Multifunctional Alkaline Pulping with Enzyme Treatments:

How to Convert Paper-grade Pulps into High-quality Dissolving Pulps

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Abstract

The world demand for cellulosic fibers has boomed within the last decade while the demand for paper and paper products is decreasing. Regenerated cellulosic fibers from the raw material wood can be produced by the lyocell process or the viscose process and represent an ecological alternative to cotton fibers. Thus, today the production of dissolving pulp instead of paper-grade pulp is of high economical interest for many market players.

Post-refining of paper-grade pulp is a new strategy for the production of a high-yield and high-quality dissolving pulp. The commercial benefit of dissolving pulps produced from paper-grade pulps is based on the high selectivity of the hemicellulose extraction step.

It was the scope of this thesis to develop efficient process steps for the conversion of paper-grade pulps into dissolving pulps. Therein, the main challenge was to ensure the selective removal of hemicelluloses and the precise adjustment of pulp viscosity, while resulting in a highly reactive dissolving pulp as required for viscose application. For this purpose, the utilization of enzymes for hemicellulose extraction and for pulp depolymerization was investigated.

Hemicellulose removal from hardwood kraft pulps was accomplished by xylanase treatment followed by cold caustic extraction (CCE) at low alkalinity. These xylanase pretreated pulps showed increased reactivity towards xanthation and higher viscose dope quality compared to pulps treated at elevated alkalinity for comparable hemicellulose removal. When xylanase treatment was performed prior to alkaline extraction, xylan in the alkaline extraction lye was found to be slightly degraded, which is, however, disadvantageous when xylan is recovered and utilized as an additional polymeric product.

The degree of polymerization (DP) of dissolving pulps is conventionally adjusted by prolonged cooking or during bleaching. Alternatively, in the described work, it was shown how to adjust the DP in a controlled way by endoglucanase (EG) post-treatment after bleaching. The influence of the wood source, the pulping process, the non-cellulosic polymeric compounds, and the cellulose morphology on the efficiency of the enzymatic degradation was examined. The accessibility of the cellulosic surface of the

pulps to the enzymes was reduced in the presence of hemicelluloses and residual lignin, and was increased after alkaline pretreatment due to increased swelling capacity of the pulp.

When CCE-treated pulps are dried, the pulp fiber microfibrils aggregate and thus, the accessible surface area and the pulp reactivity are clearly impaired. Consequently, this leads to uneven derivatization along the cellulose chain in the viscose process. As a result, undissolved and/or jellylike particles in the viscose cause filtration problems and spinning faults. It was demonstrated that adoption of an EG-treatment of the final bleached pulp can clearly enhance its reactivity and thus its applicability for the viscose process.

For direct dissolution processes, like the lyocell process, surface carboxylation by TEMPO-mediated oxidation was applied as an alternative way for pulp depolymerization while yielding highly reactive dissolving pulps. TEMPO-mediated oxidation was demonstrated to enhance homogeneous and fast dissolution of dissolving pulps prepared from paper-grade pulps.

Thus, pulp treatments like EG-depolymerization or surface derivatization are promising techniques aiming at an increase in pulp reactivity which is the key criterion for the processability of pulps in current regenerated fiber processes.

Kurzfassung

Die weltweite Nachfrage nach zellulosischen Fasern stieg innerhalb des letzen Jahrzehnts enorm, der Bedarf an Papierprodukten sinkt hingegen. Zellulosefasern aus dem Rohmaterial Holz werden als Regeneratfasern nach dem Lyocell- oder dem Viskose-Verfahren hergestellt und stellen eine ökologische Alternative zur Baumwollfaser dar. Daher ist aktuell die Herstellung von Chemiezellstoff anstatt von Papierzellstoff von hohem ökonomischem Interesse für viele Produzenten.

Die Nachveredelung von Papierzellstoff zu Chemiezellstoff ist eine neue Methode, die auf hohe Ausbeuten und hohe Qualitäten bei der Herstellung von Chemiezellstoff abzielt. Der wirtschaftliche Vorteil der Produktion von Chemiezellstoff aus Papierzellstoff liegt in der hohen Selektivität der Extraktionsschritte für Hemizellulosen.

Im Rahmen der dargelegten Arbeit wurden effiziente Prozessschritte zur Weiterverarbeitung von Papierzellstoff zu Chemiezellstoff entwickelt. Die Herausforderungen darin umfassten das selektive Entfernen von Hemizellulosen und die kontrollierte Einstellung der Zellstoff-Viskosität sowie im Zuge dessen, einen hoch-reaktiven Chemiezellstoff herzustellen, wie er für den Viskose-Prozess benötigt wird. Zu diesem Zweck wurde der Einsatz von Enzymen zur Entfernung von Hemizellulosen und zur Depolymerisation der Zellulose untersucht.

Hemizellulosen aus Laubholz-Kraft-Zellstoffen wurden mittels Xylanase-Behandlung und anschließender Kalt-Alkali-Extraktion (CCE) bei niedriger Alkalinität entfernt. Die mit Xylanase vorbehandelten Zellstoffe zeigten verbesserte Reaktivität im Zuge der Xanthogenierung und erhöhte Viskose-Qualitäten im Vergleich zu denen nach einer CCE bei höherer Alkalität. Wurde vor der CCE-Stufe eine Xylanase-Behandlung vorgeschaltet, so lag das Xylan in der Lauge leicht abgebaut vor. Dies stellt einen Nachteil dar, wenn das Xylan als zusätzliches polymeres Produkt wieder gewonnen wird.

Üblicherweise wird der Polymerisationsgrad (DP) von Chemiezellstoffen durch verlängertes Kochen oder im Zuge der Bleiche abgesenkt. Als Alternative wurde in der dargelegten Arbeit aufgezeigt, wie der DP mittels Endoglukanase-Behandlung (EG) nach der Bleiche adjustiert werden kann. Im Zuge dessen wurde der Einfluss der

Holzart, des Kochprozesses, nicht-zellulosischer Bestandteile und der Zellulose-Morphologie auf die Effizienz des enzymatischen Abbaus untersucht. Die Zugänglichkeit der zellulosischen Oberfläche des Zellstoffs war in der Gegenwart von Hemizellulosen oder Restlignin eingeschränkt. Nach einer alkalischen Behandlung hingegen waren die Quellfähigkeit des Zellstoffs und somit dessen Zugänglichkeit gesteigert.

Werden Zellstoffe nach einer CCE-Behandlung getrocknet, aggregieren die Mikrofibrillen der Zellstofffasern, wodurch die Oberflächen-Zugänglichkeit und somit die Reaktivität für den Viskose-Prozess reduziert werden. Die geminderte Zellulose-Zugänglichkeit führt zu ungleichmäßiger Derivatisierung entlang der Zelluloseketten und zu ungelösten und/oder gelartigen Partikeln in der Viskose, die Filtrationsprobleme und Spinnfehler verursachen. Es wurde nun gezeigt, dass die Durchführung einer EG-Behandlung am endgebleichten Zellstoff deutlich dessen Reaktivität und dessen Anwendbarkeit für den Viskose-Prozess erhöht.

Für Direkt-Löseprozesse wie dem Lyocell-Verfahren wurde Oberflächen-Carboxylierung mittels TEMPO-Oxidation als alternative Möglichkeit zur Zellstoff-Depolymerisation und zur gleichzeitigen Herstellung hoch-reaktiver Chemiezellstoffe aus Papierzellstoffen aufgezeigt. TEMPO-Oxidation steigerte die Lösequalität hinsichtlich Homogenität der resultierenden Lösung und reduzierte die Lösedauer des Zellstoffs deutlich.

Daher kann zusammengefasst werden, dass sowohl EG-Behandlung als auch Oberflächen-Derivatisierung vielversprechende Methoden sind, die Reaktivität von Zellstoffen zu steigern und somit deren Anwendbarkeit in den aktuellen Regeneratfaser-Prozessen deutlich zu verbessern.

List of Publications

The following thesis is based on four journal papers, three of them peer reviewed (Paper 1, Paper 3, Paper 4), and one poster contribution given to an abstract-citing conference (Poster 3) dealing with the production of dissolving pulps from paper-grade pulps following the multifunctional alkaline pulping process. One additional peer reviewed journal paper about structural clarification of residual lignin from pulp was achieved within the time period of the thesis (Paper 5).

Paper

1. Gehmayr, V, Schild, G, Sixta, H (2011) A precise study on the feasibility of enzyme treatments of a kraft pulp for viscose application. Cellulose 18(2), 479-491
2. Gehmayr, V, Sixta, H (2011) Dissolving pulps from enzyme treated kraft pulps for viscose application. Lenzinger Berichte 89, 152-160
3. Gehmayr, V, Sixta, H (2012) Pulp properties and their influence on enzymatic degradability. Biomacromolecules 13(3), 645-651
4. Gehmayr, V, Potthast, A, Sixta, H (2012) Reactivity of dissolving pulps modified by TEMPO-mediated oxidation. Cellulose 19(4), 1125-1134
5. Gehmayr, V, Gütsch, J, Leschinsky, M, Zuckerstätter, G, Sixta, H (2010) New aspects of residual lignin isolation. Holzforschung 64(4), 417-420

Contribution of the author:

Paper 1-4: Experiments, analyses in part, first version of the manuscript
Paper 5: Experiments, analyses in part, first version of the manuscript; in cooperation with co-author Gütsch, J.

Poster Presentations

1. Gehmayr, V, Sixta, H (2010) Feasibility of enzymatic bio-bleaching techniques of a kraft pulp for viscose application. International Workshop on Wood Biorefinery and Tree Biotechnology, Örnsköldsvik, Sweden, Juni 21-24

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3. Gehmayr, V, Sixta, H (2011) Controlled cellulose depolymerization by enzymatic treatment. Abstracts of Papers, CELL-113, 241st ACS National Meeting & Exposition, Anaheim, CA, United States, March 27-31

Oral Contributions

1. Schild, G, Sixta, H, Gehmayr, V (2010) Production of a novel generation of sulphur-free dissolving pulps. Zellcheming Cellulose Symposium, Wiesbaden, Germany, June 29-July 01

2. Gehmayr, V (2010) Upgrading of paper-grade pulps to dissolving pulps. Wood School, Gaming, Austria, September 29-30

3. Gehmayr, V, Sixta, H (2011) Pulp reactivity enhancement by enzymatic treatment. 14th Austrian Chemistry Days, Linz, Austria, September 26-29

4. Sixta, H, Gehmayr, V, Schild, G, Hummel, M, Hauru, L, Kilpelainen, I, King, A (2011) Novel aspects in dissolving pulp chemistry. 1st Avancell Conference, Göteborg, Sweden, October 18-19

Table of Contents

1 Introduction 1

1.1 Background 1

1.1.1 The World Demand for Cellulosic Fibers 1

1.1.2 The Cell Wall Structure of Wood 1

1.1.3 Market Pulps and Pulping Technology 2

1.1.4 The Multifunctional Alkaline Pulping Process 4

1.1.5 Reactivity of Dissolving Pulps 6

1.1.6 Pulp Reactivity Enhancement 7

1.2 Objectives of this Work 11

2 Experimental Part 12

2.1 Materials 12

2.1.1 Pulp Substrates 12

2.1.2 Enzyme Preparations 13

2.2 Analysis 14

2.2.1 Standard Analytical Methods 14

2.2.2 FT-Raman 14

2.2.3 WAXS Analysis 15

2.2.4 Specific Surface Area (BET-Surface) 15

2.2.5 Carbonyl Group and Carboxyl Group Determination with Fluorescence Labeling 15

2.2.6 FE-SEM 16

2.2.7 Pulp Reactivity Measurements 16

2.3 Pulp Treatments Following the Multifunctional Alkaline Pulping Process 18

2.3.1 Enzymatic Hydrolysis of Hemicelluloses from a Paper-grade Kraft Pulp 19

2.3.2 Alkaline Extraction of Hemicelluloses from a Paper-grade Kraft Pulp 20

2.3.3 TCF-Bleaching 20
2.3.4 Enzymatic Depolymerization with Endoglucanase 21
2.4 Pulp Properties and their Influence on Enzymatic Degradability 24
2.4.1 Hemicellulose Removal from a Sulfite Pulp 24
2.4.2 Influence of Cellulose Morphology on Enzymatic Degradability 25
2.5 The Effect of Enzymatic Depolymerization on Pulp Reactivity 26
2.5.1 Acid-catalyzed Hydrolysis of a Paper-grade Kraft Pulp 26
2.5.2 Acid-catalyzed Hydrolysis of a Paper-grade Sulfite Pulp 27
2.6 TEMPO-mediated Oxidation of a Dissolving Pulp 27
2.6.1 Pretrials of TEMPO-mediated Oxidation 28
2.6.2 TEMPO-mediated Oxidation and CCE 29
2.6.3 Viscose Preparation from a Direct-xanthation-process 30
2.6.4 Swelling and Dissolution Behavior of the Oxidized Pulps Observed with Optical Microscopy 31
3 Results and Discussion 32
3.1 Production of a Dissolving Pulp by the Multifunctional Alkaline Pulping Process 32
3.1.1 Removal of Hemicelluloses and TCF-bleaching 32
3.1.2 Enzymatic Depolymerization with Endoglucanase 34
3.1.3 Pulp Reactivity 36
3.2 Pulp Properties and their Influence on Enzymatic Degradability 39
3.2.1 Influence of Cellulose Morphology 39
3.2.2 Influence of Residual Hemicelluloses 41
3.2.3 Influence of Residual Lignin 43

3.3 The Effect of Different Methods of Pulp Depolymerization on their Reactivity 46

3.3.1 Spruce Sulfite Pulps 46

3.3.2 Eucalypt Kraft Pulps 49

3.4 Reactivity of TEMPO-oxidized Dissolving Pulps 50

3.4.1 Pulp Properties after TEMPO-oxidation and CCE 50

3.4.2 Viscose Reactivity of the TEMPO-oxidized Pulps 52

3.4.3 Dissolution Behavior of the TEMPO-oxidized Pulps in NMMO-water 53

3.4.4 Swelling and Dissolution Behavior of the TEMPO-oxidized Pulps in Cuen-solution 55

4 Conclusion and Outlook 59

5 References 62

6 Appendix 72

6.1 List of Abbreviations 72

6.2 List of Figures 74

6.3 List of Tables 77

1 Introduction

1.1 Background

1.1.1 The World Demand for Cellulosic Fibers

Demands for dissolving pulp and cellulosic fibers have boomed world-wide within the last decade due to world population growth and wealth growth. Cotton probably represents the best-known natural grown cellulosic fiber. Cotton mainly consists of cellulosic material and due to its natural fiber length (20-40 mm) it can be directly used in yarn manufacture after some purification steps. For cotton production, huge growing areas are required, and high watering demand of the plants makes the production more and more expensive. Additionally, climate variations like floods and heat periods often threaten cotton harvest unforeseeably. Thus, the market price for cotton has risen intensely within the last years and demands for alternative cellulosic fibers have increased. Cellulosic fibers from wood raw material represent an ecological alternative to cotton fibers. As the cellulosic material in wood is incorporated in a complex matrix with other polymeric compounds, wood chips have to be subjected to pulping processes in order to liberate the cellulosic fibers. The resulting dissolving pulp is used in fiber processes, like the lyocell process or the viscose process. In the lyocell process, the dissolving pulp is directly dissolved in an organic melt and regenerated from this solution. In the viscose process instead, pulp fibers are converted into spinning dope (viscose) by steps of derivatization and dissolution, and endless cellulose fibers are spun thereof. As the world demand for paper and paper products tends to decrease, nowadays some paper producers put more and more effort on converting existing paper pulp mills into dissolving pulp mills.

1.1.2 The Cell Wall Structure of Wood

Wood cell walls are composed of three main components, namely cellulose, hemicelluloses and lignin (Fig. 1). Cellulose acts as framework within the wood cell wall and is arranged in microfibrils with sub-structured elementary fibrils. Hemicelluloses act as matrix substances forming complexes with cellulose or lignin molecules. Lignin works as intercellular cement and is responsible for strength of lignified plants enabling height growth. Thus, lignin makes up about 80 % of the middle lamella region and the primary wall of wood cell walls (Panshin and de Zeeuw 1980).

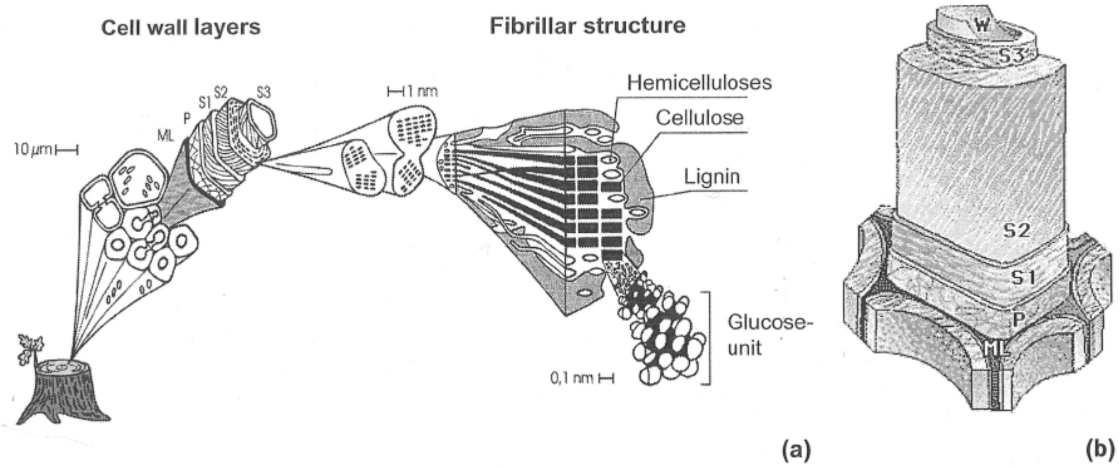

Fig. 1 (a) Illustration of the cell wall layers and their chemical composition (Hoffmann and Jones 1990); (b) The cell wall structures of an individual wood cell. ML = middle lamella, P = primary wall, S = secondary wall (S1, S2, S3), W = warty layer (Côté et al. 1964)

1.1.3 Market Pulps and Pulping Technology

Pulping processes aim to liberate fibers from wood or other lignocellulosic material. In principal, the process of defibration can be accomplished mechanically or chemically. Modern mechanical and chemical pulping processes have been available since the 1850s[1]. Mechanical methods demand a lot of electric power, but make use of the whole wood material. In the resulting high-yield pulps, the bulk amount of lignin and hemicelluloses are preserved besides the cellulosic material. These pulps are of low brightness due to the high residual lignin content. In chemical pulping instead, only about half of the wood mass is found in the pulp. The removed half of the wood mass, composed of hemicelluloses, extractives and inorganic compounds, is dissolved during pulping as delignification is not a selective process and about 90 % of the wood lignin needs to be removed to have the wood cells chemically separated. The most commonly applied chemical pulping processes, depending on the wood type and the aimed pulp quality, comprise the alkaline sulfate process, also called kraft process, and the acid sulfite process. For pulping of annual plants, the alkaline soda process using aqueous

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[1] www.pulpandpaper.org

sodium hydroxide is primarily used. However, when catalytic amounts of anthraquinone (AQ) are added, also hardwood pulps can be produced by the soda-AQ process, representing an ecologically friendly alternative for processes using sulfur-containing cooking liquors (Bränvall 2009; Sixta et al. 2006).

1.1.3.1 Paper-grade Pulp

Paper-grade pulp mainly consists of cellulose and 15-25 % hemicelluloses. The hemicelluloses in the pulp are necessary because they contribute to the majority of bonding forces in the final paper product. Mechanical pulping processes like e.g. TMP (thermomechanical pulping) and chemical pulping processes like the kraft process, are mainly applied for the production of pulps for all kinds of unbleached paper products, like packaging paper or linerboards. Bleached pulps are also used for the production of writing paper and tissue paper.

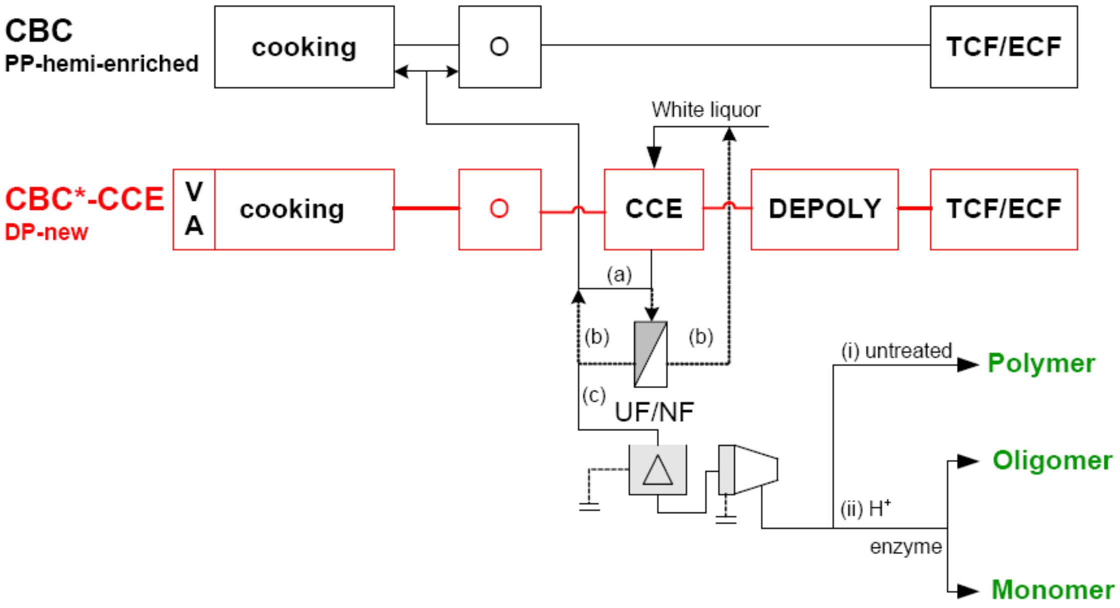
1.1.3.2 Dissolving Pulp

Dissolving pulp instead is the main source for the manufacture of viscose rayon, cellulose yarns, and cellulose derivatives, such as cellulose esters and cellulose ethers. The most commonly applied processes for dissolving pulp production are the acid sulfite cooking and the alkaline prehydrolysis kraft (PHK) cooking.

In the sulfite process, hemicelluloses and lignin are removed from the wood chips in the same process step, which impedes the recovery of hemicelluloses from cooking liquor. Nevertheless, about 60 % of the market volume on dissolving pulp is produced by the sulfite technology. The PHK process instead uses an alkaline cooking step. Hemicelluloses are preextracted from the wood chips in big quantities in a step of prehydrolysis prior to cooking. During prehydrolysis, short-chain carbohydrates, preferably hemicelluloses, are hydrolyzed owing to autohydrolysis initiated by the release of acetic acid. Besides the advantages of high pulp quality and high cooking capacity, PHK cooking also bears a big technological drawback. The main problem is the formation of highly reactive degradation intermediates in the resulting prehydrolyzate. These undergo condensation reactions and form sticky precipitates during drainage. These pitch-like compounds present a big technological challenge and have been discussed frequently in the focus of prevention, reduction or economical handling (Annergren et al. 1965; Gütsch et al. 2011; Gütsch and Sixta 2011; Leschinsky et al. 2008a; Leschinsky et al. 2008b; Sixta et al. 2006). In order to avoid the problem of precipitation, the adaption of steam activation followed by a neutralization step prior to alkaline cooking was realized as an alternative process to the PHK cooking (Wizani et al. 1994).

1.1.4 The Multifunctional Alkaline Pulping Process

Nowadays much effort is put on the production of dissolving pulps from paper-grade pulps besides the dissolving pulp production from the commonly applied technologies. Quite recently, the multifunctional alkaline pulping process was suggested, which allows the production of a broad range of pulp grades and the recovery of sugar-based products (Schild et al. 2010b; Sixta et al. 2007). In a second parallel production line, the production of a high-yield paper-grade pulp is enabled.

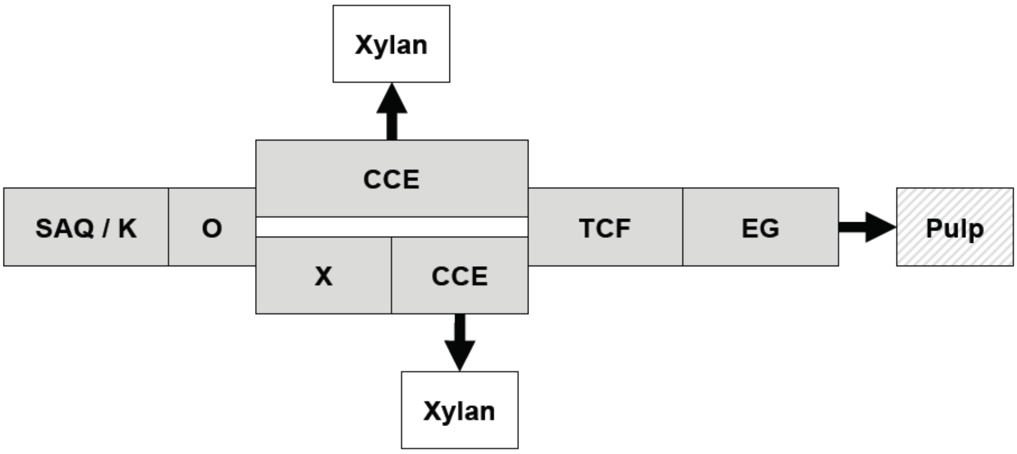


CBC	Continuous batch cooking process	DEPOLY	Depolymerization treatment
PP	Paper-grade pulp	TCF	Total chlorine free bleaching
DP	Dissolving pulp	ECF	Elemental chlorine free bleaching
O	Oxygen delignification	UF	Ultrafiltration
VA	Vapor activation	NF	Nanofiltration
CCE	Cold caustic extraction		

Fig. 2 Concept of the multifunctional alkaline pulping process according to Sixta et al. (2007)

In the concept of multifunctional alkaline pulping (Fig. 2), short-chain carbohydrates are removed from wood chips by autohydrolysis initiated under the conditions of vapor activation. High molecular weight (MW) hemicelluloses may be isolated from the pulp by cold caustic extraction (CCE, see 2.3.2). The resulting alkaline CCE-lye represents a pure source for carbohydrates for the conversion into building block chemicals. Alternatively, high-WM xylan can be utilized as additive for paper pulp production, to improve yield and fiber-fiber bonding capability in the paper product (Schild et al. 2010b).

A simplified concept of the multifunctional alkaline cooking process concentrating on the dissolving pulp production line is shown in Fig. 3. This scheme describes the conversion of paper-grade pulps into dissolving pulps by post-extraction of hemicelluloses from the pulp, TCF-bleaching and adjustment of final pulp viscosity. The commercial benefit of this new so-called modified paper pulps (MPPs) compared to conventional PHK pulps is constituted by a substantial higher yield, ranging between 4 wt % and 8 wt % on oven dried wood depending on the wood source (Schild et al. 2010a). The yield advantage is based on the difference in hemicellulose extraction between the conventional dissolving pulp production and the novel dissolving pulp production. In the latter, hemicelluloses are removed from the pulp under cold and alkaline conditions without affecting the cellulose yield. Especially for viscose application, but also for any other alkaline conversion process as for the manufacture of cellulose ethers, the removal of the alkali soluble hemicelluloses from the paper-grade pulp is necessary, because otherwise they are dissolved in the steeping lye and impair the viscose process. Depending on the type of hemicelluloses that needs to be removed from pulp, different alkaline extraction methods are applicable following physical and chemical reaction pathways, respectively. Prehydrolysis instead is a chemical process step at acidic and hot conditions initiating carbohydrate degradation reactions. Thus, the aforementioned yield advantage of the novel dissolving pulp production is based on the high selectivity of the CCE-treatment towards cellulose.



SAQ	Soda-AQ pulping	X	Xylanase treatment
K	Kraft pulping	CCE	Cold caustic extraction
O	Oxygen delignification	TCF	Total chlorine free bleaching
		EG	Endoglucanase treatment

Fig. 3 Concept for the production of modified paper pulps

1.1.5 Reactivity of Dissolving Pulps

The drawback of MPPs, however, is the lower reactivity as compared to conventional PHK pulps. The reactivity of a pulp is determined by the accessibility of the hydroxyl groups on C_6 and C_2/C_3 of the glucose monomer units to the reactants (Sixta 2006b) and determines the processability of a dissolving pulp for the viscose process (Sixta 2006a). A working hypothesis for the reduced reactivity of kraft pulps after purification by CCE compared to PHK pulps is based on the structure of the residual hemicelluloses in the pulp. The residual hemicelluloses in PHK pulps are of low molecular weight and show many functional groups as side chains. Because of their mobility, these short-chain hemicelluloses are supposed to act as spacer in-between the cellulose microfibrils, reducing their tendency to aggregate. CCE-treated kraft pulps instead, contain long-chained hemicelluloses with less functional groups (Schild and Sixta 2011). Thus, the cellulose microfibrils tend to aggregate upon intense dewatering or drying, which may play a key-role in the relation of hornification and pulp reactivity (Fig. 4).

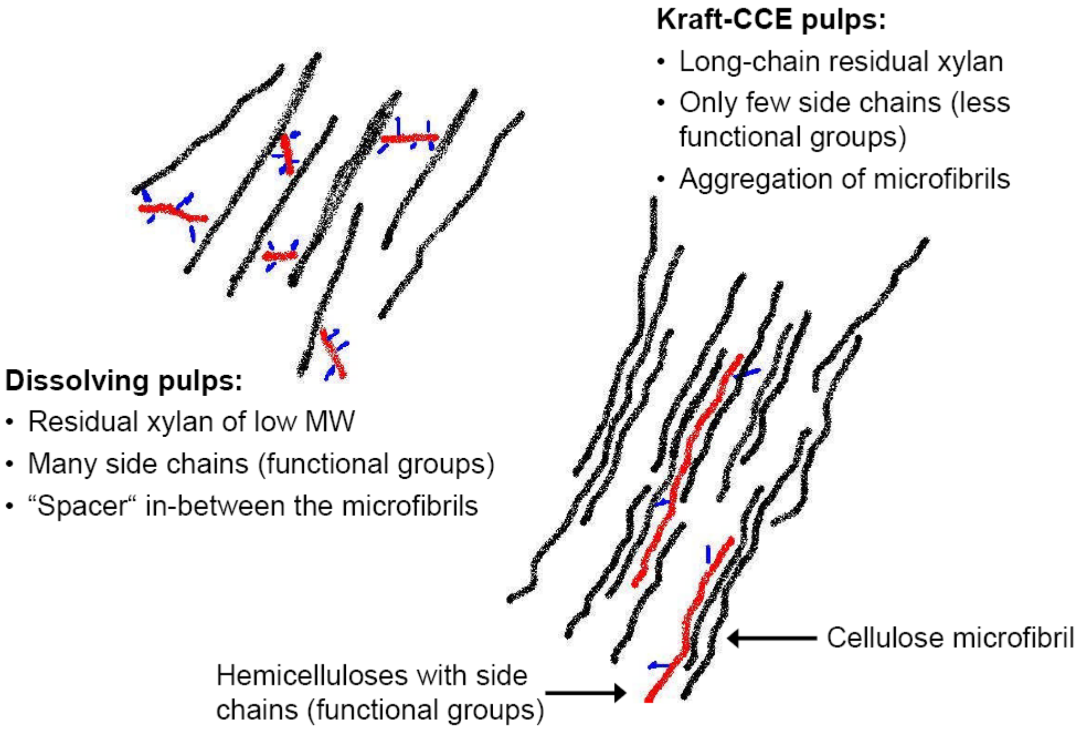


Fig. 4 Model structures of different dissolving pulps: PHK pulp (left), CCE-refined kraft pulp (right)

Hornification refers to the stiffening of polymer structures in lignocellulosic material that takes place upon drying or water removal. Upon drying, the internal pore volume shrinks because of structural changes in pulp fibers (Fernandes Diniz et al. 2004). This is especially known for pulps after CCE-purification. Applying CCE shows effects on the morphology of cellulose attributed to the alkalinity of this treatment. If the alkali concentration in the extraction lye exceeds 8 wt % (Sixta 2006b), the crystal lattice of cellulose I gradually changes to that of Na-cellulose I and, upon regeneration, to the cellulose II modification (Krässig 1984). The cellulose II crystal lattice is characterized by intersheet O-H…O hydrogen bonds which have not been detected for cellulose I_β (Langan et al. 1999; Nishiyama et al. 2002). This additional hydrogen bond in cellulose II compared to native cellulose might give a contribution to the reduced pulp reactivity towards derivatization of mercerized dried pulps (Jayme and Schenck 1949; Kleinert 1975; Kyrklund and Sihtola 1963; Roffael 1972; Samuelson et al. 1954).

1.1.6 Pulp Reactivity Enhancement

Hornification still represents an important challenge in dissolving pulp production. Thus, many treatments for increasing the reactivity of a pulp such as swelling, solvent exchange, degradation procedures (Krässig 1993a), alternative solvents for extraction of hemicelluloses such as cupriethylene diamine (Cuen) and nitren (Puls et al. 2006), and chemical modification (Sears et al. 1982) have been investigated. Weightman et al. (2009) presented advanced methods to achieve a more homogeneous distribution of the xanthate substituent in viscose within an anhydroglucose unit and along the cellulose chain. They suggested the activation of the dissolving pulp prior to steeping by e-beaming, liquid ammonia or steam explosion treatments in order to open the structures of the cellulose fibers of the pulp. Another aspect of pulp activation concerns the introduction of spacers, such as polyethylene glycol of specified molar mass. These spacers are thought to be capable of entering the voids within the fiber wall which are formed when hemicelluloses are dissolved upon alkaline treatments. In this way, the pore volume is preserved ensuring better accessibility (Weightman et al. 2009).

1.1.6.1 Endoglucanase Treatment

Within the last few years, the pulp treatment with a monocomponent endoglucanase (EG) has been studied extensively aiming at the improvement of pulp reactivity (Engström et al. 2006; Henriksson et al. 2005; Ibarra et al. 2009; Ibarra et al. 2010a; Köpcke et al. 2008; Köpcke et al. 2010; Kvarnlöf et al. 2007; Kvarnlöf et al. 2008b). Endo-1,4-β-glucanase randomly cleaves glycosidic bonds within polysaccharide chains and typically acts on less ordered cellulose structures, as illustrated in Fig. 5, Atalla (1979) and Rahkamo et al. (1998) observed enhanced degradation of cellulose II compared to cellulose I upon EG-treatment. They considered the decrease in cellulose II morphology to be a key element for the activation of pulp. Another suggested aspect of pulp activation is the degradation of amorphous cellulose located on the fiber surface and between the microfibrils. Removing these amorphous fractions may lead to increased reactivity of the pulp due to elevated crystalline surface accessibility for e.g. xanthation.

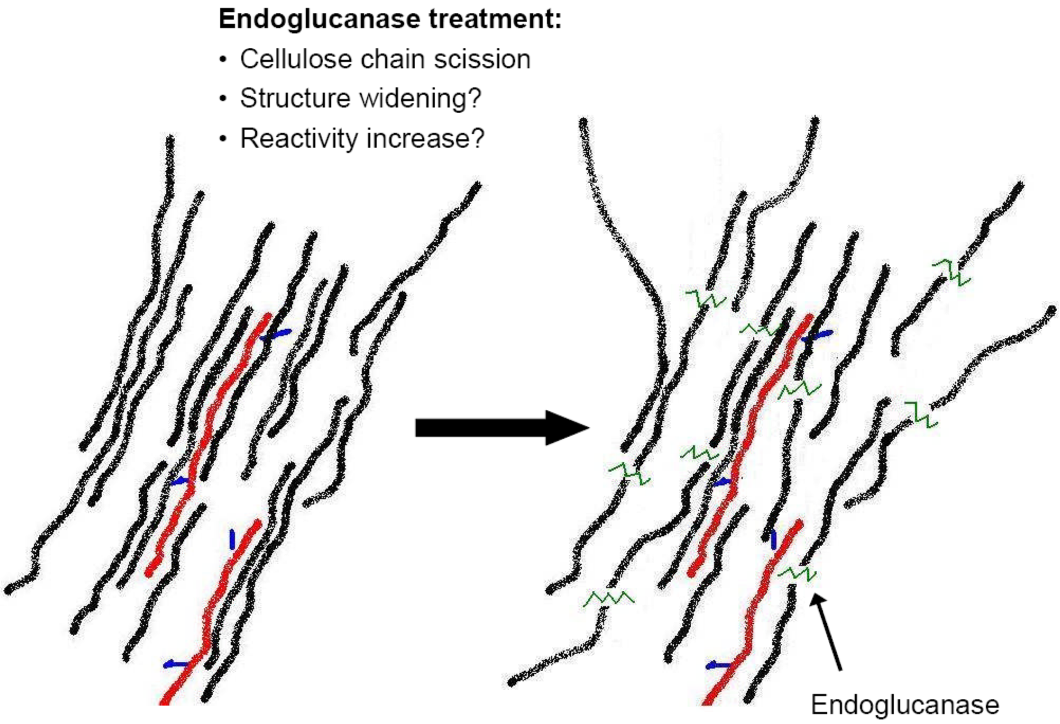


Fig. 5 Illustration of how enzyme treatment may increase pulp reactivity

1.1.6.2 Modification of Cellulose Surface

Weigert et al. (1998) demonstrated that derivatization of pulp fibers with acrylamide derivatives lowers the hornification tendency of pulps upon drying. In this case, the bulky cellulose substituents may act as spacer and show an increasing effect on pulp accessibility and reactivity as short-chain hemicelluloses in PHK pulps, as discussed before in a working hypothesis. Another approach for the formation of voluminous functional groups by modification of the cellulose molecule is TEMPO-oxidation. TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) is a water-soluble, stable nitroxyl radical. TEMPO-mediated oxidation converts the C_6 primary hydroxyl groups present on the microfibril surfaces of native cellulose to carbonyl and further to carboxyl groups under mild conditions (Hirota et al. 2009; Isogai and Kato 1998). In pulp and paper industry, the treatment of kraft pulps for increased paper strength represents an important task of TEMPO-mediated oxidation (Dang et al. 2007; Kitaoka et al. 1999; Saito and Isogai 2005). In the case of cellulosic nanofiber production, high contents of carboxyl groups are introduced into cellulosic substrates in order to increase the repulsive strength of negatively charged carboxylate groups, which promotes the separation of the microfibrils (Fujisawa et al. 2011). A similar approach can be made for reducing hornification tendency of dissolving pulps. The newly formed -COOH group of the glucose monomer unit shows increased hydrophilic character compared to the -CH_2-OH group and may act as a kind of spacer in-between the cellulose microfibrils, reducing their tendency to aggregate.

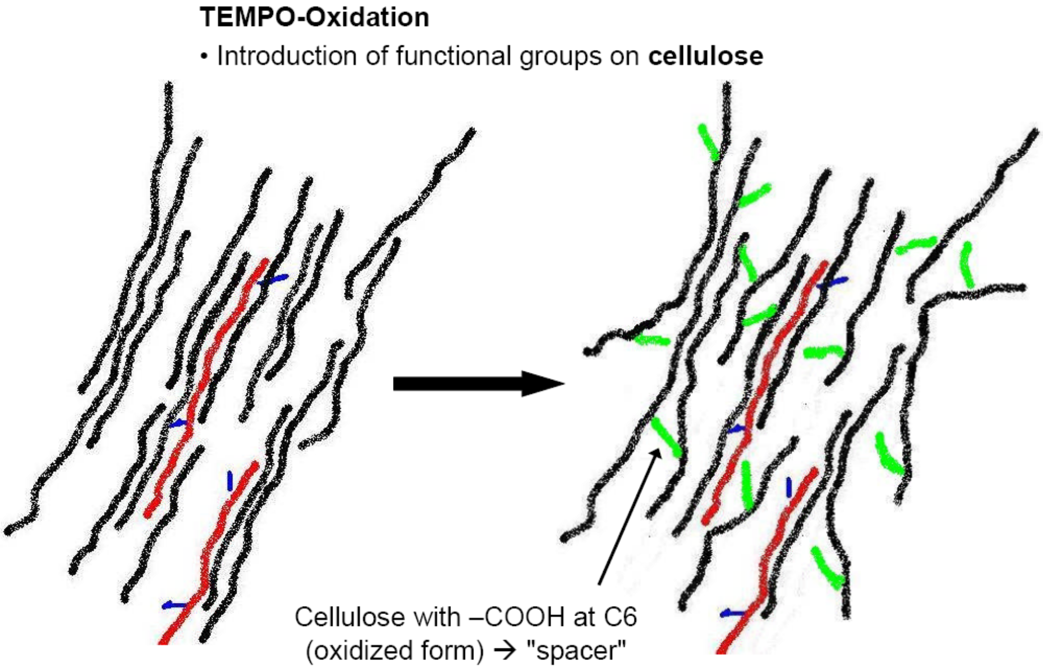


Fig. 6 Carboxyl groups introduced by TEMPO-mediated oxidation form spacer between microfibrils

1.2 Objectives of this Work

The development of efficient process steps to convert paper-grade into dissolving pulps was investigated. Therein, the challenge of pulp refinement comprised the selective removal of hemicelluloses and the precise adjustment of the pulp viscosity, while resulting in a highly reactive dissolving pulp as required for viscose application.

The effect of the utilization of enzymes for hemicellulose extraction and pulp depolymerization on the final dissolving pulp quality was investigated. Therefore, a commercial oxygen-delignified *Eucalyptus globulus* paper-grade kraft pulp was refined in the course of a TCF-bleaching sequence following the multifunctional alkaline pulping process. Xylanase treatment was adopted prior to CCE, and its influence on chemical consumption in the final bleaching process was examined. Controlled adjustment of final pulp viscosity was accomplished utilizing endoglucanase post-treatment. The pulps were characterized in terms of purity and pulp reactivity in the viscose process and compared to conventionally-manufactured dissolving pulps (Paper 1, Paper 2).

For the purpose of promoting the commercial applicability of endoglucanase, the effects of the wood source, the pulping process, and the non-cellulosic polymeric compounds present in different hardwood and softwood pulps on the efficiency of the EG-treatment were analyzed. Further, the influence of the cellulose II content of a pulp on the enzymatic degradability and the associated pulp activation were studied (Paper 3).

In order to elucidate the influence of enzyme treatments on the resulting pulp reactivity, the reactivity of enzymatically hydrolyzed pulps was compared to pulps degraded by acid-catalyzed hydrolysis (Paper 2, Paper 3).

TEMPO-mediated oxidation was used to convert the alcoholic C_6-group of the glucose monomer units in native cellulose to carboxyl groups. It was examined, whether this hydrophilic group could act as spacer in-between the cellulose microfibrils, reducing the hornification tendency upon dewatering by testing the reactivity as well as the swelling and dissolution behavior of the TEMPO-oxidized pulp (Paper 4).

2 Experimental Part

2.1 Materials

All chemicals used in the experiments and analytical procedures were commercially available and purchased from common suppliers like Merck, Sigma-Aldrich, Fluka and others.

2.1.1 Pulp Substrates

2.1.1.1 *Eucalyptus globulus* Kraft Pulps

Three different *E. globulus* paper-grade kraft pulps (Table 1) were kindly supplied by Ence, Spain.

K-ECF: Dried ECF-bleached kraft pulp from Ence, Huelva, produced from wood chips from Uruguay.

K-TCF: Dried TCF-bleached kraft pulp from Ence, Pontevedra.

K-O: Never-dried oxygen-delignified kraft pulp from Ence, Huelva, produced from wood chips from Uruguay.

2.1.1.2 Spruce Sulfite Pulps

Spruce sulfite paper-grade pulps (Table 1) after different bleaching stages in the never-dried state were kindly supplied by Lenzing AG, Lenzing, Austria. The pulps were produced at the Biocel pulp mill in Paskov, Czech Republic.

S-15: Unbleached brownstock sulfite pulp, residual kappa number ~ 15

S-5: Partly bleached sulfite pulp, residual kappa number ~ 5

S-1: ECF-bleached sulfite pulp, residual kappa number ~ 1

2.1.1.3 Cotton Linters Pulp

CLP: Dried cotton linters pulp (Table 1)

Table 1 Pulp specifications

Pulp properties	**K-ECF**	**K-TCF**	**K-O**	**S-15**	**S-5**	**S-1**	**CLP**
Kappa	0.5	6.0	10.3	14.8	5.0	1.2	0.1
Brightness (%)	91.7	90.1	58.8	62.2	71.1	87.6	82.0
[η] (mL g^{-1})	830	815	895	1165	1080	795	405
R10 (%)	90.1	87.9	86.2	85.5	86.1	86.3	96.7
R18 (%)	92.9	91.5	89.5	86.9	87.6	88.4	98.5
DCM-Extractives (%)	0.06	0.06	n.d.	0.58	0.16	0.08	0.03
Glucan (% odp)	72.1	79.3	70.0	79.7	84.3	87.1	96.5
Xylan (% odp)	22.1	19.1	22.5	3.6	3.9	3.5	0.6
Mannan (% odp)	0.1	0	0	6.9	6.6	6.1	0
Mn (kg $mole^{-1}$)	49.2	61.4	33.4	n.d.	n.d	n.d.	72.2
Mw (kg $mole^{-1}$)	366	336	416	n.d.	n.d.	n.d.	146
Mz (kg $mole^{-1}$)	1037	892	1288	n.d.	n.d.	n.d.	230
PDI	7.4	5.5	12.5	n.d.	n.d.	n.d.	2.0

2.1.2 Enzyme Preparations

The enzyme preparations were kindly supplied by Novozymes, Denmark. The xylanase preparation Pulpzyme® HC is produced by submerged fermentation of a genetically modified *Bacillus licheniformis* microorganism and its concentration is standardized by the manufacturer to 1000 AXU g^{-1} (active xylanase units). The monocomponent endoglucanase preparation Novozym® 476 is produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and its concentration is standardized by the manufacturer to 4500 ECU g^{-1} (endocellulase units). The mannanase preparation Mannaway® is produced by submerged fermentation of a genetically modified *Bacillus licheniformis* microorganism and its concentration is standardized by the manufacturer to 4 MIUM g^{-1}.

2.2 Analysis

2.2.1 Standard Analytical Methods

Kappa number was determined according to TAPPI T 236 cm-85 (1993a), brightness according to ISO 2470-1 (2009), alkali resistance in 10 wt % (R10) and 18 wt % (R18) NaOH solutions, respectively, according to DIN 54355 (1977b). Intrinsic viscosity [η] was measured according to SCAN-CM 15:99 (1999) and dichloromethane (DCM)-extractives according to DIN 54354 (1977a). The degree of polymerization DPv was calculated from the intrinsic viscosity according to Marx-Figini (1978). Carbohydrate content was measured after a two stage total hydrolysis by high performance anion exchange chromatography with pulsed amperometric detection (Sixta et al. 2001). Carboxyl group content (COOH) with methylene blue was determined according to Philipp et al. (1965) and beta-cellulose referring to TAPPI T 203 om-93 (1993b). Water retention value (WRV) was measured according to Zellcheming Merkblatt IV/33/57 (1957). The WRV is determined as the weight gain of a sample after swelling in water and centrifugation, and high values indicate a highly hydrophilic accessible surface. Total bound nitrogen was determined according to DIN 38409 (1992). Molecular weight distribution (MWD) was measured by size exclusion chromatography (SEC) with multi-angle light scattering detection (MALLS) in LiCl/DMAc solution according to Schelosky et al.(1999).

2.2.2 FT-Raman

The degree of crystallinity (CrI) and the cellulose II content (Cell II) were determined with Fourier transform Raman spectroscopy (FT-Raman) (Röder et al. 2006; Ruland 1961). FT-Raman measurements were done using a Bruker IFS66 with Raman module FRA106, Nd:YAG Laser 500 mW; Laser wavenumber 9394 cm^{-1} (1064 nm), liq. N_2 cooled Ge-Detektor, 3500-100 cm^{-1}, resolution 4 cm^{-1}, 100 scans, 4 measurements of each sample. The sample of 300 mg was pressed with 6 t cm^{-1} for approximately 10 min. Analysis was performed with a chemometric model, wide angle X-ray scattering (WAXS) data were used as a standard for calibration and comparison.

2.2.3 WAXS Analysis

WAXS measurements were performed for the determination of the degree of crystallinity (CrI), the cellulose II content (Cell II) and the crystal size of pulps. The crystal size can be determined from the distances $D_{(hkl)}$ between parallel atomic layers of lattice planes that are defined with the Miller indices *hkl* for a given crystal system like the monoclinic system for cellulose (Will 1978). For WAXS measurements, air-dried pulp samples were suspended in liquid N_2 and grinded in a centrifugal mill (ZM100, Fa. Retsch), sieve size 1 mm. The isotropic powder was dissected between PET-foils and sealed air-tight with silicon. The WAXS measurements were conducted with a D5000 two-circle diffractometer (Fa. Bruker-AXS) in symmetrical transmission geometry with a focusing Ge(111) primary monochromator with Cu-Kα-beam ($\lambda = 0.15406$ nm) at 30 mA and 40 kV. The diffractogram was recorded in a 2θ-angle range of 4°-104° (interval $\Delta 2\theta = 0.2°$, recording time / $\Delta 2\theta$: 55 s). The sample was rotated perpendicularly at 15 rpm and each sample was measured three times. The results were analyzed with the IAP-software WAXS7.

2.2.4 Specific Surface Area (BET-Surface)

The specific surface area $a_{s,\,BET}$ according to Brunauer, Emmett and Teller is defined as the accessible area of solid surface per unit mass of material (Barrett et al. 1951; Brunauer et al. 1938). This specific surface area is calculated from the BET-isotherm, which is gained from a model for physical adsorption describing multilayer formation of gaseous particles on a solid surface (Atkins 1996). The measurements of $a_{s,\,BET}$ were conducted by a volumetric method with a Belsorp mini II using N_2 gas.

2.2.5 Carbonyl Group and Carboxyl Group Determination with Fluorescence Labeling

The carboxyl group content was determined with FDAM/SEC analysis, where the carboxyl groups are labeled with the diazomethane derivative FDAM, carried out as off-line heterogeneous derivatization, and converted into fluorescent fluoren-2-yl methyl esters. The carboxyl groups are quantified with SEC measurement employing refractive index, MALLS, and fluorescence detection (Bohrn et al. 2006). The carbonyl group content (CO) was measured by fluorescence labeling with CCOA and SEC according to Röhrling et al. (2002a; 2002b).

2.2.6 FE-SEM

Pulp preparation for field emission scanning electron microscopy (FE-SEM) was performed according to Duchesne et al. (2001) and Okamoto and Meshitsuka (2010). The pulp samples were freeze-dried from a water suspension, mounted on a stub with a double-sided adhesive tape and fully degassed in a vacuum oven at 60 °C for 1 h at 200 mbar. Finally the samples were coated with a thin layer of Au/Pd. The samples were examined by high-resolution scanning electron microscopy up to a 30.000 × magnification with a Hitachi S4000 FE-SEM using an acceleration voltage of 6 kV and a working distance of 8 mm.

2.2.7 Pulp Reactivity Measurements

The pulp reactivity was determined according to two different lab-scale viscose processes. Reactivity was measured according to the Fock-method (Fock 1959), and following a modified protocol by Treiber et al. (1962) as described by Hüpfl and Zauner (1966) for viscose preparation and characterization in terms of particle content and filterability, respectively.

The Fock-method imitates the viscose process without the ageing step on lab-scale. A viscose-like solution is prepared from an alkaline pulp suspension and carbon disulfide (Fig. 7). Cellulose is regenerated from this solution by acidification and quantified via oxidation and titration. The reactivity according to the Fock-test describes the amount of pulp (wt %) which was dissolved in the viscose-like solution. Consequently, high values obtained from the Fock-test indicate high pulp reactivity but might be misleading because the result also includes the amount of alkali soluble hemicelluloses.

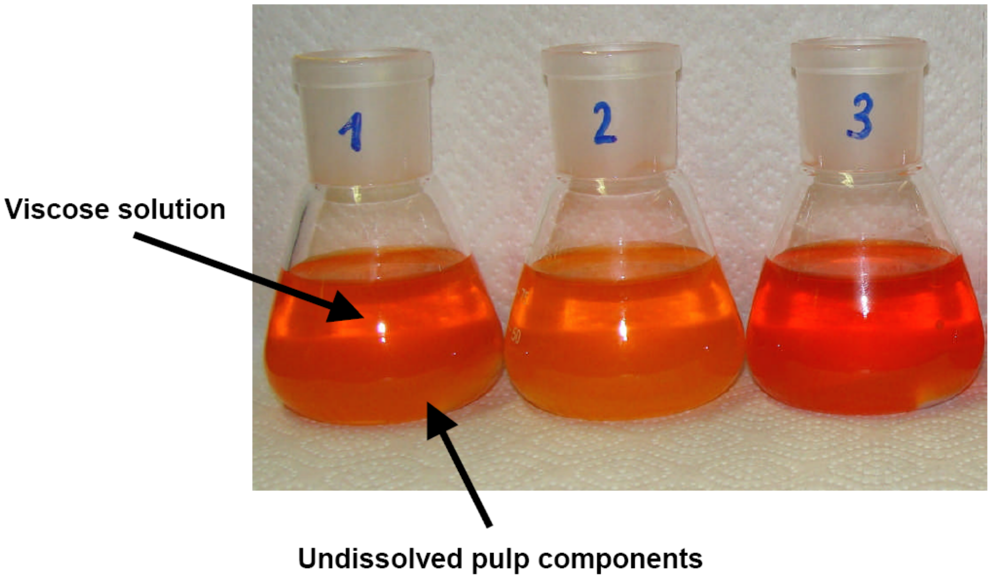


Fig. 7 Viscose-like solution obtained from the Fock-test

In contrast to the Fock-test, the Treiber-method mimics the entire industrial viscose process. The viscose process comprises the steps of pulp steeping, ageing, derivatization with carbon disulfide, and dissolution of the xanthate in alkaline solution (Fig. 8). Fibers are spun from the spinning dope after filtration and ripening. In the lab scale Treiber-method, the quality of the viscose solution is evaluated by its filterability (filter value) in a well-defined filter bed (deep filtration) and its particle content. In this way, a reliable prediction of the applicability of a pulp for a commercial viscose process is obtained.

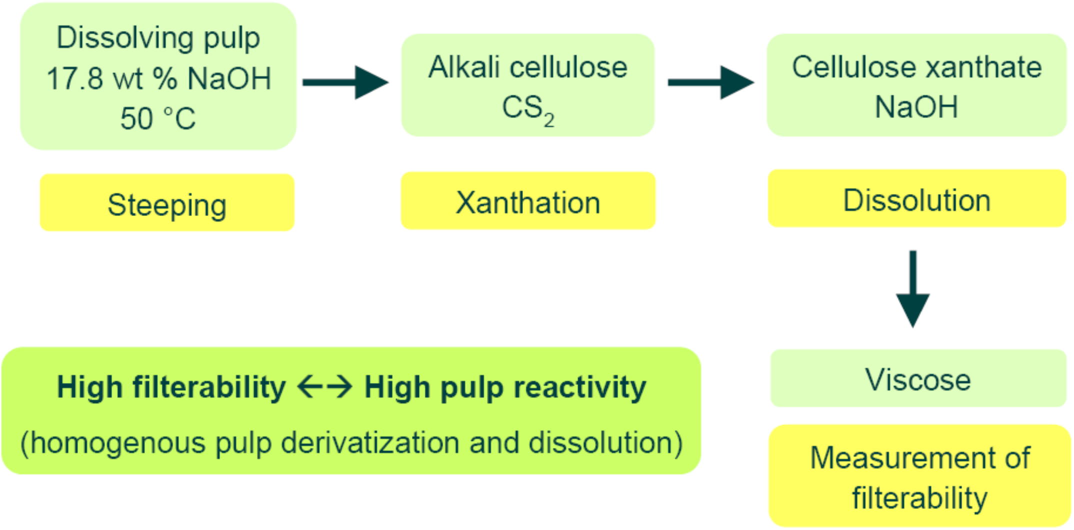


Fig. 8 Scheme of the Treiber-method following the industrial viscose process

The filter value is calculated from the permeated mass of viscose after 100 min and 150 min of filtration at 20 °C and 2 bar pressure (Fig. 9) and thus high filter values are interpreted as high pulp reactivity. Particles in a range of 3 to 155 µm were measured on a Pamas device operating according to the light blockade principle.

Fig. 9 Filtration device for measuring the filter value following the Treiber-method; the picture was kindly supplied by Lenzing AG

2.3 Pulp Treatments Following the Multifunctional Alkaline Pulping Process

The effects of various enzyme treatments on the final dissolving pulp quality were investigated. A commercial oxygen-delignified *Eucalyptus globulus* paper-grade kraft pulp was refined in the course of a TCF-bleaching sequence following the principle of the modified paper pulp production. Xylanase pretreatment was adopted to achieve the applicability of the final pulp for the viscose process besides the reduction of chemical consumption in alkaline and ozone bleaching steps. Controlled adjustment of final pulp viscosity was accomplished utilizing endoglucanase post-treatment. The effects of xylanase pretreatment on the efficiency of CCE in xylan removal, and on the MWD of the pulp after CCE and of the solubilized xylan fraction were examined. Furthermore, the effects of xylanase pretreatment on the final bleaching performance, the process of chain scission during subsequent endoglucanase treatment as well as the final quality and viscose reactivity of the pulp were investigated.

2.3.1 Enzymatic Hydrolysis of Hemicelluloses from a Paper-grade Kraft Pulp

Xylan removal from wood- and non-wood-pulps with the help of enzymes and in combination with CCE has been reported manifoldly (Bajpai and Bajpai 2001; Ibarra et al. 2010b; Jackson et al. 1998; Köpcke et al. 2008; Köpcke et al. 2010). Xylanase acts on the accessible surface of microfibrils and removes surface xylan, which is re-deposited during the final stage of kraft cooking at decreased alkalinity. Besides the main purpose of xylan removal, xylanase pretreatment prior to elemental chlorine free (ECF)- or total chlorine free (TCF)-bleaching is a promising technique to reduce bleaching chemical consumption. This aspect of xylanase pretreatment has been reported as a “bleach boosting” effect and allows the removal of xylan from lignin-carbohydrate-complexes, thereby allowing the enhanced leaching of entrapped lignin subsequently (Bhat 2000). This effect is seen from the perspective of significant brightness gain and kappa number reduction during xylanase treatment (Allison and Clark 1994; Ates et al. 2009; Brown et al. 1994; Shatalov and Pereira 2008a; Shatalov and Pereira 2008b; Yang et al. 1993). Valchev and Tsekova (2008) adopted the step of xylanase post-treatment to the bleaching processes of pulps in order to remove chromophoric structures connected to the accessible surface xylan. Besides pretreatment of pulp, xylanase pretreatment was also adapted to eucalypt wood chips and was reported to result in increased pulp yields in kraft cooking for a given kappa number compared to kraft cooks of non-pretreated wood chips (Bertaud et al. 2008).

In the described work, xylanase (X)-treatment of the paper-grade kraft pulp K-O and the washing procedure for enzyme inactivation were performed according to Köpcke et al. (2008). X-treatment was conducted at 3 % consistency in a phosphate buffer pH 7 (11 mM NaH_2PO_4, 9 mM Na_2HPO_4; deionised water) at 60 °C for 120 min at an enzyme concentration of 500 AXU g^{-1} odp. In order to reach a homogeneous enzyme distribution, the enzyme substrate was mixed with the buffer solution prior to addition to the pulp. Enzyme treatment was conducted in polyethylene flasks, being shaken strongly every 30 min. In order to stop the enzyme treatment and remove the enzyme solution, the pulp was filtered with a commercial suction drainer of pore size 2 and was quickly washed with hot deionised water (90 °C). The pulp cake was put back into the flask and hot deionised water was added (consistency 3 %) for inactivation of the residual enzymes. The flask was stored in a 90 °C water bath for 30 min for complete enzyme inactivation. Subsequently, the pulp was filtered, washed with cold deionised water and drained to a dry content of about 25-30 %.

2.3.2 Alkaline Extraction of Hemicelluloses from a Paper-grade Kraft Pulp

Alkaline post-extraction of hemicelluloses at temperatures from 25 to 45 °C, called cold caustic extraction (CCE), represents an effective method for dissolution of hemicelluloses, especially xylan (Jayme and Roffael 1969; Wallis and Wearne 1990). During CCE-purification, physical phenomena are dominating the extraction of hemicelluloses and almost no cellulose degradation occurs because peeling reactions are very slow at temperatures applied during CCE (Glaus and van Loon 2008). Intermicellar and intramicellar swelling permit short-chain celluloses and especially xylan to dissolve (Sixta 2006b). Mannan instead, shows a highly ordered structure compared to xylan, initiated through co-crystallization with cellulose (Annergren and Rydholm 1959) and thus is resistant to physical dissolution under the conditions of CCE. Thus, CCE is more effectively used for treatment of hardwood pulps compared to pulps from softwood.

In this thesis, CCE of the eucalypt paper-grade kraft pulp K-O was conducted at a NaOH concentration of 100 g L^{-1} (CCE100). CCE of the X-pretreated pulp K-O-X was performed at an alkali concentration of 70 g NaOH L^{-1} (CCE70). CCE-treatments were conducted in polyethylene flasks (Wallis and Wearne 1990) at 30 °C for 30 min at 10 % consistency.

2.3.3 TCF-Bleaching

The following eucalypt kraft pulps with reduced hemicellulose content

- K-O-X-CCE70
- K-O-CCE100

were subjected to a full TCF-bleaching sequence (A-Z-P).

Hot acid treatment (A) was conducted for removal of hexenuronic acid side chains at pH 3.5 adjusted with sulfuric acid and was performed at 90 °C for 60 min in polyethylene flasks at 10 % consistency according to Vuorinen et al. (1999).

Final pulp bleaching comprised an ozone (Z) stage followed by hydrogen peroxide bleaching (P). Ozone bleaching was carried out in a medium-consistency high-shear mixer at 50 °C for 10 s at pH 2.5 and 10 % consistency with an ozone consumption of 3.3 kg t^{-1} odp with X-pretreatment and 2.9 kg t^{-1} odp without X-pretreatment.

P-bleaching was conducted at standard conditions applying 10 kg NaOH t^{-1} odp, 8 kg H_2O_2 t^{-1} odp and 1 kg $MgSO_4.7H_2O$ t^{-1} odp at 80 °C for 120 min at 10 % consistency. The final bleached pulps were acidified in deionised water with diluted sulfuric acid prior to testing and analytical characterization.

2.3.4 Enzymatic Depolymerization with Endoglucanase

DP-adjustment of the following bleached eucalypt kraft pulps

- K-O-X-CCE70-TCF
- K-O-CCE100-TCF

was accomplished with endoglucanase as the final treatment step aiming at about 450 mL g^{-1} (DPv = 1050), the target viscosity for viscose staple fiber kraft pulps.

EG-treatment was conducted at 3 % consistency in a phosphate buffer pH 7 (11 mM NaH_2PO_4, 9 mM Na_2HPO_4; deionised water) at 50 °C for 60 min at an enzyme concentration of 20 ECU g^{-1} odp for the X-treated pulp and at an enzyme concentration of 15 ECU g^{-1} odp for the reference pulp. The washing procedure was performed as described previously for the xylanase treatment (2.3.1).

2.3.4.1 Quantification of Enzyme Removal from the Pulp

Cellulase enzymes must bind to the surface of insoluble carbohydrates prior to hydrolysis, which is demonstrated by an increased rate of cellulose hydrolysis initiated by enzymes containing a cellulose binding domain (Zhang and Lynd 2004). Enzymes must be removed from the pulp completely after depolymerization for economic aspects regarding the reutilization of the enzyme preparation, and the quality and skin compatibility of the final fiber product. Recently, Mollerup (2012) investigated different methods for enzyme removal and deactivation, such as cooling to 4 °C and washing of the pulp in a centrifuge, or filtration with hot water. Both methods resulted in residual enzyme activity on the pulp as determined on carboxymethyl cellulose with color assays using dinitrosalicylic acid measuring reducing end groups of carbohydrates. Only when a step of hot incubation was performed after hot filtration, no residual enzyme activity was found on the pulp.

Thus within this thesis, the enzymes were removed from the pulp by a multi-step treatment. At first, the pulp was filtrated over a suction drainer in order to receive the filtrate of the depolymerization treatment with the active enzymes simulating recycling purposes in industrial scale. Subsequently, the pulp was washed with hot water (> 90 °C) in order to denaturize the remaining enzymes on the pulp and stop further enzymatic hydrolysis reactions. Afterwards, the pulp was resuspended in hot water, placed in a 90 °C water bath for 30 min for complete enzyme denaturation and washed again in a suction drainer with cold water.

Different attempts of analyses were taken for ensuring complete removal of enzymes from the pulp. On the one hand, analytical methods for complete recovery of the enzymes in the filtrate and washing filtrate were investigated. On the other hand, detection of residual enzymes on the pulp was examined.

Nitrogen determination according to DIN 38409 (1992) was performed on the filtrate and the washing filtrate aiming at complete nitrogen recovery from the peptide amine bonds. The aqueous samples were decomposed at 700 °C under oxygen atmosphere and quantitatively converted into nitrogen monoxide NO, which was detected via chemiluminescence. With this method, the nitrogen from the enzyme preparation that was brought into the pulp suspension was completely recovered in the filtrate and the washing filtrate. As shown in Fig. 10, for EG-treatments with 250 and 500 ECU g^{-1} odp, respectively, about 50 wt % of all nitrogen was found in the filtrate and 50 wt % in the washing filtrate. The nitrogen concentration in the filtrate was only about 65 % of the concentration in the suspension during the treatment, which indicates enzymes remaining sticking on the pulp during drainage. Thus, an intense washing procedure was applied using about 5-6 times the amount of washing water compared to the filtrate volume (Fig. 10), and all enzymes could be removed from the pulp.

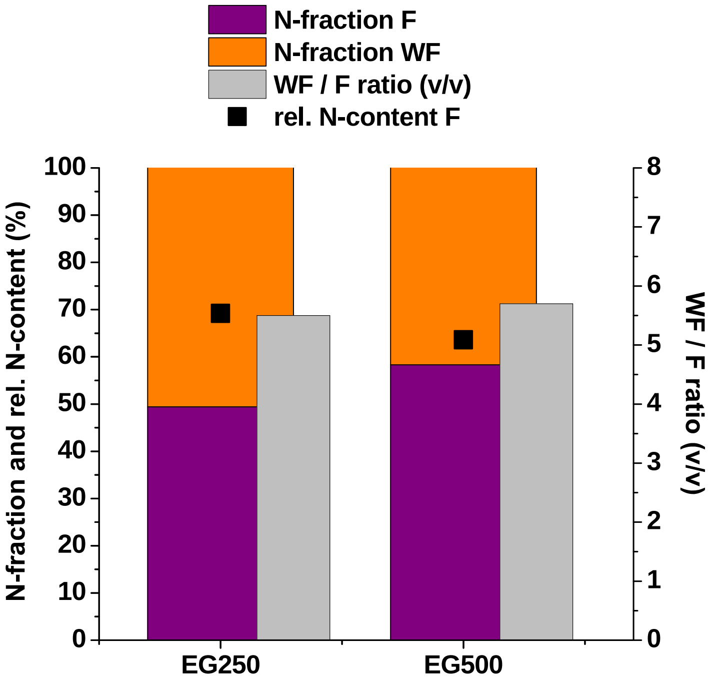


Fig. 10 Nitrogen fraction recovered in the filtrate (F) and the washing filtrate (WF), relative nitrogen content of the filtrate, and volumetric ratio of the washing filtrate and the filtrate

Alternatively, the recovery of the enzyme in the filtrate and the washing filtrate was tested with the Bradford-assay. This assay is a method for protein determination that involves the binding of a color reagent (Coomassie Brilliant Blue G 250) to a protein. The binding of the dye to the protein causes a shift in the absorption maximum of the dye from 465 to 595 nm, and it is the increase in absorption at 595 nm that is monitored (Bradford 1976). This detection method failed as the enzyme was to highly dilute in the filtrate and the washing filtrate of the depolymerization treatment.

The detection of residual enzymes sticking on the pulp was based also on the analyses of nitrogen on the pulp deriving from the amide bonds of the enzymes. The applied detection method accomplished burning of the pulp under oxygen atmosphere at 950 °C, subsequent reduction to elemental nitrogen N_2 with copper catalysis in a helium flow at 750 °C, and adsorption of other gaseous molecules. The N_2 content was determined via heat conductivity but the results were clearly below the detection limit of the nitrogen-analyzer apparatus (250 ppm).

2.4 Pulp Properties and their Influence on Enzymatic Degradability

For the purpose of promoting the commercial applicability of endoglucanase, the effects of the wood source, the pulping process, and the non-cellulosic polymeric compounds on the efficiency of the EG-treatment were analyzed. Therefore, hemicelluloses were removed from a spruce paper-grade sulfite pulp by alkaline extraction methods and hemicellulase treatments, respectively. The pulps with reduced hemicellulose content were subjected to EG-depolymerization and compared to eucalypt kraft pulps. Additionally, the effects of the cellulose II content and the residual lignin content on the enzymatic degradability of pulps were studied utilizing cotton linters pulps and spruce paper-grade sulfite pulps, respectively.

2.4.1 Hemicellulose Removal from a Sulfite Pulp

Short-chain carbohydrates can be removed from pulp also with alkali at higher temperatures (80 °C), in contrast to CCE, using weaker alkaline solutions. In hot caustic extraction (HCE), the removal of hemicelluloses is mainly governed by chemical degradation reactions involving endwise depolymerization reactions, called peeling reactions. The peeling reaction becomes the dominant pathway for the degradation of pulp carbohydrates with increasing temperature (Sixta 2006b). During HCE, large amounts of mannan are removed from the pulp. Xylan instead, is chemically stabilized because of uronic acid side chains which initiate stopping reactions (Sjöström 1993).

Hemicelluloses from the spruce paper-grade sulfite pulp S-5 were removed by HCE, CCE, X-treatment and M-treatment, respectively. In order to reduce the residual content of both main hemicellulose types of the spruce pulp, mannan and xylan, HCE and CCE were also performed as subsequent treatment steps. HCE is known to form chromophoric structures from carbohydrate degradation products on the cellulose surface (Forsskåhl et al. 1976) and a decrease in brightness upon HCE was expected. Thus, the partly bleached pulp S-5 with a residual kappa number ~ 5 was chosen for these experiments. In the case of this pulp, a post-bleaching stage after HCE is unavoidable and would be necessary also under process conditions.

HCE of the spruce paper-grade sulfite pulp S-5 was conducted in a medium-consistency high-shear mixer at 110 °C for 60 min at 10 % consistency and 3 bar relative

N_2-pressure with 4 wt % and 8 wt % NaOH odp, respectively (HCE_4% and HCE_8%). CCE of the pulps S-5 and S-5-HCE_4% was carried out in the same mixer at 30 °C for 30 min at 10 % consistency and 3 bar relative N_2-pressure with 80 g NaOH L^{-1} (CCE80). X-treatment was conducted at 3 % consistency in a phosphate buffer pH 7 (11 mM NaH_2PO_4, 9 mM Na_2HPO_4; deionised water) at 60 °C for 120 min at an enzyme concentration of 500 AXU g^{-1} odp. Mannanse (M)-treatment was performed at 3 % consistency in a phosphate buffer pH 8 (0.05 M KH_2PO_4, 0.05 M NaOH; deionised water) at 45 °C for 170 min at 10 MIUM g^{-1} odp. The resulting pulp substrates and the pulps S-15, S-5 and S-1 with different residual lignin contents were subjected to enzymatic hydrolysis by EG at different enzyme concentrations.

2.4.2 Influence of Cellulose Morphology on Enzymatic Degradability

The influence of the cellulose II content of a pulp on the enzymatic degradability was studied. Owing to the lack of non-cellulosic impurities, CLP was steeped at stepwise increasing alkali concentrations to adjust different cellulose II contents prior to EG-treatment, as confirmed by WAXS measurements (Fig. 11). Steeping of CLP was conducted with a steeping ratio (mass-ratio odp to steeping lye) of 1:18 at varying alkali concentrations in the steeping lye from 11.9-17.8 wt % NaOH at 50 °C for 15 min. Subsequently, the pulp was filtrated and pressed to a dry content of ~ 35 %. The filter cake was resuspended in deionised water and acidified to neutral conditions with acetic acid and phenolphthalein as color indicator. Finally, the pulp was filtered again and washed thoroughly. The resulting pulp substrates were subjected to enzymatic hydrolysis by EG at different enzyme concentrations.

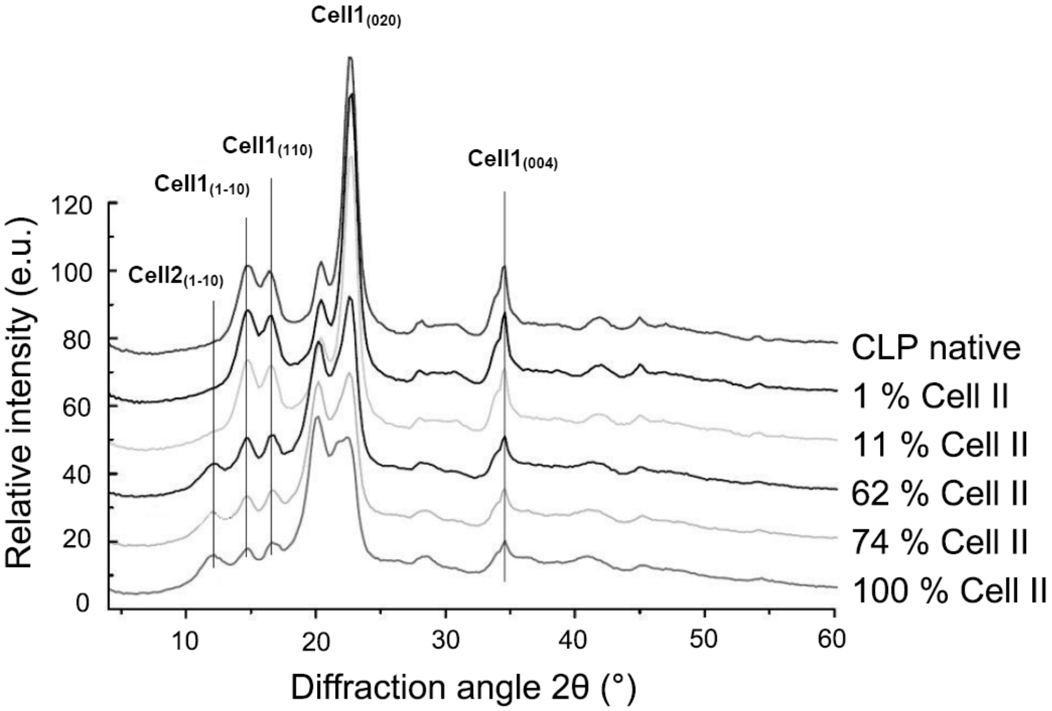


Fig. 11 WAXS diffractogram of differently alkalized cotton linters pulps

2.5 The Effect of Enzymatic Depolymerization on Pulp Reactivity

In order to elucidate the influence of enzyme treatments on the resulting pulp reactivity of sulfite as well as kraft pulps, the reactivity of enzymatically hydrolyzed pulps was compared to pulps degraded by acid-catalyzed hydrolysis.

2.5.1 Acid-catalyzed Hydrolysis of a Paper-grade Kraft Pulp

The eucalypt paper-grade kraft pulp K-TCF was subjected to the following treatment sequence:

- K-TCF-CCE100-P-$A_{Hydr.}$

Hemicelluloses from the kraft pulp K-TCF were removed by CCE100, performed with 100 g NaOH L^{-1} at 30 °C for 30 min at 10 % consistency in a polyethylene flask. P-bleaching was performed at 70 °C for 120 min at 10 % consistency applying 5 kg NaOH t^{-1} odp, 4 kg H_2O_2 t^{-1} odp and 1 kg $MgSO_4.7H_2O$ t^{-1} odp. Acid-catalyzed hydrolysis ($A_{Hydr.}$) of the pulp was performed at 90 °C for 60 min at 3 % consistency at varying pH values from 1-3 (target intrinsic viscosity [η] ~ 410 mL g^{-1}) (Hakansson and Ahlgren 2005; Vehviläinen et al. 2010). The final treated pulp was compared to EG-treated pulps (see 2.3) in terms of pulp reactivity determined by the Fock-test.

2.5.2 Acid-catalyzed Hydrolysis of a Paper-grade Sulfite Pulp

In order to examine the effect of different degradation methods on pulp reactivity also for sulfite pulps, the sulfite pulp S-1 was also degraded to a preset pulp viscosity (~ 600 mL g^{-1}) by $A_{Hydr.}$ at pH 1.5 adjusted with sulfuric acid at 90 °C for varying periods of 30-180 min at 3 % consistency and compared to the EG-treated pulp S-1 (see 2.4.1).

2.6 TEMPO-mediated Oxidation of a Dissolving Pulp

TEMPO-mediated oxidation at neutral pH conditions was applied to the paper-grade kraft pulp K-ECF in order to oxidize the C_6 primary hydroxyl groups present on the microfibril surface of native cellulose to carboxyl groups (Hirota et al. 2009). Cellulose oxidation with the system TEMPO/NaOCl/$NaClO_2$ was applied in the process of refining the pulp K-ECF prior to and subsequently to CCE for utilization as dissolving pulp. In this oxidation system, $NaClO_2$ acts as the primary oxidant (Fig. 12). The effect of the voluminous carboxyl groups on preserving the accessibility of the cellulose surface by reducing the aggregation of microfibrils was investigated.

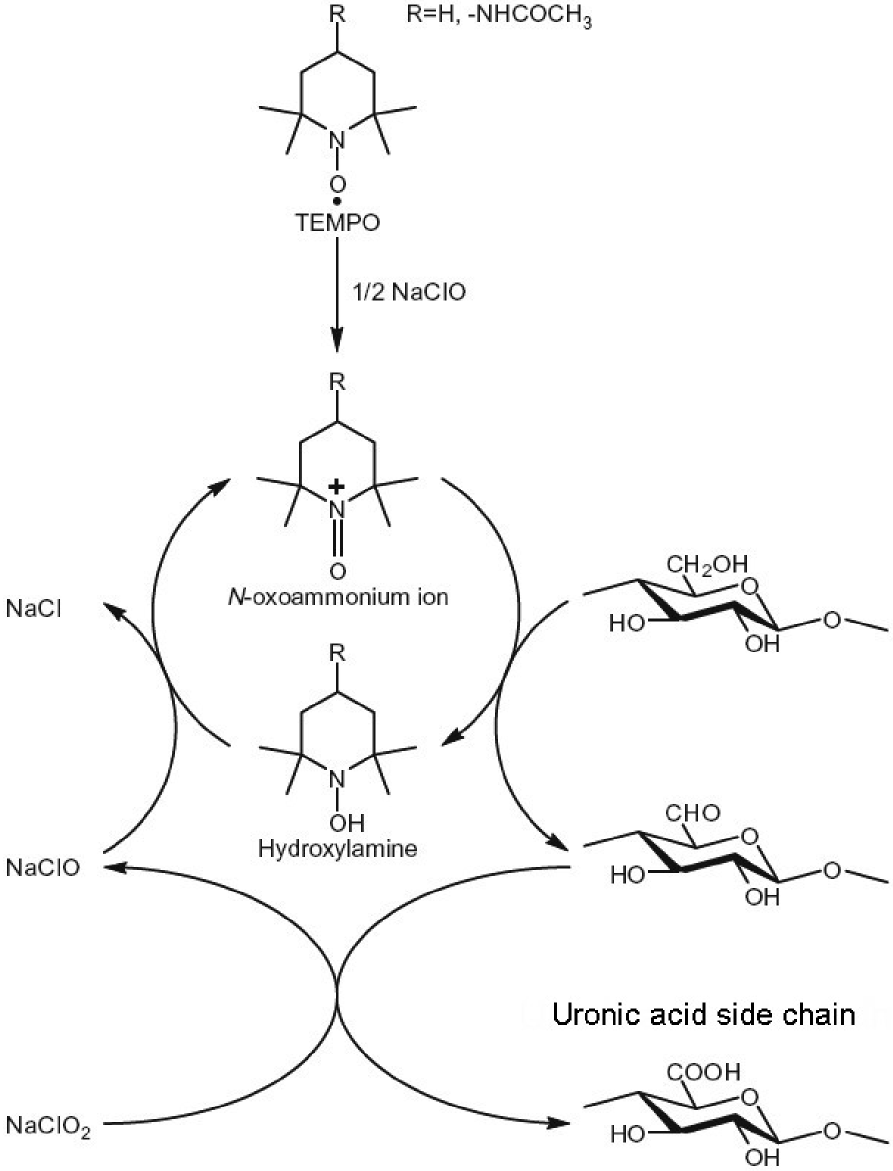


Fig. 12 Oxidation of primary hydroxyls to carboxyls by the TEMPO/NaOCl/NaClO$_2$ system under weakly acidic or neutral conditions (Hirota et al. 2009)

2.6.1 Pretrials of TEMPO-mediated Oxidation

Oxidation reactions were carried out at 60 °C in PE-bottles in a shaking water bath. The pulp was preheated in the bottles in the water bath prior to addition of the reagents. The oxidation was accomplished at 1.5 % consistency in 0.05 M phosphate buffer of pH 6.8 (prepared from 0.05 M $Na_2HPO_4.2H_2O$ and HCl conc.). The buffer was heated to 60 °C in a beaker, and TEMPO (156.25 g mole^{-1}; 0.1 mmole g^{-1} odp) and NaClO$_2$ (7.5 mmole g^{-1} odp) were dissolved in the buffer. The solution was added to the pulp and the bottle was strongly shaken. NaOCl (0.75 mmole g^{-1} odp) was used as

1 wt % solution diluted with phosphate buffer and was immediately added to the pulp suspension. The bottles were stored in the shaking water bath (130 rpm) for reaction times of 3-48 h (Fig. 13).

Fig. 13 TEMPO-mediated oxidation of pulps performed in PE-bottles stored in a shaking water bath

After the reaction time, ethanol (20 mL g^{-1} odp) was added to the pulp suspension for quenching the reaction (Hirota et al. 2009). The pulp was filtered with a suction drainer of pore size 2, washed thoroughly with deionised water and put back into the PE-bottle. Post-oxidation ensuring the complete conversion of carbonyl groups to carboxyl groups was performed with $NaClO_2$ (3 mmole g^{-1} odp) in 0.1 M acetate buffer of pH 4.5 (50 mL g^{-1} odp) prepared from 0.1 M acetic acid and 0.1 M sodium acetate solution. The sodium chlorite solution was added to the pulp and shaken for 48 h at room temperature. Finally, the pulp was filtered again with a suction drainer of pore size 2 and washed with hot deionised water. The pulp cake was resuspended in deionised water and drained again three times.

2.6.2 TEMPO-mediated Oxidation and CCE

TEMPO-mediated oxidation of the kraft paper pulp was performed prior to and subsequently to CCE, respectively. In both cases, CCE was conducted for hemicellulose removal at an alkali concentration of 100 g NaOH L^{-1} (CCE100), 30 °C, and 30 min at

10 % consistency in PE-bottles. Oxidation reactions were carried out as described before (see 2.6.1) for 48 h reaction time. After post-oxidation with $NaClO_2$ and washing with deionised water, the pulp was resuspended in deionised water and acidified to a pH < 3 with sulfuric acid. The suspension was stirred at room temperature for 10 min. Subsequently, the pulp was filtered, washed with hot deionised water and drained to a dry content of about 30 %. A reference pulp without TEMPO-mediated oxidation was prepared from the CCE100-treated kraft pulp with acid catalyzed hydrolysis ($A_{Hydr.}$) and a final alkaline peroxide (P) bleaching step. $A_{Hydr.}$ was performed for cellulose depolymerization at pH 2 adjusted with sulfuric acid at 90 °C for 60 min at 3 % consistency (Hakansson and Ahlgren 2005; Vehviläinen et al. 2010). P-bleaching was conducted at standard conditions applying 6 kg NaOH t^{-1} odp, 5 kg H_2O_2 t^{-1} odp and 1 kg $MgSO_4.7H_2O$ t^{-1} odp at 70 °C for 120 min at 10 % consistency. For reactivity measurements, bigger pulp charges were prepared separately according to the described protocol.

2.6.3 Viscose Preparation from a Direct-xanthation-process

The TEMPO-oxidized pulps could not be converted into viscose according to the viscose process at laboratory scale following the procedure of the Treiber test (Hüpfl and Zauner 1966; Treiber et al. 1962). Due to the enhanced hydrophilic character of the oxidized pulps, the alkali cellulose after steeping could not be dewatered to the required cellulose content of ~ 35 wt %, which made it impossible to convert the pulps into viscose. Alternatively, viscose was prepared as described by Götze (1967) in a direct-xanthation-process in a batch reactor. Therefore, the TEMPO-oxidized pulps were also used in the wet state in order to avoid hornification. For a scale of 100 g viscose, wet pulp (5 wt % α-cellulose in viscose) was treated in a 250 mL steel reactor with alkali used as 100 g L^{-1} solution (5 wt % alkali in viscose, meaning an alkali-to-cellulose ratio of 1) and Berol 388 (0.2 wt % on α-cellulose) as surfactant. Steeping was conducted under manual shaking for 5 min. Afterwards, the steel reactor was evacuated for 5 min and CS_2 (40 wt % on α-cellulose) was added with a syringe by a frit. Xanthation was continued for 2.5 h in a rotating water bath at 30 °C. After completed reaction, the residual amount of cold water was added and the cellulose xanthate got dissolved in the cold (fridge) under shaking.

2.6.4 Swelling and Dissolution Behavior of the Oxidized Pulps Observed with Optical Microscopy

The swelling and dissolution behavior of the TEMPO-oxidized pulps was investigated in NMMO-water (*N*-methylmorpholine-*N*-oxide) and Cuen-solution, respectively.

For all experiments, pulp fibers from an aqueous suspension were dissected between two glass plates and air-dried over night. The dissolution and swelling behavior of the pulp samples was observed by transmission optical microscopy with an Olympus BH2 microscope equipped with an OSIS Color View 2 camera with an Olympus Soft Imaging system. For dissolution experiments in NMMO, the camera was additionally equipped with a Linkam CO 102 heating device. The pulp samples were preheated to 90 °C on the sample table. 87 wt % NMMO (NMMO.H_2O) preheated to 90 °C was added to the pulp fibers with a pipette to the glass plates and was introduced by capillary forces (Chaudemanche and Navard 2011). Swelling experiments were performed according to the same procedure but at room temperature with 0.5 M Cuen-solution.

3 Results and Discussion

3.1 Production of a Dissolving Pulp by the Multifunctional Alkaline Pulping Process

→ Paper 1, Paper 2

Following the concept of the modified paper pulp production, the eucalypt paper-grade kraft pulp K-O was refined to accomplish the utilization as dissolving pulp for the viscose process. The main treatment steps comprised the extraction of hemicelluloses from the pulp by xylanase treatment and CCE, TCF-bleaching, and adjustment of final pulp viscosity by endoglucanase treatment.

3.1.1 Removal of Hemicelluloses and TCF-bleaching

X-treatment of the pulp K-O solubilized almost half of the initial xylan. In the subsequent CCE70-treatment, the xylan content was reduced to a comparable level as after a one-stage CCE100-treatment (Table 2).

Table 2 Residual xylan content of the eucalypt paper-grade kraft pulp K-O after X-treatment and alkaline extraction

Pulp	Xylan (% odp)	
K-O	22.5	
K-O-X	12.1	
K-O-CCE70	8.4	
K-O-X-CCE70	4.8	→ TCF-bleaching
K-O-CCE100	4.0	

The MWD of the pulps (Fig. 14) confirmed the extraction of notable amounts of xylan during X-treatment. Chain degradation of the residual hemicelluloses of the pulp can be seen from the arising shoulder in the MWD of the pulp K-O-X at a MW of ~ 10 kg mole^{-1}. Xylan chain degradation during X-treatment was further confirmed by the analysis of the beta-cellulose fractions from the CCE-lyes (Table 3). Beta-cellulose is the alkali-soluble fraction of a pulp, which contains mainly hemicelluloses that are insoluble at acidic conditions (pH 2.5). The weight-average molecular weight (Mw), determined by SEC, of the beta-cellulose fraction isolated from the CCE70-lye after X-treatment was clearly lower as compared to the beta-cellulose fraction derived from the CCE100-lye of the starting pulp. The main carbohydrate component of both beta-cellulose fractions was xylan. This shows that the low-MW cellulose fraction did not re-precipitate upon acidification. Thus, caustic extraction lye after X-treatment and filtrates of enzymatic hydrolysis are valuable xylan sources for the production of e.g. xylo-oligosaccharides or xylitol.

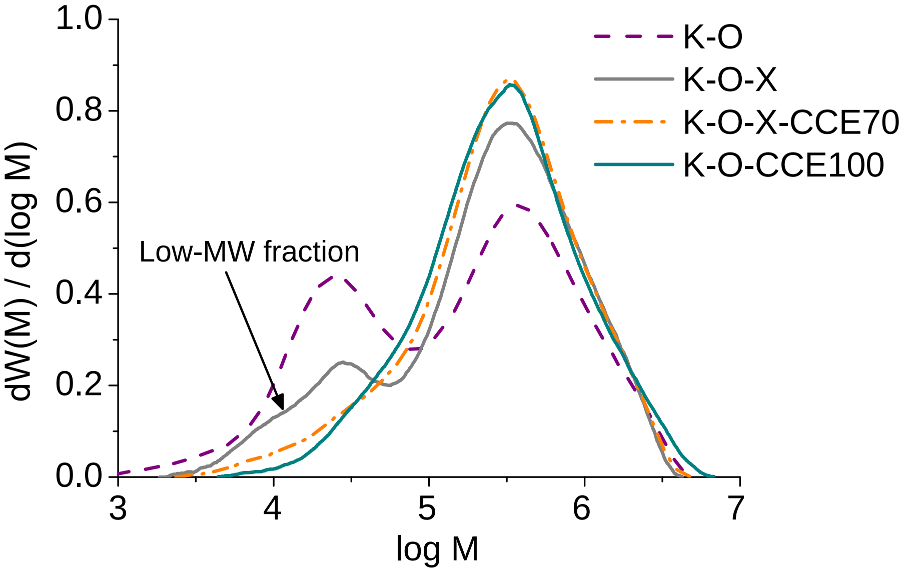


Fig. 14 MWD of the pulp K-O, after X-treatment and CCE70, and after CCE100 without previous enzyme treatment

Table 3 Carbohydrate composition and Mw of the beta-cellulose fractions precipitated from the caustic lyes of the CCE70-treatment after X-treatment, and from the CCE100-treatment

	Glucan (% opd)	**Xylan (% opd)**	**Beta-cellulose Mw (kg mole^{-1})**
X-CCE70	0.1	99.5	11.4
CCE100	0.5	98.9	21.8

TCF-bleaching comprised the steps of A-treatment for selective removal of hexenuronic acids, Z-bleaching for depolymerization, and final P-bleaching for chemical stabilization of cellulose chains and brightness adjustment. The X-treated pulp showed an increase in final brightness of 1.6 %.

3.1.2 Enzymatic Depolymerization with Endoglucanase

EG-treatment at different concentrations revealed different depolymerization behavior of the bleached pulps (Fig. 15).

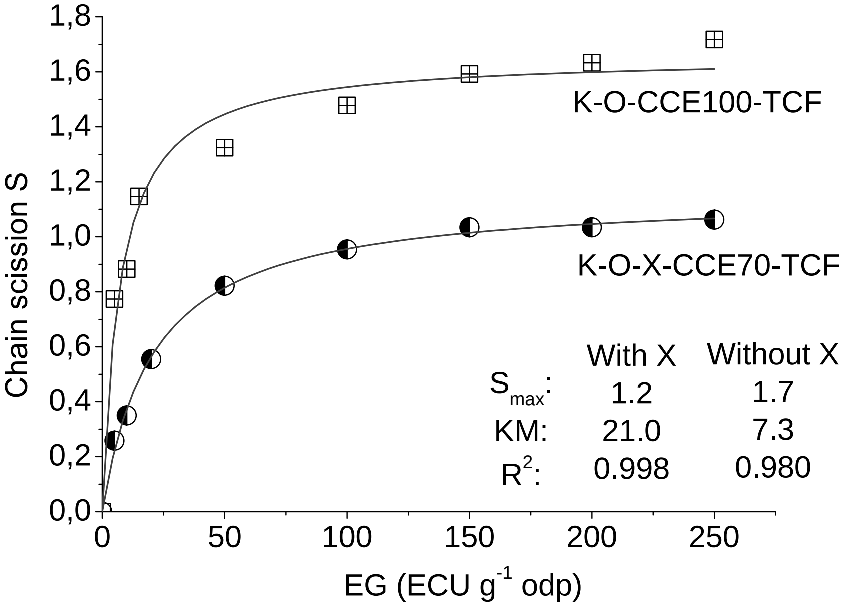


Fig. 15 Chain scission at increasing EG-concentrations after hemicellulose removal and TCF-bleaching of kraft pulps

The rate of cellulose chain scission S (Equ. 1) was calculated from the degree of polymerization of the pulp prior to (DP_0) and after (DP_t) the step of depolymerization. The chains scission represents the number of chain cleavage steps per initial cellulose chain.

$$S = \frac{DP_0}{DP_t} - 1$$

Equ. 1 Calculation of the cellulose chain scission S (Calvini et al. 2008)

The curve shape of the chain scission S in dependence of the applied enzyme concentration was fitted with Equ. 2 in similarity to the concept of Michaelis-Menten kinetics (Grassl et al. 1987). S_{max} represents the maximal chain scission according to this model and the constant KM gives the enzyme concentration EG, at which half of the maximal chain scission is reached (Grassl et al. 1987). EG acts as hydrolysis catalyst. Limiting factors for hydrolysis are, on the one hand, end-product inhibition especially by cellobiose (Radeva et al. 2012). On the other hand, limited mobility of cellulose binding modules entail that hydrolyzation terminates (Mollerup 2012).

$$S = \frac{S_{max} * EG}{EG + KM}$$

Equ. 2 Curve fitting equation for the cellulose chain scission S in similarity to Michaelis-Menten kinetics; S_{max} = maximal chain scission, KM = constant

The MWD of the pulp K-O-X-CCE70-TCF-EG at varying EG-concentrations is shown in Fig. 16. The peak maxima of all EG-treated pulps shifted towards lower values with increasing EG-concentrations. The fraction of low-MW components (DP < 50) increased at EG-concentrations > 50 ECU g^{-1} odp. This observation can be traced back to the preferred cleavage of loose cellulose chain ends and became apparent as a slight shoulder in the MWD (Kvarnlöf et al. 2008a; Medve et al. 1998). The pulps without X-treatment gave an analogous MWD.

Final EG-treatment decreased the viscosity of the X-treated pulp to 420 mL g^{-1} at an enzyme concentration of 20 ECU g^{-1} and the viscosity of the reference pulp to 330 mL g^{-1} at an enzyme concentration of 15 ECU g^{-1} (Table 4). The increased chain scission of the pulp K-O-CCE100-TCF can be ascribed to the elevated cellulose II content of the pulp and the enhanced swelling ability (see 3.2.1).

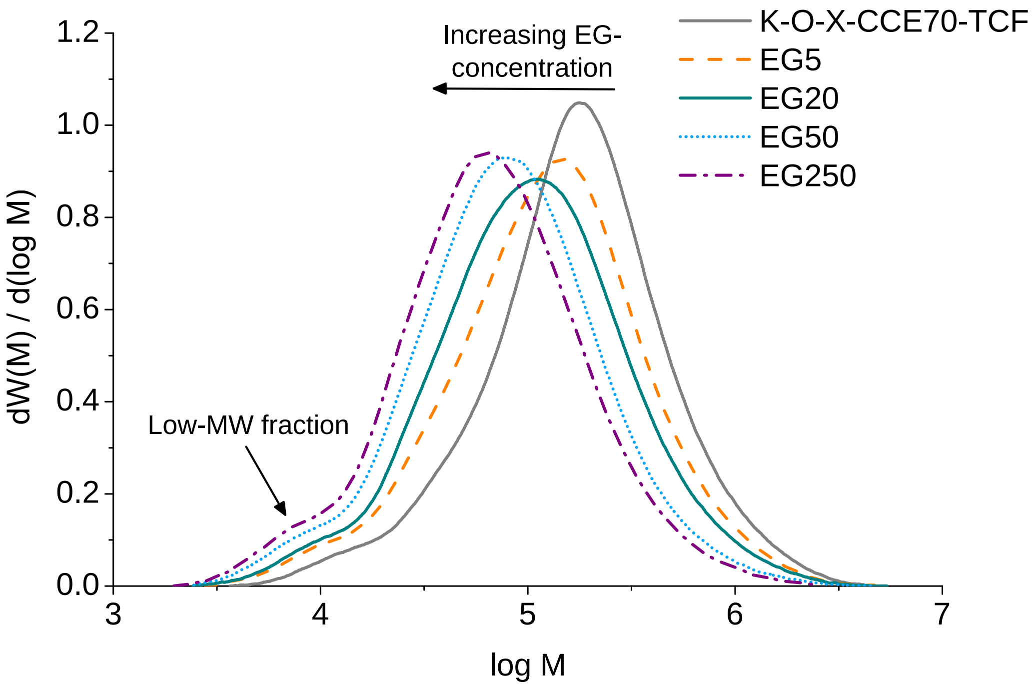


Fig. 16 MWD of pulps treated with endoglucanase up to a concentration of 250 ECU g^{-1} opd; the TCF-bleached pulp represents the X-pretreated substrate for EG-treatment

Table 4 Chain scission during EG-treatment of bleached kraft pulps of different cellulose II contents; Cell II determined by FT-Raman

Pulp	**[η] (mL g^{-1})**	**DPv**	**Chain scission**	**Cell II (%)**
K-O-X-CCE70-TCF	585	1485	--	8
K-O-X-CCE70-TCF-EG20	420	955	0.55	8
K-O-CCE100-TCF	645	1685	--	15
K-O-CCE100-TCF-EG15	330	785	1.15	13

3.1.3 Pulp Reactivity

The reactivity of a pulp is determined by the accessibility of the hydroxyl groups on C_6 and C_2/C_3 of the glucose monomer units to the reactants. For application in the viscose process, the reactivity of the pulp towards xanthation is the most relevant factor.

Pulp reactivity measurements according to Treiber (1962) were performed from the prepared MPPs:

- K-O-X-CCE70-TCF-EG20
- K-O-CCE100-TCF-EG15

When X-treatments and CCE70 were applied subsequently for hemicellulose removal, the pulp showed a higher reactivity (filter value 171) compared to the CCE100-treated pulp (filter value 119). Both enzyme treated pulps revealed higher filter values in comparison to other alkali extracted kraft pulps which were not enzyme treated (Table 5). This indicates an increase in reactivity caused by the enzyme treatment. Similar results were reported by Wollboldt et al. (2010), who investigated the properties of an *E. globulus* kraft pulp after CCE100 and after subsequent EG-treatment (K-TCF-CCE100; -EG75). EG-treatment increased the filter value of the pulp significantly from 49 to 108 (Table 5).

Prerequisites for a low particle content of a viscose dope are homogeneous penetration of the dissolving lye into the pulp, uniform sorption and swelling of the pulp. Drying or intense dewatering of pulps after a treatment at high alkalinity impair these processes because of the formation of molecular aggregates by inter- and intra-fibrillar hydrogen bonding of the cellulose microfibrils, which is known as hornification. When acetylation is applied for further derivatization, this aggregate formation clearly reduces the reactivity of the pulp towards the acetylation reagent because of the decreased accessible surface area (Sixta 2006a). In terms of reactivity towards xanthation in the viscose process, this effect is not completely clear yet. As can be seen from Table 5, the particle content of each enzyme treated kraft pulps was in the high-quality range of the PHK pulp. In order to quantify hornification, the WRV as an indicator of the swelling capacity and the accessible hydrophilic surface of a pulp was determined. A high swelling capacity of a pulp is assumed to promote the homogeneous penetration of the dissolving lye into the capillary system. In good agreement with these assumptions, an indirect correlation of the WRV of pulps and the viscose dope particle content (Table 5) was observed for the enzyme treated pulps.

Table 5 Results of the reactivity tests of the differently treated pulps and analysis of the hydrophilic character determined as WRV and COOH-content

	FV	**Particle (ppm)**	**WRV (%)**
K-TCF-CCE90 [a]	94	65	102
K-TCF-CCE100 [b]	49	71	n.a.
K-TCF-CCE100-EG75 [b]	108	23	n.a.
K-O-CCE100-TCF-EG15	119	18	120
K-O-X-CCE70-TCF-EG20	171	23	113
PHK [b]	429	18	n.a.
	Xylan (% odp)	**Xylan in AC (% odp)**	**COOH (µmole g^{-1})**
K-TCF-CCE90 [a]	4.0	n.a.	34
K-TCF-CCE100 [b]	5.7	3.1	n.a.
K-TCF-CCE100-EG75 [b]	5.5	2.1	n.a.
K-O-CCE100-TCF-EG15	4.5	1.4	29
K-O-X-CCE70-TCF-EG20	4.7	1.1	28
PHK [b]	4.7	0.8	n.a.

[a] Schild and Sixta (2011); [b] Wollboldt et al. (2010)

After steeping of the pulps, the xylan content in the alkali cellulose (AC) was measured. Wollboldt et al. (2010) concluded an indirect correlation between the content of alkali-resistant xylan in AC and the reactivity towards viscose formation measured as filter value. As shown in Table 5, they reported increased filter values and decreased xylan contents in AC from 3.1 % to 2.1 % after EG-treatment of the aforementioned CCE-treated kraft pulp (K-TCF-CCE100; -EG75). The lowest xylan content in AC (0.8 %) was found for a highly reactive conventionally-cooked PHK pulp. In the described work, 1.1 % alkali-resistant xylan was detected in the X-treated pulp K-O-X-CCE70-TCF-EG20 and 1.4 % in the pulp K-O-CCE100-TCF-EG15 at a similar residual xylan content of both pulps. In the case of alkaline extracted kraft pulps, endoglucanase post-treatment seems to have a positive effect on the reactivity of pulps by lowering the alkali-resistant xylan fraction. This could be induced by cleavage of bonds between residual xylan and cellulose during EG-treatment and solubilization of the xylan fraction.

3.2 Pulp Properties and their Influence on Enzymatic Degradability

→ Paper 3

Several pulps of different chemical composition and cellulose morphology were subjected to EG-treatment. EG-treatment has been reported to show an increasing effect on the final pulp reactivity due to morphological structures that are opened upon enzyme treatment, as discussed before (see 1.1.6). In the concept of the modified paper pulp production, EG-treatment is applied for the purpose of depolymerization besides reactivity enhancement. In order to promote the commercial applicability of endoglucanase, the effect of the wood source, the pulping process as well as the non-cellulosic polymeric compounds, such as hemicelluloses and residual lignin, on the enzymatic degradability of pulp was examined.

3.2.1 Influence of Cellulose Morphology

Pulps with an increasing proportion of cellulose II experienced an increasing extent of chain scission upon EG-treatment. This may be attributed to enhanced cellulose accessibility, as demonstrated for cotton linters pulps with different cellulose II contents (Fig. 17).

The accessibility of cellulose needs to be discussed in respect of its molecular, supramolecular, and fibrillar structure, respectively. On the one hand, WAXS measurements revealed rising lateral cellulose I crystal dimensions with increasing cellulose II contents (Table 6) which are ascribed to the dissolution of small or less ordered crystallites during mercerization (Fink et al. 1985; Fink and Walenta 1994). Thus, the increased cellulose accessibility of the mercerized pulps can be attributed to the changed unit cell dimensions as occurred during the conversion from cellulose I to cellulose II. The exocyclic hydroxymethyl groups at C_5 of anhydroglucose units of the corner chains but also of center chains of microfibrils convert from the *trans-gauche* position to the *gauche-trans* position, which loosens the rigid cellulose lattice (Blackwell et al. 1978; Kolpak and Blackwell 1976; Kroon-Batenburg and Kroon 1997; Langan et al. 1999; Nishiyama et al. 2002). This change in orientation introduces an additional intersheet H-bond, which results in a decreased specific surface area $a_{s,\,BET}$ of dried pulps. This was confirmed for the alkalized CLPs by gas sorption measurements according to Brunauer, Emmet and Teller (Table 6) (Brunauer et al. 1938; Zeronian 1985).

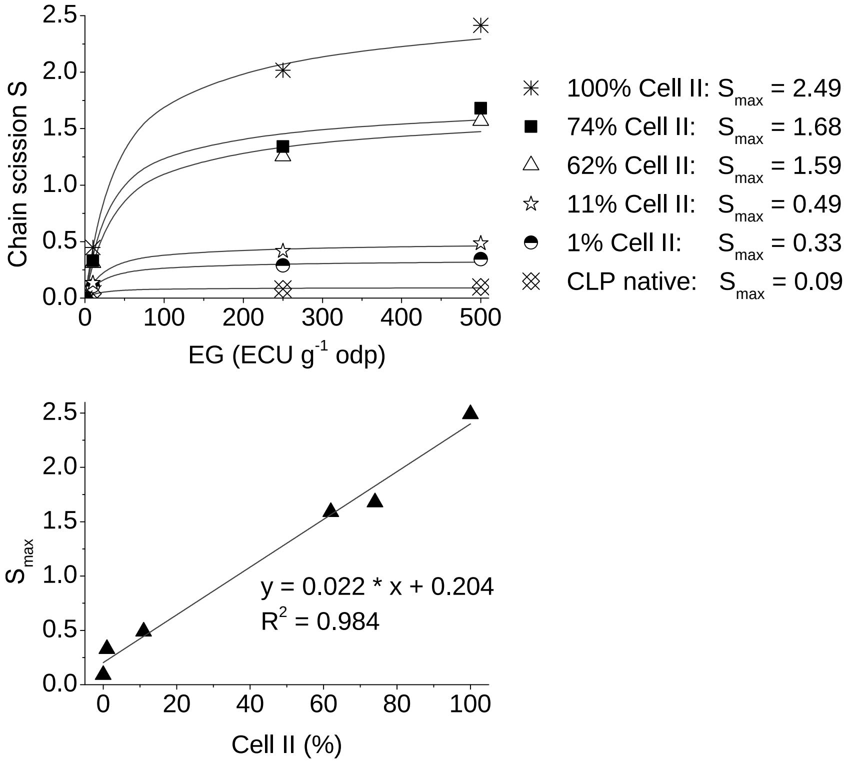


Fig. 17 Upper diagram: chain scission of differently alkalized CLPs upon EG-treatment; lower diagram: linear correlation of S_{max} and the Cell II content

On the other hand, the overall availability of OH-groups and thus the hydrophilic character of cellulose II is higher than in cellulose I (Krässig 1993b). This can be observed from an increased swelling capacity (Jayme and Schenck 1949) of pulps containing cellulose II, as determined by WRV measurements (Table 6), and explains higher reactivity of cellulose II in heterogeneous reactions like derivatization or hydrolysis. However, also the different stacking of the cellulose planes in the structures of cellulose I and cellulose II contribute to the difference in the swelling behavior, as shown with molecular dynamics simulations by Kroon-Batenburg et al. (1997). Cellulose II shows higher average sorption energy compared to cellulose I. Therefore, in cellulose II the water monolayer adsorbs more readily and desorbs less readily than with cellulose I (Pizzi and Eaton 1987; Pott 2004).

Table 6 WAXS results and wet chemical analysis of differently alkalized cotton linters pulps

NaOH (wt %) [a]	**Cell II (%)**	**CrI (%)**	**$D_{(hkl)}$ Cell I (nm)**				**WRV (%)** [b]	**$a_{s,\,BET}$ ($m^2\,g^{-1}$)**
			(1-10)	(110)	(020)	(004)		
--	0	60	4.9	6.2	6.7	13.7	52	0.47
11.9	1	58	5.2	6.7	6.9	14.9	57	0.41
12.9	11	53	5.5	7.1	6.9	13.0	62	0.37
14.8	62	40	8.2	9.6	7.3	11.4	82	0.24
15.2	74	38	8.0	8.9	7.0	10.6	83	0.24
17.8	100	39	--	--	--	--	95	0.17

[a] NaOH concentration in the steeping lye; [b] WRV measured from never-dried pulps

3.2.2 Influence of Residual Hemicelluloses

The influence of the residual hemicellulose fraction on the accessibility of cellulose upon enzymatic depolymerization was investigated on spruce sulfite and eucalypt kraft pulps.

In the case of the eucalypt kraft pulp K-O, X-treatment removed almost half of the initial xylan (Table 2) and clearly increased the degradability of the pulp during EG-treatment, as shown in Fig. 18 (a). Alkaline extraction CCE70 resulted in even lower residual xylan content and thus led to a higher extent of enzymatic hydrolysis.

In the case of the spruce sulfite pulp S-5, X-treatment of the pulp removed only little amounts of xylan from the pulp (Table 7) compared to its effect on the eucalypt kraft pulp. Enzymes removed accessible xylan located on the fibril surface of the kraft pulp. Xylan in softwood cells like spruce instead, is mainly located in the inaccessible S3 cell wall layer (Sjöström 1993). Also M-treatment didn’t show any relevant effect on the residual hemicellulose content of the pulp.

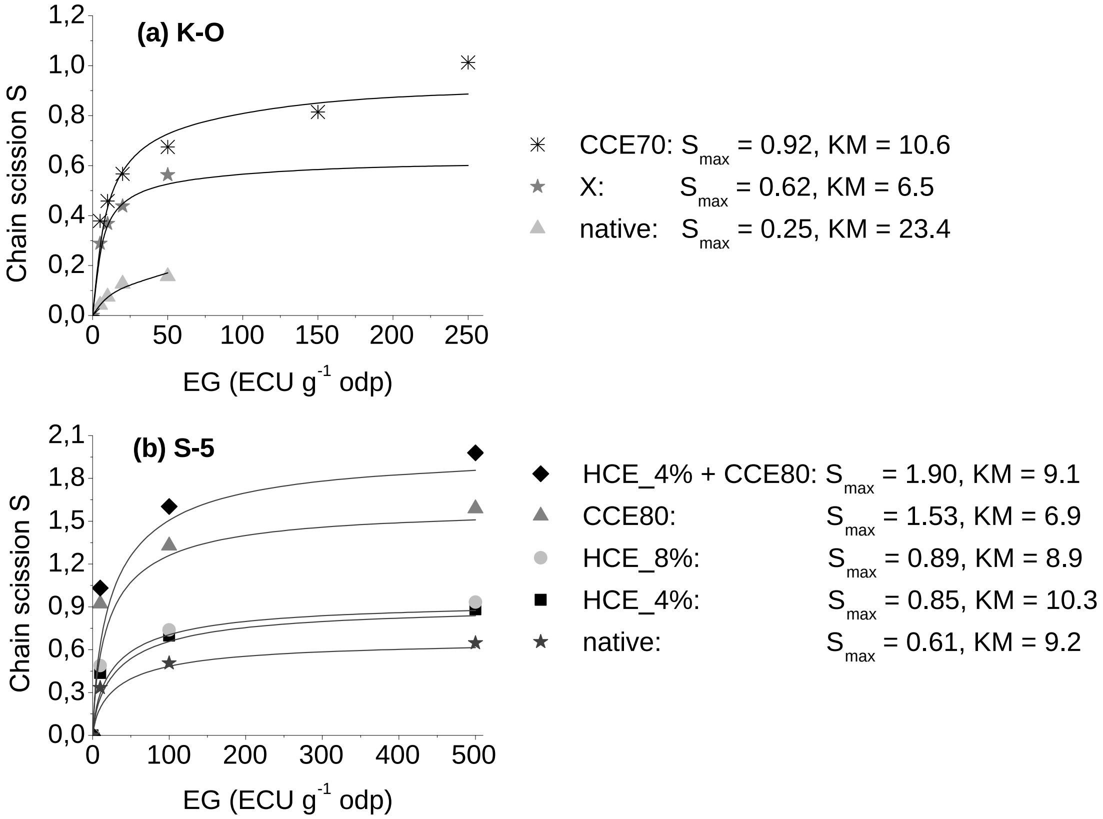


Fig. 18 Chain scission at increasing EG-concentrations after removal of hemicelluloses from the pulps (a) K-O and (b) S-5

Table 7 Pulp properties of the spruce paper-grade sulfite pulp S-5 after enzyme treatments, HCE, and CCE for hemicellulose removal

Pulp	**Xylan (% odp)**	**Mannan (% odp)**	**R18 (%)**	**Brightness (%)**
S-5	3.9	6.6	87.6	71.1
S-5-X	3.1	6.3	n.d.	n.d.
S-5-M	3.5	6.3	n.d.	n.d.
S-5-HCE_4%	3.2	2.8	93.2	69.2
S-5-HCE_8%	2.9	1.5	95.0	68.1
S-5-CCE80	1.0	4.2	94.5	73.4
S-5-HCE_4%-CCE80	1.0	2.2	96.2	70.8

HCE with an alkali charge of 4 % on odp (HCE_4%) of the semi-bleached pulp S-5 mainly removed mannan from the pulp by peeling reactions at temperatures > 80 °C.

Only little amounts of xylan were removed owing to the presence of the uronic acid side chains which initiate stopping reactions (Sjöström 1993). The treatment HCE_8% at increased alkali charge was slightly more efficient in the overall removal of hemicelluloses. A totally different final carbohydrate composition of the pulp S-5 was obtained after CCE. During CCE80 at only 30 °C, peeling reactions are very slow (Glaus and van Loon 2008) and mannan is resistant to physical dissolution due to its highly ordered structure, initiated through co-crystallization with cellulose (Annergren and Rydholm 1959). Thus, only one third of the mannan was extracted from the pulp upon CCE80. In contrast, almost 75 % of the pulp's xylan was removed because of its good solubility under the conditions of CCE. The sequential application of HCE and CCE (HCE_4% and CCE80) further lowered the residual mannan and xylan contents. HCE-treatments of the pulp caused a slightly enhanced chain scission upon EG-treatment compared to the starting pulp S-5 due to improved cellulose accessibility, Fig. 18 (b). An even higher extent of chain degradation was observed for the CCE80-treated pulp. The higher chain scission rate may be attributed to the presence of the cellulose II conformation after CCE80 (11 % Cell II). The sequential application of HCE and CCE affected the highest cellulose accessibility and pulp swelling capacity at a cellulose II content of 8 %. These combined treatments obviously caused a significant increase of the efficiency of the EG-treatment. Thus, the cellulose II content and the swelling capacity of pulps were found to show the major influence on the accessibility of cellulose to enzymes.

3.2.3 Influence of Residual Lignin

Mansfield et al. (1999) summarized the influence of lignin distribution on the rate of enzymatic hydrolysis by cellulases. They reported lignin to act as physical barrier which impedes the accessibility of cellulose (Ucar and Fengel 1988) and also described adsorption of cellulase enzymes on lignin and thus prevented action on cellulose (Ahlgren et al. 1971). The spruce paper-grade sulfite pulps with residual kappa number 1-15 (brownstock: kappa 15; semi-bleached pulp: kappa 5; final bleached pulp: kappa 1) were subjected to EG-treatment. Removing lignin from the sulfite pulp increased its porosity (Ahlgren et al. 1971; Fock 1959), which can be seen from the increasing specific surface area $a_{s,\ BET}$ of the pulps S-15 and S-5 (Fig. 19) and the increased enzymatic hydrolysis rate after lignin removal. In the case of the final bleached pulp S-1 instead, the specific surface area $a_{s,\ BET}$ was lower compared to the other pulps probably because of cell wall compaction upon chemical industrial treatments. This lower specific surface area was also manifested in reduced accessibility of the cellulose to enzymatic hydrolysis. FE-SEM pictures of the pulp S-1 before and after EG-treatment were recorded and revealed massive structural changes of the pulp fibers (Fig. 20).

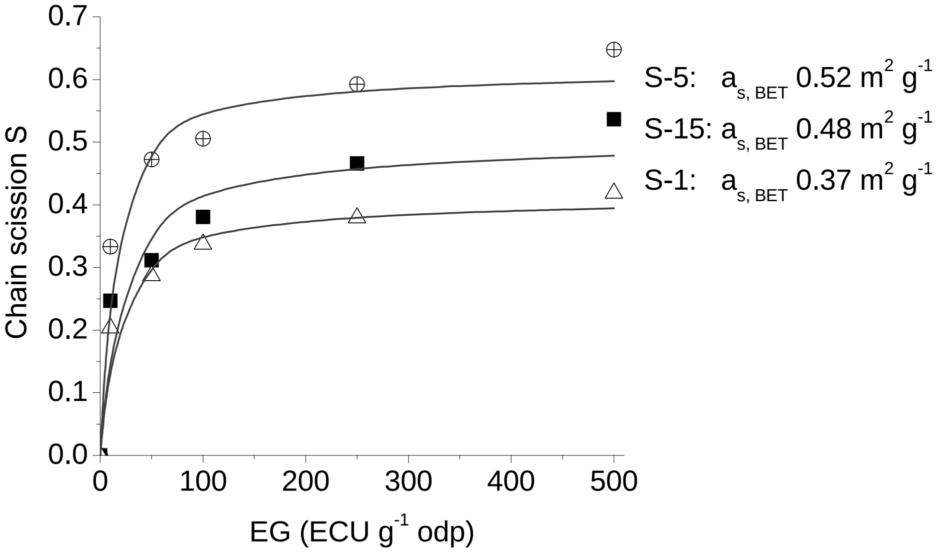


Fig. 19 Chain scission at increasing EG-concentrations after delignification of the spruce sulfite pulps; $a_{s,\ BET}$ = specific surface area

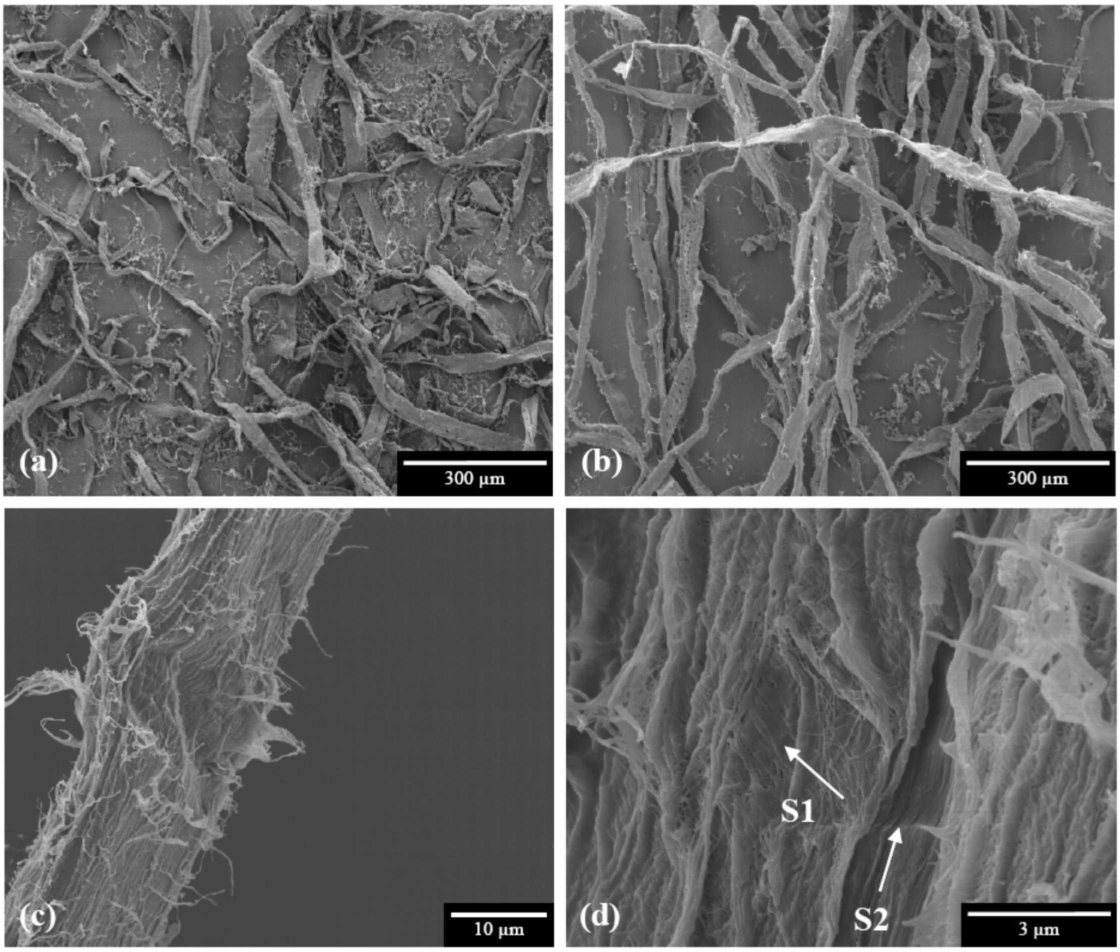


Fig. 20 FE-SEM pictures of the pulp S-1; (a) S-1 before and (b)-(d) after EG-treatment (concentration 500 ECU g^{-1} odp); (b): fine and thin pulp components are removed by enzymes; (c): pulp fiber damage derived from enzymatic hydrolysis; (d): cracked S1 cell wall layer and S2 identified from the different orientations of the microfibrils (arrows)

3.3 The Effect of Different Methods of Pulp Depolymerization on their Reactivity

→ Paper 2, Paper 3

EG-treatment was reported to show an increasing effect on the reactivity of pulps. In an attempt to elucidate the effect of different degradation methods on pulp reactivity, enzymatic degradation and acid-catalyzed hydrolysis $A_{Hydr.}$, respectively, were performed on pulps.

3.3.1 Spruce Sulfite Pulps

The spruce sulfite pulp S-1 was degraded to a preset pulp viscosity (~ 600 mL g^{-1}) by both EG-treatment, and $A_{Hydr.}$ at pH 1.5, as shown in Fig. 21 (a). Reactivity measurements according to the methods of Fock (1959) and Treiber et al. (1962) were performed. The Fock-reactivity after EG-treatment was 93.3 % and after $A_{Hydr.}$ 92.9 %, respectively. These very high values are comparable to those found by Henriksson et al. (2005) for a similar softwood sulfite pulp after EG-treatment. It is well known that the outer cell wall layers of sulfite dissolving pulps contain cellulose of low MW and short-chain hemicelluloses with many carboxyl groups (Jayme and von Köppen 1950). These fractions show high swelling and dissolution tendency. Thus, the Fock-test indicated very high reactivity of both pulps independent from the depolymerization treatment. In contrast to the Fock-test, the reactivity measurement according to Treiber et al. gives the filterability (filter value) and particle content of a viscose dope, and thus allows a reliable prediction of the pulp applicability for the viscose process. The viscose dope prepared from the sulfite pulp S-1 after EG-treatment showed a filter value of 179 and an average particle volume of 13.8 ppm. Unexpectedly, the viscose prepared from the same pulp after $A_{Hydr.}$ revealed a filter value of 330 and an average particle volume of 12.0 ppm, which represents a clearly better viscose quality as compared to the viscose prepared from the EG-treated pulp S-1.

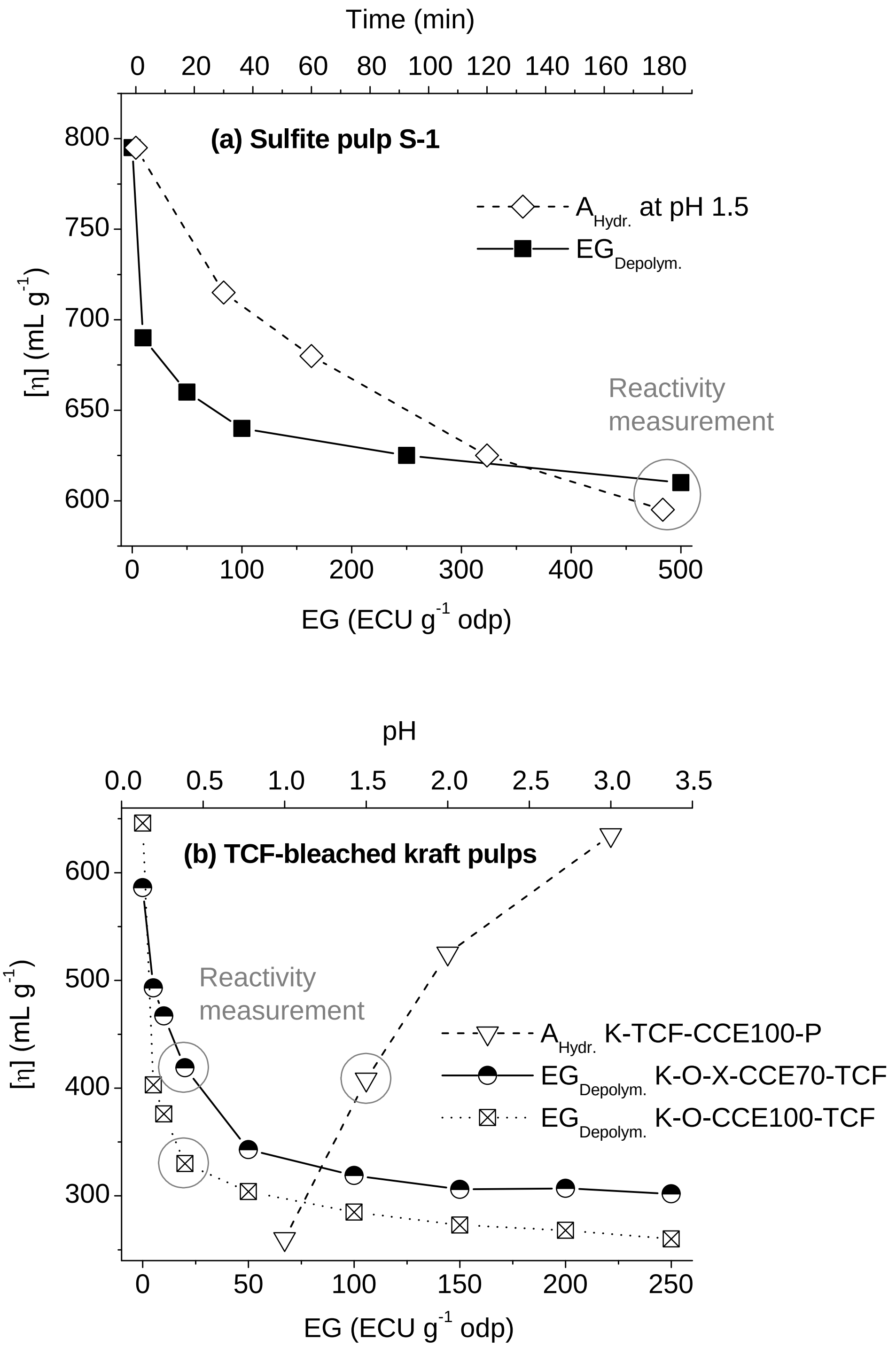


Fig. 21 (a) Viscosity decrease of the pulp S-1 during acid-catalyzed hydrolysis $A_{Hydr.}$ at pH 1.5 (varying hydrolysis time, upper abscissa) and EG-treatment (varying enzyme concentration, lower abscissa); (b) Viscosity decrease of TCF-bleached kraft pulps during acid-catalyzed hydrolysis $A_{Hydr.}$ for 60 min at 90 °C at varying pH (upper abscissa) and EG-treatment (varying enzyme concentration, lower abscissa); encircled: pulps for reactivity measurement

In this case, the results from Treiber-test seem to be industrially more reliable. Thus, it can be concluded that the pulp reactivity for viscose application was seemingly not improved by the EG-treatment. The EG-treatment is obviously more effective on pulps with limited accessibility of the fiber surface as observed for kraft and soda-AQ pulps after CCE-treatment (Wollboldt et al. 2010). Alternatively, the enhanced reactivity after $A_{Hydr.}$ of the sulfite pulp may be attributed to the massive fiber fibrillation of the pulp, as can be seen from Fig. 22.

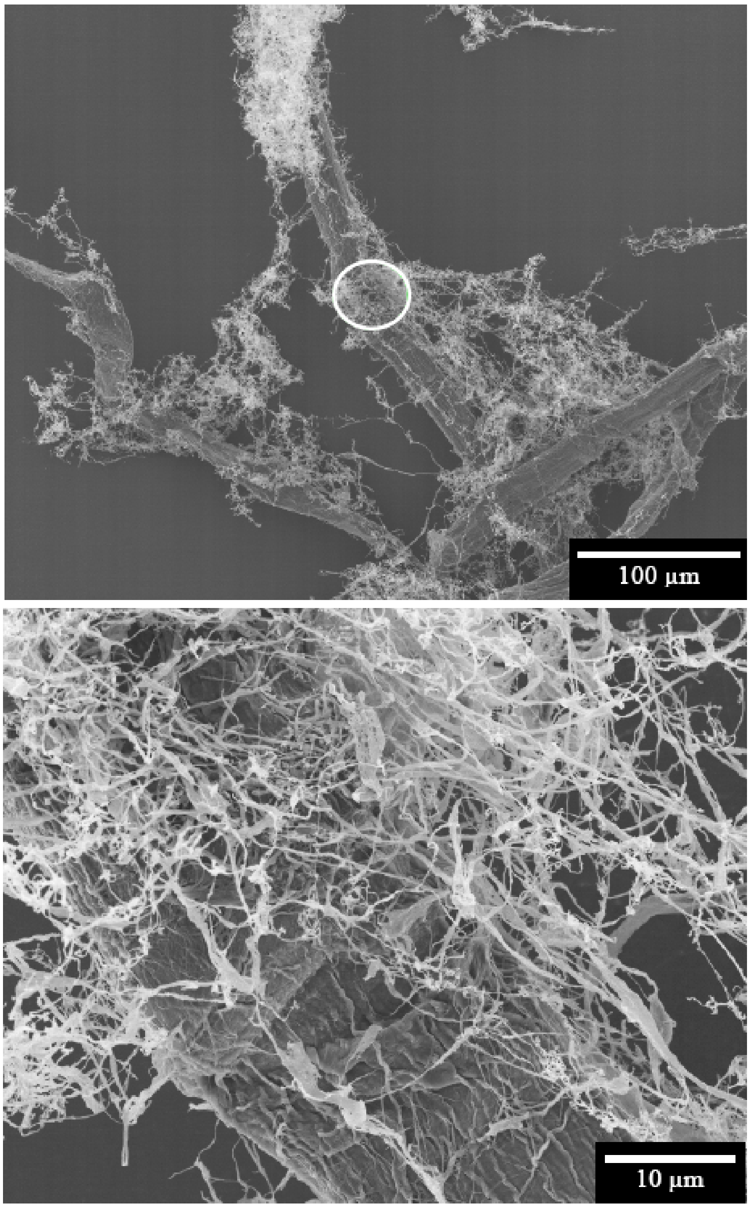


Fig. 22 FE-SEM pictures of the pulp S-1 after $A_{Hydr.}$ at pH 1.5 for 180 min; intense fiber fibrillation visible

3.3.2 Eucalypt Kraft Pulps

CCE-treated kraft pulps clearly experienced an increase in pulp reactivity upon EG-treatment as confirmed by filter value measurements (see 3.1.3). As shown in Fig. 21 (b), the TCF-bleached kraft pulps after EG-treatment were compared to a TCF-bleached kraft pulp after CCE100 and a peroxide step (K-TCF-CCE100-P) during acid-catalyzed hydrolysis $A_{Hydr.}$. Following the trend of the filter values, the pulp K-O-X-CCE70-TCF-EG20 showed a higher Fock-reactivity (26.8 %) compared to K-O-CCE100-TCF-EG15 (22.9 %). The pulp degraded by $A_{Hydr.}$ at pH 1.5 had an even lower Fock-reactivity of 19.1 %. Thus, the increasing effect of EG-treatment on pulp reactivity was confirmed for alkaline cooked pulps after CCE-purification but not for sulfite pulps.

3.4 Reactivity of TEMPO-oxidized Dissolving Pulps

→ Paper 4

TEMPO-mediated oxidation was applied in the process of refining a kraft paper pulp K-ECF prior to and subsequently to CCE for utilization as dissolving pulp. The effect of the voluminous carboxyl groups on preserving the accessibility of the cellulose surface by reducing the aggregation of microfibrils was investigated.

3.4.1 Pulp Properties after TEMPO-oxidation and CCE

Pretrials of TEMPO-mediated oxidation were performed for oxidation times of 3-48 h. The viscosity of the TEMPO-oxidized pulp decreased fast during the oxidation procedure to a similar level for all pulp preparations (Table 8).

Table 8 Pretrials of TEMPO-mediated oxidation of the kraft pulp K-ECF with the system TEMPO/NaOCl/$NaClO_2$ at pH 6.8 at 60 °C, reaction time 3-48 h

	Kraft pulp	**TEMPO-oxidation**			
	K-ECF	3 h	10 h	24 h	48 h
[η] (mL g^{-1})	830	525	505	505	485
$COOH_{MB}$ (µmole g^{-1}) [a]	88	615	716	791	820
$COOH_{FDAM}$ (µmole g^{-1}) [b]	78	459	492	493	535
CO (µmole g^{-1}) [c]	8.2	20.3	21.0	18.4	12.1

[a] determined with methylene blue; [b] determined with FDAM/SEC;
[c] determined with CCOA/SEC

To minimize depolymerization reactions starting from carbonyl groups in the subsequent alkaline CCE-treatment, called β-elimination, the TEMPO-oxidized pulps were subjected to an oxidative post-treatment with chlorite in order to convert all carbonyl groups into carboxyl groups. The carbonyl group content of the TEMPO-treated pulps after chlorite oxidation was determined with CCOA/SEC analysis and showed slightly increased values compared to the starting pulp within the range of other commercially available dissolving pulps (Table 8). The carboxyl group content was determined with methylene blue and FDAM/SEC analysis (Fig. 23), respectively, and both methods revealed a similar trend over the reaction time of 3-48 h (Table 8). The absolute values are disputable because the analyses were out of the standard calibration range for both methods. Nevertheless, these absolute numbers give an indication of the degree of oxidation along the cellulose chain of ~ 12-16 mole %. As shown in the FDAM/SEC spectra in Fig. 23, the carboxyl groups of the starting pulp are mainly located in the hemicellulose and cellulose fraction of the pulp with a molecular weight < 100 kg mole^{-1} (log M = 5; ~ cellulose DP < 600). For the oxidized pulps instead, less than 50 % of all carboxyl groups are located in the DP < 500 fraction and up to 20 % are located in the high-molecular weight fraction with a DP > 2000. This strong increase of the carboxyl groups in the high-WM fractions of the pulps show that also highly-polymerized cellulose chains were accessible to TEMPO-oxidation.

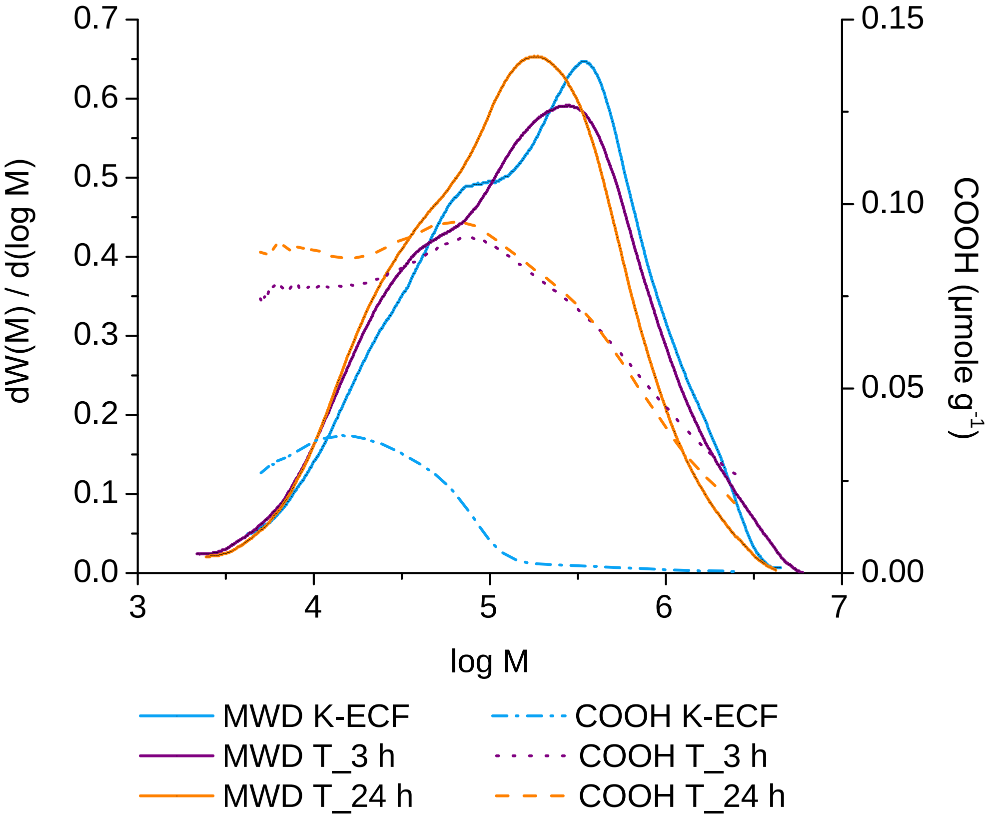


Fig. 23 Molecular weight distribution (MWD) and carboxyl group content (COOH) determined from FDAM/SEC of the kraft pulp (K-ECF) and of the TEMPO-oxidized pulps (T) after 3 h and 24 h reaction time, respectively

When TEMPO-mediated oxidation was performed subsequently to CCE, the alkaline pretreated pulp K-CCE100 showed higher reactivity as compared to the native kraft pulp, and thus showed a higher final carboxyl group content (Table 9). This effect of an alkaline pretreatment can be ascribed to higher accessibility of regenerated or mercerized cellulose, owing to a lower crystallinity compared to native cellulose. The elevated hydrophilicity of both TEMPO-treated pulps was reflected in similar, very high water retention values (Table 9). The WRV is an indicator of the swelling capacity of a pulp and is influenced by the content of hydrophilic groups, such as carboxyl groups, and by the cellulose II content, which shows a higher availability of the hydroxyl groups as compared to cellulose I (Krässig 1993b). As far as the WRV is affected, the higher carboxyl group content obviously compensated for the lack of cellulose II crystal structures as shown for the K-CCE100-TEMPO treated pulp in comparison to the K-TEMPO-CCE100 treated pulp (Table 9).

Table 9 Properties of the kraft pulps treated with TEMPO-mediated oxidation prior to and after CCE100, respectively, and of a reference pulp with acid-catalyzed hydrolysis $A_{Hydr.}$ applied for depolymerization

	K-TEMPO-CCE100	**K-CCE100-TEMPO**	**K-CCE100-$A_{Hydr.}$-P**
[η] (mL g^{-1})	450	390	450
$COOH_{MB}$ (μmole g^{-1}) [a]	821	1516	30
WRV (%)	133	133	98
Cell II (%) [b]	11	0	14
Gamma value	14.2 [c]	20.9 [c]	36.2 [d]
Dissolution time (min) [e]	7	>> 12	7

[a] determined with methylene blue; [b] determined with FT-Raman; [c] viscose from the direct-xanthation-process; [d] viscose prepared according to the Treiber method; [e] in NMMO.H_2O at 90 °C starting from the time of pulp fiber saturation

3.4.2 Viscose Reactivity of the TEMPO-oxidized Pulps

The TEMPO-oxidized pulps were subjected to a direct-xanthation-process in a batch-reactor. This process did not include an ageing step like it is usually done in the viscose process. The reference pulp was subjected to the viscose process at laboratory scale according to the procedure of the Treiber test (Hüpfl and Zauner 1966; Treiber et al.

1962). The degree of xanthation of the viscose samples was analyzed as gamma value (Table 9) and was found to be lower for the oxidized pulps compared to the reference pulp. This can be attributed to the partially oxidized C_6 carbons that are not available for xanthation reactions with CS_2. Usually, the C_6-hydroxyl group reacts first in xanthation reactions as it possesses the highest mobility and accessibility, and is thus sterically favored in the derivatization process (Schwaighofer et al. 2011). From the low gamma values after TEMPO-oxidation it can be concluded that xanthation is not redirected to the C_2/C_3-hydroxyl groups when the C_6-hydroxyl group is blocked. Thus, the intense oxidation treatment caused an inverse effect on pulp reactivity and clearly reduced the applicability of the TEMPO-oxidized pulps for the viscose process.

3.4.3 Dissolution Behavior of the TEMPO-oxidized Pulps in NMMO-water

The dissolution behavior of the TEMPO-oxidized pulps was investigated in NMMO.H_2O as ideal solvent. The pulp with the lower carboxyl group content and the reference pulp dissolved much faster compared to the highest oxidized pulp, Table 9 and Fig. 24.

According to Spinu et al. (2011), the limiting factor for dissolution in NMMO-water is not the penetration of the solvent inside the fiber but the local concentration of NMMO, as investigated on softwood pulp fibers. Thus, the dissolution quality is reduced when NMMO is locally diluted with the water around and inside the fibers. Therefore, one explanation for the clearly lower solubility of the highest oxidized pulp K-CCE100-TEMPO could be a higher residual water content of the pulp sample. All pulp fibers were prepared from aqueous pulp suspensions and air-dried afterwards. Upon the mild drying conditions, water molecules could be fixed by an increased amount of hydrogen bonds in the sample K-CCE100-TEMPO. The physicochemical process of cellulose dissolution in NMMO-water is not comprehensively understood today, but the hydroxyl groups of cellulose are assumed to act as acids in donor-acceptor interactions with NMMO (Rosenau et al. 2001). According to the proposals of Michels and Kosan (2005), two NMMO.H_2O molecules are incorporated into inter-sheet hydrogen bonds with the OH-groups on C_6 and C_3 in cellulose upon dissolution in NMMO-water (Fig. 25). Therefore, an increased content of C_6-carboxylate groups with lack of protons for forming hydrogen bonds seems to reduce the solubility of pulp fibers in NMMO-water.

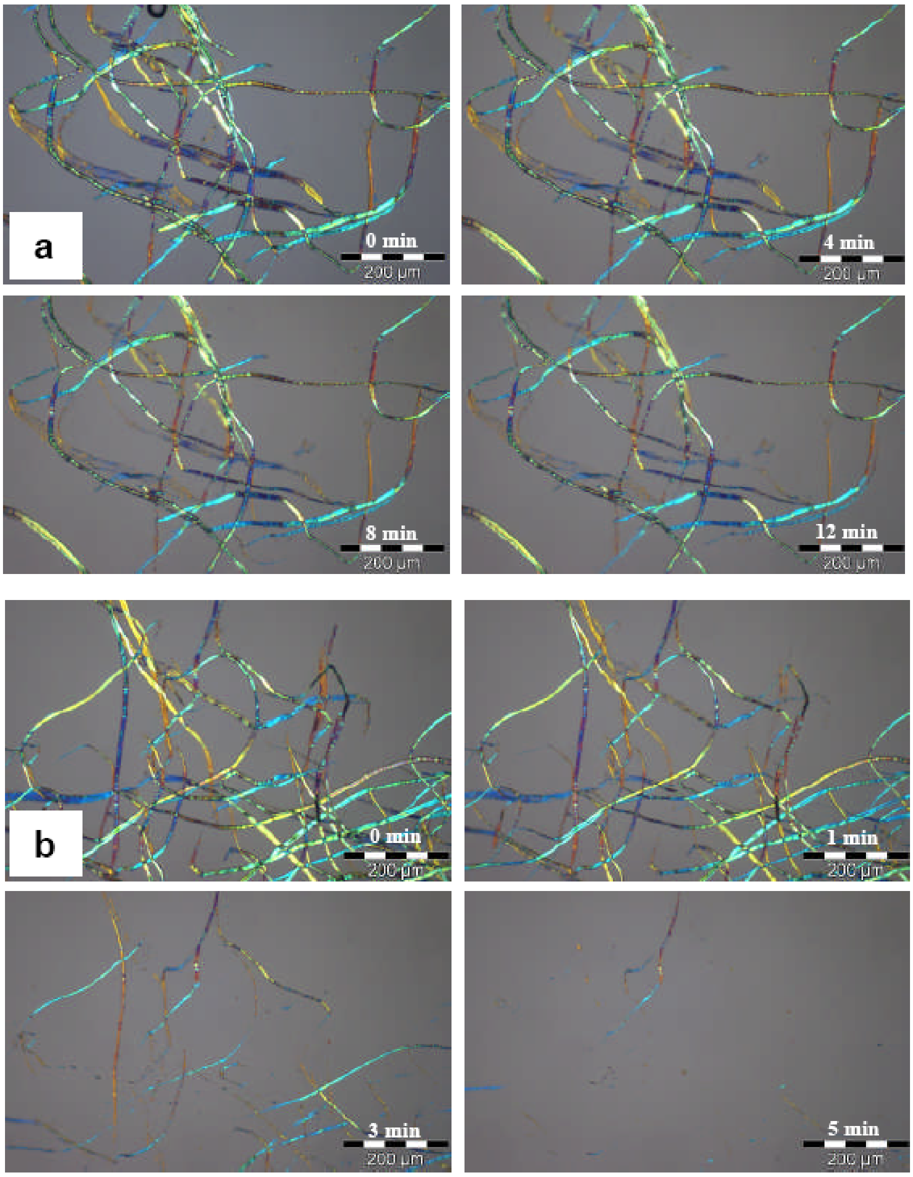


Fig. 24 Dissolution of pulp fibers in NMMO.H_2O at 90 °C; time of fiber saturation is chosen as starting time “0 min”; a) K-CCE100-TEMPO from 0-12 min; b) K-TEMPO-CCE100 from 0-5 min

From the dissolution times found for the TEMPO-oxidized pulps (Table 9), it can be speculated that there is a critical content of C_6-carboxylate groups above which dissolution is impeded as compared to the reference pulp. As shown in Fig. 24, in agreement with the results obtained by Cuissinat et al. (2006) and Chaudemanche et al. (2011), the mechanism of wood pulp fibers dissolution in NMMO-water (≤ 17 wt %

water) comprises fractionation and disintegration into spindle parts, and fast dissolution without ballooning.

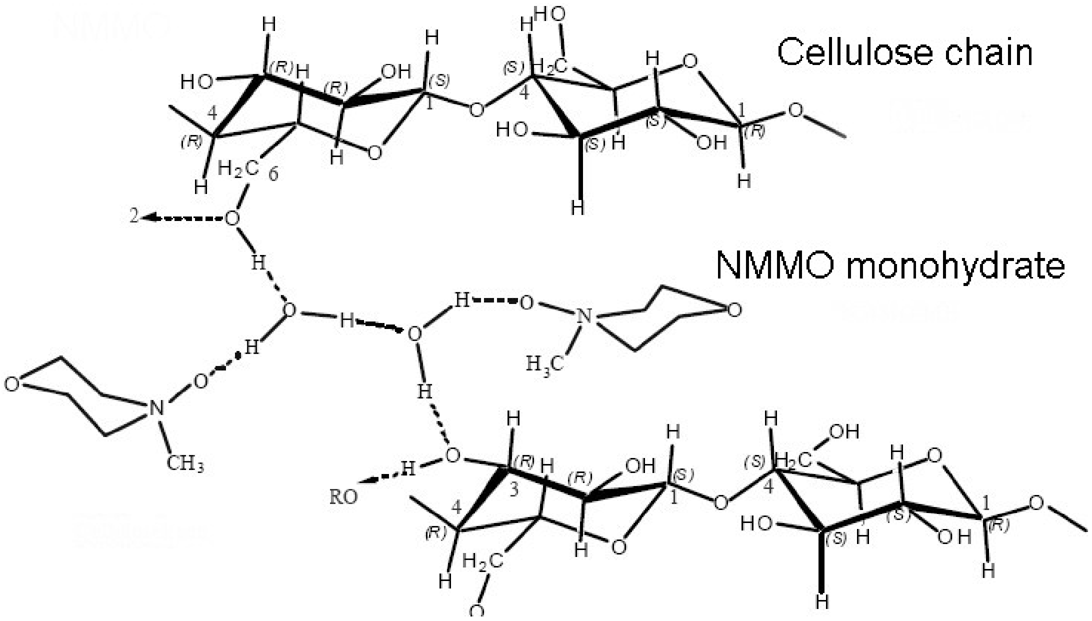


Fig. 25 Cellulose dissolution process in NMMO-water; illustration adopted from Michels and Kosan (2005)

3.4.4 Swelling and Dissolution Behavior of the TEMPO-oxidized Pulps in Cuen-solution

In an attempt to understand the differences in solubility of the prepared pulps, the mechanism of swelling of the pulp fibers referring to their fiber morphology was investigated. In this study, 0.5 M Cuen-solution was applied as non-ideal solvent for investigating the influence of the carboxyl group content on the pulp solubility. The outer cell wall layers S1 and PW (primary wall) of CCE-refined dissolving pulps produced from paper-grade pulps are preserved to a greater extent compared to conventionally-cooked dissolving pulps (Schild and Sixta 2011). Additionally, the S2 layer shows increased swelling capacity due to the cellulose II morphology and the reduced crystallinity after CCE. Nevertheless, swelling of the S2 layer is restricted by the presence of the S1 and the PW, which results in ballooning phenomena and the formation of collars from the pulp fiber upon dissolution (Cuissinat and Navard 2006). This behavior was expected and partly observed for the non-oxidized reference pulp, which showed intense ballooning in the swelling agent but did not dissolve at the chosen concentration of the alkaline Cuen-solution (Fig. 26).

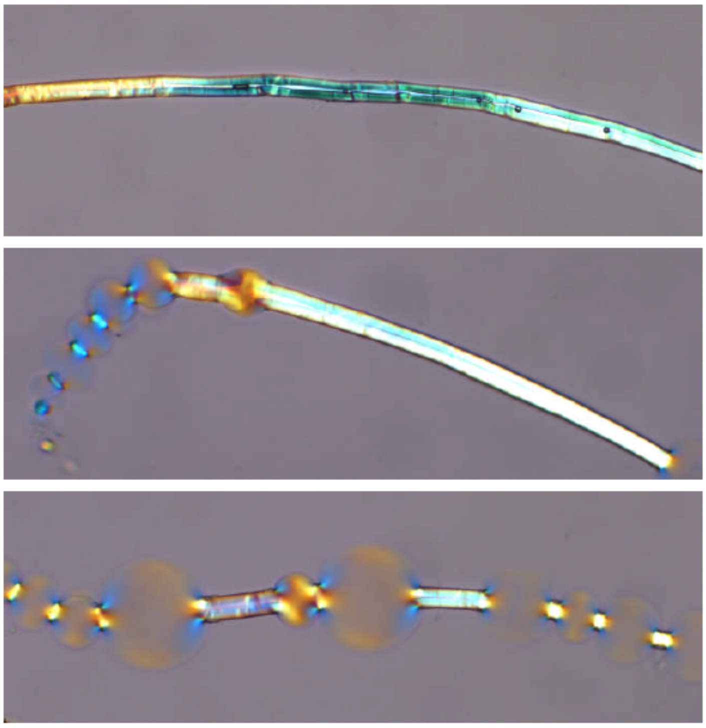


Fig. 26 K-CCE100-$A_{\text{Hydr.}}$-P pulp fiber swollen by intense ballooning in 0.5 M Cuen-solution at room temperature; the highly-ballooned structure was the final swelling state after 45 s

Both TEMPO-oxidized pulps instead, dissolved homogeneously by unwinding of the internal helical cellulose structure, as shown for the highest oxidized pulp in Fig. 27. Navard and co-worker (2006) explained the visible left handed untwisting of the cellulose to the release of internal stresses, which is necessary for dissolution. This dissolution mechanism is similar to the one of commercial PHK pulps and sulfite market dissolving pulps without swelling restrictions due to S1/PW-removal (Fig. 28). Consequently, the introduction of carboxyl groups by TEMPO-oxidation increased the swelling capacity of the S1 layer and the PW in the way that ballooning is avoided and homogenous dissolution is enabled.

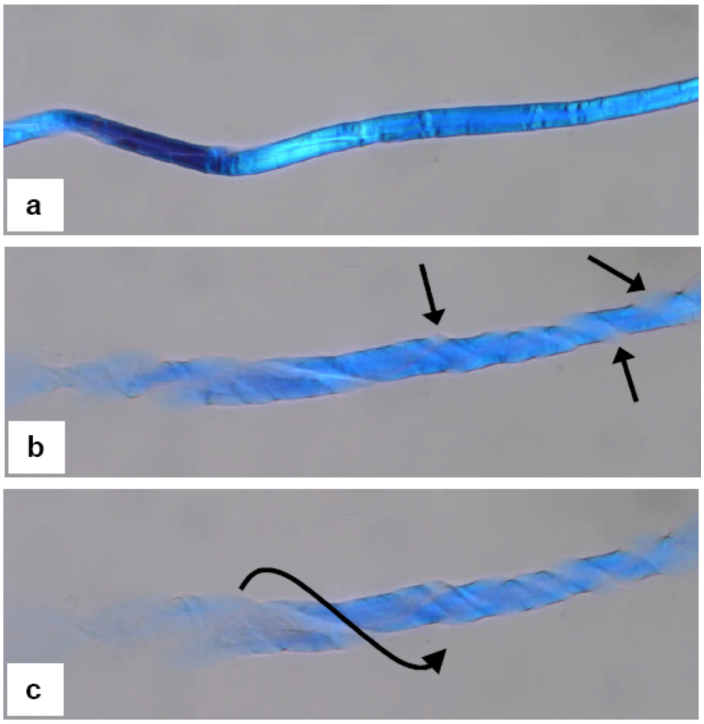


Fig. 27 TEMPO-oxidized pulp fiber dissolving homogeneously by unwinding of the internal helical cellulose structure; fiber completely dissolved after 30 s; a) saturated fiber K-CCE100-TEMPO; b) release of dissolved cellulose from the surface (arrows); c) direction of left handed untwisting

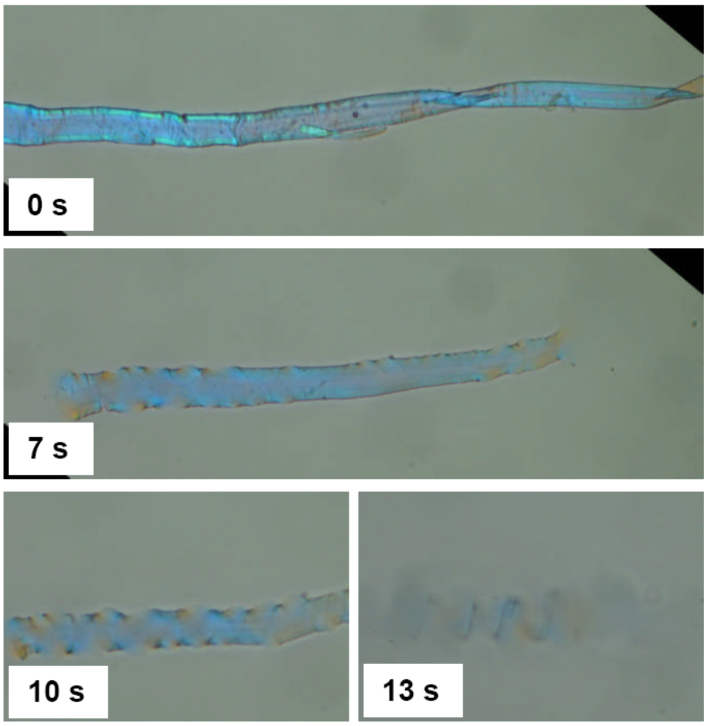


Fig. 28 Dissolution mechanism of conventionally-produced eucalypt sulfite dissolving pulps; fiber completely dissolved after 20 s

In contrast to the acid-base interaction of cellulose and NMMO in the dissolution process, cellulose forms complexes with the transition metal copper in Cuen-solution. Only the hydroxyl groups on C_2 and C_3 are incorporated in this complex (Fig. 29). Therefore, the C_6-carboxylate group doesn't hamper the dissolution process even at very high contents. Instead, homogeneous dissolution is even promoted because of the increased hydrophilic character of the S1 layer and the PW. Thus, mild TEMPO-mediated oxidation is suggested as a means for increasing the swelling capacity of the cell wall layers S1 and PW of MPPs, enabling homogenous dissolution.

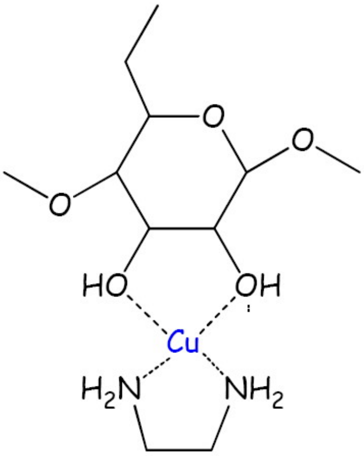


Fig. 29 Cellulose dissolution process in cupriethylene diamine; illustration adopted from Sixta (2011)

4 Conclusion and Outlook

The conversion of paper-grade pulps into dissolving pulps is currently of high economical interest for dissolving pulp producers. The commercial benefit of the new modified paper pulps compared to a conventional prehydrolysis kraft pulp is constituted by a substantial higher yield at a given R18 content. This yield advantage is based on the high selectivity of the cold caustic extraction treatment towards cellulose, while prehydrolysis of wood initiates a substantial cellulose yield loss owing to peeling reactions. Upon the applied cooking and refinery stages in the multifunctional alkaline pulping process, the reactivity of the resulting high-purity pulps is often reduced as compared to conventionally-cooked dissolving pulps. In this work, the development of efficient process steps to convert paper-grade pulps into dissolving pulps was investigated. The challenge of pulp refinement comprised the selective removal of hemicelluloses and the precise adjustment of pulp viscosity, while resulting in a highly reactive dissolving pulp as required for viscose application.

A commercial *Eucalyptus globulus* paper-grade kraft pulp was purified in the course of a total chlorine free bleaching sequence following the principle of the modified paper pulp production. Thereby, the effects of various enzyme treatments on the final dissolving pulp quality were investigated. A two-step treatment for hemicellulose removal was applied, comprising xylanase pretreatment combined with cold caustic extraction at reduced alkalinity as compared to a conventional one-step alkaline extraction step. This method efficiently removed the hemicelluloses from the pulp, and a lower cellulose II content and clearly increased pulp brightness were obtained. Xylan recovered from the alkaline extraction lye after xylanase treatment was slightly degraded. Thus, xylanase pretreatment is only recommended to be applied when the xylan fraction in the caustic lye is not further needed as polymeric reusable material and/or when the formation of cellulose II needs to be prevented (e.g. cellulose acetate-grade pulp). Instead, caustic extraction lye after xylanase treatment or filtrates of enzymatic hydrolysis can be used as sources for low molar mass xylan for the production of xylo-oligosaccharides (prebiotic food additive) or xylitol (sweetener) after appropriate enzymatic hydrolysis and Raney Nickel reduction, respectively. The xylanase pretreated pulp showed increased reactivity towards xanthation and high viscose dope quality in terms of particle content.

Controlled adjustment of final pulp viscosity was accomplished utilizing endoglucanase post-treatment. For the purpose of promoting the commercial applicability of endoglucanase, the effects of the wood source, the pulping process, the presence of non-cellulosic polymeric compounds as well as the extent of cellulose II in the pulp caused by alkaline pretreatment on the efficiency of the endoglucanase treatment were analyzed. Removing non-cellulosic compounds from pulps clearly increased the accessibility of the surfaces of the cellulose fibers to endoglucanase. This was confirmed by an increased chain scission upon enzymatic hydrolysis when hemicelluloses were removed from a eucalypt kraft pulp by alkaline extraction and xylanase treatment, respectively. The same effect was observed when lignin was removed from a spruce sulfite pulp by ECF-bleaching. Pulps with increased cellulose II content showed enhanced accessibility of cellulose towards endoglucanase because of their increased swelling capacity compared to pulps of native cellulose.

In order to elucidate the influence of enzyme treatments on the resulting pulp reactivity, the reactivity of enzymatically hydrolyzed pulps was compared to pulps degraded by acid-catalyzed hydrolysis. Thereby, viscose reactivity of sulfite pulps was not improved by endoglucanase treatment. Cold caustic extracted kraft pulps instead, experienced an increase in pulp reactivity upon endoglucanase treatment as confirmed by both viscose filterability and Fock-test. The endoglucanase treatment is obviously more effective on pulps with limited accessibility of the fiber surface as observed for kraft and soda-anthraquinone pulps after cold caustic extraction.

The reactivity of a pulp towards derivatization and dissolution is mainly determined by the accessibility of the hydroxyl groups, which can be increased by introducing a spacer as accomplished by chemical derivatization. These spacers also act as inhibitors of the coalescence of microfibrils during drying.

We selected TEMPO-mediated oxidation as a means of efficient surface carboxylation of a CCE-treated kraft paper pulp. The effect of the partial oxidation of the C_6-hydroxyl groups into carboxyl groups on pulp reactivity in derivatization processes, and dissolution in complexing and non-complexing solvents, respectively, was examined. In the course of viscose manufacture, the enhanced hydrophilic character and swelling capacity of the oxidized pulps inhibited dewatering of the alkali cellulose to the required cellulose concentration. Thus, the intense oxidation treatment caused an inverse effect on pulp reactivity and made the pulp impractical for the standard viscose application. Direct dissolution of oxidized pulps in *N*-methylmorpholine-*N*-oxide monohydrate, exceeding a certain degree of carboxylate groups at C_6, was impeded obviously due to a decreased ability to form hydrogen bonds with the solvent. In the complexing solvent cupriethylene diamine, however, the dissolution mechanism of cellulose was not deteriorated by the high content of C_6-carboxylate groups because only the hydroxyl groups on C_2/C_3 are incorporated in the complex formation with the transition metal. Instead, the oxidation procedure increased the hydrophilicity and thus the swelling capacity of the cell wall layers S1 and PW. Dissolution restrictions by the outer cell wall layers as commonly observed as ballooning phenomena were prevented, and homogeneous dissolution was achieved. Therefore, mild TEMPO-mediated oxidation is suggested as a means for increasing the swelling capacity of the cell wall layers S1 and PW of refined paper pulps promoting homogenous dissolution.

So far, the processability of pulps in current regenerated fiber processes is the key criterion for the applicability of dissolving pulps produced from paper-grade pulps. Thereby, filtration difficulties caused by undissolved pulp particles represent the most challenging task to be solved.

Thus, the most challenging task for future research work on dissolving pulps remains to develop strategies enhancing pulp reactivity with reference to the direct dissolution and heterogeneous derivatization of a pulp, resulting in high quality spinning dopes. Pulp treatments like endoglucanase depolymerization or surface derivatization are promising techniques aiming at an increase in pulp reactivity. However, future research work is needed to develop commercially attractive and, at the same time, even more efficient reactivity enhancing methods.

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6 Appendix

6.1 List of Abbreviations

[η]	Intrinsic viscosity
A	Acid treatment
AC	Alkali cellulose
$A_{Hydr.}$	Acid-catalyzed hydrolysis
AQ	Anthraquinone
$a_{s,\ BET}$	Specific surface area according to Brunauer, Emmet and Teller
AXU	Active xylanase unit
B	Brightness
CCE	Cold caustic extraction
CCOA	Carbazole-9-carboxylic acid [2-(2-aminooxyethoxy)ethoxy]amide
Cell II	Cellulose II content
CLP	Cotton linters pulp
CO	Carbonyl
COOH	Carboxyl
CrI	Crystallinity index
Cuen	Cupriethylene diamine
DCM	Dichloromethane
DMAc	Dimethylacetamide
DP	Degree of polymerization
ECF	Elemental chlorine free
ECU	Endocellulase unit
EG	Endoglucanase
Equ.	Equation
FDAM	9*H*-Fluoren-2-yl-diazomethane
FE-SEM	Field emission scanning electron microscopy
FT-Raman	Fourier transform Raman spectroscopy
FV	Filter value
HCE	Hot caustic extraction

hkl	Miller indizes
K	Kraft pulp
M	Mannanase
MALLS	Multi-angle laser light scattering
MB	Methylene blue
MPP	Modified paper pulp
MW	Molecular weight
Mw	Weight-average molecular weight
MWD	Molecular weight distribution
n.d.	Not determined
NMMO	*N*-Methylmorpholine-*N*-oxide
O	Oxygen bleaching
odp	Oven dried pulp
OH	Hydroxyl
P	Peroxide bleaching
PHK	Prehydrolysis kraft
PW	Primary wall
S	Chain scission
SEC	Size exclusion chromatography
TCF	Total chlorine free
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
WAXS	Wide angle X-ray scattering
WRV	Water retention value
X	Xylanase
Z	Ozone bleaching

6.2 List of Figures

Fig. 1 (a) Illustration of the cell wall layers and their chemical composition (Hoffmann and Jones 1990); (b) The cell wall structures of an individual wood cell. ML = middle lamella, P = primary wall, S = secondary wall (S1, S2, S3), W = warty layer (Côté et al. 1964) 2

Fig. 2 Concept of the multifunctional alkaline pulping process according to Sixta et al. (2007) 4

Fig. 3 Concept for the production of modified paper pulps 6

Fig. 4 Model structures of different dissolving pulps: PHK pulp (left), CCE-refined kraft pulp (right) 7

Fig. 5 Illustration of how enzyme treatment may increase pulp reactivity 9

Fig. 6 Carboxyl groups introduced by TEMPO-mediated oxidation form spacer between microfibrils 10

Fig. 7 Viscose-like solution obtained from the Fock-test 17

Fig. 8 Scheme of the Treiber-method following the industrial viscose process 17

Fig. 9 Filtration device for measuring the filter value following the Treiber-method; the picture was kindly supplied by Lenzing AG 18

Fig. 10 Nitrogen fraction recovered in the filtrate (F) and the washing filtrate (WF), relative nitrogen content of the filtrate, and volumetric ratio of the washing filtrate and the filtrate 23

Fig. 11 WAXS diffractogram of differently alkalized cotton linters pulps 26

Fig. 12 Oxidation of primary hydroxyls to carboxyls by the TEMPO/NaOCl/$NaClO_2$ system under weakly acidic or neutral conditions (Hirota et al. 2009) 28

Fig. 13 TEMPO-mediated oxidation of pulps performed in PE-bottles stored in a shaking water bath 29

Fig. 14 MWD of the pulp K-O, after X-treatment and CCE70, and after CCE100 without previous enzyme treatment 33

Fig. 15 Chain scission at increasing EG-concentrations after hemicellulose removal and TCF-bleaching of kraft pulps 34

Fig. 16 MWD of pulps treated with endoglucanase up to a concentration of 250 ECU g^{-1} opd; the TCF-bleached pulp represents the X-pretreated substrate for EG-treatment 36

Fig. 17 Upper diagram: chain scission of differently alkalized CLPs upon EG-treatment; lower diagram: linear correlation of S_{max} and the Cell II content 40

Fig. 18 Chain scission at increasing EG-concentrations after removal of hemicelluloses from the pulps (a) K-O and (b) S-5 42

Fig. 19 Chain scission at increasing EG-concentrations after delignification of the spruce sulfite pulps; $a_{s,\ BET}$ = specific surface area 44

Fig. 20 FE-SEM pictures of the pulp S-1; (a) S-1 before and (b)-(d) after EG-treatment (concentration 500 ECU g^{-1} odp); (b): fine and thin pulp components are removed by enzymes; (c): pulp fiber damage derived from enzymatic hydrolysis; (d): cracked S1 cell wall layer and S2 identified from the different orientations of the microfibrils (arrows).. 45

Fig. 21 (a) Viscosity decrease of the pulp S-1 during acid-catalyzed hydrolysis $A_{Hydr.}$ at pH 1.5 (varying hydrolysis time, upper abscissa) and EG-treatment (varying enzyme concentration, lower abscissa); (b) Viscosity decrease of TCF-bleached kraft pulps during acid-catalyzed hydrolysis $A_{Hydr.}$ for 60 min at 90 °C at varying pH (upper abscissa) and EG-treatment (varying enzyme concentration, lower abscissa); encircled: pulps for reactivity measurement 47

Fig. 22 FE-SEM pictures of the pulp S-1 after $A_{Hydr.}$ at pH 1.5 for 180 min; intense fiber fibrillation visible 48

Fig. 23 Molecular weight distribution (MWD) and carboxyl group content (COOH) determined from FDAM/SEC of the kraft pulp (K-ECF) and of the TEMPO-oxidized pulps (T) after 3 h and 24 h reaction time, respectively 51

Fig. 24 Dissolution of pulp fibers in NMMO.H_2O at 90 °C; time of fiber saturation is chosen as starting time “0 min”; a) K-CCE100-TEMPO from 0-12 min; b) K-TEMPO-CCE100 from 0-5 min 54

Fig. 25 Cellulose dissolution process in NMMO-water; illustration adopted from Michels and Kosan (2005).. 55

Fig. 26 K-CCE100-$A_{Hydr.}$-P pulp fiber swollen by intense ballooning in 0.5 M Cuen-solution at room temperature; the highly-ballooned structure was the final swelling state after 45 s 56

Fig. 27 TEMPO-oxidized pulp fiber dissolving homogeneously by unwinding of the internal helical cellulose structure; fiber completely dissolved after 30 s; a) saturated fiber K-CCE100-TEMPO; b) release of dissolved cellulose from the surface (arrows); c) direction of left handed untwisting........ 57

Fig. 28 Dissolution mechanism of conventionally-produced eucalypt sulfite dissolving pulps; fiber completely dissolved after 20 s 57

Fig. 29 Cellulose dissolution process in cupriethylene diamine; illustration adopted from Sixta (2011) 58

6.3 List of Tables

Table 1 Pulp specifications 13

Table 2 Residual xylan content of the eucalypt paper-grade kraft pulp K-O after X-treatment and alkaline extraction 32

Table 3 Carbohydrate composition and Mw of the beta-cellulose fractions precipitated from the caustic lyes of the CCE70-treatment after X-treatment, and from the CCE100-treatment 33

Table 4 Chain scission during EG-treatment of bleached kraft pulps of different cellulose II contents; Cell II determined by FT-Raman 36

Table 5 Results of the reactivity tests of the differently treated pulps and analysis of the hydrophilic character determined as WRV and COOH-content 38

Table 6 WAXS results and wet chemical analysis of differently alkalized cotton linters pulps 41

Table 7 Pulp properties of the spruce paper-grade sulfite pulp S-5 after enzyme treatments, HCE, and CCE for hemicellulose removal 42

Table 8 Pretrials of TEMPO-mediated oxidation of the kraft pulp K-ECF with the system TEMPO/NaOCl/$NaClO_2$ at pH 6.8 at 60 °C, reaction time 3-48 h 50

Table 9 Properties of the kraft pulps treated with TEMPO-mediated oxidation prior to and after CCE100, respectively, and of a reference pulp with acid-catalyzed hydrolysis $A_{Hydr.}$ applied for depolymerization 52

Paper 1

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Abstract The development of efficient process steps to convert paper-grade to dissolving pulps was investigated as part of the work programme to improve the process economics. The challenge of pulp refinement comprises the selective removal of hemicelluloses and the precise adjustment of the pulp viscosity, while maintaining the reactivity of the pulp as required for viscose application. The purpose of this study was to investigate the effects of various enzyme treatments on a commercial oxygen-delignified *Eucalyptus globulus* paper-grade kraft pulp in the course of a total chlorine free bleaching sequence in combination with refining techniques following the principle of Modified Kraft Cooking (Sixta et al. 2007). The objectives were to assess its applicability as viscose pulp besides the reduction of chemical consumption in alkaline and ozone bleaching steps by means of xylanase pre-treatment and the controlled adjustment of final pulp viscosity utilizing endoglucanase post-treatment. Xylanase pre-treatment combined with cold caustic extraction at reduced alkalinity efficiently removed the hemicelluloses from the pulp and clearly increased the pulp brightness by extensive removal of hexenuronic acid side chains. The xylanase pre-treated pulp showed increased reactivity towards xanthation and high viscose dope quality in terms of particle content. The dependence of cellulose chain scission on the applied endoglucanase concentration was analyzed in detail, and this allowed precise viscosity reduction as well as reactivity increase. The differently treated pulps, with and without xylanase pre-treatment, were of very narrow molecular weight distribution and the quality of the spun fibers were very similar to those viscose fibers from commercial dissolving pulps.

Keywords Degree of polymerization · Dissolving pulp · Viscose fibers · Endoglucanase · Enzyme · Filterability · Xylanase

Abbreviations

[η]	Intrinsic viscosity
A	Acid bleaching
AC	Alkali cellulose
AXU	Active xylanase unit
B	Brightness
CCE	Cold caustic extraction
DP	Degree of polymerization
ECF	Elemental chlorine free
ECU	Endoglucanase unit
EG	Endoglucanase
FV	Filter clogging value

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HexA	Hexenuronic acid
MWD	Molecular weight distribution
n.d.	Not determined
O	Oxygen bleaching
odp	Oven dried pulp
P	Peroxide bleaching
PHK	Pre-hydrolysis kraft pulp
SEC	Size exclusion chromatography
TCF	Total chlorine free
WRV	Water retention value
X	Xylanase
Z	Ozone bleaching

Introduction

Dissolving pulps consisting of wood-derived celluloses are the main source for the manufacture of viscose rayon and cellulose derivatives, such as cellulose esters and cellulose ethers. Dissolving pulps are generally characterized by high cellulose content, low hemicellulose content and only very minor amounts of residual lignin, extractives and minerals, as well as high brightness and very uniform molecular weight distribution (Hinck et al. 1985). The most commonly applied processes for dissolving pulp production are the acid sulphite cooking and the alkaline pre-hydrolysis kraft (PHK) cooking.

In the sulphite process, hemicelluloses and lignin are removed from the wood chips in the same process step, which impedes the recovery of hemicelluloses from cooking liquor. Nevertheless, about 60% of the market volume on dissolving pulp is produced by the sulphite technology. The pre-hydrolysis kraft process instead uses an alkaline cooking step, where hemicelluloses are extracted from the wood chips in big quantities prior to cooking. The technological implementation of this step of pre-extraction is the so-called pre-hydrolysis, in which the short-chain carbohydrates, preferably hemicelluloses, are hydrolyzed owing to autohydrolysis initiated by the release of acetic acid. Besides the advantages of high pulp quality and high cooking capacity, the PHK cooking also bears a big technological drawback. This main problem is the formation of highly reactive degradation intermediates in the resulting pre-hydrolyzate and these undergo condensation reactions and form sticky precipitates during drainage. These pitch-like compounds present a big technological challenge and have been discussed frequently in the focus of prevention, reduction or economical handling (Annergren et al. 1965; Leschinsky et al. 2008a, b; Sixta et al. 2006). In order to avoid the problem of precipitation, the adaption of steam activation followed by a neutralization step prior to alkaline cooking was realized as an alternative process to the pre-hydrolysis kraft cooking to produce high quality dissolving pulps (Wizani et al. 1994).

In recent years several studies focused on developing efficient process steps to convert paper-grade to dissolving pulps in the drive to increase the process economics. These studies aimed at maintained or even improved pulp quality, particularly for viscose application (Jackson et al. 1998; Köpcke et al. 2008). The main challenges in this process comprise the selective removal of hemicelluloses and the precise adjustment of pulp viscosity, and, most importantly, the increase in pulp reactivity as required for viscose application. Especially for the viscose application, but also for any other alkaline conversion process as for the manufacture of cellulose ethers, the removal of the alkali soluble hemicelluloses is necessary, because otherwise they are dissolved in the steeping lye and impair the conversion process. The difference between the conventional dissolving pulp production and the novel dissolving pulp production is that the latter is able to remove hemicelluloses without affecting the cellulose yield. However, at the same time the polydispersity of the molar mass distribution of the novel pulps is lower compared to conventionally produced dissolving pulps which implies a higher purity.

The removal of hemicelluloses from PHK pulps to attain very high cellulose purity as requested for cellulose acetate pulps is commonly performed by alkaline post-extraction and provides caustic lyes, which are further used for the recovery of hemicellulose fractions. Alkaline extraction at moderate temperatures from 20 to 40 °C is known as cold caustic extraction (CCE) (Jayme and Roffael 1969; Wallis and Wearne 1990). Alkaline treatment at elevated alkali concentration induces the conversion of the cellulose modification from cellulose I (native cellulose) to cellulose II (Na-cellulose I and regenerated cellulose after acidification) (Krässig 1984). Mercerized cellulose is less crystalline and more accessible to reagents (Sears et al. 1982), and thus, is known to show enhanced reactivity over native cellulose. The cellulose II modification is characterized by an increased number of inter- and intra-planar hydrogen bonds compared to cellulose I. As a consequence, mercerized pulps become relatively inert towards derivatization when they are dried because of the collapse of interfibrillar spaces (Kleinert 1975; Krässig 1984; Kyrklund and Sihtola 1963; Oksanen et al. 1997; Sears et al. 1982). The resulting fiber aggregates of a mercerized dried pulp show decreased surface area and pore volume, which is called hornification, and are thus less accessible and less reactive to esterification agents (El-Din and El-Megeid 1994; Jayme and Schenck 1949).

In order to avoid the problem of hornification, alternative solvents for extraction of hemicelluloses such as cupriethylenediamine and nitren (Puls et al. 2006) or organic solvents were also investigated.

Anyhow, a small fraction of alkali-resistant hemicelluloses still remains in the intermicellar pulp material after alkaline cooking and alkaline post-extraction (Rydholm 1965; Sixta 2006b). These residual hemicelluloses are thought to compete with cellulose in the step of xanthation in the viscose process, becoming apparent in decreased pulp reactivity (Sixta 2006a). Thus, gentle methods are needed for the maximum removal of hemicelluloses in order to yield highly reactive pulps with low residual xylan content. In this context, the utilization of enzymes for pulp pre-treatment in combination with alkaline post-extraction at reduced alkalinity has turned out to be of interest.

Jackson et al. (1998) investigated xylan removal from a bleached hardwood kraft pulp by combining xylanase (X) treatment and CCE with sodium hydroxide (NaOH) concentrations from 80 to 100 g L^{-1}. They found a lower residual xylan content of the pulp after X-treatment followed by a CCE-stage applying a caustic solution containing 80 g NaOH L^{-1} compared to the pulp after extraction at an elevated alkali concentration of 100 g NaOH L^{-1}. Further information about xylan removal from wood- and non-wood-pulps with the help of enzymes was given by Bajpai and Bajpai (2001), Köpcke et al. (2008, 2010) as well as Ibarra et al. (2010). Roncero et al. (2005) used scanning electron microscopy to characterize the surface of xylanase treated fibers and found external fibrillation. They ascribed this aspect to the removal of surface xylan, which is re-deposited during the final stage of kraft cooking at decreased alkalinity. Similar observations were reported by Spence et al. (2009).

Besides the main purpose of xylan removal, xylanase pre-treatment prior to ECF and TCF bleaching is a promising technique to reduce bleaching chemical consumption. This aspect of xylanase pre-treatment has been reported as a "bleach boosting" effect and allows the removal of xylan from lignin–carbohydrate-complexes, thereby allowing the enhanced leaching of entrapped lignin subsequently (Bhat 2000). This effect is seen from the perspective of significant brightness gain and kappa number reduction during xylanase treatment (Allison and Clark 1994; Ates et al. 2009; Brown et al. 1994; Shatalov and Pereira 2008a; Shatalov and Pereira 2008b; Yang et al. 1993). Valchev and Tsekova (2008) adopted the step of xylanase post-treatment to the bleaching processes of pulps in order to remove chromophoric structures connected to the accessible surface xylan. Besides pre-treatment of pulp, xylanase pre-treatment was also adapted to eucalypt wood chips and was reported to result in increased pulp yields in kraft cooking for a given kappa number compared to kraft cooks of non-pre-treated wood chips (Bertaud et al. 2008).

Besides the high demand for dissolving pulp with low residual content of hemicelluloses, the reactivity is one of the most important quality parameters. The reactivity of a pulp concerning derivatization reactions such as esterification, etherification or xanthation is mainly determined by the accessibility of the hydroxyl groups on C_6 and C_2 of the glucose monomer units to the reactants (Sixta 2006b). This parameter determines the processability of a dissolving pulp for the viscose process (Sixta 2006a). Thus, detailed characterization of the fiber surface in terms of hemicellulose residues and fibrillation as well as fibril structure in terms of hornification, accessible inner surface and pore volume is required to fully understand the term of reactivity (Krässig 1993a). Many treatments such as swelling, solvent exchange, degradation procedures (Krässig 1993b) and chemical modification (Sears et al. 1982) were investigated for increasing the reactivity of a pulp.

Within the last few years, the monocomponent endoglucanase (EG) treatment to increase pulp reactivity and, as a side effect, precisely adjust the viscosity of a pulp has been the focus of much work (Engström et al. 2006; Henriksson et al. 2005; Ibarra et al. 2010; Köpcke et al. 2008; Luo 2009).

Endoglucanase preferably degrades amorphous cellulose located on the fiber surface and in-between the microfibrils, which leads to increased crystalline surface exposure and to increased swelling ability and reactivity of the pulp (Henriksson et al. 2005). Additionally, cellulose II is attacked by endoglucanase (Atalla 1979; Rahkamo et al. 1998), which was speculated to play a role in reactivity increase of the pulp after endoglucanase treatment (Engström et al. 2006; Henriksson et al. 2005). Associated with the preferred degradation of certain cellulose modifications, an increased crystallinity index of the pulp after endoglucanase treatment was presented (Cao and Tan 2006). Krässig (1993a) explained this relative increase in crystallinity by the degradation of amorphous and easily accessible structures or the re-crystallization of amorphous regions when textural stress is released from the pulp. Medve et al. (1998) found the release of significant amounts of low-DP (degree of polymerization) products in the pulp after endoglucanase treatment and assumed some processivity or pseudo-processivity of the enzyme. Hilden et al. (2005) further confirmed the formation of a low-molecular weight fraction by scanning electron microscopy of enzyme degraded pulps and revealed the preferred cutting of loose ends of cellulose chains by the enzymes. Strey et al. (2009) investigated the influence of endoglucanase treatment on fiber stability and found reduced strength properties due to decreased fibrillation and inter-fiber bonding.

In this study, the applicability of the steps of xylanase pre-treatment and endoglucanase post-treatment within a full bleaching sequence in relation to process conditions and analytics was thoroughly investigated. Xylanase pre-treatment of a commercial *Eucalyptus globulus* kraft pulp was applied after oxygen delignification and prior to CCE, TCF bleaching and final endoglucanase post-treatment. We examined the effects of xylanase pre-treatment on the efficiency of CCE in xylan removal and the molecular weight distribution (MWD) of the pulp after CCE and of the solubilized xylan fraction. Furthermore we investigated the effects of xylanase pre-treatment on the final bleaching performance, the process of chain scission during subsequent endoglucanase treatment as well as the final quality and viscose reactivity of the pulp. The bleaching and purification sequence O-(X)-CCE-A-Z-P-EG was chosen to evaluate the effect of the X-pre-treatment.

Experimental

Starting pulp

Oxygen delignified *Eucalyptus globulus* kraft pulp from the pulp mill in Huelva, Spain, was prepared from wood chips from Uruguay and was kindly supplied by ENCE. This pulp was used for all bleaching experiments. The characteristics of the starting pulp are listed in Table 1.

Enzyme preparations

The enzyme preparations were kindly supplied by Novozymes, Denmark. The xylanase preparation Pulpzyme® HC, in the following referred to as xylanase, is produced by submerged fermentation of a genetically modified *Bacillus licheniformis* microorganism and its concentration is standardized by the manufacturer to 1000 AXU g^{-1} (active xylanase units). The monocomponent endoglucanase preparation Novozym® 476, in the following referred to as endoglucanase, is produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and its concentration is standardized by the manufacturer to 4,500 ECU g^{-1} (endoglucanase units).

Table 1 Parameters of a commercial O-treated *E. globulus* kraft pulp, used as starting pulp

Kappa number (–)	10.3
HexA [a] (μmol g^{-1} odp)	49.2
Brightness (%ISO)	58.8
R10 (%)	86.2
R18 (%)	89.5
[η] (mL g^{-1} odp)	895
WRV (%)	151
Ash at 850 °C (%)	0.8
Glucan (%)	70.0
Xylan (%)	22.5
Mannan (%)	0.0
Arabinan (%)	0.0
Rhamnan (%)	0.0
Galactan (%)	0.1
M_w (kg $mole^{-1}$)	452

The given M_w is calculated from GPC-MALLS measurements

[a] Hexenuronic acid (HexA) content is accounting for ≈4.2 kappa number units (Li and Gellerstedt 1997)

Bleaching and enzyme treatments of the kraft pulp

All chemicals were of analytical grade.

Enzyme treatments

Enzyme treatments and the washing procedure for enzyme inactivation were performed according to Köpcke et al. (2008) at 3% consistency in a phosphate buffer pH 7 (11 mM NaH_2PO_4, 9 mM Na_2HPO_4; deionised water) at 60 °C for 120 min (xylanase treatment) and at 50 °C for 60 min (endoglucanase treatment), respectively. In order to reach a homogeneous enzyme distribution, the enzyme substrate was mixed with the buffer solution prior to addition to the pulp. Enzyme treatment was conducted in polyethylene flasks, being shaken strongly every 30 min. In order to stop the enzyme treatment and remove the enzyme solution, the pulp was filtered with a commercial suction strainer of pore size 2 and was quickly washed with hot deionised water (90 °C). The pulp cake was put back into the flask and hot deionised water was added (consistency 3%) for inactivation of the residual enzymes. The flask was stored in a 90 °C water bath for 30 min for complete enzyme inactivation. Subsequently, the pulp was filtered, washed with cold deionised water and drained to a dry content of about 30%.

Caustic extraction and TCF-bleaching

The substrate pulp was subjected to cold caustic extraction for hemicellulose removal. Pre-trials in the stage of cold caustic extraction (40-70 g NaOH L^{-1}, 30-70 min, 30 °C, 10% consistency) were carried out in polyethylene flasks (Wallis and Wearne 1990) to determine the required alkali concentration for the adjustment of the residual xylan content (target xylan content 4.5-5%). Based on these results, the appropriate conditions were chosen for final CCE (NaOH concentrations of 70 g L^{-1} with X-pre-treatment and 100 g L^{-1} without X-pre-treatment; 30 min).

Hot acid treatment was conducted for removal of hexenuronic acid side chains at pH 3.5 adjusted with sulfuric acid and was performed at 90 °C for 60 min in polyethylene flasks at 10% consistency according to Vuorinen et al. (1999).

Final pulp bleaching comprised an ozone stage followed by hydrogen peroxide bleaching. Pre-trials in Z-bleaching were performed to determine the required amount of ozone for the adjustment of the intrinsic viscosity $[\eta]$ to a level of 700–750 mL g^{-1}. Ozone bleaching was carried out in a medium-consistency high-shear mixer at 50 °C for 10 s at pH 2.5 and 10% consistency. The ozone charge was adjusted to the viscosity target (ozone consumption of 3.3 kg t^{-1} odp with X-pre-treatment and 2.9 kg t^{-1} odp without X-pre-treatment).

Peroxide bleaching was conducted at standard conditions applying 10 kg NaOH t^{-1} odp, 8 kg H_2O_2 t^{-1} odp and 1 kg $MgSO_4 \cdot 7H_2O$ t^{-1} odp at 80 °C for 120 min at 10% consistency. The final bleached pulps were acidified with diluted sulphuric acid prior to testing and analytical characterization.

Viscose preparation

Viscose preparation and characterization in terms of particle content and filterability (filter clogging value) were performed according to a modified method by Treiber et al. (1962). Particles in a range of 3–155 μm were measured on a Pamas device operating according to the light blockade principle (sensor model HCB25/25, serial number W-2525-2).

Viscose fiber production

Viscose solution was extruded through a 20-hole spinneret into a spinning bath on a bench-scale unit. The fibers were cut to staples with a length of 40 mm and were washed to remove acid, salts and occluded sulfur.

Analytical methods

Carbohydrate content was measured after a two stage total hydrolysis by high performance anion exchange chromatography with pulsed amperometric detection (Sixta et al. 2001). Ox-Dem kappa number was determined according to Li and Gellerstedt (2002), HexA content according to Gellerstedt and Li (1996), water retention value (WRV) according to Zellcheming (1957) and carboxylic group content COOH according to Philipp et al. (1965). Kappa number was determined according to TAPPI (1993b), beta-cellulose according to TAPPI (1993a), freeness of pulp following the Canadian standard method (CSF) according to TAPPI (2004), brightness according to ISO (2009), intrinsic viscosity according to SCAN-CM (1999), ash content at 850 °C according to DIN (1981) and alkali resistance according to DIN (1977). Alkaline-soluble lignin was determined by UV-absorption at 280 nm with an extinction coefficient of 26.3 L g^{-1} cm^{-1} calibrated for alkaline pre-extraction lye. The degree of polymerization was calculated from the intrinsic viscosity according to Marx-Figini (1978).

Molecular weight distribution was measured by size exclusion chromatography (SEC) with multi-angle light scattering (MALLS) detection in LiCl/DMAc solution according to Schelosky et al. (1999). The degree of crystallinity (CrI) and the cellulose II content (Cell II) were determined with FT-Raman (Röder et al. 2006; Ruland 1961). FT-Raman measurements were done using a Bruker IFS66 with Raman module FRA106, Nd:YAG Laser 500 mW; Laser wavenumber 9,394 cm^{-1} (1,064 nm), liq. N_2 cooled Ge-Detektor, 3,500-100 cm^{-1}, resolution 4 cm^{-1}, 100 scans, 4 measurements of each sample. The sample of 300 mg was pressed with 6 t cm^{-2} for approximately 10 min. Analysis was performed with a chemometric model, WAXS data were used as a standard for calibration and comparison.

Results and discussion

Eucalyptus globulus kraft pulp was treated following the sequence O-(X)-CCE-A-Z-P-EG, with and without xylanase pre-treatment. The main analytical data of the bleached pulps after each treatment step are summarized in Table 2.

Xylanase pre-treatment and caustic extraction

Xylanase pre-treatment of the starting pulp solubilized 46% of the initial xylan to a content of 12.1%, which is in good agreement with the results found by Köpcke et al. (2008). Kappa number decreased by 3.9 units (kappa 6.4), whereas brightness increased by 8.6% ISO. Shatalov and Pereira (2008b) showed, that brightness increase during X-pre-treatment is

Table 2 Parameters of the differently bleached pulps with optional xylanase pre-treatment

	Kappa (–)	HexA (µmol g^{-1})	B (%)	[η] (mL g^{-1})	R18 (%)	Xylan (%)	Y (%)
Starting pulp	10.3	49.2	58.8	895	89.5	22.5	–
With X-pre-treatment[a]							
X	6.4	25.4	67.4	945	90.9	12.1	88.7
CCE70	3.6	10.0	70.6	1025	96.8	4.9	89.8
A	3.6	9.1	71.7	1020	96.9	n.d.	98.9
Z-3.3	0.9	n.d.	85.3	720	96.8	n.d.	100.0
P	0.4	0.2	91.8	585	96.9	4.8	99.5
EG20	0.4	0.0	91.7	420	95.9	4.7	99.3
						Y_{tot} (%)	77.9
Without X-pre-treatment[b]							
CCE100	3.9	12.1	68.0	1015	98.3	4.2	80.4
A	3.4	6.9	68.7	945	98.2	n.d.	97.5
Z-2.9	0.9	n.d.	84.2	675	97.9	n.d.	99.9
P	0.5	0.1	90.2	645	97.8	4.0	98.9
EG15	0.5	0.3	90.6	330	96.8	4.5	95.1
						Y_{tot} (%)	73.7

Yield, Y, was determined gravimetrically for each bleaching stage. The total yield throughout the bleaching sequence, Y_{tot}, was calculated by multiplying the single stage yields

[a] CCE with 70 g NaOH L^{-1}, Z-bleaching with 3.3 kg ozone t^{-1}, EG-treatment with 20 ECU g^{-1}

[b] CCE with 100 g NaOH L^{-1}, Z-bleaching with 2.9 kg ozone t^{-1}, EG-treatment with 15 ECU g^{-1}

fully ascribed to the removal of hexenuronic acids. The HexA content of the starting pulp was reduced by almost 49% during X-pre-treatment. According to Li and Gellerstedt (1997), the removed amount of HexA corresponds to a kappa number reduction of about 2 units. Thus, a significant proportion of the xylanase-induced kappa number reduction can be attributed to mechanisms distinct from HexA removal, which is in line with the results published by Jeffries et al. (1998). They analyzed material that was solubilized upon X-treatment and found no methoxy groups in the material. Thus, they assumed that the residual delta in kappa number derived from the removal of degraded carbohydrate components but not lignin.

During X-pre-treatment, the molecular weight of the xylan was substantially decreased, as can be verified by SEC. The MWD of the pulps revealed the extraction of notable amounts of xylan and chain degradation of the residual hemicelluloses of the pulp upon X-pre-treatment (Fig. 1). In the subsequent CCE70 treatment, the xylan content was reduced to a comparable level as after the one-stage CCE100 treatment (residual xylan 4.9% after X-CCE70 and 4.2% after CCE100). The difference in the R18 alkali resistance observed for the two pulps (96.8% after X-CCE70 and 98.3% after CCE100) may be attributed to the higher solubility of the residual xylan of the CCE70 pulp owing to its lower molecular weight.

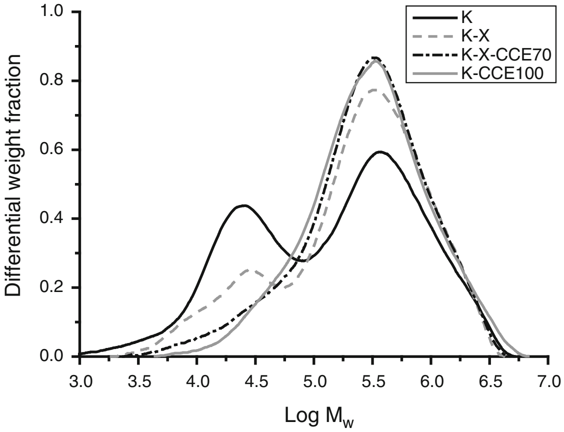

Fig. 1 Molecular weight distribution of the starting pulp (K), after xylanase treatment (K-X) and alkaline extraction with 70 g NaOH L^{-1} (K-X-CCE70) and after alkaline extraction with 100 g NaOH L^{-1} without previous enzyme treatment (K-CCE100)

Xylan chain degradation during X-pre-treatment was further confirmed by the analysis of the beta-cellulose fractions from the CCE-lyes (Table 3). Beta-cellulose is the alkali-soluble fraction of a pulp, which contains mainly hemicelluloses that are insoluble under slightly acidic conditions (pH 2.5). The molecular weight of the beta-cellulose isolated from the CCE70-lye after X-pre-treatment was clearly lower ($M_w = 11.4$ kg mole^{-1}) as compared to the beta-cellulose derived from the CCE100-lye of the starting pulp ($M_w = 21.8$ kg mole^{-1}). The main carbohydrate component of both beta-cellulose fractions was xylan, which showed that the low-DP cellulose fraction did not re-precipitate on acidification. Further on, only minor amounts of lignin were detected in the lyes (Table 3). Consequently, owing to the high degree of xylan chain degradation, xylanase pre-treatment is only recommended for use when the xylan fraction in the caustic lye is not further needed as polymeric reusable material.

Bleaching and endoglucanase treatment

Subsequent to optional xylanase pre-treatment and CCE, hot acid treatment (A) was applied for effective and selective removal of HexA, as reported by Vuorinen et al. (1999) and Jiang et al. (2000).

In the case of the X-pre-treated pulp, hardly any removal of HexA upon the A-stage was seen (Δ HexA < 1 μmol g^{-1}). Furthermore, A-bleaching did not cause any kappa number reduction (Δ kappa = 0), which is in good agreement with the assumption, that easily available HexA was already removed during xylanase pre-treatment (Shatalov and Pereira 2008b). In the case of the CCE100 treated pulp, kappa number decreased by 0.5 units, indicating HexA removal of 5.8 μmol g^{-1} according to the calculations of Li and Gellerstedt (1997). HexA determination revealed a

Table 3 Composition of the caustic lyes of the CCE70 treatment after X-pre-treatment and of the CCE100 treatment

Fraction	CCE-Lye	Beta-cellulose		M_w (kg mole^{-1})
	Lignin (mg L^{-1})	Carbohydrate composition		
		Glucan (%)	Xylan (%)	
X-CCE70	130	0.1	99.5	11.4
CCE100	320	0.5	98.9	21.8

decrease by 5.2 μmol g^{-1} upon A-bleaching, meaning that kappa number change was fully attributable to HexA removal.

Ozone bleaching conditions were optimized to reach a degree of polymerization (DPv) of ~2,000 (target [η] 750 mL g^{-1}). The DPv designates the average number of anhydroglucose units linked together by β-glucosidic linkages in the long cellulose chain molecule (Krässig 1993c). Cellulose chain degradation was measured in terms of decreasing viscosity and was expressed as chain scission S [$S = (DP_0/DP_t) - 1$], which represents the number of chain cleavage steps per initial cellulose chain. Ozone bleaching proceeded similarly for both pulps in regard to cellulose chain degradation and chemical consumption. The X-pre-treated pulp consumed 3.3 kg ozone t^{-1} odp, resulting in a chain scission $S = 0.58$. Ozone treatment affected a comparable chain scission on the pulp without enzyme treatment, $S = 0.55$, consuming slightly less amount of ozone (2.9 kg t^{-1} odp).

Final brightness adjustment was performed with peroxide bleaching and revealed an increase in brightness of 1.6% ISO of the X-pre-treated pulp.

DP-adjustment with endoglucanase was applied as the final treatment step aiming at about 450 mL g^{-1} (DPv = 1,050), the target viscosity for viscose staple fiber kraft pulps. Pre-trials were carried out at different EG-concentrations in the range of 5–250 ECU g^{-1} odp and revealed different depolymerization behavior of the pulps. Chain scission S in dependence of the applied enzyme concentration followed the concept of Michaelis–Menten kinetics for both pulps (Fig. 2). *S*_max represents the maximal chain scission according to this model and the Michaelis-constant KM gives the enzyme concentration EG, at which half of the maximal chain scission is reached (Grassl et al. 1987). Final EG-treatment decreased the viscosity of the X-pre-treated pulp to 420 mL g^{-1} odp ($S = 0.55$) at an enzyme concentration of 20 ECU g^{-1} (EG20) and the viscosity of the reference pulp without X-pre-treatment to 330 mL g^{-1} ($S = 1.15$) at an enzyme concentration of 15 ECU g^{-1} (EG15).

Figure 3 shows the MWD of the pulp bleached in the sequence O-X-CCE-A-Z-P-EG at varying EG-concentrations. The peak maxima of all EG-treated pulps shifted towards lower values with increasing EG-concentrations. The fraction of low molecular weight components (DP < 50) increased at EG-concentrations >50 ECU g^{-1} odp. This observation can be traced back to the preferred cleavage of loose cellulose chain ends and became apparent as a slight shoulder in the MWD (Kvarnlöf et al. 2008; Medve et al. 1998). The pulps without xylanase treatment gave an analogous MWD (not shown).

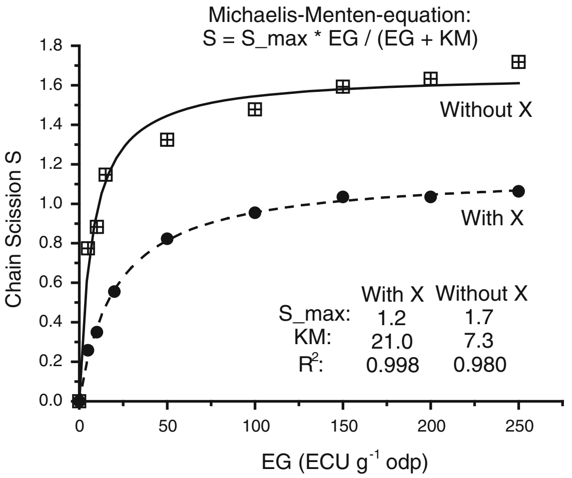

Fig. 2 DP-adjustment with endoglucanase treatment expressed as chain scission *S* at different enzyme concentrations (EG). *S*_max = maximal chain scission, *KM* Michaelis-constant

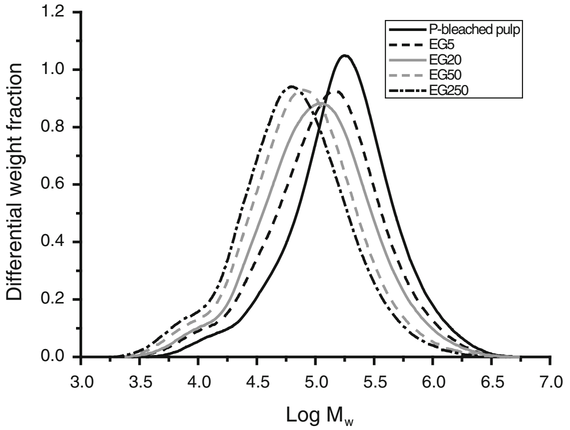

Fig. 3 MWD of pulps treated with endoglucanase up to a concentration of 250 ECU g^{-1} opd. The P-bleached pulp represents the X-pre-treated substrate for EG-treatment

Final pulp quality and reactivity

The X-pre-treated pulp, here referred to as K-X-CCE70-EG20, and the pulp without xylanase treatment, here referred to as K-CCE100-EG15, were analyzed regarding their properties as required for viscose application. The pulps were compared with an *E. globulus* kraft pulp extracted with 90 g NaOH L^{-1} (CCE90), referred to as K-CCE90 (Table 4).

K-X-CCE70-EG20 showed an increased molar mass M_w but a broader MWD (183 kg $mole^{-1}$, PDI 3.3) compared to K-CCE100-EG15 (128 kg $mole^{-1}$, PDI 2.3) (Table 4). As emphasized previously, the CCE100 treated pulp showed a very low polydispersity index because of the efficient extraction of the degraded cellulose material in the step of cold caustic extraction at an alkali concentration showing the maximum cellulose solubility (Sixta 2006b).

The endoglucanase treated pulps showed a slightly higher degree of crystallinity CrI (54%) compared to the alkaline extracted kraft pulp K-CCE90 (52%). As described previously by Cao and Tan (2006), the crystallinity of the substrates was not affected by the X-pre-treatment.

Caustic extraction induced the formation of the cellulose II modification and yielded 8% cellulose II after CCE70 and 13% cellulose II after CCE90 and CCE100. Cellulose II was reported to be more susceptible to hydrolysis by endoglucanase (Atalla 1979; Rahkamo et al. 1998). For this reason, the favoured DP-decrease of K-CCE100-EG15 upon endoglucanase treatment might be attributable to the enzymatic hydrolysis of cellulose II besides amorphous cellulose. This can be manifested by the fact, that the cellulose II content of the pulp K-CCE100-EG15 decreased by 2% upon EG-treatment. Additionally, the yield of the EG-stage of the pulp K-CCE100-EG15 was significantly lower (99.3% with X-CCE70, 95.1% without X and CCE100), resulting in a decreased overall yield, Y_{tot}, throughout the sequence by 4.2% (Table 2). Anyhow, the preliminary results concerning the yield of an EG-stage will be a question of further investigations, but the achieved yields were in a satisfying range for commercial application.

The reactivity of the pulps was determined by measuring the filterability of the viscose dope as filter clogging value (FV). The filter clogging value is calculated from the permeated mass of viscose after 100 min and 150 min of filtration at 20 °C and 2 bar pressure. High filter values are interpreted as high pulp reactivity. The X-pre-treated pulp K-X-CCE70-EG20 had a higher reactivity (171) compared to K-CCE100-EG15 (119). Both enzyme treated pulps revealed higher filter values in comparison to other alkali extracted kraft pulps (Fig. 4), indicating an increase in reactivity caused by enzyme treatment.

Table 4 Structural properties and results of the application tests of the differently treated pulps, analysis of the viscose dopes and fiber characteristics (given for stretching 60%)

	K-CCE90	K-X-CCE70-EG20 With X	K-CCE100-EG15 Without X
Cell II (%)	13	8	13
CrI (%)	52	54	54
M_w (kg $mole^{-1}$)	216	183	128
PDI (–)	2.6	3.3	2.3
DP <50 (%)	0.6	1.5	0.9
FV (–)	94	171	119
Particle (ppm)	65	23	18
Xylan in AC (%)	n.d.	1.1	1.4
WRV (%)	102	113	120
COOH (μmol g^{-1})	n.d.	27.5	28.7
Tenacity (cN tex^{-1})	25.7	24.7	25.1
Elongation (%)	16.7	17.2	17.5
Working capacity (cN tex^{-1} %)	431	430	440

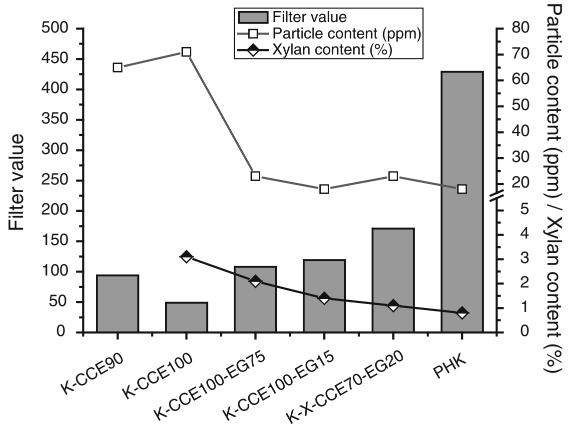

Fig. 4 Filter value, particle content and alkali-resistant xylan content of alkali cellulose of differently treated kraft pulps after alkaline extraction (K-CCE90, K-CCE100), xylanase and endoglucanase treatments (K-CCE100-EG75, K-CCE100-EG15, K-X-CCE70-EG20) in comparison to an *E. globulus* pre-hydrolysis kraft pulp (*PHK*)

Wollboldt et al. (2009) investigated the properties of an *E. globulus* kraft pulp after CCE100 (K-CCE100) and after subsequent EG-treatment with 75 ECU g^{-1} odp, referred to as K-CCE100-EG75. EG-treatment was shown to increase the filter value of the pulp from 49 to 108 and reduced the dope particle content significantly from 71 to 23 ppm. The particle content of the viscose dopes from the enzyme treated pulps were comparable (18–23 ppm) and in the high-quality range of the PHK pulp (18 ppm).

Drying or intense dewatering of pulps after treatment at high alkalinity is known to induce the formation of molecular aggregates by inter- and intra-fibrillar hydrogen bonding of the cellulose microfibrils, which is known as hornification. When acetylation is applied as further derivatization, this aggregate formation clearly reduces the reactivity of the pulp towards the acetylation reagent because of the decreased accessible surface area (Sixta 2006a). In terms of reactivity towards xanthation in the viscose process, this effect is not yet clear. In order to quantify hornification, the water retention value (WRV) as an indicator of the swelling capacity of a pulp might be determined. The WRV provides information about the integral pore volume of a pulp and the content of hydrophilic groups (carboxylic group content COOH). The WRV is determined as the weight gain of a sample after swelling in water and centrifugation, and high values are assumed to indicate a capacious capillary system. This enables homogeneous penetration of the dissolving lye into the pulp, uniform sorption and swelling of the pulp and a low particle content of the final viscose dope. This hypothesis is in good agreement with the results of this study. The never-dried pulp K-X-CCE70-EG20 showed a slightly lower hydrophilic behaviour (WRV 113%, COOH 27.5 μmol g^{-1}) in comparison to the pulp K-CCE100-EG15 (WRV 120%, COOH 28.7 μmol g^{-1}). The WRV of the K-CCE90 pulp was 102%, indicating a lower accessibility (Table 4).

The enzyme treated pulps were subjected to the viscose process at laboratory scale. After steeping, the xylan content in the alkali cellulose (AC) was measured. Wollboldt et al. (2009) concluded an indirect proportional correlation between the content of alkali-resistant xylan in AC and the reactivity towards viscose formation measured as filter clogging value (FV). The accumulated data of the current study confirmed this assumption. At a similar residual xylan content of the pulps (4.5–4.7%), 1.1% alkali-resistant xylan was detected in the X-pre-treated pulp K-X-CCE70-EG20 and 1.4% in the pulp K-CCE100-EG15. The previously mentioned PHK pulp showed a xylan content (4.7%) comparable to the pulps of this study, but only 0.8% of xylan remained in the AC after steeping (Wollboldt et al. 2009). In the case of alkaline extracted kraft pulps, endoglucanase post-treatment seemed to have a positive effect on the alkali-resistant xylan fraction and the reactivity of the pulp. EG-treatment of the kraft pulp K-CCE100 (5.7% xylan) was reported to reduce the xylan content of AC from 3.1 to 2.1% (Wollboldt et al. 2009). The correlation between the filter values and the xylan contents of AC of the differently treated kraft pulps is shown in Fig. 4.

The characteristics of the spun fibers made from the enzyme treated pulps and the kraft pulp K-CCE90 at 60% stretching are listed in Table 4. Mechanical fiber properties expressed in terms of tenacity, elongation and working capacity of the staple fibers made thereof were comparable, also to those made of commercial PHK pulps (not shown).

Conclusion

The present study revealed the beneficial effects of xylanase pre- and endoglucanase post-treatments in the course of a TCF-bleaching sequence to full brightness for the production of a novel high-purity dissolving pulp. A commercial oxygen delignified *E. globulus* kraft pulp was subjected to a (X)-CCE-A-Z-P-EG treatment, with and without xylanase pre-treatment.

We first examined the effects of xylanase pre-treatment on CCE and demonstrated that almost 50% of the initial xylan of the pulp was removed upon X-pre-treatment. Subsequent CCE at reduced alkalinity (70 g NaOH L^{-1}) was adequate to reach the target residual xylan content of the pulp. The pulp showed a broader molecular weight distribution and the xylan recovered from CCE-lye was highly degraded compared to the pulp extracted at an elevated alkalinity of 100 g NaOH L^{-1}. Thus, xylanase pre-treatment is only recommended to be applied when the xylan fraction in the caustic lye is not further needed as polymeric reusable material.

Furthermore we examined the effects of xylanase pre-treatment on final bleaching and the process of chain scission during subsequent endoglucanase treatment. Xylanase pre-treatment caused a substantial brightness increase of the pulp, which was fully attributable to the removal of hexenuronic acids. Thus, the step of acid treatment became obsolete in the case of the X-pre-treated pulp. The adjustment of the average degree of polymerization was followed in terms of chain scission and pursued the Michaelis–Menten kinetics for both pulps but with considerably increased chain cleavage for the pulp extracted at a high alkalinity of 100 g NaOH L^{-1}, possibly due to the higher proportion of cellulose II which is known to be more reactive towards endoglucanase treatment.

The opposite is known for the pulp reactivity towards chemical derivatization reactions. Thus, the xylanase pre-treated pulp showed an increased reactivity towards xanthation also because of the lower degree of fiber hornification. The differently treated pulps fulfilled the main characteristics as required for viscose application and were of narrow molecular weight distribution. The enzyme treated pulps showed clearly increased filterability and a reduced particle content of the viscose dopes in comparison to kraft pulps after CCE. These properties might be attributed to a more homogeneous penetration of carbon disulfide and dissolving lye into the fibers after enzyme treatment due to an enlarged capillary system, this being apparent in increased water retention values. This even penetration resulted in uniform swelling and dissolution of the pulp during viscose production.

In summary, this study gave very promising results and should promote further consideration about the applicability of enzymes in the process of dissolving pulp production. Xylanase-pre-treatment was demonstrated to be a very beneficial tool for effective removal of hemicelluloses, gain in brightness and brightness stability without a hot acid treatment (A) step and reduction in chemical consumption, while endoglucanase post-treatment was successfully applied for the precise adjustment of the degree of polymerization of the pulp and the improvement of viscose pulp reactivity. The commercial benefit of the new modified paper pulps compared to a conventional prehydrolysis-kraft pulp is constituted by a substantial higher yield at a given R18 content, ranging between 4 and 8% on oven dried wood depending on the wood source (Schild et al. 2010). During the CCE-purification physical phenomena are dominating the extraction of hemicelluloses and almost no cellulose degradation occurs, while prehydrolysis is a chemical process step involving carbohydrate degradation reactions. Thus, the yield advantage is based on the high selectivity of the CCE treatment towards cellulose, while prehydrolysis of wood introduces new reducing end groups through cellulose depolymerization which in turn initiates a substantial cellulose yield loss during subsequent alkaline cooking owing to peeling reactions.

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Paper 2

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DISSOLVING PULPS FROM ENZYME TREATED KRAFT PULPS FOR VISCOSE APPLICATION

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The purpose of this study was to illuminate enzymatic treatments as process steps for the efficient conversion of paper-grade pulps to dissolving pulps in terms of process economics. The challenge of pulp refinement comprises the selective removal of hemicelluloses and the precise adjustment of the pulp viscosity, while maintaining the reactivity of the pulp as required for viscose application.

A commercial oxygen-delignified *E. globulus* paper-grade kraft pulp was subjected to xylanase (X) pre-treatment combined with cold caustic extraction (CCE) at reduced alkali concentration and to endoglucanase (EG) post-treatment after TCF-bleaching. The xylanase pre-treated pulp showed increased reactivity towards xanthation and high viscose dope quality in terms of particle content in comparison to the pulp after cold caustic extraction at elevated alkali concentration.

The dependence of cellulose chain scission upon endoglucanase treatment on certain pulp qualities was analyzed in detail, and this allowed precise viscosity reduction as well as reactivity increase. The qualities of the spun fibers from the differently treated pulps were very similar to those viscose fibers from commercial dissolving pulps.

Keywords: *Dissolving pulp, endoglucanase, enzyme, filterability, reactivity, viscose fiber, xylanase*

Introduction

Demands for dissolving pulp and fibers have boomed world-wide within the last decade and thus nowadays much effort is put into the production of dissolving pulps from paper-grade pulps besides the dissolving pulp production from the commonly applied technologies such as the acid sulfite process and the alkaline pre-hydrolysis kraft process.

The main challenges in the conversion of a paper-grade pulp to overcome are the selective removal of hemicelluloses while ensuring high pulp reactivity.

For the viscose application the removal of the alkali soluble hemicelluloses is necessary, because otherwise they are dissolved in the steeping lye and impair the viscose process. A well-known method for the removal of hemicelluloses is the cold caustic extraction, CCE, which is an alkaline extraction at moderate temperatures from 25 to 45 °C and alkali concentrations from 5-10 % (w/w) NaOH in the lye [1]. Anyhow, pulp treatment at elevated alkali concentration induces the formation of the cellulose modification II [2]. The cellulose II crystal lattice is characterized by an increased number of inter-planar hydrogen bonds compared to cellulose I [3, 4]. Upon drying, inter-fibrillar spaces of mercerized pulps collapse and thus mercerized dried pulps show intensely decreased surface area and pore volume [2, 5-8]. This results in reduced pulp reactivity towards derivatization and is called hornification [9, 10]. The reactivity of a pulp is determined by the accessibility of the OH-groups at C_6 and C_2 of the glucose monomer units to the reactants [11] and the reactivity determines the processability of a dissolving pulp for the viscose process [12]. Many treatments such as swelling, solvent exchange, degradation procedures [13] and chemical modification [8] were investigated for increasing the reactivity of a pulp.

Within the last few years, the monocomponent endoglucanase (EG) treatment to increase pulp reactivity and, as a side effect, precisely adjust the viscosity of a pulp has been the focus of much work [14-19].

Endoglucanase preferably degrades amorphous cellulose located on the fiber surface and in-between the microfibrils, which leads to increased crystalline surface exposure and to increased swelling ability and reactivity of the pulp [15]. Additionally, cellulose II is attacked by endoglucanase [20, 21], which was speculated to play a role in reactivity increase of the pulp after endoglucanase treatment [14, 15].

In this study, the applicability of enzyme treatment steps within a full bleaching sequence in relation to process conditions was thoroughly investigated. For removal of hemicelluloses, a commercial oxygen-delignified *E. globulus* kraft pulp was treated with xylanase (X) and CCE prior to TCF (total chlorine free) bleaching. Finally, the pulp was subjected to an endoglucanase (EG) treatment in order to adjust the final viscosity and to increase the pulp reactivity. The experimental bleaching and purification sequence of choice was O-(X)-CCE-A-Z-P-EG, with and without xylanase treatment. Additionally, an ECF-bleached (elemental chlorine free) *E. globulus* kraft pulp was subjected to acid hydrolysis for comparative reasons concerning final pulp reactivity.

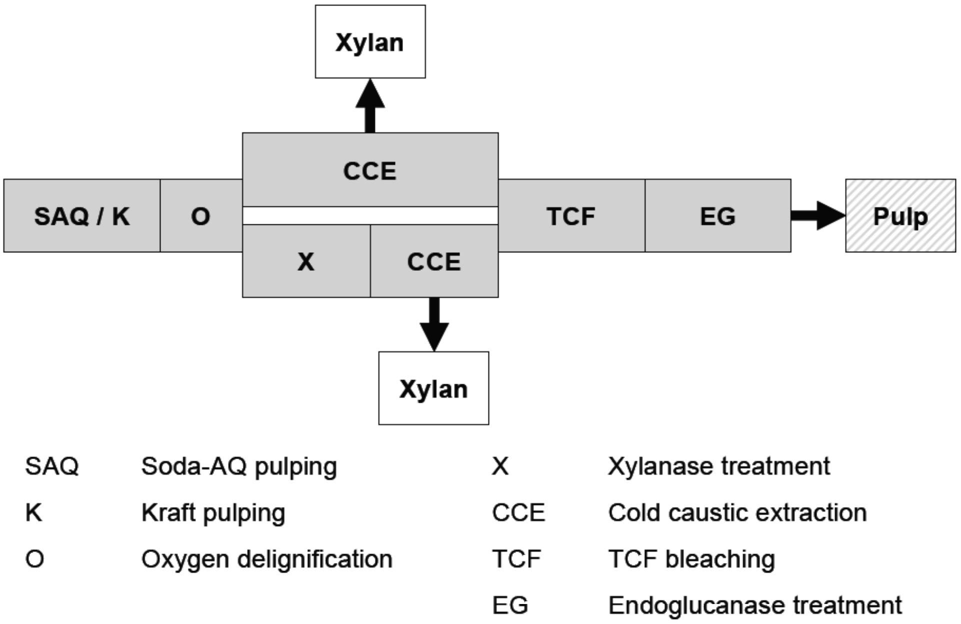

Figure 1. Concept of the Hem-Extra-process.

Table 1. Parameters of the HW-O and HW-ECF starting pulps and differently bleached pulps with optional xylanase pre-treatment.

	Kappa (--)	B (%)	[η] (mL g^{-1})	R18 (%)	Xylan (%)
HW-O	10.3	58.8	895	89.5	22.5
With X-pre-treatment [a]					
X	6.4	67.4	945	90.9	12.1
CCE70	3.6	70.6	1025	96.8	4.9
A	3.6	71.7	1020	96.9	n.d.
Z-3.3	0.9	85.3	720	96.8	n.d.
P	0.4	91.8	585	96.9	4.8
EG20	0.4	91.7	420	95.9	4.7
Without X-pre-treatment [b]					
CCE100	3.9	68.0	1015	98.3	4.2
A	3.4	68.7	945	98.2	n.d.
Z-2.9	0.9	84.2	675	97.9	n.d.
P	0.5	90.2	645	97.8	4.0
EG15	0.5	90.6	330	96.8	4.5
HW-ECF	6.0	90.1	750	91.5	19.1
CCE100	1.4	92.3	860	97.1	6.3
P	2.0	93.3	695	97.1	7.0
A (pH 1.5)	1.2	n.d.	410	n.d.	7.1

[a] CCE 70 g NaOH L^{-1}, Z-bleaching 3.3 kg ozone t^{-1}, EG-treatment 20 ECU g^{-1}

[b] CCE 100 g NaOH L^{-1}, Z-bleaching 2.9 kg ozone t^{-1}, EG-treatment 15 ECU g^{-1}

Experimental

Pulps and enzyme preparations

E. globulus kraft pulps after oxygen-delignification (HW-O) and after final ECF-bleaching (HW-ECF) were kindly supplied by ENCE (Table 1). Both pulps were prepared from wood chips from Uruguay in the pulp mill in Huelva, Spain. The xylanase preparation Pulpzyme® HC and the monocomponent endoglucanase preparation Novozym® 476 were kindly supplied by Novozymes, Denmark. Both preparations are produced by submerged fermentation of genetically modified microorganism.

Enzyme treatments

Enzyme treatments and the washing procedure for enzyme inactivation were performed according to Köpcke *et al.* [16] at 3 % consistency in a phosphate buffer pH 7 (11 mM NaH_2PO_4, 9 mM Na_2HPO_4; deionised water) at 60 °C for 120 min (xylanase treatment) and at 50 °C for 60 min (endoglucanase treatment), respectively. A detailed description of the handling of the enzyme treatments is given in our previous work [19].

Caustic extraction and TCF-bleaching of HW-O

The substrate pulp was subjected to cold caustic extraction with a target residual xylan content of 4.5-5 %. After xylanase-treatment, alkaline extraction with 70 g NaOH L^{-1} (CCE70) was performed while CCE100 (100 g NaOH L^{-1}) was necessary without enzyme pre-treatment to reach the target hemicellulose content (30 min, 30 °C, 10 % consistency). Hot acid treatment (A) was conducted at pH 3.5 adjusted with sulfuric acid at 90 °C for 60 min at 10 % consistency [22]. Final pulp bleaching comprised an ozone stage (Z) followed by hydrogen peroxide bleaching (P). Ozone bleaching was carried out in a medium-consistency high-shear mixer at 50 °C for 10 s at pH 2.5 and 10 % consistency. The ozone charge was adjusted to the viscosity target of 700-750 mL g^{-1}. Peroxide bleaching was performed at 80°C for 120 min at 10 % consistency applying 10 kg NaOH t^{-1} odp, 8 kg H_2O_2 t^{-1} odp and 1 kg $MgSO_4.7H_2O$ t^{-1} odp. The final bleached pulps were acidified with diluted sulfuric acid prior to testing and analytical characterization.

Caustic extraction and acid hydrolysis of HW-ECF

Alkaline extraction was performed with 100 g NaOH L^{-1} at 30 °C for 30 min at 10 % consistency. Peroxide bleaching was performed at 70 °C for 120 min at 10 % consistency applying 5 kg NaOH t^{-1} odp, 4 kg H_2O_2 t^{-1} odp and 1 kg $MgSO_4.7H_2O$ t^{-1} odp. Hot acid treatment of the pulp was performed at 90 °C for 60 min at 3 % consistency and at varying pH values from 1-3.

Viscose preparation and fiber production

Viscose preparation and characterization in terms of particle content and filterability were performed according to a modified method by Treiber *et al.* [23]. Particles in a range of 3 to 155 µm were measured on a Pamas device operating according to the light blockade principle (sensor model HCB25/25, serial number W-2525-2). Viscose solution was extruded through a 20-hole spinneret into a spinning bath on a bench-scale unit. The fibers were cut to staples with a length of 40 mm and were washed to remove acid, salts and occluded sulfur.

Table 2. Structural properties and results of the application tests of the differently treated pulps, analysis of the viscose dopes and fiber characteristics (given for stretching 60 %). The crystallinity index CrI represents the crystallinity of the samples containing cellulose I and II.

	HW-O	HW-O X-CCE70-EG20	HW-O CCE100-EG15
Cell II (%)	n.d.	8	13
CrI (%)	n.d.	54	54
M_w (kg $mole^{-1}$)	450	183	128
PDI (--)	4.9	3.3	2.3
FV (--)	n.d.	171	119
Particle (ppm)	n.d.	23	18
WRV (%)	151	113	120
Tenacity (cN tex^{-1})	n.d.	24.7	25.1
Elongation (%)	n.d.	17.2	17.5
Working capacity (cN tex^{-1} %)	n.d.	430	440

Analytical methods

Carbohydrate content was measured after a two stage total hydrolysis by high performance anion exchange chromatography with pulsed amperometric detection [24]. Water retention value (WRV) was determined according to Zellcheming [25], kappa number according to TAPPI [26], brightness according to ISO [27], intrinsic viscosity [η] according to SCAN-CM [28] and alkali resistance according to DIN [29]. The degree of polymerization (DPv) was calculated from the intrinsic viscosity according to Marx-Figini [30]. Fock-reactivity was measured according to a slightly modified method of Fock *et al.* [31, 32].

Molecular weight distribution was measured by size exclusion chromatography (SEC) with multi-angle light scattering (MALLS) detection in LiCl/DMAc solution according to Schelosky *et al.* [33]. The degree of crystallinity (CrI) and the cellulose II content (Cell II) were determined with FT-Raman [34, 35]. FT-Raman measurements were done using a Bruker IFS66 with Raman module FRA106, Nd:YAG Laser 500 mW; Laser wavenumber 9394 cm^{-1} (1064 nm), liq. N_2 cooled Ge-Detektor, 3500-100 cm^{-1}, resolution 4 cm^{-1}, 100 scans, 4 measurements of each sample. The sample of 300 mg was pressed with 6 t cm^{-1} for approximately 10 min. Analysis was performed with a chemometric model, WAXS data were used as a standard for calibration and comparison.

FE-SEM pictures were accomplished at the Wood Analytics Centre Lenzing (WAL), Lenzing, Austria. The sample was coated with a thin layer of Au/Pd and examined by high-resolution scanning electron microscopy (10,000 x magnification) with a Hitachi S4000 FE-SEM using an acceleration voltage of 10 kV and a working distance of 12 mm.

Results and discussion

Converting the paper-grade pulp HW-O into a dissolving pulp

Xylanase (X) pre-treatment of the starting pulp solubilized 46 % of the initial xylan to a content of 12.1 % (Table 1) which is in good agreement with the results found by Köpcke *et al.* [16]. During X-pre-treatment, the molecular weight of the residual xylan in the pulp was substantially decreased, as can be verified by SEC of the pulps. Xylan chain degradation during X-pre-treatment was further confirmed by the analysis of the beta-cellulose fractions from the CCE-lyes (data not shown) [19]. Consequently, owing to the high degree of xylan chain degradation, xylanase pre-treatment is only recommended for use when the xylan fraction in the caustic lye is not further needed as polymeric reusable material. In contrast, xylan is solubilized in CCE-lyes as high molecular weight polymeric material and thus can be utilized for many additional applications, as described by the principles of modified kraft cooking [36] and the Hem-Extra-process (Figure 1). These process-concepts describe the conversion of paper-grade pulps into dissolving pulps by extraction of hemicelluloses, TCF-bleaching and adjustment of final pulp viscosity and reactivity increase by endoglucanase treatment. The extracted hemi-fraction might be purified and separated into monomers and oligomers by filtration or re-utilized in the cooking or O-bleaching stage of a kraft paper pulp cook in order to attain increased paper pulp fiber strength. Subsequent to TCF-bleaching, DP-adjustment with endoglucanase (EG) was applied as the final treatment step aiming at about 450 mL g^{-1} (DPv = 1050), the target viscosity for viscose staple fiber kraft pulps. Final EG-treatment decreased the viscosity of the X-pre-treated pulp to 420 mL g^{-1} odp at an enzyme concentration of 20 ECU g^{-1} (EG20) and the viscosity of the reference pulp without X-pre-treatment to 330 mL g^{-1} at an enzyme concentration of 15 ECU g^{-1} (EG15). The elevated chain degradation of the CCE100-treated pulp might be traced back to its increased cellulose II content, which is preferably degraded by the enzymes. The final pulp properties, spinning dope qualities and the fiber characteristics are shown in Table 2. A FE-SEM picture of the pulp HW-O-X-CCE70-EG20 is shown in Figure 2. The CCE100-treated pulp was of high purity (R18 96.8 %) and thus showed a narrower molecular weight distribution, expressed as very low polydispersity index. The reactivity of the pulps was determined by measuring the filterability of the viscose dope as filter clogging value (FV). The X-pre-treated pulp HW-O-X-CCE70-EG20 had a higher reactivity (171) compared to HW-O-CCE100-EG15 (119). Both enzyme treated pulps revealed higher filter values in comparison to other alkali extracted kraft pulps [37, 38], indicating an increase in reactivity caused by enzyme treatment. Drying or intense dewatering of pulps after treatment at high alkalinity is known to induce the formation of molecular aggregates by inter- and intra-fibrillar hydrogen bonding of the cellulose microfibrils, which is known as hornification. In terms of reactivity towards xanthation in the viscose process, the influence of drying is not yet clear. In order to quantify hornification, the water retention value, which provides information about the integral pore volume of a pulp and the content of hydrophilic groups, was determined as an indicator of the swelling capacity of a pulp. High swelling capacity enables homogeneous penetration of the dissolving lye into the pulp, uniform sorption and swelling of the pulp and a low particle content of the final viscose dope. This hypothesis is in good agreement with the results of this study. The never-dried pulp HW-O-X-CCE70-EG20 showed a slightly lower WRV (113 %), but a higher dope particle content (23 ppm) in comparison to the pulp HW-O-CCE100-EG15 (WRV 120 %, particle 18 ppm). Mechanical fiber properties expressed in terms of tenacity, elongation and working capacity of the staple fibers made thereof were comparable (Table 2), also to those made of commercial PHK pulps (not shown).

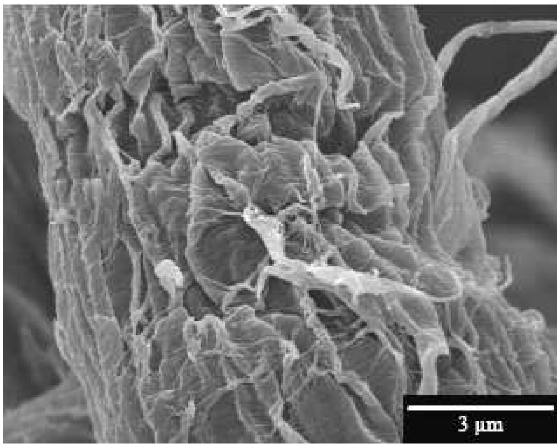

Figure 2. FE-SEM picture of the endoglucanase treated pulp HW-O-X-CCE70-EG20; destroyed surface structure visible.

Comparison of enzyme and acid degradation

In order to investigate the activating effect of an endoglucanase treatment, the reactivity according to Fock [31] of the enzymatically degraded pulp was measured and compared to an acidly degraded industrial HW-ECF paper-grade pulp of same viscosity after CCE100- and P-treatment (Table 1). The change in viscosity is shown in Figure 3. The Fock-method describes a lab-scale viscose process upon which an alkaline pulp suspension is mixed with carbon disulfide in order to get a viscose-like solution. From this solution, cellulose is regenerated by acidification and quantified by an oxidation- and titration-procedure. The Fock-reactivity given in weight percentage describes the amount of pulp which was dissolved in the viscose-like solution. Consequently, high Fock-reactivity indicates high pulp reactivity. Following the trend of the filter values, the pulp

HW-O-X-CCE70-EG20 showed a higher Fock-reactivity (26.8 %) compared to HW-O-CCE100-EG15 (22.9 %). The pulp degraded at pH 1.5 had an even lower Fock-reactivity of 19.1 % and a broader molecular weight distribution (PDI 2.9) compared to the other CCE100-treated pulp (PDI 2.3) was observed.

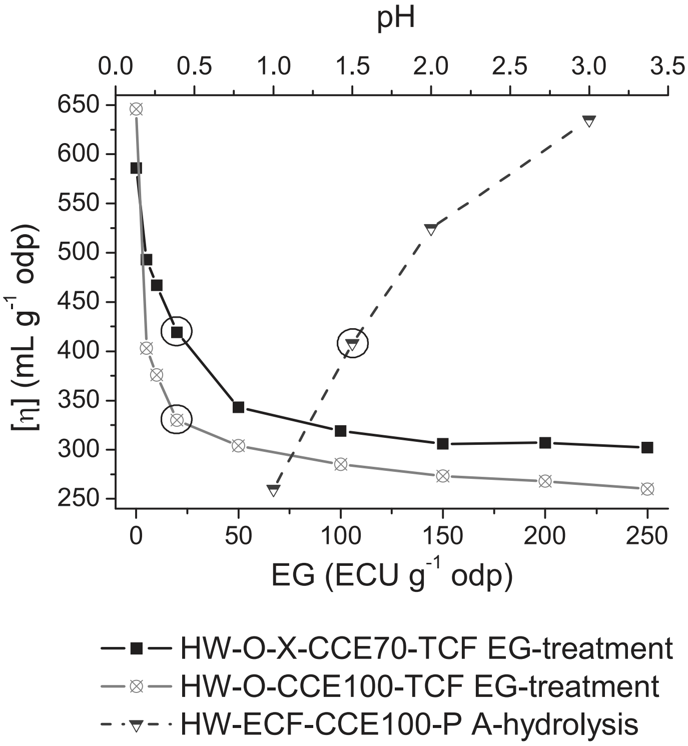

Figure 3. Comparison of cellulose degradation upon endoglucanase treatment and acid hydrolysis: EG-treatment of the dissolving pulps converted from the paper-grade pulp HW-O; A-hydrolysis of the HW-ECF paper-grade pulp after CCE100- and P-treatment; Encircled: pulps for Fock-reactivity measurement.

Conclusions

The present study revealed beneficial effects of xylanase pre-treatment and endoglucanase post-treatment in the production of novel high-purity dissolving pulps following the Hem-Extra-process scheme. A commercial oxygen delignified *E. globulus* kraft pulp was subjected to a (X)-CCE-A-Z-P-EG treatment, with and without xylanase pre-treatment, revealing the following effects:

- Xylanase (X) pre-treatment removed almost 50 % of the initial xylan of the pulp and thus subsequent CCE at reduced alkalinity was adequate to reach the target residual xylan content of the pulp.
- Xylan recovered from CCE-lye after X-pre-treatment was highly degraded and so the xylan fraction in the caustic lye was not useable anymore as polymeric material.
- The adjustment of the final average degree of polymerization was accomplished by endoglucanase treatment and a direct correlation between the cellulose II content and the chain cleavage of the pulps upon enzymatic degradation was confirmed.
- The xylanase pre-treated pulp showed an increased reactivity towards xanthation determined as

filter value and as Fock-reactivity because of the lower degree of fiber hornification.

- A comparison of enzymatically degraded pulps with an acidly hydrolyzed pulp showed higher Fock-reactivity for the enzyme-treated pulps, indicating an opening effect of the enzyme treatment on the fiber pore structures - this being apparent in increased water retention values, resulting in a more homogeneous penetration of carbon disulfide and dissolving lye into the fibers after enzyme treatment due to an enlarged capillary system.
- The differently treated pulps fulfilled the main characteristics as required for viscose application and the mechanical fiber properties expressed in terms of tenacity, elongation and working capacity of the staple fibers made thereof were comparable to those made of commercial PHK pulps.

The commercial benefit of the new Hem-Extra-pulps compared to a conventional prehydrolysis-kraft pulp is constituted by a substantial higher yield at a given R18 content, ranging between 4 % and 8 % on oven dried wood depending on the wood source [39]. This yield advantage is based on the high selectivity of the CCE treatment towards cellulose, while prehydrolysis of wood introduces new reducing end groups through cellulose depolymerization which in turn initiates a substantial cellulose yield loss during subsequent alkaline cooking owing to peeling reactions.

Acknowledgements

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Paper 3

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Biomacromolecules 13(3), 645-651

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Pulp Properties and Their Influence on Enzymatic Degradability

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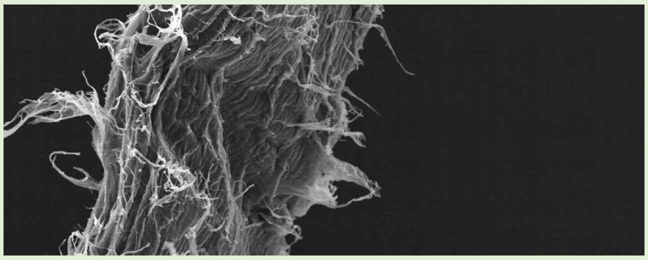
Supporting Information

ABSTRACT: Endoglucanase treatment of pulp for the adjustment of viscosity and the increase in pulp reactivity is a promising step in the concept for the beneficial production of dissolving pulps from paper grade pulps. To promote the commercial applicability of these enzymes, the influence of pulp properties such as carbohydrate composition, pulp type and cellulose morphology on the enzymatic degradability of a pulp was examined. High contents of hemicelluloses and lignin were shown to impair the accessibility of the cellulose to the enzymes. Due to the elevated swelling capacity of cellulose II, conversion of the cellulose morphology from I to II upon alkaline treatments showed a large increasing effect on the cellulose accessibility, and enzymatic degradability. Reactivity measurements of softwood sulfite pulps after enzymatic degradation and acid-catalyzed hydrolysis, respectively, revealed elevated reactivity for the pulp after acid treatment. This is in contrast to effects of enzyme treatments reported for CCE treated kraft pulps.



INTRODUCTION

In recent years, several studies investigated the applicability of enzymes in the production of dissolving pulps.[1−10] Therein, the main fields of enzyme application are the selective removal of hemicelluloses, increased pulp bleachability and brightness stability, adjustment of pulp viscosity, and the increase in pulp reactivity. Now, the influence of cellulose morphology, supramolecular pulp structure, and noncellulosic components on the efficiency of an enzyme treatment are not fully understood and therefore need to be evaluated for each individual pulp. It is the objective of this study to examine the effect of the most important pulp properties on the efficiency of an enzyme treatment and its effect on pulp reactivity toward viscose manufacture.

Regarding the removal of hemicelluloses from pulps, cold caustic extraction (CCE)[11,12] represents an effective method for the dissolution of hemicelluloses, especially xylan. If the alkali concentration in the extraction lye exceeds 8 wt %,[13] the crystal lattice of cellulose I gradually changes to that of Na-cellulose I and, upon regeneration, to cellulose II modification.[14] The cellulose II crystal lattice is characterized by additional intersheet O−H···O hydrogen bonds,[15,16] which might be responsible for the reduced pulp reactivity toward derivatization of mercerized dried pulps.[17−21] This behavior, referred to as hornification, is attributed to a decreased surface area and pore volume of pulps owing to the aggregation of microfibrils and still represents an important challenge in dissolving pulp production.

The reactivity of a pulp is defined as accessibility of the hydroxyl groups at C_6 and C_2/C_3 of the glucose monomer units to the reactants[13] and determines the processability of a dissolving pulp for the viscose process.[22] Thus, many pulp treatments aiming at increasing the accessibility of the hydroxyl groups of cellulose were investigated. Those comprise methods for preserving the pore volume of pulp fibers upon chemical treatments[23−25] and reopening collapsed structures of cellulose fibers prior to alkaline steeping.[25] Within the past few years, the pulp treatment with a monocomponent endoglucanase (EG) has been studied extensively aiming at the improvement of pulp reactivity.[1,3−5,7,9,26−28] *endo*-1,4-*β*-Glucanases randomly cleave glycosidic bonds within polysaccharide chains and typically act on less-ordered cellulose structures.[29] One suggested aspect of pulp activation upon EG-treatment is enhanced chain scission as observed for cellulose II compared to cellulose I.[1,4,27,30,31] Another key element may be the degradation of amorphous cellulose located on the fiber surface and between the microfibrils which leads to increased crystalline surface exposure allowing increased swelling ability and pulp reactivity.[27] As cellulase enzymes must bind to the surface of insoluble carbohydrates prior to hydrolysis, current working hypothesis describe the accessibility of the cellulose substrate and the size and shape of the enzyme to be crucial for enzymatic hydrolysis.[30,32]

In our previous work, it was shown that cellulose treatment with a monocomponent EG allows the controlled and selective adjustment of molar mass, which is essential in dissolving pulp

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manufacture and application.[9,33] To promote the commercial applicability of these enzymes, we investigated the effects of the wood source, the pulping process as well as the noncellulosic polymeric compounds, hemicelluloses and residual lignin, on the efficiency of the EG-treatment. Further, the influence of the cellulose II content of a pulp on the enzymatic degradability and the associated pulp activation was thoroughly studied. Thereby, cotton linters pulp (CLP) was steeped at stepwise increasing alkali concentrations to adjust different cellulose II contents. The removal of hemicelluloses from the wood pulps was accomplished by hot caustic extraction (HCE), CCE, and xylanase (X) treatments. Finally, we compared the reactivity of enzymatically hydrolyzed pulps with pulps degraded by acid-catalyzed hydrolysis (A-treatment).

■ EXPERIMENTAL SECTION

All chemicals were of analytical grade.

Raw Materials. Oxygen delignified *Eucalyptus globulus* kraft pulp (hardwood, HW-O) from the pulp mill in Huelva, Spain, was prepared from wood chips from Uruguay and was kindly supplied by ENCE (kappa number 10.3, R18 89.5%, intrinsic viscosity $[\eta]$ 895 mL g^{-1}, xylan 22.5%). Furthermore, softwood (SW) sulfite pulps of different residual kappa numbers, (a) SW-1, kappa number 1.2, (b) SW-5, kappa number 4.8, (c) SW-15, kappa number 14.8, and CLP were used for the experiments. The SW and HW pulps were used in the never-dried state to exclude hornification upon drying. For complete pulp characteristics, see Supporting Information.

Enzyme Preparations. The enzyme preparations were kindly supplied by Novozymes, Denmark. The X-preparation Pulpzyme HC is produced by submerged fermentation of a genetically modified *Bacillus licheniformis* microorganism and its concentration is standardized by the manufacturer to 1000 AXU g^{-1} (active xylanase units). The monocomponent EG-preparation Novozym 476 is produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and its concentration is standardized by the manufacturer to 4500 ECU g^{-1} (*endo*-cellulase units).

Enzyme Treatments. Enzyme treatments and the washing procedure for enzyme inactivation were performed according to Köpcke et al.[3] at 3% consistency in a phosphate buffer pH 7 (11 mM NaH_2PO_4, 9 mM Na_2HPO_4; deionized water) at 60 °C for 120 min (X-treatment with 500 AXU g^{-1} calculated on oven-dried pulp, odp) and at 50 °C for 60 min (EG-treatment at varying enzyme concentrations), respectively. For the EG-preparation Novozym 476, cellulose degradation can be considered as almost completed after 60 min without any major effect when the reaction time is further increased.[26] A detailed description of the handling of the enzyme treatments is given in our previous work.[9]

Caustic Extractions and Acid Treatment. HCE of the sulfite pulp SW-5 was conducted in a medium-consistency high-shear mixer at 110 °C for 60 min at 10% consistency and 3 bar relative N_2 pressure with 4 and 8 wt % NaOH on odp, respectively (HCE_4% and HCE_8%). CCE of the native sulfite pulp SW-5 and after HCE_4% was carried out in the same mixer at 30 °C for 30 min at 10% consistency and 3 bar relative N_2-pressure with 80 g NaOH L^{-1} (CCE80). CCE of the kraft pulp HW-O was conducted in a polyethylene flask[12] at 30 °C for 30 min at 10% consistency with 70 g NaOH L^{-1} (CCE70). A-treatment of the sulfite pulp SW-1 was performed at pH 1.5 adjusted with sulfuric acid at 90 °C for varying periods from 30 to 180 min at 3% consistency.[34,35]

Steeping. Steeping of the CLP was conducted with a steeping ratio (mass-ratio od pulp to steeping lye) of 1:18 at varying alkali concentrations in the steeping lye from 11.9 to 17.8 wt % NaOH at 50 °C for 15 min. Subsequently, the pulp was filtrated and pressed to a dry content of ~35%. The filter cake was resuspended in deionized water and acidified to neutral conditions with acetic acid and phenolphthalein as color indicator. Finally the pulp was filtered again and washed thoroughly.

Analytical Methods. Carbohydrate content was measured after a two stage total hydrolysis by high performance anion exchange chromatography with pulsed amperometric detection.[36] Water retention value (WRV) was determined according to a Zellcheming method,[37] Kappa number was determined according to TAPPI T 236 cm-85,[38] brightness according to ISO 2470−1,[39] intrinsic viscosity according to SCAN-CM 15:99,[40] and alkali resistance according to DIN 54355.[41] The degree of polymerization DPv was calculated from the intrinsic viscosity $[\eta]$ according to Marx−Figini.[42] The pulp reactivity toward xanthation was determined by both the Fock method[43] and a modified Treiber method,[44] as described by Hüpfl and Zauner.[45] The viscose samples derived from the latter were tested regarding their filterability (filter value) and their particle content. The filter value is calculated from the permeated mass of viscose after 100 and 150 min of filtration at 20 °C and 2 bar pressure and high filter values are interpreted as high pulp reactivity. Particles in a range of 3 to 155 μm were measured on a Pamas device operating according to the light blockade principle (sensor model HCB25/25, serial number W-2525-2). The degree of crystallinity (CrI), the cellulose II content (Cell II), and the crystal size of the CLPs were determined with wide-angle X-ray scattering (WAXS). The crystal size was calculated from the distances $D_{(hkl)}$ between parallel atomic layers of lattice planes as defined by the Miller indices *hkl* for a given crystal system.[46] For WAXS measurements, air-dried pulp samples were suspended in liquid N_2 and grinded in a centrifugal mill (ZM100, Fa. Retsch), sieve size 1 mm. The isotropic powder was dissected between PET-foils and sealed airtight with silicon. The WAXS measurements were conducted with a D5000 two-circle diffractometer (Fa. Bruker-AXS) in symmetrical transmission geometry with a focusing Ge(111) primary monochromator with Cu Kα beam (λ = 0.15406 nm) at 30 mA and 40 kV. The diffractogram was recorded in a 2θ-angle range of 4−104° (interval $\Delta 2\theta$ = 0.2°, recording time/$\Delta 2\theta$: 55 s). The sample was rotated perpendicularly at 15 rpm and each sample was measured three times. The degree of crystallinity and the cellulose II content of the HW and SW pulps were determined with FT-Raman.[47,48] FT-Raman measurements were done using a Bruker IFS66 with Raman module FRA106, Nd:YAG Laser 500 mW; Laser wavenumber 9394 cm^{-1} (1064 nm), liquid N_2 cooled Ge-detector, 3500−100 cm^{-1}, resolution 4 cm^{-1}, 100 scans, four measurements of each sample. The sample of 300 mg was pressed with 6 t cm^{-1} for approximately 10 min. Analysis was performed with a chemometric model, WAXS data were used as a standard for calibration and comparison. The specific surface area $a_{s,BET}$ according to Brunauer, Emmett, and Teller is defined as the accessible area of solid surface per unit mass of material.[49,50] This specific surface area is calculated from the BET isotherm, which is gained from a model for physical adsorption describing multilayer formation of gaseous particles on a solid surface.[51] The measurements of $a_{s,BET}$ were conducted by a volumetric method with a Belsorp mini II using N_2 gas. Field emission scanning electron microscopy (FE-SEM) pictures were accomplished at the Wood Analytics Centre Lenzing (WAL), Lenzing, Austria. Pulp preparation for FE-SEM was performed according to Duchesne et al.[52] and Okamoto and Meshitsuka.[53] The pulp samples were freeze-dried from a water suspension, mounted on a stub with a double-sided adhesive tape and fully degassed in a vacuum oven at 60 °C for 1 h at 200 mbar. Finally the samples were coated with a thin layer of Au/Pd. The samples were examined by high-resolution scanning electron microscopy up to a 30.000× magnification with a Hitachi S4000 FE-SEM using an acceleration voltage of 6 kV and a working distance of 8 mm.

■ RESULTS AND DISCUSSION

Influence of Cellulose II on the Enzymatic Degradability of Pulp. The increased degradation of cellulose II upon EG-treatment and its impact on pulp activation has been reported repeatedly.[1,4,27,30,31] Owing to the lack of non-cellulosic impurities, CLP was subjected to steeping processes at varying alkali concentrations in order to induce a stepwise conversion of cellulose I to cellulose II, as confirmed by WAXS measurements (Figure 1). The resulting pulp substrates (0/1/11/62/74/100% cellulose II) were subjected to enzymatic hydrolysis by EG at different enzyme concentrations. Cellulose depolymerization was followed by the number of chain scission, S [$S = (DP_0/DP_t) - 1$][54] calculated from DPv. The chain scission represents the number of chain cleavage steps per initial cellulose chain. Chain scission as a function of the applied enzyme concentration EG was fitted with the equation $S = S_{max} \times EG/(EG + K)$: S_{max} is the maximal chain scission and K is a constant. Pulps with an increasing proportion of cellulose II experienced an increasing extent of maximal chain scission (see Supporting Information), ranging from 0.1 for the native CLP (0% cellulose II) to 2.5 for the fully mercerized CLP (100% cellulose II). This effect may be attributed to enhanced accessibility of the cellulose II modification. Slightly decreasing crystallinity indices of the pulps with increasing cellulose II content were found (see Supporting Information) but are supposed to play only a minor role for the enhanced degradability.[55] The accessibility of cellulose needs to be discussed in respect of its molecular, supramolecular, and fibrillar structure, respectively. WAXS measurements (Figure 1) revealed rising lateral cellulose I crystal dimensions with increasing cellulose II contents which are ascribed to the dissolution of small or less ordered crystallites during mercerization[56,57] (see Supporting Information). Thus, the increased cellulose accessibility of the mercerized pulps can be attributed to the changed unit cell dimensions as occurred during the conversion from cellulose I to cellulose II. The exocyclic hydroxymethyl groups at C_5 of anhydroglucose units of the corner chains but also of center chains of microfibrils convert from the *tg* (*trans–gauche*) position to the *gt* (*gauche–trans*) position, which can be interpreted as loosening of the rigid cellulose lattice.[15,16,58−60] This change in orientation introduces an additional intersheet H-bond, which results in a decreased surface area of dried pulps. This was confirmed by a decrease in the specific surface area $a_{s,BET}$ from 0.47 $m^2\ g^{-1}$ for the native CLP to 0.17 $m^2\ g^{-1}$ for the pure cellulose II substrate (see Supporting Information).[61] Nevertheless, the overall availability of OH groups and thus the hydrophilic character of cellulose II is higher compared to cellulose I.[62] This can be observed from an increased swelling capacity[19] of pulps containing cellulose II, as determined by WRV measurements, and explains higher reactivity of cellulose II in heterogeneous reactions like derivatization or hydrolysis.[62] WRV measured from the never-dried CLP after steeping increased from 52% for the native CLP (0% cellulose II) to 95% for the fully mercerized CLP (100% cellulose II, see Supporting Information). However, also the different stacking of the cellulose planes in the structures of cellulose I and cellulose II contribute to the difference in the swelling behavior, as shown with molecular dynamics simulations by Kroon-Batenburg et al.[60] Cellulose II shows higher average sorption energy compared to cellulose I. Therefore, in cellulose II the water monolayer adsorbs more readily and desorbs less readily than with cellulose I.[63,64]

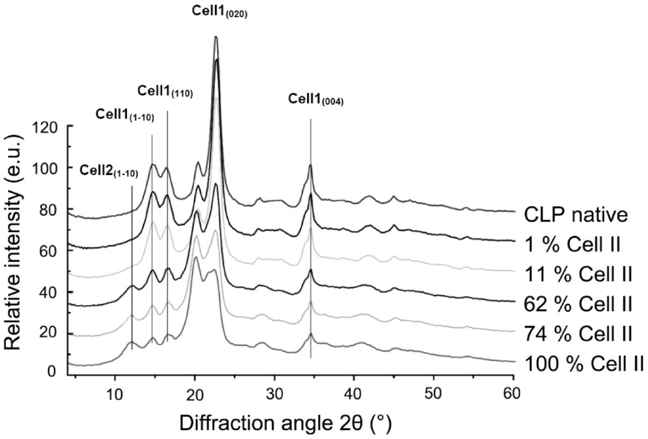

Figure 1. WAXS diffractogram of differently alkalized CLPs.

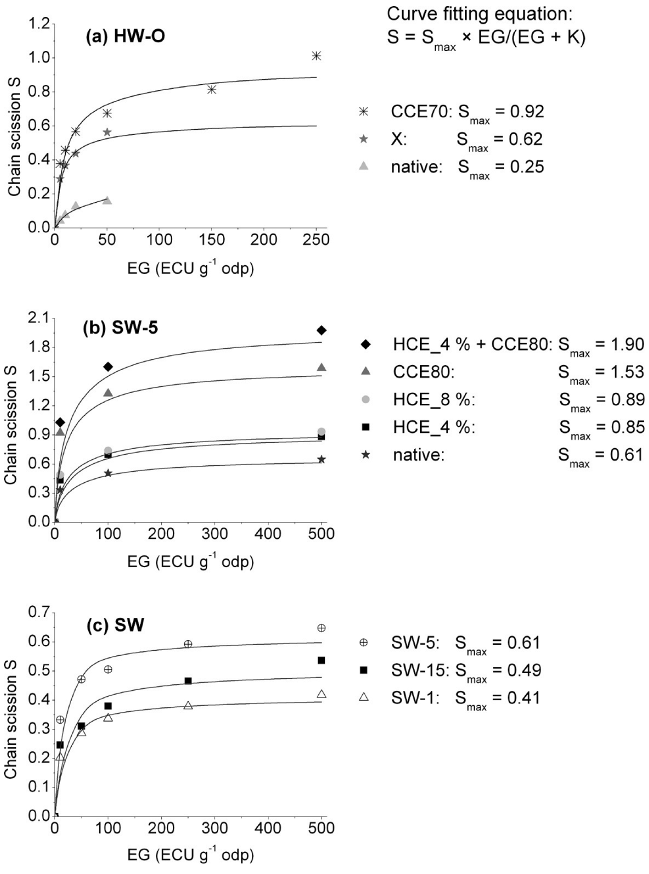

Figure 2. Chain scission at increasing EG-concentrations after removal of hemicelluloses from the pulps (a) HW-O and (b) SW-5 and (c) after delignification of the SW pulp at residual kappa numbers ∼15−1; S_{max} is the maximal chain scission, K is a constant, and incubation time is 60 min.

Influence of Chemical Pulp Composition on the Enzymatic Degradability of Pulp: (1) Residual Hemicelluloses. The hemicelluloses from the kraft pulp HW-O were removed by X-treatment and CCE, respectively, while those from the sulfite pulp SW-5 were removed by HCE, CCE, and by a combination of both methods prior to EG-treatment (see Supporting Information). X-treatment of HW-O removed 43% of the initial xylan (HW-O: 22.5% xylan; HW-O-X: 12.8% xylan) and clearly increased the enzymatic degradability of HW-O during EG-treatment (Figure 2a). CCE70 of HW-O, instead, decreased the xylan content by 62% (HW-O−CCE70: 8.6% xylan) and yielded high pulp purity (R18 96.2%), which was connected with an even higher degree of cellulose chain scission upon the subsequent EG-treatment. HCE of the pulp SW-5 mainly removed mannan by peeling reactions at

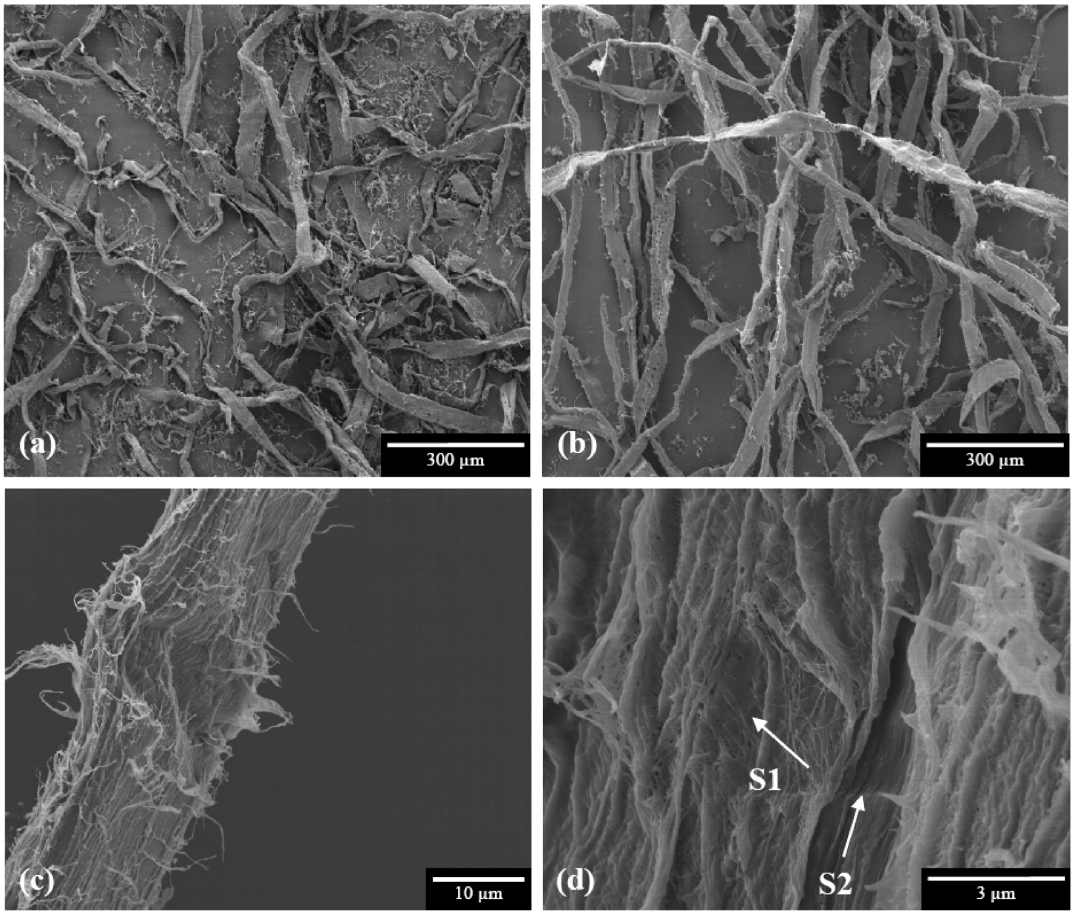

Figure 3. FE-SEM pictures of the pulp SW-1; (a) SW-1 before and (b–d) after EG-treatment (concentration 500 ECU g^{-1} odp): (a, b) fine and thin pulp components are removed by enzymes; (c) pulp fiber damage derived from enzymatic hydrolysis; (d) cracked S1 cell wall layer and S2 identified from the different orientations of the microfibrils (arrows).

temperatures >80 °C, but only little amounts of xylan were removed owing to the presence of the uronic acid side chains that initiate stopping reactions[65] (SW-5: 6.6% mannan, 3.9% xylan; SW-5-HCE_4%: 2.8% mannan, 3.2% xylan; SW-5-HCE_8%: 1.5% mannan, 2.9% xylan). A totally different final carbohydrate composition of the pulp SW-5 was obtained after CCE. During CCE80 at only 30 °C, peeling reactions are very slow[66] and mannan is resistant to physical dissolution due to its highly ordered structure, possibly because of cocrystallization with cellulose.[67] Thus, only one-third of the mannan was extracted from the pulp upon CCE80, while almost 75% of the pulp xylan was removed because of its good solubility under these conditions (SW-5-CCE80: 4.2% mannan, 1.0% xylan). HCE treatment of the pulp caused a slightly enhanced chain scission upon EG-treatment, probably due to improved cellulose accessibility (Figure 2b). The treatment HCE_8% was more efficient in the removal of hemicelluloses than CCE80, as demonstrated by the lower total hemicellulose content of the pulp (4.4 and 5.2%, respectively). Nevertheless, the extent of chain degradation upon EG-treatment was higher for the CCE80 treated pulp as compared to the HCE_8% treated pulp. The higher degree of chain scission may be attributed to the presence of the cellulose II conformation after CCE80 (11% Cell II). The sequential application of HCE and CCE further lowered the residual mannan (2.2%) and xylan (1.0%) contents, while at the same time the cellulose II content turned out to be slightly lower (8% Cell II) compared to the CCE80 treated pulp. The combined extractions resulted in a high purity pulp (R18 96.2%) showing a high cellulose accessibility and pulp swelling capacity. These combined effects obviously caused a significant increase of the efficiency of the EG-treatment (Figure 2b). HCE also created significant amounts of chromophores from carbohydrate degradation products,[68] which, in reference to the untreated pulp SW-5, can be deduced from the reduced pulp brightness despite the lower kappa number (SW-5: kappa number 5.0, 71.1% brightness; SW-5-HCE_4%: kappa number 3.8, 69.2% brightness; SW-5-HCE_8%: kappa number 3.1, 68.1% brightness).

Influence of Chemical Pulp Composition on the Enzymatic Degradability of Pulp: (2) Residual Lignin. Mansfield et al.[55] summarized the influence of lignin distribution on the rate of enzymatic hydrolysis by cellulases. They reported lignin to act as physical barrier which impedes the accessibility of cellulose[69] and also described adsorption of cellulase enzymes on lignin and thus prevented action on cellulose.[70] In this study, the reduction of the lignin content of SW pulps from kappa 14.8 to kappa 5.0 notably increased the accessibility of cellulose resulting in increased cellulose chain degradation upon EG-treatment, Figure 2c. Removing lignin from the SW pulp increased its porosity,[71,72] which can be seen from the increasing specific surface area $a_{\mathrm{s,BET}}$ of the pulps SW-15 (0.48 m^2 g^{-1}) and SW-5 (0.52 m^2 g^{-1}) and the increased enzymatic hydrolysis after lignin removal. In the case of the final bleached pulp SW-1 instead, the specific surface area $a_{\mathrm{s,BET}}$

was lower (0.37 m^2 g^{-1}) compared to the other SW pulps probably because of cell wall compaction upon chemical industrial treatments. This lower specific surface area was also manifested in reduced accessibility of the cellulose to enzymatic hydrolysis, shown in Figure 2c. FE-SEM pictures of the pulp SW-1 before and after EG-treatment were recorded (Figure 3) and revealed massive structural changes of the pulp fibers.

EG-Treatment and Its Influence on Pulp Reactivity. EG-Treatment was reported to show an increasing effect on the reactivity of pulps. Especially pulps containing cellulose II, show high swelling capacity and, thus, high enzymatic degradability. New cellulose fiber structures are opened upon EG-treatment, which consequently results in increased pulp reactivity upon derivatization, such as xanthation in the viscose process.

In an attempt to elucidate the effect of different degradation methods on pulp reactivity, SW-1 was degraded to a preset pulp viscosity (~600 mL g^{-1}) by both EG-treatment and A-treatment at pH 1.5 (Figure 4) and reactivity measurements according to the methods of Fock[43] and Treiber[44,45] were performed. The Fock method imitates the viscose process on lab-scale, upon which a viscose-like solution is prepared from an alkaline pulp suspension and carbon disulfide. Cellulose is regenerated from this solution by acidification and quantified via oxidation and titration. The reactivity according to the Fock test (wt %) describes the amount of pulp which was dissolved in the viscose-like solution. Consequently, the results are strongly influenced by the pulp viscosity and the amount of alkali soluble hemicelluloses and might be misleading in terms of cellulose reactivity. The Fock reactivity after EG-treatment was 93.3% and after A-treatment 92.9%, respectively, measured in two parallel determinations each. These very high values are comparable to those found by Henriksson et al.[27] for a similar SW sulfite pulp after EG-treatment. It is well-known that the outer cell wall layers of sulfite dissolving pulps contain cellulose of low molecular weight and short-chained hemicelluloses with many carboxylic groups.[73] These fractions show high swelling and dissolution tendency. Thus, the Fock test indicated very high reactivity of both pulps independent from the depolymerization treatment. In contrast to the Fock test, the Treiber method mimics the entire industrial viscose process. There, the quality of the viscose solution is evaluated by its filterability (filter value) and its particle content ranging from 3 to 155 μm. In this way, a reliable prediction of the applicability of a pulp for a commercial viscose process is obtained. The viscose dope prepared from the sulfite pulp SW-1 after EG-treatment showed a filter value of 179 (double determination) and an average particle volume of 13.8 ppm. Unexpectedly, the viscose prepared from the same pulp after an A-treatment revealed a filter value of 330 (±31, triple determination) and an average particle volume of 12.0 ppm, which represents a clearly better viscose quality as compared to the viscose prepared from the EG-treated pulp SW-1. Following the results from the industrially more reliable Treiber test, it can be concluded that the pulp reactivity for viscose application was seemingly not improved by the EG-treatment. Contrary to sulfite pulps, CCE treated kraft pulps clearly experienced an increase in pulp reactivity upon EG-treatment, as confirmed by both viscose filterability and Fock test.[6,9] The EG-treatment is obviously more effective on pulps with limited accessibility of the fiber surface as observed for kraft and soda-AQ pulps after CCE treatment.[6] Alternatively, the enhanced reactivity after the A-treatment of the sulfite pulp may be attributed to the massive fiber fibrillation of the pulp, as can be seen from Figure 5.

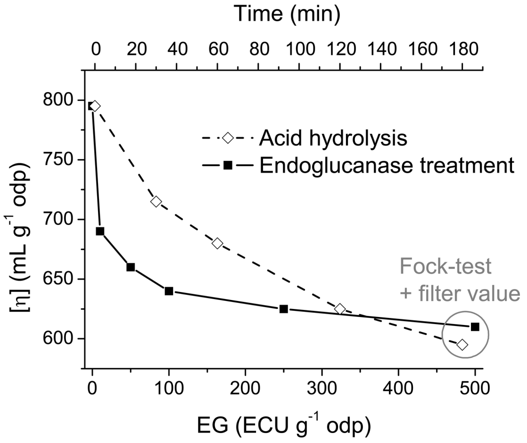

Figure 4. Viscosity decrease of the pulp SW-1 during A-treatment at pH 1.5 (varying hydrolysis time, upper abscissa) and EG-treatment (varying enzyme concentration and 60 min incubation time, lower abscissa), respectively.

CONCLUSION

Removing noncellulosic compounds from pulps clearly increased the accessibility of the surfaces of the cellulose fibers to endoglucanases. Thus, increased chain scission upon

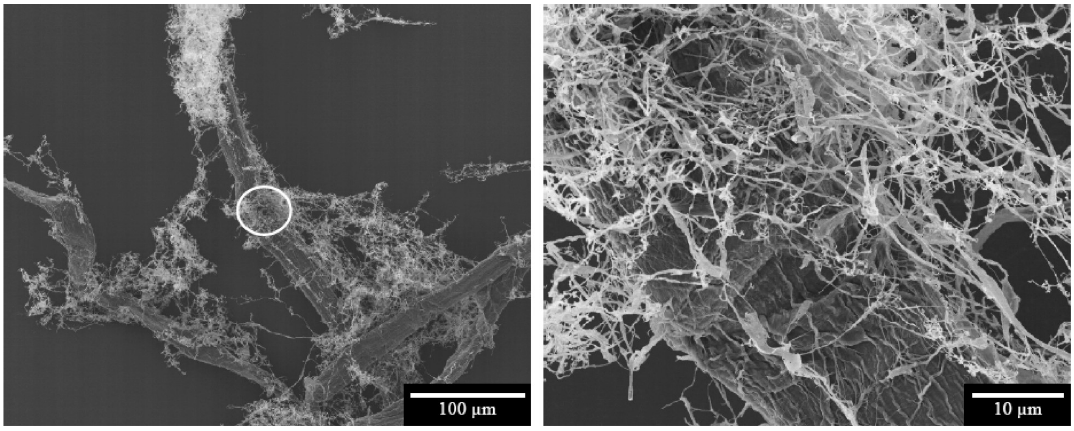

Figure 5. FE-SEM pictures of the pulp SW-1 after A-treatment at pH 1.5 for 180 min; intense fiber fibrillation visible.

enzymatic hydrolysis was observed when hemicelluloses were removed from a hardwood kraft pulp by alkaline extraction and xylanase treatment, respectively, and when lignin was removed from a softwood sulfite pulp by elemental chlorine free bleaching. Pulps with increased cellulose II content showed a greater accessibility of cellulose toward endoglucanases because of their increased swelling capacity compared to pulps of native cellulose. Reactivity of sulfite pulps was determined as filterability of the derived viscose samples and was not improved by endoglucanase treatment, whereas this was reported for CCE treated kraft pulps in literature.

ASSOCIATED CONTENT

Supporting Information

Table listing the main characteristics of the hardwood and softwood starting pulps, of the pulps with reduced hemicellulose content (xylanase treatment, HCE, CCE) and reduced lignin content; Table summarizing the steeping parameter of the CLPs and final pulp properties determined with WAXS and wet chemical analyses, and intrinsic viscosity after EG-treatment at different enzyme concentrations; and Figure demonstrating the different extent of chain scission observed for the CLPs of different cellulose II contents and linear correlation of the maximal chain scission and the cellulose II content. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Paper 3

Supporting information

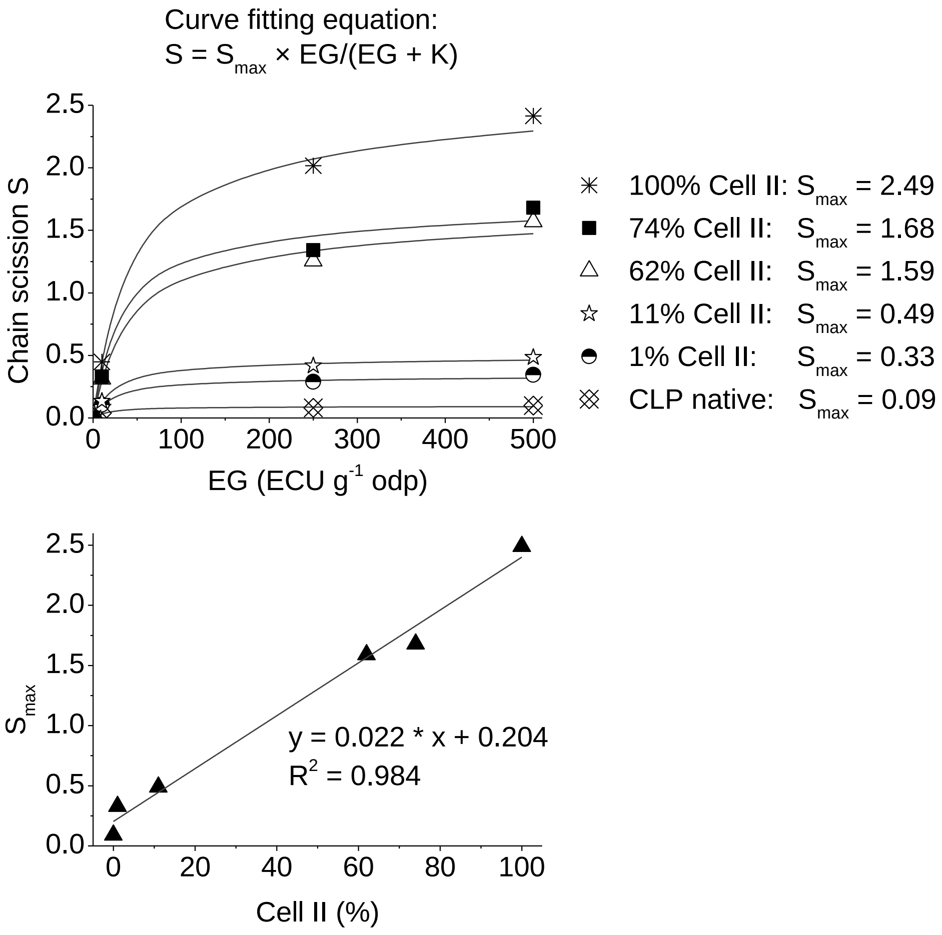
Pulp properties of the oxygen-delignified hardwood kraft pulp (HW-O) and with reduced hemicellulose content (X: xylanase treatment; CCE70: cold caustic extraction with 70 g NaOH L^{-1}); Pulp properties of the softwood sulfite pulps (SW) with different residual kappa numbers and with reduced hemicellulose content (HCE: hot caustic extraction with 4 wt % and 8 wt % NaOH on odp, respectively; CCE80: cold caustic extraction with 80 g NaOH L^{-1}).

	HW-O	HW-O		SW-15	SW-5	SW-5				SW-1
		X	CCE70			HCE_4 %	HCE_8 %	CCE80	HCE_4 % + CCE80	
Kappa number (--)	10.3	6.4	5.0	14.8	5.0	3.8	3.1	4.4	3.5	1.2
Brightness (%)	58.8	67.9	n.d.	62.2	71.1	69.2	68.1	73.4	70.8	87.6
R10 (%)	86.2	n.d.	94.7	85.5	86.1	91.7	93.4	92.9	94.1	86.3
R18 (%)	89.5	90.9	96.2	86.9	87.6	93.2	95.0	94.5	96.2	88.4
$[\eta]$ (mL g^{-1} odp)	895	945	1010	1165	1080	1135	1155	1145	1150	795
WRV (%)	151	n.d.	n.d.	108	104	121	90	114	110	110
Glucan (%)	70.0	80.2	86.5	79.9	84.3	91.7	94.3	94.6	92.8	87.1
Xylan (%)	22.5	12.8	8.6	3.6	3.9	3.2	2.9	1.0	1.0	3.5
Mannan (%)	0.0	0.1	0.2	6.9	6.6	2.8	1.5	4.2	2.2	6.1
$a_{s, BET}$ (m^2 g^{-1})	n.d.	n.d.	0.82	0.48	0.52	0.72	0.66	0.55	0.41	0.37
CrI (%)	n.d.	n.d.	n.d.	n.d.	53	55	56	53	54	54
Cell II (%)	n.d.	n.d.	n.d.	n.d.	4	5	6	11	8	3

WAXS results and wet chemical analyses of cotton linters pulps steeped at increasing alkali concentration and intrinsic viscosity [η] after EG-treatment at concentrations of 10/250/500 ECU g^{-1} odp. [η] of all pulps prior to enzyme treatment ~390 mL g^{-1} odp.

NaOH (%)[a]	Cell II (%)	CrI (%)	$D_{(hkl)}$ Cell I				WRV (%)[b]	$a_{s, BET}$ (m^2 g^{-1})	[η] (mL g^{-1})		
			(1-10)	(110)	(020)	(004)			EG10	EG250	EG500
--	0	60	4.9	6.2	6.7	13.7	52	0.47	381	365	360
11.9	1	58	5.2	6.7	6.9	14.9	57	0.41	354	305	292
12.9	11	53	5.5	7.1	6.9	13.0	62	0.37	346	278	264
14.8	62	40	8.2	9.6	7.3	11.4	82	0.24	297	173	151
15.2	74	38	8.0	8.9	7.0	10.6	83	0.24	292	165	144
17.8	100	39	--	--	--	--	95	0.17	262	125	111

[a] NaOH concentration in the steeping lye; [b] water retention value measured from never-dried pulps.



Upper diagram: chain scission of differently alkalized cotton linters pulps upon EG-treatment; S_{max} is the maximal chain scission and K is a constant, incubation time 60 min; lower diagram: linear correlation of S_{max} and the Cell II content of the pulps.

Paper 4

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ORIGINAL PAPER

Reactivity of dissolving pulps modified by TEMPO-mediated oxidation

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Abstract The reactivity of dissolving pulps towards derivatization or dissolution is a crucial quality parameter and is mainly determined by the accessibility of the hydroxyl groups. When dissolving pulps are produced from paper-grade pulps by cold caustic extraction (CCE), their reactivity is often inferior as compared to commercial prehydrolysis kraft dissolving pulps. It was hypothesized that pulp reactivity can be enhanced by the introduction of small amounts of substituents to facilitate interchain accessibility. In this study, CCE-treated *Eucalyptus globulus* kraft paper pulp was subjected to TEMPO-mediated oxidation to initiate partial oxidation of the C_6-hydroxyl groups to carboxyl groups. The effect of this pulp modification on the reactivity towards xanthation and the subsequent dissolution in diluted aqueous alkali solution (viscose process) as well as the dissolution in complexing and non-complexing solvents, respectively, was thoroughly examined. The results revealed that the oxidized pulps rich in C_6-carboxylate groups impeded the xanthation reaction obviously because of the reduced availability of hydroxyl groups. When *N*-methylmorpholine-*N*-oxide monohydrate was used as a direct solvent, a very high content of C_6-carboxylate groups was found to reduce the solubility of the pulp fibers as less hydrogen bonds can be formed with $NMMO \cdot H_2O$. In the case of dissolution in the complexing solvent cupriethylenediamine, the dissolution mechanism of cellulose was not deteriorated by the high content of C_6-carboxylate groups. Instead, the oxidation procedure increased the hydrophilic character and the swelling capacity of the outer cell wall layers allowed homogeneous dissolution.

Keywords Cuen · Dissolving pulp · NMMO · Reactivity · Spacer · TEMPO-oxidation

Abbreviations

[η]	Intrinsic viscosity
A	Acid catalyzed hydrolysis
AHG	Anhydroglucose
CCE	Cold caustic extraction
CCOA	Carbazole-9-carboxylic acid [2-(2-aminooxyethoxy)ethoxy]amide
Cell II	Cellulose II content
Cuen	Cupriethylenediamine
DP	Degree of polymerization

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FDAM	9*H*-fluoren-2-yl-diazomethane
FT-IR	Fourier transform infrared spectroscopy
FT-Raman	Fourier transform Raman spectroscopy
MB	Methylene blue
MW	Molecular weight
MWD	Molecular weight distribution
NMMO	*N*-methylmorpholine-*N*-oxide
odp	Oven dried pulp
P	Peroxide bleaching
PHK	Prehydrolysis kraft
PW	Primary wall
SEC	Size exclusion chromatography
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
WRV	Water retention value

Introduction

Nowadays, dissolving pulp is mainly produced by acid sulfite and alkaline prehydrolysis kraft (PHK) cooking. To increase process economy, also the conversion of paper-grade pulps into dissolving pulps by post-extraction of hemicelluloses is currently the research focus of many dissolving pulp producers. The commercial benefit of this refinement process is constituted by a substantial higher yield compared to a conventional PHK pulp (Schild et al. 2010). The drawback however, is the lower reactivity towards xanthation and thus viscose preparation. A working hypothesis for the reduced reactivity of kraft pulps after purification by cold caustic extraction (CCE) compared to PHK pulps is based on the structure of the residual hemicelluloses of the pulp. The residual hemicelluloses of PHK pulps are of low molecular weight (MW) and show many functional groups as side chains. Because of their mobility, these short-chain hemicelluloses are supposed to act as spacer in-between the cellulose microfibrils preventing them from aggregation and allowing better surface accessibility. CCE-treated kraft pulps instead, contain long-chained hemicelluloses with less functional groups (Schild and Sixta 2011). Thus, the cellulose microfibrils tend to aggregate upon intense dewatering or drying, resulting in additional hydrogen bonds and ester formation upon crosslinking (Fernandes Diniz et al. 2004). The reactivity of a pulp towards derivatization and dissolution processes is determined by the accessibility of the hydroxyl groups of the glucose monomer units. Thus, long range ordering and interaction between cellulose fibers, as e.g. derived from drying procedures, reduce the reactivity of a pulp (Le Moigne and Navard 2010). Several physical and chemical activation methods have been developed to increase the accessibility of solvents or reagents to the whole cellulosic structures in pulps. These treatments aim at disrupting aggregated cellulosic microstructures and comprise treatments such as swelling, solvent exchange, degradation procedures (Krässig 1993b), chemical modification (Sears et al. 1982), the activation of the dissolving pulp prior to steeping by e-beaming, liquid ammonia or steam explosion treatments (Weightman et al. 2009), and enzymatic treatment with cellulase, as first reported by Rahkamo et al. (1996). Another approach deals with the introduction of spacers, such as polyethylene glycol of specified molar mass. These spacers are thought to be capable of entering the voids within the fiber wall which are formed when hemicelluloses are dissolved upon alkaline refinery steps. In this way, the pore volume is preserved ensuring better accessibility of the cellulose surface and the hydroxyl groups (Weightman et al. 2009). As demonstrated by Weigert and Gruber (1998), derivatization of pulp fibers with acrylamide derivatives also lowers the hornification tendency of pulps upon drying. In this case, the bulky cellulose substituents may also form spacer and show an increasing effect on pulp accessibility and reactivity as short-chain hemicelluloses in PHK pulps.

In this study, TEMPO-mediated oxidation at neutral pH was applied to paper-grade kraft pulps prior to and subsequent to CCE-treatment. In pulp and paper industry, the treatment of kraft pulps for increased paper strength represents an important task of TEMPO-mediated oxidation (Dang et al. 2007; Kitaoka et al. 1999; Saito and Isogai 2005). In the case of cellulosic nanofiber production, high contents of carboxyl groups are introduced into cellulosic substrates in order to increase the repulsive strength of negatively charged carboxylate groups, which promotes the separation of the microfibrils (Fujisawa et al. 2011). A similar approach can be made for reducing hornification tendency of dissolving pulps. The newly formed carboxyl group of the glucose monomer unit shows increased hydrophilic character compared to the hydroxyl group and may act as a kind of spacer in-between the cellulose microfibrils, reducing the aggregation tendency. Thus, cellulose oxidation with the system TEMPO/NaOCl/$NaClO_2$ was applied in the process of refining a kraft paper pulp for utilization as a dissolving pulp. The oxidized pulps were characterized in terms of reactivity towards xanthation, dissolution behavior in *N*-methylmorpholine-*N*-oxide monohydrate ($NMMO{\cdot}H_2O$) and in alkaline Cuen-solution (cupriethylenediamine), respectively.

Experimental

Starting pulp

Elemental chlorine free bleached *Eucalyptus globulus* paper-grade kraft pulp from the pulp mill in Huelva, Spain, was prepared from wood chips from Uruguay and was kindly supplied by ENCE as industrially dried bale sheets. Prior to any oxidation or extraction treatment, the kraft paper pulp was suspended in deionised water, acidified to a $pH < 3$ with sulfuric acid, and stirred at room temperature for 10 min. Subsequently, the pulp was filtered, washed with hot deionised water and drained to a dry content of about 30 %.

Pretrials of TEMPO-mediated oxidation

Oxidation reactions were carried out at 60 °C in PE-bottles in a shaking water bath. The pulp was preheated in the bottles in the water bath prior to addition of reagents. The oxidation was accomplished at 1.5 % consistency in 0.05 M phosphate buffer of pH 6.8 (prepared from 0.05 M $Na_2HPO_4{\cdot}2H_2O$ and HCl conc.). The buffer was heated to 60 °C in a beaker, and TEMPO ($M = 156.25$ g mole^{-1}; 0.1 mol g^{-1} odp) and $NaClO_2$ (7.5 mol g^{-1} odp) were dissolved in the buffer. The solution was added to the pulp and the bottle was strongly shaken. NaOCl (0.75 mol g^{-1} odp) was used as 1 wt% solution diluted with phosphate buffer and was immediately added to the pulp suspension. The bottles were stored in the shaking water bath (130 rpm) for reaction times from 3–48 h.

After the reaction time, ethanol (20 mL g^{-1} odp) was added to the pulp suspension for quenching the reaction (Hirota et al. 2009). The pulp was filtered with a suction drainer of pore size 2, washed thoroughly with deionised water and put back into the PE-bottle. Post-oxidation ensuring the complete conversion of carbonyl groups to carboxyl groups was performed with $NaClO_2$ (3 mol g^{-1} odp) in 0.1 M acetate buffer of pH 4.5 (50 mL g^{-1} odp) prepared from 0.1 M acetic acid and 0.1 M sodium acetate solution. The sodium chlorite solution was added to the pulp and shaken for 48 h at room temperature. Finally, the pulp was filtered again with a suction drainer of pore size 2 and washed with hot deionised water. The pulp cake was resuspended in deionised water and drained again for 3 times.

TEMPO-mediated oxidation and CCE

TEMPO-mediated oxidation of the kraft paper pulp was performed prior to and subsequent to CCE, respectively. In both cases, CCE was conducted for hemicellulose removal at an alkali concentration of 100 g NaOH L^{-1} (CCE100), 30 °C, and 30 min at 10 % consistency in PE-bottles. Oxidation reactions were carried out as described before for the pretrials for 48 h reaction time. After post-oxidation with $NaClO_2$ and washing with deionised water, the pulp was resuspended in deionised water and acidified to a $pH < 3$ with sulfuric acid. The suspension was stirred at room temperature for 10 min. Subsequently, the pulp was filtered, washed with hot deionised water and drained to a dry content of about 30 %. A reference pulp without TEMPO-mediated oxidation was prepared from the CCE100-treated kraft pulp with acid catalyzed hydrolysis (A) and a final alkaline peroxide (P) bleaching step. A-treatment was performed for cellulose depolymerization at pH 2 adjusted with sulfuric acid at 90 °C for 60 min at 3 % consistency (Hakansson and Ahlgren 2005; Vehviläinen et al. 2010). P-bleaching was conducted at standard conditions applying 6 kg NaOH t^{-1} odp, 5 kg H_2O_2 t^{-1} odp and 1 kg $MgSO_4{\cdot}7H_2O$ t^{-1} odp at 70 °C for 120 min at 10 % consistency. For reactivity measurements, bigger pulp charges were prepared separately according to the described protocol.

Viscose preparation from a direct-xanthation-process

The TEMPO-oxidized pulps were subjected to a direct-xanthation-process in a batch reactor (Götze 1967a). For a scale of 100 g viscose, wet pulp (5 wt% α-cellulose in viscose) was treated in a 250 mL steel reactor with alkali used as 100 g L^{-1} solution (5 wt% alkali in viscose, meaning an alkali-to-cellulose ratio of 1) and Berol 388 (0.2 wt% on α-cellulose) as surfactant. Steeping was conducted under manual shaking for 5 min. Afterwards, the steel reactor was evacuated for 5 min and CS_2 (40 wt% on α-cellulose) was added with a syringe by a frit. Xanthation was continued for 2.5 h in a rotating water bath at 30 °C. After completed reaction, the residual amount of cold water was added and the cellulose xanthate got dissolved in the cold (fridge) under shaking.

Analytical methods

Xylan content was measured after a two stage total hydrolysis by high performance anion exchange chromatography with pulsed amperometric detection (Sixta et al. 2001). Water retention value (WRV) was determined according to Zellcheming IV/33/57 (1957). Intrinsic viscosity [η] was determined according to SCAN-CM 15:99 (1999) and alkali resistance in 10 wt% (R10) and 18 wt% (R18) NaOH solutions, respectively, according to DIN 54355 (1977). The cellulose II content (Cell II) was determined with FT-Raman (Röder et al. 2006; Ruland 1961). FT-Raman measurements were done using a Bruker IFS66 with Raman module FRA106, Nd: YAG Laser 500 mW; Laser wavenumber 9,394 cm^{-1} (1,064 nm), liq. N_2 cooled Ge-Detektor, 3,500–100 cm^{-1}, resolution 4 cm^{-1}, 100 scans, 4 measurements of each sample. The sample of 300 mg was pressed with 6 t cm^{-1} for approximately 10 min. Analysis was performed with a chemometric model, wide-angle X-ray scattering data were used as a standard for calibration and comparison. FT-IR spectra were recorded on a Bruker Tensor 27 spectrometer using a golden gate single reflection diamond ATR from Specac. Pulp reactivity was measured following a modified protocol by Treiber et al. (1962) as described by Hüpfl and Zauner (1966) for viscose preparation and characterization in terms of filterability and particle content. Particles in a range of 3–155 µm were measured on a Pamas device operating according to the light blockade principle. The xanthate group content of viscose per 100 anhydroglucose (AHG) units, known as gamma value (Götze 1967b) was determined referring to Zellcheming III/21/72 (1972).

Carboxyl group and carbonyl group determination

The carboxyl group content (COOH) was determined (1) with methylene blue (MB) according to Philipp et al. (1965) where both the uronic acids and the aldonic acids are measured, and (2) with FDAM/SEC (size exclusion chromatography) analysis which only determines the uronic acids. Thus, the results of the FDAM/SEC method are usually slightly lower compared to the results of the MB-method. The MB-method is based on the reaction of the anionic carboxylate group with the cationic MB. Because of the ionic interaction of the color reagent and the functional group of the pulp, the color intensity of the pulp suspension decreases, which is measured by photometry at $\lambda = 655$ nm. With FDAM/SEC analysis, the carboxyl groups are labeled with the diazomethane derivative FDAM, carried out as off-line heterogeneous derivatization, and converted into fluorescent fluoren-2-yl esters. The carboxyl groups are quantified with SEC measurement employing refractive index, multi-angle laser light scattering, and fluorescence detection (Bohrn et al. 2006). The carbonyl group content (CO) was measured as copper number according to TAPPI T 430 om-49 (1994), and quantified by fluorescence labeling with CCOA and SEC according to Röhrling et al. (2002a, b).

Dissolution and swelling behavior observed with optical microscopy

For all experiments, pulp fibers from aqueous suspensions were dissected between two glass plates and air-dried over night. The dissolution and swelling behavior of the pulp samples was observed by transmission optical microscopy with an Olympus BH2 microscope equipped with an OSIS Color View 2 camera with an Olympus Soft Imaging system. For dissolution experiments in NMMO, the camera was additionally equipped with a Linkam CO 102 heating device (maximum temperature 99 °C). The pulp samples were preheated to 90 °C on the sample table. 87 wt% NMMO (NMMO·H_2O) preheated to 90 °C was added to the pulp fibers with a pipette to the glass plates and was introduced by capillary forces (Chaudemanche and Navard 2011). Swelling experiments were performed according to the same procedure but at room temperature with 0.5 M Cuen-solution.

Results and discussion

Pulp properties after TEMPO-oxidation

The viscosity of the TEMPO-oxidized pulp decreased fast during the oxidation procedure to a similar level

Table 1 Pretrials of TEMPO-mediated oxidation of a bleached kraft paper pulp with the system TEMPO/NaOCl/$NaClO_2$ at pH 6.8 at 60 °C, reaction time 3–48 h

	Kraft pulp	TEMPO-oxidation			
	K	3 h	10 h	24 h	48 h
[η] (mL g^{-1})	830	525	505	505	485
COOH_{MB} (µmole g^{-1})[a]	88	615	716	791	820
$\text{COOH}_{\text{FDAM}}$ (µmole g^{-1})[b]	78	459	492	493	535
COOH (%) in DP < 500	84.7	42.7	46.9	46.1	46.0
COOH (%) in DP $< 2{,}000$	96.7	79.6	84.1	85.3	86.4
COOH (%) in DP $> 2{,}000$	3.3	20.4	15.9	14.7	13.6
CO (µmole g^{-1})[c]	8.2	20.3	21.0	18.4	12.1

[a] Determined with methylene blue

[b] Determined with FDAM/SEC

[c] Determined with CCOA/SEC

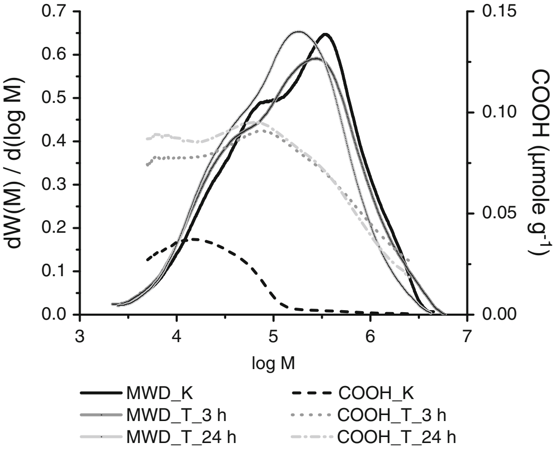

Fig. 1 Molecular weight distribution (MWD) and carboxyl group content (COOH) determined from FDAM/SEC of the kraft pulp (K) and of the TEMPO-oxidized pulps (T) after 3 and 24 h reaction time, respectively

for all pulp preparations, treated for 3–48 h, respectively (Table 1). To minimize depolymerization reactions starting from carbonyl groups in the subsequent alkaline CCE-treatment (β-elimination) the TEMPO-oxidized pulps were subjected to an oxidative post-treatment with chlorite in order to convert all carbonyl groups into carboxyl groups. The carbonyl group content of the TEMPO-treated pulps after chlorite oxidation was determined with CCOA/SEC analysis and showed slightly increased values compared to the starting pulp within the range of other commercial dissolving pulps (Table 1). The carboxyl group content was determined with the MB-method and FDAM/SEC analysis, respectively, and both methods revealed a similar trend over the reaction time of 3–48 h (Table 1). The absolute values are disputable because the analyses were out of the standard calibration range for both methods. Nevertheless, these absolute numbers give an indication of the degree of oxidation along the cellulose chain of ~12–16 mole%. As shown in the FDAM/SEC spectra in Fig. 1, the carboxyl groups of the starting pulp are mainly located in the hemicellulose and cellulose fraction of the pulp with a MW < 100 kg mole^{-1} (log $M = 5$; $\sim$DP < 600). For the oxidized pulps instead, < 50 % of all carboxyl groups are located in the DP < 500 fraction and up to 20 % are located in the high-MW fraction with a DP $> 2{,}000$ (Table 1). The strong increase of the carboxyl groups in the high-MW fractions of the pulps shows that also cellulose chains of high molar mass were accessible to TEMPO-oxidation.

Pulp properties after TEMPO-oxidation and CCE

The kraft paper pulp (K) was subjected to TEMPO-mediated oxidation prior to and subsequent to CCE, respectively. In the case of the alkaline pretreated pulp K–CCE100, TEMPO-treatment initiated enhanced oxidation compared to the untreated paper pulp K, resulting in a higher final carboxyl group content (Table 2). This effect of an alkaline pretreatment can be ascribed to higher accessibility of regenerated or mercerized cellulose, owing to a lower crystallinity compared to native cellulose (Isogai and Kato 1998). The increasing carboxyl content of the pulps was also observed with FT-IR at the stretching frequency of the carbonyl band at 1,724 cm^{-1} (Fig. 2). The elevated hydrophilicity of both TEMPO-treated pulps was reflected in similar, very high water retention values (Table 2). The WRV is an indicator of the swelling capacity of a pulp and is influenced by the content of hydrophilic groups, such as carboxyl groups, and by the cellulose II content, which shows a higher availability of the hydroxyl groups as compared to cellulose I (Gehmayr and Sixta 2012; Krässig 1993a). As far as the WRV is concerned, the higher carboxyl group content obviously compensates for the lack of cellulose II crystal structures as shown for the

Table 2 Properties of the kraft pulps treated with TEMPO-mediated oxidation prior to and after CCE100, respectively, and of a reference pulp with A-treatment for depolymerization

	Kraft pulp	K–TEMPO–CCE100		K–CCE100–TEMPO		K–CCE100–A–P		
	K	TEMPO	CCE100	CCE100	TEMPO	CCE100	A	P
[η] (mL g^{-1})	830	480	450	930	390	930	455	450
R10 (%)	90.2	n.d.	86.9	96.7	63.3	96.7	n.d.	94.1
R18 (%)	92.9	87.3	94.8	98.0	87.4	98.0	96.9	96.8
Xylan (%)	22.1	17.3	4.7	6.9	5.5	6.9	7.1	7.0
WRV (%)	99.7		133.3		132.6			97.6
Copper number (%)	0.62	1.15	1.08	n.d.	1.04	n.d.	n.d.	0.84
$COOH_{MB}$ (µmole g^{-1})[a]	88	975	821	32	1,516	32	30	30
Cell II (%)		0	11	12	0	12		14
Filter value								235
Particle content (ppm)								25.8
Gamma value			14.2[b]		20.9[b]			36.2[c]
Dissolution time (min)[d]			7		>> 12			7

[a] Determined with methylene blue

[b] Viscose from the direct-xanthation-process

[c] Viscose prepared according to the Treiber method

[d] In NMMO.H_2O at 90 °C from the time of pulp fiber saturation

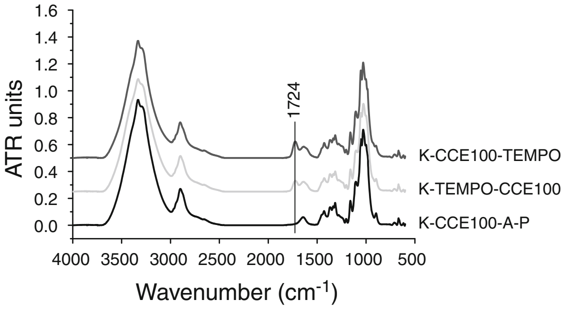

Fig. 2 FT-IR spectra of the TEMPO-oxidized pulps and the reference pulp showing an increasing carbonyl band at 1,724 cm^{-1} (C=O stretching frequency) from the carboxyl acid group with increasing degree of oxidation

K–CCE100–TEMPO treated pulp in comparison to the K–TEMPO–CCE100 treated pulp (Table 2).

Viscose reactivity of the TEMPO-oxidized pulps

The TEMPO-oxidized pulps and the reference pulp were subjected to the viscose process at laboratory scale according to the procedure of the Treiber test (Hüpfl and Zauner 1966; Treiber et al. 1962). The viscose prepared from the wet reference pulp was analyzed in terms of filterability, particle content and gamma value (Table 2). The TEMPO-oxidized pulps instead could not be converted to viscose according to this method. Due to the enhanced hydrophilic character of the oxidized pulps, the alkali cellulose after steeping could not be dewatered to the required cellulose content of ~35 %, which made it impossible to convert the pulps into viscose. Alternatively, viscose was prepared as described by Götze (1967a) in a direct-xanthation-process in a batch reactor. Therefore, the TEMPO-oxidized pulps were also used in the wet state in order to avoid enhanced hornification because of the high carboxyl group content. From the derived viscose solutions, the gamma values were determined and were found to be lower for the oxidized pulps compared to the reference pulp (Table 2). This can be attributed to the partially oxidized C_6 carbons that are not available for xanthation reactions with CS_2. Usually, the C_6-hydroxyl group preferably reacts first in xanthation reactions as it possesses the highest mobility and accessibility and is thus sterically favored in the derivatization process (Schwaighofer et al. 2011). From the low gamma values after TEMPO-oxidation it can be concluded that xanthation is not redirected to the C_2/C_3-hydroxyl groups when the C_6-hydroxyl group is blocked. Thus, the intense oxidation treatment causes an inverse

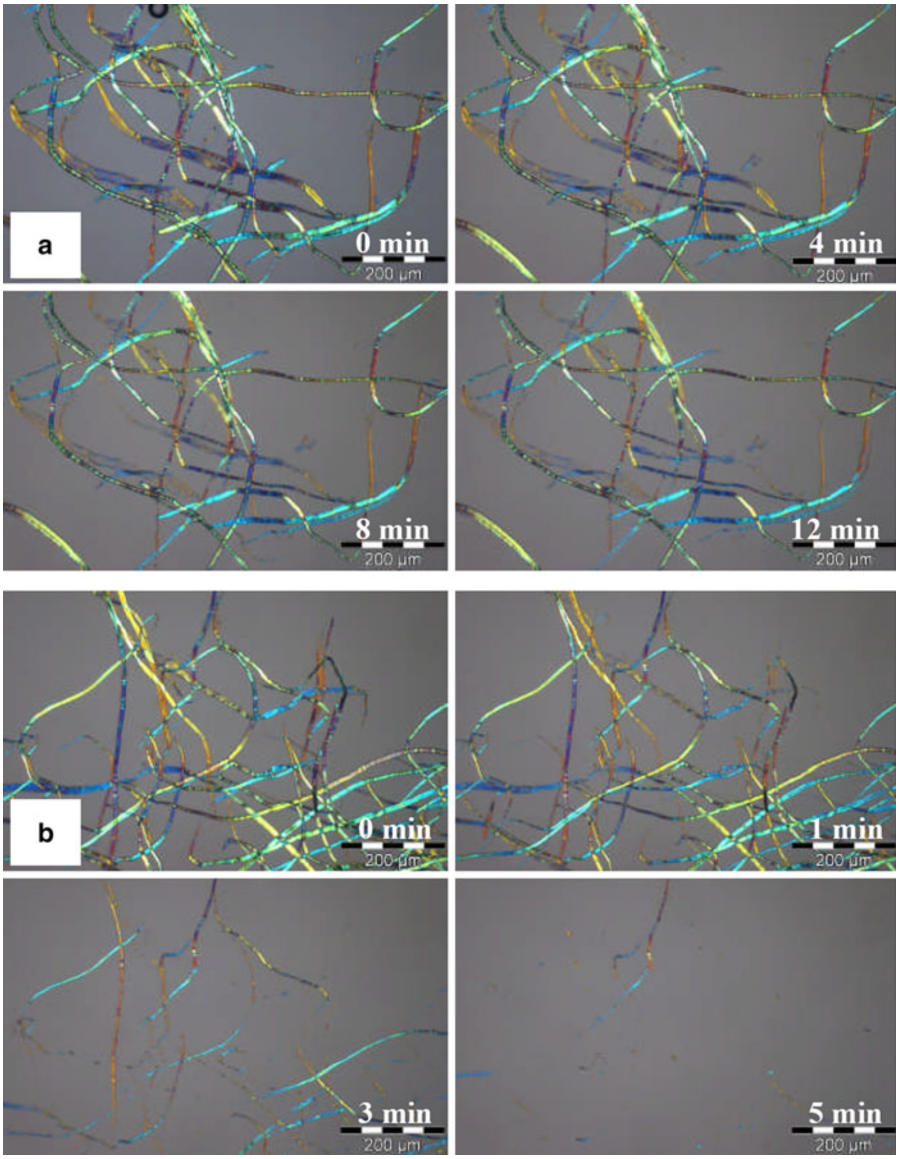

Fig. 3 Dissolution of pulp fibers in NMMO·H_2O at 90 °C; time of fiber saturation is chosen as starting time "0 min"; **a** K–CCE100–TEMPO from 0–12 min; **b** K–TEMPO–CCE100 from 0–5 min

effect on pulp reactivity and clearly disqualifies TEMPO-oxidized pulps for the viscose process.

Dissolution behavior of the TEMPO-oxidized pulps in NMMO-water

The dissolution behavior of the TEMPO-oxidized pulps was investigated in NMMO·H_2O as a direct solvent. The pulp with the lower carboxyl group content and the reference pulp dissolved much faster compared to the highest oxidized pulp, Table 2 and Fig. 3. According to Spinu et al. (2011), the limiting factor for dissolution in NMMO-water is not the penetration of the solvent inside the fiber but the local concentration of NMMO, as investigated on softwood pulp fibers. Thus, the dissolution quality is reduced when NMMO is locally diluted with the water around and inside the fibers. Therefore, one explanation for the clearly lower solubility of the highest oxidized pulp K–CCE100–TEMPO could be higher residual

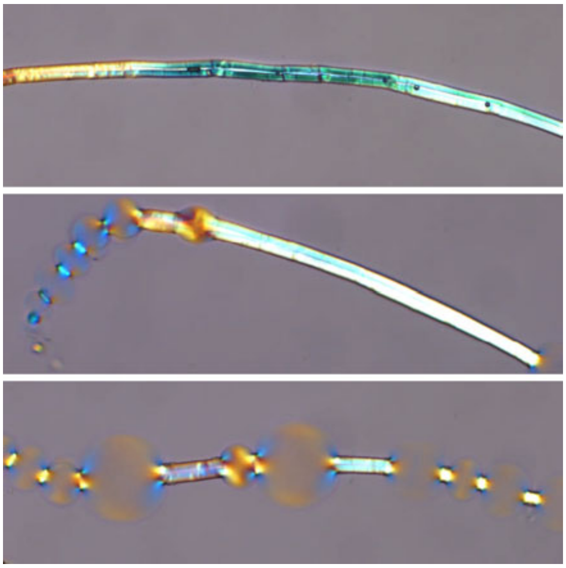

Fig. 4 K–CCE100–A–P pulp fiber swollen by intense ballooning in 0.5 M Cuen-solution at room temperature; the highly-ballooned structure was the final swelling state after 45 s

water content of the pulp sample. All pulp fibers were prepared from aqueous pulp suspensions and air-dried afterwards. Upon the mild drying conditions, water molecules could be fixed by an increased amount of hydrogen bonds in the sample K–CCE100–TEMPO. The physicochemical process of cellulose dissolution in NMMO-water is not comprehensively understood today, but the hydroxyl groups of cellulose are assumed to act as acids in donor–acceptor interactions with NMMO (Rosenau et al. 2001). According to the proposals of Michels and Kosan (2005), two NMMO·H_2O molecules are incorporated into inter-sheet hydrogen bonds with the OH-groups on C_6 and C_3 in cellulose upon dissolution in NMMO-water. Therefore, an increased content of C_6-carboxylate groups with lack of protons for forming hydrogen bonds seems to reduce the solubility of pulp fibers in NMMO-water. From the dissolution times found for the TEMPO-oxidized pulps (Table 2), it can be speculated that there is a critical content of C_6-carboxylate groups above which dissolution is impeded as compared to the reference pulp. As shown in Fig. 3, in agreement with the results obtained by Cuissinat et al. (2006) and Chaudemanche et al. (2011), the mechanism of wood pulp fibers dissolution in NMMO-water ($\leq$17 wt% water) comprises fractionation and disintegration into spindle parts, and fast dissolution without ballooning.

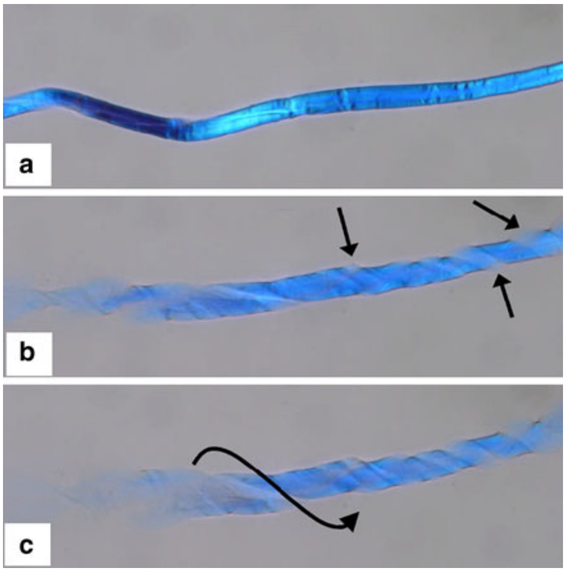

Fig. 5 TEMPO-oxidized pulp fiber dissolving homogeneously by unwinding of the internal helical cellulose structure; fiber completely dissolved after 30 s; **a** saturated fiber K–CCE100–TEMPO; **b** release of dissolved cellulose from the surface (*arrows*); **c** direction of left handed untwisting

Swelling behavior of the TEMPO-oxidized pulps in Cuen-solution

In an attempt to understand the differences in solubility of the prepared pulps, the mechanism of swelling of the pulp fibers referring to their fiber morphology was investigated. In this study, 0.5 M Cuen-solution was applied as non-ideal solvent for investigating the influence of the carboxyl group content on the pulp solubility. The outer cell wall layers S1 and PW (primary wall) of CCE-refined dissolving pulps produced from paper-grade pulps are preserved to a greater extent compared to conventionally-cooked dissolving pulps (Schild and Sixta 2011). Additionally, the S2 layer shows increased swelling capacity due to the cellulose II morphology and the reduced crystallinity after CCE. Nevertheless, swelling of the S2 layer is restricted by the presence of the S1 and the PW, which results in ballooning phenomena and the formation of collars from the pulp fiber upon dissolution (Cuissinat and Navard 2006). This behavior was expected and partly observed for the non-oxidized reference pulp, which showed intense ballooning in the swelling agent but did not dissolve at the chosen concentration of the alkaline Cuen-solution (Fig. 4). Both TEMPO-oxidized pulps instead, dissolved homogeneously by unwinding of the

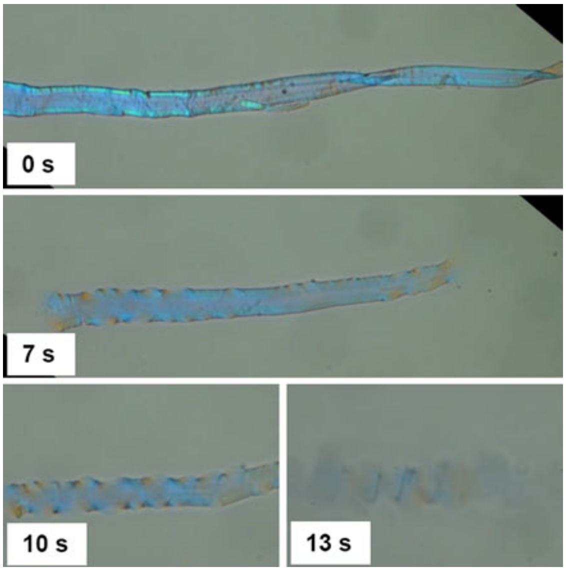

Fig. 6 Dissolution mechanism of conventionally-produced eucalypt sulfite dissolving pulps; fiber completely dissolved after 20 s

internal helical cellulose structure, as shown for the highest oxidized pulp in Fig. 5. Navard and Cuissinat (2006) explained the visible left handed untwisting of the cellulose to the release of internal stresses, which is necessary for dissolution. This dissolution mechanism is similar to the one of commercial market dissolving pulps without swelling restrictions from S1/PW (Fig. 6). Consequently, the introduction of carboxyl groups by TEMPO-oxidation increased the swelling capacity of the S1 layer and the PW in the way that ballooning is avoided and homogenous dissolution is enabled. In contrast to the acid–base interaction of cellulose and NMMO in the dissolution process, cellulose forms complexes with the transition metal copper in Cuen-solution. Only the hydroxyl groups on C_2 and C_3 are incorporated in this complex. Therefore, the C_6-carboxylate group doesn't hamper the dissolution process even at very high contents. Instead, homogeneous dissolution is even promoted because of the increased hydrophilic character of the S1 layer and the PW.

Conclusion

Introducing carboxyl groups at C_6 of untreated and CCE-treated *E. globulus* kraft pulps by TEMPO-mediated oxidation resulted in a reduced degree of xanthation at about the level of the substitution by carboxyl group. Thus, the hypothesis that blocking the C_6 position through the conversion to carboxyl groups will direct xanthation to the C_2/C_3 positions to achieve a more even distribution within the AHG units was not satisfied. Therefore, it can be concluded that the reactivity of the C_2 and C_3 positions in the AHG units was not enhanced by TEMPO-mediated oxidation. Direct dissolution of oxidized pulps in $NMMO{\cdot}H_2O$, exceeding a certain degree of carboxylate groups at C_6, was impeded obviously due to decreased ability to form H-bonds with the solvent. Instead, dissolution mechanism of the oxidized pulps in the complexing solvent cupriethylenediamine was not deteriorated by the high content of C_6-carboxylate groups because only the hydroxyl groups on C_2/C_3 are incorporated in the complex formation with the transition metal. Instead, the oxidation procedure increased the hydrophilicity and the swelling capacity of the cell wall layers S1 and PW. Dissolution restrictions from the outer cell wall layers as commonly observed by ballooning phenomena were prevented, and homogeneous dissolution as for commercial dissolving pulp was achieved. Therefore, mild TEMPO-mediated oxidation is suggested as a means for increasing the swelling capacity of the cell wall layers S1 and PW of refined paper pulps promoting homogenous dissolution.

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Paper 5

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New aspects of residual lignin isolation.

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Short Note

New aspects of residual lignin isolation

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Keywords: acidolysis; alkaline extraction; hexenuronic acid; NMR; Ox-Dem kappa; residual lignin; soda-anthraquinone (soda-AQ).

Introduction

Isolation of lignin from its polysaccharide matrix in wood or pulp is an inevitable prerequisite for its complete structural characterization. A commonly applied method of lignin isolation from wood feedstock is the extraction of milled wood lignin (Björkman 1956). The isolation of dissolved wood lignin is a rather new method (Fasching et al. 2008). Isolation of lignin from wood and pulp by acidolysis is simple and rapid (Gellerstedt et al. 1994; Evtuguin et al. 2001) and the resulting acidolysis lignin (AL) is of high purity. Enzymatic hydrolysis (Yamasaki et al. 1981; Chang 1992) and the combined method of enzymatic mild acidolysis (Argyropoulos et al. 2000) are suited for lignin isolation from pulp.

The structure of AL from *Eucalyptus globulus* kraft pulp was intensely investigated by Duarte et al. (2000) and Pinto et al. (2002). AL contained less etherified units and more free phenolic functions as compared to native lignin and to enzymatically isolated lignin from *E. globulus* kraft pulps. Accordingly, acidolysis could have split some β-O-4 bonds (Duarte et al. 2000). Changes in lignin structure during isolation were further manifested in decreased molar mass of residual lignins isolated by acidolysis of softwood pulps (Al-Dajani and Gellerstedt 2002; Jääskeläinen et al. 2003). Additionally, AL from wood as well as kraft pulp contained some unsaturated structures and non-lignin materials of polysaccharide origin and condensed tannins of catechin and gallocatechin types (Gellerstedt et al. 1994; Evtuguin et al. 2001). Thus, applying an alkaline extraction (AE) prior to acidolysis of wood was suggested by Evtuguin et al. (2001) for removing these undesirable compounds. Rutkowska et al. (2009) applied this approach also for kraft pulp. Tannins are effectively removed during kraft pulping. At the end of washing, however, some lignin is reprecipitated on the pulp as a result of reduced alkalinity. Therefore, an alkaline extraction prior to lignin isolation might remove this precipitated lignin and render the isolated lignin more uniformly.

Within this study, the influence of AE prior to acidolysis as a means of increasing the comparability of lignins from differently pretreated sources was investigated. Residual lignin from a soda-anthraquinone (soda-AQ) pulp was isolated applying an acetone extraction (AcE) step to ensure complete removal of tannins and lipophilic extractives. Then an optional AE step followed before acidolysis. To investigate the degradation of lignin during the isolation in detail, lignin removal was followed in terms of kappa number, Ox-Dem kappa number, and hexenuronic acid (HexA) content. The high purities of the extracted lignins enabled reliable comparison of their structural compositions, which were evaluated by means of qualitative and quantitative NMR experiments, FT-IR spectroscopy, determination of sugar content, and wet chemical methoxy group determination, as well as elemental analysis. The results were recalculated in the C_{900} formula and discussed.

Experimental

Cooking experiments

Eucalyptus globulus wood chips from plantations in Uruguay were supplied by ENCE for soda-AQ continuous batch cooking (CBC) (Sixta et al. 2006) in a 10-l digester (H-factor 700, 94 min at T_{max} of 160°C) with synthetic cooking liquor (30.9 g l^{-1} effective alkali, 0.2% AQ on oven dry (o.d.) wood, alkali consumption 21%).

Lignin isolation and purification

Soda-AQ pulp (kappa 14) was subjected to AcE with a liquid-to-solid ratio (L/S) of 10 (SCAN-CM 2003). An optional AE was performed (0.3% NaOH, L/S 50, 1 h reflux, nitrogen atmosphere) and lignin was isolated via multistep acidolysis (Evtuguin et al. 2001).

Standard characterization

Carbohydrate content was evaluated after two-stage total hydrolysis by high performance anion exchange chromatography with pulsed amperometric detection (Sixta et al. 2001). Elemental analyses were performed by the Fraunhofer IAP. Standard protocols were used for determination of methoxy group content (Vieböck and Schwappach 1930), kappa number (Tappi 1993), Ox-Dem kappa number (Li and Gellerstedt 2002), and HexA content (Gellerstedt and Li 1996).

Table 1 Kappa, Ox-Dem kappa, and HexA contents at different extraction stages.

	Pulp preparation				
				Acidolysis	
	Soda-AQ Pulp	AcE	AE	With AE	Without AE
Total kappa	14.0	13.5	11.5	2.6	2.4
Ox-Dem kappa[a]	7.4	6.5	6.0	0.8	1.4
HexA[b]	27.2	27.9	29.3	–	–
($\approx$kappa)[c]	(2.3)	(2.4)	(2.5)		

[a]Kappa attributed to lignin (Li and Gellerstedt 2002). [b]HexA given in μmol g^{-1} o.d. pulp. [c]Kappa attributed to HexA (Li and Gellerstedt 1997). AcE, acetone extraction; AE, alkaline extraction.

Advanced lignin characterization

FT-IR spectra were recorded on a Bruker Tensor 27 spectrometer using golden gate ATR. NMR spectra of the acetylated lignins (Lundquist 1992) were measured in $CDCl_3$ ($c=100$ mg ml^{-1} for ^1H and $^1H/^{13}C$ HSQC NMR experiments and $c=200$ mg ml^{-1} for quantitative ^{13}C NMR experiments) on a Bruker Avance DPX300 spectrometer (^1H 300 MHz, ^{13}C 75 MHz) using a 5-mm ^1H/BB inverse probe with z-gradient support, at 300 K. Chemical shifts were referenced to the residual solvent signals at $\delta(^1H)=7.26$ ppm and $\delta(^{13}C)=77$ ppm. Chromium(III)acetylacetonate (0.01 M) was added as relaxation agent for quantitative ^{13}C NMR experiments (Capanema et al. 2004). ^{13}C T_1 relaxation times were monitored with the inversion recovery technique for each sample. To ensure quantitative conditions, inverse-gated ^1H decoupled ^{13}C NMR spectra were recorded with 5 s relaxation delay, 0.4 s acquisition time, and 245 ppm spectral width. For each spectrum, 65 536 transients were accumulated. Quantitative ^1H NMR experiments were recorded with 7 s relaxation delay, 4.2 s acquisition time, and 26 ppm spectral width. In total, 128 scans were acquired from a sample with no added relaxation reagent. Processing of quantitative 1D spectra involved exponential window multiplication (LB = 0.3 Hz for ^1H and LB = 5 Hz for ^{13}C, respectively) of the free induction decay prior to Fourier transform. 2D $^1H/^{13}C$ HSQC NMR experiments were recorded with 2048 data points in the ^1H dimension, 512 time domain increments in the ^{13}C dimension, 256 scans, 0.5 s relaxation delay, 0.21 s acquisition time, and 16 ppm (^1H)/165 ppm (^{13}C) spectral width. 2D time domain data were multiplied with exponential (LB = 3 Hz; ^1H dimension) and shifted square sine bell (SSB = 2; ^{13}C dimension) functions and zero-filled to 2048 times 1024 real data points prior to Fourier transform. All 1D and 2D NMR data were processed and analyzed by Bruker software TopSpin 2.1.

Results and discussion

AcE led to a slight decrease of the soda-AQ pulp kappa number from 14 to 13.5. Subsequent AE yielded in a clear kappa number reduction of 2 units (Table 1). Regardless of whether AE was applied or not, kappa numbers of the pulps after acidolysis dropped to an average value of 2.5. As kappa number is not only affected by lignin, but by aliphatic double bonds, free aldehyde groups as well as by α-keto-carboxylic acids (Li and Gellerstedt 1998), the Ox-Dem kappa number has been introduced and represents the kappa number attributed to lignin only (Li and Gellerstedt 2002). Ox-Dem kappa dropped only slightly during AcE. AE reduced Ox-Dem kappa of the pulp by 0.5 units.

Within acidolysis of the pulps, similar amounts of lignin were removed as shown by equivalent Ox-Dem kappa reductions (Δ Ox-Dem kappa 5.2 with AE and 5.1 without AE). The pulp with AE finally showed a slightly lower Ox-Dem kappa number after acidolysis (Ox-Dem kappa 0.8) than the pulp without AE (Ox-Dem kappa 1.4). During acidolysis, the reduction in kappa number (Δ kappa 8.9 with AE, Δ kappa 11.1 without AE) was higher than the total Ox-Dem kappa value before acidolysis (Ox-Dem kappa 6.0 with AE and 6.5 without AE) (Table 1). Thus, substantial amounts of substances, which did not contribute to Ox-Dem kappa, were removed in addition to lignin within the step of acidolysis. Although similar amounts of lignin were removed from both pulps within acidolysis, the lignins showed different precipitation behavior in water during the recovery procedure. Lignin from alkaline extracted pulp precipitated only in minor amounts.

Thus, the step of AE mostly influenced the yield of lignin isolation and resulted in significant yield losses of 31% (Table 2). HexA contributed to approximately 2.5 kappa number units of each pulp, but generally do not contribute to Ox-Dem kappa value (Gellerstedt and Li 1996; Li and Gellerstedt 1997). The content of HexA remained unaltered during AcE and AE (Table 1), whereas it can be assumed that HexA was completely removed within acidolysis as the hexenuronic acid group is already liberated at slightly acidic conditions (Vuorinen and Alén 1998). Therefore, determination of HexA content after acidolysis was not accomplished.

Structural analyses of both lignin fractions with prior AE and without prior AE showed high similarities in lignin structure as can be seen from NMR (Table 3) and FT-IR spectra (Figure 1). Both isolation procedures yielded rather pure lignins with very low sugar content of approximately 1%, mainly glucose and xylose. Wet chemical methoxy group determination showed 1.12 (with AE) and 1.18 (without AE) methoxy groups per C_9 lignin unit. Similar tendencies were found in NMR determination of methoxy groups, but higher absolute values (1.22 with AE and 1.35 without

Table 2 Characterization of the isolated lignins with AE and without AE.

Lignin	Sugar (%)	Elemental analysis (%) C	H	N	O	S	OMe (%)	C_{900} formula[a]	Lignin yield (mg g^{-1})
With AE	<1	60.4	5.9	0.2	33.3	0.2	17.3	$C_{900}H_{852}O_{306}(OCH_3)_{112}$	4.62
Without AE	1.2	58.4	5.7	0.2	29.7	0.2	17.6	$C_{900}H_{833}O_{270}(OCH_3)_{118}$	6.68

[a]Values are corrected for sugar impurities.

Table 3 Chemical shifts of resonances for integration of peaks in quantitative ^{13}C NMR spectra (Robert 1992; Ralph et al. 2004; Fasching et al. 2008).

	Chemical shift (ppm)		Lignin[a]	
Functional group	From	To	With AE	Without AE
Prim. aliph. OH	171.0	169.8	0.67	0.64
Sek. aliph. OH	169.8	168.8	0.29	0.29
Phenol. OH	168.8	167.5	0.42	0.49
O-subst. arom. C[b]	162.0	142.0	2.26	2.25
C-subst. arom. C[c]	142.0	125.0	1.82	1.84
H-subst. arom. C	125.0	101.5	1.91	1.91
β-β/α	86.0	85.2	0.04	0.02
β-O-4/β	81.0	79.8	0.13	0.13
Methoxy group	57.0	54.5	1.22	1.35

[a] ^{13}C integral area of the acetylated sample per aromatic unit normalized to the integral area corresponding to methoxy groups. [b]Non-etherified phenolic C-O-Ac added to integral area. [c]Non-etherified phenolic C-O-Ac deduced from integral area.

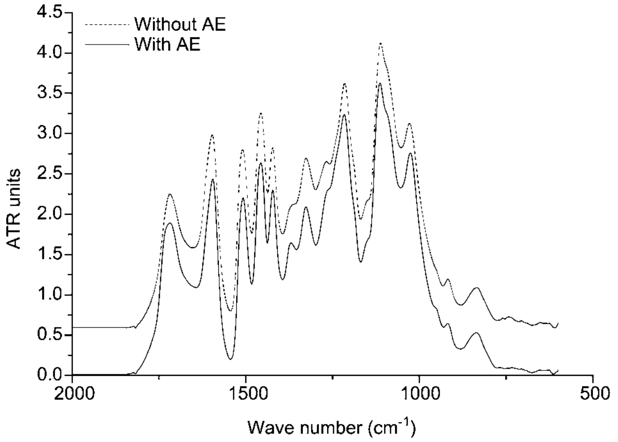

Figure 1 Attenuated total reflectance (ATR) FT-IR spectra of the isolated lignins with AE and without AE scaled on the 1503–1515 cm^{-1} band representing aromatic skeletal vibrations.

AE) were obtained. These values were in good agreement with those found by Duarte et al. (2000) for *E. globulus* kraft AL (1.2 methoxy groups per aromatic unit). The C_{900} formula was calculated from methoxy group content and elemental analysis and showed slightly higher oxygen and hydrogen contents for lignin with AE [$C_{900}H_{852}O_{306}(OCH_3)_{112}$] than the C_{900} formula $C_{900}H_{833}O_{270}(OCH_3)_{118}$ of lignin without AE (Table 2).

Structural analysis via HSQC and quantitative ^{13}C NMR experiments did not reveal any further differences between the isolated lignin fractions (Table 3). In addition to the common structural elements of lignins, NMR resonances at 7.24/105.6 ppm showed α-oxidized syringyl units, as were also found for lignins in soda-AQ pulping liquors (Venica et al. 2008). High intensities of carbonylic FT-IR bands at 1710 cm^{-1} (Figure 1) supported these findings. The presence of stilbene structures was indicated by NMR resonances in the ^{13}C range of 115–131 ppm and the ^{1}H range of 6.5–8 ppm (Ralph et al. 2004), as described by Rutkowska et al. (2009) for AL of *E. globulus* kraft pulp and Venica et al. (2008) for lignins in soda-AQ pulping liquors.

Conclusion

The effect of different treatments (acetone extraction and optional alkaline extraction) was studied in the context of residual lignin isolation from a *E. globulus* soda-AQ pulp by acidolysis. The results were monitored by determination of kappa number, Ox-Dem kappa number, and HexA content. During acetone extraction and alkaline extraction, lignin components were removed from the pulp. The residual lignins were of high purity and similar in chemical structure and composition with and without previous alkaline extraction. Alkaline extraction reduced the yield of isolated lignin significantly. Therefore, acetone extraction is a better and more efficient pretreatment step for acidolysis of *E. globulus* pulp.

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