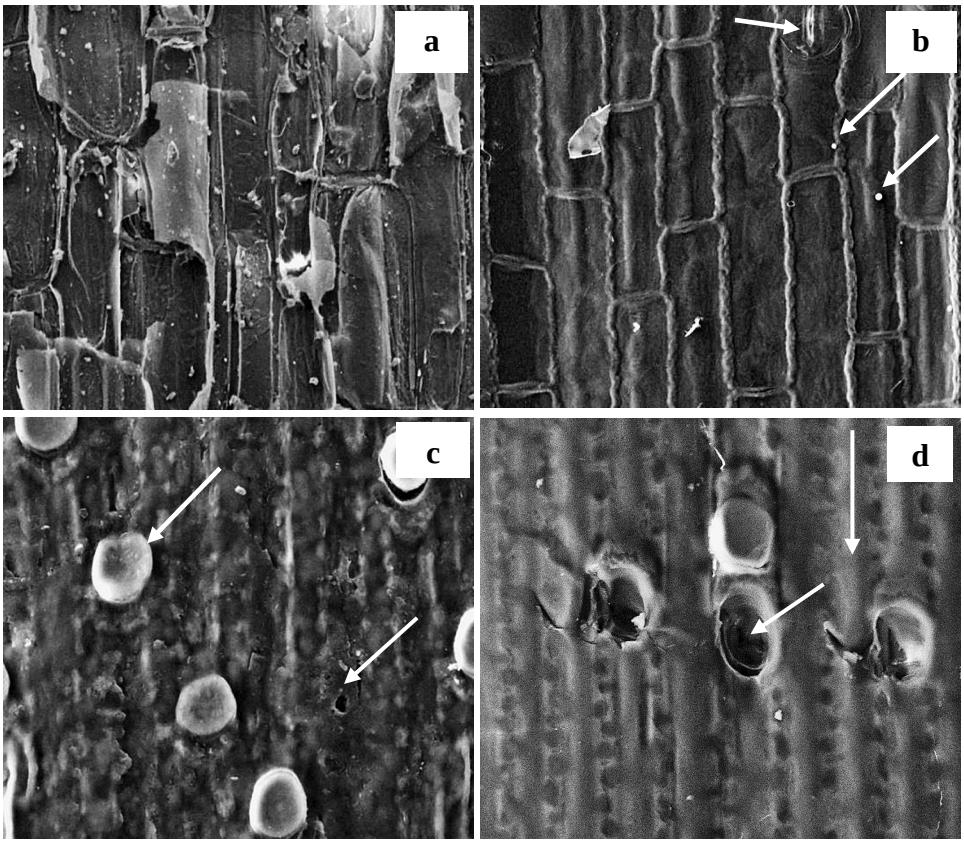


Figure 22. *Morphology of reed samples after pretreatment, reed reference (a), RH (b), RN (c), Rsxs (d).*

3.3.5. Glucan and xylan yield after enzyme hydrolysis

Enzymatically, hydrolyzed glucan and xylan yields of bagasse and reed was studied after the alkaline, alkaline peroxide and hydrotropic pretreatment (Figures 23-24).

The maximum glucan yield of bagasse samples BN, BH and Bsxs was 78.3%, 73% and 83.9%, respectively. The glucan yield achieved from low temperature alkaline pretreatment at 60 ^{o}C for 2 h was better than that from the alkaline-hydrogenperoxide pretreatment at room temperature for 24 h. This was because of the less content of lignin and hemicelluloses in BN (Table 14), as both lignin and hemicelluloses are key barriers for glucan conversion (Leu and Zhu, 2013).

According to the results in Figure 23a, the highest glucan yield could be achieved by hydrotropic pretreatment. This is due to the lower lignin and xylan content (Table 14) as well as the less surface coverage by lignin and xylan (Table 15) on bagasse fibres after hydrotropic pretreatment. The low xylan yield for the case of hydrotropic pretreatment (Figure 23b) was because of the low xylan recovery (Table 14).

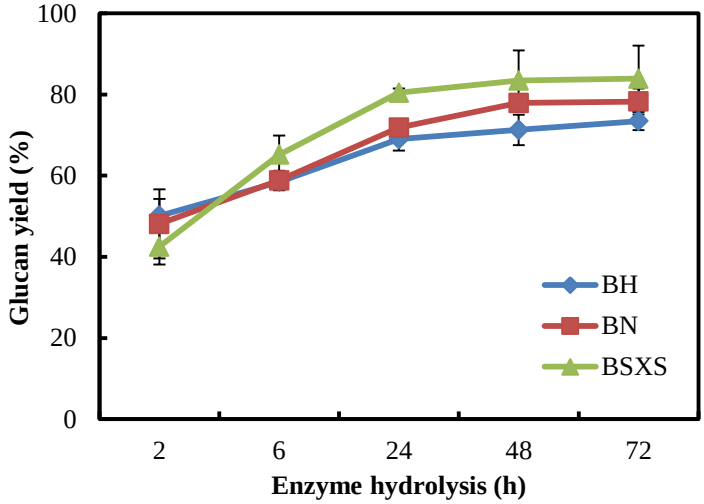


Figure 23a. *Glucan yield of bagasse after enzyme hydrolysis.*

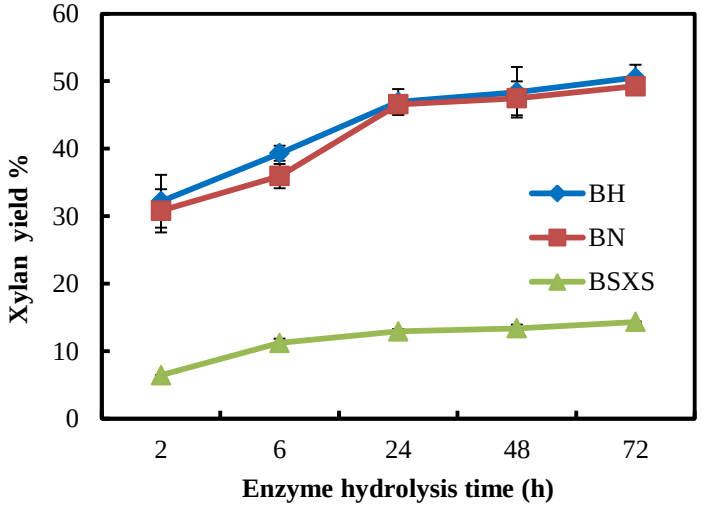


Figure 23b. *Xylan yield of bagasse after enzyme hydrolysis.*

For reed samples, as shown in Figure 24, the maximum yield of glucan from the samples RN, RH and Rsxs were 89%, 85% and 93% respectively, after 72 h

enzyme hydrolysis. In the initiate 6 hours of the enzyme hydrolysis, the glucan yield for the hydrotropic pretreated sample was lower than that for the alkaline and alkaline-hydrogen peroxide pretreated samples which correlated with the remained total lignin amount (Table 14). However, as the hydrolysis time was longer than 6 hours, the glucan yield of Rsxs increased rapidly compared to that of RN and RH. It could be speculated that the enzyme adsorption behaviour to the RN and RH fibre was negatively affected by the high content of surface lignin, as the surface coverage by lignin for RN (about 100%) and RH (91.5%) was higher than that of Rsxs (16.5%). Same as xylan yield of bagasse, the xylan yield of Rsxs presented in Figure 24b was lower than that of alkaline and alkaline-hydrogen peroxide pretreated reed samples. This is due to the fact that the hydrotropic pretreatment can remove large part of hemicellulose. Therefore, hydrotropic method is more efficient to improve the enzymatic hydrolysis of reed compared to alkaline and alkaline-hydrogen peroxide pretreatments.

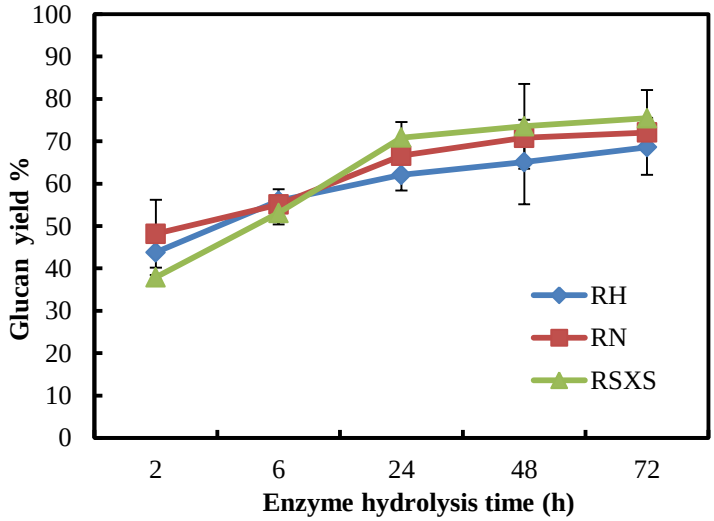


Figure 24a. *Glucan yield of reed after enzyme hydrolysis.*

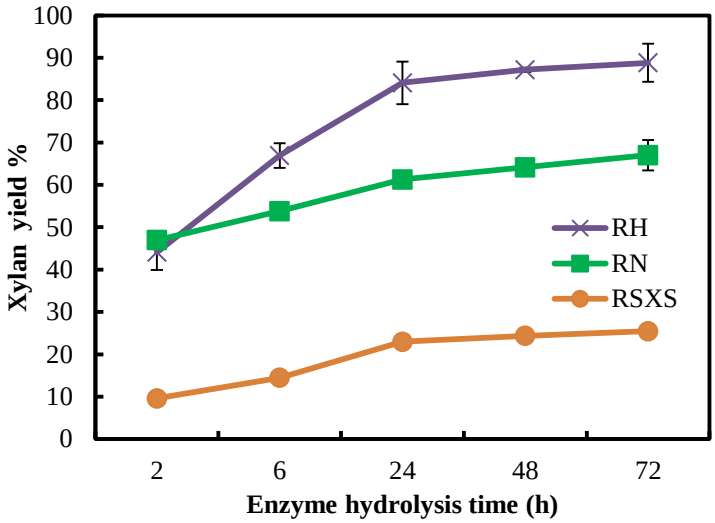


Figure 24b. *Xylan yield of reed after enzyme hydrolysis.*

Based on the results obtained, it could be concluded that among the studied pretreatment techniques, hydrotropic method is the most efficient one to significantly improve the glucan conversion for nonwood biomass; in addition to morphology change, the surface distribution of lignin and xylan is also of critical importance to enzymatic hydrolysis.

3.3.6. Surface chemical characterization of bagasse and reed samples after enzymatic hydrolysis by ToF-SIMS

The samples after pretreatment and enzymatic hydrolysis were investigated by ToF-SIMS. Signal from lignin and carbohydrates were detected and the ratio value was calculated by dividing the sum of the peak intensities of the characteristic mass fragments of carbohydrates (pentoses and hexoses) by the sum of the peak intensities of the characteristic mass fragments of lignin according to literatures (Saito et al., 2005; Fardim and Durán, 2003; Koljonen et al., 2003), as number of counts per number of counts. The ratio value between carbohydrates and lignin was higher in the pretreated samples than in reference bagasse and reed (Table 16).

For bagasse, after hydrotropic pretreatment, the ratio value between carbohydrates and lignin was increased about 3 times compared with the reference in Table 16. This supports the finding that lignin on surface was profusely removed by hydrotropic pretreatment. The peak intensities of hexose

monomer fragments and pentose monomer fragments in the ToF-SIMS spectra were also compared (Table 16). These values show similar tendency, referring to carbohydrate exposure during the pretreatments. After the hydrotropic pretreatment, the ratio of hexoses was raised to four times to that of the reference.

Similar as bagasse, carbohydrates distributed on reed fibre surface were increased by alkaline, alkaline-hydrogen peroxide and hydrotropic pretreatment. However, the lignin removal by alkaline and alkaline-hydrogen peroxide pretreatment was not as efficient as by the hydrotropic pretreatment. Another way to see the same trend is to compare the normalized intensities of the hexose peaks, that is, the number of counts from hexoses divided by the total number of counts in the spectrum. Also, the hexose ratio was increased after the pretreatments, compared to the untreated reference. Hexoses were not sufficiently exposed on reed surface after alkaline and alkaline-hydrogen peroxide pretreatment. However, the ratio value of hexose/total ions of Rsxs increased by hydrotropic pretreatment. Both for bagasse and reed, the biggest increase was detected in the SXS pretreated samples. Results from ToF-SIMS are in good agreement with XPS in Table 15 and is in conformity to the results shown in Table 14 as well.

ToF-SIMS gives the evidence that the outstanding surface delignification of hydrotropic pretreatment enhanced the distribution of cellulose on fibre surface, thus facilitating the downstream enzymatic hydrolysis. In addition, the surface chemical characterization of enzymatically hydrolysed samples was also studied and the results are showed in Table 16.

After enzymatic hydrolysis, the ratio value of carbohydrates to lignin was decreased following the hydrolyzation and removal of carbohydrates from the sample surfaces. This was not detected in the reference bagasse sample. It can be seen as a clear indication on the impact of the pretreatments in the hydrolysis efficiency, reducing the biomass recalcitrance. The value had no large difference between the different pretreatment methods. Hexose and pentose ratios follow the same trend. Especially for the hydrotropic pretreated bagasse, the hexose ratio was reduced from 4.8 to 0.8. Most of the glucose was hydrolysed by hydrotropic pretreatment. In the case of BH and BN, xylan hydrolysis was also contributing to the decreasing ratio between carbohydrates and lignin. Thus, also ToF-SIMS demonstrated the high efficiency of hydrotropic pretreatment on delignification and enzyme saccharification.

Table 16. *The ratio of the peak intensities of the characteristic mass peaks of carbohydrates and lignin by ToF-SIMS*

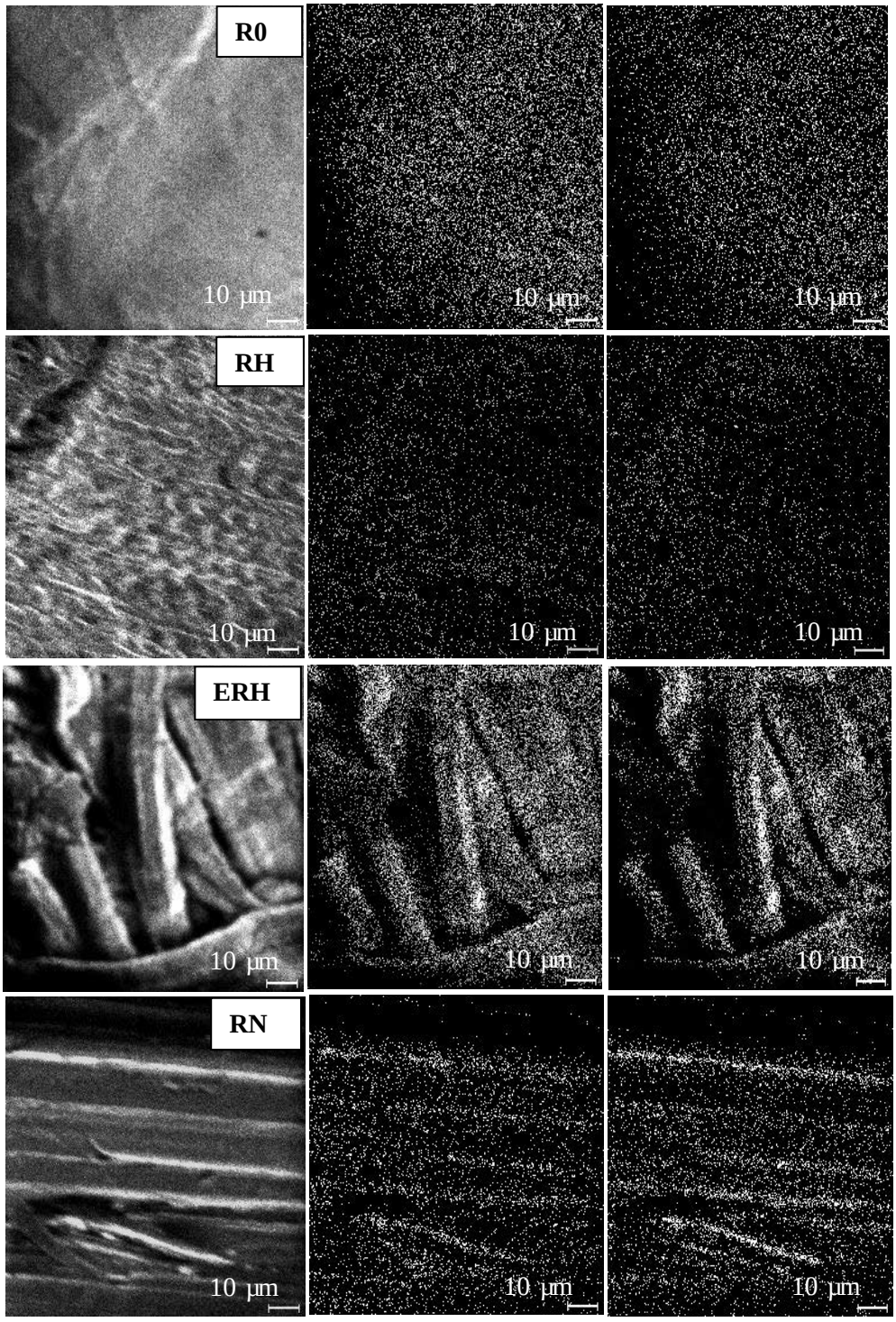
Sample	**Carbohydrates/Lignin**	**Hexose/Total *10^3**	**Pentose/Total *10^3**
Bref	0.67 (0.07)	1.1 (0.3)	1.7 (0.6)
BN	1.09 (0.51)	2.3 (1.4)	3.1 (1.8)
BH	1.27 (0.48)	2.6 (1.1)	3.1 (1.8)
Bsxs	1.82 (0.43)	4.8 (1.3)	3.7 (0.7)
EB0	1.02 (0.27)	3.1 (0.7)	2.7 (0.2)
EBN	0.36 (0.01)	0.7 (0.1)	1.2 (0.1)
EBH	0.47 (0.04)	0.6 (0.3)	1.0 (0.4)
EBsxs	0.48 (0.12)	0.8 (0.3)	2.2 (0.9)
Rref	0.63 (0.08)	0.9 (0.3)	1.4 (0.5)
RN	0.82 (0.44)	1.0 (0.7)	1.7 (1.2)
RH	1.09 (0.09)	1.0 (0.4)	2.8 (0.9)
Rsxs	1.05 (0.30)	2.2 (0.9)	2.3 (0.9)
ERref	0.73 (0.12)	2.5 (0.6)	2.9 (0.5)
ERN	0.41 (0.05)	1.3 (0.1)	2.4 (0.2)
ERH	0.67 (0.09)	2.8 (0.5)	4.7 (0.6)
ERsxs	0.44 (0.02)	1.0 (0.1)	1.8 (0.3)

The decrease of hexose compared to reference reed was due to the hydrolysis of cellulose to glucose monomers in liquid phase especially for RN and Rsxs. The reduction of carbohydrates/lignin ratio indirectly refers that the surface of fibre contained relatively more lignin after hydrolysis. From the results presented in Table 16, the hydrolysis efficiency order was Rsxs>RN>RH which is in line with the results identified from Figure 24. During enzyme hydrolysis, the G/aromatic ratio and G/Lignin ratio of RN samples was increased while that of RH and Rsxs has no difference with ERref. This could be due to removal of redeposited lignin on RN during the hydrolysis.

However, the distribution of surface lignin and carbohydrates on fibre after pretreatment and enzyme hydrolysis was assessed by ToF-SIMS imaging. Take reed as an example, selected results are shown in Figure 25. Bright pixel represents high amount of detected secondary ions in that spot. In the total ion image, all mass fragments detected in the sample in the investigated mass range in the positive ion mode have contributed to the image brightness. Topographic features can be distinguished. Correspondingly, in the lignin images, only signal from the G, S, and H fragments are included, and in the carbohydrate images, only counts from hexose and pentose monomer fragments are included.

Lignin distribution was more intense than carbohydrates on reference fibre, and after pretreatment, the carbohydrates intensity was relatively increased because of lignin removal. Combining total ion mass image and lignin image of Rsxs, it can be estimated that there was less lignin by the pores. Change in the even lignin and carbohydrate distributions was not shown in RN, RH, or Rsxs compared with reference. Based on the visual estimation of the signal brightness, it could be illustrated that the carbohydrates started to expose by alkali-peroxide pretreatment.

During enzymatic hydrolysis, the distribution of lignin and carbohydrates was changed. Disintegration of the structure can be seen in the total ion images. The lignin distribution was obviously more intense than carbohydrates on surface from the images of ERH, ERN, ERsxs as a result of cellulose hydrolysis by enzyme. The images detected by ToF-SIMS could demonstrate the change of lignin and carbohydrates during pretreatment and hydrolysis in direct viewing and confirmed the results from XPS as well. Based on the results from ToF-SIMS, the distribution and the location of residual lignin on fibre surfaces were changed by pretreatments, and the carbohydrates were exposed while surface coverage of lignin decreased. Therefore, the removal of surface lignin is more important than the influence of the total amount of residual lignin for enzymatic saccharification.


Total ion image
Lignin
Carbohydrates
R0
RH
ERH
RN
10 µm

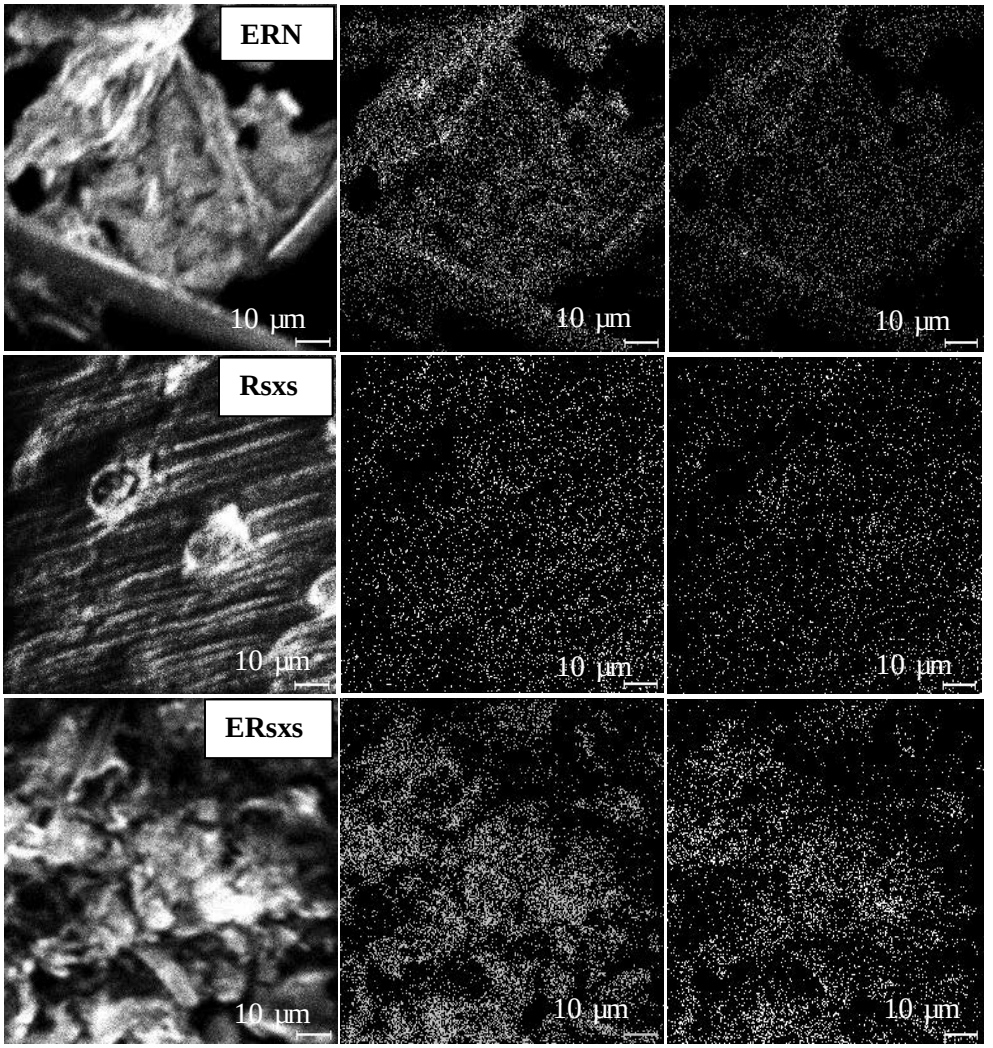


Figure 25. *Imaging of carbohydrates and lignin distribution on fibre after pretreatments and after enzymatic hydrolysis by ToF-SIMS.*

4. Conclusions

Eucalyptus and pine pulp fibres were modified by low consistency (LC) refining. Refining affects not only the morphology but also the surface chemical composition of the whole pulp in a way that possibly has significance for the final paper properties especially for optical properties. With refining, fibre shape changed from tubular to flat, and external fibrillation and fines generation took place. Refining could lead to the increase of the surface coverage by extractives, and to the decrease of surface lignin distribution. The results from XPS and ToF-SIMS were in good agreement with respect to the surface chemical composition and it supplied the evidence supporting the release of xylan and its subsequent adherence to the fibre surface after refining. However, the change of chemical composition and fibrils generation could further impact the adsorption performance of chemical agents.

In the case of refined eucalyptus ECF pulp, it was found that refining can improve the adsorption of fluorescent whitening agent (FWAs) by increasing the specific surface area and electrostatic forces (via the generation of fines) as well as the hydrophobic interactions by the change of surface chemical composition of fibres. The fines contribute more anionic charges which are beneficial for the adsorption of di-FWA (DS) but not for tetra-FWA (P01). Although, the addition of FWAs can partially compensate for the loss of optical properties resulted from LC refining, but the addition point in the process is important for FWAs adsorption. High refining energy could promote adsorption when FWAs are added after refining, but is not favourable when they are added before refining. Calcium sulphate added in appropriate dosages was retained on the refined fibres and could benefit FWAs adsorption.

XPS and ToF-SIMS used for studying the chemical pretreatment of biomass for the enhancement of enzymatic hydrolysis could give a new insight of explanation on the mechanism study. The hydrothermal hot water pretreatment, ionic liquid and hydrotropic pretreatment were used on wood biomass prior to enzymatic hydrolysis. According to the results obtained from XPS and ToF-SIMS, it was demonstrated that hydrothermal pretreatment, ionic liquid and hydrotropic pretreatment worked differently on surface lignin on birch and pine chopped wood. Hydrothermal treatment did not directly remove lignin. Ionic liquid could

swell the fibre to increase the surface area, without removal of lignin. Hydrotropic pretreatment was an efficient way to improve enzyme accessibility through lignin removal, especially syringyl from birch. At the same time, hydrotropic pretreatment also preserved carbohydrates.

The surface chemical composition played important role on nonwood biomass enzymatic hydrolysis after pretreatments, which was proved by XPS and ToF-SIMS also. Alkaline and alkaline-peroxide pretreatments were known as an efficient method for the enhancement of enzymatic hydrolysis for nonwood biomass. After Alkaline, alkaline-hydrogen peroxide pretreatments and hydrotropic pretreatments, the total lignin was reduced obviously. By XPS and ToF-SIMS determination, through comparison with alkaline, alkaline-hydrogen peroxide pretreatments, it was found that hydrotropic pretreatment method was more efficient to reduce and re-localize lignin from bagasse and reed fibre cell wall and the fibre surface. After hydrotropic pretreatment, surface lignin was removed and carbohydrates were sufficiently exposed on fibre surface leading to morphology changes and enhancing enzyme accessibility to fibres. Consequently, the maximum glucan yield was achieved from hydrotropic treatment. However, ToF-SIMS could map the chemical composition distribution on fibre surface, thereby helping to explain the mechanism of pretreatment and enzymatic hydrolysis. Therefore, from chemistry and surface chemistry aspect, hydrotropic pretreatment is an efficient method of boosting enzymatic hydrolysis of nonwood and wood materials. The recovery of SXS, as well as the fractionation of lignin and hemicellulose could be promising for controlling cost and supplying a platform for the development of high value-added biomaterials and biochemical besides cellulosic ethanol.

In conclusion, the change of surface chemical composition due to the different type of treatment was an important impact factor in the further utilization stage. Studying on surface chemistry could help the comprehensive understanding of the reaction and treatment mechanism. For lignocellulosic biomass-based study, surface chemistry could supply more concise and detailed information for better understanding and optimizing of the technology.

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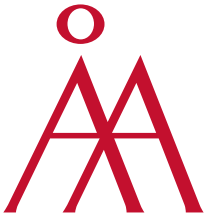

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ISBN 978-952-12-3061-5