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Influence of chemical and enzymatic treatments on a variety of wood pulps on their dissolution in NaOH-water

Influence de traitements chimiques et enzymatiques sur la dissolution de pâtes de bois dans NaOH-eau

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T H È S E

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to my parents

“ gib niemals auf ”

acknowledgement

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Nuno

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[C_4mpy]Cl – 3-methyl-1-butyl-*N*-pyridine chloride

µm - Micrometer

µmol – Micro mole

^{13}C NMR – Solid state nuclear magnetic resonance using the carbon isotope 13

Å – Angstrom

AECL - Atomic Energy of Canada Limited

Be-S – Beech Bleached Sulfite Pulp

Be-S_419 – Beech Bleached Sulfite Pulp GVZ 419

Be-S_435 – Beech Bleached Sulfite Pulp GVZ 435

Be-S_ND – Beech Bleached Sulfite Pulp Never Dried

C3 – Cabon in position 3

C6 – Carbon in position 6

CCD - Charge-coupled device

CCM – Biopectinase CCM

CEMEF – Centre de mise en forme des matériaux

CH_3COOH – Acetic acid

CL – Cotton Linter

CL_ND – Cotton Linter Never Dried

CMC – Carboxy-methyl cellulose

cNdtex^{-1} – Tensile strength unit

Cr_i – Crystallinity index

Cuen – Copper-ethylene-diamine

DBD – Dielectric barrier discharger

DMAc – Dimethyl acetamide

DMSO – Dimethyl sulfoxide

DP – Degree of polymerization

ECF – Elemental clorine free

EG – Endoglucanase

Eu_Ac-GR – Eucalyptus acetate grade (good reactivity)

Eu_Ac-PR – Eucalyptus acetate grade (poor reactivity)

Eu_Lyo – Eucalyptus lyocell grade

Eu_MCC – Eucalyptus micro crystalline cellulose grade

Eu_Vis – Eucalyptus viscose grade

Eu-KP – Eucalyptus bleached kraft pulp (paper grade)

FEG-SEM - Field Emission Gun - Scanning Electron Microscopy

g – G-force unit

g – Gram

G – Lignin units guaiacylpropane

G1 – Sintered glass porosity grade 1 (pore size 90-150 microns)

gg – Gauche-gauche

G-layer – Gelatinous layer

gt – Gauche-trans

H – Lignin units p-hydroxyphenylpropane

H_Ac – Hardwood dissolving pulp (Acetate Grade)

H_2O – Water

H_2SO_4 – Sulphuric acid

HG – Homogalacturonan

HPSEC - High pressure size exclusion chromatography

ICP-OES – Inductive coupled plasma - atomic emission spectroscopy

Ins – NaOH insoluble fraction

IS – Intercellular space

ISO –International standard organization

kat – Katal

kV – Kilo Volt

Kw – Filterability unit

L – Lumen

L40 – Pektinase L40

LiCl – Lithium cloride

LiOH – Lithium hydroxide

m - Meter

M – Middle lamella

mA - Miliampere

mbar – Milibar

MEB-FEG – Microscopie electronique à balayage - Field Emission Gun

MHz – Mega Hertz

min – Minutes

MxH-KP – Mixed hardwood bleached kraft pulp (paper grade)

ml - Mililiter

mm - Milimeter

NaOH –Sodium hydroxyde

NC-IUB - International Union of Biochemistry and Molecular Biology

$Ni(OH)_2$ – Nickel hydroxide

Ni-Eu-KP_A – Nitren Extracted EucBKP_A Never Dried

Ni-Eu-KP_B – Nitren Extracted EucBKP_B Never Dried

nm – Nanometer

NMMO – *N*-methylmorpholine-*N*-oxide

NS-S – Northern softwood sulphite pulp

NS-S_1 – Northern softwood sulphite pulp

O_3-H – Hydrogen atom bonded with the oxygen in position 3

ºC – Degrees Celcius

OH – Hydroxyl groups

pH - decimal logarithm of the reciprocal of the hydrogen ion activity, a_{H+}, in a solution

PHK – Pre-hydrolysis kraft

ppm – Parts per million

R_{10} – Resistance test in 18% NaOH for dissolving pulps

R_{18} – Resistance test in 18% NaOH for dissolving pulps

R^2 – Regression coefficient

RG-I – Rhamnogalacturonan-I

RG-II – Rhamnogalacturonan-II

RI – Refractive inde

rpm – Revolutions per minute

S – Lignin units syringylpropane

S1 – Layer form the secondary wall bonded with primary wall

S2 - Layer form the secondary wall found between S1 and S3 layers

S3 – Inner layer form the secondary wall

SEC-MALLS - Size exclusion chomatography - Multi-angle laser light scattering

SI – "*Système internationale*"

SoI – NaOH soluble fraction

S-PhK – Softwood pre-hydrolisys kraft pulp

S-PhK_ND – Softwood pre-hydrolisys kraft pulp, never dried

SPL – Pectinex Ultra SP-L

Sp-S – Spruce bleached sulfite pulp

Sp-S_ND – Spruce bleached sulfite pulp, never dried

SS-S – Southern softwood sulphite pulp

SS-S_1 – Southern softwood sulphite pulp (lower viscosity)

TCF – Total chlorine free

TEMPO - 2,2,6,6-tetramethylpiperidine-1-oxyl

tg – Trans-gauche

TI – Thünen Institut (Hamburg)

U - Enzymatic activity unit

W – Wart layer

w/v – Ratio weight per volume

w/w – Ratio weight per weight

XGA – Xylogalacturonan

ZnO – Zinc oxide

η – Intrinsic viscosity

λ – Wave length

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general introduction

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Contexte et objectifs de la thèse

Dans un contexte de pénurie programmée de pétrole et d'augmentation de la demande sociale et environnementale pour un développement durable, l'utilisation de matériaux préparés à partir de ressources renouvelables semble être l'un des moyens pour maintenir un niveau de vie acceptable dans un avenir proche.

La cellulose, le composant principal de la paroi cellulaire des plantes, peut jouer un rôle important dans ce contexte. Elle est présente dans toutes les plantes, certaines algues et est aussi produite par des bactéries. En plus d'être le biopolymère le plus abondant dans la nature, la cellulose ne représente pas une source directe d'alimentation pour l'homme, est biodégradable et est facilement décomposé par les champignons et les bactéries du sol. En outre, ce polysaccharide est une ressource naturelle renouvelable, se formant dans un cycle naturel utilisant le dioxyde de carbone et la lumière du soleil comme énergie infinie, gratuite et propre. Cela signifie que ce biopolymère peut être géré de manière durable [Gavillon 2007; Pinkert 2010].

En fait, la cellulose a toujours fait partie de la culture humaine. Il y a plusieurs millénaires, l'homme utilisait déjà les excellentes propriétés de la cellulose comme matériau composite, dans ce cas un bio -composite, le bois. Les propriétés mécaniques de ce bio -composite permettaient de l'utiliser comme matériau de construction, armes et plus tard pour la construction de bateaux. Son potentiel comme combustible a également été découvert et bientôt l'énergie de la combustion du bois, le feu, a été largement utilisée. En outre, depuis longtemps, la cellulose extraite du coton et d'autres fibres végétales a été utilisée pour produire des vêtements et en tant que matériau de renforcement dans des blocs de terre utilisés dans la construction de bâtiments. Plus tard, les égyptiens l'ont utilisée pour préparer des papyrus, le précurseur de ce qui est aujourd'hui l'un des matériaux les composites les plus fascinants, le papier.

Depuis le milieu du 19e siècle, l'intérêt de l'utilisation de la cellulose comme matière première pour l'industrie chimique a augmenté, de même que la connaissance scientifique et technologique sur ce sujet. Dans les années 1850, le premier polymère thermoplastique synthétique a été préparé à base de cellulose pour former la nitrocellulose. Ce polymère a été inventé à Birmingham par Alexander Parkes, qui l'a breveté sous le nom de " Parkesine " en 1862. Plus tard, celui-ci a été industrialisé à New York par la Société Hyatt, ajoutant du camphre comme plastifiant et le nommant « Celluloid » en 1870. Ce matériau était hautement inflammable et dégradable et aujourd'hui, malgré l'incorporation d'additifs pour améliorer sa stabilité et réduire son inflammabilité, son utilisation est limitée à la production de certains matériaux tels que les balles de ping-pong. Cette application initiale a montré que de nouveaux matériaux pourraient être produits par modification chimique de la cellulose. L'intérêt et de développement technologique de l'utilisation de fibres synthétiques à base de cellulose pour des applications

textiles et techniques a alors augmenté. Des exemples de ces utilisations sont les fibres produites à partir des procédés à base d'hydroxyde de cuprammonium, de viscose ou plus récemment par le procédé Lyocell [Klemm 2005].

Aujourd'hui le bois est toujours utilisé comme un bio –composite "prêt à l'emploi", principalement dans la construction. De plus, il est utilisé sous la forme de pâte de bois en tant que matière première pour l'industrie de transformation de la cellulose. Malgré l'intérêt de l'utilisation de la cellulose comme matière première, seulement 2% de la pâte de bois est utilisés à cette fin.

Le principal inconvénient et le défi sont que la cellulose comprend un réseau de forces intermoléculaires fortes (surtout des liaisons hydrogène) qui confèrent une forte cohésion de la structure supramoléculaire. Ceci fait que la cellulose ne peut pas être utilisée à l'état fondu et qu'il n'est pas possible de la dissoudre dans de l'eau ou les solvants organiques classiques [Navard 2013].

Ainsi, afin d'être utilisée comme une source de matières premières pour des applications autres que le papier ou les panneaux de fibres, elle doit être dérivée chimique (comme ce fut le cas pour le Celluloid mentionné ci-dessus) ou dissoute en utilisant des procédés coûteux ou toxiques. Pour cela, la pâte de bois doit répondre à des exigences spécifiques qui sont présentes dans la pâte à dissoudre. Celle-ci présente une accessibilité élevée aux groupes réactifs des molécules de cellulose, ce qui permet par exemple une substitution plus homogène des radicaux hydroxyles lors de la dérivation.

La production de pâtes à dissoudre génère des contraintes élevées de pureté, avec de très faibles quantités de lignine, hémicelluloses et extractifs résiduels. Par conséquent, les pâtes à dissoudre sont plus chères que les pâtes à papier. Ce fait et les coûts économiques et environnementaux élevés de l'industrie de la cellulose dérivée et régénérée, nécessite d'effectuer des efforts supplémentaires pour optimiser les procédés actuels et explorer d'autres façons pour améliorer la réactivité et l'accessibilité de cette bio - ressource.

Une grande partie de la recherche sur la chimie, la physique et la technologie de la cellulose traite de son gonflement et de sa dissolution. Néanmoins, certaines questions importantes doivent encore être étudiées et comprises. L'une d'elles porte sur les mécanismes de biosynthèse dans les cellules végétales, où la cellulose est polymérisée et réticulée avec d'autres polysaccharides et des composés chimiques. Un autre sujet est la recherche de l'organisation des structures de cellulose entre substances naturelles et l'origine de sa variabilité.

Un troisième sujet de recherche de grande importance scientifique et technologique (et d'intérêt industriel) est de comprendre comment les structures de cellulose se dissolvent et quelles propriétés physico- chimiques jouent un rôle dans ces processus et comment. La recherche que nous avons menée lors de cette thèse traite de ce sujet, plus précisément pour

mieux comprendre la dissolution des fibres de cellulose, un travail déjà commencé au Cemef par Cuissinat 2006; Le Moigne 2008 et Spinu 2010.

Au milieu du 19e siècle, Nägeli [Nägeli 1864] a commencé étudié les mécanismes de gonflement et de dissolution des pâtes de bois, un travail continué par un large groupe de scientifiques jusqu'à nos jours. Récemment, Cuissinat et al. ont montré que selon la qualité du solvant et de l'accessibilité de la cellulose, les fibres de cellulose native peuvent présenter cinq modes de mécanismes d'interaction avec le solvant [Cuissinat 2006].

Il a pu également être observé qu'en général ce gonflement n'est pas homogène, mais qu'il se produit sur des sections différentes de la fibre conduisant à des formations semblables à des ballons gonflés (sections gonflées) séparées par des sections non gonflées (Figure 1F).

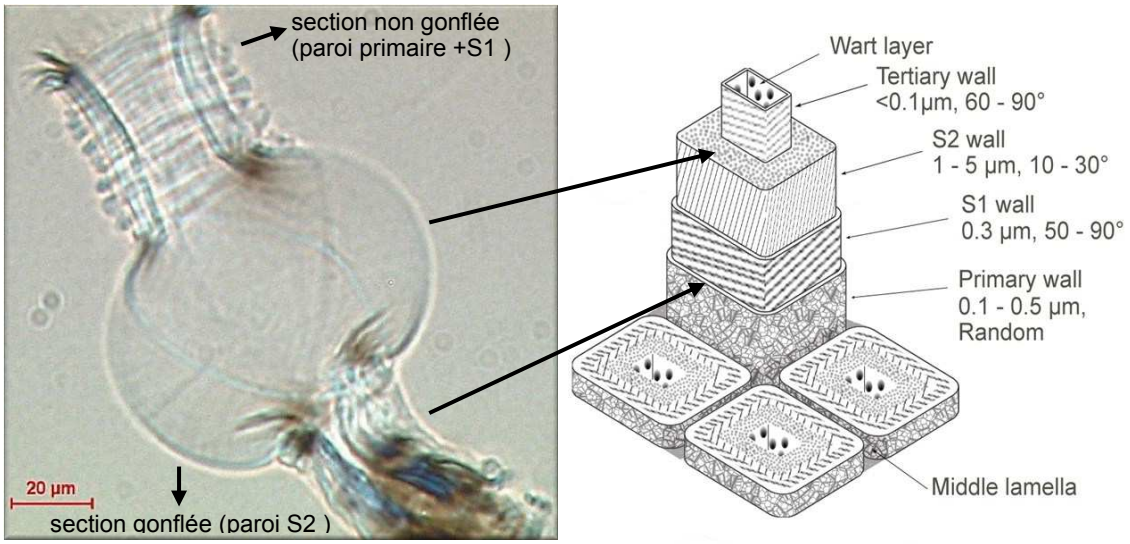

Figure 1F – Gauche: Vue partielle d'une fibre de bois gonflé dans une solution aqueuse de NMMO, avec des sections non gonflées et une section gonflée (ballon) (photographie de l'auteur, CEMEF 2010) Droite : structure de la paroi cellulaire du bois avec les différentes couches et l'orientation des fibrilles (dessin de l'auteur et Oscar Silva Design, 2011).

La plupart des auteurs qui ont étudié ce phénomène de ballonnement l'ont expliqué comme un gonflement de la paroi de la fibre S2 bloqué par la paroi primaire et, dans certains cas, la paroi S1, ce qui est le cas dans l'image ci-dessus. Cela se produit principalement en raison de l'orientation fibrillaire des fibres sur les différentes couches [Ott 1954; Le Moigne 2008]. Le Moigne et al. ont montré que l'enlèvement par un traitement enzymatique de la paroi primaire évite la formation de ballons, améliorant ainsi l'accessibilité des fibres de cellulose et leur dissolution [Le Moigne 2008]. Ceci explique ce qui se passe au niveau microscopique, mais apporte d'autres questions concernant ce qui se passe au niveau moléculaire. Par exemple, pourquoi est-ce que la paroi primaire est plus difficile à dissoudre ?

Notre travail se propose de contribuer à clarifier cette question, afin de mieux comprendre l'accessibilité des fibres de cellulose, les mécanismes de dissolution de ces fibres et les facteurs

qui peuvent jouer un rôle dans ces processus. Ayant à l'esprit la composition chimique des différentes couches de la paroi cellulaire, il est connu que la paroi primaire est, en comparaison avec les autres couches de la paroi cellulaire intérieure, enrichie en lignine, hémicelluloses et pectines.

Le premier objectif de ce travail est la compréhension fondamentale du rôle des composants mineurs de la paroi cellulaire, en particulier le xylane et les pectines, sur la dissolution de la cellulose.

Le deuxième objectif, qui est relié au premier, concerne l'application technique permettant l'utilisation de l'extraction par le nitren suivie par des traitements de pectinases pour convertir des pâtes à papier en pâte à dissoudre.

Ce projet a été mené dans le cadre d'une coopération internationale. Le travail a été effectué dans deux groupes de recherche qui travaillent depuis des années dans le domaine de la cellulose, Cemef - Mines ParisTech à Sophia Antipolis, en France, avec le Dr Patrick Navard et Université Hambourg / TI (Thünen Institut), en Allemagne, avec le Professeur Bodo Saake et le Dr Jürgen Puls. Ce projet a été financé par un consortium industriel: Sappi (Afrique du Sud), Tembec (Canada / France), Lenzing (Autriche), Viskase (USA / France) et Spontex (USA / France), et a reçu l'appui d'EPNOE (European Polysaccharidique Network of Excellence).

Résumé de la thèse et principaux résultats

Ce manuscrit est divisé en cinq chapitres.

Le **premier chapitre** est une revue de la littérature sur le bois, la structure de la paroi de la fibre de bois et les procédés de production de pâte à dissoudre, en accordant une attention particulière à la structure de la cellulose comme polymère et à la structure de la paroi cellulaire. Après cela, est présentée une brève description de la réactivité des fibres de cellulose, le chapitre se terminant par un examen exhaustif de la littérature sur la dissolution de la cellulose.

Ce chapitre permet au lecteur d'avoir une introduction des thèmes abordés lors de la thèse.

Le **deuxième chapitre** consiste en une description de tous les échantillons (échantillons de cellulose et solvants), des méthodes utilisées dans les différents traitements (extraction avec le nitren, lyophilisation ,traitements enzymatiques et dissolution dans NaOH) et des méthodes d'analyse (teneur en nickel ,analyse des glucides , distribution des poids moléculaires, viscosité intrinsèque Cuen ,analyse dimensionnelle de la fibre, diffraction des rayons X, microscopie optique et analyse MEB-FEG).

Avec cette description, le lecteur peut avoir un aperçu des échantillons utilisés, en comparant leurs caractéristiques et leurs différents prétraitements. En outre, la description des méthodes permet la reproduction de nos travaux pour es appliquer à d'autres échantillons afin de comparer les résultats à ceux que nous avons trouvé.

Le **chapitre III** étudie l'effet de l'élimination sélective de xylane de la paroi cellulaire sur les propriétés des fibres et sur la dissolution de la cellulose dans des solutions aqueuses de NaOH. Plusieurs pâtes à dissoudre commerciales ont été sélectionnées. Afin de diminuer le xylane résiduel, chaque pâte a été traitée avec des solutions aqueuses contenant 3, 5 et 7 % de nitren. À de faibles concentrations, ce solvant peut être utilisé pour extraire sélectivement les xylanes. L'influence de ces traitements de nitren sur la dissolution de la pâte a été évaluée en étudiant les rendements de dissolution dans des solutions de NaOH à basse température et les mécanismes de dissolution. Toutes les fractions issues des traitements de pâte à papier et les résidus non dissous provenant du procédé de dissolution ont été analysés en ce qui concerne leurs structures chimiques et macromoléculaires.

Les résultats de ce chapitre montrent que le traitement à base de nitren supprime une grande partie des xylanes présents dans les pâtes mais élimine également les mannanes et influence la cellulose de deux façons, en l'extrayant (avec d'autant plus d'intensité que la concentration de nitren est élevée), et en diminuant sa masse moléculaire moyenne. Les extractions de nitren sont favorables à la dissolution dans NaOH, et ce d'autant plus que la concentration de nitren est élevée. Cette modification chimique de la surface des fibres conduit à l'élimination partielle et à une destructuration de la paroi primaire. Ceci permet un accès plus facile de NaOH à des régions non accessibles pour les fibres initiales, ce qui, associé avec la diminution de la masse molaire moyenne cellulose, permet une dissolution plus facile et induit des mécanismes de dissolution différents.

Le **chapitre IV** est consacré à la description de l'effet de la pectinase en tant que prétraitement de plusieurs pâtes à dissoudre et d'une pâte à papier avant leur dissolution dans NaOH. Il est divisé en trois parties:

- Une première étude initiale à l'aide d'une pectinase commerciale (CCM) a montré qu'un tel traitement avait un effet positif évident sur la dissolution. Le fait que l'enzyme ne soit en fait pas une pure pectinase a nécessité la mise en place d'une seconde phase de l'étude où des endopectinase et endoglucanase pures ont été utilisées.
- Dans cette deuxième phase, une endopectinase pure (L40) et une endoglucanase pure (EG) ont été utilisées avec un seul échantillon de pâte et comparées à la CCM, dans le but de trouver si l'effet de CCM était dû à sa teneur en endoglucanase. Le résultat a montré que l'endopectinase pure améliore la dissolution dans la soude, sans trop diminuer la masse molaire moyenne des pâtes.
- La troisième phase a été consacrée à appliquer le traitement optimisé pour L40 et CCM à cinq pâtes à dissoudre très différentes et une pâte à papier et à en évaluer leurs effets sur la dissolution dans la soude.

Le résultat de notre travail montre que bien que la quantité ou la présence de la pectine sur les pâtes de départ était trop faible pour être déterminée, l'incubation des différentes pâtes avec des préparations de pectinases conduit à une augmentation de l'accessibilité des fibres, atteignant des augmentations de rendements de dissolution supérieurs à 150 %. Les traitements enzymatiques n'affectent pas toutes les pâtes étudiées de la même manière, montrant que les

prétraitements des pâtes ont une influence sur l'efficacité des traitements enzymatiques. Les traitements enzymatiques des fibres de cellulose induisent une légère diminution de la masse molaire moyenne. L'utilisation d'un mélange d'endoglucanase et d'endopectinase a montré un effet synergique de ces deux enzymes, le mélange étant plus efficace que les enzymes poris séparément pour l'activation de la cellulose. Nous proposons que les endopectinases augmentent l'accessibilité des fibres de deux manières: (1) en décomposant la matrice de polysaccharide de la paroi primaire (où la pectine est présente), favorisant le gonflement de cette paroi, ce qui permettra une capacité de dissolution élevée des fibres, (2) en modifiant le réseau de liaisons hydrogène à partir de l'ensemble de fibres de cellulose, en diminuant légèrement la masse molaire moyenne et en aidant la diffusion des ions de solvatation de NaOH dans la structure cellulosique, facilitant de ces façons la dissolution.

Le **chapitre V** a consisté à appliquer les deux traitements, extraction avec une solution de nitren et traitement avec des pectinase, à des pâtes à papier. L'extraction avec une solution de nitren supprime de manière sélective l'hémicellulose, augmentant la teneur en cellulose de la pâte à papier, tandis que le traitement avec la pectinase active la pâte et augmente sa solubilité dans la soude caustique à froid. Il est également discuté un bilan de masse pour la pâte et la teneur totale en glucose sur la base de la pâte et du bois.

Ce travail a montré que les pâtes à papier extraites avec le nitren ont une accessibilité moindre aux ions sodium, ce qui implique une plus faible capacité de dissolution dans la soude caustique à froid. Cela pourrait s'expliquer par une hornification partielle due à l'élimination de l'hémicellulose. Les traitements avec la pectinase permettent d'améliorer le rendement de dissolution dans NaOH de 10% à 60 %. Cela peut s'expliquer par les mêmes mécanismes décrits dans le chapitre IV. En outre, cette étude a montré que pour le mode de dissolution utilisé, l'utilisation de pâtes à papier traité avec la combinaison de ces deux traitements a le potentiel d'accroître les rendements de dissolution de la cellulose, calculés à partir du bois, en comparaison avec les pâtes à dissoudre actuelles.

Enfin, une **conclusion générale** est présentée, résumant les principaux résultats de ce travail et proposant des **perspectives** pour de futures recherches sur ce sujet.

Les résultats intéressants obtenus et les techniques utilisées et développées dans ce projet permettent de définir de nouvelles orientations de recherche, à la fois au niveau fondamental (comme par exemple l'étude sur les mécanismes à l'origine de l'effet des pectinases sur le comportement des pâtes et leurs dissolutions selon les différents types de cellules du bois) et au niveau technologique (comme par exemple l'utilisation de pectinases dans l'activation des pâtes à papier permettant la conversion en pâtes à dissoudre).

Anglophone version

Background and objectives of the thesis

In a context of oil shortage and increasing social and environmental demand for a sustainable development, the need for production of biodegradable materials from renewable resources seems to be one of the ways for keeping a reasonable standard of living in the near future.

Cellulose, the main component of the plant cell walls, can play an important role in this context. It is present in all plants, some algae and is also produced by some bacteria. Besides being the most abundant biopolymer available in nature, cellulose does not represent a direct food source of humans, and is biodegradable, being easily decomposed by fungi and soil bacteria. In addition, this polysaccharide is a renewable natural resource, breeding itself through a natural cycle collecting carbon dioxide and using sunlight as energy, which is infinite, free and clean. This means that this biopolymer can be managed in a sustainable manner [Gavillon, 2007; Pinkert, 2010].

In fact, cellulose was always part of the human culture. Several millennia ago the Humankind was already applying the excellent properties of cellulose as a composite material, in this case a bio-composite, the wood. The mechanical properties of this bio-composite allowed using it as housing construction material, weapons and later on construction of boats. Its heating value potential was also discovered and soon the use energy from wood combustion, known as fire, was widely used. Also since long time, cellulose from cotton and other plant fibers was used to produce clothing and as reinforcement material in earth blocks used in building construction. Later on, the Egyptian started to use it to produce papyrus, the precursor of what is today one of the most fascinating composite materials, the paper.

Since the mid-19th century, the interest of the use of cellulose as a chemical raw material has been growing, together with scientific and technological knowledge on this topic. In the 1850's the first man-made thermoplastic polymer was made out of nitrocellulose. This was invented in Birmingham by Alexander Parkes, who patented it as "Parkesine" in 1862, and later on it was industrialized in New York by the Hyatt Manufacturing Company, adding camphor to the process as a plasticizer and registering it as "Celluloid" in 1870. This material was highly flammable and degradable and today, with incorporation of additives to increase stability and reduce flammability, its use is limited to the production of some materials such as ping pong balls. This initial application has shown that new materials could be produced by chemical modification of cellulose. With this knowledge, came the interest and technological development for the use of synthetic fibers based on cellulose for textile and technical applications. Examples of this are the regenerated cellulose fibers or materials from the Cuprammonium hydroxide, Viscose or, more recently, the Lyocell processes [Klemm, 2005].

Nowadays wood is still used as a “ready to use” bio-composite”, mostly in construction. Besides this, it is used in the form of wood pulp as raw material for the cellulose processing industry. Despite the interest on the potential use of cellulose as chemical feedstock, only 2% of the wood pulp is used for this purpose.

The main drawback and challenge is that cellulose comprises a network with strong intermolecular forces (mostly hydrogen bonds) which confers a high cohesion of the supramolecular structure. This makes that cellulose cannot be used in the molten state and is not possible to dissolve it in water or conventional organic solvents [Navard, 2013].

Thus, in order to be used as a source of materials other than paper or fiber boards it has to be chemically derivatized (as was the case for the above mentioned Celluloid) or dissolved using either expensive or toxic processes. For this purpose the wood pulp should meet specific requirements, which industry can find in dissolving pulp. This chemical pulp presents a higher accessibility to the reactive groups of the cellulose molecules, allowing for instance a more homogeneous substitution of hydroxyl groups during derivatization.

Dissolving pulp production involves special requirements in purity, demanding very low amounts of residual lignin, residual hemicelluloses, and residual extractives. Consequently, dissolving pulps are more expensive compared to paper pulps. This fact and the high economic and environmental costs of the derivative and regenerated cellulose industry, makes it necessary to spend additional effort on the process optimization, exploring different ways to improve the reactivity and accessibility of this bio-resource.

A large part of the research on cellulose chemistry, physics and technology deals with the swelling and dissolution of native cellulose. Nevertheless, some important questions have still to be investigated and understood. One of them deals with the biosynthetic mechanisms of the plant cell, where cellulose is polymerized and cross linked among other polysaccharides and chemical components. Another topic open for research is the organization of the cellulose structures among natural substances and the origin of its variability.

A third one, of large scientific and technological importance (and industry interest) is the way cellulose structures dissolve and which physic-chemical properties play a role in this process and how. The presented research falls into this topic, more specifically in the dissolution of cellulose fibers, already started in Cemef by Cuissinat, 2006; Le Moigne, 2008 and Spinu, 2010.

Back in the mid-19th century, Nägeli [Nägeli 1864] started reporting the swelling and dissolution mechanisms of wood pulps, being followed by a wide group of scientists until the present days. Recently, Cuissinat et al., showed that depending on the solvent quality and on the cellulose accessibility, the fibers of native cellulose may present five modes of interaction mechanisms with the solvent [Cuissinat, 2006].

It can be also observed that in general, this swelling is not homogeneous, but occurs on different sections of the fiber leading to formations similar to balloons (swollen sections) separated by unswollen sections (Figure 1A).

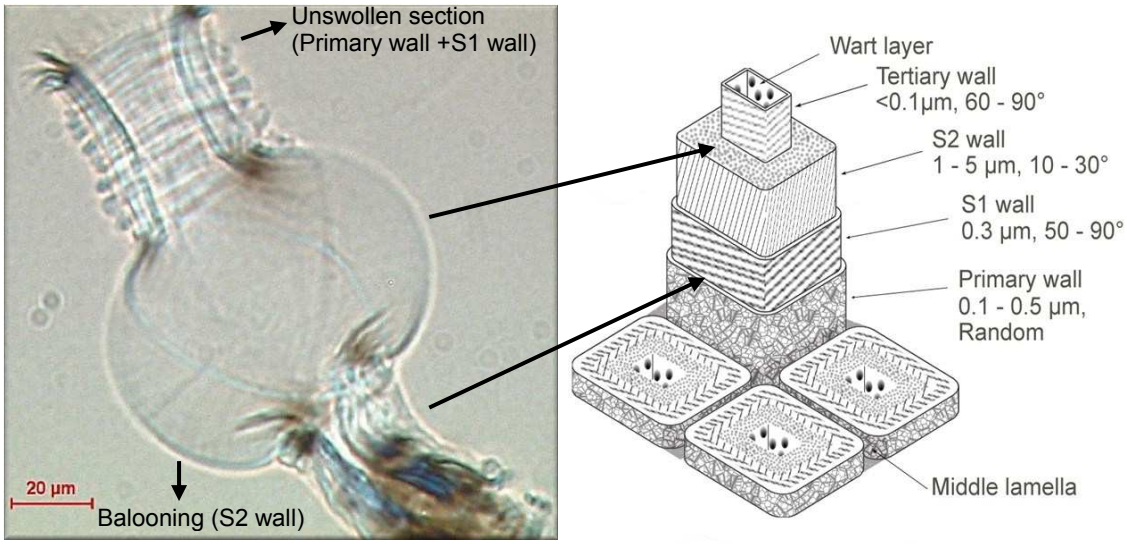

Figure 1A – Left: Partial view of a wood cell swollen in NMMO aqueous solution, with unswollen sections and swollen section (balloon) (Photography from the author, CEMEF, 2010). Right: Wood cell wall structure with the different layers and fibril orientation (Picture from the author and Oscar Silva Design, 2011).

Most of the authors that documented this ballooning phenomenon explained it as a swelling constriction of the S2 fiber wall, promoted by the primary wall and in some cases the S1 wall, which is the case in the picture. This happens mainly due to the fibrillar orientation of the fibers on the different layers [Ott, 1954; Le Moigne, 2008]. Cuissinat and Le Moigne showed that with enzymatic removal of the primary wall it was possible to avoid the formation of ballooning, improving this way the cellulose fibers accessibility and dissolution [Cuissinat, 2006; Le Moigne, 2008].

This explains what happens at the microscopic level, but brings other questions concerning what happens at the molecular dimensional level. For instance, why is the primary wall more difficult to dissolve?

This work intends to contribute to clarify this question, in order to further understand the fiber accessibility, dissolution mechanisms of the cellulose fibers and which factors can play a role in this process. Having in mind the chemical composition of the different cell wall layers, it is known that the primary wall is, in comparison with the inner cell wall layers, enriched in lignin, hemicelluloses and pectins.

This way one of the objectives of this work is related with fundamental research, and falls into the question about the role of minor components from the cell wall, particularly xylan and pectins on the cellulose dissolution.

The second objective, which surged with the results from the first, is more related with technical application and has the intention of studying the potential of use of nitren extraction followed by pectinase treatments on the conversion of paper grade pulps into dissolving pulps.

This project consists of an international cooperation. The work was performed in two major research groups working for years in the area of cellulose, Cemef - Mines ParisTech, Sophia Antipolis, France, with Dr Patrick Navard and TI / Hamburg University, Germany, with Dr Bodo Saake and Dr Jürgen Puls. This project was financed by an industry consortium: Sappi (South Africa), Tembec (Canada/France), Lenzing (Austria), Viskase (USA/France) and Spontex (USA/France), and had the support from EPNOE (European Polysaccharide Network of Excellence).

Outline of the thesis and main results

This manuscript is divided into five chapters.

The **first chapter** deals with a literature review about wood, wood fiber wall structure, and dissolving pulp production processes, giving special attention to the structure of cellulose as a polymer and to the cell wall structure. After this, is presented a brief description about cellulose fibers reactivity, ending the chapter with a comprehensive literature review on cellulose dissolution.

This chapter allows the reader to have an introduction to the topics discussed along the dissertation.

The **second chapter** consists of a description of all the samples (cellulose samples and solvents) and methods used in the different treatments (nitren extraction, freeze drying, enzymatic treatments and dissolution in NaOH) and analytical methods (nickel content, carbohydrate analysis, molecular weight distribution, cuen intrinsic viscosity, fiber dimensional analysis, X-Ray diffraction, optical microscopy and FEG-SEM analysis).

With this description, the reader can have an overview on the samples used, comparing their characteristics and manufacturing pretreatments history. In addition, the methodology description allows the reproduction of the work and its application in other samples for comparison with the results presented in this work.

Chapter III focuses on the effect of the selective removal of xylan from the cell wall on the fiber properties and on the cellulose dissolution in NaOH-water. Several commercial dissolving pulps were selected. In order to decrease the residual xylan, each pulp was treated with aqueous solutions containing 3, 5 and 7% nitren. In low concentrations, this solvent can be used to selectively extract xylans. The influence of these nitren treatments on the pulp dissolution was assessed by studying the dissolution yields in cold NaOH and the dissolution mechanisms. All fractions from the pulp treatments and the undissolved residues from the dissolution process were analyzed regarding their chemical and macromolecular structure.

The results of this chapter show that nitren treatment is removing a large part of the xylan present in a dissolving pulp but is also removing mannans and influencing the cellulose structure in two ways, (1) extracting it, with more intensity for higher nitren concentrations, and (2) decreasing its mean molecular mass, also more evident with nitren concentration increase. The nitren extractions are favorable for the dissolution in cold NaOH–water, being more effective with higher concentrations. This chemical modification of the fiber surface leads to the disassembly and partial removal of the primary wall. This allows an easier access of the NaOH reagent to regions not accessible on the initial fibers, which with the decrease of the cellulose average molar mass allows an easier dissolution and gives different dissolution mechanisms.

Chapter IV is devoted to describing the effect of pectinase as a pretreatment of several dissolving pulps and one paper grade pulp prior to dissolution in NaOH, being divided in 3 phases:

- A first initial study using a commercial pectinase enzyme (CCM), which showed that there was a clear, positive effect on dissolution. The fact that the enzyme was not a pure pectinase prompted the second phase of the study where pure endopectinase and endoglucanase were used.
- In this second phase, a pure endopectinase (L40) and a pure endoglucanase (EG) were used in only one pulp sample and compared to CCM, with the aim of finding whether the CCM effect was due to its endoglucanase content. The result showed that the endopectinase is improving the dissolution in cold soda without decreasing too much the average molar mass of pulps.
- The third phase was devoted to apply the optimized treatment for L40 and CCM to five very different dissolving pulps and one paper grade pulp and to assess their effect on dissolution in cold soda.

As results, despite the amount or presence of pectin on the starting pulps was too low to be determined, the incubation of different pulps with pectinase preparations lead to an increase of the accessibility of the fibers, reaching dissolution yields increase higher than 150%. The enzymatic treatments were not affecting all the studied pulps on the same manner, showing that the pretreatments history of the dissolving pulps have an influence on the enzymatic efficiency. With the enzymatic activation of the cellulose fibers, a slight decrease of the average molar mass was verified. Using a mixture of endopectinase and endoglucanase showed that the synergistic effect of these two enzymes is more effective on cellulose activation. This study proposed that this enzyme is promoting the accessibility of the fibers in two ways: (1) decomposes the polysaccharide matrix from the primary wall (where pectin is present), promoting the swelling of this wall, which will allow a higher dissolution capacity of the fibers; (2) changes the hydrogen bonding network from the whole cellulose fiber, slightly decreasing the average molar mass and promoting the diffusion of the NaOH solvating ions into the cellulosic structure, facilitating this way the dissolution.

The **chapter V** consisted on applying the combination of both nitren extraction and pectinase treatment in paper grade pulps. The nitren extraction can selectively remove the hemicellulose, increasing the cellulose content on the pulp, while the pectinase treatment is activating the pulp, increasing its solubility in cold caustic soda. It is also presented a discussion on the mass balances for pulp material and total glucose content based on pulp and based on wood.

This work package showed that paper grade pulps extracted with nitren have a lower accessibility to NaOH ions, meaning lower dissolution capacity in cold caustic soda. This might be explained by the partial hornification due to hemicellulose removal. The further treatment with pectinase was able to improve the dissolution yield in NaOH from 10% to 60%. This might be explained by the same mechanisms described in for the chapter IV. In addition, this study showed that, for the dissolving system used, the use of paper grade pulps treated with the combination of these treatments has the potential of increasing the overall cellulose (total glucose) yields based on wood, in comparison with current dissolving pulps.

Finally, a **general conclusion** is presented, summarizing the main results of this work and proposing some **perspectives** for further research on the topic.

The interesting results achieved and the techniques used and further developed with this project allowed to define new research directions, of both fundamental character (e.g. study on the mechanisms behind the pectinase effect on the pulp and dissolution behavior of the different wood cell types) and technological applications (e.g. use of pectinase in the activation of paper grade pulps after hemicellulose extractions, allowing the conversion to dissolving grade pulps).

Lusophone version

Enquadramento e objectivos da tese

Num contexto de escassez de petróleo e aumento da pressão social e ambiental para um desenvolvimento sustentável, a produção de materiais biodegradáveis a partir de fontes renováveis é visto como um dos caminhos a seguir para permitir mantermos um nível de vida razoável num futuro próximo.

A celulose, sendo o principal componente da parede celular das plantas, poderá ter um papel fundamental nesta conjuntura. Esta está presente em todas a plantas, algumas algas e também é produzida por algumas bactérias. Para além de ser o bio-polímero mais abundante na natureza, a celulose não representa uma fonte directa de alimento para o Homem e é biodegradável, sendo facilmente decomposta por fungos e bactérias. Este polissacarídeo natural é também uma matéria-prima renovável, regenerando-se através de um ciclo natural em que se colecta dióxido de carbono da atmosfera e utiliza como fonte energética a luz solar, a qual é limpa, gratuita e ilimitada. Isto significa que este bio-polímero pode ser gerido de forma sustentável [Gavillon, 2007; Pinkert, 2010].

De facto, a celulose sempre esteve presente na cultura humana. Há alguns milhares de anos atrás, a humanidade já fazia uso das excelentes propriedades da celulose como material compósito, neste caso bio-compósito, a madeira. As propriedades mecânicas deste material permitiram a sua utilização como material de construção, armas e mais tarde na construção naval. Quando o potencial do poder calorífico da madeira foi descoberto, o uso da energia da sua combustão, o fogo, passou a ser utilizado de forma habitual. Também desde há muito tempo, as fibras celulósicas do algodão e outras fibras vegetais foram utilizadas na produção de vestuário e como material de reforço no fabrico de blocos de argila (adobe) usados na construção. Mais tarde, os Egípcios começaram a usar fibras celulósicas de papiro para produzir folhas de papiro, que foi o percursor do que é hoje um dos materiais compósitos mais fascinantes, o papel.

Por meados do século XIX verificou-se um aumento no interesse pelo uso da celulose como matéria-prima para a indústria química, acompanhando o crescimento do conhecimento científico e tecnológico neste tema. Por volta de 1950 foi produzido o primeiro polímero termoplástico a partir de nitrocelulose. Este feito concretizou-se em Birmingham e deve-se a Alexander Parkes, que patenteou este novo material como “Parkesine” em 1862. Mais tarde o processo foi industrializado pela companhia Hyatt Manufacturing Company em Nova Iorque, adicionando ao processo cânfora como plastificante e registando o material em 1870 como “Celluloid”. Este material era extremamente inflamável e facilmente degradado. Hoje, com a incorporação de aditivos para aumentar a estabilidade e reduzir a inflamabilidade, este material está ainda limitado à produção de alguns materiais, como por exemplo bolas de ténis de mesa. Esta processo e aplicações inovadoras iniciais mostraram que se poderiam produzir novos

materiais a partir de celulose modificada quimicamente. Este conhecimento despertou o interesse que levou ao desenvolvimento tecnológico para utilizar fibras sintéticas de celulose na produção têxtil e outras aplicações tecnológicas. Como exemplo, poderão enumerar-se materiais celulósicos sintetizados através de vários processos como Hidróxido de cupramónio, Viscose ou, mais recentemente o processo Lyocell® [Klemm, 2005].

Hoje em dia madeira continua a ser utilizada sob a forma natural de bio-compósito, principalmente na construção. No entanto, uma grande parte é utilizada sob a forma de pasta de celulose como matéria-prima na indústria da celulose (pasta para papel/cartão e pasta solúvel). Apesar do grande interesse no potencial da utilização de celulose como matéria-prima da indústria química, apenas cerca de 2% da pasta celulósica produzida é utilizada para este fim como pasta solúvel.

A principal desvantagem e desafio é o facto de a celulose ter na sua estrutura uma rede de fortes ligações intermoleculares (maioritariamente ligações de hidrogénio), as quais conferem uma grande coesão na estrutura supra molecular. Isto faz com que a celulose não possa ser processada no estado de fusão, assim como não se dissolve em água nem em solventes orgânicos convencionais [Navard, 2013].

Assim, para poder ser usada na produção de outros materiais que não o papel ou placas de fibras, a celulose tem que ser derivatizada (como é o caso referido acima para o “Celluloid”) ou então dissolvida utilizando processos tóxicos ou muito dispendiosos. Para poder ser usada nestes processos, a celulose (na forma de pasta) deve atender a requisitos específicos, os quais são encontrados na indústria nas pastas solúveis. Neste tipo de pasta química, os grupos reactivos apresentam uma acessibilidade mais alta, permitindo por exemplo uma substituição mais homogénea dos grupos hidroxilo durante uma derivatização.

A produção de pastas solúveis envolve requisitos especiais em termos de pureza, tais como baixo teor de lenhina residual e presença residual de hemiceluloses e extractivos. Consequentemente, as pastas solúveis têm um custo de produção mais alto e um rendimento mais baixo quando comparadas com pastas para papel. Este facto, aliado aos elevados custos económicos e ambientais verificados na indústria da derivatização e regeneração de celulose, conduzem à necessidade de despender esforços adicionais em optimização dos processos, explorando diferentes formas de aumentar a reactividade e acessibilidade.

Uma grande parte da pesquisa em química, física e tecnologia da celulose está ligada aos mecanismos de dilatação ou inchamento e dissolução de fibras de celulose naturais. No entanto, algumas questões importantes permanecem ainda por investigar e entender. Uma delas está relacionada com os mecanismos da biossíntese das células das plantas, mais especificamente a parede celular, onde a celulose é polimerizada e interligada com outros componentes químicos. Outro tema aberto para pesquisa prende-se com o estudo da organização das variadas estruturas celulósicas nas varias fontes naturais e as origens dessa variabilidade.

Uma terceira área de pesquisa com largo interesse científico e tecnológico (e portanto industrial) está relacionado com os mecanismos de dissolução das fibras celulósicas e quais as propriedades físico-químicas que influenciam este processo, e de que forma. A pesquisa apresentada nesta dissertação enquadra-se neste tema, mais especificamente na dissolução de fibras de celulose naturais, sendo uma continuação do trabalho já elaborado no CEMEF (Centre de mise em forme des materiáux) por Cuissinat, 2006; Le Moigne, 2008 e Spinu, 2010.

Em meados do século IXX, Nägeli [Nägeli 1864] começou a descrever a dilatação e mecanismos de dissolução de fibras de celulose, sendo seguido por inúmeros cientistas até aos dias de hoje. Recentemente, Cuissinat et al. mostraram que dependendo da qualidade do solvente utilizado e da acessibilidade da rede celulósica, as fibras de celulose nativas podem apresentar cinco modos de mecanismos de interacção com o solvente [Cuissinat, 2006].

Também pode ser observado que geralmente a dilatação das fibras não é homogéneo, ocorrendo em diferentes secções da fibra, conduzindo assim à formação de estruturas semelhantes a balões cheios (secções dilatadas ou inchadas), intercaladas com secções não dilatadas (Figura 1P).

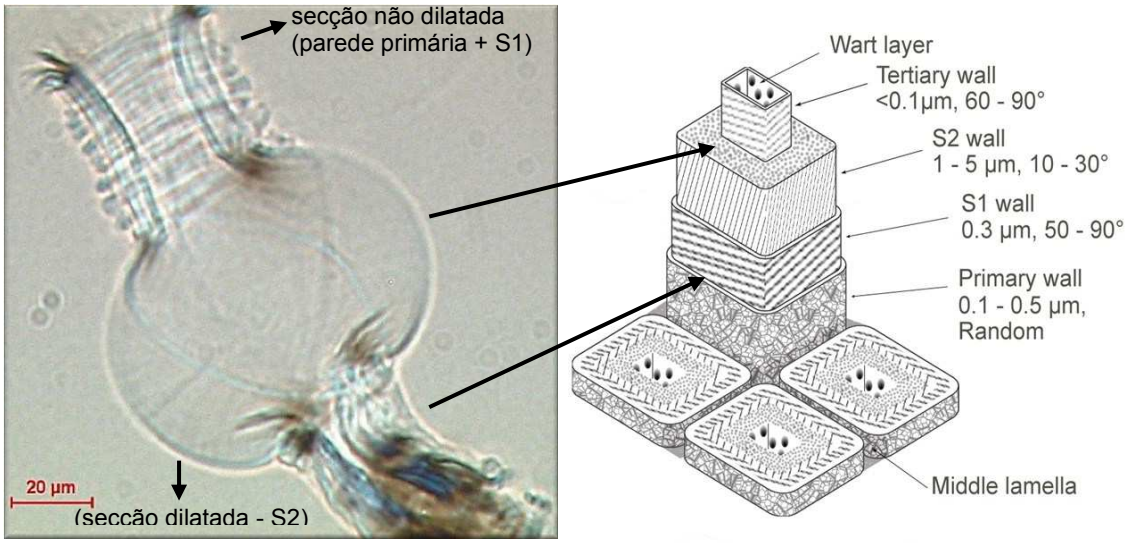

Figure 1P – Esquerda: Vista parcial de uma célula de madeira dilatada numa solução aquosa de NMMO, apresentando secções não dilatadas e uma secção dilatada (balão) (fotografia do autor, CEMEF, 2010). Direita: Estrutura da parede celular de células da madeira, evidenciando as diferentes camadas (paredes) com as respectivas espessura e orientação fibrilar (Esquema desenhado pelo autor e Oscar Silva Design, 2011).

A maioria dos autores que documentaram este fenómeno de dilatação heterogénea (balonamento), explicaram-no como uma dilatação da parede secundária S2 bloqueada pela parede primária e por vezes também pela parede secundária S1, como é o caso da imagem acima. Isto acontece principalmente devido à orientação fibrilar verificada nas diferentes camadas (paredes) da parede celular das fibras [Ott, 1954; Le Moigne, 2008]. Cuissinat e Le Moigne demonstraram que através da remoção da parede primaria com tratamentos enzimáticos

é possível evitar o fenómeno de formação de "balões" (*"balooning"*) descrito, aumentando assim a acessibilidade e performance de dissolução [Cuissinat, 2006; Le Moigne, 2008].

Isto explica o que ocorre ao nível microscópico, mas levanta outras questões acerca do que se passa ao nível molecular. Por exemplo, porque é que a parede primaria é mais difícil de dissolver?

Este trabalho pretende contribuir para a clarificação desta questão, de forma a perceber melhor a acessibilidade e os mecanismos de dissolução das fibras celulósicas, e quais os factores que podem influenciar este processo. Tendo em conta a composição química das diferentes camadas (paredes) da parede celular, verifica-se que a parede primaria é, em comparação com as restantes, enriquecida em lenhina, hemiceluloses e pectinas.

Desta forma um dos objectivos deste trabalho está relacionado com pesquisa fundamental, focando-se na questão acerca do papel que os componentes residuais da parede celular, particularmente xilanas e pectinas, têm na dissolução das fibras de celulose.

O segundo objectivo, que surgiu a partir dos resultados do anterior, está mais relacionado com aplicações tecnólogicas, tendo por finalidade estudar o potencial da utilização de uma combinação de tratamentos (extracção com nitreno seguida de incubação com pectinases) na conversão de pastas de papel em pastas solúveis.

Este projecto consiste numa cooperação internacional. O trabalho laboratorial foi efectuado em dois centros de investigação com larga experiência na área da celulose, Cemef - Mines ParisTech, Sophia Antipolis, França, com o Doutor Patrick Navard e Thünen Institut / Universität Hamburg, Alemanha, com o Doutor Bodo Saake e o Doutor Jürgen Puls. O projecto foi financiado por um consorcio de empresas multinacionais: Sappi (África do Sul), Tembec (Canada / França), Lenzing (Áustria), Viskase (EUA / França) e Spontex (EUA / França), tendo também o apoio da organização europeia EPNOE (European Polysaccharide Network of Excellence).

Plano geral da tese e principais resultados

Esta dissertação está dividida em cinco capítulos.

O **primeiro capítulo** consiste numa revisão bibliográfica acerca da madeira, parede celular das células da madeira e processos de produção de pasta solúvel, dando especial atenção à estrutura da celulose como polímero e à estrutura química e física da parede celular. Seguidamente, é apresentada uma breve descrição acerca reactividade e acessibilidade de fibras celulósicas, terminando com uma revisão bibliográfica compreensiva sobre dissolução de celulose.

Este capitulo permite ao leitor ter uma introdução aos temas abordados ao longo da dissertação.

No **segundo capítulo** é feita uma descrição de todas as amostras (amostras de celulose e solventes), dos métodos utilizados nos diferentes tratamentos (extracção com nitreno, liofilização, incubações enzimáticas e dissolução em hidróxido de sódio) e dos métodos analíticos (teor de níquel, análise de açucares, distribuição de peso molecular, viscosidade intrínseca, análise dimensional das fibras, difracção de raios-X e microscopia óptica e electrónica de varrimento (FEG-SEM).

Com esta descrição, o leitor tem uma vista geral das amostras utilizadas, com uma comparação das suas propriedades e historial de processamento ou pré-tratamentos. Para além disso, a descrição das metodologias, permite uma reprodução do trabalho e aplicação a outras amostras para uma comparação com os resultados aqui apresentados.

O **capítulo III** está focado no efeito que a remoção selectiva das xilanas da parede celular tem nas propriedades das fibras celulósicas e na capacidade de dissolução destas fibras em soluções aquosas de hidróxido de sódio. Algumas pastas solúveis disponíveis no mercado foram seleccionadas. De forma a reduzir o teor de xilanas, cada uma das pastas foi tratada com uma soluções aquosas de nitreno, com concentrações de 3, 5 e 7%. Quando usado em baixas concentrações, este solvente pode ser usado para remover xilanas. A influencia deste tratamento na dissolução de fibras celulósicas (pasta solúvel) foi estudada através da avaliação do rendimento de dissolução em soluções aquosas de hidróxido de sódio a baixas temperaturas (-6 ºC) e dos mecanismos de dissolução. Todas as fracções obtidas nos tratamentos e os resíduos sólidos da dissolução foram analisados em termos de estrutura química e molecular.

Os resultados deste capitulo mostram que as extracções com nitreno removem uma grande parte das xilanas presentes nas pastas solúveis. Para além disso verificou-se que também removem mananas e influenciam a estrutura da celulose de duas formas: (1) extraindo-a, com mais intensidade para concentrações de nitreno mais altas, e (2) diminuindo a sua massa molar média, sendo esta diminuição mais acentuada também para concentrações mais elevadas. As extracções com nitreno são favoráveis à dissolução em NaOH-água a frio, sendo mais eficiente utilizando concentrações mais altas. Esta alteração da superfície das fibras provoca uma desestruturação e remoção parcial da parede primária. Isto permite um acesso mais fácil dos iões de NaoH a regiões não acessíveis nas fibras iniciais, o que, associado à diminuição da massa molar média, permite uma dissolução mais fácil e induz mecanismos de dissolução diferentes.

O **capítulo IV** é dedicado à descrição dos efeitos de incubações enzimáticas com pectinase em várias pastas solúveis e uma pasta de papel como pré-tratamentos para dissolução em soda cáustica, estando dividido em três fases:

- Um estudo inicial, utilizando uma pectinase comercial (CCM), que mostrou claramente haver um efeito positivo na capacidade de dissolução. O facto de a enzima não ser uma pectinase pura levou à formulação da segunda fase do estudo em que foram utilizadas uma endopectinase e uma endoglucanase puras.

- Na segunda fase, uma endopectinase pura (L40) e uma endoglucanase pura (EG) foram utilizadas no tratamento de apenas uma pasta solúvel. Os resultados foram comparados com aqueles obtidos anteriormente com CCM, com a finalidade de verificar se o efeito verificado com a enzima CCM se deve à endoglucanase presente na sua formulação. Os resultados mostraram que o tratamento com endopectinase favorece a dissolução em hidróxido de śodio, sem diminuir significativamente a massa molar média da pasta solúvel estudada.
- A terceira fase consistiu na aplicação das condições optimizadas para L40 e CCM em cinco pastas solúveis diferentes e uma pasta de papel, avaliando também os efeitos destes tratamentos na dissolução das pastas em NaOH aquoso.

Como resultado, apesar da quantidade de pectinas presente nas pastas iniciais ser muito baixa para uma determinação eficiente, a incubação das diferentes pastas com pectinase levou a uma melhoria da acessibilidade nas fibras, verificando-se aumentos nos rendimentos de dissolução em NaOH superiores a 150%. Os tratamentos enzimáticos não afectaram todas a pastas da mesma forma, demonstrando que o historial dos pré-tratamentos das pastas tem influência tanto na eficiência do tratamento enzimático como no processo de dissolução. Com a activação enzimática das fibras celulósicas, verificou-se um ligeiro decréscimo na massa molar média. O uso de uma mistura de endopectinase e endoglucanase mostrou que o efeito sinergístico destas duas enzimas é mais eficiente na activação de celulose. Este estudo propôs que esta enzima (endopectinase) promove a acessibilidade nas fibras de duas fromas: (1) desestrutura a rede de polissacarídeos na parede primária (onde a pectina está presente), promovendo assim a dilatação ou inchamento desta parede, o que vai permitir uma maior capacidade de dissolução das fibras; (2) altera a rede de ligações de hidrogénio da estrutura da fibra celulósica, diminuindo ligeiramente a massa molar média e facilitando a difusão dos iões de NaOH para o interior da estrutura celulósica, facilitando assim a dissolução.

O **capítulo V** consistiu na aplicação de ambos os tratamentos com nitreno e pectinase em pastas para papel. A extracção com nitreno consegue remover selectivamente as hemiceluloses, aumentando o teor de celulose na pasta, enquanto que a incubação com pectinase vai activar a pasta celulósica, aumentando a sua solubilidade em hidróxido de sódio. É também apresentada uma discussão sobre os balanços de massa em termos de quantidade de pasta e quantidade de glucose total, baseados na quantidade de pasta inicial e na quantidade de madeira inicial.

Este trabalho mostrou que pastas de papel após extracção com nitreno têm uma menor acessibilidade para os iões de NaOH, traduzindo-se numa menor capacidade de dissolução em soda cáustica. Isto pode ser explicado por uma hornificação ou colapsamento parcial da estrutura da fibra devido à extracção das hemiceluloses. O tratamento conseguinte com pectinase permitiu um aumento no rendimento de dissolução em NaOH de 10% (depois do tratamento com nitreno) para 60%. Isto poderá ser explicado pelos mesmos mecanismos descritos para os resultados obtidos no capítulo IV. Para além disto, este estudo mostrou que, para o sistema de dissolução utilizado, o uso de pastas de papel tratadas com a combinação dos dois tratamento referidos tem

o potencial de conseguir aumentar o rendimento global de celulose (glucose total), baseado na madeira, em comparação com o uso das convencionais pastas solúveis.

Finalmente, uma **conclusão geral** é apresentada, resumindo os resultados principais deste trabalho e propondo também algumas **perspectivas** para futura pesquisa neste tópico.

Os resultados interessantes obtidos e as técnicas utilizadas e desenvolvidas neste projecto permitiram a definição de novas linhas de investigação, de ambos os carácteres fundamental (como por exemplo o estudo sobre os mecanismos que originam os efeitos da pectinase nas pastas celulósicas e os mecanismos de dissolução nos diferentes tipo de células da madeira) e carácter tecnológico (por exemplo uso de pectinase na activação de pasta de papel, depois de extraídas as hemiceluloses, permitindo assim a conversão para pasta solúvel).

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chapter I

State of the art review on wood cell wall structure, cellulose reactivity and dissolution

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I.1. – Introduction

Although several sources of cellulose are available, the predominant source of this raw material in industry is wood. This is due to the large amount available, and the fact that wood can be obtained all year round and that it can be easily stored in order to ensure a continuous production on the mills. The different morphological characteristics and chemical composition of the wood have as a consequence the need for different conditions in the pulping processes. In addition, the final products will show different properties according to the characteristics of the original raw material. Due to this fact, one of the main parameters to keep in mind while processing wood, pulp or cellulose is the original wood that is used [Sjöström, 1993; Ferreira, 2000]. The same is applied for the regenerated and derivative cellulose industry, once that the reactivity and solubility are varying significantly with the cellulose origin and fiber properties.

This way, this chapter will start with a concise review on the wood, fiber structure, and dissolving pulp production processes, giving special attention to the structure of cellulose as a polymer and to the cell wall structure. After this, is presented a brief description about cellulose fibers reactivity, ending the chapter with a comprehensive literature review on cellulose dissolution.

I.2. - Wood cell wall structure

In order to have a systematic and coherent understanding on the chemical nature, structure and morphology of the cellulose fibers, one must analyze it at different dimension levels, which are described in the following and illustrated in Figure I.1 [Ferreira, 1993; Cuissinat, 2006; Le Moigne, 2008]:

- Molecular level (Å), relating to the chemical composition, conformation, functional groups, molecular mass, intra- and intermolecular interactions, degree of polymerization, etc.
- Supramolecular level (nm), focusing on the aggregation of cellulose macromolecules to form fibrils, fibrillar angle orientation related to the fiber axis, degree of order within and around the fiber, etc.
- Ultrastructure level (nm to µm), regarding the spatial disposition of the aggregated fibrils in the fiber wall, the dimension of the fiber walls, presence of pits, etc.
- Macrostructure level (µm to m), describing the morphology of the fibers, tissues, stem and tree.

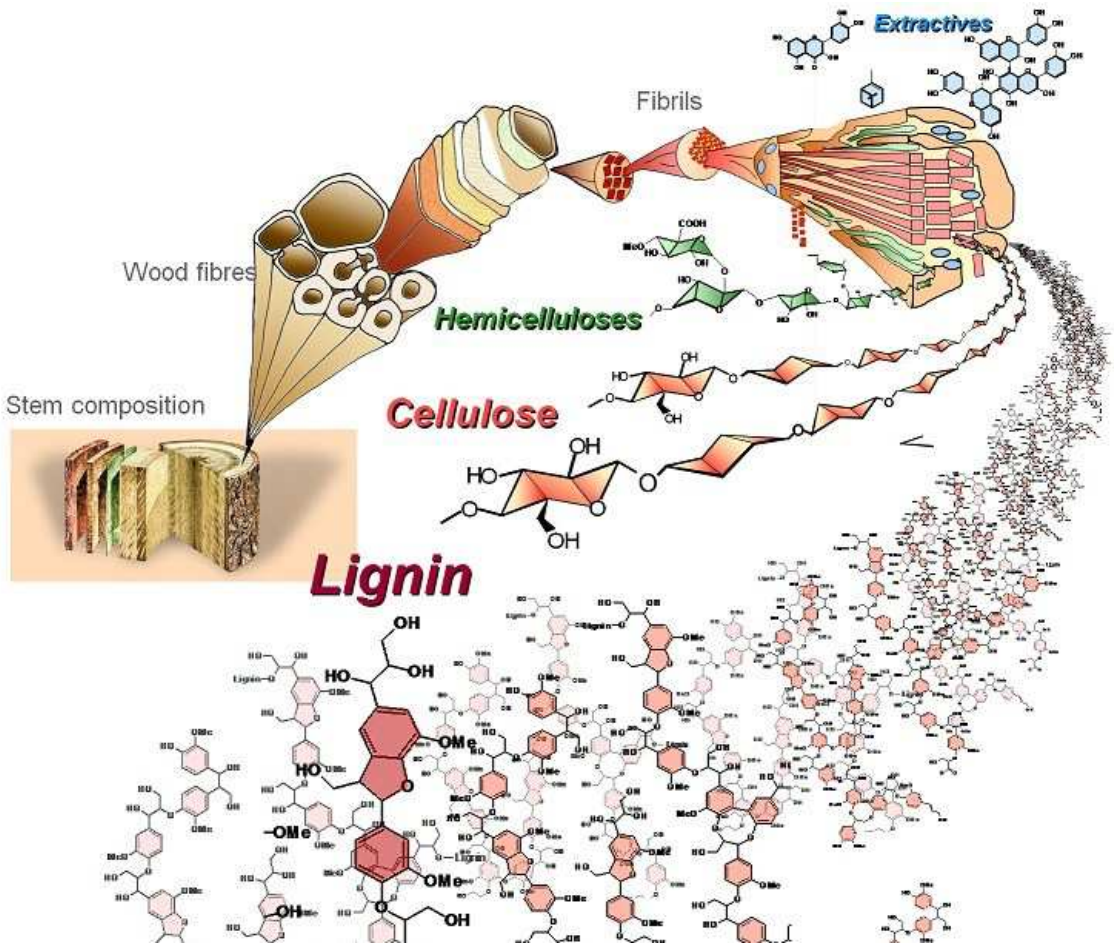

Figure I.1: Analysis of wood (taken from Lehnen, TI Hamburg).

I.2.1. - Molecular structure (Chemical composition)

Wood is made of a highly dynamic composite material composed mainly by structural components. Its architecture and molecular composition changes dramatically during growth and cellular differentiation [Laine, 2005; Johansen, 2006].

The ultimate (elemental) composition of wood is approximately 50% of carbon, 43% of oxygen, 6% of hydrogen and less than 1% of nitrogen, with some variation between species, especially due to different lignin contents. The major cell wall constituents are cellulose, hemicelluloses, and lignin. Other polymeric constituents, present in lower and often varying quantities, are starch, pectin and polyphenols. In addition, inorganic substances are present in different amounts [Timell, 1967; Smook, 1992; Ferreira, 2000; Xavier, 2005].

The next table presents a general distribution of the major compounds (in average) in cotton, hardwood and softwood.

Table I.1: Average chemical composition (dry wood %, w/w) of softwood and hardwood [Smook, 1992; Koch, 2006].

	Hardwood	**Softwood**
Cellulose	45±2	42±2
Hemicelluloses	30±5	27±2
Lignin	20±4	28±3
Other compounds	5±3	3±2

I.2.1.1. - Lignin

Lignin is the second most abundant component in the wood cell walls. Although most of the lignin is found in the secondary wall, where it imparts rigidity to the cell wall, this component is highly concentrated in the middle lamella compound, where its physical role is to reinforce the wood structure, by taking part in the matrix which binds the cellulose fibers. Due to its hydrophobic properties, lignin limits the water permeability across the cell walls, playing an important role in the internal transport of water, nutrients, and metabolites. In addition, it contributes to the protection of lignified tissues against microorganisms attack by impeding penetration of destructive enzymes into the cell wall [Hon, 2001; Lin, 2002; Xavier, 2005; Gandini, 2008].

Lignin is an amorphous three-dimensional heteropolymer composed of hydroxylated phenyl-propane units. These units are named p-coumaryl, p-coniferyl and p-sinapyl alcohols (Figure I.2), corresponding in the lignin structure to the units p-hydroxyphenylpropane (H), guaiacylpropane (G) and syringylpropane (S) [Gandini, 2008; Hon, 2001; Xavier, 2005].

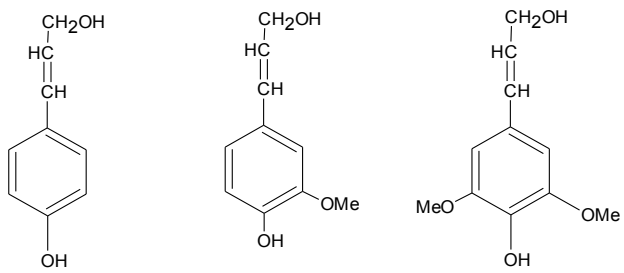

Figure I.2: Lignin structural units, from left to right (p-coumaryl, p-coniferyl and p-sinapyl alcohols) [Xavier, 2005].

These molecular units are linked together mainly with carbon-oxygen (ether) bonds but also with carbon-carbon bonds. The exact composition of lignin depends widely on the wood species, but normally, softwood contains mainly coniferyl units whereas hardwood is composed of coniferyl and sinapyl building blocks [Vander Wielen, 2004; Viikari, 2009].

Lignin is the most complex natural polymer with regard to structure and heterogeneity. Because of this, no definite lignin structure is possible, although numerous models, representing an "average" structure, have been proposed [Lin, 2002]. Proposed structural models of softwood and beech (hardwood) lignin are shown in Figures I.3 and Figure I.4, respectively.

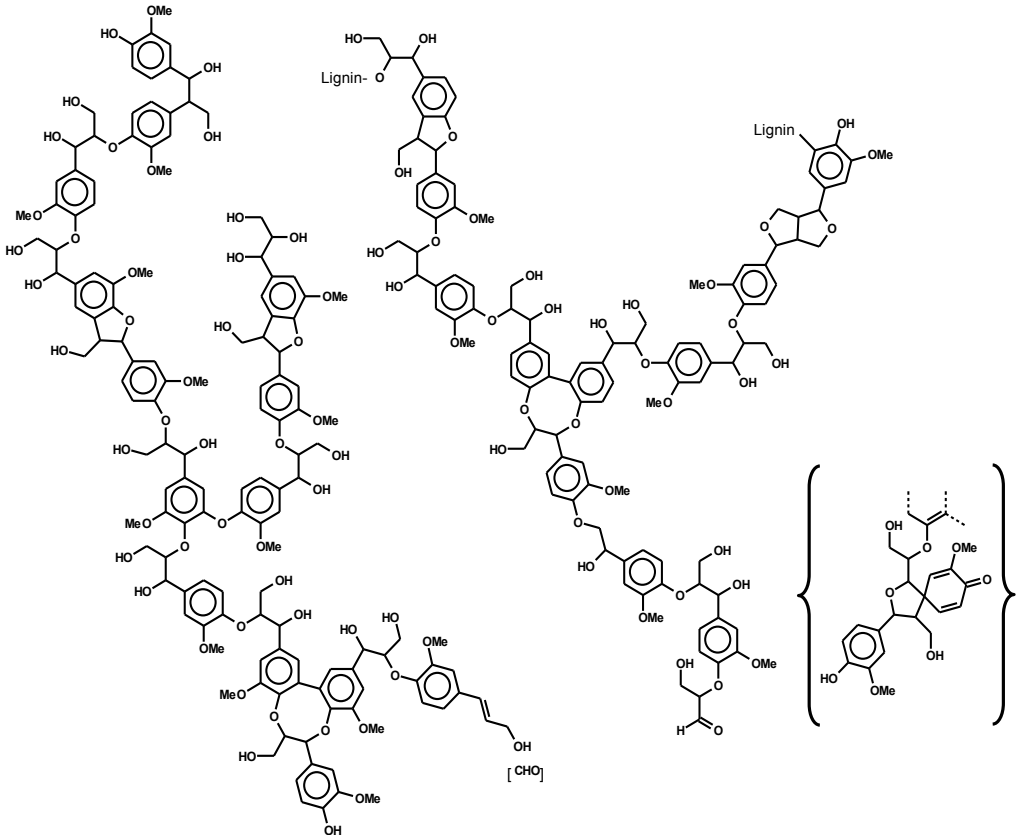

Figure I.3: Partial structure of softwood lignin [Brunow, 1998].

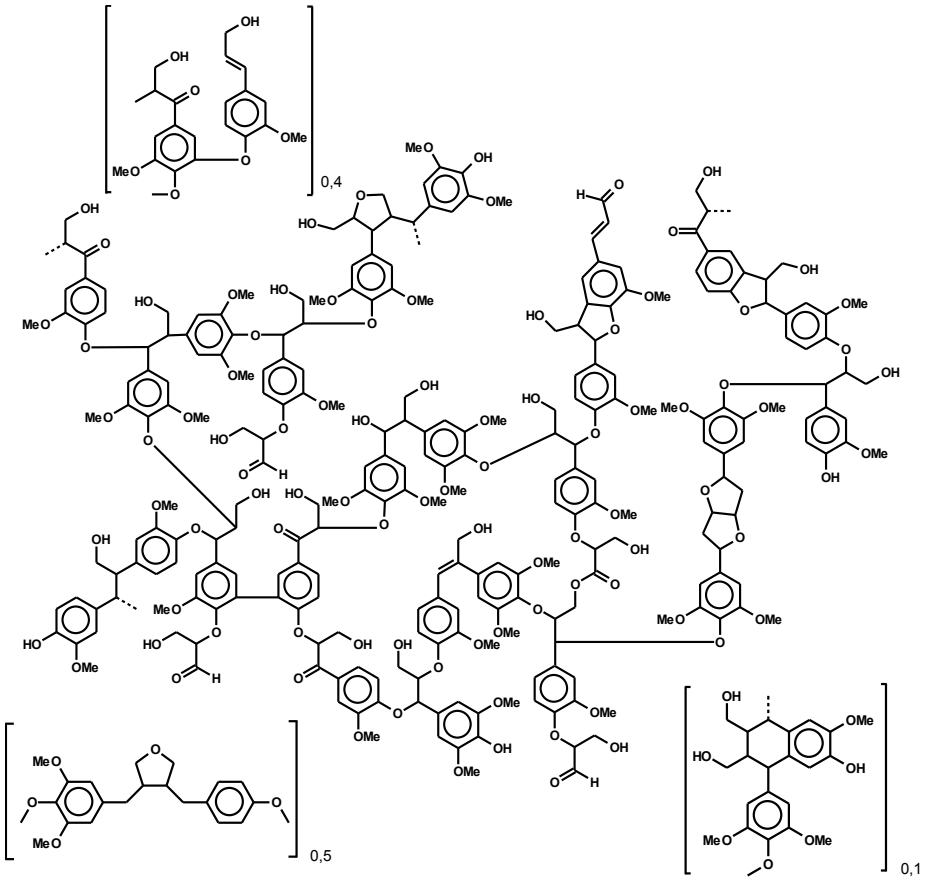

Figure I.4: Proposed structure of hardwood (Beech) lignin [Nimz, 1974].

I.2.1.2. - Extractives

This fraction of wood is composed of a wide number of different low molecular mass compounds, which, due to being hydrophobic or hydrophilic, can be extracted by means of polar and non-polar solvents [Fengel, 1984; Sjöström, 1993]. As for the other wood components, the amount and distribution of extractives varies by species and as well as by the location within an individual tree. Usually, the amount of extractives accounts only for a low percent of the wood, but it can be significantly higher in parts like branches and bark and can also increase in wounded wood [Vander Wielen, 2004; Jansson, 2009].

One fraction of the extractives is termed resin, in which the following compounds are included: terpenes, lignans, stilbenes, flavonoids and other aromatics. In addition to these substances other organic compounds are present in the extractives: fats, waxes, fatty acids and alcohols, steroids, higher hydrocarbons [Fengel, 1984].

The extractives can be regarded as nonstructural wood components and present various functions. For example, terpenoids, resin acids, and phenolic substances protect the wood against insect and microbiological attack, while fats provide energy for the wood cell metabolism [Sjöström, 1993; Vander Wielen, 2004].

I.2.1.3. - Inorganic components

Inorganic components are often determined as ash content. The inorganic components are minor compounds in wood, and their qualitative and quantitative composition depends much on the species, mineral composition of soils and environmental conditions. The amount of inorganic material is considerably low, between 0.1 and 1% (except for some tropical species, where the ash content can reach 5%), but some of them are crucial for the plant development [Fengel, 1984]. These compounds are mainly sulphates, phosphates, silicates, oxalates, and calcium, potassium and magnesium carbonates. Iron and manganese occur in small amounts and among other transition metals, copper and cobalt are present only as traces [Sjöström, 1999; Nimz, 2002].

I.2.1.4. - Hemicelluloses

Hemicelluloses, also known as polyoses, are a heterogeneous group of branched polysaccharides constituted of several monosaccharide units. They are one of the main components of the wood, accounting for 20 to 35% of total dry mass [Fengel, 1984; Teleman, 2009]. These compounds show an amorphous structure and have a degree of polymerization between 100 and 200, which makes them susceptible to chemical attack [Vander Wielen, 2004; Viikari, 2009]. Compared to cellulose, the chemical and thermal stability of hemicelluloses is generally lower [Teleman, 2009].

The hemicelluloses main building units are hexoses (D-glucose, D-mannose and D-galactose) and pentoses (D-xylose and L-arabinose), although small amounts of deoxy-hexoses (L-rhamnose and L-fucose) and certain uronic acids (4-*O*-methyl-D-glucuronic acid, D-galacturonic acid and D-glucuronic acid) are also found. In Figure I.5 and Figure I.6, partial structures of xylans and glucomannans respectively are drawn, being these the two main hemicelluloses in wood [Teleman, 2009; Viikari, 2009].

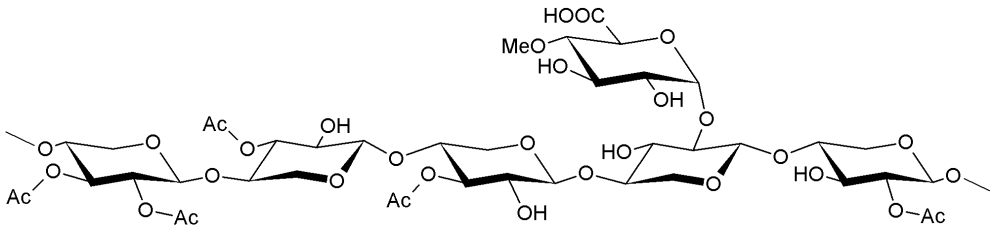

Figure I.5: Partial structure of hardwood 4-*O*-methyl-D-glucurono-D-Xylan [taken from TI, Hamburg]

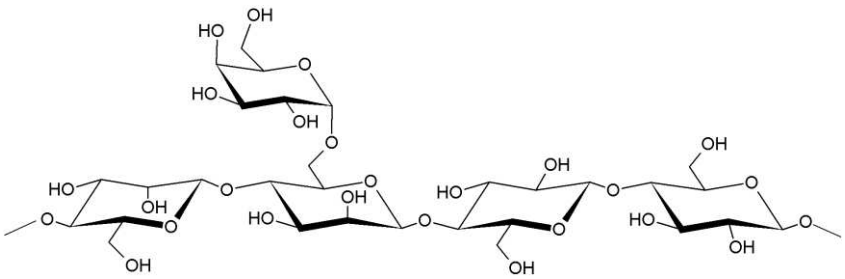

Figure I.6: Partial structure of softwood D-galacto-D-gluco-D-mannan [taken from TI, Hamburg]

The type, amount and distribution of hemicelluloses in wood fibers vary broadly, depending on plant species, type of tissues, growth stage and growth conditions. In general hardwoods contain higher amounts of hemicelluloses than softwoods. In hardwoods the main hemicelluloses are xylans and glucomannans, while in softwoods galactoglucomann, glucomannan and arabinoglucuronoxylan predominate [Fengel, 1984; Rowel, 2005; Teleman, 2009; Viikari, 2009]. In softwoods, the outer layer of secondary wall (S2) and the warty layer (W) are usually richer in xylan than the middle of the secondary wall; while the glucomannan content increases steadily from the outer parts to the inner parts of cell wall. In hardwoods, the outermost layers of secondary fiber wall are rich in xylan, whereas the content of glucomannan remains low and constant through the fiber walls [Viikari, 2009].

The hemicellulose units are bound strongly in the cell wall matrix between cellulose fibrils and other fiber wall components. Their function, although not fully understood, is believed to be the strengthening of rigidity by acting as an interface between the cellulose and lignin; and is supposed to take part in regulation of wall elongation, modification and porosity [Teleman, 2009; Viikari, 2009].

I.2.1.5. - Pectin

Pectin (also termed pectic substances), described by the first time in the eighteenth century [Vauquelin, 1790], consist of an acidic heterogeneous carbohydrate polymer and is one of the major plant cell wall components. After cellulose and hemicelluloses, pectin is the third group of polysaccharides, and is characterized by relatively high extractability using acid or chelators and a high content of galacturonic acid [Mohnen, 1999; Coenen, 2007; Harholt, 2010; Agoda-Tandjawa, 2012].

The structures of discrete pectic elements and complex pectins formed from them are not fully known, what makes pectin probably the most complex macromolecule in nature. It can be composed of as many as 17 different monosaccharides containing more than 20 different linkages [Vincken, 2003; Coenen, 2007, Yapo, 2011]. Nevertheless, it is widely accepted that it can be classified in three to four main structural classes, namely, homogalacturonan (HG), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II) and/or xylogalacturonan (XGA), which are covalently inter-linked to form different pectin-complexes [Caffall, 2009; Yapo, 2011; Harholt, 2010; Gu, 2013]. Pectic substances can also be classified as protopectin, pectic acid, pectinic acid and pectin depending on the type of modifications that occur on the backbone chain [Kashyap, 2001].

Homogalacturonans, are linear 1,4-linked α-D-galactopyranosyluronic acid chains, in which some of the residues can be methyl esterified and/or partially O-acetylated. This structural class is considered the “smooth region” of pectin, in contrast with the other three classes, considered the “hairy regions”. The galacturonic acid backbone of xylogalacturonan (XGA) contains branches of xylose residues of different lengths depending on the plant species and tissues. RGII presents also a galacturonan backbone but contains four different side chains consisting of different sugar residues, including rare sugars such as apiose, aceric acid, 3-deoxy-D-lyxo-heptulosaric acid and 2-keto-3-deoxy-D-mannooctulosonic acid. With a different backbone configuration, the rhamnogalacturonan I (RGI) consists of a disaccharide backbone with alternating rhamnose and galacturonic acid. The galacturonic residues can be acetylated and both residues can carry side chains of neutral sugars as galactose, arabinose and xylose [Ridley, 2001; Pérez, 2003; Vorwerk, 2004; Pedrolli, 2009]. In Figure I.7, there is a schematic illustration of the primary structure of pectin, where a differentiation of the four mentioned classes is shown.

In the pectin network, several levels of crosslinking are found. These include, among others, backbone glycosidic linkages, calcium and borate ester crosslinking, and covalent linkages to phenolic and possibly other compounds. These polysaccharides are also reported to bind with hemicelluloses, proteins and cellulose [Zykwinska, 2005; Caffal, 2009].

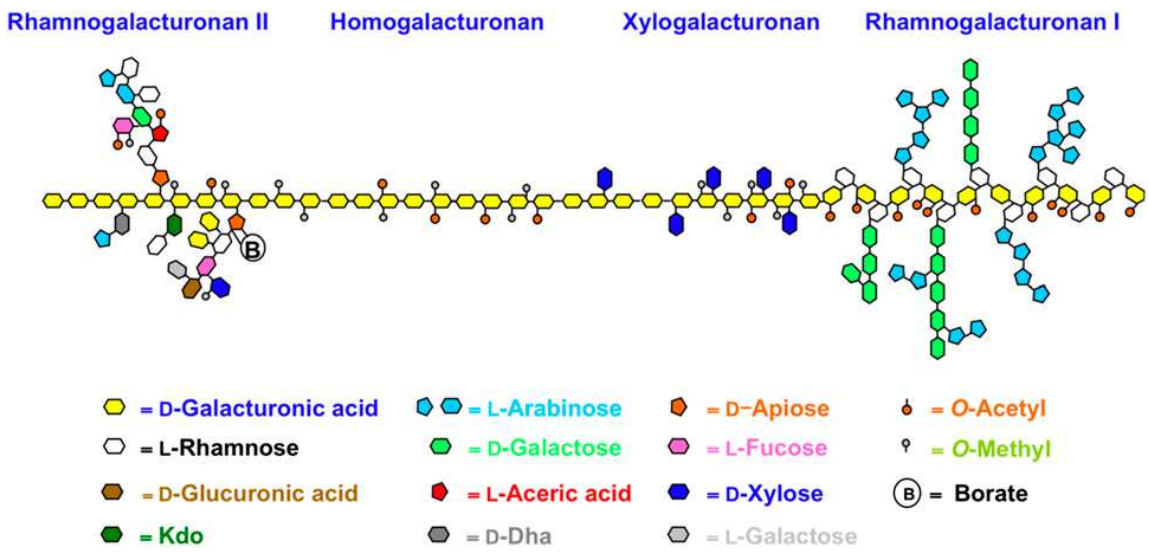

Figure I.7: Schematic representation of pectin, ilustrating the four different types of pectic polysaccharides, and the main structural units [taken from Harholt, 2010]

Considering the self-association, two main crosslinks can be found: one between unesterified carboxyl groups of the galacturonosyl residues of two HG chains, by insertion of Ca^{2+} ions, forming the so called "egg boxes" (Figure I.8); and the other between RG-II chains, in this case by borate-diol esters, which can be formed between the apiofuranosyl residues of the 2-O-methyl-D-Xylcontaining side chains (Figure I.9) [Vincken, 2003; Caffal, 2009].

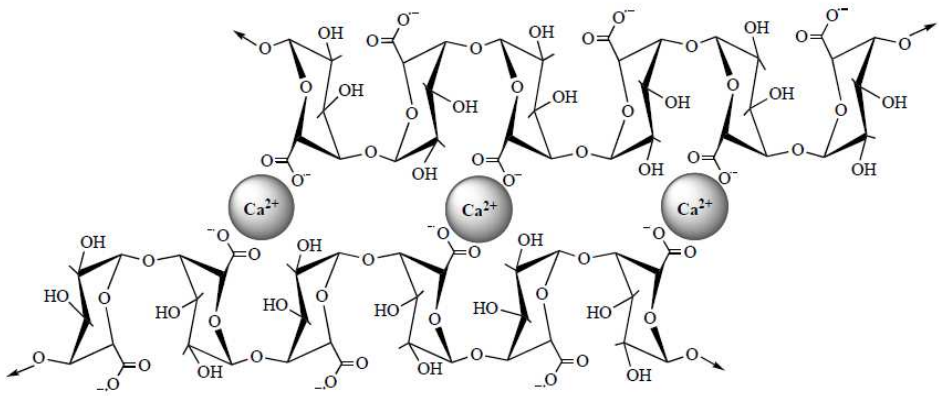

Figure I.8: Schematic representation of the "egg box" model for calcium crosslinking between unesterified HG pectin [Vincken, 2003].

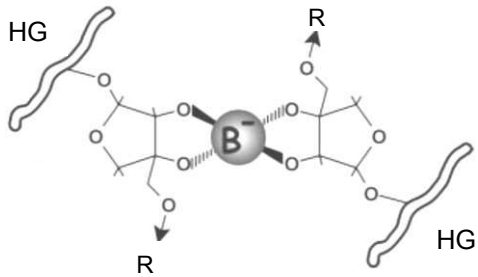

Figure I.9: Schematic representation of a borate ester crosslink in RG-II pectin [Vincken, 2003].

Alike the other plant components, the amount, distribution and functions of the pectic substances varies along the different parts of the plant and depends greatly on the species and development stage [Coenen, 2007].

Pectin is one of the main components of dicotyledon, being present mostly in the middle lamella compound (middle lamella and primary wall) [Fengel, 1984; Zykwinska, 2005; Caffall, 2009]. Here, they can play a wide number of functions with regard to cell strength, cell adhesion, stomatal function, storage, metal ions binding, regulators in defense response, ripening, and seed germination. Pectic substances can also play a determining role in structuring the plant cell walls by providing a proper environment for the deposition, slippage and further extension of the cellulosic-glycan network [Vincken, 2003; Caffall, 2009; Teleman, 2009; Harholt, 2010].

With the lignification of softwood and hardwood, the amount of pectin decreases considerably, as well as its functionality. In wood, the amount of this material accounts for a few percent, being present mostly in the middle lamella compound, in the pits membranes and when present, in the warty layer and warts. Here, they contribute mostly to the cell adhesion and mechanical properties of the tissues [Baird, 1974; Fengel, 1984; Teleman, 2009].

I.2.1.6. - Cellulose

Anselme Payen, a French chemist, was the first scientist to isolate and describe cellulose. After treating different woods with nitric acid, he obtained a fibrous substance common to all which he had also found in cotton and other plants. His analysis revealed the chemical formula of the substance to be $C_6H_{10}O_5$. He reported the discovery and the first results of this classic work in 1838 in Comptes Rendus. The name "cellulose" was coined and introduced into the scientific literature one year later, in 1839 [Payen, 1838; Krassig, 1993; Klemm, 2005]. Nevertheless, only almost one century later the polymeric structure of this material started to be unveiled, with the contribution of the Nobel prized Hermann Staudinger in 1920 [Staudinger, 1920; Klemm, 2005]. Cellulose is the basic compound of the cell wall in all plants, although can also be found in some algae, fungi, bacteria and even in animals [O'Sullivan, 2005].

I.2.1.6.1. - Cellulose primary structure

Cellulose is a high molecular weight linear homo-polysaccharide, composed of β-D-glucopyranose units, which are ~5 Å long and are linked by covalent β-1,4-glycosidic bonds between the carbon atoms C_1 and C_4 (Figure I.10). The repeating unit in a cellulose chain is cellobiose, consisting of a disaccharide unit built of two β-D-glucopyranose units, which are oriented 180° in relation to each other. At each end of the cellulose chain, two different terminal hydroxyl groups are found: the non-reducing end contains an alcoholic hydroxyl group, while the

reducing end contains an aldehyde hydrate group [Smook, 1992; Krassig, 1993; Alén, 2000; Köpcke, 2010; Srndovic, 2011].

Despite the cellulose structure repeating unit is the cellobiose, composed of two β-D-glucopyranose units, the degree polymerization (DP) is given by the number of these β-D-glucopyranose units. The chain length varies significantly, depending on the treatment and origin of the cellulosic material. The degree of polymerization can be of 150-300 for microcrystalline cellulose; 300-1700 for wood pulps and 800-12,000 for cotton fibers and other plants [Krassig, 1993; Klemm, 2005].

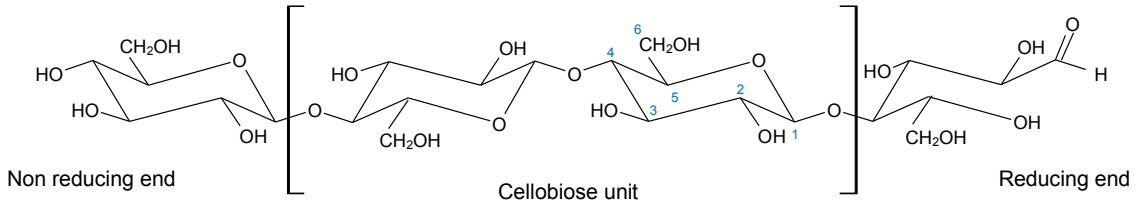

Figure I.10: Chemical structure of cellulose and a cellobiose residue (length 1.03 nm) with the β-1,4-glycosidic bond. The β-D-glucopyranose units are in a chair conformation. They are at an angle of 180° in relation to each other [Hon, 2001; Xavier, 2005; Srndovic, 2011].

I.2.1.6.2. - Cellulose secondary structure

I.2.1.6.2.1. - Hydrogen bonding

The presence of a large amount of OH groups in the cellulose chain (three in each glucose unit) is responsible for some of its properties: hydrophilicity, degradability and broad chemical variability. These hydroxyl groups are also the basis of the extensive hydrogen bond networks, which give cellulose a multitude of partially crystalline fiber structures, morphologies and stiffness [Krassig, 1993; Cuissinat, 2006c]. There are two kinds of hydrogen bonding: intermolecular, hydrogen bonds between OH-groups of adjacent glucose units in the same cellulose molecule; and intramolecular, between OH-groups of adjacent cellulose molecules, what will bond several cellulose chains, forming sheets. Two major intramolecular hydrogen bonds in native cellulose are described: one is between the C_3 hydroxyl group and the pyranose ring oxygen of an adjacent glucose residue (O_3 - H···O_5) with bond length of 2.707 Å; and the other one is between the C_2 OH and the C_6 oxygen of a neighboring glucose residue (O_2 - H···O_6') with bond length of 2.802 Å. The major intermolecular hydrogen bond is between the C_6 OH and C_3 oxygen (O_6 - H···O_3) with a bond length of 2.874 Å [Gardner, 1974; Fengel, 1984; Sjöström; 1993; O'Sullivan, 1997; Klemm, 1998; Wang, 2008]. The mentioned hydrogen bonds are illustrated in Figure I.11.

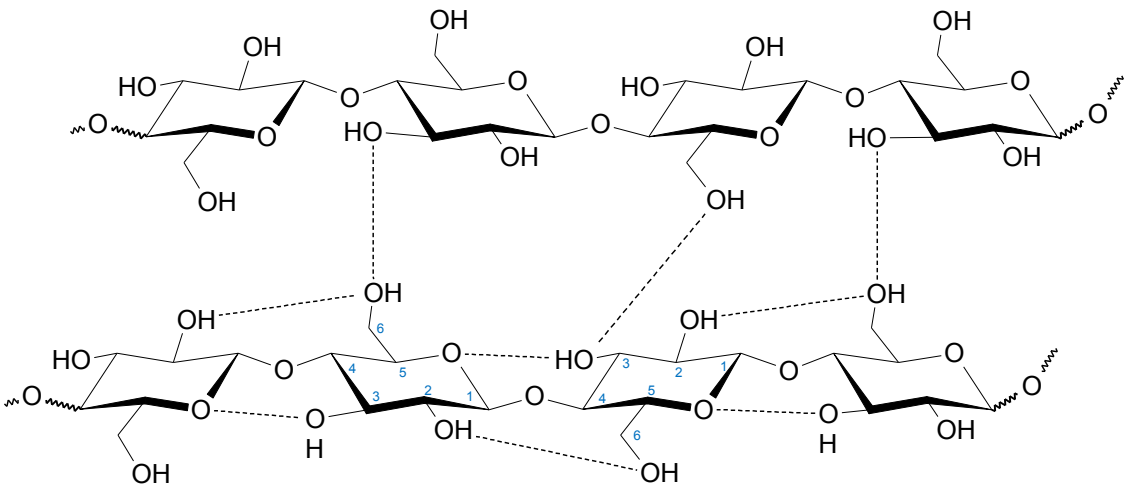

Figure I.11: Two segments of cellulose chains illustrating the most probable intra- and inter-molecular hydrogen bonds (dashed lines) [Klemm, 1998; Xavier, 2005].

Because of the strong tendency for intra- and intermolecular hydrogen bonding, bundles of cellulose molecules aggregate to microfibrils, which form either highly ordered (crystalline) or less ordered (amorphous) regions. This microfibrils are further aggregated to fibrils and finally to cellulose fibers [Sjöström, 1993]. The surfaces of isolated wood cells or fibers in the non-dried state are also able to form hydrogen bonds with each other, being these bonds that define the mechanical properties of a pulp or paper sheet. Hydrogen bonds do not only exist between cellulose OH-groups but also between cellulose-OH and water-OH. Depending on the water content single water molecules or clusters can be linked to the cellulosic surfaces [Fengel, 1984].

I.2.1.6.2.2. - Crystal structure

Due to the β-D-glucopyranose unit conformation, the hydroxyl groups in cellulose are arranged in radial orientation while the aliphatic hydrogen atoms in axial positions. Therefore, as discussed above, strong interchain hydrogen bonds between neighboring chains are easily formed. With the formation of sheets by the aggregation of cellulose chains, both sheet surfaces will be saturated with the aliphatic hydrogen atoms in axial position, which is creating a hydrophobic character on these surfaces and because of this, cellulose sheets are positioned over each other and interact with van der Waals bonds and χ-interactions, but no hydrogen bonds. This way, in addition to hydrogen bonding, van der Waals forces and χ-interactions (also called hydrophobic interaction) are also important for the cellulose network structure, especially between the non-polar sheets [Wang, 2008; Henriksson, 2009]. Studies with computer modeling of cellulose crystallite show that van der Waals force contributes to the major part of lattice energy between the non-polar sheets [Wakelyn, 1998]. Some researchers proposed that the cellulose resistance to acid hydrolysis is due to the hydrophobic face of cellulose sheets where a dense

layer of water formed near the hydrated cellulose surface and the resistance to enzyme hydrolysis is due to the inter-chain hydrogen bonding [Wang, 2008].

Cellulose exists in several crystal modifications, differing in unit cell dimensions and, possibly, in chain polarity. The different polymorphs of cellulose and its derivatives have been well documented. These are cellulose I, II, III and IV [O'Sullivan, 1997; Klemm, 2003].

The crystalline native cellulose, or cellulose I, is the main form found in nature, but its crystalline structure description is not unanimous. Around the 1930s, Meyer, Mark, and Misch proposed a model that assumes a monoclinic unit cell with two anti-parallel cellobiose chain segments running in opposite directions along the fiber axis. Later on, in 1974, Gardner and Blackwell proposed for cellulose the same monoclinic lattice packing but with two parallel running chains, assumed this to be valid for cellulose I in general. In the same year, Sarko and Muggli proposed a triclinic lattice cell with two cellulose chain segments running parallel along the fiber axis [Klemm, 2003].

Besides this, is widely accepted that cellulose I can be ordered in two different crystal forms, cellulose I_α and I_β. This is due to the displacement verified in the position of the chains in the adjutant cellulose sheets. The third layer can be displaced in the same direction as the second, forming cellulose I_α, or in the opposed direction, forming cellulose I_β (Figure I.12). There are also differences in the hydrogen-bonding pattern of cellulose I_α and I_β. As a result, cellulose I_α and I_β have different unit cells. Cellulose I_α has a one-chain triclinic unit cell and cellulose I_β a two-chain monoclinic unit cell. Although cellulose I_α is produced mostly by primitive organisms and cellulose I_β by higher plants both crystal forms can co-exist in the cellulosic material. Cellulose I_α is meta-stable and can be converted to the more stable cellulose I_β at high temperature and pressure in alkaline or acidic conditions [O'Sullivan, 1997; Henriksson, 2009].

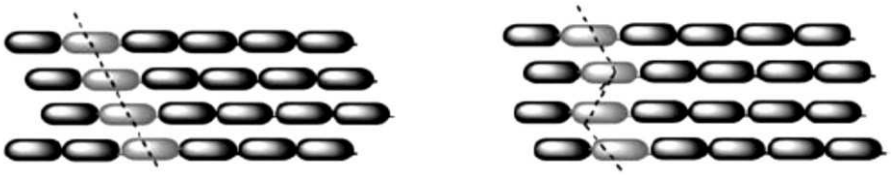

Figure I.12: Cellulose I_α (left) and I_β. (right). The figure shows the lateral view of cellulose layers, positioned over each other [Henriksson, 2009].

Besides cellulose I, the cellulose II crystalline form is of great importance from a technical and commercial point of view. This polymorph is naturally produced by a mutant strain of *Gluconacetobacter xylinum* and occurs in the marine alga *Halicystis*, or can be prepared either by regeneration of dissolved cellulose, or by the so-called mercerization process [Krassig, 1993; Klemm, 2003].

In comparison with Cellulose I, in Cellulose II the cellulose chains align in an anti-parallel manner and may present a folded chain structure, meaning that every second chain has opposite polarity to the next. In the cellulose II polymorph, the backbones of these two chains have the same conformation, but they differ in the conformation of their hydroxylmethyl groups (Figure I.13). These groups are near the gt conformation for the glycosyl residues located at the origin ('up' chain) of the cell. In contrast, the center chain hydroxylmethyl moieties adopt the tg conformation ('down' chain) [Langan, 1999; Granström, 2009; Yamane, 2013].

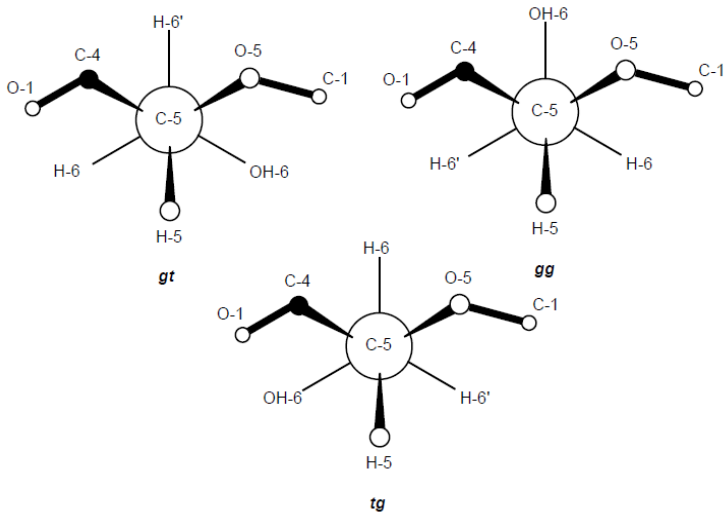

Figure I.13: Schematic presentation of the hydroxylmethyl conformations showing the orientation of the C6-O6 bond as gauche-trans (gt), gauche-gauche (gg) and trans-gauche (tg) [Granström, 2009].

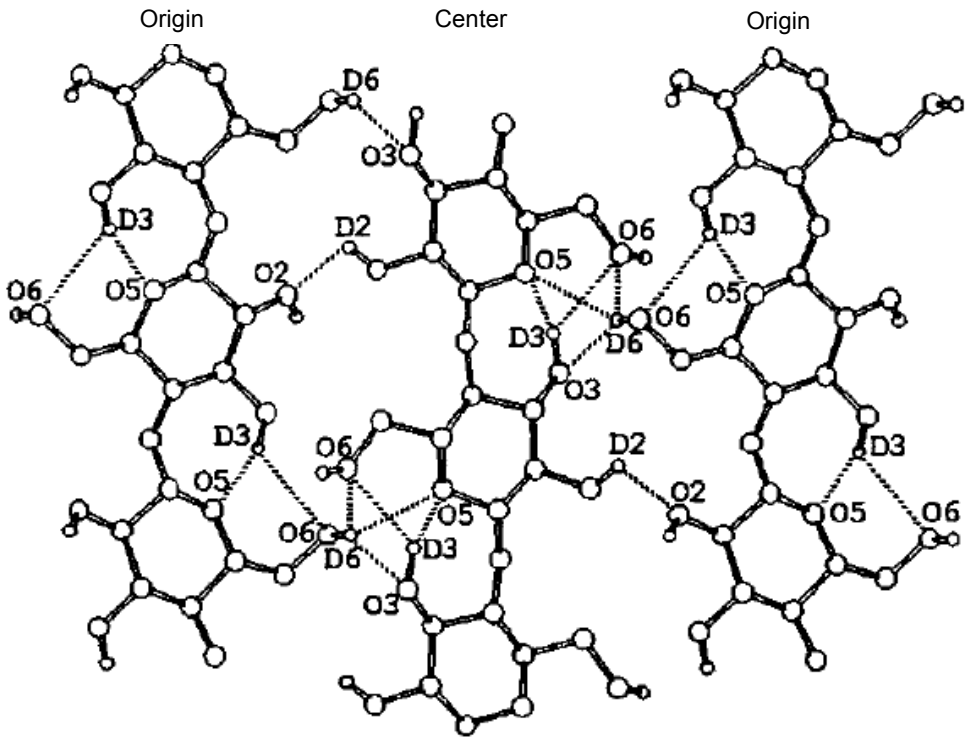

Figure I.14: A schematic representation of the hydrogen bonds in cellulose II. Only atoms involved in hydrogen bonding are labeled. Hydrogen bonds are represented by dotted lines [Langan, 1999].

Therefore the hydrogen bond pattern is different, and there is a 3D network of hydrogen bonds between origin and center chains in crystalline fibers of cellulose II (Figure I.14). This might explain the fact that cellulose II is the thermodynamically more stable form of cellulose [O'Sullivan, 1997; Langan, 1999; Klemm, 2003; Henriksson, 2009].

The polymorphs of cellulose III and IV are less important structural forms. As cellulose II they are with few exceptions only made by man and does not occur in nature to large extent. The crystalline modification of cellulose III is obtained by treating native cellulose with liquid ammonia (below -30 ºC) or an organic amine, followed with evaporating the ammonia and washing with alcohol. Small differences in lattice dimensions exist between the two submodifications cellulose III_I and III_{II} which are originated from cellulose I and cellulose II respectively. Cellulose IV can be synthetized by treatment of other cellulose polymorphs in a suitable liquid at high temperature. As for cellulose III, two allomorphs IV_I and IV_{II} are produced from cellulose I and cellulose II respectively, and both present an orthogonal unit cell. In Figure I.15, a scheme of the conversion of the different cellulose polymorphs is shown. Cellulose III has some similarities with cellulose II although it is believed to have parallel chains. Cellulose IV is rather similar to cellulose Iβ [Klemm, 1998; Klemm, 2005; Egal, 2006; Henriksson, 2009].

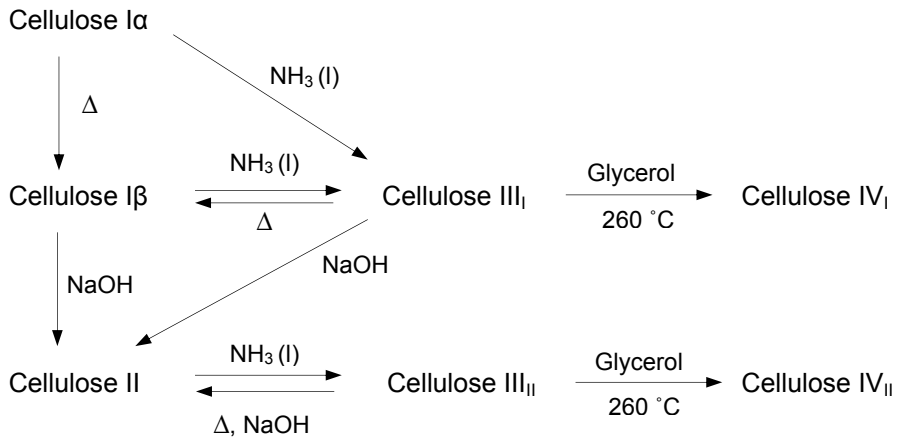

Figure I.15: Diagram of the conversion of various crystalline forms of cellulose. Cellulose I is converted to cellulose II by treatment in strong alkali. Treating both cellulose I and II with liquid ammonia form cellulose III, which can be converted to cellulose IV by treatment III with glycerol at high temperature [Henriksson, 2009].

As mentioned before, the aggregation of cellulose macromolecules is not uniform and is widely assumed the presence of amorphous regions with low order and crystalline regions with high order. This is represented by a two phase model, known as fringed fibril model (Figure I.16). The relative amount of crystalline cellulose in the structure is defined as crystallinity index or degree of crystallinity. This degree can show different values, depending on the origin and treatment of the cellulose sample, and as well as on the analytical method used for its determination [Fengel, 1984; Klemm, 1998].

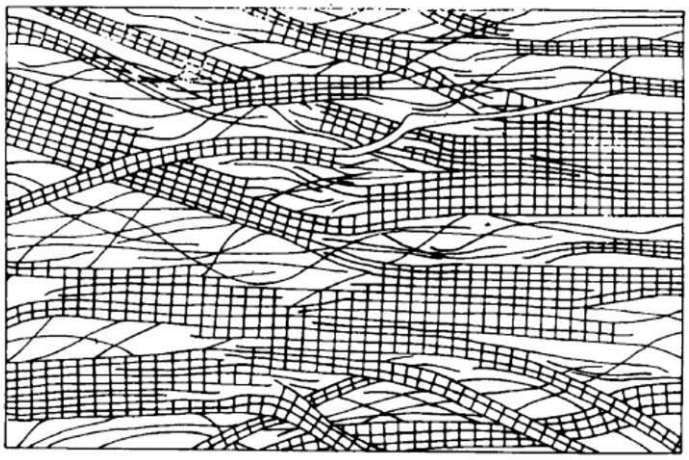

Figure I.16: Two phase “fringed fibril model” of the supramolecular structure of cellulose. The lattice work represents the highly ordered (crystalline) region while elongated lines represent the low ordered (amorphous) regions [Hearle, 1958; Granström, 2009].

The aggregation of cellulose chains in crystallites linked by less ordered cellulose (amorphous regions) is forming micro-fibrils or elementary fibrils. Due to the presence of van der Waals forces, hydrogen bonds and hydrophobic interactions these fibrils can further aggregate into larger fibrils called “macro-fibrils” (Figure I.17) [Fengel, 1984; Kihlman, 2012]. Further discussion at this level is done in the “Supramolecular structure” section.

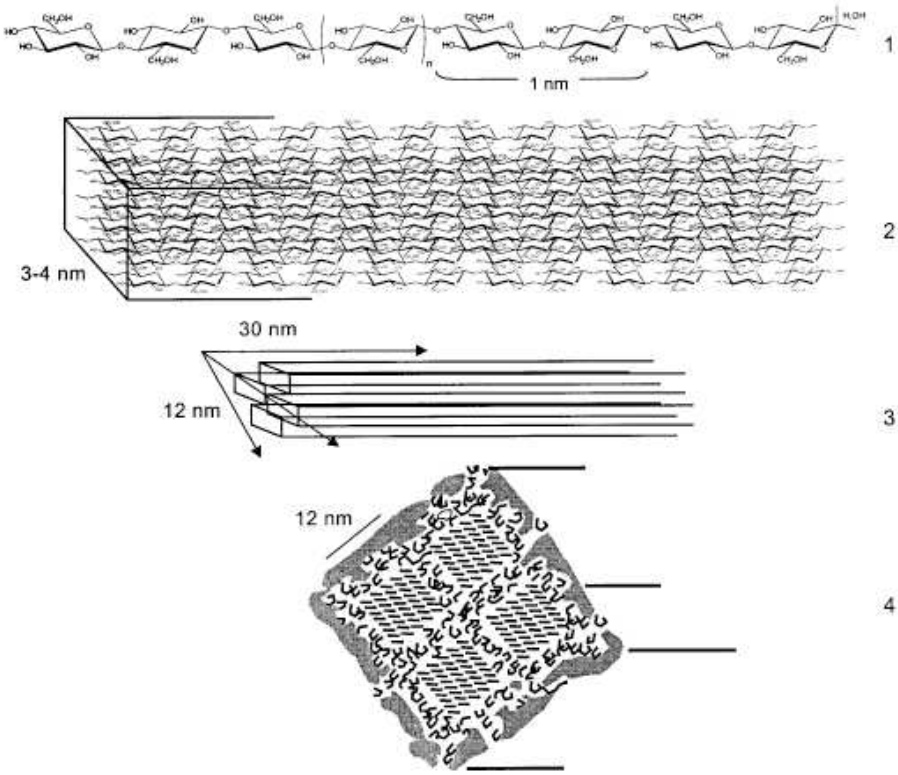

Figure I.17: Different levels of cellulose aggregation: (1) the cellulose chain (2) framework of cellulose chains in the elementary fibril; (3) cellulose crystallite; (4) microfibril cross section, showing strands of cellulose molecules embedded in a matrix of hemicellulose and protolignin [Fengel, 1984; Ramos, 2003].

I.2.2. - Supramolecular structure

I.2.2.1. - Polymeric arrangements in the cell wall structure

In Figure I.18, the disposition and interaction of the various chemical components in the cell wall is depicted. The crystalline structure of the cellulose β-1,4-glucan chains can be easily differentiated from the “cementing” amorphous phase composed by the remaining chemical species (mostly lignin, hemicelluloses and pectin).

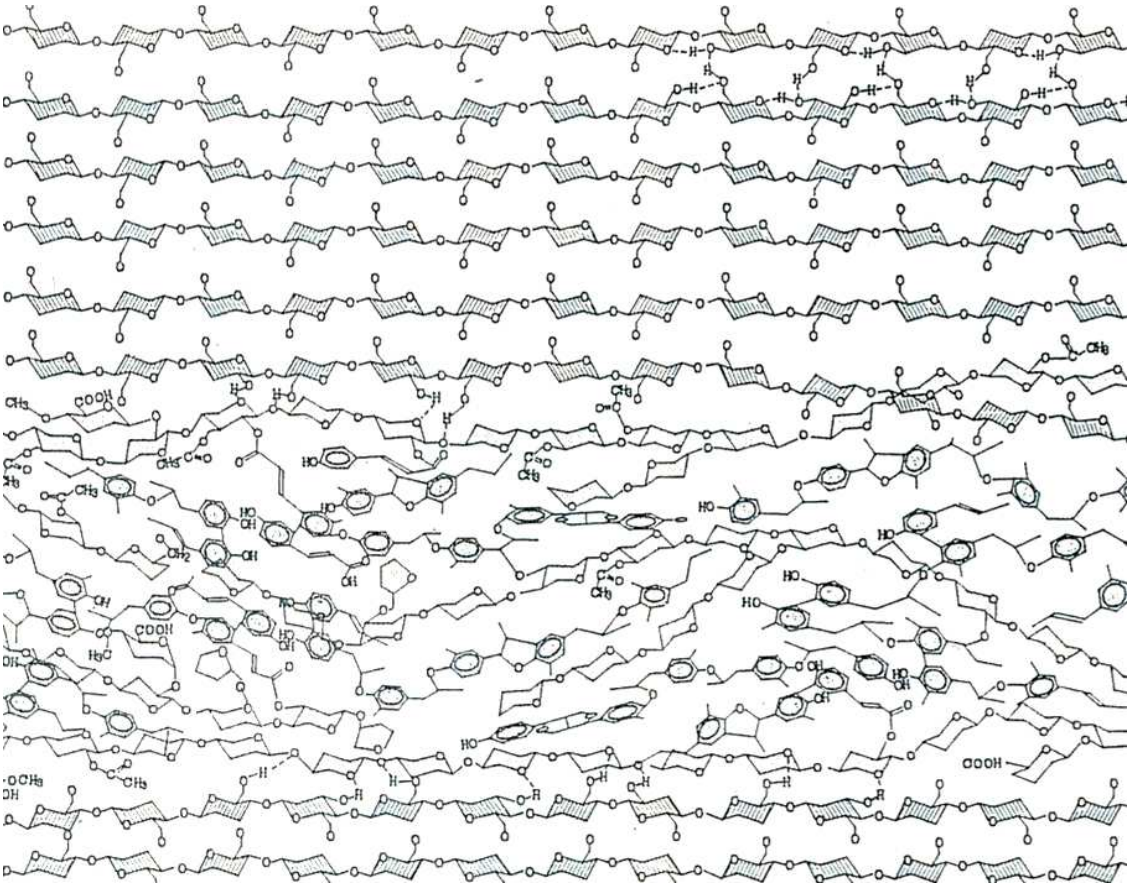

Figure I.18: A tentative structural model of lignified plant cell wall [Terashima, 1993]

I.2.2.2. - Pore structure

Besides to the matter network there is also a network of void spaces, pores, capillaries and interstices. This network is extremely important for the reactivity of the cellulosic material, once it regulates the accessibility or diffusion of the solvents into the fiber structure. The size distribution, porosity and the spatial distribution depends on the origin of the cellulosic material, the pre-treatments and analytical methods. For example: inefficient packing of the micro fibril induces the formation of intra-fibrillar pores of range from 2 ~ 7 nm, while large pores of up to 150 nm may be present between the hydrogen bonding fibrillar network [Ramos, 2005; Wang, 2008].

If the fibers are submitted to mercerization or ammonia treatments, the pore diameter decreases. The same occurs when the material is dried, in which the small pores collapse and new hydrogen bonds are formed (process known as hornification). In the other hand, the use of treatments that dissolve some of the material will increase the micropore surface, which is the case for enzymatic treatments or acid hydrolysis [Wang, 2008; Le Moigne, 2008].

I.2.3. - Macrostructure (wood)

I.2.3.1. - Structure of wood cell

The morphology of the cell wall is characterized by a concentric structure of several different wall layers. This layered structure is caused by differences in chemical composition as well as different orientations of the structural elements, microfibrils, which are synthesized during cell division, cell differentiation and cell elongation. In a mature cell, the S layers are well differentiated (Figure I.19), with variable thickness and fibrillar angle [Koch, 2006].

Between the cells, there is the compound middle lamella, which is responsible for keeping the cells together. This compound consists of the middle lamella and the primary wall, which are difficult to differentiate. At an early stage of growth, is mainly composed of pectic substances and later on becomes highly lignified [Koch, 2006; Sjöström, 1993].

The primary wall is a thin layer composed of cellulose, hemicelluloses, pectin and protein in a matrix of lignin. In the outer portion of this wall, the microfibrils form an irregular network, while, in the inner side, they are oriented almost perpendicularly with the cell axis [Sjöström, 1993].

The secondary wall is composed of two thin layers (S1 and S3) and one thick middle layer (S2). The S1 layer, next to the primary wall has about 3-4 lamellae where the microfibrils form a Z or S helix with microfibril angles of about 50 to 70 º (related to the cell axis). The S2 layer forms the main portion of the cell wall and it can contain between 30 to 150 lamellae, with fibrillar angles between 5 and 30 º. The S3 wall is a thin layer of helically arranged microfibrils similar to the S1 and constitutes the interface with the cytoplasm in living cells and the lumen in dead cells [Koch, 2006; Sjöström, 1993]. In some cases the inner cell wall is covered by the warty layer. This structure consists of an amorphous structure containing numerous protuberances, the warts. It is highly lignified and is also composed of hemicelluloses and pectic substances [Liese, 1963; Baird, 1974; Parham, 1974; Fengel, 1984; Fujita, 2001].

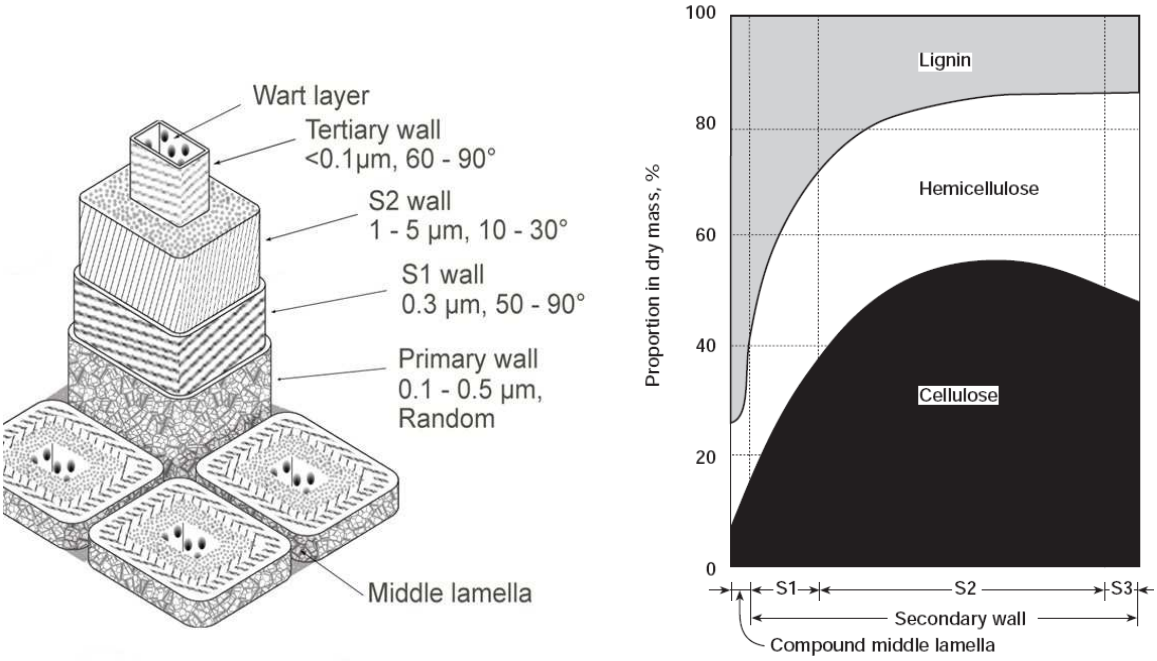

Figure I.19: Left: schematic drawing of the macrostructural morphology of wood cell wall with: middle lamella, primary wall, secondary wall - S1 and S2 (main body), tertiary wall, wart layer and lumen. For each wall, the thickness and the microfibrils orientation are drawn according to Abe, 2005; Fengel, 1984 and Egal, 2006. On the right the distributions of the chemical components along the fiber wall is plotted [Panshin, 1980].

Generally, the secondary layers show specific apertures in their structure, called pits (Figure I.20). These structures are usually located in adjacent cells, forming pit pairs separated by the pit membrane. This semi-porous membrane is composed of the middle lamella compound, being enriched in lignin and pectin material. Its structure is different for hardwood and softwood, occurring a differentiation in torus and margo in the second case (Figure I.20 c)). The whole structure acts as valve-like canals allowing the flow of liquids both laterally and vertically through the cell walls. The pit structure varies according to the type of cell they are linking as well as the wood species. Three different types of pits are characterized: simple pits, connecting two parenchyma cells; bordered pits, connecting vascular cells; and half-bordered pits, connecting parenchyma cells to vascular cells [Sjöström, 1993; Fahlén, 2005; Wiedenhoeft, 2005; Koch, 2006; Geoffrey, 2009].

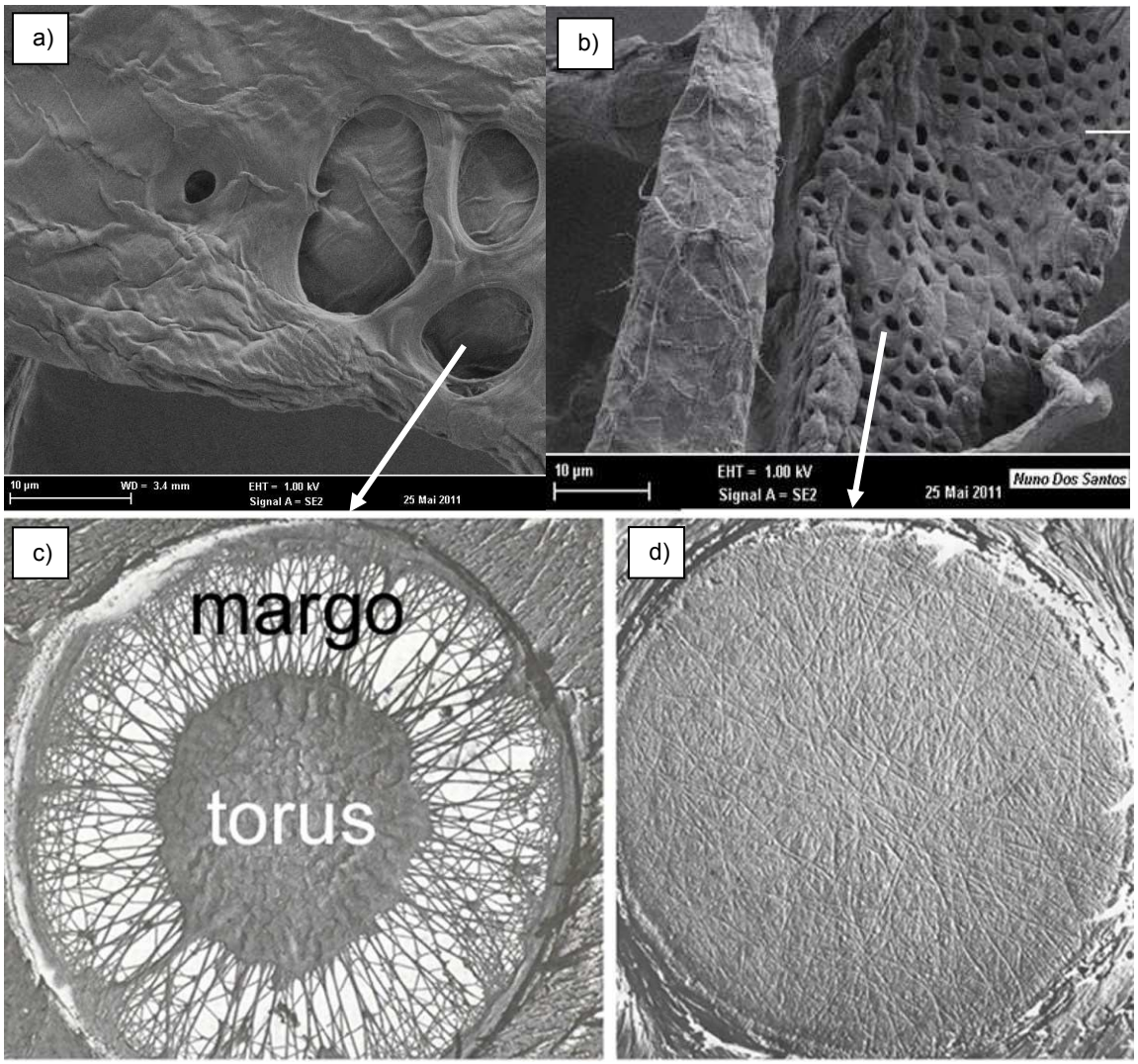

Figure I.20: Upper pictures: Pulp fibers showing the pits localization for softwood a), and hardwood b) (the pits were removed during delignification) [photos from the author].
Pictures below: Detailed structure of the pits of softwood (with margo and torus) c), and hardwood d) [photo credit: Springer Science and Business Media, G.L. Comstock, W.A. Côté and E. Wheeler].

I.2.3.2. - Wood cell types

Wood contains different types of cells (Figure I.21) and all have specific genetically predetermined functions. This function determines the size, form, wall thickness, and perforation of a cell. During growth and maturation, from the two initial meristematic cell types known as the fusiform and ray initials, they will change in shape, size, and structure to fulfill the specific requirements of their function in the living tree [Alén, 2000; Koch, 2006; Geoffrey, 2009]. Depending on their individual functionality and morphology, wood cells can be classified in four main groups: tracheids, fibers, parenchyma cells and vessels [Koch, 2006; Geoffrey, 2009]:

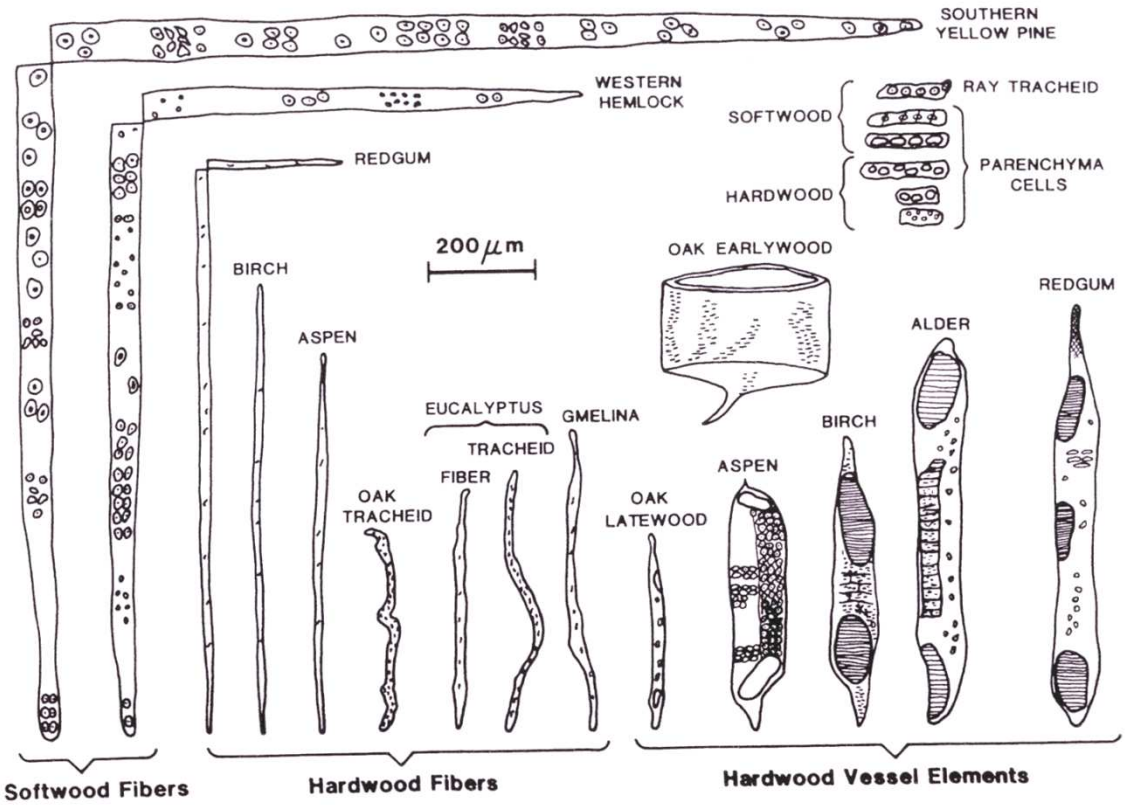

Figure I.21: Representation of major cell types in hardwood and softwood [Mimms, 1989].

Tracheids are the principal axial cell elements in the softwoods, serving as conduction paths for water and nutrients (thin-walled earlywood tracheids) and providing the necessary mechanical stiffness (thick-walled latewood tracheids). These cells can also be present in hardwoods, being located adjacent to the vessels (vasicentric tracheids) or resembling vessels (vascular tracheids) with the function of water transport.

Fibers are found exclusively in hardwood. In most species, fibers occupy 40-75% of wood volume (note that the term “fiber” is commonly used in the concept of all fibers). The primary function of fibers is to support the structures of the tree, although they can also conduct water. These cells are long, narrow, with closed ends and very thick walls. Two types of fibers exist: tracheid fibers, with bordered pits and libriform fibers, with simple pits. The length of the fibers is 0.7-1.2 mm, the width is 10-30 mm, and the length-to-diameter ratio is around 1:50 [Alén, 2000; Koch, 2006].

The parenchyma cells have two different stages with different functions. In the sapwood, these cells have cytoplasm and are engaged in several processes such as storage of nutrients, metabolic pathways and short distance transport. These cells have a thin secondary wall, can be axially oriented but also transversally disposed in cell network, forming horizontal bands (rays).

Another anatomical characteristic of hardwoods is the presence of vessel elements. They are short, with a large diameter and thin-walled cells and in some cases with perforated ends known as perforation plates. They link end-to-end along the longitudinal axis of the stem to form tubelike structures of indefinite length. In the cross-section of wood, vessels appear as larger pores (Figure I.23), and for this, sometimes hardwoods have the designation of porous woods. These cells are specialized in the long range transport of water and minerals. Just as the tracheids, vessels only achieve functionality after the loss of their cytoplasm. The proportion of vessels in most hardwoods is 10%-40% of the volume, but less of the mass. Usually, vessel elements are shorter than hardwood fibers [Alén, 2000; Koch, 2006].

In Table 2, the different cell types are classified according to their function and wood type. The same wood cell types are depicted and identified in wood tissue in Figure I.22 for softwoods and Figure I.23 for hardwoods.

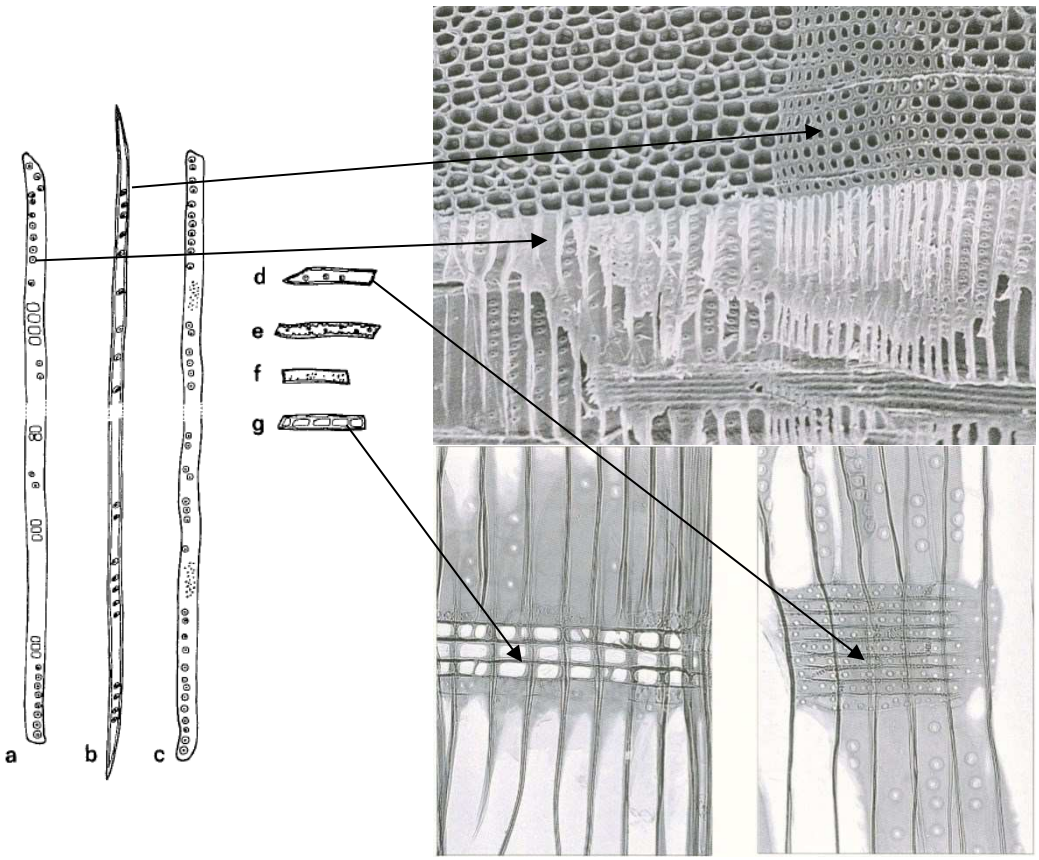

Figure I.22: Cells of softwood. An earlywood (a) and latewood (b) pine tracheid, an earlywood spruce tracheid (c), ray tracheid of spruce (d) and of pine (e), ray parenchyma cell of spruce (f) and pine (g) [Aitken, 1988; Sjöström, 1993].

Table I.2: Classification of the different fibers from softwoods and hardwoods according to their function [Fengel, 1984]

	Mechanical function	Conducting function	Storing function	Secreting function
Softwoods	Latewood tracheids	Earlywood teacheids Ray tracheids	Ray parenchyma Longitudinal parenchyma (Resin canals)	Epithelial cells
Hardwoods	Libriform fibers Fiber tracheids	Vessels Vessel tracheids	Ray parenchyma Longitudinal parenchyma (Resin canals)	Epithelial cells

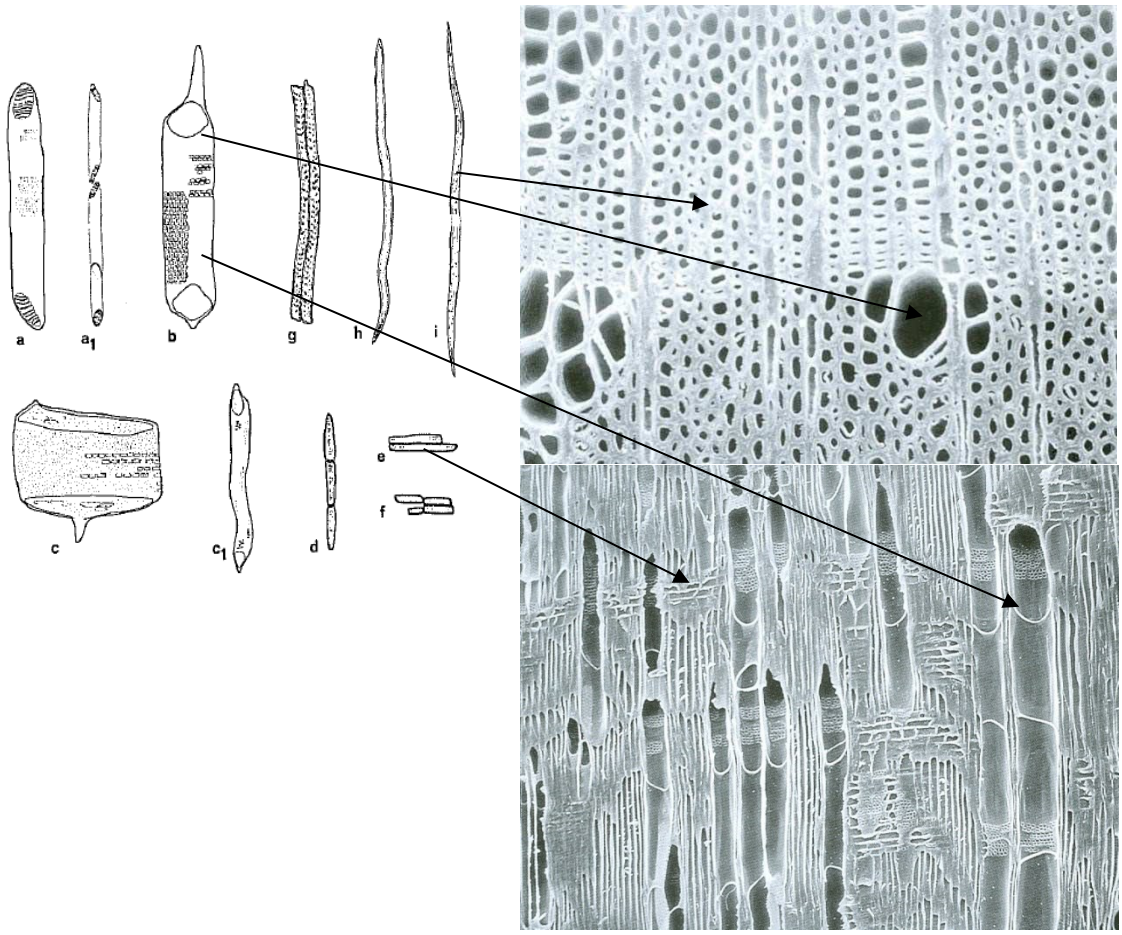

Figure I.23: Hardwood cells. Vessel elements of birch (a), of aspen (b), and oak in earlywood (c) and latewood (c1), as well as latewood birch vessel (a1). Longitudinal parenchyma of oak (d), ray parenchyma of aspen (e) and birch (f). Tracheids of oak (g) and birch (h) and birch libriform fiber (i) [Aitken, 1988; Sjöström, 1993].

I.2.3.3. - Reaction Wood

When the natural equilibrium of the tree is disturbed it will react in order to reestablish it. For example, when the tree is growing on a mountain slope, or is continuously exposed to strong wind from one preferential direction or heavy snow fall, the tree counteracts these external forces by producing a special tissue known as reaction wood. The function of this tissue is to absorb the external forces and stabilize the vertical statics of the tree. [Fengel, 1984; Sjöström, 1993; Fujita, 2001; Plomion, 2001].

Softwoods and hardwoods have a different strategy, forming individual types of reaction wood. Coniferous trees develop compression wood in the compressed ranges, that pushes a stem or a branch up, and deciduous trees develop tension wood in the tensive ranges, that pulls them up. Compression and tension tissues differ in chemical, physical and anatomical properties from each other as well as from the normal tissue of wood [Coté, 1965; Fengel, 1984; Sjöström, 1993; Fujita, 2001].

Comparing with normal wood, compression wood is heavier, harder and denser, which is explained by the presence of short and thick-walled tracheids. The S1 layer is thicker than in normal wood, while the S3 layer is not present. The S2 wall is characterized by the presence of helical cavities. The cells have a round shape, as if they are swollen, creating the formation of intercellular spaces, which is a microscopic criteria for microscopic identification of this kind of tissue (Figure I.24). Chemically, the cellulose content in this tissue is lower and the lignin content higher than for normal wood [Coté, 1965; Fengel, 1984; Sjöström, 1993; Fujita, 2001].

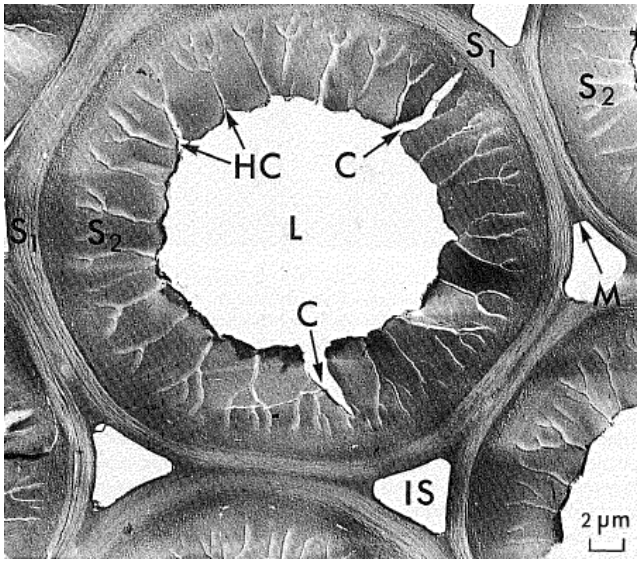

Figure I 24: Transverse section of compression wood tracheids, showing intercellular spaces (IS), middle lamella (M), outer (S1) and inner (S2) layers of the secondary wall, and the lumen (L). The S2 layer contains branched helical cavities (HC) and two wide drying checks (C) [Sjöström, 1993].

Concerning tension wood, this tissue is characterized by the presence of thick-walled fibers, with a gelatinous layer known as G-layer is composed of pure and highly crystalline cellulose oriented in the same direction as the fiber axis (Figure I.25). This layer may be present instead of the S2, the tertiary wall or additionally to the normal wall layers. Comparing with other tissues, tension wood contains fewer and smaller vessels than normal wood. The cellulose content in tension wood is higher than in normal wood, while the lignin content is lower, which confers a lighter color to the tissue [Coté, 1965; Fengel, 1984; Sjöström, 1993; Fujita, 2001].

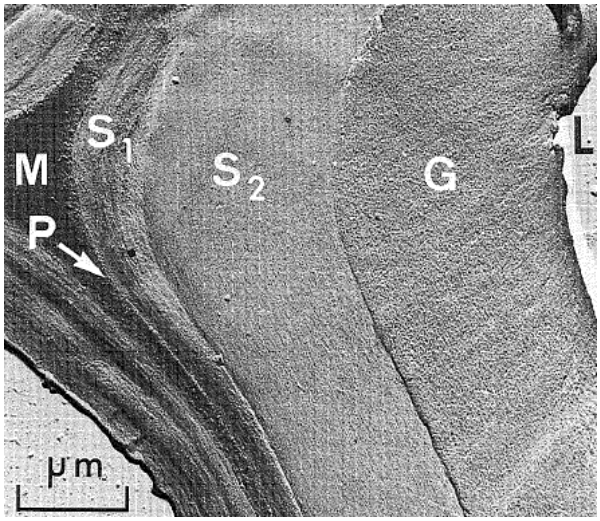

Figure I.25: Transverse section of a tension wood fiber, showing the middle lamella (M), primary wall (P), outer (S1) and middle (S2) layers of the secondary wall, the G-layer (G) and the lumen (L) [Sjöström, 1993].

I.2.3.4. - Wood structure

In the wood trunk of different species the various cells are forming different microscopic and macroscopic structures. In the following, the main structures are presented as well as the major differences between softwoods and hardwood.

In Figure I.26, the macroscopic structure of a tree stem with its several layers is revealed. The bark consists of two layers: the outer dead bark (cork or rhytidome) and the inner living bark (phloem). The function of the outer bark is to protect the tree against mechanical damage and microbiological attack and limit evaporative water loss, while the phloem is the tissue through which sugars produced by photosynthesis are transported from the leaves to the roots or growing portions of the tree [Alén, 2000; Wiedenhoeft, 2005].

The vascular cambium is a thin layer of cells between the bark and the secondary xylem, being the region where the growth both these tissues takes place, allowing this way the radial growth of the tree [Sjöström, 1993; Wiedenhoeft, 2005; Geoffrey, 2009; Henriksson, 2009].

The outer section of the secondary xylem is termed "sapwood." It gives structural support, acts as a storage reservoir, and transports water and nutrients from the roots to the tree foliage. Although the majority of the cells in sapwood are dead, the parenchyma cells are metabolically active. Hence, sapwood is physiologically active [Alén, 2000; Fujita, 2001; Wiedenhoeft, 2005; Lourenço, 2012].

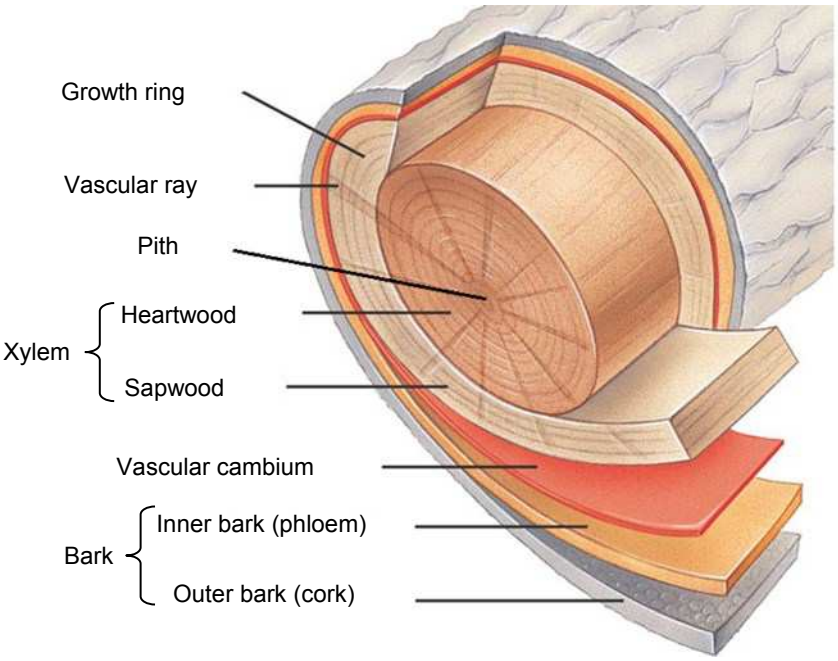

Figure I.26: Macroscopic stem structure of a mature tree [adapted from http://hbio6gbs1112.blogspot.de, (accessed in October 2013) and Alén, 2000]

The inner part of the xylem usually consists of physiologically inactive "heartwood", which may have a distinct colour and odour as well as lower moisture content and higher density than sapwood. Heartwood consists of dead cells that no longer take any part in transporting water or nutrients and functions in the support role and long term storage of biochemicals known as extractives [Alén, 2000; Fujita, 2001; Wiedenhoeft, 2005; Lourenço, 2012].

In the radial centre of the stem or branches is the pith, this tissue is formed during the first year of growth, before the wood formation [Sjöström, 1993; Wiedenhoeft, 2005]

The vascular rays system is orientated perpendicularly to the tree axis and form horizontal files of cells extending from the bark to the pith (primary rays) or to specific annual rings (secondary rays). The major function of the ray cells is to store and redistribute storage materials from the phloem to the living cells of the cambium and sapwood [Sjöström, 1993; Biermann, 1996; Geoffrey, 2009; Henriksson, 2009].

As mentioned before, between the two mentioned classes, softwood and hardwood, the main wood tissue structural difference is the presence of vessels on the second one (Figure I.27).

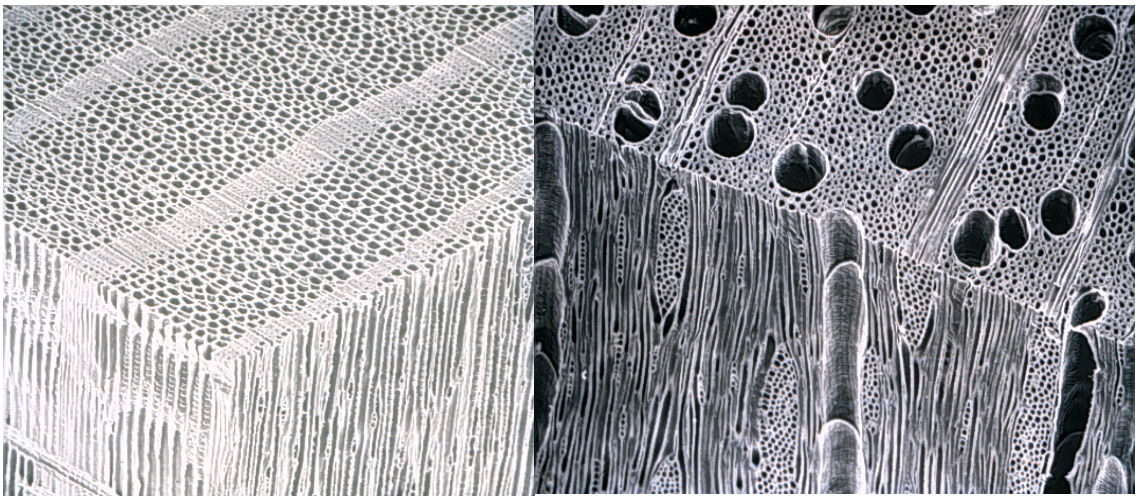

Figure I.27: Electronic microscope images from wood tissue, softwood on left, and hardwood (with vessels) on right [Jameel, 2005].

I.2.4. - Dissolving pulp

Dissolving pulp, also named as chemical pulp, consists of a wood pulp with high purity of cellulose (90-98%) and low amounts of hemicellulose, lignin and extractives. Compared to a paper grade pulp, dissolving pulp is characterized by properties such as a low pulp yield (35 to 40%), a high α-cellulose content, narrow molecular weight distributions and low ash content [Christoffersson, 2005]. In Table I.3 is a simplified list of the main chemical and physical properties required for some representative dissolving pulps.

Table I.3: Physical and chemical properties of selected dissolving pulps [Sixta, 2006].

Main application		Viscose	Ether	Viscose	Acetate	Ether
Raw material		Hardwood	Softwood	Hardwood	Hardwood	Cotton linters
Delignification process	Cooking	Sulfite	Sulfite	PHK	PHK	Soda
	Bleaching	ECF	ECF	TCF	ECF	ECF
Brightness	% ISO	93	85	89	92.5	85
R_{18}	%	95	95	96.5	98.2	99.2
R_{10}	%	89	93.8	93.5	97.7	98.5
Glucan	%	97	94.8	96.3	98.8	99.6
Xylan	%	2.5	3.2	3.5	1.0	0.4
Carbonyl	$\mu mol\ g^{-1}$	14	6	6	4	3
Carboxyl	$\mu mol\ g^{-1}$	30	50	28	16	10
Viscosity	$mL\ g^{-1}$	500	1500	450	730	2000

Presently, the production of dissolving pulp is using two main delignification processes, the alkaline pre-hydrolysis kraft (PHK) and the acid sulfite process. Nevertheless, although none is currently used on an industrial scale, other processes to manufacturing dissolving pulps were proposed as alternatives: including several types of organosolv [Sixta, 2004], pre-hydrolyzed soda/anthraquinone [Reguant, 1997], and pre-hydrolyzed-alkaline/sulfite [Kordsachia, 2004].

The pre-hydrolysis kraft delignification process is a two-stage process. First, the wood chips are steamed during 1 to 3 hours with temperatures between 140 and 170 ºC. This will liberate organic acids such as acetic and formic acid from the wood, which will promote a selective acid hydrolysis of hemicelluloses into soluble sugars. After this, the wood chips are cooked with sodium hydroxide and sodium sulfide (alkaline "Kraft" process) which will reduce the lignin content [Saka, 2004; Strunk, 2012].

The acid sulfite process can vary by the use of different cations, temperature and pH, depending on the wood used and targeted pulp properties. Initially, it was mostly used the calcium sulfite process, but later, the calcium was replaced by other cations such as ammonia, sodium or magnesium. The use of two- or three-cooking stages in the sulfite process allows improving the extraction of hemicelluloses and lignin by alternating between different pH levels. It is used for example to overcome the problems associated with the lignin condensation verified with the conventional sulfite process when pine is used. The amorphous state of the hemicelluloses as well as their weaker glucosidic bonds and lower DP, makes that the hemicelluloses are easily depolymerized compared to cellulose, being dissolved in the cooking liquor as monosugars. The main degradation or decrease of DP for the cellulose occurs in the last stages of delignification [Strunk, 2012].

For both delignification processes, further ECF (elemental clorine free) or TCF (total clorine free) bleaching stages will further increase the α-cellulose, by further removing residual lignin, and are also used to decrease the pulp viscosity to targeted values by controlled cellulose hydrolysis.

I.3. - Cellulose fibers reactivity and dissolution

I.3.1. - Reactivity of cellulose

The reactivity of dissolving pulp has been widely studied since it started to be used industrially. The main objective is to correlate the pulps reactivity with their performance and behavior during industrial processing. Besides this, it allows also to correlate the pulps properties with their reactivity and study the effect that changing these properties by different pre-treatments will have in the industrial application [Roffael, 1998]. The reactivity of cellulose pulps depends essentially on the availability of the hydroxyl groups from the cellulose chains to react. This is influenced by different factors like fiber origin, accessibility and pretreatment history. It has been reported that cellulose reactivity is related to the dissociation of fibril aggregates into elementary

fibrils [Fahmy, 1971]. According to Krässig, the structure and morphology of the cellulose fiber determine how reactive and accessible the cellulose is to chemicals [Krässig, 1993; Elg-Christoffersson, 2002]. Hornification, an irreversible collapse of the polymer structure, also affects the pulp reactivity and accessibility [Diniz, 2004; Ponni, 2013].

The reactivity can be evaluated using different methods, but it is not a simple parameter that can be measured in an objective manner and caution should be taken on using the same method for comparison of different samples. Roffael et all describes a wide number of methods used for measuring cellulose pulps reactivity [Roffael, 1998].

In recent years several studies have been made on increasing the reactivity of pulp [Engström, 2006; Kvarnlöf, 2007; Köpcke, 2008; Ibarra, 2010; Ostberg, 2012]. In these works, the reactivity was measured either by the Fock Test or as filterability, Kw, in accordance with Treiber [Treiber, 1962]. Despite this method is laborious and complex, it is the most used, since it resembles the industrial viscose process. In this work, due to the high amount of samples analyzed, the reactivity of the samples was measured using a simple and quick method of dissolution in cold caustic soda [Le Moigne, 2010; Spinu, 2011; Santos, 2013]. The dissolution yield was calculated gravimetrically, which allowed a quantitative assessment of the reactivity. Besides this, the fraction of undissolved material was observed with optical microscopy. A systematic evaluation of the reactivity capacity is also possible by comparing the residues morphology with the swelling degree scale for cellulose fibers established by Stawitz and Kage (Figure I.28) [Stawitz, 1959].

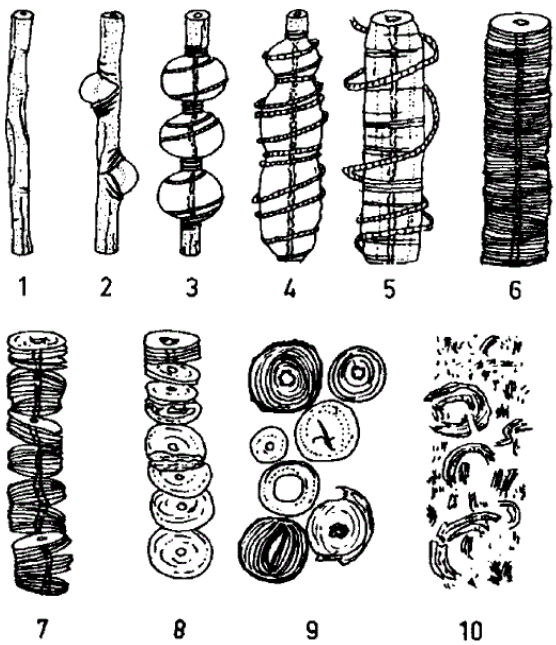

Figure I.28: Swelling scale for CMC cellulose fibers, established by Stawitz and Kuga [Stawitz, 1959].

I.3.3. - Cellulose dissolution

Since cellulose cannot melt, dissolution has been a very important issue [Warwicker, 1966; Liebert, 2010; Navard, 2012]. Cellulose solutions are used for processing cellulose directly in the form of fibers, films, membranes, sponges, or for performing chemical derivatization. Finding easy and cheap ways to dissolve cellulose has been a hot research topic since cellulose was isolated in the 19th century.

Many compounds have been tested, with a very successful decade of research in the 1930's where phosphoric acid-based solvents [British Celanese 1925; Turbak, 1980; Boerstoel, 2001; Northolt, 2001], ionic liquids [Graenacher 1934; Swatloski, 2002; Zhang, 2005; Kosan, 2008], amine oxide [Graenacher, 1939; Johnson 1969; Franks, 1977; Mc Corsley, 1979], and NaOH-water [Davidson 1934, 1936] were discovered. As a derivatizing dissolution method, the carbamate process for cellulose was investigated [Hill, 1938; Sprague, 1961; Kunze, 2005].

However, aside the cuprammonium process which has a very low production, only two methods have been implemented in a large scale to produce regenerated cellulose objects. The main process is still the viscose process dating from first patent on the use of cellulose xanthate preparation and subsequent regeneration by C. Cross, E. Bevan and C. Beadle in 1892 [Cross, 1893]. As an alternative the younger lyocell process is now in use [Firgo, 1994; Fink, 1998; Fink, 2001].

The viscose process goes through a derivatization step, based on the treatment of cellulose with sodium hydroxide and carbon disulfite to form cellulose xanthate which is soluble in sodium hydroxide-water mixtures. The solution can then be shaped, followed by an acid or a thermal treatment that reverts the cellulose derivative back to cellulose. The viscose process produces a variety of reagents and side-products that need to be isolated and re-processed at high de-pollution costs. Lyocell is operated by a direct dissolution pathway with a simple recovery cycle. However, there exists a risk of explosions which has to be considered by adequate process conditions and the addition of stabilizers [Rosenau, 2001].

Present time research is focusing on several other solvents, mainly ionic liquids and sodium hydroxide. The ionic liquid dissolution method is still expensive and not well understood, but has the potential of dissolving large amounts of cellulose for the production of fibers [Kosan, 2008], cellulose composites [Zhao, 2009]; porous aerogels [Gavillon, 2007; Tsioptsias, 2008; Sescousse, 2009; Sescousse, 2011a], beads [Sescousse, 2011b], bioactive films [Turner, 2004], and functionalized microparticles [Lin, 2009]. The sodium hydroxyl-water pathway has the advantage to be very simple, cheap and without environment concerns, but the dissolution is difficult.

The first reports that cellulose is soluble in NaOH-water mixtures in a certain range of rather low NaOH concentrations and low temperatures are from Davidson [Davidson, 1934]. Sobue published a full phase diagram of cellulose interactions with NaOH-water (Figure I.28) [Sobue, 1939]. Untreated cellulose samples were not dissolving well in this system. Already in the 1930's, it was found that the addition of compounds like ZnO or urea was helping dissolution [Davidson, 1937]. Apparently, the discoveries of these cellulose dissolution processes were not considered as important and these discoveries were somehow forgotten.

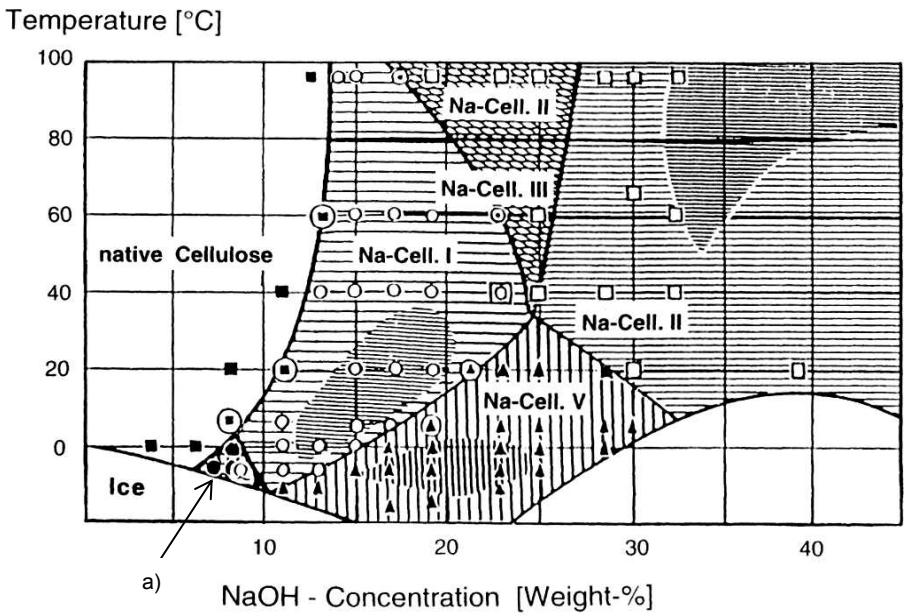

Figure I.29: Phase diagram of the system cellulose/NaOH/water, cellulose being natural ramie fibers. This graph reveals zones of different sodium celluloses as a function of NaOH concentration and temperature. a) condition in which cellulose can be dissolved [Sobue, 1939].

A revival of an interest in cellulose dissolution in NaOH-water came from Japan in the middle of the 1980's. A team of Japanese researchers from Asahi Chemical Industry Co made a breakthrough in the dissolution of cellulose in dilute aqueous solutions of sodium hydroxide. In a series of papers [Kamide, 1984; Kamide, 1990; Yamashiki, 1988; Kamide, 1992; Yamane, 1996], they report an extensive study of cellulose dissolution in NaOH-water. The first paper by Kamide et al. [Kamide, 1984] reported that regenerated cellulose from a cupramonium solution and ball milled amorphous cellulose dissolves in aqueous alkali and that the solution is stable over a long period of time. They pointed out that the requirement for dissolution is the breaking or weakening of the intramolecular O_3-H$\cdots O_5$ hydrogen bond. The authors did not indicate crystallinity as a main factor of dissolution. However, cellulose of higher crystallinity tends to be more difficult to dissolve, as found already by Davidson in 1934. The lack of a strong correlation between the amount of amorphous phase and solubility was confirmed later [Kamide, 1992].

The same authors turned then towards steam-explosion as a pre-treatment and the solubility of steam-exploded pulp was found to be very high. The maximum solubility was found in a temperature range from -10 °C to 0 °C using NaOH concentrations from 6-9%. This corresponds to the previously described conditions by Sobue and Davidson (Figure I.28) [Sobue, 1939]. Kamide and coworkers developed an industrial method for dissolving cellulose starting from steam exploded cellulose pulp, with wet pulverization to increase the surface of cellulose particles, pre-treatment in NaOH-water with 2-6% NaOH at -2 °C, high speed mixing followed by a dissolution in 6-9% NaOH solutions at -2 °C at 12000 rpm for 1 min [Yamane, 1996]. However, this process did not find any industrial application.

Isogai and Atalla [Isogai, 1998] investigated the dissolution of a large variety of cellulose samples from different origins. They reported improved dissolution after freezing cellulose in 8.5% NaOH at -20 °C. Subsequently samples were brought to room temperature and the NaOH concentration was adjusted to 5%. With this procedure, solutions produced from microcrystalline cellulose were stable at room temperature. Other cellulose samples were partially soluble. The authors concluded that the presence of hemicellulose did not influence dissolution since most hemicellulose fractions were soluble in NaOH-water mixtures. Molar mass was supposed to be the key point for explaining solubility, the higher molar masses being more difficult to dissolve.

The dissolution mechanisms of native cellulose proceed through several steps depending on the solvent [Cuissinat, 2006a; Cuissinat 2006b]. In NaOH-water [Cuissinat, 2006b], a native fiber shows a ballooning (Figure I.29) followed by an incomplete dissolution of the primary wall, typical for bad solvents.

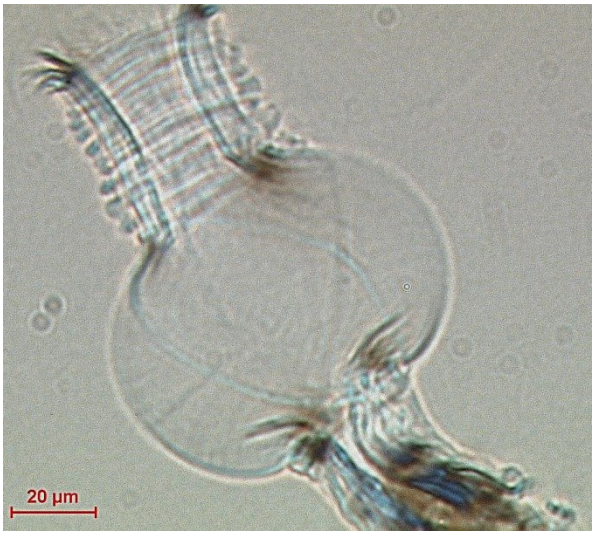

Figure I.30: Example of an incomplete dissolution of the primary wall, which constricts the swelling of the S2 wall, promoting this way the occurrence of balooning. Optical microscopy picture of a softwood bleached sulfite fiber frangment after dissolution treatment with a bad solvent (aqueous solution of NMMO with a 16% H_2O), the dissolution yield was ~27% [picture from the author].

Interactions between cellulose, water and NaOH in the region of dissolution studied by calorimetry [Roy, 2001; Egal, 2007], showed that dissolution requires at least four NaOH molecules per one anhydroglucose unit, leading to an almost equal weight ratio of cellulose and NaOH. In addition cellulose can be dissolved only in a narrow range of sodium hydroxide concentrations, from 6-10%. As a consequence the amount of cellulose that can be dissolved in NaOH-water solutions is 10%.

Efforts to dissolve cellulose in NaOH-water continued. It was rather straightforward to investigate the addition of compounds like urea, thiourea or ZnO in order to improve the state of cellulose dissolution [Harrison, 1928]. The first to report such attempts with thiourea is Laszkiewicz [Laszkiewicz, 1993], followed by the use of urea [Laszkiewicz, 1998]. A mixture of activation by hydrothermal or enzymatic treatments with the addition of ZnO was later on developed and termed Biocelsol [Ciechanska, 1996; Wawro, 2009; Vehviläinen, 2008]. Typical conditions are cellulose concentration of 6%, in 7.8% NaOH and 0.84% of ZnO. The best fibers spun with this method have tenacity of 1.8 $cNdtex^{-1}$ with 15% elongation at break. Recent results [Liu, 2011] showed that ZnO is quite efficient in delaying gelation of cellulose-NaOH-water solutions. It does not have much influence on the kinetic order of gelation or the junction zone structure of cellulose gels. In addition, the addition of ZnO does not change the properties of cellulose at the molecular level and does not improve the thermodynamic property of solvent towards cellulose.

Starting in 2000, the group of L. Zhang revisited the dissolution of cellulose in NaOH-water using derivatives [Zhou, 2000; Cai, 2005]. They used the preparation method of Isogai and Atalla [Isogai, 1998] and added first urea and then thiourea, inspired by the work of Laszkiewicz [Laszkiewicz, 1990; Laszkiewicz, 1993; Laszkiewicz, 1998]. Solutions with 4-8% were prepared and were stable enough to allow fiber spinning and membrane production.

Cellulose cannot be dissolved at temperatures above 10°C in the NaOH-urea-water system. Furthermore lower molar mass improves the dissolution yield [Qi, 2008]. The addition of urea [Zhou, 2000] has two advantages. It increases the dissolution yield and the solution stability. However, the addition of urea does not change the interactions between cellulose and NaOH and the addition of cellulose does not change the urea-water interactions [Egal, 2008]. These authors suggested that the addition of urea is decreasing the amount of free water, thus helping cellulose chains to stay in solution. Cai et al. [Cai, 2008] proposed a model where NaOH hydrates are hydrogen-bonded to cellulose molecules, and urea hydrates are bonded to NaOH hydrates at the surface of the cellulose-NaOH complex (Figure I.29). This structure, called inclusion complex, prevents the agglomeration of cellulose chains in solution, however, with time and increasing temperature, this arrangement is slowly displaced, leading to the formation of large aggregates with radius of gyration larger than 200 nm [Lu, 2011; Lue, 2011].

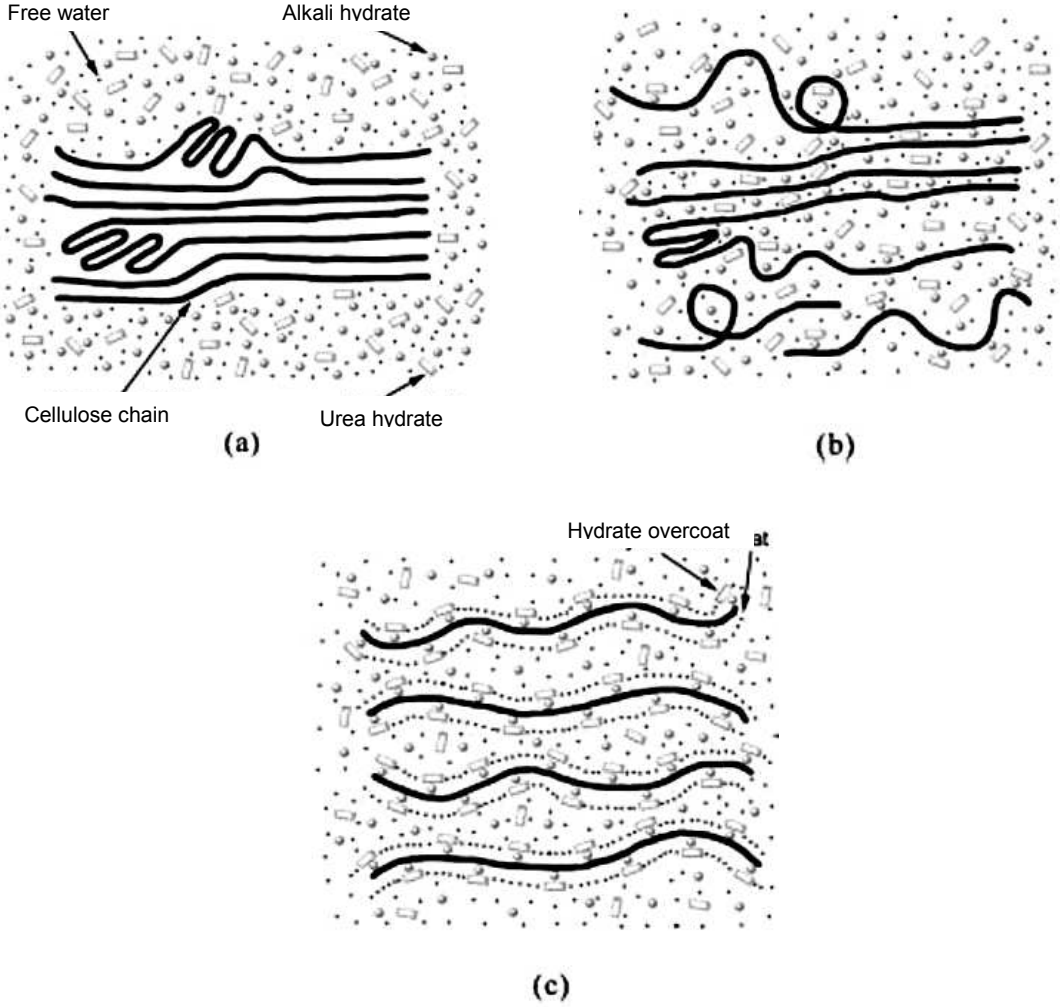

Figure I.31: Illustration from the dissolution process of cellulose in LiOH/urea and NaOH/urea aqueous solutions according to Cai et al.: (a) cellulose bundle in the solvent, (b) swollen cellulose in the solution, (c) transparent cellulose solution [Cai, 2005].

Fibers processed from the NaOH-water-system have properties lower than the ones of lyocell and viscose due to the low cellulose concentration (7-8%) [Egal, 2007], and the low molar masses required for this process. In addition, gelling creates problems especially at high temperatures, concentrations, and molar masses [Roy, 2003; Egal, 2006, Gavillon, 2008].

These difficulties are hampering industrial developments of this process and accordingly it is necessary to improve the dissolution of cellulose in NaOH-water.

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chapter II

Materials and methods

Materials and methods

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II.1. - Introduction

During this project, cellulose fibers from different production processes were extracted with nitren or treated enzymatically. The treated pulps were compared with the initial pulps concerning dissolution capacity in cold caustic soda, as well as the mechanisms and kinetics of dissolution. The different fractions were physically and chemically analyzed. Figure II.1 illustrates a simple flow chart of the research strategy, showing the different processes, fractions and analysis steps. In all procedures, when H_2O is mentioned, it refers to de-ionized H_2O.

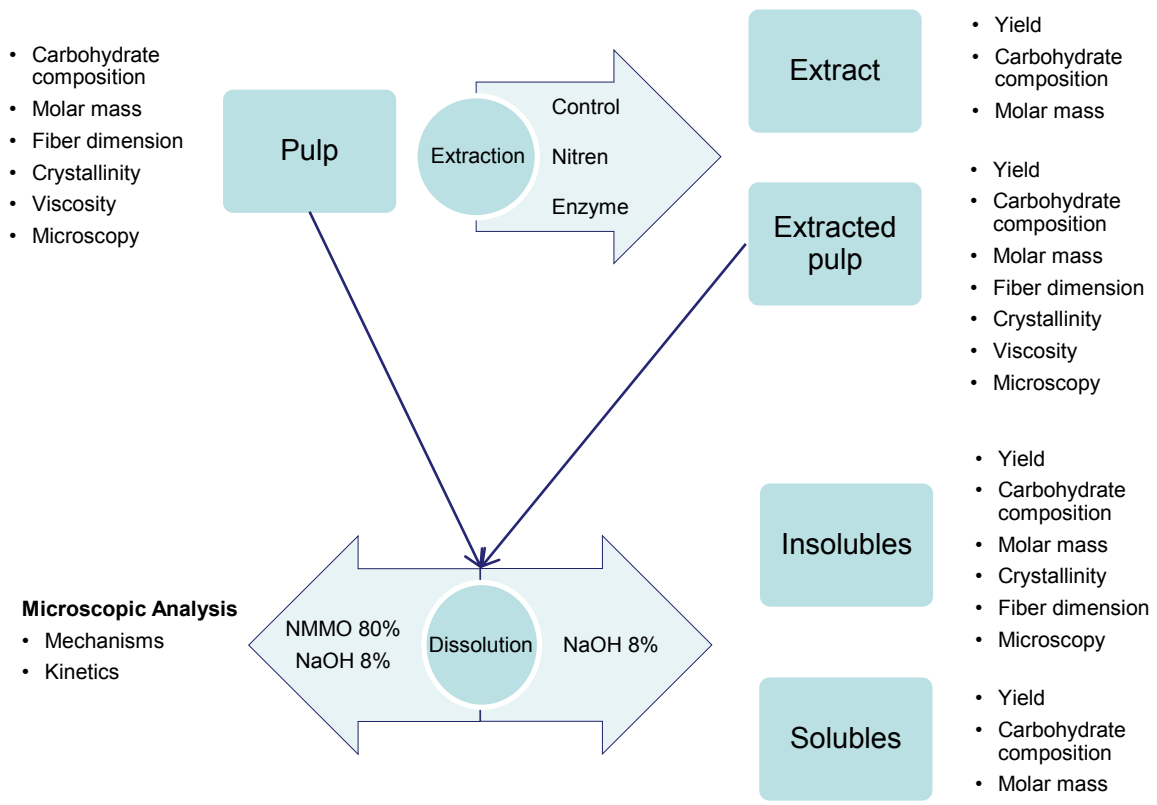

Figure II.1: Flow chart of the strategy drawn for the research work. The "Extract" is the part of the mixture of cellulose in nitren that went in solution. The "Extracted pulp" is the part that did not went in solution. The Extracted pulp is the initial pulp without the material that went in solution (the "Extract").

II.2. - Fibers analyzed

The origin and production process of different pulps are listed in Table II.1. In order to facilitate the identification of the samples, a short name was attributed to each sample and will be used along the text.

Not all the listed samples were subjected to the full sequence of tests described in Figure II.1 and some of them were only analyzed as reference samples.

Table II.1: List of pulps studied

#	Description	Short name	Provider	Country
01	**Be**ech Bleached **S**ulfite Pulp **N**ever **D**ried	**Be-S_ND**	Lenzing AG	Austria
02	**Be**ech Bleached **S**ulfite Pulp	**Be-S**	Lenzing AG	Austria
03	**Sp**ruce Bleached **S**ulfite Pulp **N**ever **D**ried	**Sp-S_ND**	Borregaard Schweiz AG*	Switzerland
04	**Sp**ruce Bleached **S**ulfite Pulp	**Sp-S**	Borregaard Schweiz AG*	Switzerland
05	**C**otton **L**inter **N**ever **D**ried	**CL_ND**	Milouban (M.C.P.) LTD	Israel
06	**C**otton **L**inter	**CL**	Milouban (M.C.P.) LTD	Israel
07	**S**oftwood **P**re-**h**ydrolisys **K**raft Pulp **N**ever **D**ried	**S-PhK_ND**	Buckeye	USA
08	**S**oftwood **P**re-**h**ydrolisys **K**raft Pulp	**S-PhK**	Buckeye	USA
09	**N**orthern **S**oftwood **S**ulphite Pulp	**NS-S_1**	Tembec (Canada)	Canada
10	**N**orthern **S**oftwood **S**ulphite Pulp	**NS-S**	Tembec (Canada)	Canada
11	**S**outhern **S**oftwood **S**ulphite Pulp	**SS-S**	Tembec (France)	France
12	**S**outhern **S**oftwood **S**ulphite Pulp	**SS-S_1**	Tembec (France)	France
13	**Euc**alyptus (**M**icro **C**rystalline **C**ellulose Grade)	**Eu_MCC**	Sappi (Saiccor)	South Africa
14	**Euc**alyptus (**Lyo**cell Grade)	**Eu_Lyo**	Sappi (Saiccor)	South Africa
15	**Euc**alyptus (**Vis**cose Grade)	**Eu_Vis**	Sappi (Saiccor)	South Africa
16	**Euc**alyptus (**Ac**etate Grade - **G**ood **R**eactivity)	**Eu_Ac-GR**	Sappi (Saiccor)	South Africa
17	**Euc**alyptus (**Ac**etate Grade - **P**oor **R**eactivity)	**Eu_Ac-PR**	Sappi (Saiccor)	South Africa
18	**Euc**alyptus Bleached **K**raft **P**ulp (Paper Grade)	**Eu-KP**	Sappi (Ngodwana)	South Africa
19	**Nit**ren **Ex**tracted **EucBKP_A** Never Dried	**Ni-Eu-KP_A**	Sappi (Ngodwana)	South Africa
20	**Ni**tren **Ex**tracted **EucBKP_B** Never Dried	**Ni-Eu-KP_B**	Sappi (Ngodwana)	South Africa
21	**H**ardwood Dissolving Pulp (**Ac**etate Grade)	**H_Ac**	Sappi (Rayonier)	USA
22	**Be**ech Bleached **S**ulfite Pulp GVZ **435**	**Be-S_435**	Lenzing AG	Austria
23	**Be**ech Bleached **S**ulfite Pulp GVZ **419**	**Be-S_419**	Lenzing AG	Austria
24	**Bact**erial **Cel**lulose	**BactCel**	FZMB GmbH	Germany
25	**Avicel**® PH-101	**Avicel**	Sigma Aldrich	Ireland
26	**Mix**ed **H**ardwood Bleached **K**raft **P**ulp (Paper Grade)	**MxH-KP**	Tembec (France)	France

*At the date of publication of this thesis, this pulp was no longer available in the market.

II.3. - Fiber preparation

Most of the pulps were received from industry in the form of dry pulp sheets. In order to facilitate the processing of the fibers, the pulp sheets had to be disintegrated. To achieve that, the pulp was soaked in water for 15 minutes, defibrillated with a disintegrator (Pendraulik, Typ 6HW(M) 4) for 5 minutes at 930 rpm. After removing most of the water with a centrifuge, the pulp was acclimated in a room with controlled atmosphere (20 ºC and 65% humidity), reaching a consistency of ~93%, and then packed hermetically in “ready to use” packs with 50 g or 30 g of fibers (dry solid). For the pulps received in the never dried state, the consistency was determined and the same packing procedure was done.

Swelling
↓
Disintegration
↓
Acclimatization
↓
“Ready to use” Packing

Figure II.2: Fiber preparation for experiments (left – dry pulp sheets, right – “ready to use” 50 g fiber packs).

II.4. - Nitren extraction

II.4.1. - Nitren solution preparation

In a 2000 ml glass reactor, 120.5 g of nickel (II) hydroxide and 146.2 g of tris(2-aminoethyl)amine (both Aldrich) were added to 2000 ml H_2O. The solution was stirred for 8.5 hours at 50 ºC. The excess of nickel (II) hydroxide was removed by centrifugation (30 min, 4400 rpm; Multifug 4 KR, Heraeus) and the solution recovered by decantation (Figure II.3).

The exact concentration of nickel was determined with an ICP-OES (Optima 3000, Perkin Elmer) at a wavelength of 231.6 nm. The plasma was maintained by inductive heating of argon gas with a 40 MHz generator. For calibration of the ICP-OES system a multi element standard (Merck 50036256) of 0.1 ppm, 1.0 ppm and 10 ppm was used [Kettenbach, 2007; Janzon, 2008].

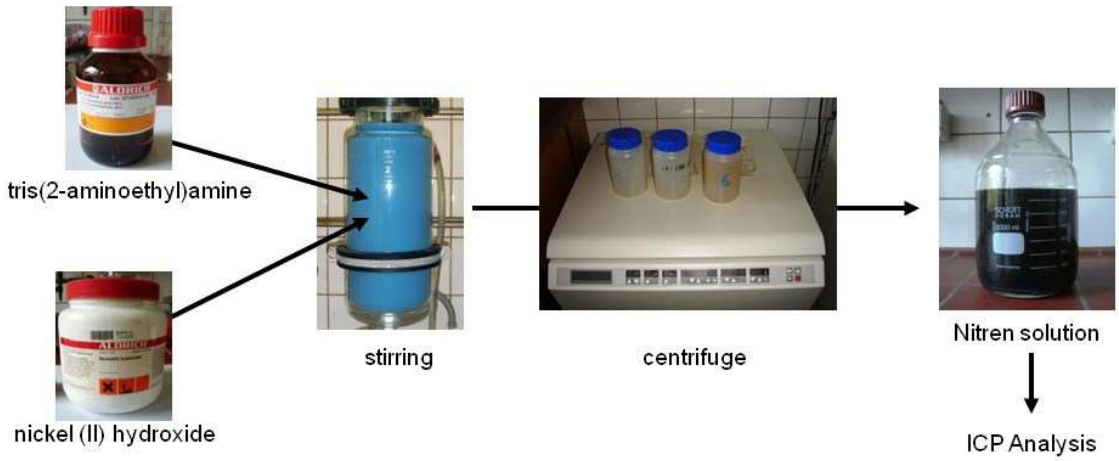

Figure II.3: Nitren solution preparation.

II.4.2. - Nitren treatment

For each nitren extraction, in a 10 l reactor, 50 g (dry weight) of pulp were added to nitren solution with concentration of 3, 5 or 7% (w/w %), with a liquid to pulp ratio of 10:1. The reaction took place for 30 minutes in a roller mixer (Roller Mixer from Ratik) with a controlled temperature chamber (Certomat® HK from B. Braun) set for 30 ºC. The fibers were separated from the Extract by filtration over a 1 l sintered glass filter (G1) under vacuum and mechanical pressure (Figure II.4). In order to remove extracts and the residual nitren complex, fibers were submitted to a three step washing procedure, with 2% NaOH, 5% lactic acid and boiling H_2O. The fibers were acclimatized, the consistency determined, weighted and hermetically stored prior to analysis. The extracted pulp (Figure II.4 d) was named with the starting pulp name, followed by the suffix "_3%", "_5%" or "_7%", according to the Nitren concentration used in the extraction (Ex.: "Be-S_5%").

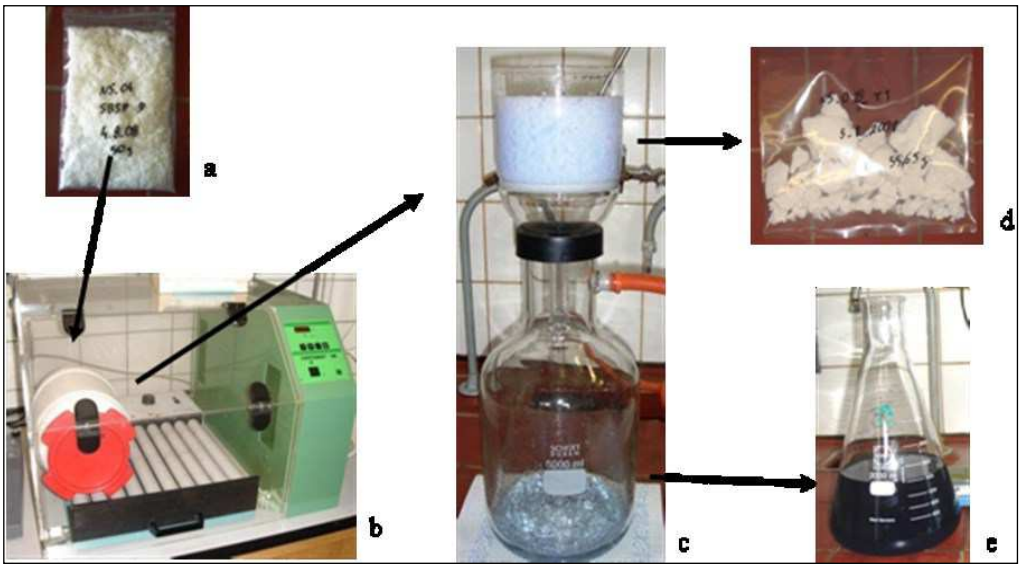

Figure II.4: Nitren extraction proceedings diagram (a - pulp, b - 10 l reactor in a chamber with a roller mixer, c - filtration and washing apparatus, d - stored extracted pulp, e – solvent with extracted fraction)

II.4.3. - Xylan recovery

For precipitation of the extracts, the liquid recovered from the nitren treatment (Figure II.4 e) was acidified with acetic acid to pH 4 and the suspension obtained was stored overnight at 5 ºC. The precipitated material was separated by centrifugation (20 minutes, 4400 rpm; Multifug 4 KR from Heraeus) and washed five times with an aqueous solution of lactic acid 5%, 2 times with H_2O, freeze dried (Alpha 2-4 LSC from Christ®), weighted and stored (Figure II.5).

This fraction of extracted material (Figure II.5 e) was named using the name of the corresponding extracted pulp, followed by the suffix "_Ext" (Ex.: Be-S_5%_Ext).

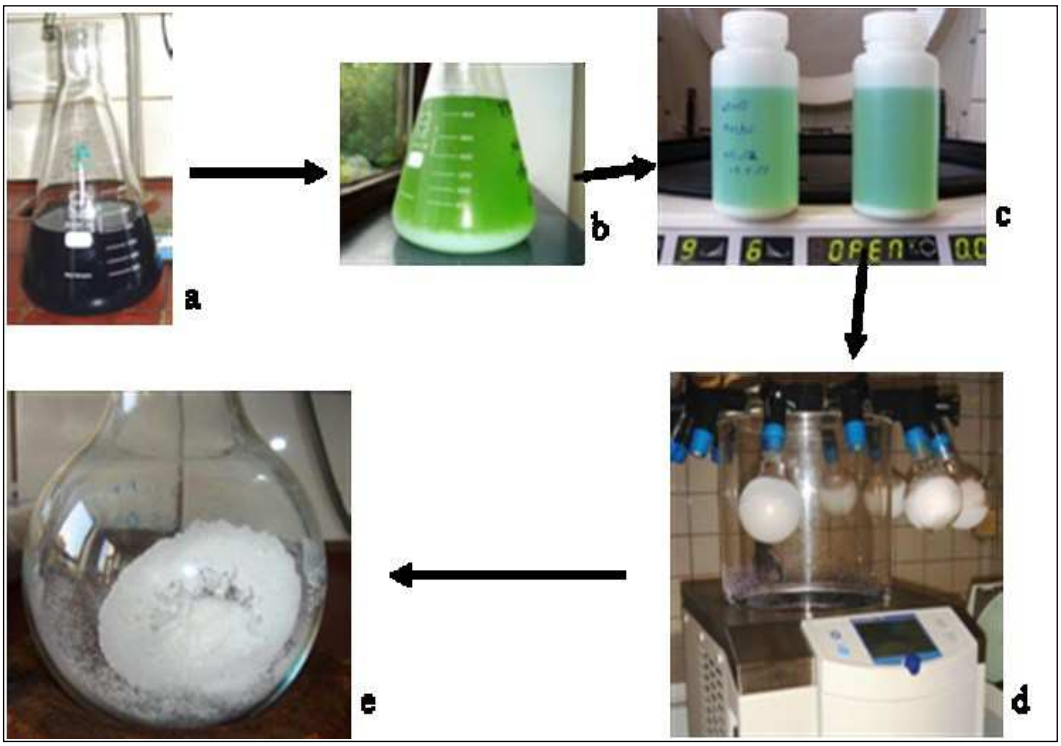

Figure II.5: Extracts recovery diagram (a - solvent with extracted fraction, b – suspension of solvent with precipitated extracts, c – centrifuged suspension, d – lyophilizator, e – extracts after freeze drying)

II.5. - Enzymatic treatment

II.5.1. - Enzymes

Table II.2: List of the enzymes used

#	Description	Short name	Provider	Country
01	BiopectinaseCCM*	CCM	Kerry Bio-science	Ireland
02	Pektinase L-40	L40	ASA Spezialenzyme GmbH	Germany
03	Cellulase (endo-β-glucanase)	EG	Megazyme	Ireland
04	Pectinex Ultra SP-L	SPL	Novozymes	USA

*At the date of publication from this Thesis, this enzyme was no longer available in the market.

II.5.2. - Enzyme solution preparation

The crude solutions were diluted in H_2O yielding a 10% (w/w %) aqueous preparation. All solutions were kept at 4 ºC.

II.5.3. - Enzymatic incubations

For each enzyme treatment, in a 1 l reactor, 20 g (dry weight) of pulp were added to the pre-heated enzyme solution prepared with a buffer at pH 3.5. The liquid to pulp ratio was 20:1. The reaction took place for 15 hours in a roller mixer within a temperature controlled chamber, set for 45 ºC. After extraction, the fibers were separated from the extract by filtration using a 1 l sintered glass filter (G1) with vacuum and pressure. In order to stop any activity of residual enzyme, the fibers were washed and the enzyme denaturized with boiling H_2O. The extracted pulp was acclimatized in a room with controlled atmosphere. The consistency determined, and the pulp was weighted and stored prior to further analysis. The extraction liquor was boiled and stored in the fridge at 4 ºC.

The extracted pulp was named with the starting pulp name, followed by the suffix "_CCM", corresponding to the biopectinase used (Ex.: Be-S_CCM), while the extracts were classified with the same nomination plus the additional suffix "_Ext" (E.: Be-S_CCM_Ext).

II.6. - Freeze drying

The samples were suspended in water, transferred into a round bottom flask, frozen in an ethylene-glycol bath at -18 ºC and transferred to a lyophilizator (Alfa 2-4 LSC from Christ®) with a condenser temperature set at -85 ºC, under a pressure of 1.03 mbar.

II.7. - Gravimetric solubility measurements in NaOH

This work was performed in the laboratories from both research institutions (CEMEF (Sophia Antipolis) and TI (Hamburg)). In order to validate the methodology, three different samples were analyzed in both laboratories, giving the same results.

II.7.1. - Solvent

The gravimetric dissolution tests were performed with an aqueous solution of NaOH 8%, using NaOH pellets with 98% purity (Sigma Aldrich).

II.7.2. - Dissolution test and observations

Two grams of pulp fibres (dry weight) were added to 66 g of H_2O, slightly stirred and stored at 4 ºC for 2 hours. 132 g of 12% NaOH solution were prepared and stored in an ethylene glycol bath set for -6.1 ºC during 2 hours. Both preparations were mixed together, giving a solution of 1% cellulose in an 8% NaOH solution. This mixture was stirred for 2 hours at -6 °C using a rotary overhead mixer at 1000 rpm. After this treatment, the soluble and insoluble fractions were separated by centrifugation at 0 °C with 9,000 rpm (equivalent to 9,050 *g*) for 8 min (Hettich Universal 320RHK centrifuge, 1620A rotor) according to Le Moigne et al [LeMoigne, 2010]. In Hamburg, was used a centrifuge Sorvall RC 5C Plus with a Sorvall HS-4 rotor at 6,860 rpm (equivalent to 9,060 *g*).

The NaOH-water insoluble fractions were washed with methanol and water prior to freeze drying. The soluble fraction was precipitated in methanol, washed with water, and recovered by freeze-drying. For each NaOH dissolution trial, three microscopic preparations of the insoluble freaction were observed by optical microscopy in transmission mode with a Metallux 3 (Leitz) optical microscope equipped with a high resolution 3-CCD camera JVC KY-F75U at CEMEF, while in Hamburg was used a Digital Microscope VHX-500F optical microscope from Keyence, with a VHZ 100 UR objective and a OP-72403 Short adapter. These procedures are schematized in Figure II.8.

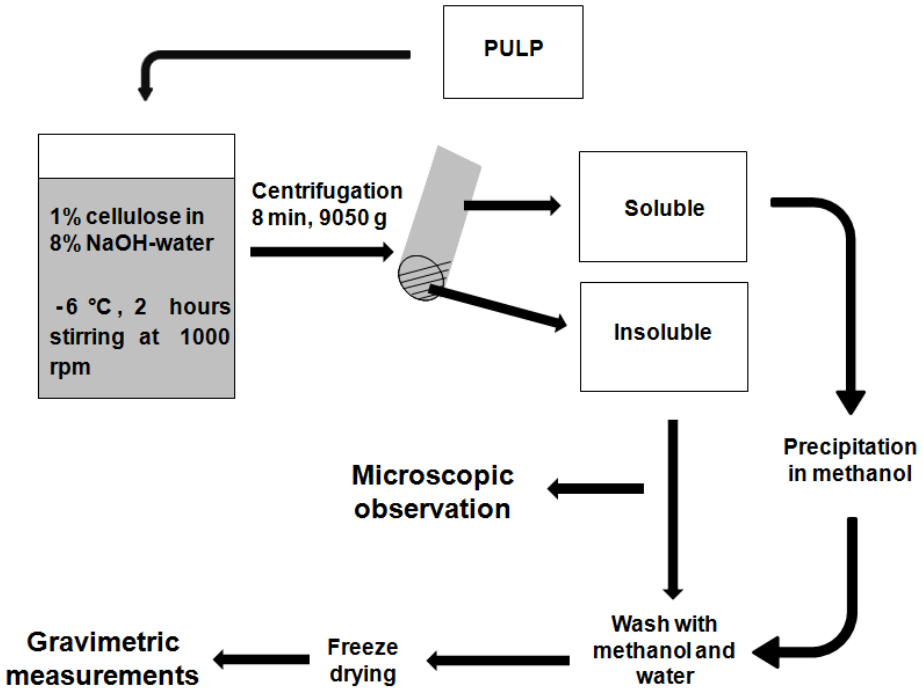

Figure II.8: Gravimetric dissolution in NaOH (flow diagram of the method)

In terms of nomenclature, the two fractions resulting from this experiment (soluble and insoluble) were named according to the name of the pulp used for dissolution, followed by the suffix "_Ins", for the Insoluble fraction (Ex.: Be-S_5%_Ins) or by the suffix "_Sol" for the Soluble fraction (Ex.: "Be-S_5%_Sol").

II.8. - Chemical analysis

II.8.1. - Nickel content analysis

Nickel concentration in the nitren extraction liquor was determined with inductively coupled plasma coupled with optical emission spectroscopy ICP-OES (Optima 3000, Perkin Elmer), according to Janzon el al. [Janzon, 2008a].

II.8.2. - Carbohydrate analysis

Pulps as well as insoluble and soluble fractions from NaOH-treatments, and the nitren-extracts recovered from the extractions with 5 and 7% nitren solutions were subjected to a two-step hydrolysis with H_2SO_4, according to the procedure described by Willför et al [Willför, 2009]. Monosaccharides were separated by borate complex anion exchange chromatography, at 60 °C using an Omnifit columns (6.6 mm * 115 mm) filled with MCI Gel CA08F (Mitsubishi). A linear gradient was run (0.7 $ml.min^{-1}$) made of A: 0.3 M potassium borate buffer pH 9.2 and B: 0.9 M potassium borate buffer pH 9.2 (0 min: 90% A and 10% B; 35 min: 10% A and 90% B). Sugar quantification was achieved by post-column derivatization with Cu-bichinconinate (0.35 ml min^{-1}) at 105 °C in a 30 m crocheted Teflon coil of 0.3 mm inner diameter followed by detection at 560 nm. Data was processed with the Dionex Chromeleon software. The value for cellulose was calculated adding the value of glucose to the value of cellobiose, applying a correction factor for cellobiose. The results were normalized and not corrected for sugar losses or water addition during hydrolysis.

II.8.3. - Molecular weight distribution

The molecular weight distributions of pulps after the various treatments and fractionations were measured by SEC MALLS after direct dissolution in DMAc/LiCl, according to Rörling et al. [Rörling, 2002]. A multi-detection HPSEC was used with the following settings: pump (Knauer Smartline Pump 1000), MALLS detector (Wyatt Dawn Helleos), RI detector (Shodex RI-101), columns: (First: PL-Gel 20 µm Mixed-A 300×7,5 mm, second and third: 2 × PL-Gel 20 µm Mixed-A LS 300×7.5 mm, Guard column: PL-Gel 20 µm Guard, 50×7.5 mm), eluent: DMAc/LiCl 0.9% w/v, flow: 1 mL/min, software: Astra 5.3, dn/dc: 0.147 mL/g. The nitren–treated extracts were analyzed as described by Janzon e al [Janzon 2008b], using HPSEC performed on GRAM columns (10; 30; 1,000; 3,000 Å, each 8×300 mm; Polymer Standard Service) using DMSO:H_2O (9:1) with addition of 0.05 M LiBr as eluent at 60 ºC and a flow rate of 0.4 mL/min. Molar masses were determined from viscosimetric (H502B, Viscotek) and refractive index detection (RI-71, Shodex) using a universal calibration with pullulan standards and the software PSS Win GPC Unity, Build 5403.

II.8.4. - Cuen intrinsic viscosity

The intrinsic viscosity of the pulp was measured using cuen (copper ethylene diamine solution), following ISO 5351 standard method.

II.9. - Fiber dimensional analysis

The kajaani FiberLab™ equipment from Metso Automation (Finland) was used to measure fiber dimensions. In 400 ml H_2O, 12 mg of hardwood fibers (16 mg for softwood) were added to make a homogeneous suspension. 50 ml of this suspension was transferred to the sample container and the automatic measurement was performed. The low consistency suspension flows thorough a narrow capillary and a laser light source in the capillary was used to control the two CCD cameras which capture images of the fibers. Once the fiber reaches the center of the optics section, a flashing Xenon lamp enabled both the cross-sectional and length CCD cameras to capture an image of the fiber. The images were then processed by the Kajaani FiberLab software to provide several fiber dimensional parameters [Copur, 2007].

II.10. - X-Ray diffraction

The equipment used for X-Ray experiments was a XPERT-PRO from PANanalytical. The Tension used to accelerate the electrons was 40 kV, with an electric Current of 30 mA and a copper anode, with a wavelength of λ = 0.1542 nm. The diffraction angles were obtained by one scan in the range 5 to 60 º, with a frequency of 0.04.

II.11. - Surface analysis - FEG-SEM

Scanning electron microscopy experiments were performed on a SEM-FEG (Field Emission Gun - Scanning Electron Microscopy) ZEISS Supra 40 at acceleration voltage of 3 or 7kV. To avoid electrical charging effects, thin layer of gold-palladium were deposited by sputtering onto the surfaces of some samples. Before analysis the smples were dried in a room with controlled atmosphere (20 ºC and 65% humidity), reaching a consistency of ~93%.

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chapter III

Improving dissolution of wood dissolving pulps by removing residual xylan with an organometallic complex (nitren) treatment

Improving dissolution of wood dissolving pulps by removing residual xylan with an organometallic complex (nitren) treatment

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III.1. - Introduction

Having in mind the chemical composition of the different cell wall layers, it is known that the primary wall is, in comparison with the inner cell wall layers, enriched in lignin, hemicellulose and pectin. During the manufacture of dissolving pulps, the lignocellulosic material is usually submitted to acid sulfite or pre-hydrolysis kraft pulping, followed by several bleaching stages. Through these treatments, the feedstock is delignified and the hemicelluloses are hydrolyzed and extracted. Nevertheless, a residual amount of xylan (about 1.3-3.6% in acid sulfite and 0.9-3.5% in pre-hydrolysis kraft pulps) remains in the fibers [Sixta, 2006].This chapter focuses on the influence of the selective removal of xylan from the cell wall on the cellulose dissolution in NaOH-water. NaOH-water is a potential good candidate for dissolving cellulose at industrial scale owing to its ease of use and low toxicity of the chemicals used but this way is hampered by the poor solubility of cellulose, which strongly limits its applicability. The present work will consider the influence of xylan on dissolution.

Considering the raw materials used in the cellulose derivative and regenerated cellulose industry, several commercial dissolving pulps were selected. In order to decrease the residual xylan, each pulp was treated with aqueous solutions containing 3, 5 and 7% nitren.

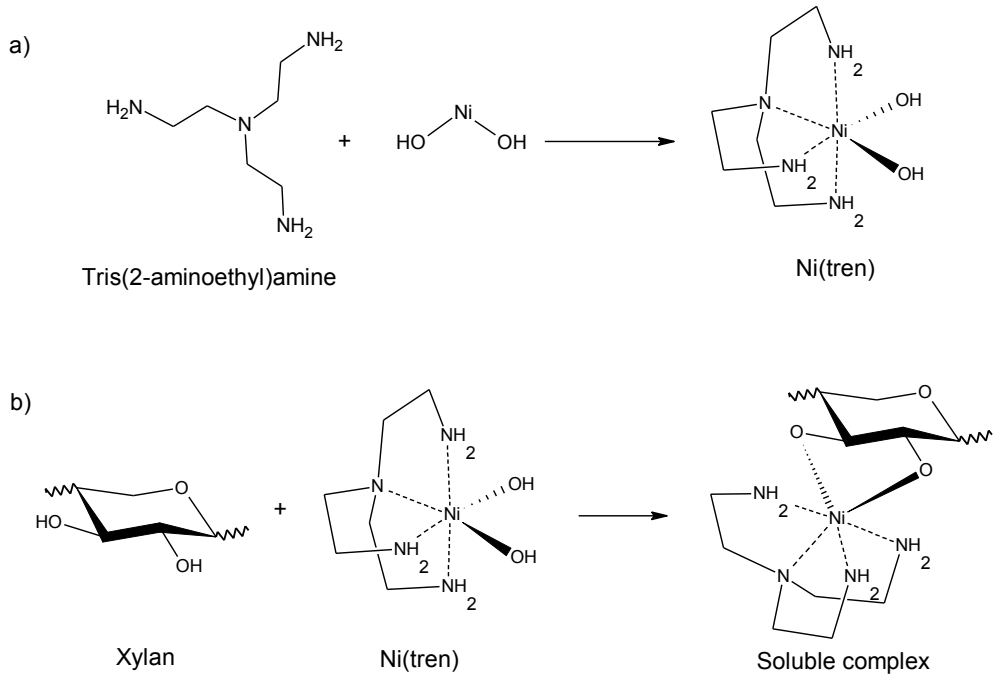


Figure III.1: a) Tris(2-aminoethyl)amine and $Ni(OH)_2$ form the complex nitren; b) nitren dissolves xylan by binding to the hydroxyl groups in position C2 and C3 (trans configuration) after deprotonation (adapted [Janzon, 2008a]).

This solvent is an organometallic complex developed to be used as a cellulose dissolving agent [Kettenbach, 2007]. However, in diluted form, it was shown that it can be used to selectively extract xylans from paper grade pulp (Figure III.1a). The Nitren complex dissolves the xylan molecules by binding to the hydroxyl groups in position C2 and C3 (trans-configuration) after deprotonation (Figure III.1b). The reverse reaction, with acidification, will release the xylan, which precipitates. Hence, the xylan can be recovered and the solvent recycled. This technique is considered to be suitable to upgrade paper grade pulps into dissolving pulps [Janzon, 2008a]. This same method was applied in the present work, following the proceedings from Janzon et al [Janzon, 2008a].

The influence of these nitren treatments on the pulp dissolution was assessed by studying the dissolution yields and the dissolution mechanisms. The solubility performance of the treated pulps was calculated by gravimetric studies (dissolution yield) in a weak solvent of 8% NaOH in H_2O at a temperature of -6 ºC. All fractions from the pulp treatments and the undissolved residues from the dissolution process were analyzed regarding their chemical and macromolecular structure (molar mass distribution, carbohydrate composition, CUEN intrinsic viscosity and fiber dimensional analysis).

III.2. - Results and discussion

III.2.1. - Sp-S - Spruce bleached sulfite pulp

The starting softwood bleached sulfite pulp (Sp-S), with intrinsic viscosity of 620 ml/g, was subjected to extractions with nitren concentrations 3, 5 or 7%. The initial pulp, as well as the nitren extracted pulps, was further studied concerning their solubility in NaOH. The whole treatment is depicted in Figure III.2.

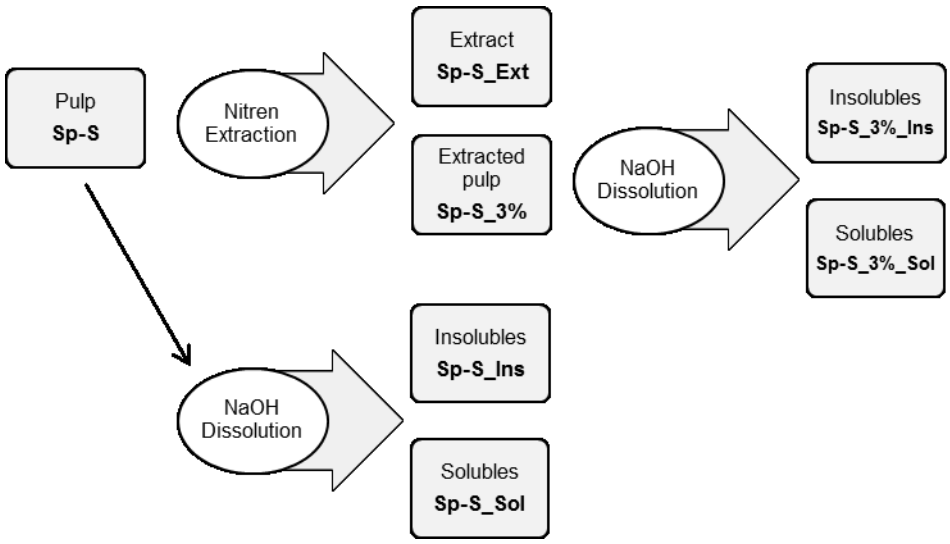


Figure III.2: Treatments diagram for nitren extraction and NaOH solubility test. For each fraction a name example is given in bold.

Nomenclature: considering this procedure, the different fractions are named in Table III.1. The initial Sp-S is dissolved in NaOH-water. It is giving two fractions, a dissolved one (named Sp-S_Sol) and an insoluble one (named Sp-S_Ins). When the pulp is treated e.g. with a 3% nitren solution, this treatment is extracting a small amount of products. This nitren-soluble extract is called Sp-S_3%_Ext. The remaining pulp residue is named Sp-S_3% (Table III.1). In resemblance with the initial pulp, when incubated with stirring in NaOH-water, it gives a soluble part, named Sp-S_3%_Sol and an insoluble part, named Sp-S_3%_Ins. The same nomenclature applies for the nitren concentrations of 5 and 7%.

In terms of analytical work, all fractions were submitted to recovery yield determination by gravimetric measurements and measurement of carbohydrate composition and molar mass distribution. The initial pulps and insoluble fractions were observed by microscopy and fiber dimensions and intrinsic viscosities were measured.

The values of the dissolution yield in NaOH have an estimated error of ±1.0% in absolute value. This ± 1.0% was verified for all the range of results, meaning that the relative error

decreased with the increase of dissolution yield. Probably this reflects an error inherent to the material/method used. In practice, this error can be illustrated by the following examples from Table III.1: Sp-S has a dissolution yield of 26.5 ± 1.0% and the Sp-S_7% has a dissolution yield in NaOH of 39.5 ± 1.0%. For the pulps recovery yield after extractions, the error is ± 2%, and the viscosity measurements show an error of ± 10ml/g. All the reported values consist of the average of at least 2 measurements that fit within the error bar.

Table III.1 gives the residue and extract yields after nitren extraction. The yields of the fractions "Pulp" and "Extracts" after nitren treatments are calculated based on the initial untreated pulp used for nitren extractions (50 g), while the yields for the "Insoluble" and "Soluble" fractions from NaOH-treatments are based on the amount of pulp used in the dissolution trials (2 g). In the following, the samples will be annotated with the names listed in Table III.1.

Table III.1: Complete set of treatments performed including the yields of extract and residue from nitren extraction and the yields from NaOH fractionation. The nomenclature from the last column will be used throughout the text.

Treatment	Nitren extraction		NaOH Dissolution		Sample name
	Fraction	Yield (%)	Fraction	Yield (%)	
None	Pulp	100.0			Sp-S
			Insoluble	73.5	Sp-S_Ins
			Soluble	26.5	Sp-S_Sol
3% Nitren	Pulp	96.5			Sp-S_3%
	Extract	0.2			Sp-S_3%_ Ext
			Insoluble	65.5	Sp-S_3%_Ins
			Soluble	34.5	Sp-S_3%_Sol
5% Nitren	Pulp	93.6			Sp-S_5%
	Extract	2.7			Sp-S_5%_ Ext
			Insoluble	65.0	Sp-S_5%_Ins
			Soluble	35.0	Sp-S_5%_Sol
7% Nitren	Pulp	81.0			Sp-S_7%
	Extract	18.0			Sp-S_7%_ Ext
			Insoluble	60.5	Sp-S_7%_Ins
			Soluble	39.5	Sp-S_7%_Sol

III.2.1.1. - Effects of nitren treatments on fiber structure and composition

After extractions with nitren, the two recovered fractions were gravimetrically analyzed and their yields calculated (Figure III.3). With the increase of nitren concentrations, the proportion of extracted material is continuously increased (Table III.1). While at 3% nitren concentration only 0.2% of extracted material is recovered, the extract amounts to 2.7% for 5% nitren and even to 18% with 7%. Considering that the initial untreated pulp contains only 6.3% hemicellulose (Table III.2), it becomes apparent that, at least with 7% nitren, substantial amounts of cellulose were extracted. Therefore, from this gravimetric analysis it can be concluded that for a nitren concentration higher than 5% the method is not effective for removing only xylans, since cellulose is also dissolved. This is similar to what was found for paper grade pulps, where the extracts after a 7% nitren treatment were containing a large amount of cellulose, while very little cellulose was solubilized in 3 and 5% nitren [Janzon, 2008a].

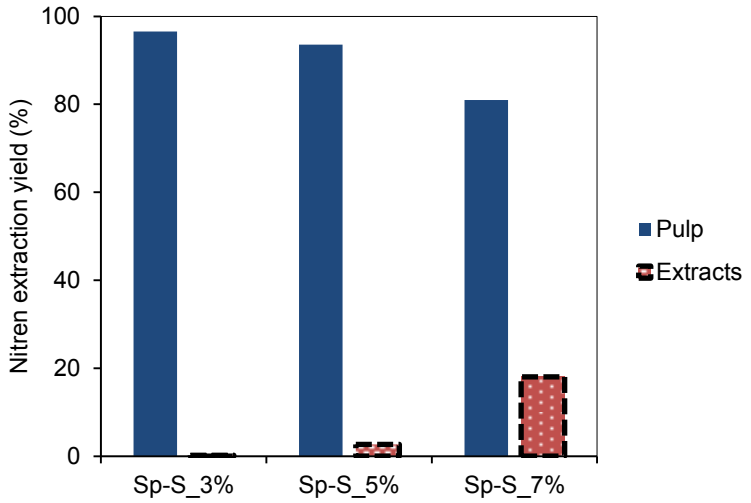


Figure III.3: Yields of the two fractions (see Table III.1) recovered after initial pulp fiber treatments with different nitren concentrations.

Table III.2 gives the results of the carbohydrate composition and the cuen viscosity of all samples. The total hemicellulose content decreases from 6.3% in the untreated pulp down to 3.5% for the 7% nitren treated pulp. The major contribution, as expected from the results on paper grade pulp, is due to the decrease of the content of xylan. Two remarks must be made. First, xylan is not fully removed even at 7% nitren since only 81% of it is removed, to be compared with 98% extracted from fully bleached eucalyptus and birch kraft pulps [Janzon, 2008a]. Second, contrary to paper grade pulp where the nitren treatment was very selective towards xylan (not extracting mannan), it is apparent that a fraction of mannan is also removed from the dissolving pulp used in this study (Table III.2).

Table III.2: Carbohydrate composition for all fractions after nitren treatments and cuen viscosity results for the different pulp fractions.

Sample	Carbohydrate Analysis (%)					Cuen
	Mannose	Xylose	Total Glucose	Total Hemicellulose	Residue	Viscosity (ml/g)
Sp-S	3.9	2.1	93.6	6.3	0.2	620
Sp-S_3%	3.5	1,0	95.1	4.6	0.3	510
Sp-S_3%_Ext	1.4	18.8	79.2	20.8	0.0	-
Sp-S_5%	3.1	0.6	96.0	3.9	0.2	480
Sp-S_5%_Ext	2.9	8.8	85.8	12.6	1.7	-
Sp-S_7%	2.8	0.4	96.5	3.5	0.0	430
Sp-S_7%_Ext	3.7	2.7	92.7	6.6	0.7	-

An increased cellulose purity of the pulps after nitren extraction can be observed (Figure III.4). The selectivity of the xylan removal can be evaluated from the carbohydrate analysis. The amount of mannose is twice the amount of xylose in the starting pulp, which is due to the high galactoglucomannan content of the softwood starting material. After nitren extractions, both mannose and xylose concentrations decrease. For nitren concentrations of 3, 5 and 7%, the mannose content in the pulps decreases by 10, 20 and 28%; while the xylose content is decreased by 52, 71 and 81%, respectively. Thus, the nitren extraction is more efficient for xylans compared to mannans.

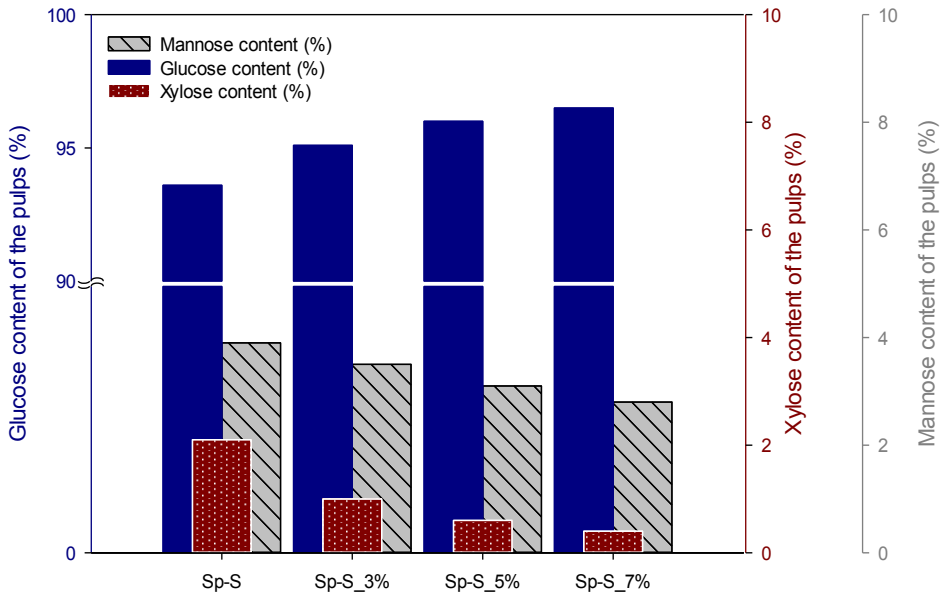


Figure III.4: Effect of the nitren extractions on the carbohydrate composition of treated pulps

The increased mannan extraction found here compared to the previous work of Janzon et al. [Janzon, 2006] might be due to the different type of pulps under investigation. Here, we used a softwood dissolving pulp from the sulfite process while Janzon used a softwood kraft paper grade

pulp. The mannans, being more concentrated in the inner cell wall layers [Janzon, 2006], are not as accessible for the nitren liquor in paper grade kraft pulps as in dissolving sulfite pulp where the outer layers of the cell wall are more degraded, leading to a greater solubility of mannans in the alkaline nitren extraction process. For all nitren concentrations, cellulose is extracted by the nitren treatment, since high glucose concentrations are obtained in the extracted material, which cannot be correlated to the glucose content of the extracted galactoglucomannans.

Increasing nitren concentration decreases the cuen viscosity of the pulp. This is surprising since previous studies on paper grade pulp showed higher cellulose stability and an increased molar mass of the overall pulp due to the removal of low molar mass components [Janzon, 2008a].

The effect of the three nitren treatments on the molecular mass distribution is depicted in Figure III.5. In the initial untreated pulp, peak intensity in the low molecular weight zone confirms the presence of hemicelluloses. All nitren treatments strongly decrease this low molar mass shoulder in the distribution, indicating the extraction of hemicellulose in accordance with the carbohydrate analysis. The main peak is shifting in the direction of lower molar masses, confirming the results of cuen viscosity (Table III.2), and showing that cellulose chains of Sp-S are partly degraded by the nitren treatment, in contrast to the paper grade pulp.

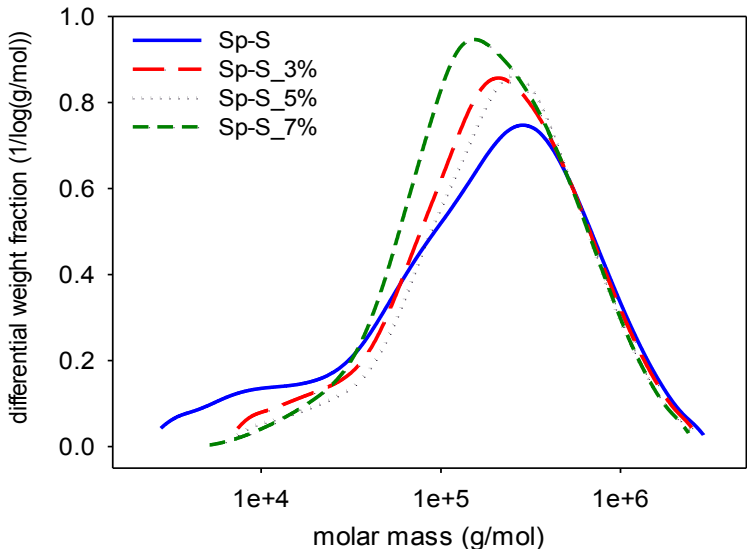


Figure III.5: Effect of nitren treatments at three nitren concentrations of 3, 5 and 7% on the molar mass distribution of the pulps.

Nitren has also the effect of increasing the crystallinity index of the pulps, as can be understood from Figure III.6. The initial pulp has a crystallinity index of 65%. After treatments with nitren concentrations of 3, 5 and 7%, the crystallinity indices are respectively 70%, 68% and 63%. By removing amorphous xylan, the crystallinity index increases (for nitren concentrations of 3 and 5%) up to the 7% concentration where cellulose is also removed and probably strongly affected by the treatment, with the consequence of a crystallinity index decrease. The crystallinity index is defined and was calculated according to Segal et al:

C_{Ir} (%) = $[(I_{220} - I_{am})/I_{200}]\times100$ [III-1]

, where I_{200} is the peak intensity corresponding to both the amorphous and crystalline fractions of cellulose I (maximum intensity between 2θ = 21 and 23 º) and I_{am} is the peak intensity of the amorphous fraction (minimum peak intensity between 2θ = 18 and 20 º) [Segal, 1959, Jonoobi, 2011, Ramos, 2005].

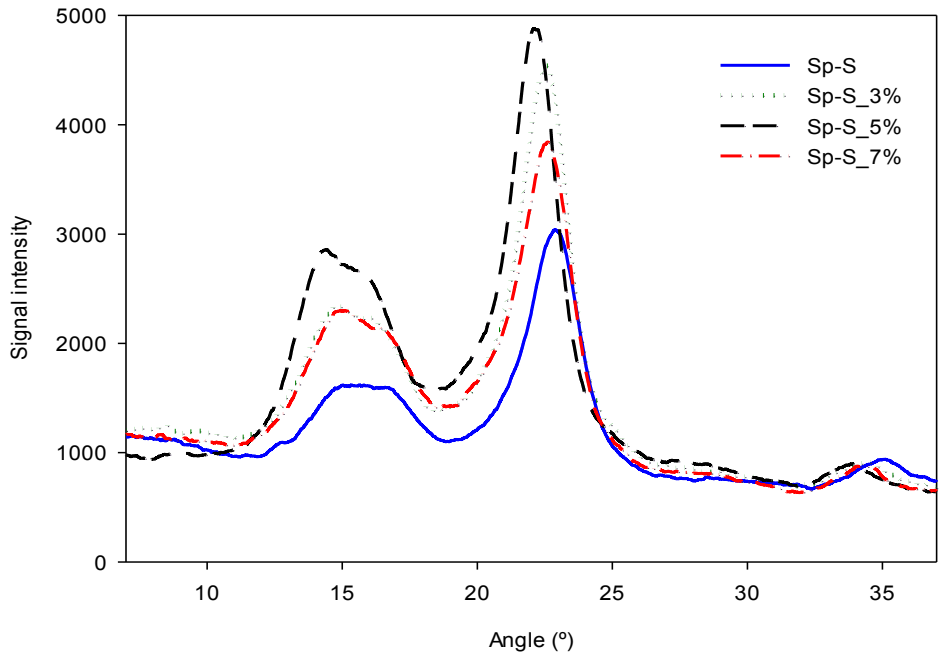


Figure III.6: X-ray diffraction diagram of Sp-S without and with nitren treatments.

In order to better understand these effects, the fiber morphology of the nitren extracted pulps was evaluated using FiberLab equipment. The fiber length and fiber width, as well as the percentage of fines and fibrillation are summarized in Figure III.7.

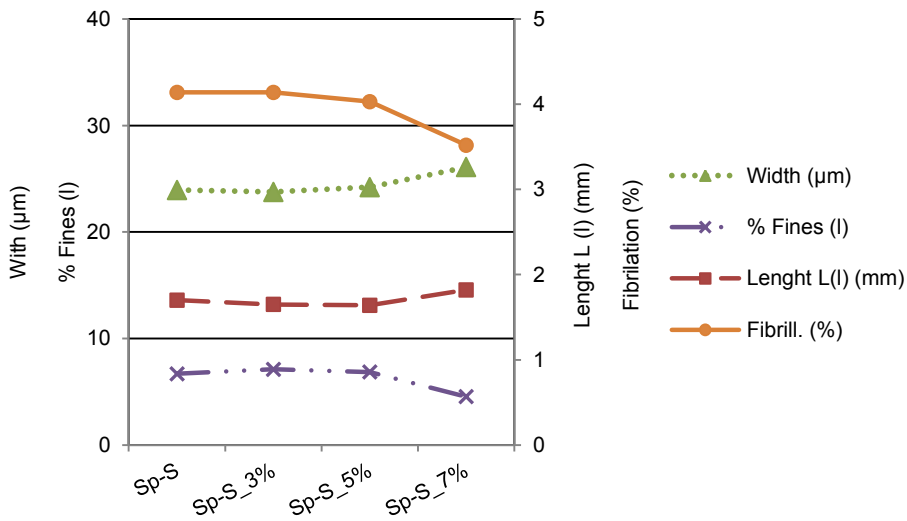


Figure III.7: Effects of nitren extraction on the fiber morphology parameters of the pulps and relation with the dissolution nitren yield.

Treatments with 3% and 5% nitren are not affecting the pulp while with 7% nitren, the fibrillation and fines fraction are reduced. This indicates that fines and fibrils are dissolved by the solvent. Consequently the average fiber length is increasing slightly after extraction with 7% nitren. The fiber width is as well increasing, most likely as a result of fiber swelling after the treatment with 7% nitren solution or due to the dissolution of the thinner fibers and fibrils. In order to have a better insight of this increase, the fiber width distributions of the pulp before and after treatment with 7% nitren were compared (Figure III.8). When overlapping the two charts (Figure III.8 b), two facts can be noted: there is a minor frequency of fibers with lower width after extraction, which verifies the dissolution of the thinner fibers and fibrils; and at the same time it can be observed a minor shift of the distribution towards higher values probably due to the occurrence of a slight swelling of the fibers with the nitren treatment.

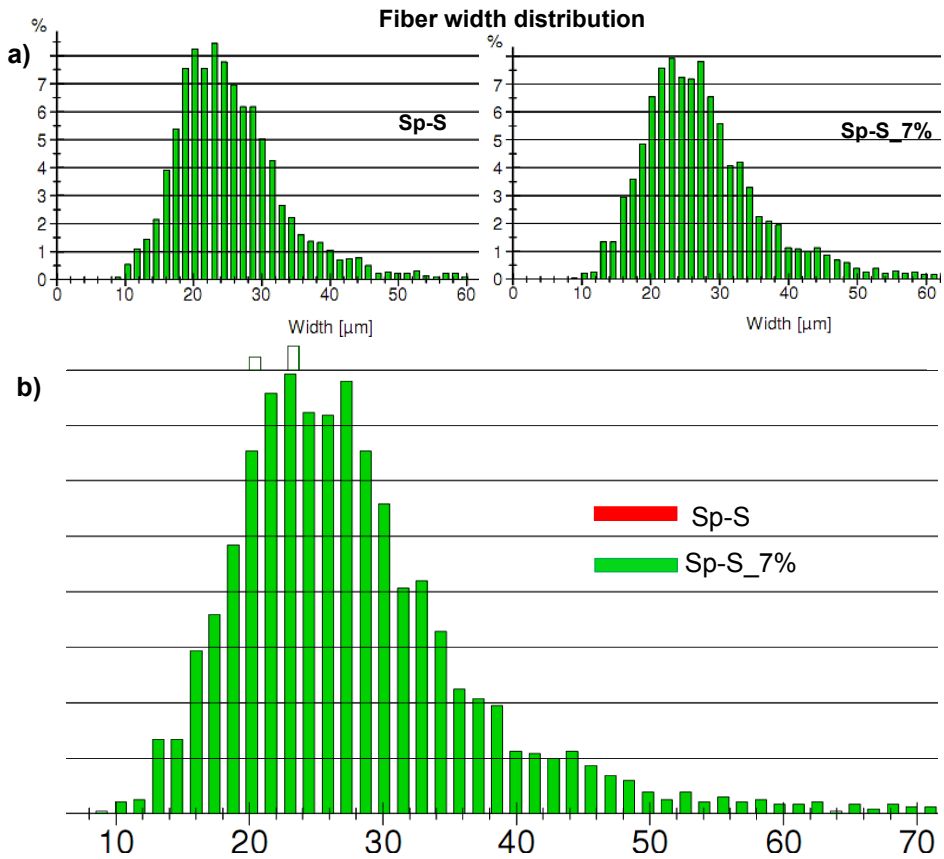


Figure III.8: Pulp fiber width distributions. a) Individual distribution for initial pulp (Sp-S) and pulp extracted with 7% nitren (Sp-S_7%). b) Overlying of fiber width distributions from both untreated and treated pulps for a better comparison.

An effect of the nitren treatments on the fiber surface and partial cellulose dissolution is observed when observing untreated and nitren-treated fibers by scanning electron microscopy (Figures III.9 - III.12). No evident difference exists between the initial untreated pulp (Figure III.9) and the pulp treated with 3% nitren (Figure III.10). After extraction with 5% nitren (Figure III.11), most of the fibrils were dissolved from the fiber surface. When pulps were treated with 7% nitren (Figure III.12), no fibrillation can be seen on the fibers surface. This microscopic observation shows also that the extraction with 7% nitren is dissolving cellulose in different parts of the fiber

wall: in the inner fiber walls, traces of what can be considered as regenerated cellulose can be seen (a) just beside a bursted balloon (b), which indicates that cellulose was dissolved at the interior of the fiber, released with the bursting of the balloon and precipitated on the fiber surface neighboring the balloon. SEM images show that the outer layers are also partially dissolved, since parts of the S2 wall are visible (d).

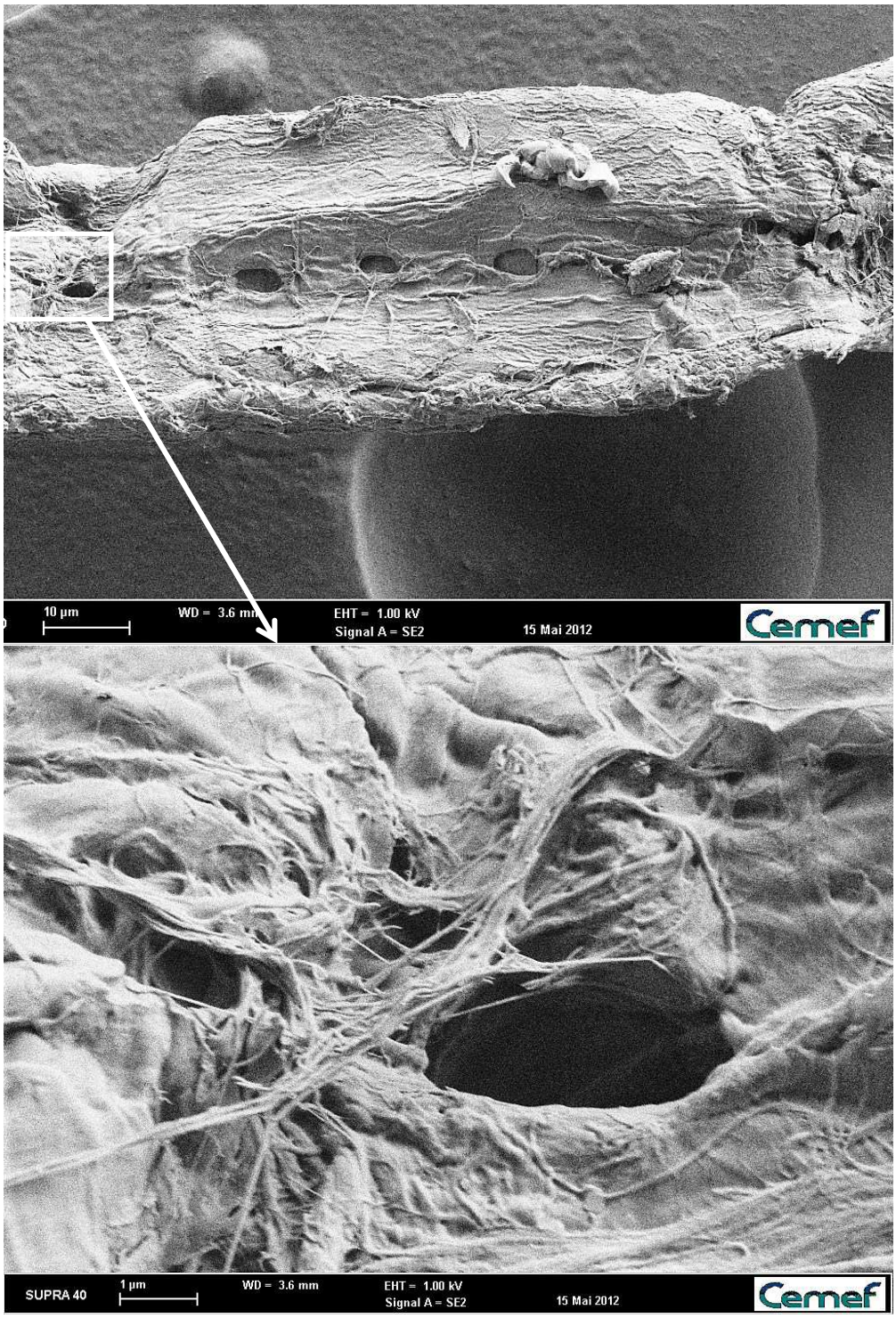


Figure III.9: Scanning electron microscopy images of untreated fibers (Sp-S), the picture below is an amplified detail of micro fibrils (with less than 100 nm thickness) attached to the surface surrounding a pit opening.

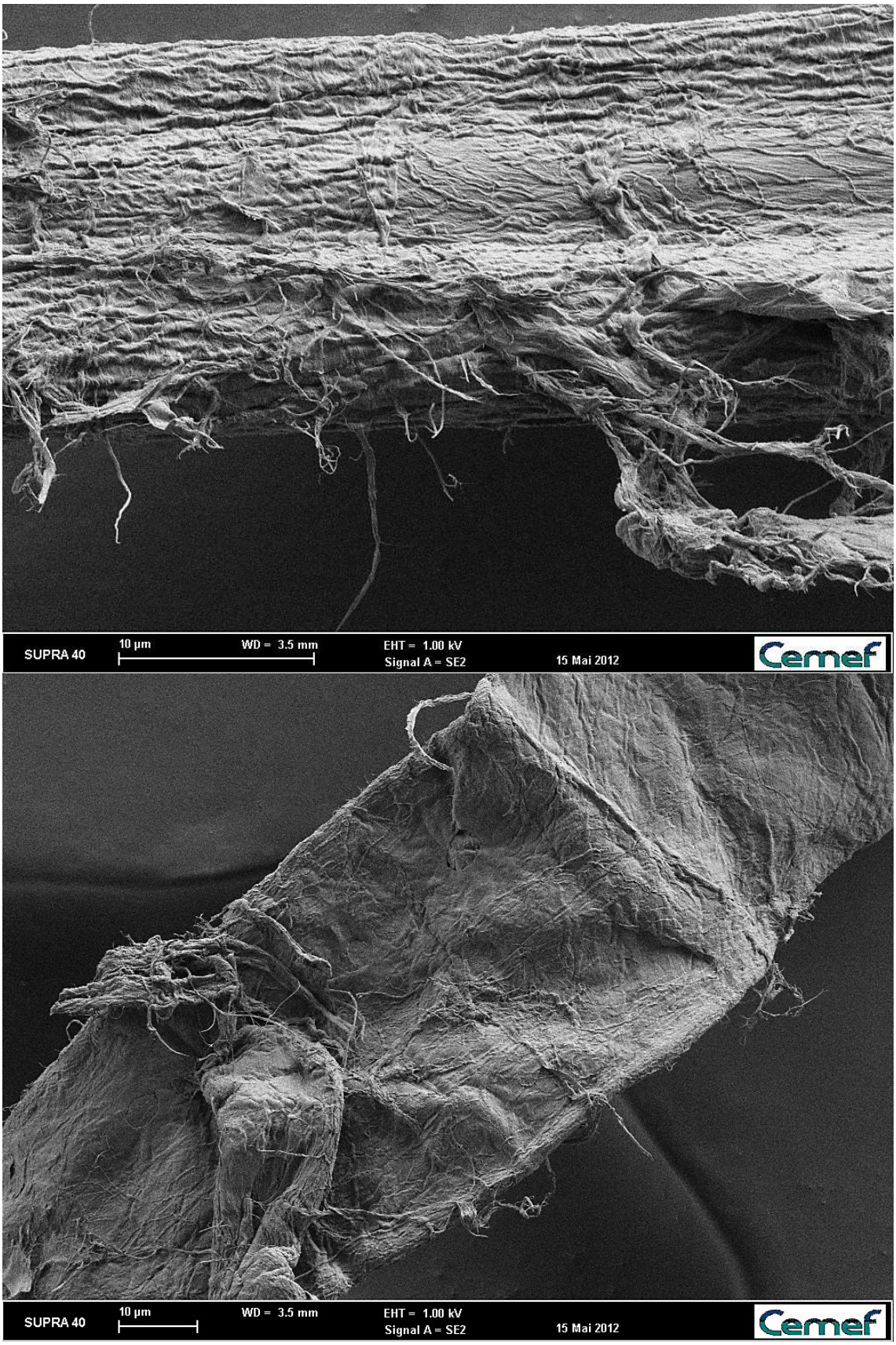


Figure III.10: SEM images of 3% nitren-treated fibers (Sp-S_3%).

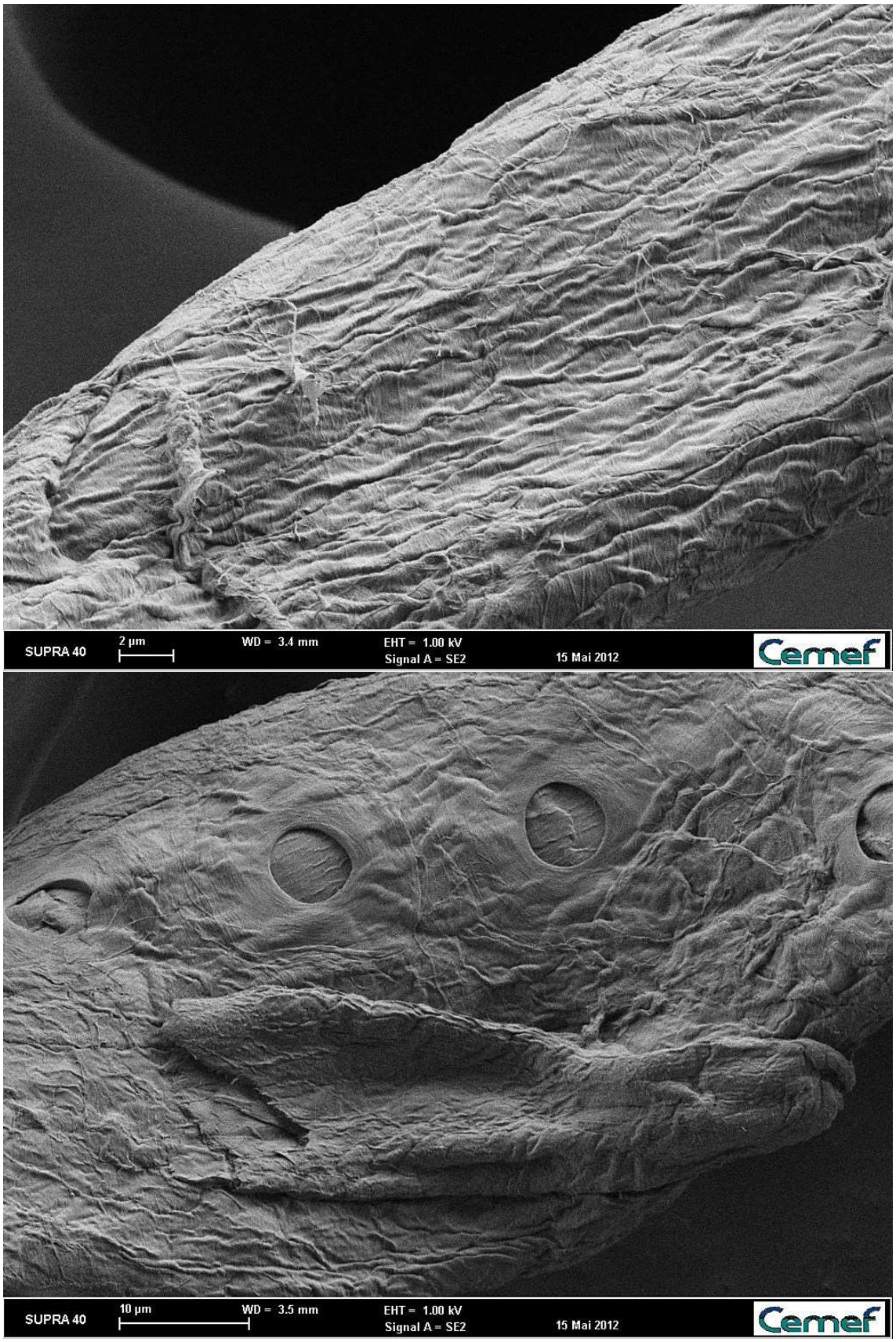


Figure III.11: SEM images of 5% nitren-treated fibers (Sp-S_5%).

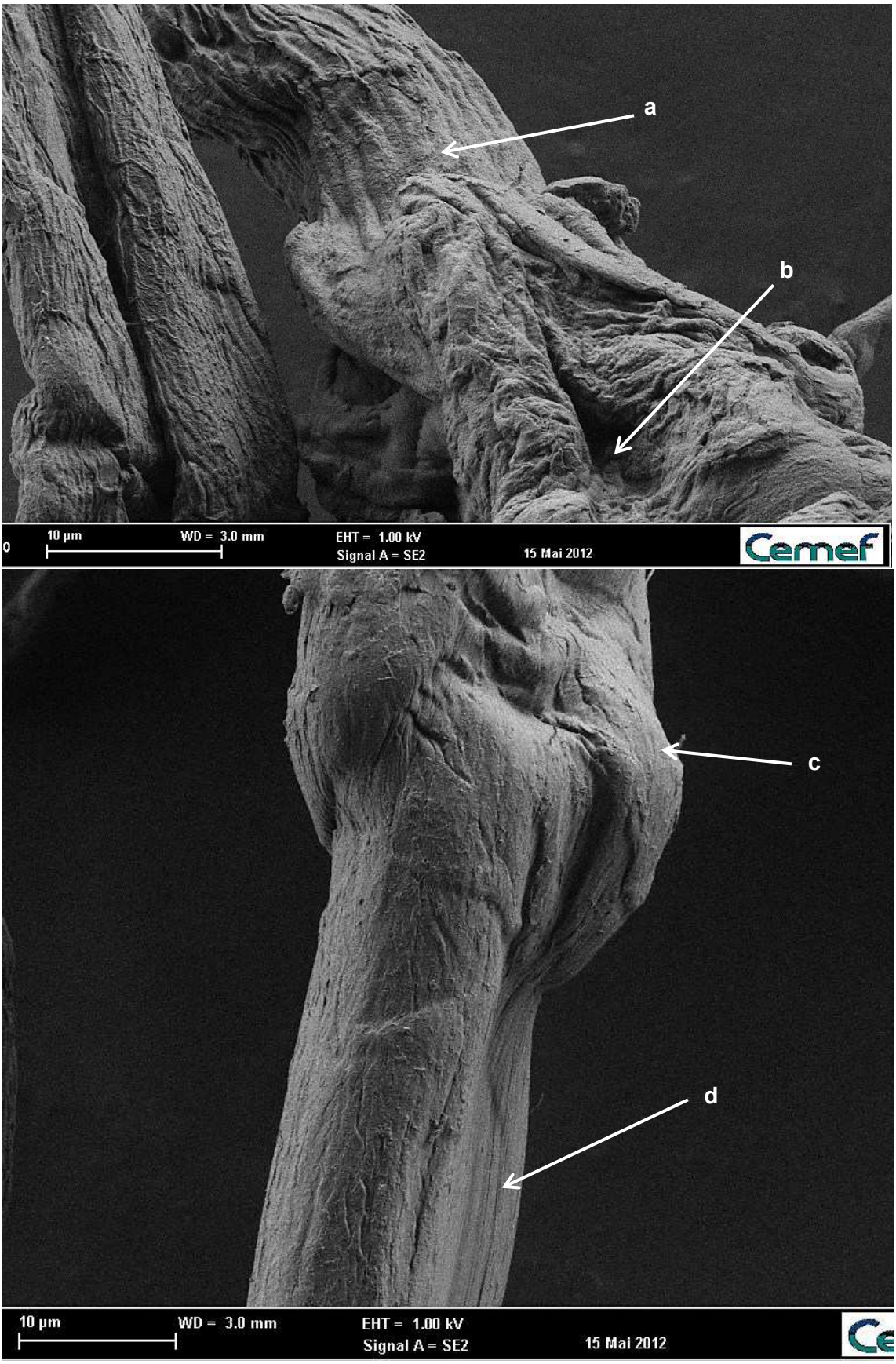


Figure III.12: SEM images of 7% nitren-treated fibers. The arrows spot regenerated cellulose materials on the fiber surface (a), one bursted balloon (b), one collapsed balloon (c) and the secondary wall (d).

III.2.1.2. - Effects of nitren treatments on the dissolution of spruce bleached sulfite pulp in NaOH-water

In order to study the effect that the physical and chemical modifications promoted by the nitren extractions have on the chemical accessibility of spruce bleached sulfite pulp fibers, pulps with and without nitren extraction were treated with NaOH-water and fractionated into a soluble fraction and an insoluble fraction. Weak solvents, such as aqueous solutions of caustic soda, have a high sensitivity for minor changes on the accessibility of the fibers, while strong solvents like monohydrated *N*-methylmorpholine-*N*-oxide will dissolve the fibers by fragmentation followed by complete dissolution (mode 5 according to Cuissinat et al [Cuissinat, 2006a]), regardless the pretreatment of the pulps.

After separation, recovery and lyophilization of the soluble and insoluble fractions, the yields of dissolution were gravimetrically measured and the results are given in Figure III.13. The dissolution yield is considered all the material which is not recovered as insoluble, according to the following formula:

$$\text{Dissolution yield (\%)} = \frac{2\text{-Ins}}{2} \times 100 \qquad \text{[III-2]}$$

, where “2” corresponds to the initial 2 grams of material, and “Ins” is the amount of recovered insoluble.

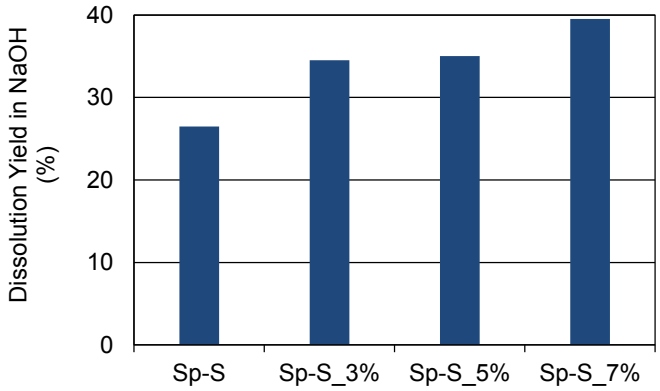


Figure III.13: Dissolution yield (fraction of dissolved pulp) for the different pulps after dissolution in NaOH-water.

There is a clear increase of NaOH dissolution yield with increasing nitren content (Figure III.13). Up to nearly 40% of the dissolving pulp can be dissolved compared to 26% with the initial untreated dissolving pulp. It is still smaller than what can be achieved by NMMO-water (monohydrated *N*-methylmorpholine-*N*-oxide) where nearly 100% of pulps are dissolved, but it is high compared to the results of Feng and Chen [Feng, 2008]. Theses authors reported for the same pulp type (spruce sulfite pulp) a dissolution yield of 37% using 3-methyl-1-butyl-*N*-pyridine chloride, [C_4mpy]Cl, as solvent.

However, it has to be remembered that materials from the pulp have been extracted by the nitren treatment (see Table III.1). Taking this extracted material during the nitren treatment into account, 26.5% of the initial pulp is solubilized if untreated with nitren, while 33% of the initial pulp is dissolved in NaOH-water when treated with 3 and 5% nitren, and 32% when treated with 7% nitren. These values were calculated according the following formula:

Overall dissolution yield (%) = NaOH dissolution yield × Nitren pulp recovery yield [III-3]

At this point, two conclusions can be drawn: first, nitren treatment is efficient for increasing the total fraction of this spruce bleached sulfite pulp that can be solubilized in NaOH-water. Second, it seems that this fraction is not depending on the nitren concentration, since whatever the concentration is, about 33% of the initial pulp is dissolved, meaning that 67% are not dissolved in part because they have been extracted by the nitren treatment (this applies mainly to the 7% nitren that is extracting nearly 18% of the pulp) and in part because it stays undissolved in NaOH-water mixture. The fact that the total soluble fraction is not depending on nitren content can well be coincidental, with no specific meaning.

An assessment on the carbohydrate composition on the different fractions yielded from the NaOH treatments (insolubles and solubles) is plotted in Figure III.14, which includes also the values from the initial and nitren-treated pulps for comparison. As expected, the insoluble fractions are enriched in glucose, once the residual hemicelluloses are easily dissolved by the caustic solvent. Thus, most of the hemicellulose is found in the soluble fraction. The increase of glucose content and decrease of hemicellulose content observed on the soluble fraction for the different nitren treated fibers is proportional to the values observed on the pulps. This is explained by the effect of these extractions on the chemical composition of the pulps: the nitren treatments will extract the hemicelluloses, therefore the relative amounts of xylose and mannose in the soluble fraction will be lower, leading to an enrichment of the material in glucose.

The fibers in the NaOH-insoluble fraction were observed by optical microscopy after the dissolution of the untreated or nitren-treated fibers in 8% NaOH-water. As expected, this solid fraction is composed of fibers or fiber fragments that were not dissolved, as shown in Figure III.15. Untreated fibers that were not solubilized in NaOH-water are not intact (Figure III.15a), but they keep their overall “fiber-like shape”. Most of them show traces of swelling, with some weak traces of ballooning. This suggests that cellulose present in these fibers was probably extracted from the inner fiber walls, but that for some reasons probably linked to the resistance of the primary wall [Cuissinat, 2006a; Cuissinat, 2006b, Le Moigne, 2010; Spinu, 2011], the fiber envelope did not dissolve. Nitren treatments change the way undissolved fibers look. When increasing nitren concentration, swelling with ballooning is more pronounced and fibers became strongly de-structured, with the presence of disk like fragments. It means that for the nitren-treated fibers, only the primary wall is not dissolving (some fibers are however resistant to treatment and are kept nearly intact).

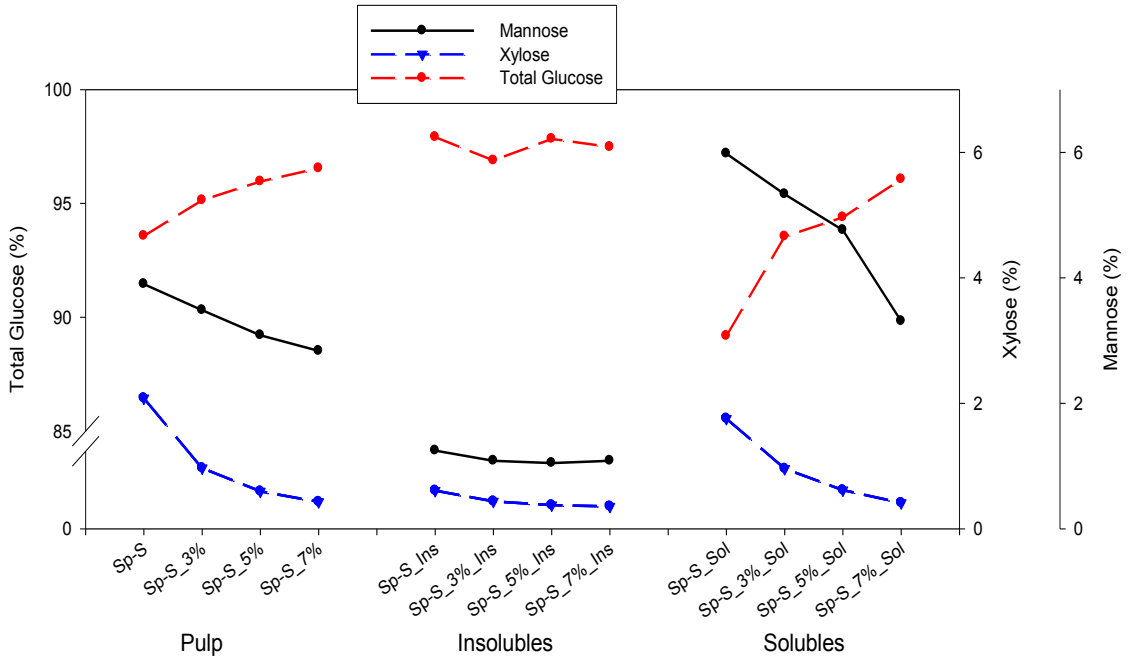


Figure III.14: Carbohydrate composition of the different fractions from the NaOH extraction.

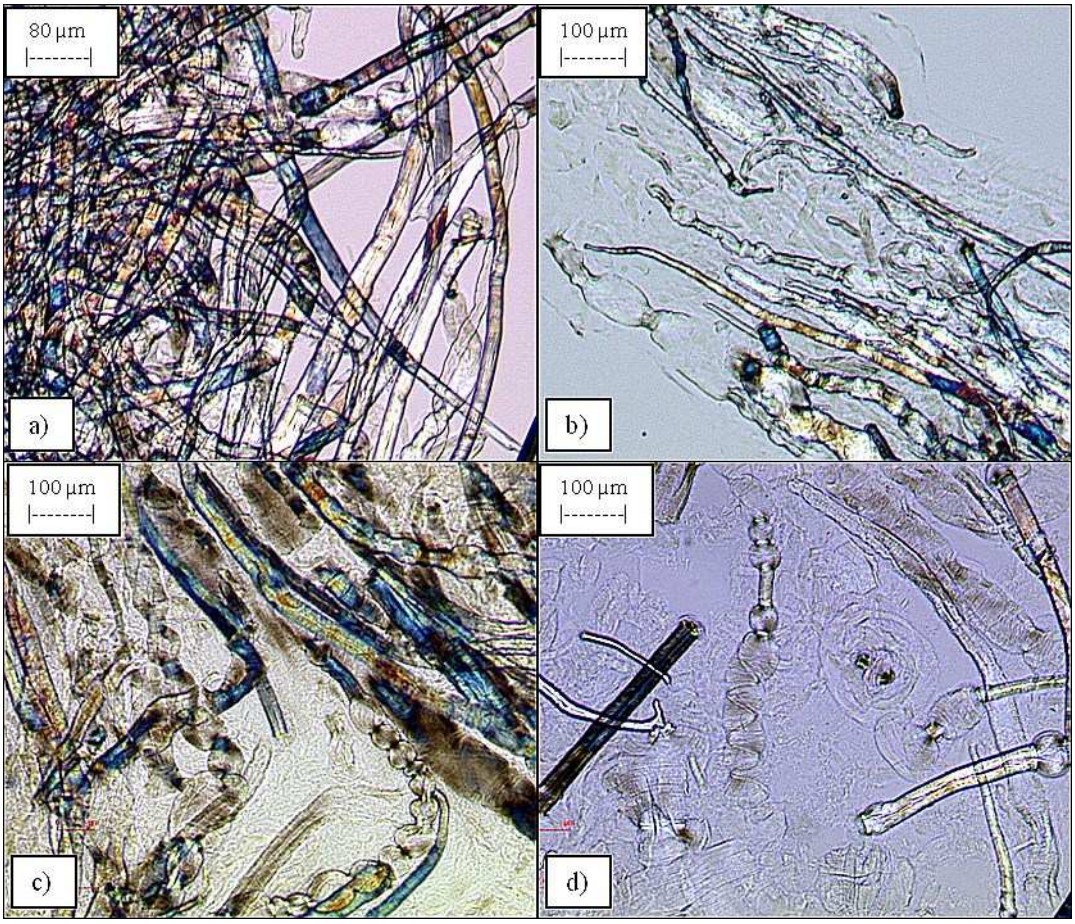


Figure III.15: Morphology of the NaOH insoluble fractions of the initial pulp (a), and treated pulps with 3% (b), 5% (c) and 7% nitren (d).

A comparison of the fiber dimensions between the fibers and the recovered insoluble is given in Figure III.16. It has already been discussed that the nitren extractions (with concentrations lower than 7%) are not changing the average fiber length of the pulp (Figure III.7, Figure III.16a). After NaOH dissolution, the mean fiber length and width of the insoluble parts are the same as for the initial pulp for the untreated Sp-S pulp. However, when the pulp is treated with nitren, it is much more easily attacked by NaOH-water and the length of the fibers that did not go into solution (insolubles) decreases quite substantially (Figure III.16a-b). This degradation of the integrity of undissolved fibers compared to initial ones can be seen also in Figure III.15. Concerning the amount of fines (Figure III.16b), for the untreated pulp, its number is lower in the insoluble fraction, while for the nitren extracted pulps, the opposite is verified. According to the microscopic observations, this can be explained as follows: for the initial pulp, when submitted to the treatment with NaOH, most of the fines are dissolved, while, as discussed above, the fibers are kept nearly intact; for the nitren extracted pulps, the number of fines is increasing proportionally with the concentration of the nitren used, representing the fiber fragments that result from the partial dissolution of the fibers. These fragments are accounted as fines by the software due to their small dimensions.

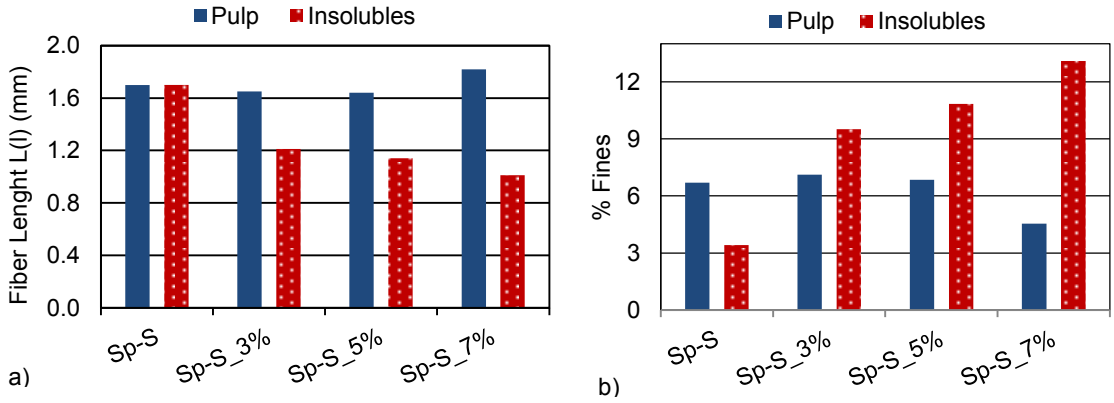


Figure III.16: Effect of the dissolution with 8% NaOH-water on the fiber length (a) and % of fines (b) for untreated and nitren treated pulp.

Considering the treatment with 3% nitren, Table III.2 shows that 4.6% of hemicellulose remains in the fibers. Although this value is lower than the 6.3% present in the initial untreated pulp, it demonstrates that the 3% nitren treatment is not so efficient in terms of hemicellulose removal. Despite this, it is increasing the dissolution yield in cold caustic soda by 25% (33% is dissolved compared to 26.5% for the initial pulp). This improvement is illustrated by comparing Figures III.15a and b. After extraction with NaOH (Figure III.15b) the fibers show a higher swelling and ballooning, due to a breaking of the primary wall [Cuissinat, 2006b, Le Moigne, 2010]. Thus, the cellulose that is additionally extracted by NaOH from the nitren treated pulp comes from the inner fiber walls that are now accessible to the solvent.

The question is then, why the removal of such a small amount of hemicelluloses is weakening so much the Sp-S fiber structure? The molar mass distributions reported on Figure III.17 shows that, as always found, the insoluble fraction has an average molar mass larger

than the one of the soluble fraction. Figure III.18 shows that there is a clear connection between the NaOH dissolution yield and the viscosity of the untreated and nitren-treated pulps.

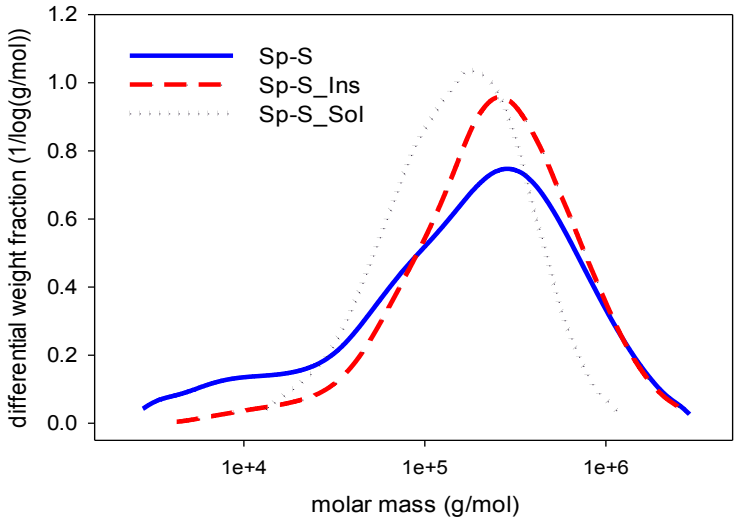


Figure III.17: Mw distribution of the initial Sp-S pulp without nitren treatment and of the insoluble and soluble fractions after dissolution in NaOH solution.

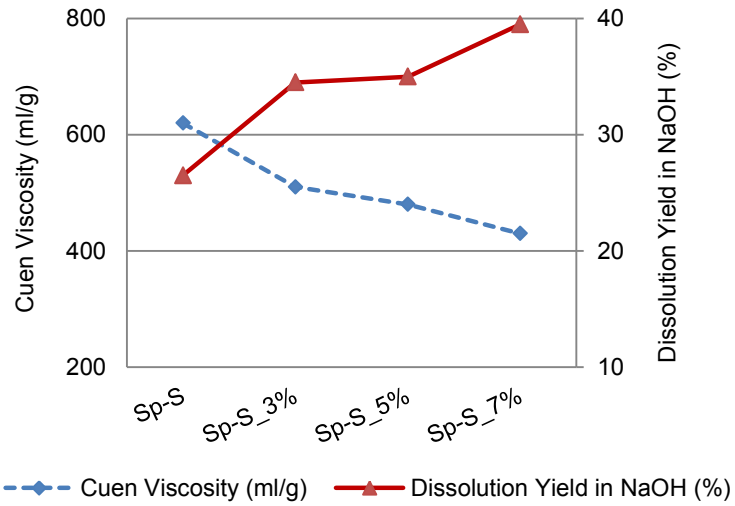


Figure III.18: Relation between the dissolution yield (right hand axis) and the viscosity (left-hand axis) of the pulp.

Thus, the effect of a nitren treatment has several different effects towards dissolution ability in NaOH water. It is decreasing the average molar mass of the cellulose present in the pulp and also increasing the accessibility of NaOH to regions that are not reachable without this treatment, as can be seen when looking at the de-structured aspects of the fibers that are not dissolving in NaOH-water. The removal of xylan is not the only factor since the 3% nitren-treated fibers are much easier to be dissolved while the amount of material removed by the nitren treatment is small. It may be that it is this removal, even very tiny, or the disassembly of the hemicellulose network that is increasing accessibility of fibers to NaOH ions.

III.2.2. - Effect of nitren treatments on other pulp samples.

Several other dissolving pulps were submitted to nitren treatments and investigated regarding their solubility in cold caustic soda. In this section, these effects are discussed in order to cover a wide range of the pulps available in the market. The following dissolving pulps and paper grade pulp were thus studied:

- Hardwood (beech) pulped with the sulfite process (Be-S), with 530 ml/g of intrinsic viscosity.
- Softwood delignified with a pre-hydrolysis kraft process (S-PhK), 450 ml/g.
- Northern softwood, pulped with a sulfite process (NS-S), 540 ml/g.
- Southern softwood, pulped with a sulfite process (SS-S), 500 ml/g.
- Mixed hardwood kraft pulp (MxH-KP), 790 ml/g.

The nomenclature of the different fractions yielded from the treatments follows the same pattern described for the precedent pulp (Section III.2.1). For each sample, a table with all the numerical results can also be consulted in Annex 1. As for the previous discussed sample, the yields of the fractions “Pulp” and “Extracts” are calculated based on the initial pulp used for nitren extractions or enzymatic treatments, while the yields for the fractions “Insoluble” and “Soluble” are based on the amount of pulp used in the dissolution trials.

The first two samples (Be-S and S-PhK) were treated with different nitren concentrations in order to confirm the influence of different loads of this chemical on the pulp properties. For the remaining pulps, only a treatment with 5% nitren was performed. In the study of the influence of nitren extractions on the dissolution in cold aqueous NaOH solution, only the initial pulps and pulps extracted with 5% nitren were used.

III.2.2.1. - Effects of nitren treatments on fiber structure and composition

In accordance with the previous results for the spruce bleached sulfite pulp sample (Sp-S), the hardwood sulfite pulp (Be-S) and softwood pre-hydrolysis pulp (S-PhK) have extracted materials that are proportional to the nitren concentration (Figure III.15). The extraction with 7% nitren for the Be-S pulp was not evaluated, since the fibers could not be recovered after extraction, due to the highly viscous solution obtained (high amount of cellulose in solution). This fact made it impossible to further analyze the material. For the S-PhK, with 7% nitren the extract amounted to 11.5%, indicating that with this concentration, cellulose is also extracted from the pulp, as for the case of the spruce bleached sulfite pulp of the previous section.

The Northern and Southern softwoods (NS-S and SS-S) with the 5% nitren extraction yield 1.3% and 1.7% extracts. With the paper grade pulp (MxH-KP) treatment with 5% nitren the extract

amounts to 14.5% (Figure III.19). This high value for the paper grade is due to the high amount of hemicelluloses present in the paper grade pulp.

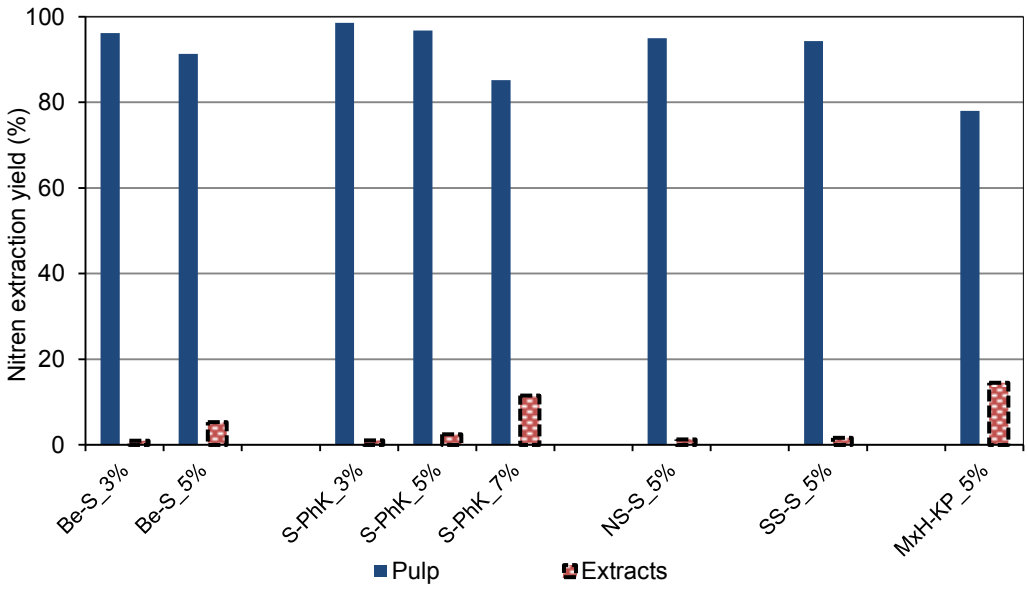


Figure III.19: Yields of the recovered pulp and extracts fractions after nitren extractions for each pulp.

Analyzing the effect of nitren extractions on the sugar composition (Figure III.20), it is evident that this treatment promotes the enrichment of cellulose on the pulps. For the two pulps treated with different concentrations of nitren, this enrichment is proportional to the concentration of the solvent, similar to what was verified for the Sp-S pulp. The selectivity of the solvent regarding the dissolution of xylans is noticeable, especially on the paper grade pulp (MxH-KP) where an increase on the relative amount of mannans occurred. For the dissolving pulps, this selectivity not so evident, considering that for 5% and 7% nitren extractions, the mannans are also partially dissolved, probably due to the delignification processes that the pulps were submitted to. The harsher conditions used on the production of dissolving pulps promotes a higher degradation of the pulp fiber integrity. This allows an easier diffusion of the solvent towards the inner walls of the wood cells, where the mannans are more concentrated.

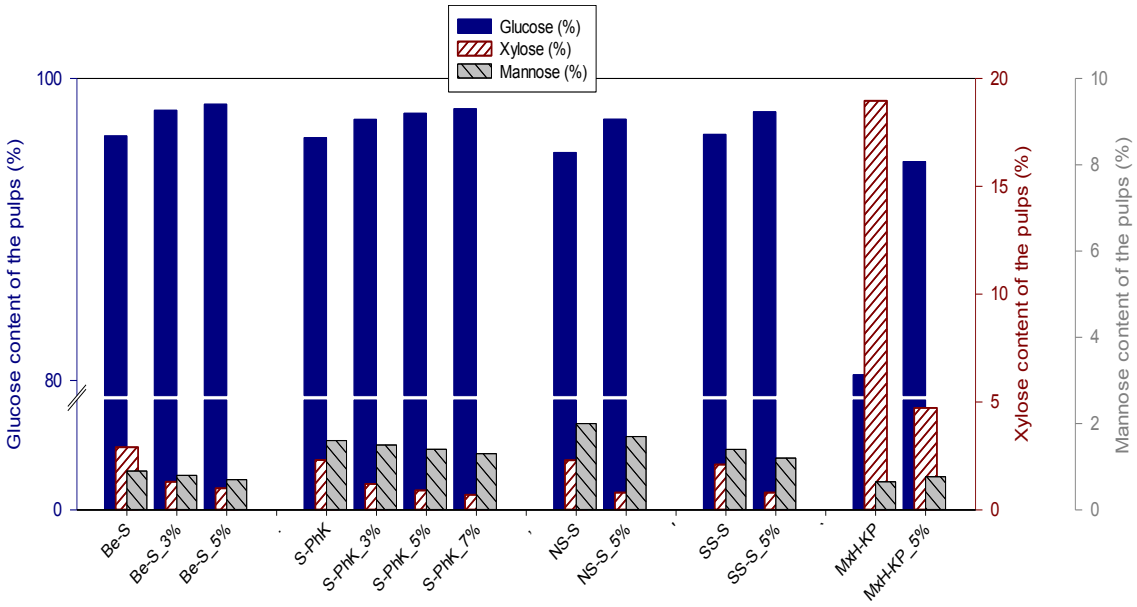


Figure III.20: Effect of nitren extraction on the carbohydrate composition of pulps.

The molar mass distributions were analyzed for the Be-S and S-PhK pulps, with and without nitren extractions (Figure III.21). For the hardwood pulp, the extraction of low molar mass molecules is evident since the low molar mass shoulder of the distribution is decreasing in intensity, proportionally to the nitren concentration. Focusing on the distribution for high molar masses, a slight shift is noticed towards lower molar masses. For the softwood pre-hydrolysis kraft, a minor decrease on the low molar mass is observed while there is a pronounced shift of the molar mass distribution towards lower molar masses after treatments with nitren. This shift is more evident for the pulps extracted with higher nitren concentrations. These observations support the results verified before, i.e. that the decrease in intensity of the distribution for lower molar masses is related with the extraction of hemicelluloses from the pulps, while the general shift of the molar mass distribution towards lower molar masses is explained by the partial hydrolysis and dissolution of the cellulosic chains. Therefore, resembling the results for the Sp-S pulp, the nitren extractions are extracting mostly the low molar mass molecules (hemicelluloses) and, when using higher nitren concentration, the cellulose chains are to some extent hydrolyzed and dissolved, decreasing the degree of polymerization of the dissolving pulps.

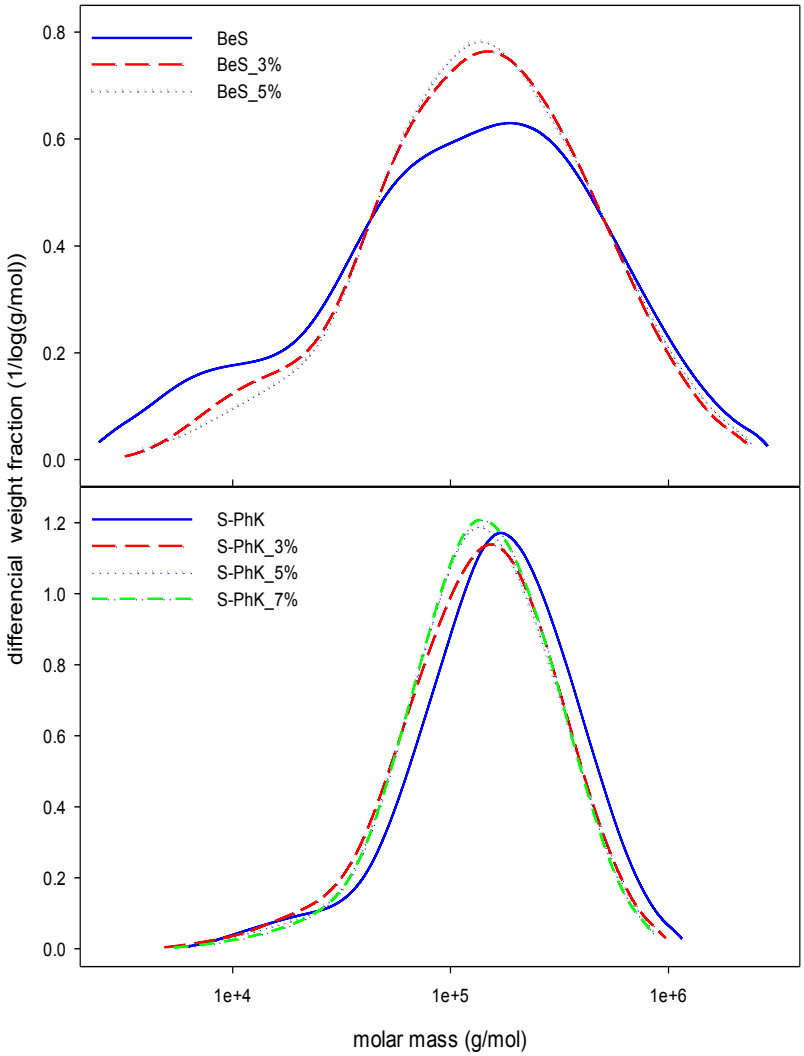


Figure III.21: Effect of the nitren extractions on the pulps molar mass distribution.

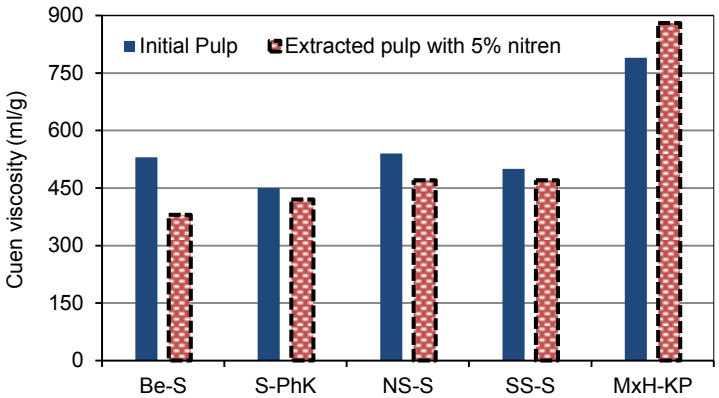


Figure III.22: Effect of the nitren extractions on the pulps intrinsic viscosity (treatment with 5% nitren).

This decrease can be also observed by the determination of the intrinsic viscosities for the pulps with and without extractions with 5% nitren (Figure III.22). For all the dissolving pulps, the degree of polymerization is decreasing, while, in contrast, the intrinsic viscosity of the paper grade

pulp is increased after treatment with 5% nitren solution. This fact, also verified by Janzon et al [Janzon, 2008a], is due to the high hemicellulose amount extracted from the initial pulp: the removal of this low molar mass material is increasing the average degree of polymerization of the pulp.

III.2.2.2 .- Effects of nitren treatments on the dissolution of pulp in NaOH-water

As in section III.2.1.2. for the spruce bleached sulfite pulp, the influence of the nitren treatments on the dissolution of the other pulp fibers in a cold aqueous solution of 8% sodium hydroxide will be discussed. This study will complement the knowledge already discussed for the Sp-S pulp. Each initial untreated pulp was compared to the same sample treated with 5% nitren. After the dissolution procedure, the dissolution yield in NaOH was gravimetrically determined; the insoluble fractions were separated and analyzed with optical microscopy.

In Figure III.23, the dissolution yields in 8% NaOH are depicted. For all the dissolving pulps, the dissolution ability of the pulp fibers increased after extraction with 5% nitren. Before extraction, the pre-hydrolysis kraft pulp (S-PhK) shows a lower accessibility for NaOH in comparison to the sulfite dissolving pulps. However, the S-PhK pulp is the one which denotes the highest improvement in chemical accessibility with the nitren extraction, showing an increase of 44.7% on the dissolution yield, compared to 19.3% increase for Be-S, 11% for NS-S, 4% for SS-S and 32.1% for the previously studied Sp-S pulp (section III.2.1.2).

The paper grade pulp (MxH-KP) shows a different behavior. Instead of promoting the fiber accessibility; the nitren extraction is yielding a pulp which is more difficult to dissolve in cold caustic soda. While 21.8% of the initial pulp could be dissolved, for the nitren treated only 8% of the material is dissolved, meaning a decrease of 63% on the pulp dissolution capacity.

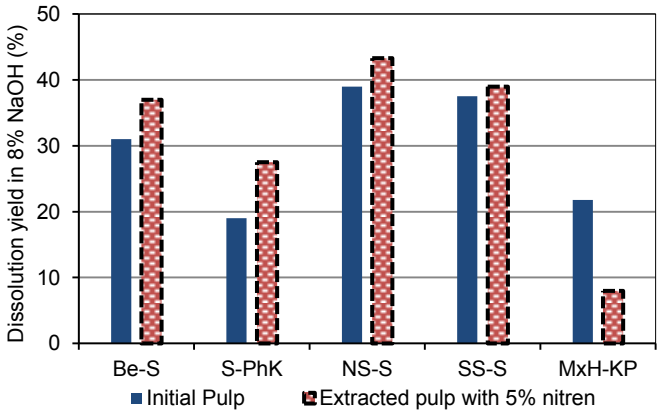


Figure III.23: Dissolution yield (fraction of dissolved pulp) for the different pulps after dissolution in NaOH-water.

Since the NaOH dissolving system is very efficient on dissolving hemicelluloses (see Figure III.14), these results can be partly explained by the presence of a high amount of hemicelluloses

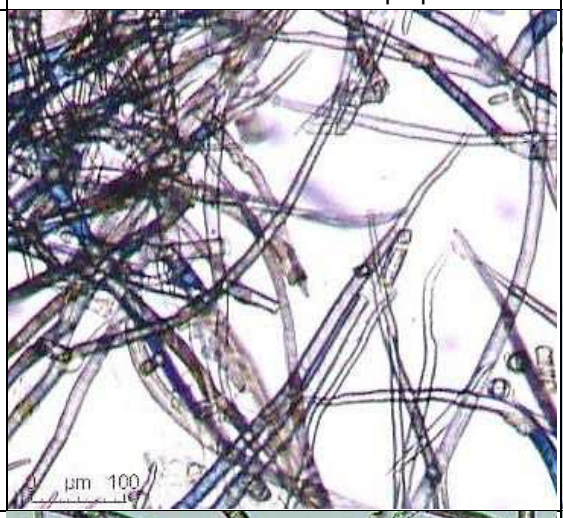
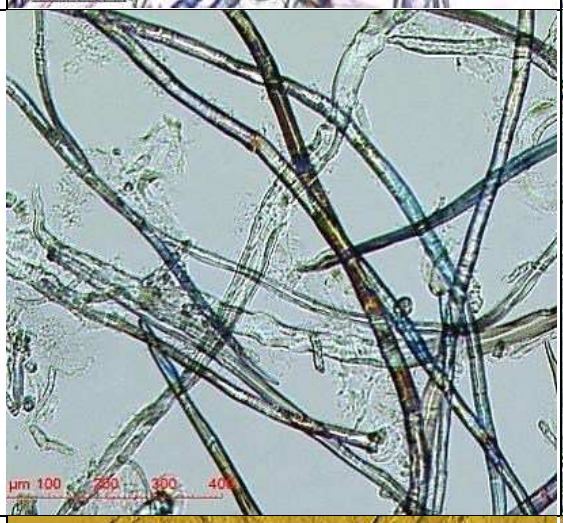
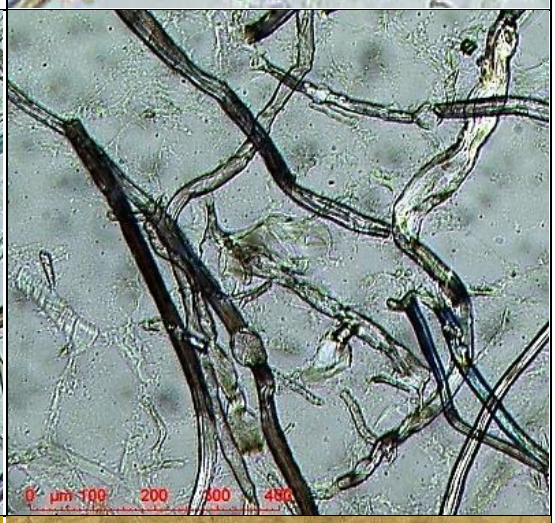
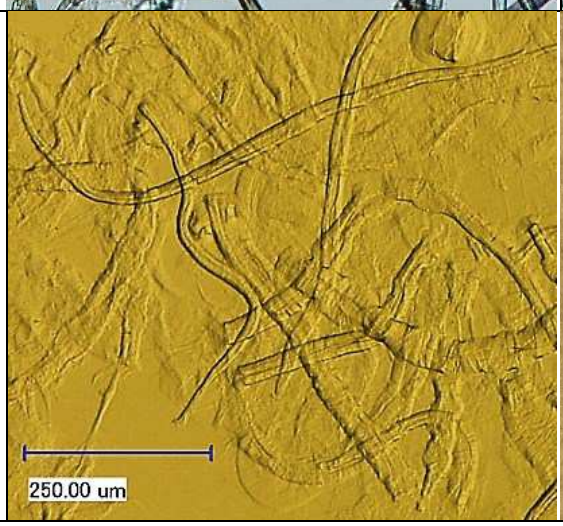
on the initial paper pulp (19.8%) compared to a low amount after extraction with nitren (5.6%). If one considers this difference (14.2%) and subtracts it from the dissolved material in NaOH for the initial pulp, it gives a value of 7.6% for the dissolution yield, which is quite similar to the 8% of dissolution yield in NaOH obtained after nitren extraction. Thus, in light of these results, one can state that the nitren extraction is not improving the cellulose dissolution of the paper grade pulp in cold sodium hydroxide.

The study of the morphology from the NaOH insoluble fractions allows a better assessment on the impact that nitren extractions have on the solubility of the pulp in cold caustic soda. In Figure III.24 this study is illustrated with pictures from the insoluble fractions obtained by optical microscopy.

For the hardwood sulfite pulp (Be-S), the insoluble fraction of the initial pulp is composed almost exclusively by intact fibers, showing minor fragmentation and ballooning. In contrast with that, the insoluble fraction from pulp treated with nitren shows increased ballooning and fragmentation as well as higher swelling. Although not so noticeable, the same can be observed for the softwood pre-hydrolysis kraft pulp. The presence of intact fibers is more evident on the insoluble fraction from the initial pulp. For the Northern softwood sulfite pulp, the nitren extracted sample will result into insolubles which are composed mostly of disintegrated and highly swollen fiber particles, while for the untreated pulp, the insolubles still remain intact after the NaOH treatment. The insoluble fraction of Southern softwood sulfite pulp is showing fibers which are slightly swollen and some ballooning can also be seen, while for the insolubles from nitren treated pulp, the fibers are more degraded, presenting a higher ballooning and swelling and large amounts of small highly swollen fiber fragments.

When it comes to the paper grade pulp, the nitren extraction impact is not so evident. Nevertheless, the initial pulp presents a slightly higher accessibility with a minor swelling and ballooning, while the insolubles from the nitren extracted pulp consists mainly on intact fibers.

These microscopic observations are in accordance with the results obtained from the gravimetric measurements.

	Insolubles from initial pulp	Insolubles from 5% nitren extracted pulp
Be-S		
S-PhK		
NS-S		

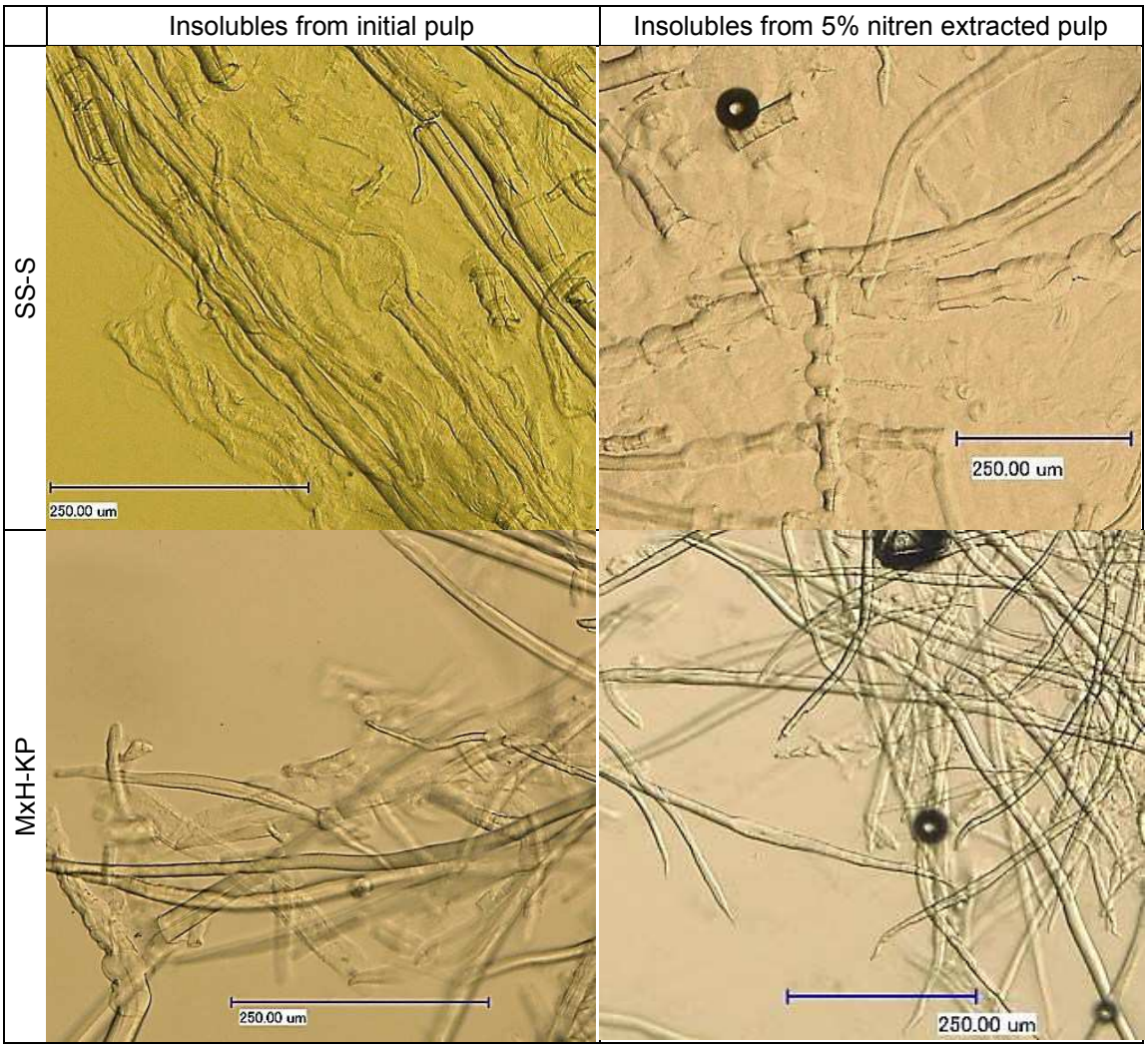


Figure III.24: Microscopic images from the NaOH insoluble fractions of the initial pulp and the pulp treated with 5% nitren.

Comparing the different dissolving pulps, the hardwood is more sensitive to the nitren treatment than the softwoods. If identical nitren concentrations, independently of the pulp viscosity, the recovered pulp yield after nitren extraction is lower for the hardwood (Figures III.3 and III.19). When comparing the three softwoods sulfite pulps, it can be stated that the Sp-S pulp, despite its higher intrinsic viscosity, yields a lower amount of recovered pulp after treatment with 5% nitren, in comparison with the NS-S and SS-S pulps. This might be explained by the fact that the Sp-S pulp has a higher amount of hemicelluloses (6.3%) when compared with the other two pulps (4.5 and 3.6% respectively). If we compare the softwood sulfite pulp with the pre-hydrolysis kraft, despite having a lower viscosity, the pre-hydrolysis kraft pulp is more resistant to the nitren treatment, yielding a higher amount of recovered pulp (e.g. in 5% nitren: S-PhK 96.8%, Sp-S 93.6%,NS-S 95.0% and SS-S 94.3%). The same tendency is verified on the dissolution in NaOH, where the amount of insoluble fraction for the S-PhK pulp is higher, when compared with the sulfite dissolving pulps.

In contrast with the dissolving pulps, the results obtained with the paper grade pulp are in accordance with the ones published by other authors [Janzon, 2008a]. With a 5% nitren solution, the selectivity for xylan is evident; the extracts consist mainly of xylan with a minor amount of cellulose and an insignificant amount of mannans. The xylans were extracted without degrading the cellulose, which explains the increase in viscosity. When it comes to the impact of the nitren extractions on the fiber accessibility towards dissolution in cold caustic soda, a positive effect is evident for the sulfite pulps, while is decreasing this accessibility on the paper grade pulp.

These observations support the known idea that the chemical accessibility depends on the history of the pulps, specifically the pulping process. With sulfite pulping, the fibers were subjected to acidic cooking conditions, promoting an opening of the cell wall structure. Thus the diffusion of the nitren solution or NaOH ions into the inner fiber walls is easier, allowing this way a partial dissolution of mannans (present mainly in the secondary wall) and cellulose degradation.

III.2.3. - Viscosity versus xylan content

With the nitren extraction approach, it was possible to decrease the content of xylan in pulps. However, this was always associated with a reduction in viscosity as it can be seen in Figure III.25. As reported for the Sp-S pulp, for all dissolving pulps, the extraction of xylan leads to an increase of the dissolution ability of the pulp fibers, however, at the same time the average molar mass of the cellulose chains is decreasing proportionally.

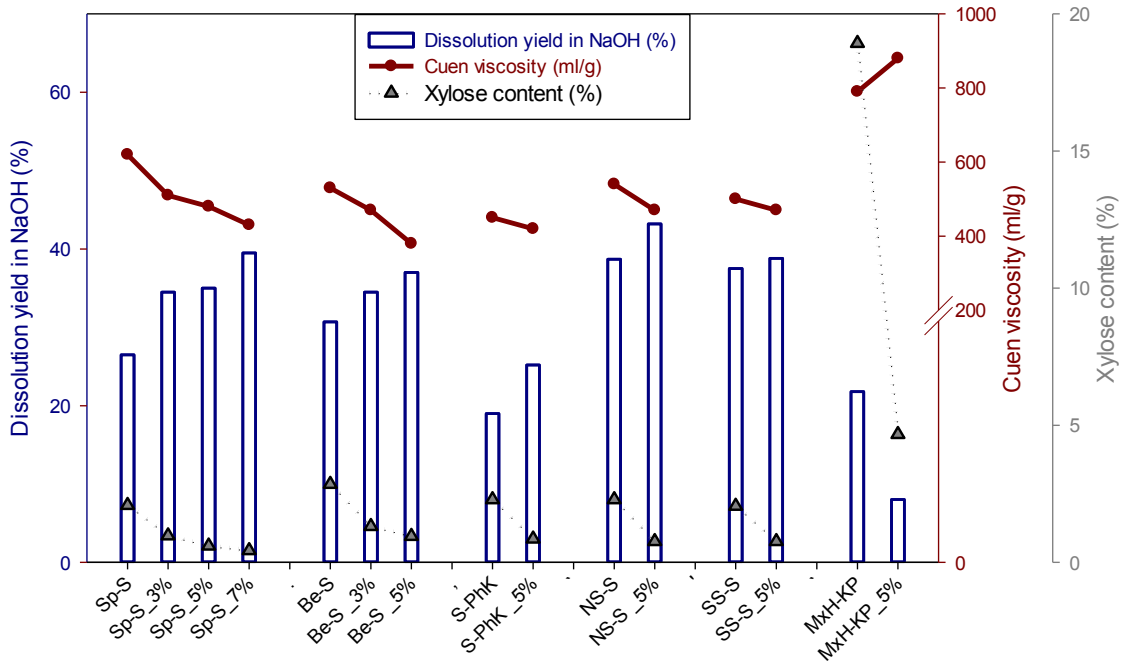


Figure III.25: Plots relating for each pulp the dissolution yield in NaOH, intrinsic viscosity and xylose content.

Only to the paper grade pulp, the observations are different, with reduced xylose content, an increased degree of polymerization and a lower dissolution yield in NaOH. This different behavior is explained by the large amount of xylans present in the initial pulp, as it was discussed above.

In order to investigate if the dissolution capability of the dissolving pulps can be independently correlated either with the xylose content or with the intrinsic viscosity, these parameters were plotted and correlated for all dissolving pulps, untreated and treated with nitren (Figure III.26).

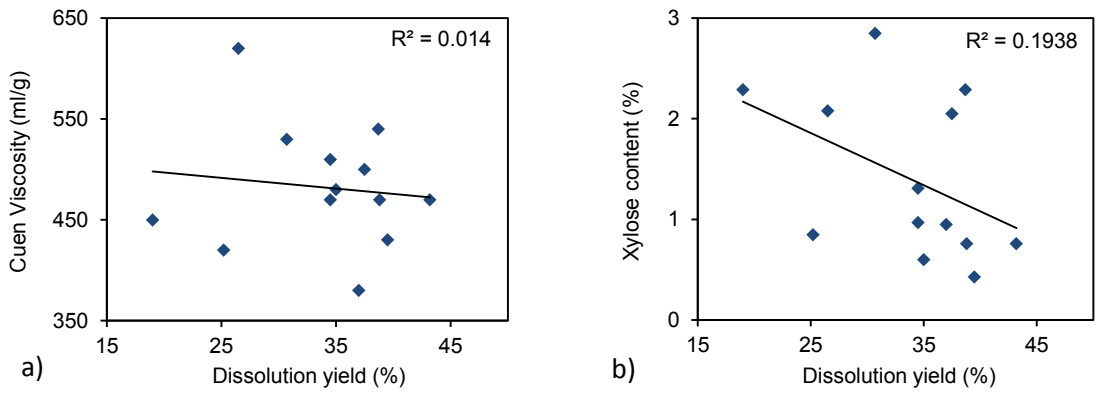


Figure III.26: a) Dissolution yield versus intrinsic viscosity; b) Dissolution yield versus xylose content.

When comparing all the pulps, it can be observed that for samples with the same viscosity, different dissolution yields can be obtained (Figure III.26a). The same can be noticed for the xylose content (Figure III.26b). Both graphs show a low R^2 value, which is not statistically significant. This leads to the conclusion that none of the two parameters can independently explain the dissolution yield of a dissolving pulp in a cold solution of 8% NaOH.

III.3. - Conclusions

It has to be stated that nitren treatments are interesting as a laboratory test for fundamental research. For industrial applications on dissolving pulps, this approach is not attractive since after extraction, a laborious process is required in order to remove nickel.

The effects of a low concentration nitren treatment on the studied dissolving pulp shows that it is quite different from what was reported before for paper grade pulps. In the case of the paper-grade pulp, 3 to 5% nitren treatments were very selective to xylan and they were not dissolving cellulose. In the case of the studied dissolving pulps, mannan and xylan are dissolved with a clear selectivity for xylan, but cellulose is also extracted. Further on, the treated dissolving pulps have lower average molar mass indicating increased degradation with increasing nitren concentration. For nitren concentrations of 5 and 7%, the fibrils attached to the fibers surface and the fines are dissolved, even promoting for the 7% nitren an increase in the average fiber length and fiber width on the pulp fibers.

Accordingly to the results, hardwood pulps show a higher sensitivity towards nitren treatment in comparison with softwood. When delignified with pre-hydrolysis kraft process, the softwood presents a higher resistance to alkaline extraction than observed for softwood sulfite pulps.

Nitren treated pulps have higher cellulose purities. However, this is not the reason for their improved processability in NaOH-water solutions. Nitren treatment is deeply changing the way NaOH can penetrate the fiber structure, reaching areas which are not accessible for the untreated pulps. Whether it is the breakage of cellulose chains (seen by the decrease of molecular weight) or the removing of small amounts of hemicellulose that are responsible for the increase of solubility remains to be understood, considering that both mechanisms have the effect of loosening the wall structure.

These results show that the performance in dissolution of cellulose fibers, depends not only on chemical composition, but also on other factors, like the morphology of the fiber, the topological arrangement of the cellulose fibrils, the process used on the delignification of the pulp or the botanic origin.

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chapter IV

Use of pectinase to modify the pectic network and study of its effect on the cellulose fibers structure and solubility

Use of pectinase to modify the pectic network and study of its effect on the cellulose fibers structure and solubility

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IV.1. - Introduction

This chapter will describe the effect of pectinase as a pretreatment of several dissolving pulps and one paper grade pulp prior to dissolution in NaOH.

This study represents the major part of this work owing to the many validation and scale up experiments. This chapter is divided into three major steps:

- A first initial study using a commercial pectinase enzyme (which commercial production has stopped during the study) which showed that there was a clear, positive effect on dissolution on three dissolving pulps after a CCM treatment. Considering that there are nearly no pectins in the used pulps, a further study showed that CCM had also a low endoglucanase activity. This prompted the second phase of the study where pure endopectinase and endoglucanase were used.
- In this second phase, a pure endopectinase called L40 and a pure endoglucanase called EG were used on only one pulp sample and compared to CCM, with the aim of finding whether the CCM effect was due to its endoglucanase content. The result showed that the endopectinase is improving the dissolution in cold soda without decreasing too much the average molar mass of pulps. Furthermore it is apparent that it is the combined effect of a large endopectinase activity with a low endoglucanase activity, is the most efficient. To perform this study, we proposed the notion of “total activity units” of enzymes taking into account a combination of time of incubation and enzyme concentration for further experiments.
- The third phase was devoted to apply the optimized treatment for L40 and CCM to five very different dissolving pulps and one paper grade pulp and to assess thei effect on dissolution in cold soda.

For simplifying the text, the enzymes will be noted as CCM, L40 and EG for Biopectinase CCM, Pektinase L40 and Cellulase (endo-1,4-ß-D-Glucanase) (*T. emersonii*) (EG) respectively. As for the nitren treatment, the sample name will be the initial pulp name plus the simplified name of the enzyme as a suffix (e.g. Be-S_CCM after incubation with CCM enzyme). After dissolution in NaOH, the two fractions will be named also as before (e.g. Be-S_CCM_Ins for the insoluble and Be-S_CCM_Sol for the soluble fractions).

IV.2. - Results and discussion

IV.2.1. - First step: initial trials

Three dissolving pulps, Sp-S, Be-S and S-PhK, were treated with Biopectinase CCM. These pulps were selected since they represent both wood types (hardwood and softwood) and both main delignification processes used in the production of dissolving pulps (sulfite and pre-hydrolysis kraft). This enzyme was selected because it was available and considered originally as a pure pectinase enzyme. As it will be shown latter, this is not the case and some endoglucanase activity can be measured for Biopectinase CCM. For these initial trials, the incubation conditions used are described in Table IV.1. These conditions were set according to the enzyme provider recommendations and literature [Ortega, 2004; Pedrolli, 2009], except for the pulp suspension consistency, which was optimized for an efficient mixing of the pulp suspension during rotation of the incubation flask.

Table IV.1: Incubation conditions used for the first enzymatic treatments.

Pulp amount (g)		20
Pulp suspension consistency (%)		5
Incubation time (hours)		15
Temperature (ºC)		45
pH		3.5
Buffer		pH adjusted with CH_3COOH
Enzyme amount (ml)		0.2
[Enzyme] Solvent based	%	0.05
	mg /g	0.5
	ppm	500
Enzymatic activity units per gram of pulp (U/g)	Endopectinase	31.8
	Endoglucanase	12.1
Total enzymatic activity units per gram of pulp (U/g) *	Endopectinase	476.3
	Endoglucanase	181.5

* See section IV.2.2.4.3.

The pH was adjusted by adding 20% (w/w) aqueous solution of acetic acid to the incubation preparation. The pH was controlled and there was no change during the incubation time.

After the enzymatic treatment, the pulp recovery, intrinsic viscosities and the carbohydrate composition of the recovered pulps as well as the pulp dissolution yields in cold NaOH were determined. These data were compared with the results obtained with the nitren treatments (Figure IV.1, Figure IV.2). The determination of the dissolution yield in NaOH has an estimated error of ±1% in absolute value for the whole range of results. Accordingly, the relative error decreased with the increase of dissolution yield. Probably this reflects an error inherent to the material/method used. In practice, this error can be illustrated by the following examples from Figure IV.1: S-PhK has a dissolution yield of 21 ± 1% and the S-PhK has a dissolution yield in NaOH of 40 ± 1%. For the pulp recovery yield after extractions, the error is ± 2%, and the viscosity measurements show an error of ± 10ml/g.

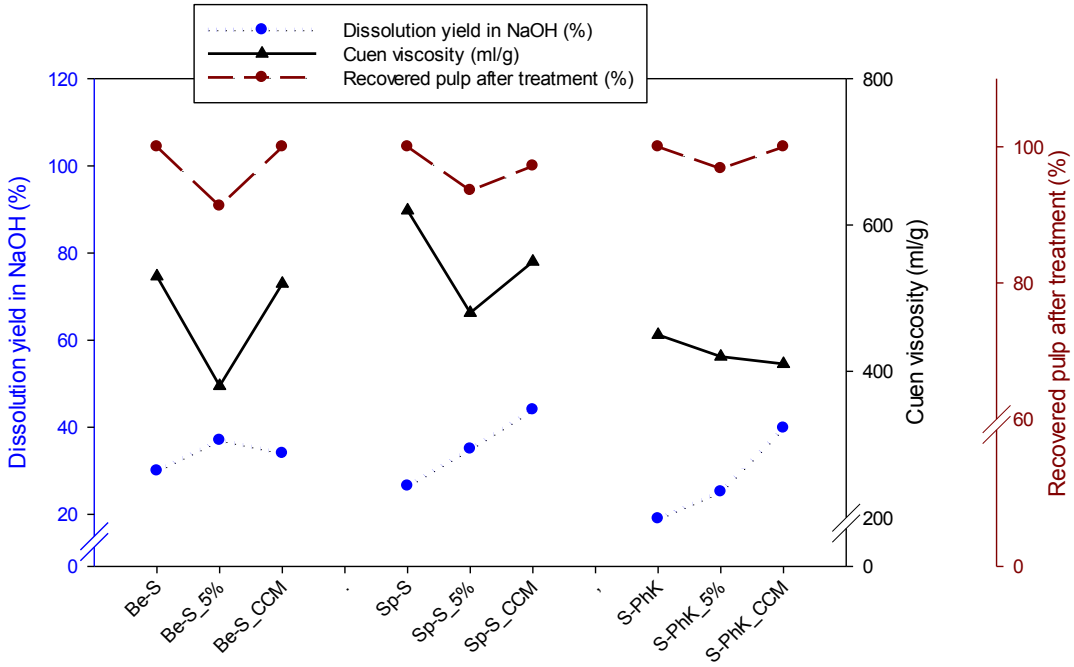

Figure IV.1: Comparison of nitren 5% and pectinase CCM treatments regarding the pulp recovery yield, viscosity and dissolution yield in NaOH.

These initial pectinase treatments shows that almost 100% of the treated pulps are recovered, contrary to the nitren treatment (Figure IV.1). There is no loss of materials. With exception for the S-PhK pulp, the enzymatic treatment is degrading the cellulose chains only slightly compared to the nitren treatment, since the intrinsic viscosity is not decreasing significantly (Figure IV.1). The initial study revealed that the dissolution yield is increasing with the CCM treatment for all samples. Engström et al, and Henriksson et al, reported also insignificant losses of material after treating a dissolving pulp with a monocomponent endoglucanase [Henriksson, 2005; Engström, 2006]. Similar results were achieved by the use of endoglucanases I and II and cellobiohydrolases I and II [Rahkamo, 1998]. Other enzymatic studies, that intended to increase the cellulose fibers accessibility by removal of the primary wall showed considerable losses of material during the treatment. For example, for reaching the highest dissolution yields, Wang et al reported a loss of material of ~20% using Celluclast 1.5 L [Wang, 2008]. Using the

same enzymatic solution in combination with Econase HC 400, losses of 25 to 30% of material were observed [Le Moigne, 2010]. From the literature, we verified that most of the studies dealing with treatment of dissolving pulp to increase its accessibility were not reporting the amount of the recovered material after treatment. From the technical application point of view, mass balances are of great importance.

Regarding the carbohydrate composition (Figure IV.2), it is evident that the incubations with CCM are not modifying the sugar composition of the pulps, the amounts of glucose and hemicelluloses being the same before and after enzymatic treatment. This is again in contrast with the nitren treatment, where hemicelluloses were extracted (Figure IV.2). Comparing with other studies for activation of dissolving pulps, Kihlman et al reported a loss of ~50% in hemicelluloses with a steam explosion extraction, while with a hydrothermal treatment the hemicellulose content decreased only by ~10% [Kihlman, 2012]. Treatment with ethanol-acid, despite removing the primary wall and decreasing the cellulose DP, is reported not to change the overall carbohydrate composition, showing only a slight decrease on the hemicellulose content when high temperatures (75 ºC) are used [Trygg, 2011]. 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) - mediated oxidation is also reported to decrease the xylan content of kraft pulp [Gehmayr, 2012b]. Östberg et al used a sequential combination of xylanase (with and without alkaline extraction) and endoglucanase. Without alkaline extraction both enzyme treatments were not changing the carbohydrate composition [Östberg, 2012].

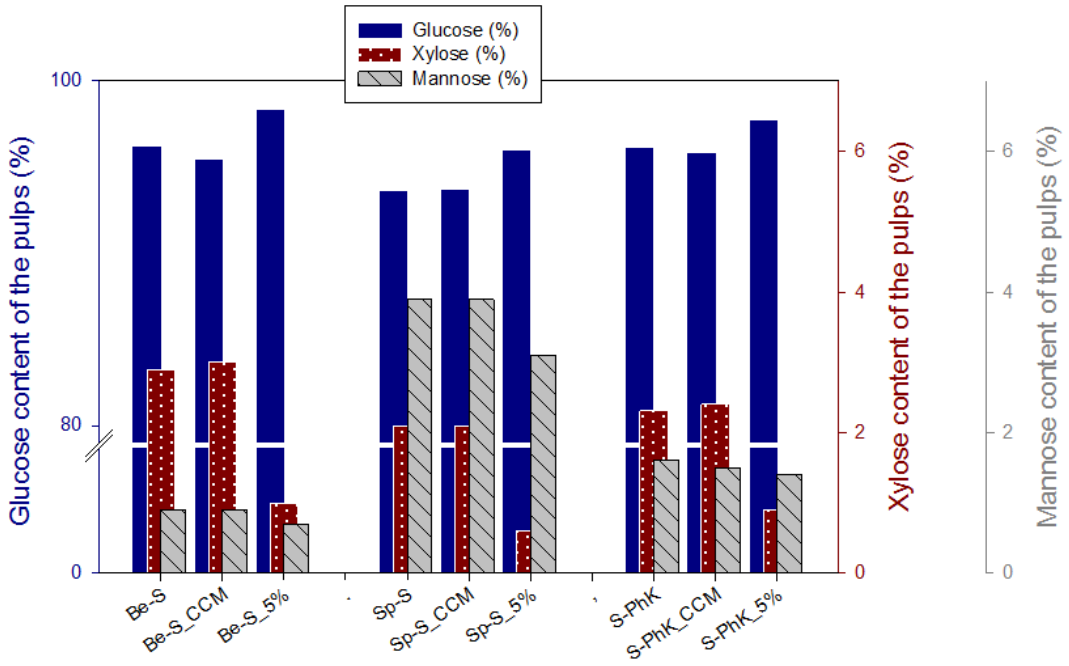

Figure IV.2: Comparison of carbohydrate composition of the pulps after 5% nitren and pectinase CCM treatments.

This preliminary study showed that two pulps (Be-S and Sp-S) treated with pectinase CCM seem to give a higher dissolution yield than with nitren treatment without degrading the pulp, since composition and cuen viscosity are not significantly changed compared to the initial pulp. One

pulp (S-PhK) is not behaving exactly in the same way since the dissolution yield in cold soda increases a lot but at the expense of a decreased cuen viscosity. This suggests that the CCM treatment, despite keeping the carbohydrate composition and not extracting material, is sensitive to the structure and composition of the pulp.

Taking in consideration these promising results, more detailed study on the effects of the pectinase treatments was planned and performed, with two phases: first a study made with one pulp of the effects of endoglucanase and endopectinase compared with CCM and an optimization of treatment conditions and, second, a study of pectinase-based enzyme treatments on different pulps.

IV.2.2. - Comparison of CCM with endopectinase and endoglucanase and optimization of enzymatic incubation

As will be seen, CCM is a mixture of endopectinase and endoglucanase. Normally, the pectinases available in the market for applications in food processing are mixtures of polygalacturonases, pectin lyases and pectin methyl esterases, and are usually derived from fungi, mainly the genera *Aspergillus* [Pedrolli, 2009]. The first part of this chapter will thus be devoted to separate the effects of these two enzymes. Then the enzymatic activities for pectinase-based enzymes will be evaluated in terms of effectiveness by varying the concentration, incubation time and buffer system.

IV.2.2.1. - Selection of the pulp sample that will be used

For the enzyme treatments optimization, only one pulp sample was used, i.e. S-PhK. This sample was selected because it presented a higher sensitivity towards enzymatic treatment (improvement of 123% on the dissolution yield in caustic soda). It was expected minor changes on the enzymatic performance can be better seen when using this sample instead other pulps with lower sensitivity to CCM.

IV.2.2.2. - Characterization of CCM

Biopectinase CCM was supposed to be pure pectinase according to the manufacturer specification. However, once the effect of the enzyme on dissolution yield was very high in first experiments the activity towards pectin and glucans was investigated. It turned out that the enzyme preparation contained indeed some endoglucanase activity (see below). This fact led to the need to clarify if the results obtained during the preliminary study were due to the endopectinase activity or due to the small endoglucanase activity. To clarify this, two other enzyme preparations sold to be “pure”, were tested, endopectinase (L40) and endoglucanase (EG).

The enzymatic activity can be expressed either in enzymatic units (U), adopted in 1961 by the International Union of Biochemistry and Molecular Biology (NC-IUB), or in katals (kat), SI unit

adopted in 1999 by the General Conference on Weights and Measures. The relation between them can be defined as 1 U = 16.67×10^{-9} kat (or 16.67 nano katal) [NC-IUB, 1979, Dybkaer, 2002]. These units are defined according to kinetics of catalytic reactions. In addition there are other units based on the physical changes of the substrate, like the decrease of intrinsic viscosity (Anson units), which are more common in the industry field [Viforr, 2008]. In this work the enzymatic unit (U) was adopted, which defines one unit as the amount of enzyme necessary to catalyze the conversion of one µmole of substrate per minute at 30 ºC and optimum substrate concentration and pH conditions [NC-IUB, 1979, Viforr, 2008). Accordingly, an enzyme solution with an endopectinase activity of 500 U is able to catalyze the conversion of 500 µmol of substrate per minute under the specified conditions.

Since all the enzymatic preparations were from commercial suppliers, the activities of these three enzymes were checked. This was done by the laboratory of a biotech company (ASA Spezialenzyme GmbH). The values of the activities are usually varying strongly depending on the methods used for its estimation. Further on, a variation can exist between different product batches For instance, for the CCM enzymatic solution, an endoglucanase activity of 1211 U/ml was determined, while previously Buchert et al have reported a much lower value of 88 U/ml [Buchert, 2005]. The cellulase EG showed in this work an endoglucanase activity higher than reported by the provider by a factor of 10. These observations demonstrate that care that must be taken when comparing results from different studies, even with the same enzyme preparation. it is essential to measure the activities of different enzymes with the same method in one lab for comparison purposes.

Table IV.2: Comparison of the cellulase and pectinase activity of the three enzymes (determined by ASA Spezialenzyme GmbH).

		Biopectinase CCM *	**Pektinase L40**	**Cellulase (EG)**
Provider		Kerry Bio-Science, Ireland	ASA, Germany	Megazyme, Ireland
Origin		*Aspergillus niger*	*Aspergillus niger*	*Talaromyces emersonii*
Optimum temperature (ºC)		35-**45**-50	20-**50**-**60**-55	40-**70**
Optimum pH		2.5-**3.5**-4.5	2.5-**4**-**5**-6	3-**4.5**-**4.6**-8
Enzymatic activity units U/ml	Endopectinase	3175	4608	0
	Pectinesterase	143	293	429
	Cellobiohydrolase (C1-Cellulase)	5	1	2
	Endoglucanase (CMCase)	1211	1	10370

* Product no longer marketed at the end of this study.

The evaluation results show that CCM had a rather high endoglucanase activity. The two other enzyme products were indeed rather pure. L40 had no significant endoglucanase activity While the EG had no endopectinase activity. For all the enzyme preparations there was no evidence for significant amount of cellobiohydrolase (C1-Cellulase) activity. Pectinesterase activity is present in all samples. This activity is not important in the present work, since we are studying cellulose dissolving pulps where, during the delignification and bleaching process, the pectic network was de-esterified. Thus, only the endopectinase and endoglucanase activities were considered for this study.

IV.2.2.3. - Comparison of endopectinase and endoglucanase effect

For a first comparison of EG and Pectinase effects, the initial pulp (S-PhK) was compared with three treated pulps: S-PhK_CCM, treated with CCM; S-PhK_L40, treated with pure pectinase L40, using the same load of endopectinase as for the S-PhK_CCM; and S-PhK_EG, treated with a pure endoglucanase EG using the same load of endoglucanase which was as side activity in the treatment with CCM (S-PhK_CCM). In addition a blank treatment with the same pH but without enzymes was performed (S-PhK_Blk). With the exception of the initial pulp which was not treated, the suspension consistency, incubation time, temperature and pH conditions were identical for all treatments (Table IV.1). Details of the applied enzyme activities are listed in Table IV.3, as well as the results of the pulp characterization in terms of cuen viscosity and dissolution yield in cold NaOH, which are also plotted on Figure IV.3.

Table IV.3: Enzymatic incubation conditions and intrinsic viscosity and dissolution yield results

Sample	Endopectinase activity units U/g (pulp)	Endoglucanase activity units U/g (pulp)	Total endopectinase activity (U*h/g pulp) *	Total endoglucanase activity (U*h/g pulp) *	Cuen viscosity (ml/g)	NaOH dissolution Yield (%)
S-PhK	-	-	-	-	450	20.7
S-PhK_3.5_Blk	0	0	0	0	440	21.0
S-PhK_CCM	250	95	3750	1436	400	54
S-PhK_L40	250	0	3750	1	410	50
S-PhK_EG	0	95	0	1436	400	28

* See section IV.2.2.4.3.

From these results (Table IV.3 and Figure IV.3) several observations can be discussed. After the results obtained with the control experiment (S-PhK_3.3_Blk), it can be stated that the buffer system used (aqueous solution acidified with acetic acid to pH 3.5) is not changing the pulp properties, which means that all changes seen with enzymatic treatments are due to the enzymes. The decrease of 10 ml/g on the intrinsic viscosity falls within the estimated error. Nevertheless, as it will be discussed later, it can be due to a slight acid hydrolysis from the acidic conditions of the

buffer. All the enzymatic treatments decreased the intrinsic viscosity in a similar magnitude, with a drop from 450 ml/g on the initial pulp to 400 ml/g with the CCM and EG treatments, and to 410 ml/g with the incubation with L40. The treatment of the pulp with CCM is increasing the dissolution yield from 21% to 54%, an improvement of 157%. When using pure pectinase (L40) with the same endopectinase dosage and without endoglucanase activity, the pulp also shows a considerable increase (140%) of the dissolution capacity in sodium hydroxide, reaching a dissolution yield of 50%. When the pulp is incubated with the pure endoglucanase, using the same endoglucanase activity units per gram of pulp used in the treatment with CCM (95 U/g), the dissolution yield achieved in cold caustic soda is 28%, representing an increase of only 26%.

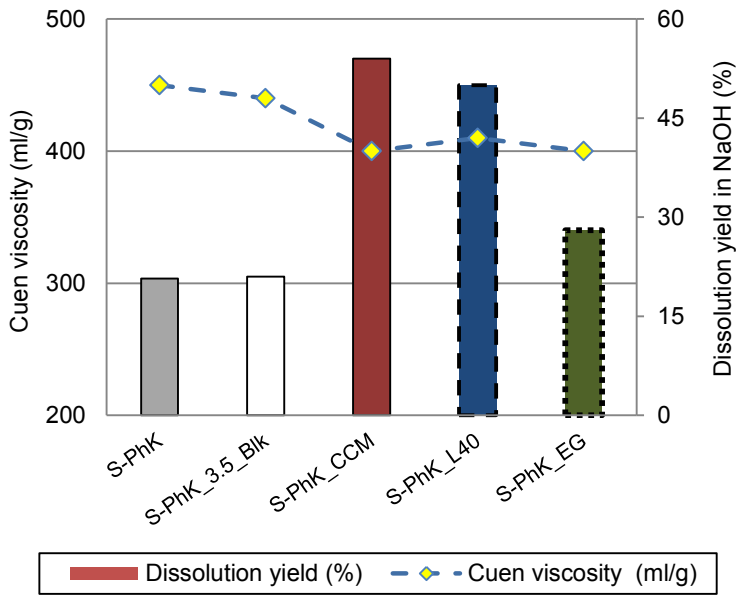

Figure IV.3: Dissolution yield in cold soda and cuen viscosity of the initial pulp, the buffer blank (S-PhK_3.5_Blk), and the pulp with CCM, L40 and EG treatments. (Identical endopectinase dosage for CCM and L40; identical endoglucanase dosage for CCM and EG).

The microscopic observation of the insoluble fraction, allows a better insight on the accessibility of the fibers, since the morphology and swelling degree of the insolubles are directly correlated with the dissolution capacity. Microscopic observations of cellulose fibers dissolution have been performed previously in order to describe the kinetics and mechanisms of dissolution [Cuissinat, 2006a; Le Moigne, 2008; Trygg, 2011]. The morphology of insoluble fractions of different cellulose fibers were also described in various studies, relating this morphology with the swelling, dissolution and accessibility capacity [Jardeby, 2004, 2005a, 2005b and 2007; Le Moigne, 2010; Spinu, 2011; Santos, 2013]. In order to allow a systematic approach for insoluble characterization, Stawitz and Kage established a scale for the swelling degree of cellulose fibers (Figure IV.4) [Stawitz, 1959]. With this “scale of swelling” one can make a better assessment on the stage of swelling of the insoluble material, which can be related with accessibility and dissolution capacity of the cellulose fibers [Cuissinat, 2006a; Le Moigne, 2008; Spinu, 2011; Santos, 2013].

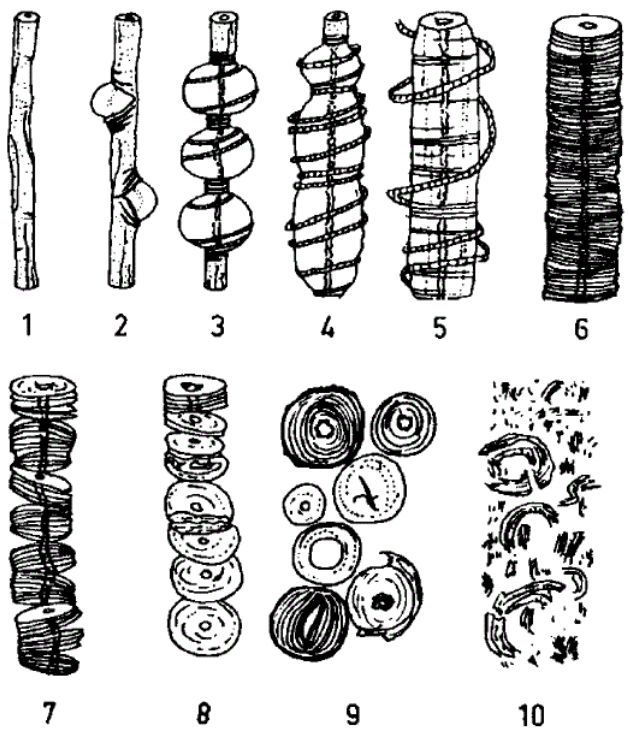

Figure IV.4: Swelling scale for CMC fibers [Stawitz, 1959]

In the present work, the observations of the morphology of the NaOH insoluble fractions after dissolution in cold NaOH (Figure IV.5) was performed and compared to the swelling scale in Figure IV.4. NaOH insolubles of samples treated with endopectinase (CCM and L40) are composed of small highly swollen fiber fragments and flat rings, corresponding mostly with the later stages of swelling according to Stawitz and Kage [Stawitz, 1959]. For the pulp treated exclusively with endoglucanase, the insoluble fractions are similar to the one seen for the untreated pulp, showing a slight swelling of the fibers and a high amount of intact fibers, corresponding to the initial swelling stages. Some of the fibers present sectional cuts, this was also reported by Le Moigne et al, and is explained by the shearing promoted by the mixing during the dissolution process [Le Moigne, 2010]. The small residues visible for the initial pulp (S-PhK) correspond to small swollen fibrils and regenerated cellulose from partially dissolved material. Concerning the results for the endopectinase treatments, similar results were reported by Le Moigne et al after enzymatic peeling. The results were explained mainly by two effects, the removal of the external wall and de-structuration of the fiber [Le Moigne, 2010]. In our case, the enzymatic incubations with L40 and CCM are improving the swelling and dissolution capacity by changing the fiber structure, on that the external wall is not being removed, as can be seen by the next results from the FEG-SEM analysis. This de-structuration was enough to eliminate the swelling resistance form the outer cell wall layers, once that these structures are the responsible to prevent the overall swelling of the fiber and subsequent dissolution [Cuissinat, 2006a; Le Moigne, 2010; Spinu, 2011; Santos, 2013]. It is still necessary to understand how and specifically in which parts of the cellulosic structure these changes are taking place.

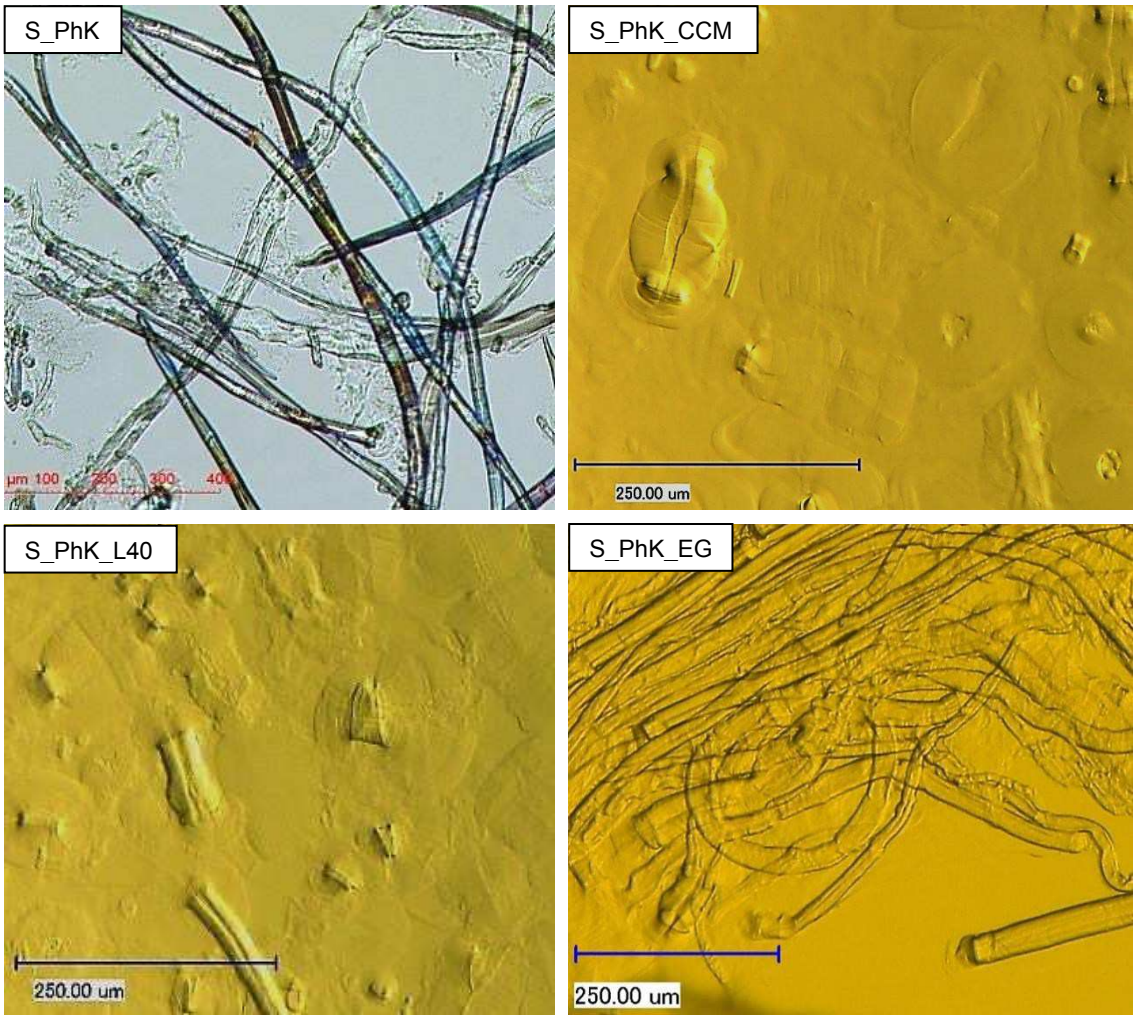

Figure IV.5: Effect of the enzymatic treatments on the morphology of NaOH insolubles from starting pulp and pulp treated with endopectinase (L40 and CCM) or endoglucanase (EG). The picture from S-PhK was taken with an optical microscope at CEMEF, Sophia Antipolis, while the others were taken with an optical microscope at Hamburg University, Hamburg.

The pulp fibers surface was further observed by FEG-SEM and the results are shown in Figures IV.6 (a and b). When the pulp is treated only with endopectinase (L40), the fiber surface shows no significant to the initial pulp, showing in both cases the presence of micro fibrils attached on the fiber surface. When the fibers are submitted to incubations where endoglucanase is present (S-PhK_CCM and S-PhK_EG), these fibrils are not observed, showing a smoother surface. Endoglucanase is hydrolyzing the small fibrils at the fiber surface. This is in accordance with the intrinsic viscosity measurements where, despite the values fall within the error (±10 ml/g), the viscosity is slightly lower (400 ml/g) if incubation is made with endoglucanase than the one after incubation with pure endopectinase (410 ml/g). The bordered pits from the fibers do not show the presence of the pit membranes (torus-margo structure), which were removed with the delignification process (pre-hydrolysis kraft). Similar microscopic observations for the endopectinase effect on the fibers surface were described by different authors [Mansfield, 1997; Chinga-Carrasco, 2010; Ibarra, 2010b; Gehmayr, 2011b and 2012]. Further discussion on the FEG-SEM observations is done in Section IV.2.3.1

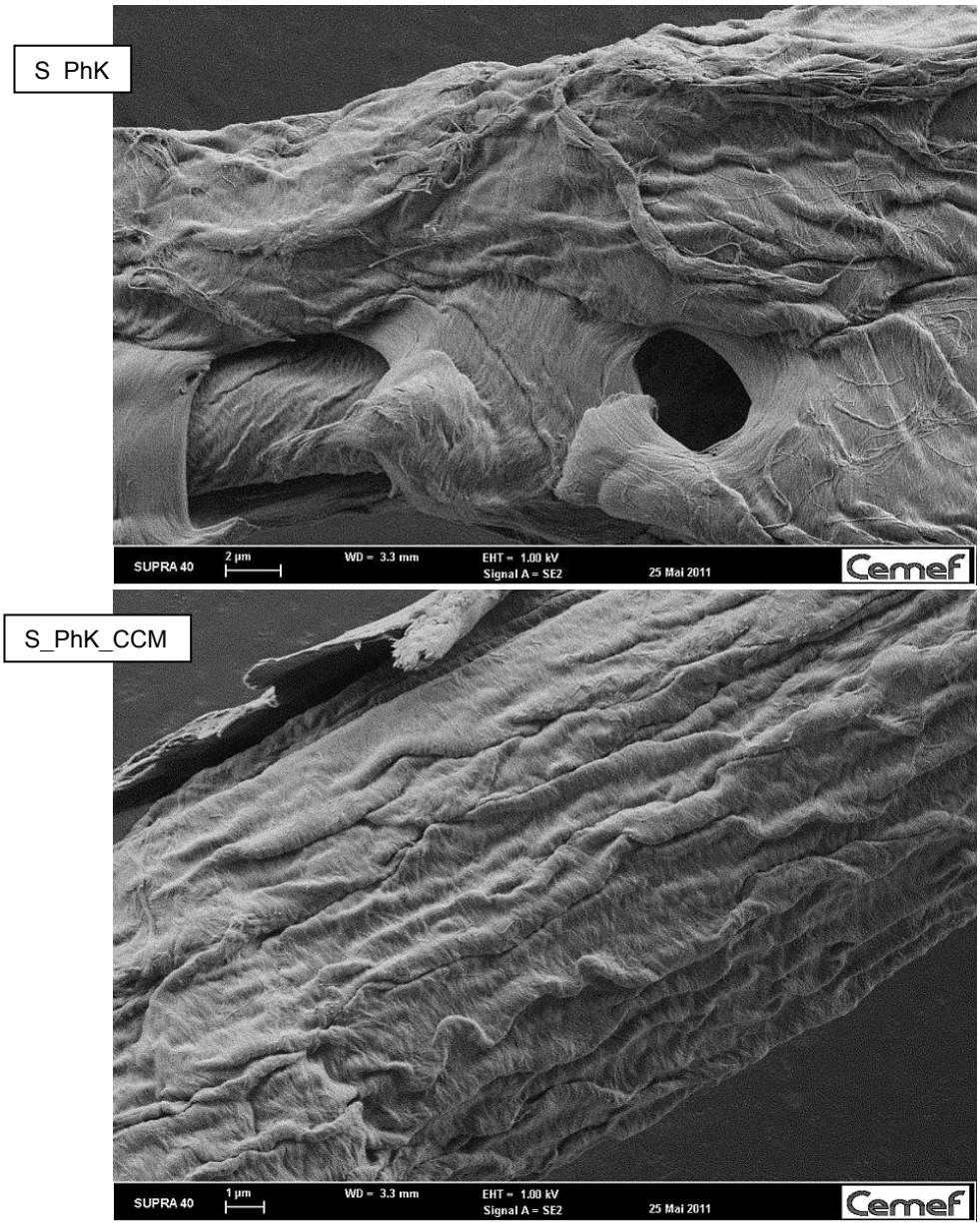

Figure IV.6a: FEG-SEM images of the fiber surface of untreated pulp (S-PhK) and pulp treated with CCM (S-PhK_CCM).

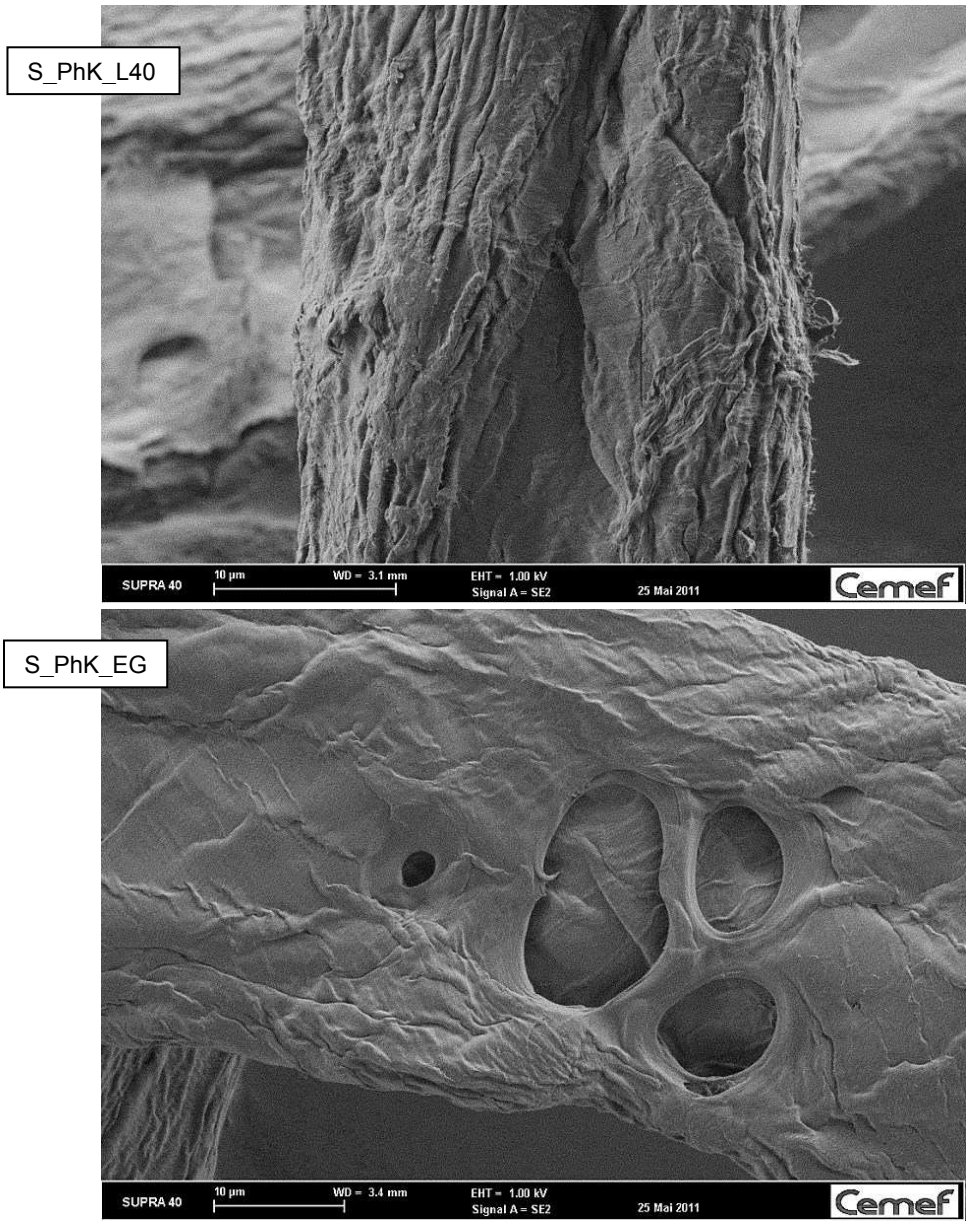

Figure IV.6b: FEG-SEM images of the fiber surface of pulp incubated with L40 (S-PhK_L40) and pulp treated with EG (S-PhK_EG).

One conclusion of this series of experiments is that pectinase itself is indeed efficient for improving dissolution of pulp in cold NaOH without decreasing significantly molar mass for the S-PhK pulp. Nevertheless, there is a 10% reduction on the intrinsic viscosity after the pectinase treatments. This might be due to some hydrolysis mechanism that was not possible to identify in this study. The major advantage of this method is that there is no loss of material during the process. In addition, such method is relatively easy to implement in industry, without significant changes in the current processes. Due to the high interest of the topic, several studies were done in order to increase the solubility of dissolving pulps in several solvating processes. AECL (Atomic Energy of Canada Limited) and Faserwerk Kelheim GmbH increased the reactivity of dissolving

pulp using electron beaming which reduced also the DP to values around 300 [Rajagopal, 1994]. Kihlman et al managed to increase dissolving pulps solubility in NaOH solutions with steam explosion and hydrothermal pretreatments, however associated with a DP decrease higher than 50% [Kihlman, 2012]. This is somehow similar to what Japanese researchers found in the 90's when they showed that steam explosion, especially for pulps with DP lower than 400 was helping to produce stable cellulose solutions [Yamashiki, 1990a, 1990b]. Takahashi et al and Kamide et al showed that the reduction of intra-molecular hydrogen bond between at C3 and C6 positions is correlated with an increase of solubility. Solid state ^{13}C NMR confirmed the destruction of intra-molecular O3-H···O'5 and inter-molecular O2-H···O'6 hydrogen bonds when in solution [Takahashi,1991; Kamide, 1992].

A removal of the primary wall, by chemically treating dissolving pulp with ethanol-hydrochloric acid for improving the solubility in a NaOH-water based solvent was investigated by Trygg et al, in this case, a material loss occurs (mostly primary wall) and the DP is significantly reduced [Trygg, 2011]. TEMPO-mediated oxidation is able to help the dissolution of cellulosic fibers, associated also with decrease of the DP [Gehmayr, 2012b]. Biologic treatments were also applied, using enzymatic incubations, mainly with cellulases. Endoglucanase treatments show similar dissolution improvements obtained in this work, but with a higher decrease in the DP [Rahkamo, 1996; Henriksson, 2005; Cao, 2006; Engström, 2006; Kvarnlöf, 2007 and 2008; Köpcke, 2008; Wang, 2008; Ibarra, 2010; Le Moigne, 2010; Östberg, 2012].

Further on, it can be concluded that the main effect on dissolution yield of CCM is due to the pectinase while the decrease of viscosity is due to the endoglucanase, as clearly shown on Figure IV.3. We saw that the endoglucanase is affecting the fiber surface structure while the incubation of pulp with endopectinase is not modifying the fiber surface as observed by electron microscopy, but acts in such way that it is either increasing the NaOH ion in the internal microstructure of the pulp fibers or having another effect so that the dissolution capacity is improved. However, the highest dissolution yield in cold sodium hydroxide is achieved with the synergistic effect of both endopectinase and endoglucanase (effect of the CCM enzymatic mixture). It is broadly known that the combined use of several enzymes brings synergistic effects. Medve et al studied the synergistic effect of endoglucanase II and cellobiohydrolase I on the hydrolysis of microcrystalline cellulose [Medve, 1998]. Synergisms between endoglucanases and exoglucanases have also been used on the saccharification of lignocellulosic material [Wood, 1979; Kanda, 1980; Azuma, 1984], which efficiency can be further increased with inclusion of β-glucosidase, also promoting a synergistic effect with the cellulases [Mansfield, 1999]. Cho et al showed the combined effect of the use of cellulase, hemicellulase and xylanase on saccharification of *Miscanthus sacchariflorus* [Cho, 2013], and a similar combination of xylanase and pectinase was used to improve the bleaching of eucalyptus kraft pulp [Beg, 2001; Ahlawatt, 2008]. In order to remove hemicelluloses from dissolving pulps, a combination of xylanase with mannanase acted synergistically on the pulp, increasing in 50% and 11% the solubility of xylan and mannan compared to the individual action of the enzymes [Gubitz, 1997]. Spagnuolo et al

showed that a pectinase, by hydrolyzing the pectic surface of a lignocellulosic substrate, favored the degradation of cellulose and hemicellulose by cellulases and hemicellulases [Spagnuolo, 1997]. A similar effect could be advocated to explain the results we have with the CCM. The endopectinase could degrade the pectic network, which gives room for the endoglucanase to reach cellulose chains that were not accessible without the pectinase action. This is leading to a higher loss in intrinsic viscosity and fiber accessibility, allowing this way to an easier diffusion of the NaOH ions into the fiber, increasing the dissolution yield. The problem in this explanation is that these pulps, according to the carbohydrate analysis, have nearly no detectable pectin present.

The objective of the next paragraphs is to further improve dissolution yields in cold NaOH by optimizing the incubation parameters with both pectinase preparations (CCM and L40).

IV.2.2.4. - Optimizing incubation parameters

In order to understand the behavior of the enzyme preparations, several parameters were studied, namely their concentration, the time of treatment and the buffer system. The constant parameters for performing this study are listed in the following table (Table IV.4). As discussed above, the pulp suspension consistency was optimized for an efficient mixing of the pulp suspension. The temperature and pH were selected according to the optimum temperature and optimum pH range for the enzymatic solutions used. The buffer system concentration was optimized to minimize any possible effect on the pulp properties without losing its buffer capacity.

Table IV.4: Parameters that were kept constant during the optimization of incubation.

Pulp	S-PhK
Pulp amount (g)	20
Pulp suspension consistency (%)	5
Temperature (ºC)	45
pH	5
Buffer	Citric/Phosphate 0.0015 M

In order to evaluate the results of each test, the intrinsic viscosity and the dissolution yield in cold caustic soda were measured.

Due to the high number of treatments performed, and since the substrate was always the same pulp, the recovered pulps are named with the name of the enzyme used, followed by a test number Txx, (Ex. CCM_T21). The parameters used in each incubation are described in Tables IV.5 to IV.9.

IV.2.2.4.1. - Influence of enzyme concentration

The four different concentrations used for each enzyme (CCM and L40) for evaluating the influence of the enzymatic charge or concentration on the effectiveness of the treatment towards cold NaOH dissolution are described in Table IV.5.

The blank (same for both enzymes) is used to evaluate if the buffer system is promoting any modification on the fiber properties. The blank for the L40 enzyme is the same as for the CCM enzyme, thus the CCM_T19* is the same experiment as the CCM_T19.

Table IV.5: Enzymatic incubation conditions used to study the influence of CCM and L40 enzyme concentration and intrinsic viscosity and dissolution yield results.

Sample	**Endopectinase activity units U/g (pulp)**	**Endoglucanase activity units U/g (pulp)**	Total endopectinase activity (U*h/g pulp) **	Total endoglucanase activity (U*h/g pulp) *	Time (hours)	Cuen viscosity (ml/g)	NaOH dissolution yield (%)
CCM_T19 (Blank)	**0**	**0**	0	0	5	450	22.6
CCM_T16	**20**	**8**	100	40		450	21.9
CCM_T12	**50**	**19**	250	95		440	26.9
CCM_T15	**100**	**38**	500	190		430	30.7
Blank (CCM_T19*)	**0**	**0**	0	0	5	450	22.6
L40_T12	**20**	**0.004**	100	0.02		450	20.7
L40_T11	**50**	**0.01**	250	0.05		450	20.7
L40_T10	**100**	**0.02**	500	0.1		430	25.2

** See section IV.2.2.4.3.

The cuen viscosities and dissolution yields are plotted in Figure IV.7, where the results from the treated fibers are grouped according to the enzyme used (CCM or L40) and compared with the untreated pulp (S-PhK). The blank (control) treatments are not changing the intrinsic viscosity of fiber but the dissolution yield is slightly increased in comparison with the untreated pulp. This increase, despite falling within the error of measurement, can be explained by the fact that salt based buffers it can improve the swelling capacity of the cellulose fibers [Weightman, 2010, Zhang, 2010], and as a consequence, slightly improve its accessibility. The CCM treatments has no effect with a concentration of 20 endopectinase units per gram of pulp, while for higher concentrations, there are both a decrease on the intrinsic viscosity and an increase on the dissolution capacity of the pulp. The L40 enzymatic solution is less efficient since there is an effect on the pulp, with an increase of the dissolution yield and a minor loss on the intrinsic viscosity only for the highest enzyme concentration (100 U/g). The ineffectiveness of the enzymes at low concentrations gives an indication that there is a minimum concentration in which the enzymes are “activated”. The dissolution yield increases and the viscosity decreases with enzyme

concentration. The CCM revealed to be more efficient than L40 since a higher dissolution yield is achieved for similar endopectinase activities, with similar viscosity losses. For instance, when using an activity of 100 U/g of pectinase, both treated pulps have the same viscosity of 430 ml/g, while the dissolution yield for the CCM treated pulp is 30.7%, higher than the 25.2% from the pulp incubated with L40. The previous result that showed an increase of dissolution yield with L40 without a decrease of viscosity is not confirmed here.

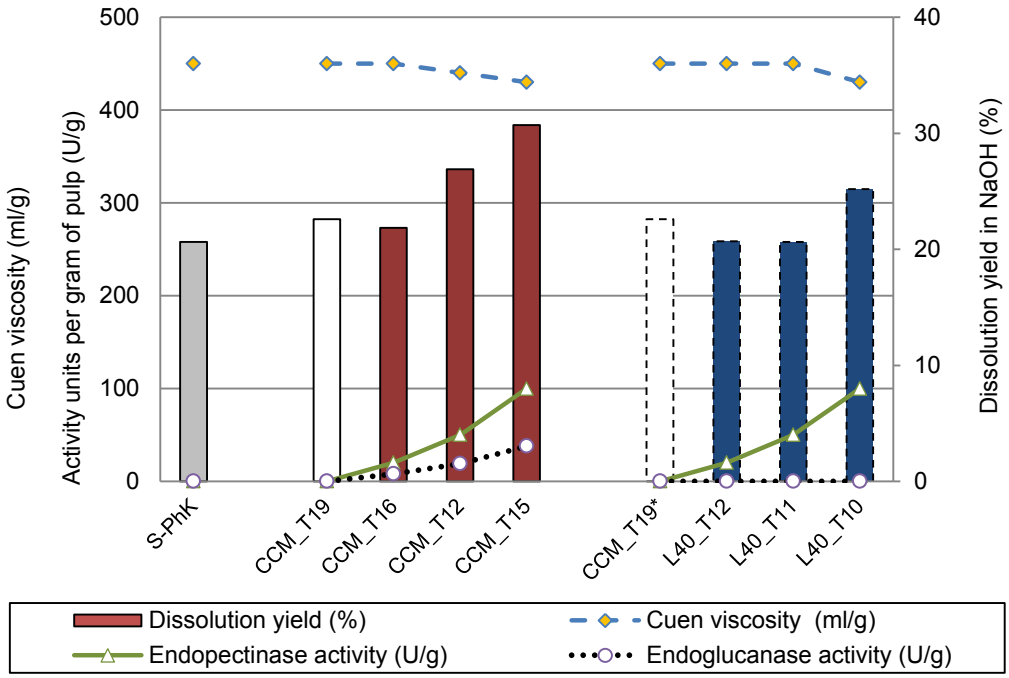

Figure IV.7: Influence of the enzyme concentration on the incubation performance.

IV.2.2.4.2. - Influence of incubation time

In order to study the influence of the incubation time on effect of the enzyme treatment, four different trials with different times were performed for each enzyme preparation. Since the L40 has shown a lower effectiveness when compared with the CCM, the initial endopectinase activity units used were twice the ones used for the CCM (Table IV.6).

In Figure IV.8, one can visualize that despite the lower concentration, the CCM treatment is promoting a much higher dissolution of the pulp in comparison with the L40. This effect is clearly amplified with the increase of the incubation time. According to Evans and Wallis, the degree of polymerization (DP) can be correlated with the limiting number of intrinsic viscosity (η) using the formula IV-1 [Evans, 1989]:

$$[\eta] = 0.606 \times \mathrm{DP}^{0.9} \qquad \left(\frac{\mathrm{ml}}{\mathrm{g}}\right) \qquad [\mathrm{IV}-1]$$

Table IV.6: Enzymatic treatment conditions used to study the influence of the incubation time on intrinsic viscosity and dissolution yield

Sample	Endopectinase activity units U/g (pulp)	Endoglucanase activity units U/g (pulp)	Total endopectinase activity (U*h/g pulp) *	Total endoglucanase activity (U*h/g pulp) *	**Time (hours)**	Cuen viscosity (ml/g)	NaOH dissolution yield (%)
CCM_T23	250	95	250	0	**1**	440	30.2
CCM_T18			500	40	**2**	440	30.9
CCM_T22			1250	95	**5**	430	36.2
CCM_T21			3750	190	**15**	420	44.1
L40_T18	500	0.1	500	0.10	**1**	440	24.7
L40_T17			1000	0.2	**2**	460	24.2
L40_T16			2500	0.5	**5**	450	26.1
L40_T15			7500	1.5	**15**	440	27.9

* See section IV.2.2.4.3.

For the presented results in Table IV.6, the highest decrease of intrinsic viscosity, from 450 to 420, corresponds to a DP reduction from 1548 to 1430, meaning a decrease of 7.6%. The L40 enzymatic solution is increasing the pulp dissolution yield in cold caustic soda, to a small extent but as well proportional to the time of treatment. The intrinsic viscosity is only slightly decreasing with during CCM incubations, while with the L40 treatments, considering the 450 ml/g from the initial pulp, the viscosity is not changing. This means that these treatments are not affecting the overall chain length of the cellulose.

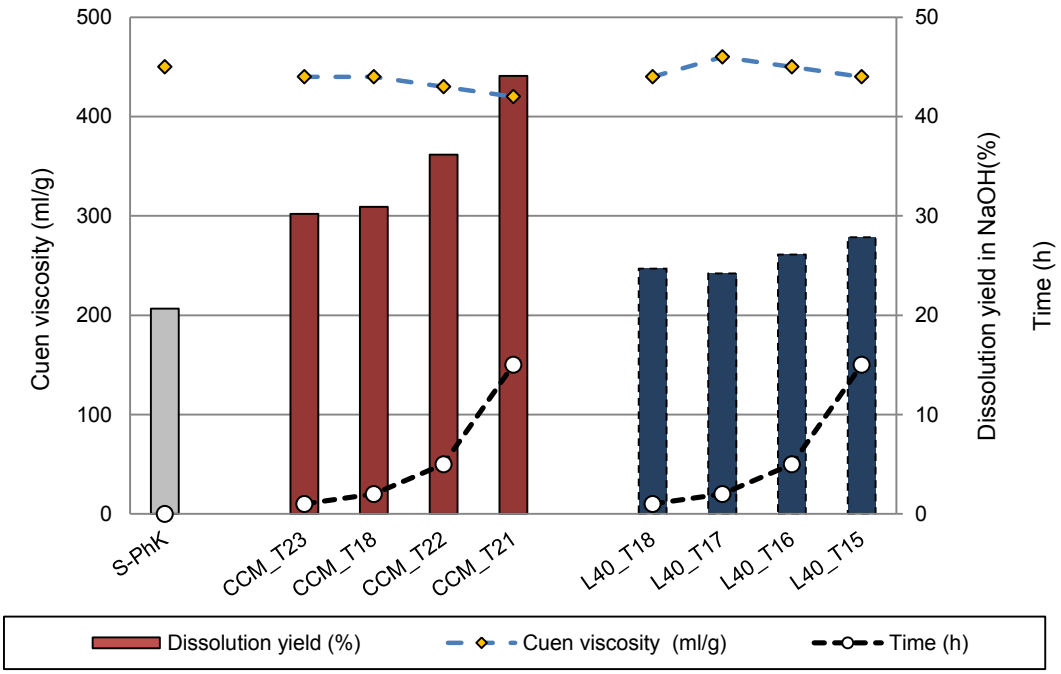

Figure IV.8: Influence of the incubation time on the effects of the enzyme treatment

IV.2.2.4.3. - Assessment of the total activity of enzymes and study of enzyme stability

Since both the enzyme dosage and the incubation time affect the treatment, a new parameter was defined and named "total activity units". This takes in consideration the initial activity units (units per gram of pulp) and the incubation time (in hours):

$$Total\ activity\ units = \frac{initial\ activity\ units\ \times incubation\ time\ (hour)}{grams\ of\ pulp} \qquad \left(\frac{\mathrm{U}}{\mathrm{g}} * h\right) \qquad [\mathrm{IV}-2]$$

This parameter allows having a numerical indication on the intensity of the treatment. We kept a "total activity units" of endopectinase at 500 U*h/g and varied concentrations and incubation times. The results are given in Table IV.7.

Table IV.7: Time and concentration conditions used for keeping a constant total pectinase activity for the L40 and CCM; intrinsic viscosity and dissolution yield results.

Sample	**Endopectinase activity units U/g (pulp)**	**Endoglucanase activity units U/g (pulp)**	Total endopectinase activity (U*h/g pulp) *	Total endoglucanase activity (U*h/g pulp) *	**Time (hours)**	Cuen viscosity (ml/g)	NaOH dissolution yield (%)
CCM_T24	**500**	**190**			**1**	440	30.9
CCM_T18	**250**	**95**			**2**	440	30.9
			500	190			
CCM_T15	**100**	**38**			**5**	430	30.7
CCM_T17	**33**	**13**			**15**	440	30.6
L40_T14	**250**	**0.05**			**2**	450	23.1
L40_T10	**100**	**0.02**	500	0.1	**5**	430	25.2
L40_T13	**33**	**0.007**			**15**	450	22.1

Figure IV.9 shows that for the pulps treated with CCM, the results are very similar, both intrinsic viscosities and dissolution yields in cold sodium hydroxide being constant whatever the time and enzyme concentration were, providing that the total endopectinase and endoglucanase activities were kept constant. It is recalled (Table IV.3) that a large total endoglucanase activity (1436 U*h/g) with a pure endoglucanase enzyme (EG,) gives a large decrease of intrinsic viscosity (400 ml/g) for a low dissolution yield (28%). This is stressing the very important effect of pectinase or the synergistic effect of pectinase and endoglucanase.

This effect is not so evident for L40 but results present the same trend as CCM, showing no significant variations on the pulp properties with concentration and time of treatment, providing the total activity is kept constant.

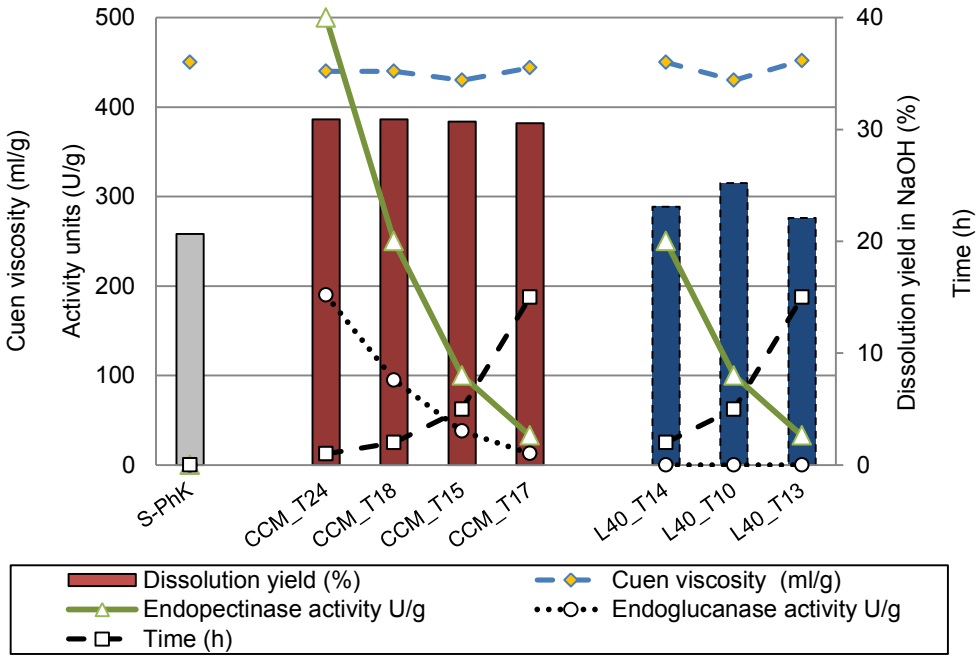

Figure IV.9: Pectinase incubation performance keeping the total endopectinase activity constant.

This means that the same results can be achieved with a short incubation time with high enzyme concentration or with a long incubation period with a low enzymatic charge. For the studied range of time and concentration, one can also say that the enzymatic activity or performance is not weakened over time, suggesting the possibility of re-utilization of the enzymatic solution, or the possibility of using continuous incubation systems.

IV.2.2.4.4. - Buffer system

In order to select the best incubation conditions to be applied on the different cellulose pulps, we studied which buffer system was yielding pulps with the best accessibility to cold caustic soda solution. For this, for both enzyme preparations, the pulps were treated with similar total activities, but with two different buffer systems, a citric/phosphate solution tuned for pH 5 and the previously used acetic acid solution adjusted pH 3.5.

Table IV.8: Enzymatic incubation conditions used to study the enzymatic stability over time, with constant total activities; intrinsic viscosity and dissolution yield results.

Sample	Endopectinase activity units U/g (pulp)	Endoglucanase activity units U/g (pulp)	Total endopectinase activity (U*h/g pulp) *	Time (hours)	**Buffer system**	Cuen viscosity (ml/g)	NaOH dissolution yield (%)
CCM_T21	**250**	**95**	3750	**15**	**pH 5 Citric/Phosphate**	420	44.1
CCM_T26					**pH 3.5 CH_3OOH**	400	54
L40_T10	**100**	**0.02**	500	**5**	**pH 5 Citric/Phosphate**	450	23.1
L40_T19	**32**	**0.007**	480	**15**	**pH 3.5 CH_3OOH**	420	34.2

Table IV.8 and Figure IV.10 show that by using a pH of 3.5 adjusted with CH_3COOH instead of the citric/phosphate buffer, the treated pulps have higher dissolution ability, although this is again associated with a loss of intrinsic viscosity. However, for a similar intrinsic viscosity (420 ml/g), the CCM in citric/phosphate buffer gives a higher dissolution yield than for the L40 in acetic acid. This suggests that the buffer system may not be a main parameter, but that what counts are the synergy of the enzymes found in CCM and the decrease of intrinsic viscosity.

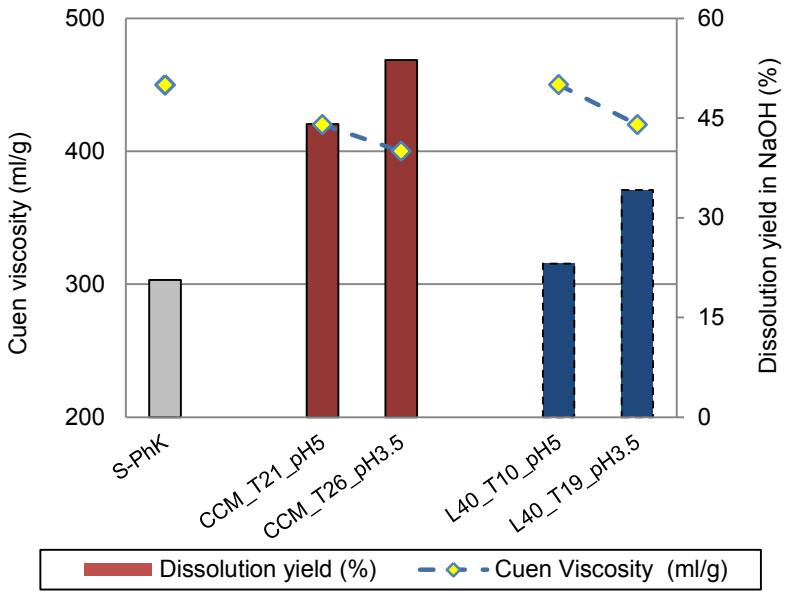

Figure IV.10: Influence of the buffer system on the enzymatic performance.

IV.2.3. - L40 and CCM enzymatic treatments of different pulps and effects on dissolution in cold soda

From the performed studies, the best incubation conditions for maximizing the cellulose dissolution yield in cold sodium hydroxide were selected. These conditions are listed in Table IV.9 and were used to study different pulps with both CCM and L40 enzymatic preparations:

Table IV.9: Incubation conditions used for the enzymatic treatments of the different cellulose pulps.

Pulp amount (g)	20
Pulp suspension consistency (%)	5
Incubation time (hours)	15
Temperature (ºC)	45
pH	3.5
Buffer	pH adjusted with CH_3COOH
Endopectinase activity units per gram of pulp (U/g)	250
Total endopectinase activity units per gram of pulp (U/g)	3750

Five dissolving pulps were used (Be-S; S-PhK; NS-S; SS-S and Eu_Vis) and one paper grade pulp (Eu-KP). A detailed description of these pulps is available in Table II.1 of Chapter II.

All samples were treated with a blank solution (only buffer), Biopectinase CCM and Pektinase L40. From these pulps, three were selected and the effect of the enzymatic extractions on the fiber properties was accessed. All samples were analyzed regarding their dissolution yield in cold NaOH. These results were compared with the initial pulps.

IV.2.3.1. - Effect of the pectinase incubations on the pulp properties

After CCM and L40 treatments, intrinsic viscosities (Figure IV.11), molar mass distributions (Figure IV.12) and X-ray diffraction (Figure IV.13) were measured.

With the control treatments, without enzyme, the pulp intrinsic viscosity is decreasing only 10 ml/g for all pulps, which is a small decrease but seems to be a true effect (seen already in Table IV.7) due to some acid hydrolysis of the pulp. The molar mass distributions (Figure IV.12), show that the distribution from the pulps submitted to the blank (control) treatments are practically coincident with the molar mass distribution from the correspondent untreated pulps, which is consistent with the low decrease of intrinsic viscosity. The buffered solution is not affecting much the weight average molar mass or the molar mass distribution of the studied pulps as also stated in section IV.2.2.3.

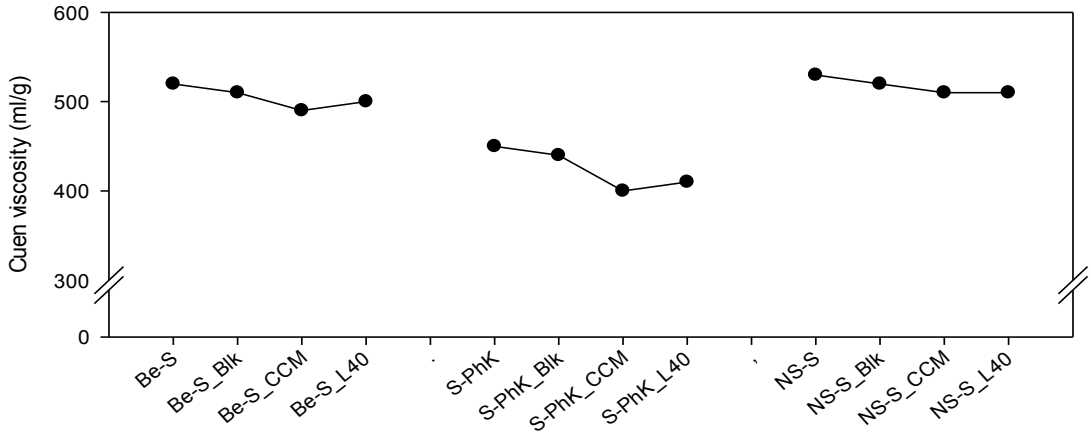

Figure IV.11: Effect of the enzymatic treatments on the intrinsic viscosity of the pulp.

The enzymatic incubations are affecting the weight average molar mass of the cellulose fibers. This degradation is more evident for softwood pulp pretreated with a pre-hydrolysis kraft delignification (S-PhK), which is also verified on the molar mass distribution, where there is a slight shift of the distributions towards lower molar mass values after enzymatic incubations. From the molar mass distributions, it can be concluded that it exists no preferred degradation of high molar mass cellulose chains and that the shape of the distribution profile is not altered, meaning that the enzymatic action is transversal to all molecules. Although the difference is not substantial, the CCM enzymatic solution is affecting the molar mass distribution more than the L40 endopectinase preparation, due to the presence of the endoglucanase component. As already discussed, it is also apparent that the pectinase treatments have a small effect on the decrease of the average molar mass of the pulps. Previous studies with other enzymatic treatments showed a high decrease of the DP [Rahkamo, 1996; Henriksson, 2005; Cao, 2006; Engström, 2006; Kvarnlöf, 2007 and 2008; Köpcke, 2008; Wang, 2008; Ibarra, 2010; Le Moigne, 2010; Östberg, 2012].

Comparing the molar mass distributions (Figure IV.12) with other studies, the low degradation of the material is confirmed. Kihlman et al reported a high decrease on the molar mass with decrease of the polydispersity for a sulfite spruce dissolving pulp after steam explosion pre-treatment. For hydrothermal pre-treatment of a pre-hydrolysis kraft southern pine dissolving pulp, the same authors presented similar trend of decrease on molar mass and polydispersity, but not so intensive as for the steam explosion [Kihlman, 2012]. The treatment of pulps with endoglucanase is shifting significantly the molar mass distribution towards lower masses [Rahkamo, 1996; Engström, 2006; Gehmayr, 2011]. Treatments with cellobiohydrolase show results similar to the endopectinases, with little effect on the molar mass distribution [Rahkamo, 1996; Mansfield, 2003]. Acid hydrolysis is also decreasing the molar mass, changing the polydispersity with a more significant degradation of the molecules with higher molar mass [Engström, 2006].

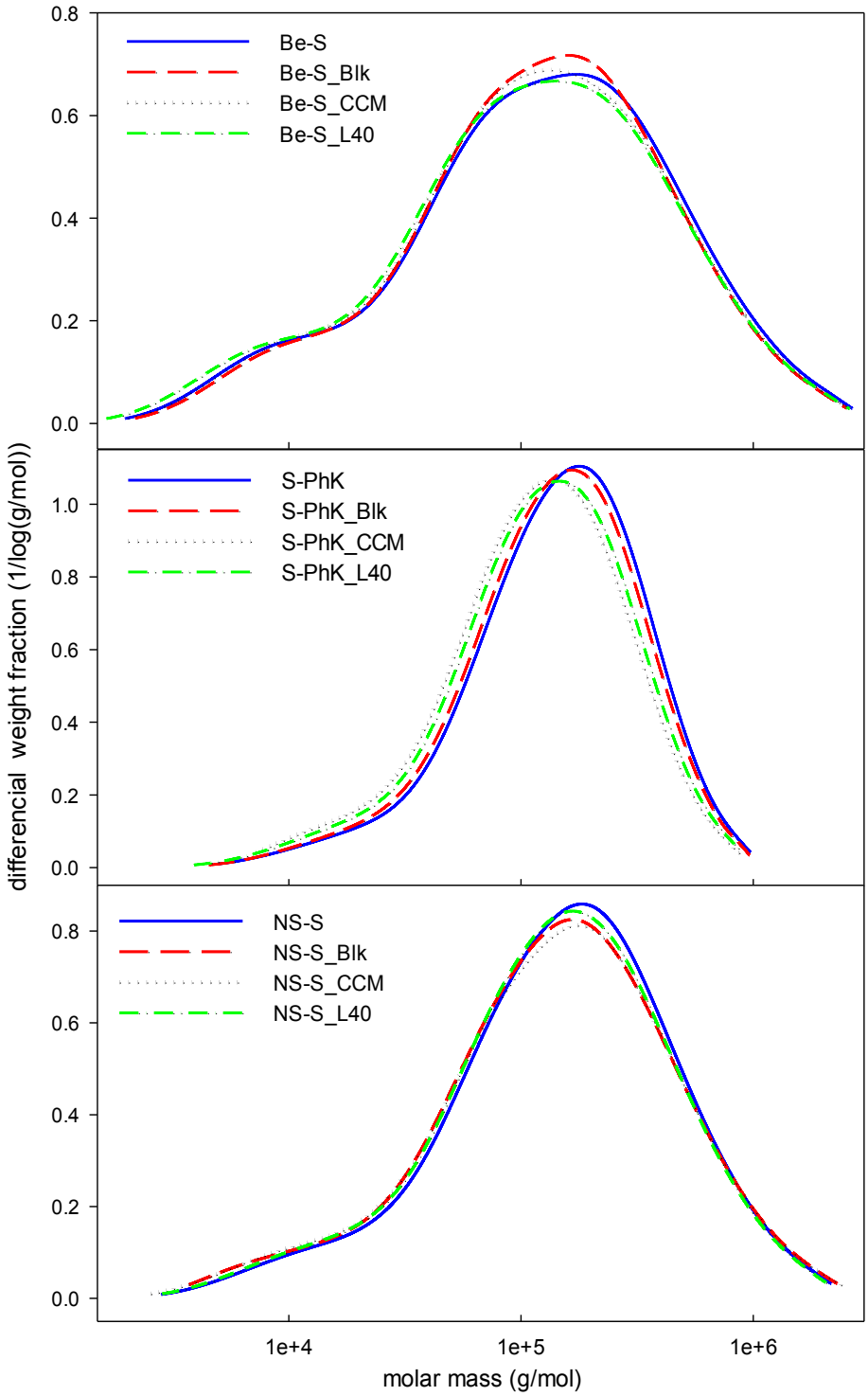

Figure IV.12: Effect of the enzymatic treatments on molar mass distribution of pulps

In general, the crystallinity of lignocellulosic material varies depending on different factors, namely, the initial amorphous content, determined by biosynthesis; the extraction or pre-treatment processes that induce changes in the cellulose chain order; and the higher mobility of cellulose

chains on the particle surfaces [Moon, 2011]. Besides this, the determination of crystallinity indexes can be also challenging due to the existence of many interfering factors such as the presence of hemicellulose, lignin, residual extractives and pectin, and the interaction between them [Kang, 2013]. For this work, as for the nitren treatment, the crystallinity index values are high, when compared with literature [Cuissinat, 2006a; Leppanen, 2009; Le Moigne 2010]. This is due to the simple method used for the measurements, the Segal-WAXS-18˚. Due to ease of use, 70 to 85% of the publications do the calculations with this method [Kang, 2013]. This empirical method was first developed by Hermans, and Weidinger [Hermans, 1948 and 1949; Röder, 2006], and further developed by Segal et al. [Segal, 1959]. This procedure is known to give high values when related with other methods like Raman or Infrared [Park, 2009; and 2010; Agarwal, 2010]. To overcome this overestimation of the crystallinity on cellulose I, as it is the case for the present samples, Agarwal et al. proposed that for the contribution of the amorphous phase, the peak height should be measured at a Bragg angle of ~21 ˚ instead of ~18 ˚.The analysis of amorphous cellulose standard samples (e.g. ball-milled cellulose), confirmed that the highest values are in the 2θ region of 21 ˚ [Agarwal, 2010]. This suggestion is supported by other authors [Teeaar, 1987; Isogai, 2009; Park, 2010] and is known as method Segal-WAXS-21˚ [Agarwal, 2010 and 2013]. However, for the present study, only a comparison of the different samples was persuit and the absolute value were not of such importance.

The diffractograms (Figure IV.13) show that the crystal type is, as expected, not affected by the treatment, since the presence of its characteristic Cellulose I peaks around 2θ = 16.5 ˚ and 22.5 ˚ is maintained [Chen, 2011]. The crystallinity index is defined according to Segal et al:

$$C_{Ir}(\%) = [(_{I220} - I_{am})/I_{200}]\times 100 \qquad \text{[IV-3]}$$

, where I_{200} is the peak intensity corresponding to both the amorphous and crystalline fractions of cellulose I (maximum intensity between 2θ = 21 and 23 º) and I_{am} is the peak intensity of the amorphous fraction (minimum peak intensity between 2θ = 18 and 20 º) [Segal, 1959, Jonoobi, 2011, Ramos, 2005].

The crystallinity, calculated from the diffractograms, was 75% for S-PhK, 80.9% for S-PhK_CCM and 79.7% for the S-PhK_L40 pulp. The effect on crystallinity index of these two enzymes is very strong. Crystallinity index for native cellulose is mainly due to the size of the crystals. The smaller the crystals are, the lower is the crystallinity index due to the widening of diffraction peaks. The interpretation of the increased crystallinity index after CCM and L40 treatments is not straightforward. Similar effects after endoglucanase treatments are usually ascribed to the fact that amorphous phases are more accessible to enzymatic destruction than the crystalline parts, with the consequence of increasing the crystallinity index [Mansfield, 2003; Cao, 2004, 2005 and 2006; Henriksson, 2005; Engström, 2006; Gehmayr, 2011]. According to Krässig, the increase of crystallinity might be also explained by the recrystallization of amorphous regions promoted by internal stress released during a treatment. [Krassig, 1993a]. Other authors attribute the increase of crystallinity due to physical annealing, aided by water plasticization. This would

involve the decrease of crystal defects and strains and also possible aggregation between smaller crystal domains or co-crystallization [Chen, 2010; Ibbett, 2013].

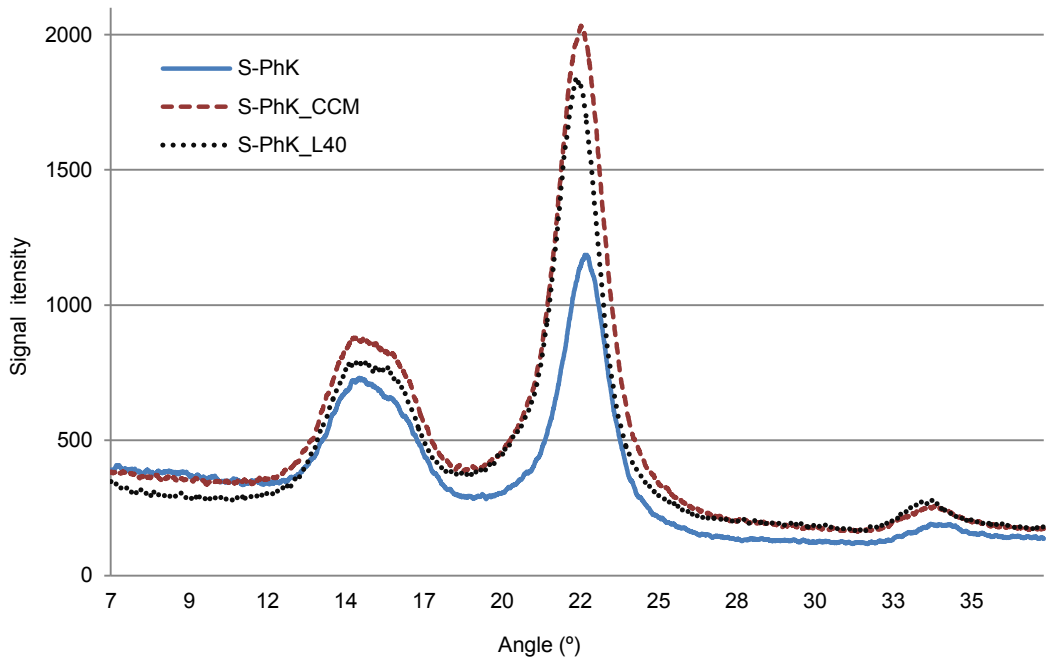

Figure IV.13: X-Ray diffractograms of S-PhK pulp untreated and incubated with CCM and L40

If in our case some amorphous phase would be digested by enzymes to an extent that it would strongly increase the crystallinity index, then there should be a strong loss of pulp after treatment, which is what we do not see (and what is giving one of the advantages of these treatments). We thus feel that this explanation is not correct in our case. Another explanation might be that the increase of crystallinity index reflects a better arrangement of crystals and an increase of their size. This could be then initiated by the decrease of molar mass which could be due to the breakage of chains that were hampering crystal merging. However this hypothesis does not fit to the small DP reduction, especially for the L40 treated pulps. The fact that microfibrils are dissolved by CCM is as well not a valid explanation for increased crystallinity index since the L40 treatment is not showing this effect on small microfibrils and is nevertheless increasing the crystallinity index. This way, the increase of the crystallinity with the pectinase treatments might be explained by reorganization and recrystallization of part of the amorphous cellulosic material due to the release of internal stress [Krassig, 1993a]. The slight swelling of the fibers that occur during the enzymatic treatments might allow a relaxation and release of the internal stress [Wakeham, 1951], allowing the rearrangement of the cellulose chains and partial recrystallization or a co-crystallization to a thermodynamically metastable form, the crystalline cellulose I [Moon, 2011]. Similar explanation was reported for a partial increase of crystallinity after hydrothermal treatments of cellulosic material [Ibbett, 2013]. Although it is known that the cellulose crystallization is favorable [Vander Wielen, 2004], this stress release is not enough to allow a transition to the thermodynamically more favorable crystalline from, the cellulose II, like occurs during the swelling with mercerization [O'Sullivan, 1997].

The observation of the fiber surface with FEG-SEM was already discussed in Figure IV.6 for the S-PhK pulp with and without enzymatic treatments. In Figures IV.14, IV.15 and IV.16 further FEG-SEM images for the dissolving pulps (Be-S and NS-S) and for paper grade pulp (Eu-KP) are depicted with and without pectinase treatments. For all samples, the cellulose pulp treatment with CCM is dissolving most of the fibrils which are attached to the fiber, promoting a smoothing of the fiber surface. The fibers which were submitted to incubation with a pure endopectinase preparation of L40, when observed with FEG-SEM show a surface structure similar to the one observed on the initial pulp.

The dissolution of the cellulose microfibrils from the fiber surface is easily explained by the presence of endoglucanase in the CCM enzymatic preparation, and one can conclude that the endopectinase is not affecting the macrostructure of the pulp fibers. As far as surface observations are concerned, dissolving pulps are behaving as paper grade pulp. The application of a pectinase in paper pulp was reported by Ahlawat et al. According to their SEM observations, the pectinase treatments created higher porosity and loss of compactness, allowing higher swelling with fibril separation and increased accessibility [Ahlawat, 2008]. In our results, this loss of compactness and increase in porosity was not possible to verify. For the endoglucanase treatment, the smoothing and “cleaning” of the fiber surface by removal of fibrils attached to the fiber surface was previously described by several authors. Similar results were verified using polarized optical microscopy [Wang, 2008], scanning electron microscopy [Mansfield, 1997; Chinga-Carrasco, 2010; Ibarra, 2010b; Gehmayr, 2011b and 2012], and atomic force microscopy [Liu, 2009].

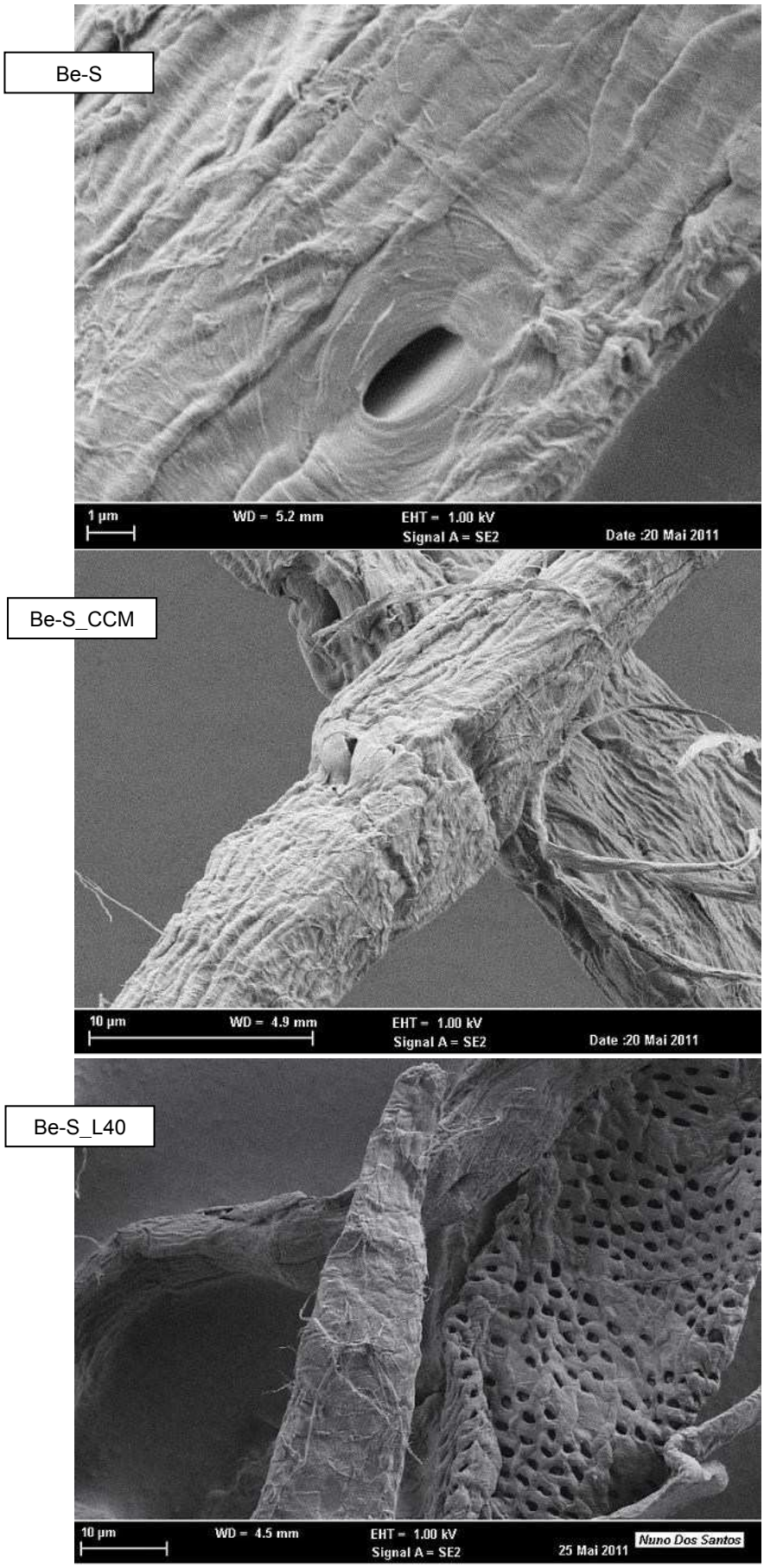

Figure IV.14: FEG-SEM surface pictures from untreated Be-S pulp fibers and treated with CCM and L40.

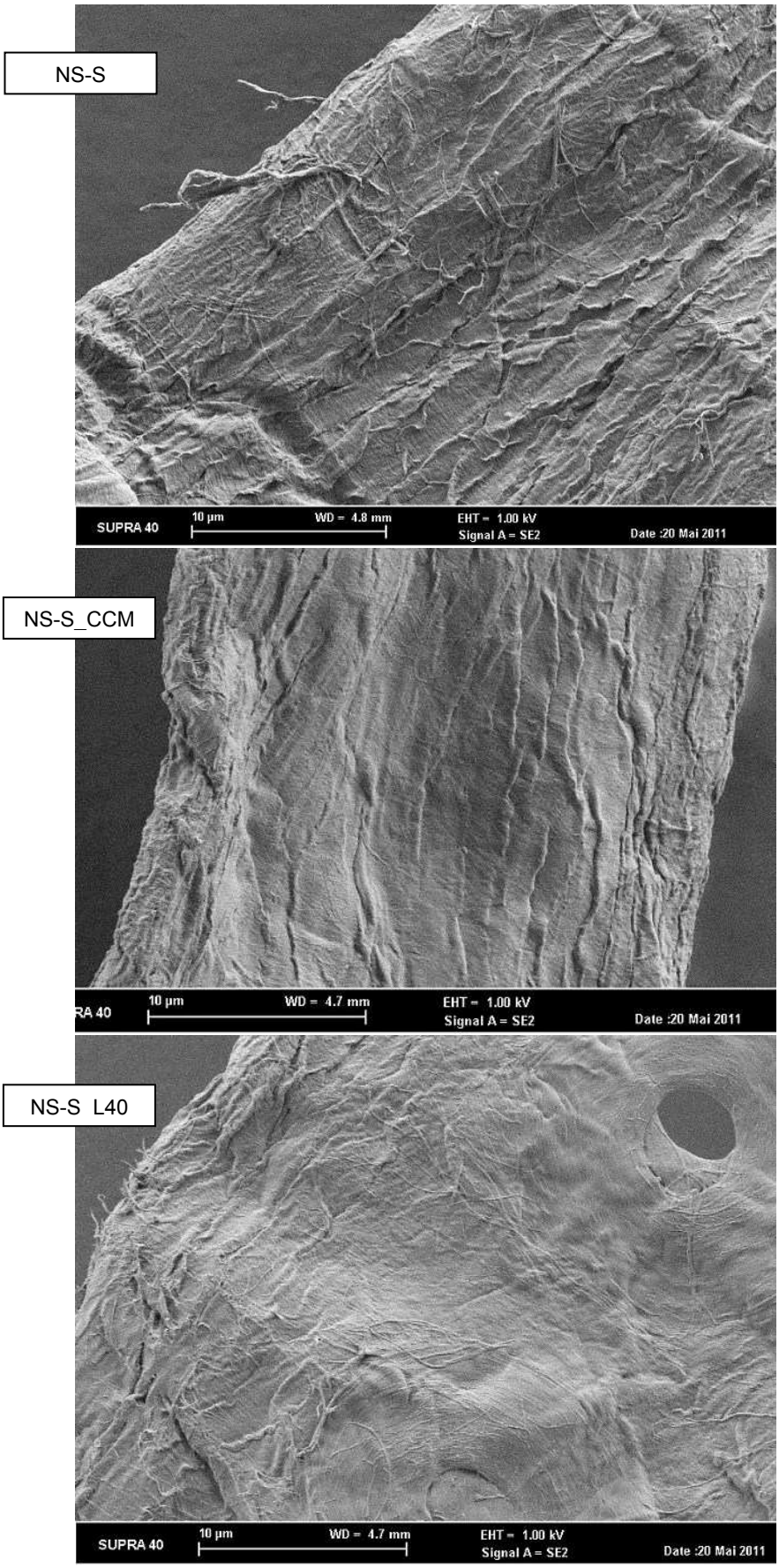

Figure IV.15: FEG-SEM surface pictures from untreated NS-S pulp fibers and treated with CCM and L40.

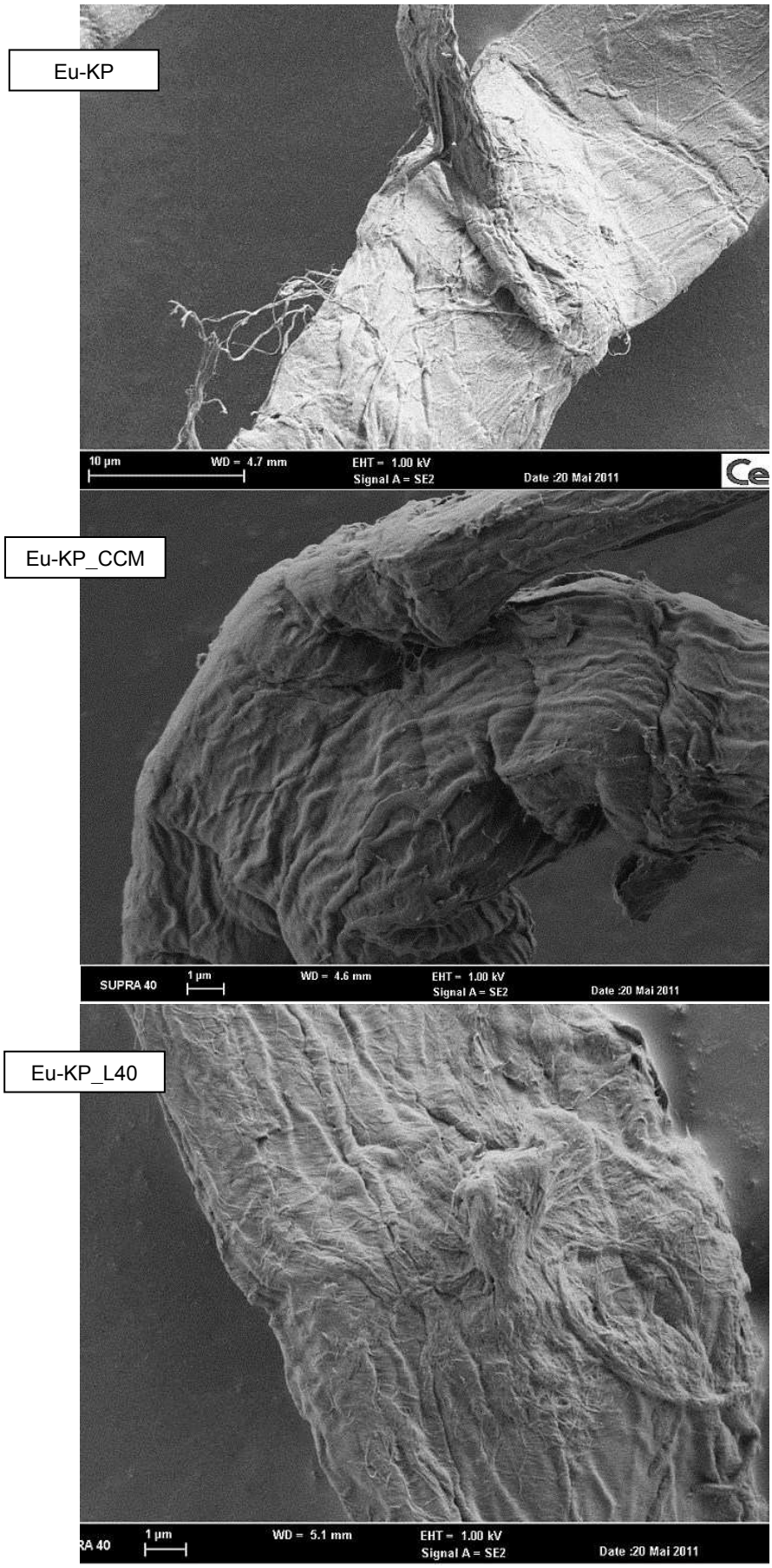

Figure IV.16: FEG-SEM surface pictures from untreated Eu-KP pulp fibers and treated with CCM and L40.

IV.2.3.2. - Effect of pectinase incubations on the dissolution of pulp in NaOH-water

In order to test the effect of the pectinase treatments on the fibers chemical accessibility, the different pulps were submitted to a dissolution treatment with cold sodium hydroxide. Similarly to the previous chapters, the dissolution yield in NaOH was gravimetrically determined after pectinase treatments of the different pulps (Figure IV.17). The recovered residues (insoluble fraction) were observed with optical microscopy, which also allows an insight on the fiber accessibility towards the NaOH solvating ions.

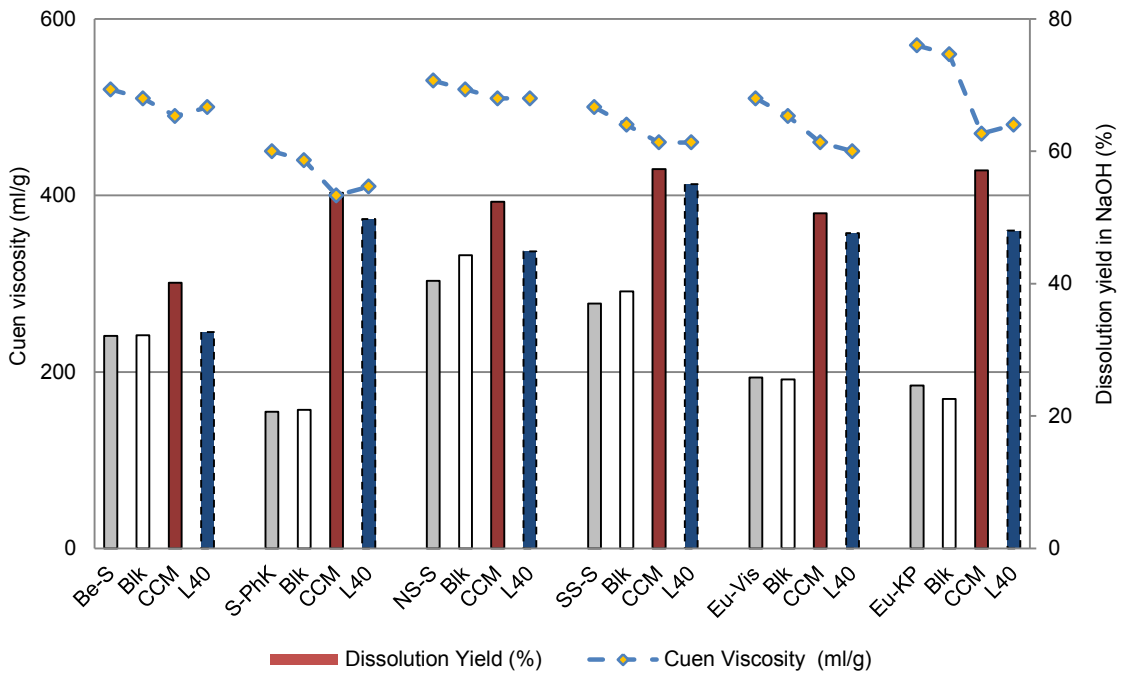

Figure IV.17: Dissolution yields of the different pulps in cold sodium hydroxide and correspondent intrinsic viscosities.

By analyzing the Figure IV.17, several considerations can be done. Comparing the untreated pulps, their dissolution capacity in cold caustic soda is very variable, even when the degree of polymerization (intrinsic viscosity) is similar, as it the case for the Be-S, NS-S and Eu-Vis pulps. For all studied pulps, the dissolution yield in NaOH is not changed after treatment with the buffered solution, confirming that the control (blank) treatments are not affecting the pulp fiber structure and properties, aside a small decrease of the intrinsic viscosity. This minor loss of DP, although in the error range of the method, is consistent for all pulps and might be explained by the acidic conditions of the incubations (pH 3.5), which can promote a slight acid hydrolysis of the cellulose chains. For all pulps, the CCM treatment is more effective for improving the dissolution yield in comparison with the L40 incubations. This effect is not strongly correlated with intrinsic viscosity changes since it is slightly increasing or decreasing between CCM and L40 treatments. In general, the different pulps are affected differently by the enzymatic treatments. For the Be-S and NS-S pulps, the dissolution yields are not improving significantly with the enzymatic

treatments. Only CCM is giving an increase of 25% for Be-S and 20% for NS-S regarding the mentioned dissolution yield in NaOH. For S-PhK pulp, CCM and L40 are improving the yield by 157% and 138% respectively. The dissolution yield for the SS-S pulp is increasing 54% with CCM and 49% with the L40 incubations. The viscose grade pulp from eucalyptus (Eu-Vis) is experiencing an increase of the dissolution yield by 96% and 85% after treatments with CCM and L40 respectively. For the hardwood paper grade pulp (Eu-KP), the CCM incubation is able to improve the dissolution yield by 128%, while for the treatment with the pure pectinase (L40) this improvement amounts to 92%. For all samples, the improved dissolution is accompanied by a decrease of the intrinsic viscosity, this being more evident for the paper grade pulp. The different results obtained for the different pulps might be explained by the pulping process used on the pulp production. Different harshness might give pulps with ultrastructure more or less de-structured. This will give different diffusion coefficient to the enzymatic liquor into the fiber, giving different effectiveness of the enzymes. The presence of residual chemicals on the pulp might also affect the enzymatic activity. Le Moigne et al described similar results, obtaining different effects of the same enzymatic treatment in different pulps [Le Moigne, 2010].

The microscopic observation of the NaOH insoluble matter (Figure IV.18 and Figure IV.5) shows that for the untreated pulps, the insolubles from are composed mainly by intact fibers, corresponding to the initial stages of swelling according to Stawitz and Kage (see Figure IV.4). The exceptions for this are the samples NS-S and SS-S, where the fibers are swollen, and exhibit ballooning and some flat rings. These insoluble fractions exhibit swelling degrees that correspond to the levels 3, 4 and 9 form the Stawitz and Kage scale. In general, the insolubles from the pulps treated with pectinases, contain highly swollen and fragmented fibers, showing also signs of ballooning, corresponding to the later levels of swelling from the Stawitz and Kage scale [Stawitz, 1959]. The insoluble residue from pulps treated with CCM are more degraded in comparison with the ones from the pulps treated with L40.This can be related to the higher effectiveness the CCM solution compared to the L40 enzymatic preparation in terms of improving the dissolution efficiency of pulps in NaOH. For the Be-S and NS-S samples, the insoluble fractions from the pulps treated with L40 are similar to the insoluble fractions of the untreated pulp, validating the fact that the L40 treatments for these two pulps are not effective, already verified in the gravimetric results.

The fact that the insoluble fractions show the presence of ballooning of the fibers is an indication that the primary wall and S1 wall are not completely removed during the delignification process and remains after the enzymatic treatments. As already discussed in section IV.2.2.3, these two cell wall layers have a major role on the cellulose fiber swelling and dissolution ability [Cuissinat, 2006a; Le Moigne, 2010; Spinu, 2011; Santos, 2013].

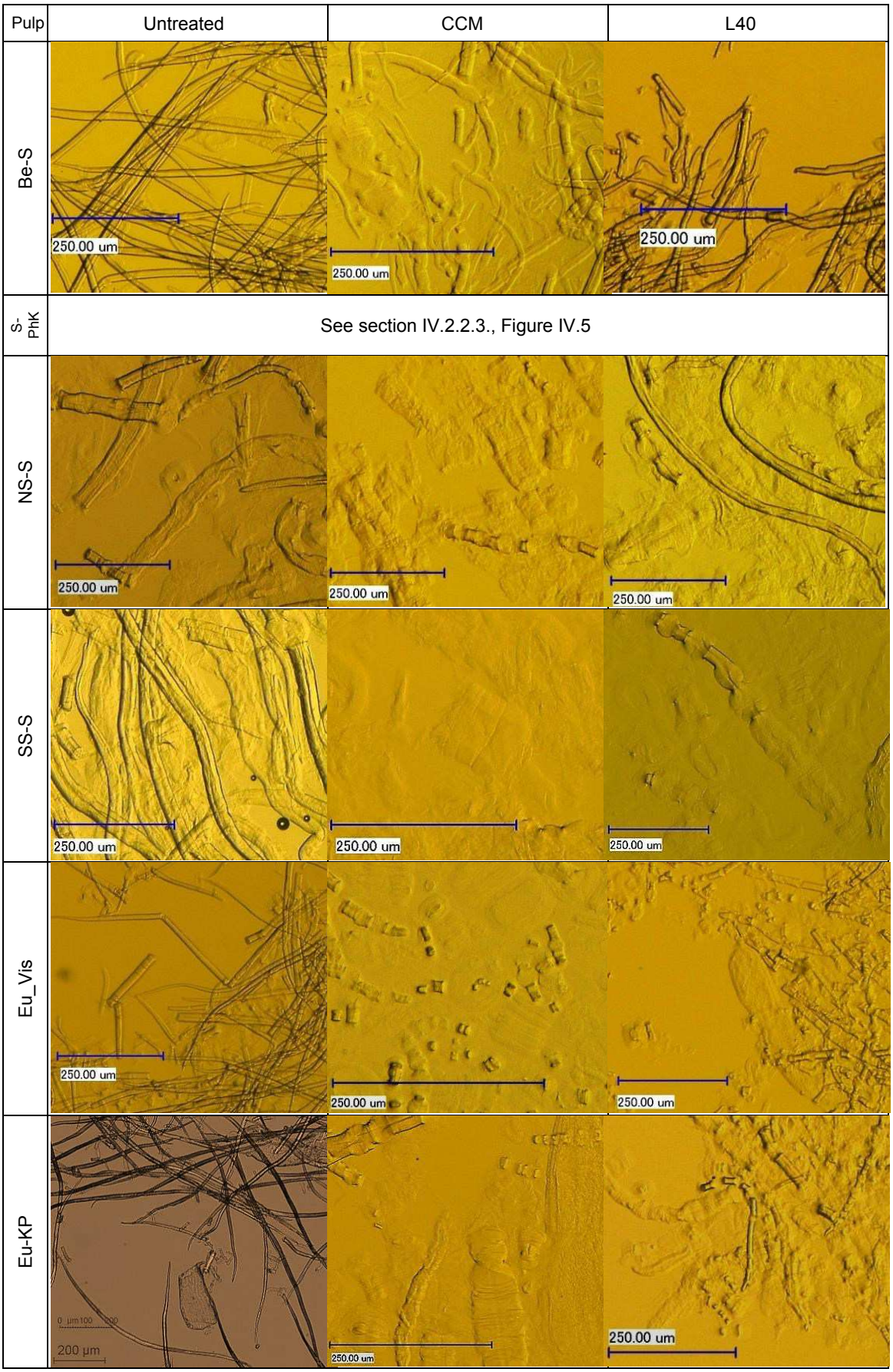

Figure IV.18: Optical microscopic pictures of the NaOH insoluble fractions.

IV.2.3.3. - Other considerations

IV.2.3.3.1. - Effect of DP on the dissolution yield in NaOH

In chapter III, we plotted the intrinsic viscosity from all the different pulps against the dissolution yield in NaOH and no correlation was found for the all set of pulps. Although for all dissolving pulps, when analyzed individually, a correlation was found, showing higher dissolution yields when the DP decreases. For the enzymatic approach, similar results are achieved. If we plot the intrinsic viscosity from all the different pulps, with and without treatments, against the dissolution yield in NaOH, no correlation is found, as can be seen in Figure IV.19.

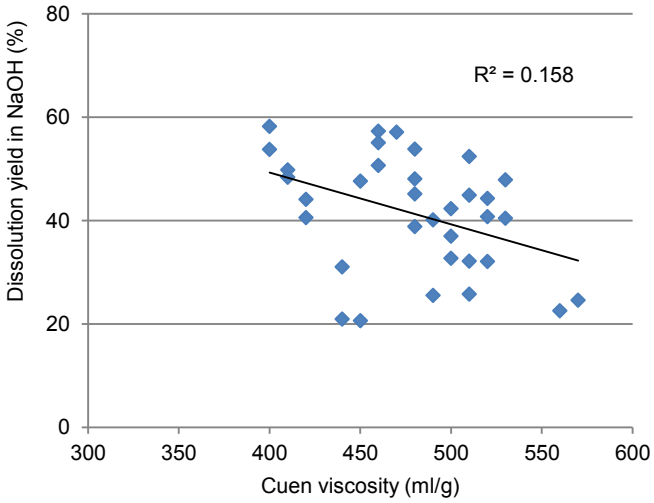

Figure IV.19: Relation between dissolution yield in NaOH and Intrinsic viscosity for all the pulps.

If we individualize the results for each pulp, the picture is different. In Figure IV.20 the results of three pulps are plotted. For all of them a correlation can be found, being more evident for the pulps S-PhK and Eu-KP, which present a higher correlation coefficient. This way, one can say that for these individual pulps, the dissolution capacity is linearly related with the mean DP of the pulp which expresses a mean destruction of the fiber structure. Direct co-relation between DP and cellulose reactivity is widely reported in literature [Cao, 2006; Rahkamo, 1996; Kopcke, 2008a; Kvarnlof, 2008; Wang, 2008; Rosenberg, 2009; Le Moigne, 2010; Kihlman, 2012].

By expanding the trend line of the linear regression it is also noticeable that these four pulps have an extrapolated viscosimetric dissolution DP, corresponding to a Cuen viscosity in the range of 350ml/g, i.e. DP range of 1100, according to Evans und Wallis. This means that hypothetically, if one reduces the viscosity to this range of values, one should achieve complete dissolution. Of course this hypothesis is only for discussion, and one must take a lot of care on these extrapolations.

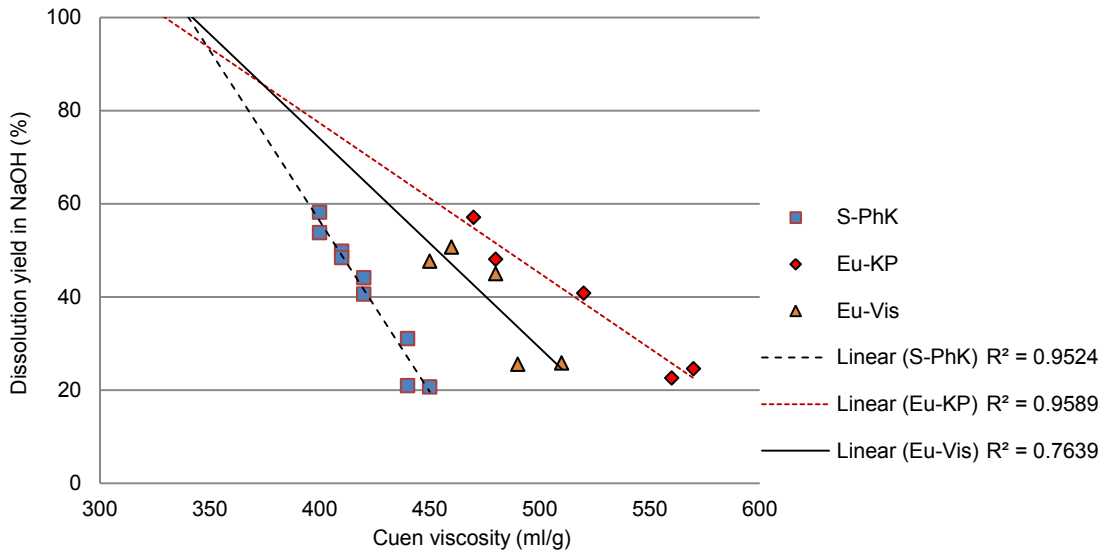

Figure IV.20: Relation between dissolution yield in NaOH and Intrinsic viscosity for each pulp.

In order to verify the aforementioned hypothesis, an attempt to reach this level of 350 ml/g was performed for the S-PhK pulp. Several incubations with CCM were done, increasing the total endopectinase activity to a maximum of 17,500 U/g. Despite the high enzyme load, the viscosity was not falling below 370 ml/g and the dissolution yield was not increasing, reaching a maximum of ~53 % independent of the intrinsic viscosity (Figure IV.21). Cao et al, when using one pulp and changing its DP with endoglucanase and cellulases, also found a correlation between the DP and alkaline solubility, until reaching a maximum plateau of 90% alkaline solubility, independent of the DP [Cao, 2006].

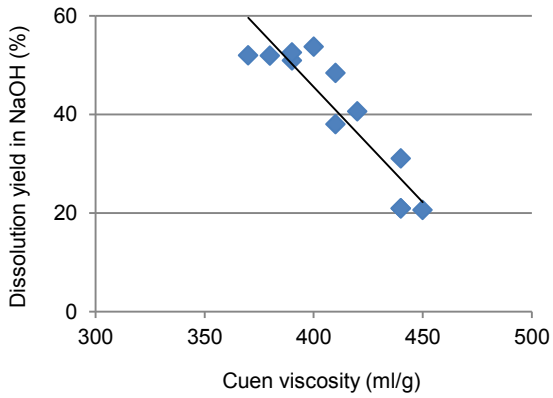

Figure IV.21: Relation between dissolution yield in NaOH and intrinsic viscosity for S-PhK.

These results bring further discussion in several directions. One is about the limit of dissolution of cellulose using the method adopted for this study. To clear this, Avicel® PH101 cellulose was tested and a dissolution yield in cold NaOH of 98% was achieved, being in agreement with the results reported by Le Moigne, where the same method was used [Le Moigne, 2008]. These results also show that there is a limit of activity from the endopectinase towards fiber modification. Besides this, and together with the results from plotting the intrinsic viscosities against the dissolution capacity for the different pulps, it is evident that the intrinsic

viscosity or degree of polymerization is not able to govern the cellulose solubility in NaOH. This was also discussed for the nitren treatments and was also described by Le Moigne et al, after submitting two different pulps to enzymatic incubations [Le Moigne, 2010].

IV.2.3.3.2. - How pectinase can change effectiveness of dissolution

As it is the case by using NaOH, the dissolution of natural cellulose fibers using direct solvents can be described in two stages: first the solvent diffusion into the fiber structure and later, the separation of the cellulosic polymer chains from each other [Cuissinat, 2006a; Trygg, 2011; Santos, 2013]. During the first stage, the diffusion of the solvent will promote the swelling of the fiber structure. In the second stage, the solvent will break the cellulose inter-molecular hydrogen bonds and form new hydrogen bonds between the cellulose chains and the solvent, bringing the molecules in solution [Cuissinat, 2006a; Lindman, 2010; Pinkert, 2010; Santos, 2013]. Takahashi et al and Kamide et al, reported that the dissolution of cellulose in NaOH is governed by the degree of destruction of the O3-H···O'5 intra-molecular and O2-H···O'6 inter-molecular hydrogen bonds [Takahashi,1991; Kamide, 1992]. Taking in consideration the inter-molecular hydrogen bonds between two parallel cellulose chains, it is also reported that cleaving the inter-molecular hydrogen bonds, associated with the C3 and C6 hydroxyl groups, will promote the cellulose dissolution [Pinkert, 2010]. The accessibility of the fiber towards the solvent is not homogenous in all the fiber structure, being lower in the primary wall and S1 wall, which are enriched in hemicelluloses and pectin. When moderate or weak solvent are used, as it is the case for NaOH, the swelling of the fiber is not homogeneous and the external walls are not affected by the solvent [Cuissinat, 2006a]. The inner walls of the fiber are dissolving first, creating a positive pressure inside the cell and increasing the swelling. This swelling is constricted by the primary and S1 walls creating the ballooning phenomenon which is preventing efficient dissolution of the cellulose [Cuissinat, 2006a; Le Moigne, 2008].

Accordingly, there are two ways of improving the dissolution of cellulose fibers: improving the accessibility to the overall fiber structure, facilitating the rupture of the inter-molecular hydrogen bonds, or increasing the accessibility and swelling capability of the primary and S1 walls. To achieve this, several studies have been conducted, focusing on these two approaches. In order to increase the overall cellulose structure accessibility, cellulose fibers can be treated enzymatically [Rahkamo, 1996; Henriksson, 2005; Cao, 2006; Engström, 2006; Kvarnlöf, 2007 and 2008; Köpcke, 2008; Wang, 2008; Ibarra, 2010; Östberg, 2012], with steam explosion [Yamashiki, 1990a and 1990b; Kamide, 1991; Kihlman, 2012], with electron beaming [Rajagopal, 1994; Kraft, 2000; Driscoll, 2009], or chemically, with acid hydrolysis [Kamide, 1991; Trygg, 2011] or TEMPO-mediated oxidation [Isogai, 2009; Gehmayr, 2012b]. In order to decrease the negative impact of the outer cell wall layers, at expense of losing material, the use of atmospheric Dielectric Barrier Discharger (DBD) Plasma treatments [Wu, 2008] or enzymatic extractions [Le Moigne, 2010] allowed the improvement on the cellulose fibers dissolution.

The application of pectinase treatments on the improvement of dissolving pulp solubility was, in our knowledge, not reported up to this date. Pectinase is one of the most used enzymes by industry and is produced in large amounts [Uenojo, 2007, Pedrolli, 2009], which makes it quite interesting from the technical point of view. This class of enzymes can be classified according to the pH in which they are active, acid or alkaline, and have a broad application spectrum in industry [Kashyap, 2001; Uenojo, 2007; Pedrolli, 2009; Arunachalam, 2010]. Alkaline pectinases are mostly used on the textile industry, pectin containing effluent treatment, vegetable oil extraction, coffee and tea fermentation, and papermaking. Acid pectinases, like the ones used in this work (CCM and L40), due to its low active pH, are suitable for use in the food industry, having applications in the fruit and juice processing, production of unicellular products and wine making industry [Kashyap, 2001; Ahlawat, 2008; Viforr, 2008; Pedrolli, 2009]. Pectinases are also used industrially in the poultry field, and in smaller scale in the purification of plant viruses [Arunachalam, 2010].

In most of the mentioned applications, the use of pectinolytic enzymes aims to de-structure the pectin network in suspension or in the middle lamella compound. Synergistically with other enzymes is able to ease the accessibility of the cellulose tissue, improving the extraction of certain chemical compounds such as vegetal oils from fruits or kernels [Uenojo, 2007; Arunachalam, 2010], or anthocyanins from red grapes during wine production [Kashyap, 2001]. This mechanism may partly explain the role of pectinase in the present work.

The fact that the pulps showed the occurrence of ballooning during dissolution proves the existence of the primary wall and S1 wall in their fibers. This way, although not detected by the presented carbohydrate composition analysis, it is quite probable that pectin is present on the mentioned outer layers of the cell wall. Assumed its presence, we consider that the pectinase treatments are improving the cellulose dissolution capacity by increasing the accessibility and swelling capability of the primary and S1 walls. The pectinase, by hydrolyzing the pectin network is creating spaces in the structure, which allow an easier diffusion of the solvent into the primary and S1 wall structure, and, at the same time, offers less resistance to the swelling of these two structures. This will avoid the occurrence of ballooning, allowing a homogeneous swelling of the fiber and consequently, and easier dissolution in NaOH. This approach allows canceling the negative effect of the presence of the outer layers without having to remove them. Besides this main effect, the pectinase extractions are slightly decreasing the average degree of polymerization. For the L40 treatments, the explanation or source for this reduction of DP could be due to the reorganization of the cellulose molecules during the verified re-crystallization, but this is not clear and requires further investigations. While for the CCM incubations this could be easily explained by the presence of endoglucanase, which is cleaving part of the amorphous cellulose chains.

IV.3. - Conclusions

The first enzymatic treatments allowed a quick survey on the potential of using pectinase in order to improve the dissolution of dissolving pulps in cold caustic soda. This approach proved to be more interesting and with a higher application potential than the nitren approach. Higher dissolution yields were achieved without losing cellulosic material or changing the carbohydrate composition and at the same time the chain length reduction of cellulose was much lower.

With the study of the enzyme preparations, it could be concluded that the endopectinase is able to improve the chemical accessibility of the pulp fibers independently of the presence of endoglucanase in the enzymatic preparation, although the synergistic effect of both enzymes is more effective. Accordingly, the Biopectinase CCM is more efficient than Pektinase L40 at similar endopectinase dosage and a higher dissolution yield is achieved with similar viscosity loss.

The increase on the dissolution yield in NaOH is directly proportional to the enzyme concentration and to the time of the enzymatic incubation. For the range of concentration and time investigated, the enzymes have shown to be stable and, when keeping constant the total endopectinase activity, the enzymatic performance is independent of the time or enzyme concentration. This allowed the definition of a new parameter that is useful on the interpretation of results, the “total activity units”, defined by the product of the initial activity units (units per gram of pulp) by the incubation time (in hours). Although the buffer solutions (blank tests) showed no effect on the pulps, the buffer system used in the incubation is important for the enzyme performance.

By treating different pulps with both enzyme preparations some of the conclusions were validated and other additional information could be established. The enzymatic treatments lead to a slight decrease of the DP. The X-Ray diffraction measurements indicate that the enzymatic treatments are increasing the crystallinity. Due to the presence of endoglucanase, the CCM product is dissolving the micro-fibrils from the fiber surface, promoting a smoothing of the pulp fibers surface.

In terms of dissolution in cold sodium hydroxide, the mixture of endopectinase with endoglucanase is more efficient for all pulps, while the pure endopectinase is not efficient on the Be-S and NS-S pulps.

Although it is clear that the endopectinase treatments are improving the chemical accessibility of the fibers, the mechanisms behind this effect is not fully understood. This is due to the facts that only a minimal residual amount of pectin is present in the pulp fibers and also because the macrostructure was not significantly affected, in contrast with the nitren treatments. Nevertheless, taking in consideration the general mechanisms for cellulose swelling and dissolution we concluded that the pectinase treatments are improving the dissolution in NaOH by hydrolyzing the pectin network in the primary and S1 walls. This is ”loosening” the structure, which allow an easier diffusion of the solvent into the primary and S1 wall structure, and, at the same

time, offers less resistance to the swelling of these two structures. This will avoid the occurrence of ballooning, allowing a homogeneous swelling of the fiber and consequently, and easier dissolution in NaOH. Since the results of the present work show that a possible modification of the pectic network has a pronounced effect on the fibers processability (in terms of dissolution in cold caustic soda), new directions of research are open: on the one hand to understand the mechanisms behind the effect of the endopectinase; and, on the other hand, to explore new applications where the fiber accessibility play a role.

The results achieved with this innovative work package, are of very high interest. The endopectinase treatments allow a great improvement on the cellulose accessibility towards cold NaOH, without significant changes of the chemical composition or macrostructure and without material losses. This, allied with the already mature market of pectinase, makes this technology very attractive from the industrial point of view.

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chapter V

Effect of nitren extraction followed by pectinase incubation on paper grade pulp

Effect of nitren extraction followed by pectinase incubation on paper grade pulp

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V.1. - Introduction

In order to be used by the regenerated cellulose and cellulose derivative industry, cellulose pulps are required to have several specific properties: uniform molar mass distribution; high cellulose content and low amounts of hemicellulose, residual lignin, extractives and minerals. Furthermore the pulps are required to have a high reactivity [Hinck, 1985; Jackson, 1998]. During the last decade the cotton linters availability decreased, while the demand for this raw material and wood dissolving pulp increased. Besides this, the projected decrease in the use of office paper is driving the paper grade pulp industry into finding new applications for their products. These facts, allied with the lower production and environmental costs of the paper grade pulps in comparison with the ones from conventional dissolving pulp production [Ribas Batalha, 2012], led to the interest on converting paper grade pulps into dissolving pulps. To this aim, different studies have been already published, using different approaches and focusing mostly on the viscose application [Gehmayr, 2011]. In order to achieve this conversion, paper grade pulps were chemically treated [Puls, 2006; Janzon, 2006; 2008a, 2008b], or subjected to chemical and/or enzymatic treatments [Jackson, 1998, 2001; Ibarra, 2009, 2010; Kopcke, 2008, 2010; Gehmayr, 2011; Li, 2012].

In Chapter III, it was shown that when treating a paper grade pulp with a nitren solution, xylans were considerably removed, without affecting the cellulose structure in the fibers. By this way, as also described by Janzon et al., a pulp with high α-cellulose content is obtained, comparable to the α-cellulose content obtained in dissolving pulp grades [Janzon, 2006; Puls, 2006]. In Chapter III, the dissolution capacity of paper grade pulp before and after a 5% nitren extraction was evaluated. It was shown that for the solvent system used (cold NaOH), the dissolution yield was decreasing, showing that the nitren treatment was decreasing the accessibility of the sodium hydroxide solvating ions into the fiber structure of paper grade pulps. With the work described in Chapter IV, it was shown that the incubation of the pulps with pectinase was increasing the dissolution yield of the fibers in cold caustic soda solution, this improvement being quite large for the paper grade pulp.

Taking these considerations into account, two questions occur: what happens when treating a paper grade pulp with a nitren solution followed by incubation with a pectinase enzymatic preparation? Would such a procedure lead to a high purity grade with a high accessibility and dissolving power in cold NaOH? This chapter is describing the work performed in order to answer to these questions.

The paper grade pulp used was a bleached eucalyptus kraft pulp (Eu-KP). This pulp was first treated with a 5% nitren solution (Eu-KP_5%), and further incubated either with a CCM solution (Eu-KP_5%_CCM) or a L40 solution (Eu-KP_5%_L40). The treatments conditions used were the same as described in Chapter III for nitren and Chapter IV (Table IV.9) for the enzymatic incubations. For comparison of the dissolution capacity in NaOH, the same initial pulp, treated with nitren by Sappi (South Africa) (Ni-Eu-KP_A), was incubated with the L40 pectinase

preparation (Ni-Eu-KP_A_L40). The next diagram (Figure V.1) shows the main samples discussed in this chapter, describing the process to which they were submitted and the name of the sample in bold.

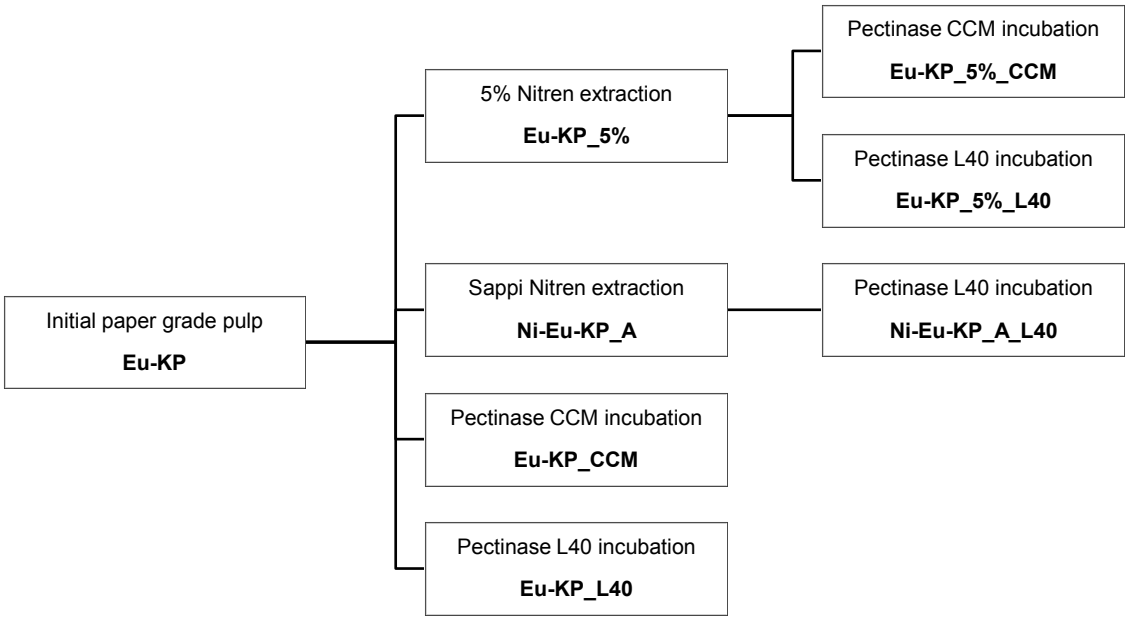

Figure V.1: Flow diagram showing the different samples and the treatments to which they were submitted.

V.2. - Results and discussion

V.2.1. - Effect of the nitren extraction followed by pectinase incubations on the pulp properties

In order to investigate the effect that the combination of the two treatments has on pulp properties, several parameters were measured and electron microscopic pictures were taken. The dimensions of the fibers were measured at Tembec laboratories in Bordeaux, France with a MorFi system (Techpap SAS, Grenoble, France). The values of the dissolution yield in NaOH have an estimated error of ±1% in absolute value. This ± 1% was verified for the whole range of results, meaning that the relative error decreased with the increase of dissolution yield. Probably this reflects an error inherent to the material/method used. In practice, this error can be illustrated by the following examples: Eu-KP has a dissolution yield of 25 ± 1% and the Eu-KP_5%_L40 has a dissolution yield in NaOH of 59 ± 1% (Figure V.6). For the pulps recovery yield after extractions, the error is ± 2%, and the viscosity measurements show an error of ± 10 ml/g. All the reported values consist of the average of at least two measurements that fit within the error.

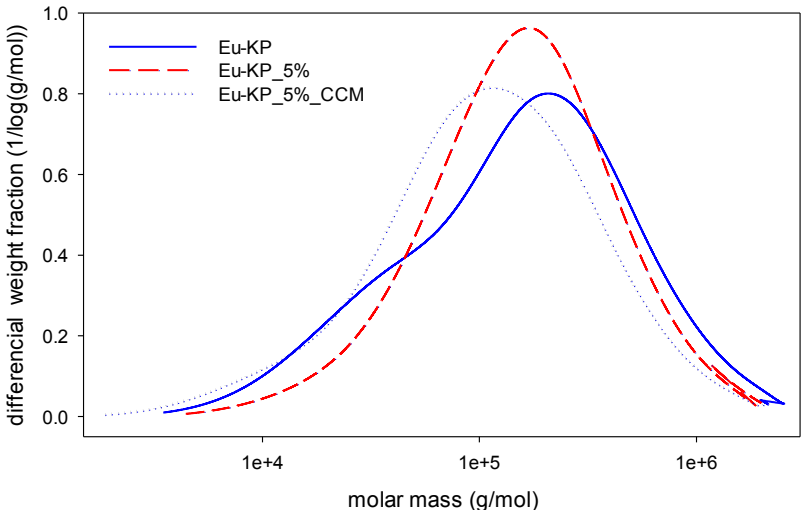

Figure V.2: Molar mass distributions of the initial and treated pulps.

At the molecular level, the molar mass distributions (Figure V.2) and the intrinsic viscosity measurements (Figure V.3) show that the nitren treatment is not lowering the average degree of polymerization of the pulp grade fiber. On one side, this treatment is degrading partially the high molar mass compounds, but on the other, this degradation is compensated by the extraction of low molar mass molecules (hemicelluloses). For this pulp (Eu-KP), this dual effect allows the pulp to preserve the same average degree of polymerization. As also verified in Chapter III, these results are in accordance with the ones reported by Janzon et al [Janzon, 2008a]. Comparing with other studies, the use of xylanase incubation followed by cold caustic extraction is also able to enrich the pulp in cellulose, with increase of the average DP and of uniformity on the molar mass distribution, but with a higher decrease of the polydispersity index [Jackson, 2001; Kopcke, 2008, 2010; Ibarra, 2009, 2010; Gehmayr, 2012].

When the nitren-extracted fibers are incubated with CCM, the cellulose chains are shortened, leading to a decrease of the pulp average degree of polymerization. This effect is clearly shown by the decrease of the intrinsic viscosity and by the shift of the molar mass distribution towards lower molar masses. However, this decrease is not as pronounced as for treatments with endoglucanase verified in literature: while the treatment with pectinase CCM is decreasing the viscosity from 570 to 400 ml/g (~30% loss), the endoglucanase treatments described in literature are decreasing the intrinsic viscosity by ~75% [Kopcke, 2008], ~70% [Ibarra, 2009], 57% [Kopcke, 2010], or ~53% [Gehmayr, 2011].

Studying the effect of the treatments on the fibers, Figure V.3 shows that nitren and the subsequent enzymatic treatments are both decreasing the fiber length and the fiber width. The decrease of the fiber length with both treatments is also clear in Figure V.4 which shows that the distributions are shifting towards lower fiber lengths, while keeping their shape. This indicates that all fibers are affected by the treatments.

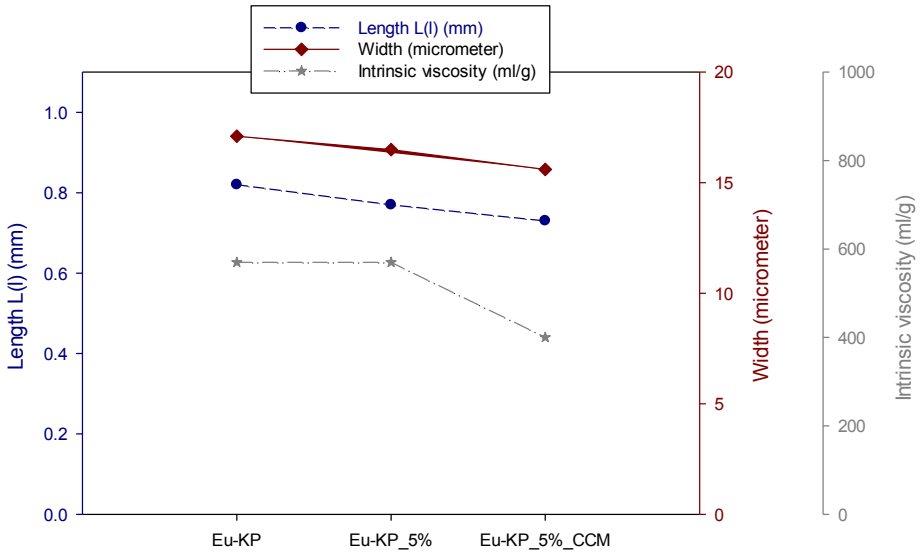

Figure V.3: Fiber dimensions and intrinsic viscosities from the initial and treated pulps.

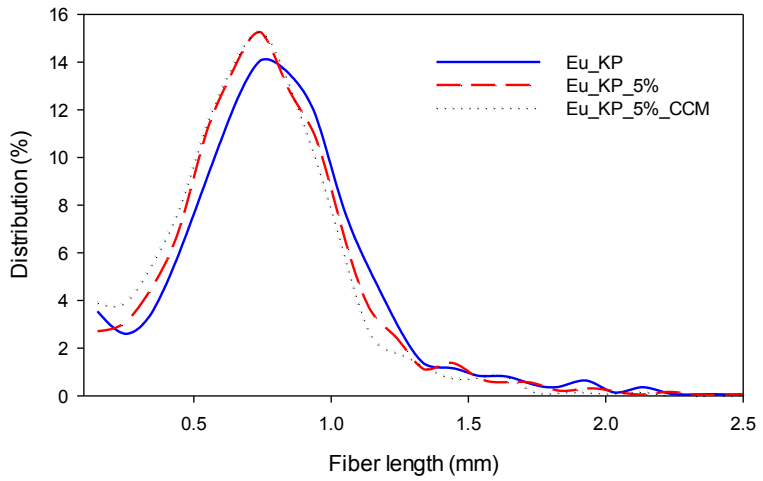

Figure V.4: Fiber length distribution from the initial and treated pulps.

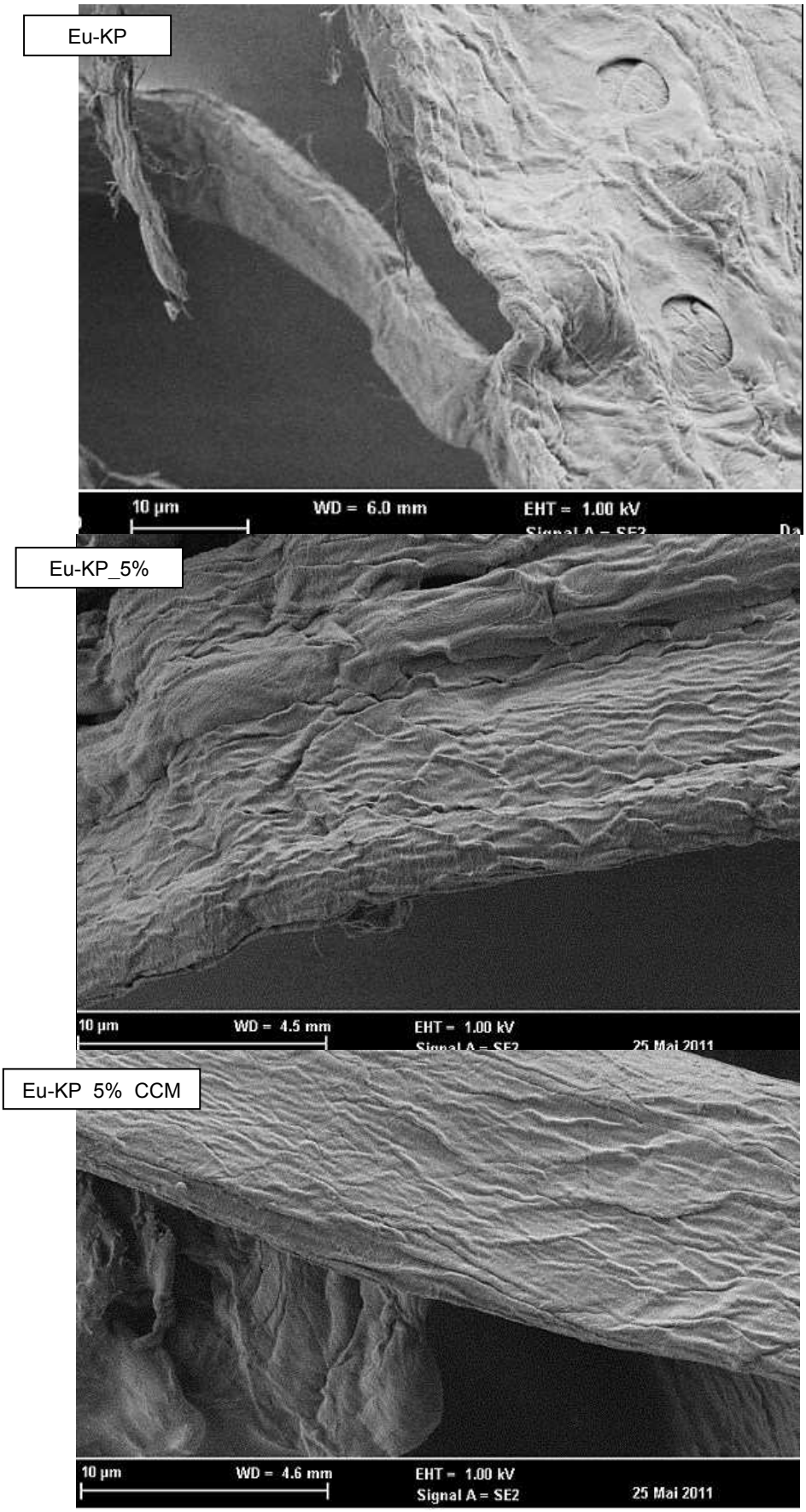

Figure V.5: FEG-SEM surface pictures from fibers of untreated Eu-KP pulp, treated with 5% nitren (Eu-KP_5%) and treated with 5% nitren followed by CCM incubation (Eu-KP_5%_CCM).

Scanning electron microscopy (FEG-SEM) allows a better insight on the effect of these treatments on the fiber surface. The pictures from Figure V.5 show that the nitren extraction is not modifying the surface of the paper grade pulp fibers, as also verified for dissolving pulp fibers for the same nitren concentration (Chapter III). The nitren solution is dissolving the fibrils partially attached to the surface. The subsequent incubation with CCM is providing a further smoothing of fiber surface, due to the presence of endoglucanase on the enzymatic preparation, also in accordance with what was found in this study for dissolving pulps (Chapter IV). In contrast, by converting a paper grade pulp into a dissolving pulp with xylanase treatment followed by cold caustic extraction and endoglucanase incubation, Gehmayr et al reported a considerable degradation of the fiber surface [Gehmayr, 2011b].

V.2.2. - Effect of the nitren extraction followed by pectinase incubations on the dissolution of pulp in NaOH-water

As discussed in Chapter III, the treatment of a paper grade pulp with a nitren solution is decreasing the accessibility of the fibers, hindering the diffusion of the NaOH ions into the fibre, evidenced by a decrease of the pulp dissolution yield in cold NaOH.

These results are confirmed for the two nitren treated pulps in this part of the study (Figure V.6). The first sample, Eu-KP_5%, was treated with the same conditions as the paper grade pulp sample discussed on Chapter III (MxH-KP). For this pulp, the nitren extraction led to a decrease of dissolution yield in cold NaOH from 25% in the untreated pulp (Eu-KP) to 11% for the nitren treated pulp (Eu-KP_5%). The second sample, Ni-Eu-KP_A, was produced by Sappi from the same starting pulp (Eu-KP) by nitren extraction. The dissolution yield was as well decreased in this case from 25% (untreated) to 10% (treated). This loss in dissolution capacity can be explained by two different factors, the hemicellulose content and the decrease on the fiber accessibility. Since the NaOH dissolving system is very efficient on dissolving hemicelluloses (see Figure V.2), these results can be partly explained by the presence of a high amount of hemicelluloses on the initial paper pulp compared to a low amount after extraction with nitren. Taking as an example the sample discussed in Chapter III, the initial pulp has 19.8% of hemicellulose, while after nitren extraction the hemicellulose content is 5.6%. If one considers this difference (14.2%) and subtracts it from the dissolved material in NaOH for the initial pulp, it gives a value of 7.6% for the dissolution yield, which is quite similar to the 8% of dissolution yield in NaOH obtained after nitren extraction. Considering this factor, we stated that the nitren extraction is not improving the cellulose dissolution of the paper grade pulp in cold sodium hydroxide. The other factor, loss in fiber accessibility, is related with the hornification of the fibers after hemicellulose removal with nitren extraction. Oksanen et al studied the role of hemicelluloses in the hornification of bleached kraft pulps (which is the case in our pulps) and found that the removal of hemicelluloses promotes the hornification of the fibers during the drying process, decreasing the swelling capacity, among other properties [Oksanen, 1997b]. Similar results were

described by other authors for wood material [Kopcke, 2008; Östberg, 2012] and non-wood material [Ibarra, 2010].

The subsequent treatment of these two nitren treated pulps with pectinase preparations is clearly promoting an activation of the cellulosic material towards its dissolution in cold NaOH (Figure V.6).

Eu-KP_5%

The pulp Eu-KP_5% was further treated with either CCM or L40.

- CCM: the pulp recovered after CCM incubation was dissolved in cold NaOH. It showed an increase of about 500% of the dissolution ability, from 11% dissolution yield for the nitren treated (Eu-KP_5%) to 65% dissolution yield after additional CCM treatment (Eu-KP_5%_CCM).
- L40: the treatment with L40 is also efficient in increasing the dissolution capability in cold NaOH. A dissolution yield of 59% is achieved, corresponding to an increase of 436% compared to the nitren treated pulp.

Sappi nitren-treated pulp Ni-Eu-KP_A

- L40: this pulp was only treated with L40. The incubation of the Sappi nitren-treated pulp Ni-Eu-KP_A with the L40 pectinase preparation is validating the activation effect of the pectinase treatment on the cellulose fibers. For this pulp, the dissolution yield in NaOH is increasing by 490%, from 10% to 59% of dissolved material in cold NaOH.

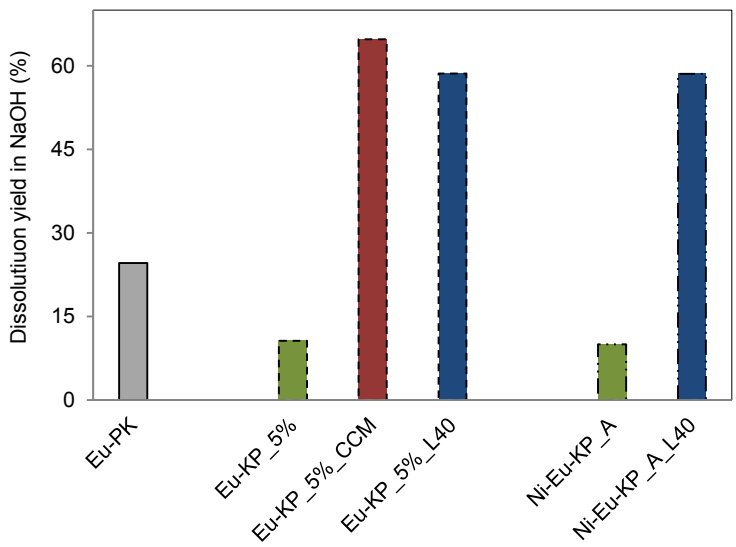

Figure V.6: Dissolution yield in NaOH for Eu-KP without any treatment, after treatment with nitren (Eu-KP_5% and Ni-Eu-KP_A), and after treatment with nitren and pectinase (Eu-KP_5%_CCM, Eu-KP_5%_L40 and Ni-Eu-KP_A_L40).

These results are in line with the reported in Chapter IV, where the pectinase treatments improved the dissolution capacity of dissolving pulps. Also for this application (conversion of paper grade pulp into dissolving pulp), this work (nitren followed by pectinase) is innovative, since so far the use of pectinase was not reported. The above-mentioned published studies also used enzymatic treatments to activate the paper grade pulps after removing the hemicelluloses, but in their case, endoglucanase was used [Ibarra, 2009, 2010; Kopcke, 2008; 2010 Gehmayr, 2011, 2011b].

This improvement of dissolution performance in cold NaOH is also evident in microscopic observations (Figure V.7). The insoluble residues from the untreated pulp show mostly intact fibers, with some fiber fragments. The same is verified for the insoluble fraction from the nitren treated pulp, where there are no sign of swelling. As discussed in Chapter IV, the swelling of fibers is a signal of solvent diffusion and dissolution of the inner fiber wall layers [Cuissinat, 2006a; Le Moigne, 2010; Spinu, 2011; Santos, 2013]. The morphology described for the insoluble from these two samples corresponds to the stage 1 from the swelling scale developed by Stawitz [Stawitz, 1959] (See Figure IV.4 from Chapter IV). For both pulps, vessels are also intact, which is as well illustrating the low dissolution capacity of the pulps in NaOH. According to our studies on cellulose fibers dissolution mechanisms (not reported nor published), for hardwoods, the vessels are the first to dissolve. This is understandable, due to the high number of pits on their structure, which eases the diffusion of the solvent into the fiber structure.

For the pulps treated with both nitren and pectinase preparations (Eu-KP_5%_CCM and Eu-KP_5%_L40), the insoluble residues consist of fiber fragments typical for pulps with high dissolution capacity, with no intact fibers: the fiber fragments are vastly de-structured showing a high swelling and “flat ring” fragments, corresponding to the Stawitz swelling stages 7, 8, 9 and 10 [Stawitz, 1959]. The slightly higher efficiency of the CCM in comparison with the L40 is also visible by the presence of ballooning (Stawitz stages 4 and 5) on the insoluble residues from the pulp treated with the pure pectinase (Eu-KP_5%_L40). These results show that the pectinase treatments are able to reverse the hornification effect of the nitren extraction, and increase the fiber structure accessibility to the solvating ions from the aqueous NaOH solvent.

For reference, pictures from the insoluble residues of the paper grade pulp Eu-KP treated only with CCM and L40 are shown (Eu-KP_CCM and Eu-KP_L40). Although not so evident, it can be seen that the undissolved residues from these pulps are not as degraded as the ones additionally treated with nitren. Accordingly, we can state that the nitren treatment is not only enriching the pulp in cellulose but as well improving the efficiency of the subsequent enzymatic treatments. These results are supported by the comparison of the dissolution yields in NaOH for the enzymatic treated pulps without and with nitren pretreatment (Figure V.8). Here it is shown that the nitren pretreatments are improving the enzymatic incubation effect, leading to an increase of the pulp dissolution capacity in NaOH.

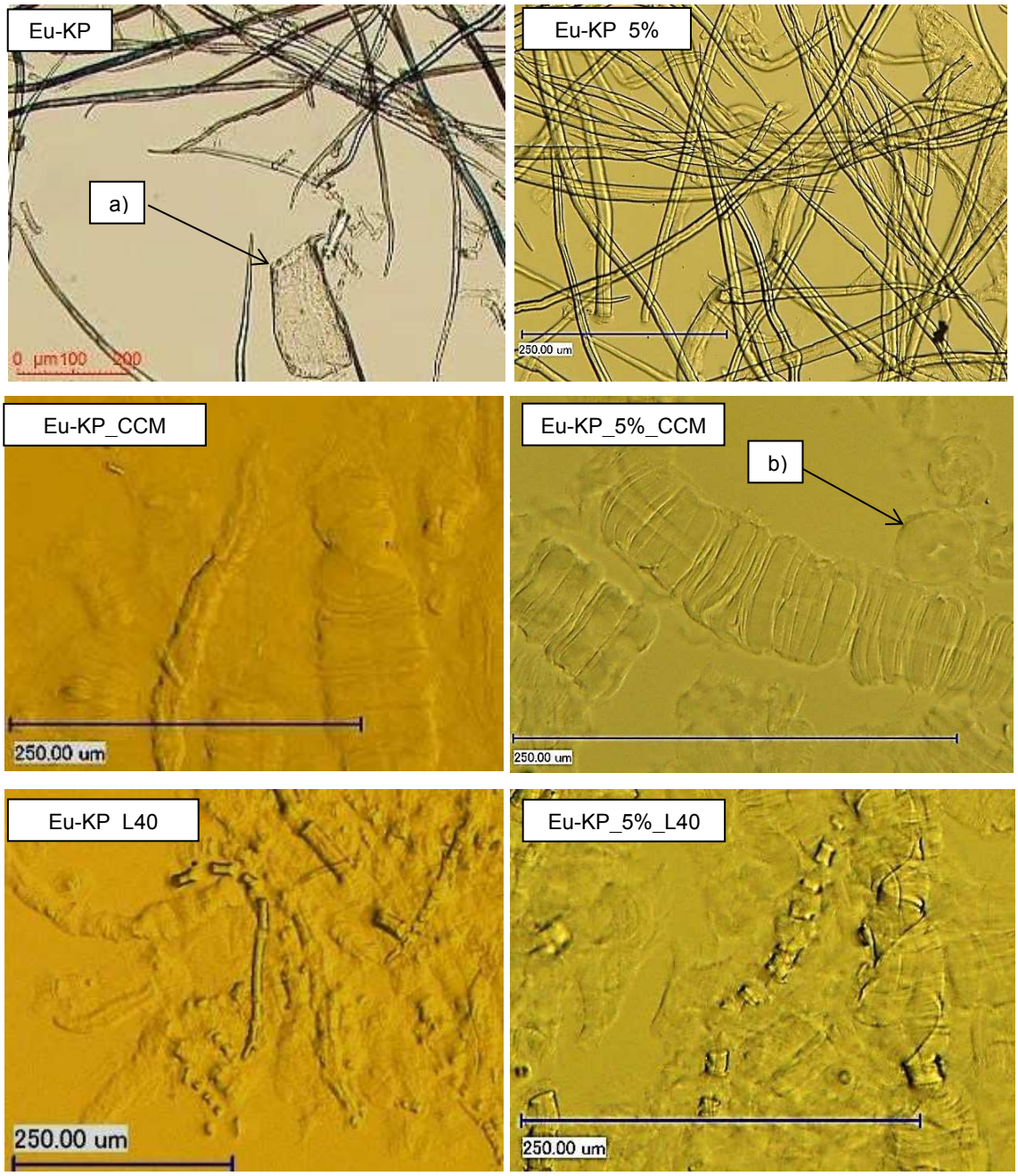

Figure V.7: Effect of the enzymatic treatments on the morphology of insolubles from the different pulps:

Eu-KP – Initial paper grade pulp (without treatment);

Eu-KP_CCM – Initial pulp treated with CCM;

Eu-KP_L40 – Initial pulp treated with L40;

Eu-KP_5% - Initial pulp extracted with nitren;

Eu-KP_5%_CCM - nitren extracted pulp treated with CCM

Eu-KP_5%_L40 - nitren extracted pulp treated with L40;

a) Vessel;

b) “Flat ring” fragment.

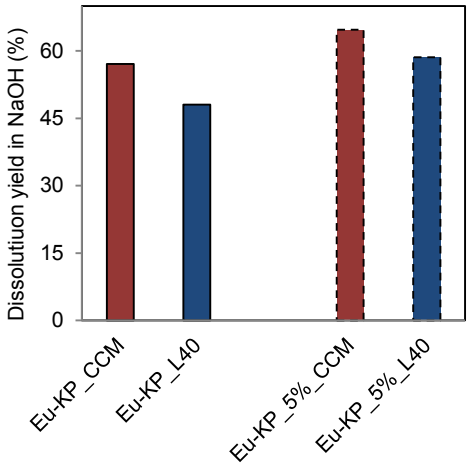

Figure V.8: Dissolution yield in NaOH for Eu-KP treated with CCM or L40 without (Eu-KP_CCM, Eu-KP_L40) and with nitren pretreatment Eu-KP_5%_CCM, Eu-KP_5%_L40).

For the dissolving pulps described in Chapter III, the nitren treatments are reducing the hemicellulose content and the degree of polymerization of the pulp, increasing this way the fiber accessibility and dissolution in NaOH [Santos, 2013]. For the paper grade pulps, the nitren treatment is essentially removing the hemicellulose, mostly xylan due to the selectivity of this method [Puls, 2006; Janzon, 2006; Santos, 2013]. By removing the hemicelluloses (xylan), the nitren extraction is opening the fiber pores and interfibrillar spaces, previously occupied by hemicelluloses [Oksanen, 1997b; Gehmayr, 2011]. These spaces collapse during drying. This hornification creates coalescence and new interfibrillar bonds which are described by some authors as irreversible [Oksanen, 1997b].This phenomena explains the decrease in fiber accessibility after nitren treatment, as discussed above.

The pectinase treatment is substantially increasing the fiber accessibility. This is not so easy to explain and is requiring further experiments to sort out what is changed in the structure which allows a better accessibility after pectinase treatment. A tentative explanation is proposed here, although without proof: the pectinase treatments could reverse the fiber hornification, by decreasing the interfibrillar bonding and coalescence, and thus regenerating interfibrillar pores and spaces. This would allow an easier diffusion of the solvent into the fiber structure, improving the dissolution in NaOH. With the extraction of hemicelluloses by nitren, the fiber porosity will be higher, which means higher accessibility. Besides this, the outer cell wall layers have a higher hemicellulose concentration and its removal will also create a higher accessibility of the solvent, decreasing this way the constriction effect during swelling described by Cuissinat et al [Cuissinat, 2006a; Le Moigne, 2008]. The higher porosity for the nitren pretreated pulps fibers and the canceling of the ballooning effect by the destructuration of the outer fiber cell walls seem able to explain the higher dissolution yield in NaOH observed for the enzymatic treated pulps with nitren pretreatment.

V.2.3. - Mass balance for the different treatments

Besides fiber accessibility and dissolution yield in NaOH, it is interesting to discuss the mass balances for the different treatments, in order to evaluate the amount of recovered material after all the treatments. This will be discussed in terms of pulp and cellulose recovery, taking the initial paper grade pulp as starting material (100%). The same is discussed considering the initial wood as base of calculation.

V.2.3.1. - Mass balance for the pulp

In Figure V.9, the pulp recovery yields after each treatment are plotted. Also, the dissolution yield of each pulp is compared with the amount of material dissolved as percentage of the starting pulp. Considering the initial pulp treated only with CCM (Eu-KP_CCM) as example, the value of the dissolution yield in cold NaOH is 57%, meaning that if 1 g of Eu-KP_CCM pulp is treated in cold NaOH, 0.57 g are dissolved. For calculating the dissolution yield in NaOH as percentage of starting pulp, the pulp recovery yield after the CCM treatment (94.8%). is taken into account. The 57% is multiplied by 0.948 giving the value of 54%. This means that starting with 1 g of initial pulp (Eu-KP), treating it with CCM and dissolving it in NaOH, only 0.541 g are going into solution. In the case of the Eu-KP_5%_CCM, the recovery yields from both nitren and CCM treatments are taken into account.

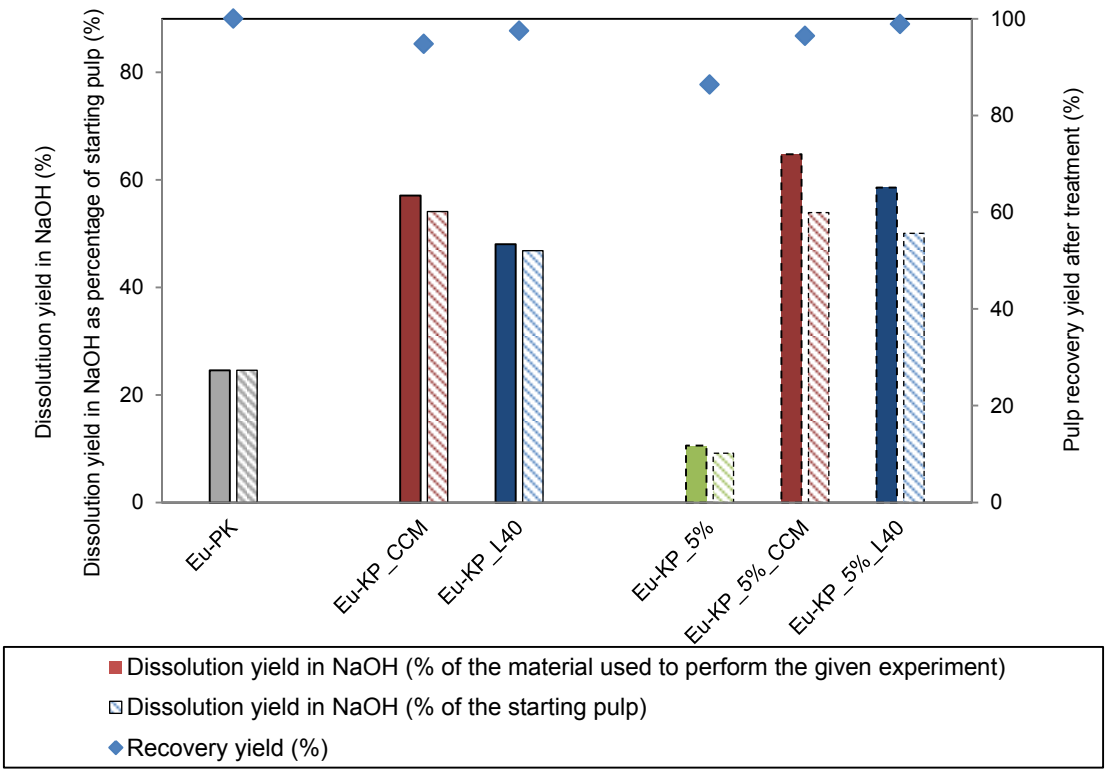

Figure V.9: Comparison of the dissolution yield in NaOH for the different pulps with the dissolved material in NaOH based on the starting pulp (Eu_KP), and pulp recovery yields after each treatment (nitren or enzymatic treatment).

Analyzing the results, the pulp recovery yield after nitren treatment is considerably lower than for all samples with an enzymatic treatment. This was already discussed in Chapter III and is mostly due to the extraction of hemicellulose. The pulp recovery yield for the enzymatic treatment with CCM (~95%) is slightly lower than for the L40 treatment (~98%). This is verified also when the pulp is pre-treated with nitren, and is explained by the presence of endoglucanase, which dissolves a small amount of cellulose.

As expected, the dissolution yield as percentage of the starting pulp is always lower, with exception for the starting pulp. Analyzing the effect of the nitren extraction before enzymatic treatment on the amount of dissolved material as percentage of the starting pulp, for CCM the same amount of material is dissolved, 54%. For the L40, 47% of material is dissolved in NaOH without nitren extraction, compared with 50% with previous nitren treatment, showing a relative increase of 6% on the dissolution yield in NaOH.

V.2.3.2. - Mass balance for the cellulose (glucose)

According to carbohydrate analysis results, the initial pulp (Eu-KP) has a total glucose content of 83%, which will be used in the following as the base of calculation (100%) of mass balances. After nitren treatment, the total glucose increases to 96.5%. As demonstrated on Chapter III, the pectinase treatments are not changing significantly the total glucose content. Having in consideration the pulp recovery yields, the total glucose content of the pulps and the fact that the insoluble fractions have in average a hemicellulose content of 2%, the total glucose dissolution yield in NaOH was calculated (with an estimated relative error of 2%) and the results are shown in Figure V.10.

Based on the starting pulp, the nitren treatment is decreasing the amount of total glucose dissolved in cold NaOH (Figure V.10). For the pectinase treated samples, the pretreatment is significantly increasing the amount of dissolved glucose in NaOH, showing a relative increase on the dissolution yield of 34% and 54% for the CCM and L40 treatments, respectively. These results show that, despite the loss of material during the nitren treatment and enzymatic treatments, the cellulose (glucose) dissolution capacity in cold sodium hydroxide is increasing. The nitren pretreatment is promoting an enormous increase of the pectinase effect on the cellulose dissolution.

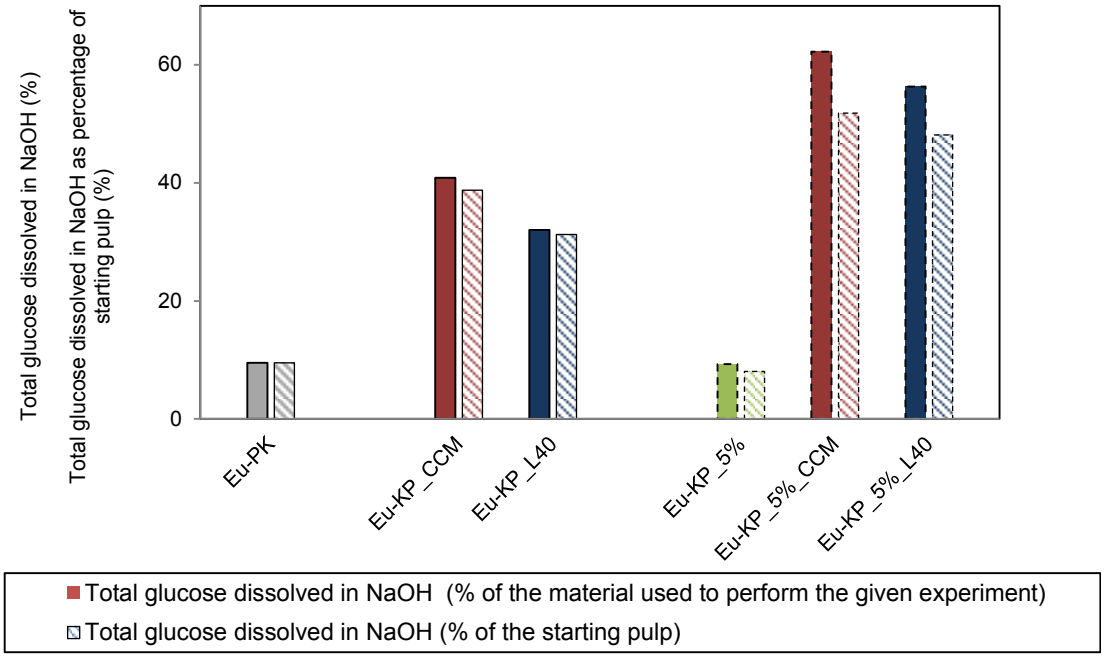

Figure V.10: Comparison of the total glucose dissolution yield in NaOH for the different pulps with the total glucose dissolved in NaOH based on the starting pulp (Eu_KP).

V.2.3.3. - Comparison of mass balance for nitren treated paper grade and dissolving pulp

In the following, the results obtained on the paper grade pulp are compared with a dissolving viscose grade pulp from the same wood source (Eucalyptus). The cellulose (total glucose) dissolution yields were compared taking the initial pulp or the initial wood as a base.

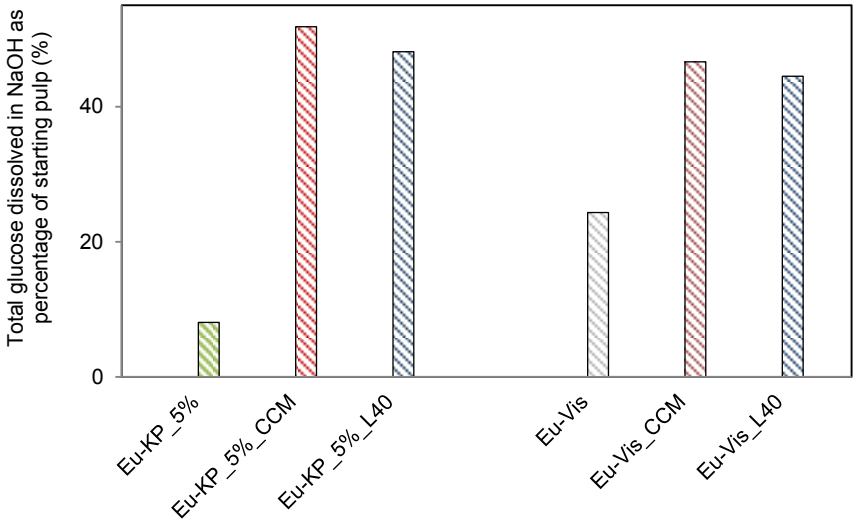

Figure V.11: Comparison of the total glucose dissolution yield in NaOH and the total glucose dissolved in NaOH based on the starting pulp (Eu_KP) for a paper grade extracted with nitren (Eu-KP_5%) and a viscose grade dissolving pulp (Eu-Vis), without and with pectinase treatments.

Considering the initial pulps, the higher cellulose dissolution ability for the viscose grade pulp is evident. The cold NaOH dissolving system is dissolving 24.3% of the cellulose (total glucose) from the Eu-Vis pulp (Figure V.11), while only 9.5% of cellulose is dissolved from the paper grade pulp Eu-KP (Figure V.10) and 8% from nitren extracted paper grade pulp Eu-KP_5% (Figure V.11). When paper grade is treated with nitren and CCM, 51.8% of the cellulose present in the initial pulp (Eu-KP) is dissolved in NaOH. 48% can be achieved in the case that L40 is applied to the nitren treated paper grade pulp. These values are higher than the values observed in the case of the dissolving pulp (Eu-Vis). When this pulp is treated with CCM or L40, respectively 46.6% and 44.5% of the cellulose present in the initial Eu-Vis pulp are going into solution.

As mentioned in the introduction, the conversion of paper grade pulps into dissolving pulp is of interest partly because of the lower production costs in comparison with dissolving pulps [Ribas Batalha, 2012]. Besides this, paper grade pulps have higher production yield, meaning that for a same amount of wood (oven-dry), a higher amount of paper grade pulp (oven-dry) can be recovered in comparison with dissolving pulp. The yields depend in different factors as pulping process, bleaching process and the raw material used (wood species) [Bierman, 1996]. The pulping yield in the production of paper grade kraft pulp varies between 45 and 55%, while the production of viscose grade dissolving pulp with a sulfite process is a low yield process with values between 30 and 35% [Biermam, 1996; Smook, 2003]. Taking this in consideration, the yields of cellulose dissolution based on the initial wood were calculated and are presented in Figure V.12. The pulping yields were reported by the pulp manufacturer (Sappi), being 47.1% for the paper grade and 39.4% for the dissolving pulp.

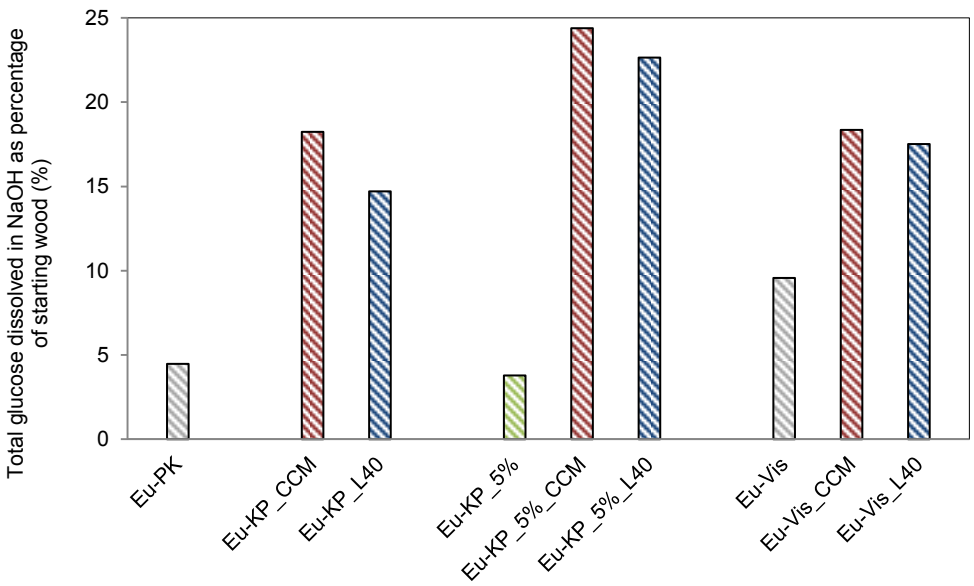

Figure V.12: Cellulose (total glucose) dissolution yield in NaOH based on the starting wood material for a paper grade untreated (Eu-KP) and treated with nitren and/or pectinase; and a viscose grade dissolving pulp untreated (Eu-Vis) and treated with pectinase.

For the untreated pulps, the dissolving pulp (Eu-Vis) shows a higher cellulose dissolution yield than the paper grade pulp (Eu-KP), also based on wood. When these two pulps are treated with pectinase CCM the relative amount of dissolved cellulose (total glucose) based on wood is equivalent ~18%, while the L40 treatment is increasing more the cellulose dissolution yield for the dissolving pulp (~17.5%) in comparison with the paper grade pulp (~14.7%). The nitren treatment of the paper grade pulp decreases the cellulose dissolution yield based on wood to 3.8%. The further treatment with pectinase increases substantially this value. After CCM treatment, it is possible to dissolve in cold NaOH 24.4% of the cellulose (total glucose) present in the wood before pulping, while for the L40 treated pulp this value accounts to 22.7%. These values are quite high, taking the dissolving pulp as reference. The paper grade pulp, treated with nitren and CCM presents a cellulose dissolution yield based on wood which is 250% of the one obtained for the dissolving pulp without treatment and 133% of the value accounted for the dissolving pulp treated with CCM. Similar results are found in the case of the L40 treatment. These results confirm the very significant effect that the combination of nitren extraction with pectinase treatment has on the cellulose dissolution in cold NaOH.

V.3. - Conclusions

The results are able to answer the two initial questions: what happens when treating a paper grade pulp with a nitren solution followed by incubation with a pectinase enzymatic preparation? Would such a procedure lead to a high purity grade with a high accessibility and dissolving power in cold NaOH?

The nitren extraction of a paper grade pulp is acting in different aspects: on one hand, it changes the chemical composition by removing the hemicelluloses which leads to an enrichment of the pulp in cellulose content. The hemicellulose removal with partial de-polymerization of the cellulose leads to an uniformization of the molar mass distribution. According to electron microscopy, the fibrils of the fiber surface are dissolved; and both fiber length and fiber width are slightly decreasing. There is a loss of ~13.5% of the initial pulp during the process. During drying, hornification occurs, which lead to an significant decrease of the dissolution capacity of the treated pulp in cold sodium hydroxide.

A subsequent treatment with pectinase, as also verified for other pulps, is not changing the chemical composition of the pulp, but is slightly decreasing the DP. In the case of a subsequent treatment with CCM, due to the presence of endoglucanase, some fibrils from the fiber surface are dissolved, conferring a further “smoothing” of the fiber surface. The fiber length and width are slightly decreased as well by this treatment. These enzymatic treatments show a residual loss of material between 1 and 4%. In terms of dissolution capacity, this treatment increases considerably the accessibility of the fiber, allowing doubling the amount of pulp dissolved in cold NaOH in comparison with the initial paper grade pulp. These effects were verified for both CCM

and L40 enzymes. Due to synergistic effects of endopectinase with a endoglucanase activity present in solution, the CCM treatment is more efficient.

The dissolution ability improvement was verified by both gravimetric measurements and assessment of the swelling degree of the insoluble residues after NaOH dissolution. These results were validated by using a second nitren treated pulp, which after pectinase treatment showed similar results.

The combination of these two treatments is yielding a pulp with a high cellulose content, low hemicellulose content, more uniform molar mass distribution and high reactivity or accessibility (in NaOH), which are parameters that characterize dissolving pulps.

The paper grade pulp treated with a combination of nitren plus pectinase was compared with the same pulp submitted only to the pectinase treatment. The results showed that the pretreatment with nitren is improving the effectiveness of the pectinase treatments, since the dissolution yields in cold sodium hydroxide are higher when a nitren pretreatment is performed.

Regarding the interest of the regenerated cellulose and cellulose derivative industry, the dissolution yields in NaOH in terms of cellulose (total glucose) based on initial pulp and initial wood were discussed and compared with the results for a viscose grade dissolving pulp, also from *Eucalyptus*. It was found that the paper grade pulp, treated with both nitren and pectinase is yielding a higher amount of cellulose in comparison with the dissolving pulp treated with pectinase.

In summary, the combination of a nitren extraction with a pectinase treatment allowed a conversion of a paper grade pulp to a pulp having the main chemical-physical parameters of a dissolving pulp. In comparison with a viscose grade dissolving pulp treated with pectinase, this converted pulp showed a higher chemical accessibility and better performances in terms of dissolution in cold NaOH.

V.4. - Bibliography

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general conclusion and perspectives

General conclusion

The present project aimed at a better understanding on the chemical accessibility of the cellulose fibers from several cellulose pulps, the mechanisms of dissolution and the role of minor chemical components (xylan and pectin) on this accessibility and dissolution ability in cold caustic soda. Different pulps were chemically and biologically treated and the influence of the treatments on the pulp properties was accessed in two ways, one by studying the changes on the chemical and physical properties, with the analysis of their chemical and macromolecular structure (molar mass distribution, carbohydrate composition, CUEN intrinsic viscosity fiber dimensional analysis and microscopy); and another by analyzing the effect of the treatments on the dissolution performance and mechanisms in cold NaOH.

Below, are briefly described the main work packages in which the work can be divided, with a short resume of the main findings:

1. Improving dissolution of wood dissolving pulps by removing residual xylan with an organometallic complex (nitren) treatment

   The results of this study show that nitren treatment has the effect of removing a large part of the xylan present in a dissolving pulp. It is also removing mannans and most important, it is influencing cellulose in two ways, (1) extracting it with more intensity when the nitren concentration increases, and (2) decreasing its mean molecular mass, also more evident with nitren concentration increase. The nitren extractions are favorable for the dissolution in cold NaOH–water, being more effective with higher concentrations. This chemical modification of the fiber surface leads to the disassembly and partial removal of the primary wall. This allows an easier access of the NaOH reagent to regions not accessible on the initial fibers, which with the decrease of the cellulose average molar mass allows an easier dissolution and gives different dissolution mechanisms.

2. Use of pectinase incubations to modify the pectic network and study of its effect on the cellulose fibers dissolution

   Despite the amount or presence of pectin on the starting pulps was too low to be determined, the incubation of different pulps lead to an increase of the accessibility of the fibers reaching dissolution yields increase higher than 150%. The enzymatic treatments were not affecting all the studied pulps on the same manner, showing that the pretreatments history of the dissolving pulps have an influence on the enzymatic efficiency. With the enzymatic activation of the cellulose fibers, a slight decrease of the average molar mass was verified. Using a mixture of endopectinase and endoglucanase showed that the synergistic effect of these two enzymes is more effective on cellulose activation. This study showed that this enzyme is promoting the accessibility of the fibers in two ways: (1) decomposes the polysaccharide matrix from the primary wall (where pectin is present),

promoting the swelling of this wall, which will allow a higher dissolution capacity of the fibers; (2) changes the hydrogen bonding network from the whole cellulose fiber, slightly decreasing the average molar mass and promoting the diffusion of the NaOH solvating ions into the cellulosic structure, facilitating this way the dissolution.

3. Study of the effect of a nitren extraction combined with a pectinase incubation in a paper grade pulp properties and dissolution capacity

   This study show that paper grade pulps extracted with nitren have a lower accessibility to NaOH ions, meaning lower dissolution capacity in cold caustic soda. This might be explained by the partial hornification due to hemicellulose removal. The further treatment with pectinase was able to improve the dissolution yield in NaOH from 10% to 60%. This might be explained by the same mechanisms described in 2. In addition, this study showed that, for the dissolving system used, the use of paper grade pulps treated with the combination of these treatments has the potential of increasing the overall cellulose (total glucose) yields based on wood, in comparison with current dissolving pulps.

As a summary, the endopectinase treatments allow a great improvement on the cellulose accessibility towards cold NaOH, without significant changes of the chemical composition or macrostructure and without material losses. This, allied with the already mature market of pectinase, makes this technology also very attractive from the industrial point of view.

Perspectives

The results achieved with this project and the different aspects observed bring new questions and new perspectives for further research and development.

The fact that the pectin is present in residual amounts, especially in wood pulps, did not allow having a clean view on the mechanisms behind the pectinase effect on the pulp. This brings the interest and opportunity for further research considering this question.

The use of nitren extraction as an agent for hemicellulose removal is interesting from the fundamental point of view. Due to its laborious and expensive proceedings and potential toxicity, it is not interesting for industry applications. Thus, considering the last work package of this project is of great interest test the combination of pectinase with other agent capable of removing the hemicelluloses, for example hemicellulase and/or cold caustic extractions.

The role of the primary wall in dissolution is already studied and well discussed. During the microscopic observations it was possible to notice that the inner wall layer of the cell wall is also not easy to dissolve. This layer is also enriched in amorphous material (lignin, hemicelluloses and pectin). It can be also interesting to study the role of this substructure of the fiber on the fiber dissolution.

During microscopic visualization of the dissolution mechanisms for different pulps (not reported in this manuscript) it was possible to verify that different wood cells have different dissolution mechanisms and different dissolution performances. This brings the interest on a comprehensive study on the dissolution behavior of different wood cells. The assessment on the dissolution mechanisms could be achieved by microscopic observations, while the dissolution performance could be studied after pulp fractionation, either with hydro-cyclones or Bauer-McNett classifiers, already used in the paper industry.

annex

Publications and communications

Publications and communications

During this project the following publications and communications were done:

Publications in peer reviewed journals:

Santos, N. M., Puls, J., Saake, B., Navard, P., (2013). "Effects of nitren extraction on a dissolving pulp and influence on cellulose dissolution in NaOH–water." Cellulose 20(4): 2013-2026

Spinu, M., Dos Santos, N., LeMoigne, N., Navard, P., (2011). "How does the never-dried state influence the swelling and dissolution of cellulose fibres in aqueous solvent?" Cellulose 18(2): 247-256

Communications in scientific conferences and meetings:

Bodo Saake, Nuno Dos Santos, Ron Janzon, Jürgen Puls, Patrick Navard, “Application of nitren for the production and modification of dissolving pulp”, 245th ACS National Meeting and Exposition, April 7-11, 2013, New Orleans, Louisiana (Oral presentation)

N. Dos Santos, P. Navard, B. Saake, J. Puls, (2010), Influence of nitren extraction prior to cellulose dissolution, XXI Tecnicelpa / VI Ciadicyp 2010, New Paradigms in the pulp and paper industry, 12-15 October 2010, Lisbon, Portugal (Poster)

N. Dos Santos, P. Navard, B. Saake, J. Puls (2010), Influence of nitren extraction on cellulose dissolution, 11th European Workshop on Lignocellulosics and Pulp, 16-19 August 2010, Hamburg, Germany (Poster)

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Influence de traitements chimiques et enzymatiques sur la dissolution de pâtes de bois dans NaOH-eau

RESUME : Différentes pâtes de bois ont été traitées chimiquement et biologiquement en vue d'améliorer leurs accessibilités chimiques et leurs dissolutions dans des mélanges froids NaOH-eau. Les effets des traitements sur les propriétés des pâtes ont été analysés par l'étude des changements sur leur structure chimique et macromoléculaire et par l'analyse des performances de dissolution dans NaOH-eau.

Le traitement à base de nitren a pour effet de supprimer une grande partie des xylanes présents dans la pâte et il élimine aussi les mannanes. L'augmentation de la concentration de nitren a pour conséquence d'extraire également de la cellulose et de diminuer sa masse molaire moyenne. Ces extractions sont favorables à la dissolution dans NaOH-eau froid, ce traitement étant plus efficace si on augmente la concentration de nitren. Une augmentation maximale de 44.7% du rendement de dissolution a été observée suite à ce traitement.

Un traitement à base de pectinase montre une grande efficacité pour promouvoir l'accessibilité des fibres aux ions NaOH (directement corrélée à la charge enzymatique), ce qui permet une augmentation maximale de 150% sur le rendement de dissolution. Une légère diminution de la masse molaire moyenne a également été observée.

Les différentes pâtes réagissent différemment aux traitements, montrant que les pré-traitements de fabrication de pâte ont une influence sur l'efficacité enzymatique. Les meilleurs résultats d'activation sont trouvés lors de l'utilisation d'un mélange de pectinase et d'endoglucanase montrant un effet synergie entre ces enzymes.

Les deux traitements nitren et pectinase améliorent l'accessibilité chimique des pâtes principalement par la modification de la structure des parois primaire et S1. Cela favorise le gonflement de ces structures cellulaires du bois, ce qui permet l'accès des ions de solvatation de NaOH dans les régions de fibres ne sont pas accessibles sur la pâte initiale. Le nitren désassemble la surface de la fibre par l'extraction de l'hémicellulose et la dégradation de la structure cellulosique.

L'utilisation de pectinase pour activer la cellulose vis-à-vis de la dissolution dans NaOH-eau est une approche novatrice. Elle présente un grand intérêt fondamental en ce qui concerne les mécanismes impliqués lors de ces traitements et un potentiel technique élevé qui pourrait ouvrir de nouvelles voies pour pré-traiter les matériaux à base de cellulose. Le travail a été effectué au Cemef (Mines ParisTech/CNRS) à Sophia Antipolis, France et l'Université de Hambourg/Thünen Institut, Allemagne et financé par les entreprises Sappi, Tembec, Viskase, Spontex et Lenzing.

Mots clés : Dissolution de la cellulose, paroi cellulaire du bois, pâte à dissoudre, nitren, pectinase, hydroxyde de sodium.

Influence of chemical and enzymatic treatments on a variety of wood pulps on their dissolution in NaOH-water

ABSTRACT : Different wood pulps were chemically and biologically treated in order to improve the chemical accessibility and dissolution capacity in cold NaOH. The effects of treatments on the pulp properties were analyzed by studying the changes on their chemical and macromolecular structure and by analyzing the dissolution performance in cold NaOH-water.

The nitren treatment has the effect of removing a large part of the xylan present in a dissolving pulp and is also removing mannans. Increasing the nitren concentrations has the consequence to extract also cellulose and decrease its mean molar mass. These extractions are favorable for the dissolution in cold NaOH–water, being more effective with higher nitren concentrations. A maximum of 44.7% increase on the dissolution yield was achieved.

A pectinase treatment shows a very high efficiency on promoting fibers accessibility to NaOH ions, (directly correlated with the enzymatic load), allowing a maximum increase of 150% on the dissolution yield. A slight decrease of the average molar mass was also seen. The different pulps reacted differently to the treatments, showing that the pulping pretreatments have an influence on the enzymatic efficiency. The best activation results are found when using a mixture of pectinase and endoglucanase giving synergistic effects between these enzymes which allows pulps to be much more suitable to dissolution in cold NaOH-water.

Both nitren and pectinase treatments are improving the pulp chemical accessibility mostly by modifying the structure of the primary and S1 walls. This promotes the swelling of these wood cell structures, allowing the access of the NaOH solvating ions into fiber regions not accessible on the original pulp. The nitren is disassembling the fiber surface with extraction of hemicelluloses and degrading the cellulosic structure.

The pectinase use on cellulose pulp activation towards dissolution in cold NaOH is importance novel approach. It presents a large fundamental interest regarding the mechanisms involved during such treatments and a high technical potential since it could open new ways to pre-treat cellulose-based materials. The work was performed in Cemef (Mines ParisTech/CNRS), Sophia Antipolis, France, and Hamburg University/Thünen Institut, Germany. Financed by the Sappi, Tembec, Viskase, Spontex and Lenzing companies.

Keywords : Cellulose dissolution, wood, nitren, dissolving pulp, nitren, pectinase, sodium hydroxide, dissolution mechanisms, wood cell wall.

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