PROPOSED NANO-SCALE COALESCENCE OF CELLULOSE IN CHEMICAL PULP FIBERS DURING TECHNICAL TREATMENTS

Raili Pönni, Tapani Vuorinen, and Eero Kontturi *

This review summarizes the proposed mechanisms for irreversible coalescence of cellulose microfibrils within fibers during various common industrial treatments for chemical pulp fibers as well as the methods to evaluate it. It is a phenomenon vital for cellulose accessibility but still under considerable debate. The proposed coalescence mechanisms include irreversible hydrogen bonding. Coalescence is induced by high temperature and by the absence of obstructing molecules, such as water, hemicelluloses, and lignin. The typical industrial processes, in the course of which nano-scale coalescence and possible aggregation of cellulose microfibrillar elements occurs, are drying and chemical pulping. Coalescence reduces cellulose accessibility and therefore, in several instances, the quality of cellulose as a raw material for novel products. The degree of coalescence also affects the processing and the quality of the products. For traditional paper-based products, the loss of strength properties is a major disadvantage. Some properties lost during coalescence can be restored to a certain extent by, *e.g.*, beating. Several factors, such as charge, have an influence on the intensity of the coalescence. The evaluation of the phenomenon is commonly conducted by water retention value measurements. Other techniques, such as deuteration combined with FTIR spectroscopy, are being applied for better understanding of the changes in cellulose accessibility.

Keywords: Coalescence; Aggregation; Cellulose; Chemical pulp; Hornification; Microfibril; Swelling

*Contact information: Department of Forest Products Technology, School of Chemical Technology, Aalto University, P.O. Box 16300, 00076 Aalto, Finland; *Corresponding author: eero.kontturi@aalto.fi*

INTRODUCTION

Cellulose is the main chemical component in wood. Lately, interest in wood biopolymers has expanded from paper products to other novel products, such as biofuels, nanomaterials, and commodity chemicals. In this respect, an interest has risen also for other components of wood, such as lignin. However, the main interest remains focused on cellulose, a biopolymer with unique properties and extensive economical potential. Cellulose molecules are able to form exceptional semi-crystalline structures, whose interiors are largely inaccessible to water. In the past, there have been several different designations for these elementary structures that cellulose forms in the fiber. In this paper, we will use the term microfibril, as it is nowadays the most commonly used term. Cellulose microfibrils in plants contribute to, *e.g.*, the resilient structure of trees.

The accessibility of cellulose hydroxyl groups is of interest in many processing steps that require chemical or enzymatic reactions or the dissolution of cellulose. Take for example the production of nanocellulose, which commonly requires a pretreatment prior to the mechanical disintegration. Many of the common pretreatments, such as 2,2,6,6-

tetramethylpiperidine-1-oxyl (TEMPO) oxidation (Saito *et al.* 2006), carboxy-methylation (Wågberg *et al.* 2008), and enzymatic hydrolysis (Pääkkö *et al.* 2007) rely on the accessibility of the hydroxyl groups. Reduced accessibility is, thus, inevitably a disadvantage for the raw material in nanocellulose production. As the accessibility of the hydroxyl groups affects the dissolution of cellulose, it is also an important parameter in biofuel production.

Several studies have proposed that cellulose microfibrils coalesce or aggregate within chemical pulp (*i.e.* kraft or sulfite) fibers during various industrial processes, such as the chemical pulping itself, as well as drying (Lyne and Gallay 1950; Klye 1961; Higgins and McKenzie 1963; Back 1967; Hult *et al.* 2001). Such coalescence is considered to reduce the accessibility of the hydroxyl groups, although there are still many open questions behind this phenomenon. In papermaking applications, coalescence of cellulose microfibrils is seen to affect the quality of the end product as well as the processing of the fiber material. In all respects, it is important to be aware of the changes taking place in the various processing steps as they will have a considerable impact on the end product qualities. In the future, cellulose microfibril coalescence can be either an advantage or disadvantage for the products made from chemical pulp fibers. Certainly, this phenomenon can be seen as a possibility for tailoring of products.

The reviews during the past decades related to the subject at hand have covered events that occur during drying (Weise 1998; Fernandes Diniz *et al.* 2004) or, from a more applied perspective, recycling (Howard 1990 and 1995; Nazhad and Pazner 1994; Nazhad 2005; Hubbe *et al.* 2007; Sheikhi *et al.* 2010). Beating, as a way to reverse the changes induced by drying, has also been reviewed (Page 1985). This review aims to provide a more comprehensive understanding of cellulose microfibrillar coalescence as an essential part of cellulosic material processing. Thus, this review aims to give a frame of reference not only for papermaking purposes but also for the needs of novel cellulosic products and processes. Concerning the cellulosic materials where microfibril coalescence is proposed to occur, the present discussion will be limited to chemical pulp fibers and, in some cases, wood which is the raw material for chemical pulp. Several fundamental issues, such as cellulose chain association in the formation of microfibrils during biogenesis, have been omitted.

The review is structured as follows: The first chapter, *The structure of a cellulose microfibril*, aims to offer a generic depiction from the cellulose molecule to the formation of microfibrils and further their alignment in a plant cell wall. This chapter also provides several examples of the size distributions between different sources of cellulose microfibrils. The chapter, *Proposed mechanisms of cellulose microfibril coalescence* includes the various proposals for this phenomenon and the debate it has given rise to. The chapter, *Consequences of cellulose microfibril coalescence* focuses on background regarding the changes taking place in the cell wall as well as the changes with respect to individual microfibrils. It also lists the most important properties lost due to this phenomenon and discusses its importance for the more traditional fiber products, such as paper, as well as for the more advanced products, such as nanocellulose. The chapter, *Treatments that induce cellulose microfibril coalescence* introduces the most common technical treatments upon which cellulose microfibril coalescence is known to occur. In addition to the treatments concerning chemical pulp fibers, this chapter also briefly covers drying of wood, as wood is the primary raw material for chemical pulp fibers and

commonly undergoes drying prior to processing. The chapter, *Variables affecting cellulose microfibril coalescence* introduces the most important variables affecting cellulose microfibril coalescence including both the fiber properties as well as the process parameters. The chapter, *Preventing and reversing cellulose microfibril coalescence* introduces processes that are applied either to regain the properties of fibers with aggregated microfibrils or to prevent the actual coalescence. The chapter, *Methods to evaluate cellulose microfibril coalescence* describes the various methods to evaluate this phenomenon either directly or indirectly. The feasibility of these methods is also given a critical view.

THE STRUCTURE OF A CELLULOSE MICROFIBRIL

Cellulose is a linear homopolymer consisting of D-anhydroglucopyranose units (AGU) that are linked together by β(1→4) glycosidic bonds. The repeating unit in this linear chain is illustrated in Fig. 1. Every AGU contains three hydroxyl groups, namely in the positions C2, C3, and C6. The degree of polymerization (DP) of cellulose is dependent on its source and the processing steps it has been subjected to. The DP of softwood and hardwood celluloses varies between 7500 and 10300 (Goring and Timell 1962). Cellulose is, however, polydisperse in native sources.

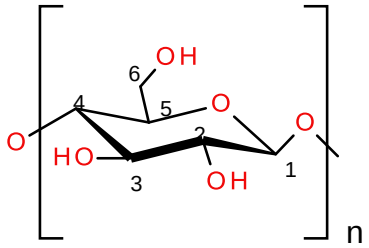

Fig. 1. Structure of cellulose (the repeating glucosyl unit)

Cellulose is observed to form highly crystalline entities by the formation of inter- and intra-molecular hydrogen bonds (Kadla and Gilbert 2000; Nishiyama *et al.* 2002; Nishiyama *et al.* 2003a; French and Johnson 2009). The intra-molecular bonds induce the high stiffness of the cellulose chain in crystalline cellulose. All of the three hydroxyl groups are involved in the formation of this hydrogen bond network. In native cellulose, there are two different crystalline forms I_α and I_β (Atalla and VanderHart 1984). The proportion of these forms is dependent on the origin of the cellulose (Atalla and VanderHart 1984). The two different crystal forms differ in cellulose chain conformation, hydrogen bonding, and different arrangement of cellulose molecules in the unit cell (Nishiyama *et al.* 2003a).

The macromolecular structure is not uniform within a cellulose microfibril as it contains both crystalline as well as less ordered (amorphous) regions (Mark 1940; Scallan 1971; Nishiyama *et al.* 2003b). The degree of crystallinity is dependent on the origin of the cellulose as well as its subsequent processing (Fink and Walenta 1994; Liitiä *et al.* 2003). In addition, different analytical techniques and methods often yield different

degrees of crystallinity (Park *et al.* 2010). For example, according to X-ray diffraction (XRD), the crystallinity of cellulose in cotton linters is 56 to 63% (Fink and Walenta 1994), while according to nuclear magnetic resonance (NMR) spectroscopy, the crystallinity of cellulose in softwood is 49 to 54% (Andersson *et al.* 2004). The crystallites within a single fiber are quite uniform in width as measured by wide-angle X-ray scattering (WAXS) technique (Hofmann *et al.* 1989). However, they differ greatly depending on the origin of the cellulose (Leppänen *et al.* 2009). For example, according to WAXS measurements, a cotton linter has a crystallite size of 17.7 nm in length and 7.1 nm in width, but in hardwood sulphite pulp, the corresponding values are 23.3 nm and 4.3 nm, respectively (Leppänen *et al.* 2009). The disordered regions are not yet understood in such detail.

The model most commonly applied to describe the longitudinal order/disorder transitions in a microfibril is termed the fringed fibril model (Hearle 1958). This model consists of a fibrillar structure, the smallest entity of which is the microfibril (Frey-Wyssling 1954; Fengel 1970). The diameter of the microfibril is dependent on the origin of the cellulose (Fink *et al.* 1990). For example, the microfibril width for hardwood kraft pulp measured by NMR spectroscopy is 3.9 nm (Wickholm *et al.* 1998). The microfibril length for hardwood cellulose is more than 2 µm (Saito *et al.* 2009). The cross-section of the smallest microfibrils, *e.g.*, those residing in wood fibers, are said to consist of 36 cellulose chains (see Fig. 2) (Okita *et al.* 2010). However, this is still being debated. An alternative model for the shape of the cross section of the microfibril is, for instance, a hexagonal model having more hydrophobic surface on the microfibril (Ding and Himmel 2006; Li and Renneckar 2011). The hydrophobicity in the cellulose microfibril is of increasing interest due to its effect on enzymatic hydrolysis (Lehtiö *et al.* 2003; Liu *et al.* 2011) and dissolution of cellulose in general (Lindman *et al.* 2010).

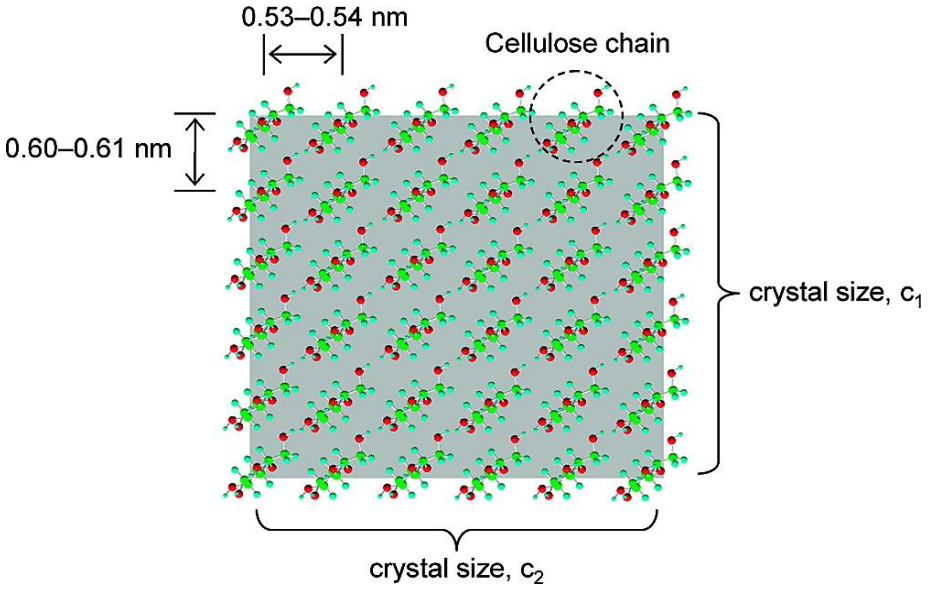

Fig. 2. Cross section of a microfibril. Reprinted with permission from Okita *et al.* 2010. Copyright 2010 American Chemical Society.

Cellulose microfibrils are said to form larger structural units, designated as cellulose microfibril aggregates or macrofibrils (Fengel 1970). Microfibrils are said to adhere to each other by lateral adhesion of crystallites already during the cell wall biosynthesis (Elazzouzi-Haffraoui *et al.* 2008). These macrofibrils can be detected in the cell wall of a fresh hardwood sample by field emission scanning electron microscopy (FE-SEM) (Awano *et al.* 2000). They are approximately 12 nm in width in the outer part of the secondary wall and approximately 15 nm in the middle layer of the secondary wall. However, it is not clearly understood in the existing studies whether or not these aggregates form as a result of sample preparation.

Individual fibrils of *ca.* 3.5 nm are also detected in fresh softwood and hardwood (Heyn 1969; Awano *et al.* 2000). The aggregate size correlates with the degree of lignification (Donaldson 2007). Thus, macrofibrils are the smallest, approximately 14 nm, in the low lignin content tension wood, and the largest, approximately 23 nm, in the highly lignified compression wood. The macrofibrils are not easily broken as they are retained even if the cell wall has been completely disintegrated into nanoscale cellulose fibrils (Pääkkö *et al.* 2007; Abe *et al.* 2007) or crystallites (Elazzouzi-Haffraoui *et al.* 2008). Hemicelluloses are also said to be partly involved in these structures (Salmén and Olsson 1998; Åkerholm and Salmén 2001).

Macrofibrils constitute the lamellar structure of the cell wall (Scallan 1974; Kerr and Goring 1975; Fahlén and Salmén 2002). The width of a lamella is said to be the magnitude of one microfibril aggregate, *i.e.*, about 20 nm (Fahlén and Salmén 2002). The cell wall of a cellulosic fiber, *e.g.*, a cotton or a wood fiber, consists of several layers (Hon and Shiraishi 1991; Klemm *et al.* 1998). The layers are formed of the lamellar structures (Kerr and Goring 1975). The microfibril orientation varies between the different cell wall layers. Within this structure, in a water-swollen state, there are also pores and voids of different sizes. Water is mainly accessible to the voids between the microfibrils and to the hemicelluloses (Alince 2002). The cell wall can be regarded as a hydrogel, the cohesive force of which is not crosslinking but the hierarchical structure of the cell wall.

PROPOSED MECHANISMS OF CELLULOSE MICROFIBRIL COALESCENCE

The fundamental phenomenon behind cellulose microfibril coalescence in chemical pulp fibers during various treatments is still under debate. The formation of irreversible hydrogen bonds between hydroxyl groups among cellulose microfibrils has been proposed in several studies over the years, although its scientific basis has never been clearly elaborated (Higgins and McKenzie 1963; Matsuda *et al.* 1994; Newman 2004). Another approach has been the lactone bridge formation, *i.e.*, the formation of bonds between hydroxyl and carboxyl groups (Back 1967; Fernandez Diniz *et al.* 2004). Other explanations over the years have included the migration of extractives to the surface (Christiansen 1990), as well as the free shrinkage that causes microcompressions in the fibers (Howard 1991).

Computational chemistry, particularly in the form of molecular dynamics and quantum mechanical studies, has recently made major contributions to an understanding of the forces within the native cellulose crystal and its solvation structures (French and Johnson 2009; Nishiyama *et al.* 2008; Gross and Chu 2010). Although these

intracrystalline forces are possibly similar to those that underlie microfibrillar coalescence, modeling is yet to develop into the stage where realistic correlations with coalescence mechanisms upon technical treatments can be made.

The hydrogen bonds formed during drying are said to be irreversible even under conditions that would normally lead to breakage of the bond (Higgins and McKenzie 1963). Irreversibility is increased according to the extent of the lateral bonding between fibers. This is due to the reduction of accessibility of the hydroxyl groups. Matsuda *et al.* propose that the changes in the fiber swelling properties are predominantly due to the formation of hydrogen bonds in non-crystalline regions of cellulose (Matsuda *et al.* 1994).

Crosslinking between crystalline cellulose domains in adjacent cellulose micro-fibrils is a possible mechanism for irreversible hydrogen bonding (Newman 2004). This phenomenon is often referred to as co-crystallization, even though the term is not universally acknowledged. The crosslinking requires the microfibrils to be parallel over a sufficient distance and the absence of other components between the microfibrils. It is considered to be favorable thermodynamically. The stiffening of the fiber as seen during drying would require a few linkages between crystallites per one microfibril. Although, according to the solid-state carbon-13 NMR spectroscopy, this crosslinking occurs during drying, it cannot explain all of the changes induced by drying (Newman 2004).

The lactone bridge formation between hydroxyl and carboxyl groups is seen as one possible route of cellulose microfibril coalescence (Back 1967; Fernandez Diniz *et al.* 2004). This theory is supported by the reduction of cellulose microfibril coalescence in alkaline solutions. In low molecular weight compounds, lactone bridges are broken in alkaline solutions. Lactones have also been successfully measured from dried cotton samples (Samuelson and Törnell 1961). The formation of bonds between carboxyl and hydroxyl groups has also been proposed by Lindström and Carlsson (1982) based on the reduction of cellulose microfibril coalescence while conducting drying with carboxyl groups in their ionized forms compared to drying in H^+-form. Ester formation is possible when carboxyl groups are in their H^+-form. However, the formation of esters in fibers is still being debated.

In addition to the bond formation between microfibrils, rearrangements within one single microfibril occur (Kontturi and Vuorinen 2009). The length distribution of the cellulose nanocrystals (CNCs), which originate from the crystalline regions of cellulose, can be determined by hydrolysis with a strong acid followed by atomic force microscopy (AFM). The acid hydrolysis breaks the microfibril structure at the amorphous regions (Battista *et al.* 1956). Drying does not change the length of the crystalline regions, but it does make the amorphous regions more susceptible to the acid hydrolysis (Steege and Philipp 1974). The more severe the drying conditions, the shorter the CNCs are after the acid hydrolysis (Kontturi and Vuorinen 2009). Kontturi and Vuorinen propose that the irreversible microfibril coalescence during drying induces tensions in the amorphous regions of the microfibrils. It has also been previously suggested that the molecular rearrangements during drying in the amorphous regions are also able to disrupt and reform the ordered regions (Sepall and Mason 1961).

CONSEQUENCES OF CELLULOSE MICROFIBRIL COALESCENCE

The major consequences of cellulose microfibril coalescence in chemical pulp fibers are pore closure and the reduced accessibility of the hydroxyl groups. Pore closure has conventionally been seen as the reduction in fiber swelling and fiber bonding capability due to the stiffness of the cell wall (Higgins and McKenzie 1963). To access the changes in the cellulosic material with respect to specific processing parameters and product qualities, one must define the concept of pore closure due to cellulose microfibril coalescence.

Cellulose microfibril coalescence within a fiber requires convergence of the microfibrils. This is hindered within a wood cell wall by the hemicellulose-lignin matrix located between the lamellae of cellulose macrofibrils. In addition, water hinders the convergence within a living tree. The removal of cell wall components from the cell wall, *e.g.*, during chemical pulping, creates larger pores into the cell wall (Stone and Scallan 1965a). This enables the convergence of the microfibrils. In many industrial processes, though, the environment is aqueous and, thus, water penetrates the pores and hinders the convergence. When water is removed from the system, the molecular segments come closer to each other (Lyne and Gallay 1950). Temperature rise increases the segmental motions and further increases the alignment and interactions between the molecular chains. In the dry state all of the hydroxyl groups in cellulose are involved in hydrogen bonding (Ellis and Bath 1940; Michell and Higgins 1999). The different stages of cell wall swelling are depicted in Fig. 3 (Scallan 1974). The fully swollen state (A) depicts the cell wall structure after the removal of the hemicellulose-lignin matrix in an aqueous environment. Drying enables the molecular segments to come closer to each other due to dehydration. Dry fibers contain almost no pores, as depicted in the dry state B (Stone *et al.* 1966). The lamellae of macrofibrils in the cell wall can coalesce in various ways and thus decrease the pore volume and accessible surface to water (Stone and Scallan 1965b). The pore size distribution will also be considerably altered due to the microfibrillar coalescence. The addition of water leads to reswelling by penetration of water between cellulose crystals, cellulose microfibrils, and the lamellae formed by macrofibrils depicted as states C and D (Gallay 1950; Stone and Scallan 1965c; Müller *et al.* 2000; Aulin *et al.* 2009). Due to the irreversibility of the cellulose microfibril coalescence during drying, the cell wall is no longer able to return to its fully swollen state (A) and, therefore, will remain in its hornified state (D). With certain restrictions, the fully swollen state can be regained by, *e.g.*, beating.

Pore closure has been the focus of significant research in the past, due to changes in the papermaking properties, *e.g.*, reduced strength properties originating from the decreased fiber deformability (Lyne and Gallay 1950; Maloney and Paulapuro 2000). At the moment, the topic of accessibility has been gathering interest with respect to the preparation of novel cellulosic products or biofuels. Regardless of one's perception of the actual mechanism behind the cellulose microfibril coalescence, it is generally accepted that this involves hydroxyl groups (Higgins and McKenzie 1963; Back 1967; Matsuda *et al.* 1994; Fernandez Diniz *et al.* 2004; Newman 2004). Coalescence of adjacent cellulosic surfaces is said to reduce the amount of accessible hydroxyl groups by creating paracrystalline cellulose, *i.e.*, an intra-fibrillar cellulose form that is inaccessible to the surrounding solvents (Wickholm *et al.* 1998).

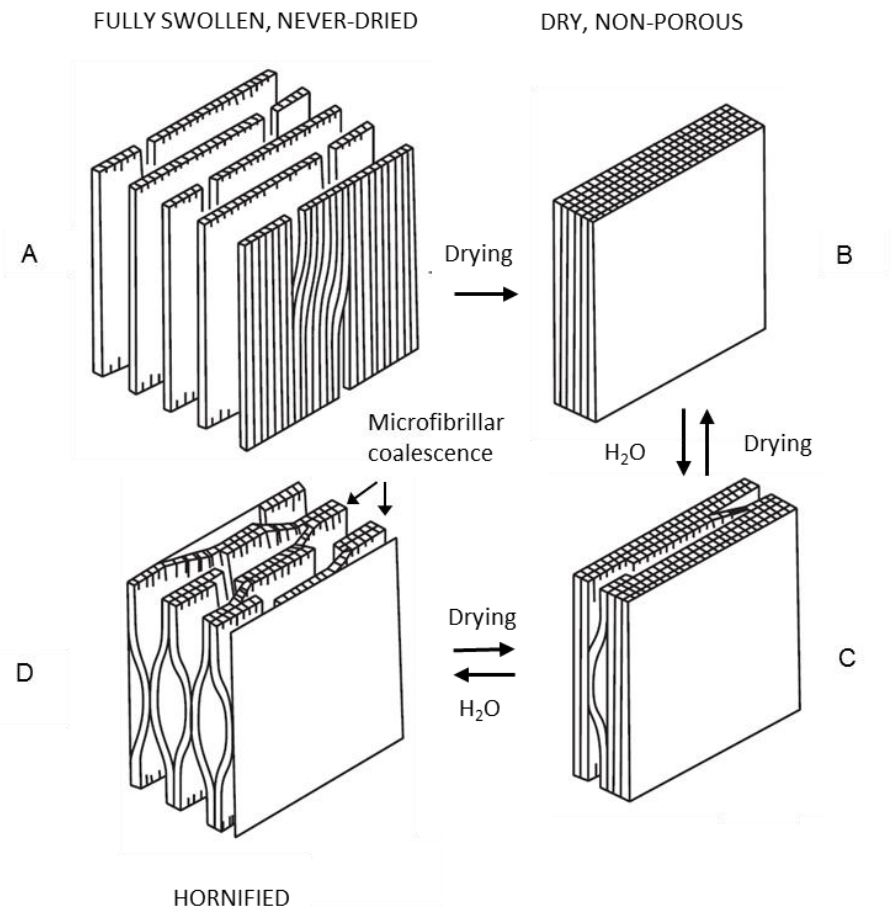

Fig. 3. Illustration of the behavior of the cell wall during drying. Adapted from Scallan 1974 with the permission from FPInnovations

The accessibility of the hydroxyl groups is important for new innovative products from wood that require chemical or enzymatic treatments. For instance, cellulose microfibril coalescence is seen to hinder both the functionality of enzyme complexes (Samejima *et al.* 1998) as well as the actual enzymatic processes (Luo *et al.* 2011). In addition, the reactivity of dissolving pulp in acetylation is said to be dependent on the lateral fibril aggregate dimension (Chunilall *et al.* 2010). Thus, this phenomenon affects most likely, *e.g.*, the chemical and enzymatic pretreatments of cellulose in nanocellulose production as well as the chemical and enzymatic degradation of cellulose into glucose for biofuel production.

TREATMENTS THAT INDUCE CELLULOSE MICROFIBRIL COALESCENCE

Drying of Wood

There are several indications that water is a structural component inside the living tree, although practically all wood fibers within a living tree are dead cells. The glass transition temperature of hemicelluloses is decreased by increasing moisture content (Olsson and Salmén 2004). This enables increased fiber elasticity and mobility in the cell

wall. Due to dehydration of wood, there are changes in the mechanical properties (Gerhards 1982), changes in the stability (Hillis 1984; Hillis and Rozsa 1985), and microscopic damage in the cell wall (Kifetew *et al.* 1998).

Some of these changes can be explained by cellulose microfibril coalescence. Cellulose microfibril coalescence in wood cells is assumed to occur due to dehydration after the tree is felled (Suchy *et al.* 2010a). The properties of dried as well as heat-treated wood are said to be altered by cellulose microfibril coalescence (Borrega and Kärenlampi 2008, 2010, 2011). This is seen as loss of mechanical properties (Borrega and Kärenlampi 2008), loss of hygroscopicity (Borrega and Kärenlampi 2010), and loss of porosity (Borrega and Kärenlampi 2011). Aggregated microfibril bundles, also referred to as macrofibrils, are found in various cellulosic fibers (Fink *et al.* 1990; Wickholm *et al.* 1998). These are partly due to the tendency of the microfibrils to form bundles already in a living organism and partly due to the drying-induced microfibril coalescence into aggregates.

At a nanometer level, WAXS shows an increase in strain and disorder as well as an increased density in the cellulose chains (Hill *et al.* 2010; Leppänen *et al.* 2011). However, controversy still remains over the changes at a molecular level during drying of wood. In the further chapters of this review, we will concentrate on the coalescence of cellulose microfibrils in cellulosic fibers disintegrated from the living organism.

Chemical Pulping

During the initial phase of kraft pulping, a large dissolution of hemicelluloses and a minor dissolution of lignin occur. According to NMR spectroscopy and AFM, the lateral fibril aggregate dimension increases simultaneously (Hult *et al.* 2001; Fahlén and Salmén 2003 and 2005). Thus, the main reasons behind cellulose microfibril coalescence during pulping are said to be the temperature rise and the removal of hemicelluloses and lignin.

The aggregates formed are larger in size than the sum of the two (or more) component fibrils. The enlargement is only approximately 4 to 5 nm, in contrast to the macrofibril size of approximately 15 to 20 nm. Therefore, it is assumed that the macrofibrils form microfibril bridges between the adjacent cellulose macrofibrils (Fahlén and Salmén 2003 and 2005). These bridges are incorporated into the resulting microfibrillar structure such as to form a slightly enlarged aggregate. The lamellar structure of the cell wall S2-layer changes during chemical pulping (Fahlén and Salmén 2005). The number of lamellae is reduced, and the width of the lamellae is increased. This is thought to be due to the addition of free microfibrils to the microfibril aggregates as well as the loss of the hemicellulose-lignin matrix lamellae. The pores formed during the dissolution of the hemicellulose-lignin matrix seem to be evenly distributed across the cell wall (Fahlén and Salmén 2005).

Although cellulose microfibril coalescence occurs mainly during kraft pulping, a moderate increase in microfibril aggregate dimensions is also present during mildly alkaline bisulphite-soda pulping (Hult *et al.* 2002). However, the fibril aggregates seem not to become enlarged during acid or neutral sulphite pulping (Hult *et al.* 2002 and 2003). This is thought to be due to the kraft pulping liquors' higher ability to cause swelling.

Drying of Pulp Fibers

The term hornification to describe the changes in chemical pulp fibers during drying was introduced by Jayme (1944). Even though this phenomenon has been known for such a long period of time, the actual mechanisms behind it are still being debated, as described in the previous chapter. The terminology and interpretation of hornification has been discussed in various reviews (Minor 1994; Nazhad and Paszner 1994; Weise 1998; Fernandez Diniz *et al.* 2004).

The removal of water from the cell wall of chemical pulp fibers entails the collapse of almost all of the pores (Stone *et al.* 1966). The absence of water also allows the formation of irreversible bonds between the microfibrils. Especially the collapse of the macropores, which, according to Maloney and Paulapuro are the pores formed by the dissolution of lignin and hemicelluloses during chemical pulping, is a significant factor in the hornification phenomenon (Maloney and Paulapuro 1999). According to the solute exclusion measurements, the amount of pores larger than 2.5 nm is decreased during drying (Stone *et al.* 1968). The amount of pores smaller than 2.5 nm is unaffected.

The assumption of the cellulose microfibril coalescence upon drying of chemical pulp fibers is supported by NMR spectroscopy (Hult *et al.* 2001; Chunilall *et al.* 2010). According to the NMR measurements, the lateral fibril aggregate dimension increases during drying. In some cases, even the lateral fibril dimension increases during drying, probably due to the addition of order by removal of residual distortion of the cellulose microfibril (Hult *et al.* 2001). Table 1 presents the changes in dimensions of fibrils as well as fibril aggregates during drying of different softwood kraft pulps.

Table 1. The Lateral Dimensions of Fibrils and Fibril Aggregates Before and After Drying *

Sample	Average lateral fibril dimension (nm)		Average lateral fibril aggregate dimension (nm)		Hemicellulose (% on dry wood)
	Never-dried pulp	Dried handsheets	Never-dried pulp	Dried handsheets	
Kraft cook (H factor 2000)	4.8	4.8	18.1	23.1	10
Kraft cook (H factor 1600)	4.5	4.8	17.9	21.4	17
Kraft cook (H factor 1300)	3.9	4.5	15.4	17.6	22

* Adapted from Hult *et al.* 2001; Copyright 2001 with permission from Elsevier

The changes in the fiber properties of chemical pulp fibers during drying have been acknowledged for quite some time. The changes relevant for papermaking purposes include reduced swelling and altered strength properties (Lyne and Gallay 1950; Maloney and Paulapuro 2000). The changes in swelling were first assessed by the centrifugation method (Jayme 1944). The decrease in pulp swelling has been later specified with other methods to evaluate the change in fiber saturation point and in the pore size distribution. These methods are described in the last chapter of this paper. The change in the strength properties is reflected in increased bulk and tear strengths, whereas tensile, burst, and fold strengths are decreased (Lyne and Gallay 1950). The loss of strength properties is due to the stiffening of the fibers that causes the decrease in the fiber-fiber bonding area

(Maloney and Paulapuro 2000). Hornification is most pronounced in the first drying and rewetting cycle (Laivins and Scallan 1993). Multiple drying and wetting cycles increase hornification only to a smaller extent (Wistara and Young 1999).

Wet Pressing

Wet pressing causes similar reduction in fiber swelling as drying (Carlsson and Lindström 1984; Luo *et al.* 2011). However, wet pressing is much less homogenous compared to drying, as the stresses are concentrated at certain parts of the fiber matrix (Carlsson and Lindström 1984). Therefore, the changes occur at a lower mean solid content level, namely at 30 to 45% compared to 50% in drying. To achieve a more uniform hornification of the sample, several wet pressing and slushing cycles are required. The reduction in fiber swelling is more pronounced in pulps with a high initial swelling capacity (Carlsson and Lindström 1984). Hornification due to wet-pressing is also seen to reduce the accessibility of cellulose in its further processing with cellulase enzyme (Luo *et al.* 2011).

Recycling

The beginning of industrial paper recycling can be assigned to the year 1800, when Matthias Koop was awarded the English patent no. 2392 for extracting ink from paper and converting such paper into pulp (California department of conservation 1997). The effect of recycling on fiber quality has also been of great interest since the 1960s. They have been summarized by Howard (1990) as well as Nazhad and Paszner (1994). The changes that fibers undergo during recycling vary notably, depending on the original papermaking procedure as well as the recycling procedure. Hornification is naturally an important factor in recycling as it involves drying and rewetting. The changes caused by hornification, such as loss of fiber bonding, were discussed in the earlier chapter. The most severe effect occurs during the first cycle of paper forming, drying, use, and recycling. Although hornification plays an important role in the changes induced by recycling, it has to be kept in mind that multiple different process variables during recycling affect the final fiber properties. Two significant factors affecting the properties of recycled fibers are the loss of fines and the loss of hemicelluloses (Wistara and Young 1999; Wistara *et al.* 1999). The sheet properties lost during recycling are primarily those related to fiber bonding, *e.g.*, bursting strength and tensile strength (McKee 1971).

High Temperature Treatments and Thermal Ageing

In general, high temperature activates and accelerates chemical reactions. In cellulosic materials it causes radical formation that enables several other reactions, *e.g.*, formation of carboxyl and carbonyl groups or depolymerisation (Back 1967). In addition, there is auto-crosslinking of cellulose that causes reduced swellability. This reaction is homogenous within the temperature range of 70 to 350 °C. In the presence of oxidants, *e.g.*, in periodate oxidation, crosslinking occurs by the formation of hemiacetal groups between carbohydrate chains. Crosslinking is enhanced by pre-oxidation prior to the heat treatments. Respectively, pre-reduction by, *e.g.*, sodium borohydrite, slows down the crosslinking. Hemiacetal bonds are broken by low as well as high pH. However, all the bonds formed at high temperature are not broken under these conditions. Even more stable crosslinking via ether-bonds may occur during heat treatments. Temperature is also seen

to enhance hornification (Laine *et al.* 2003a; Kontturi and Vuorinen 2009; Chunilall *et al.* 2010).

Kato and Cameron (1999) have reviewed the relationship between thermal ageing and hornification. Even though other reactions occur during thermal ageing, such as chain scission, the possibility for microfibril coalescence is clearly present as well (Kato and Cameron 1999). Thermal ageing of cellulose is a serious problem for preservation of historic documents and textiles as well as for paper-based electrical power transformer insulations (Kato and Cameron 2002). Ageing is said to have the same kind of effect as drying, and the consequences of each can be measured as a change in water retention value (WRV). Ageing in this context refers to ageing periods from 1000 to 1500 hours at elevated temperatures from 120°C to 160°C. The coalescence of microfibrils is partly due to the drying-induced hornification under the high temperature ageing conditions as water is lost during ageing.

VARIABLES AFFECTING CELLULOSE MICROFIBRIL COALESCENCE

Acid Groups and pH

Fibers have a negative charge within the entire pH-range of interest to papermakers (Lindström 1992). The acidic groups originate from the cell wall polymers, mainly hemicelluloses, or are introduced during chemical treatments, such as chemical pulping or bleaching. The ionizable groups in the fiber are mainly carboxylic groups, although others, such as sulphonic acid groups, may be present to a lower extent, depending on the origin and processes experienced by the pulp. The extent of hornification during drying is dependent on pH due to the carboxyl groups in the fiber (Lindström 1992). Hornification is more pronounced within the low pH range (Lindström and Carlsson 1982). Above pH 8 in the presence of Na^+-ions, the effect of pH reaches a plateau value as all the carboxyl groups have been transformed to their Na^+-form (Lindström 1992). It is assumed that carboxyl groups in their H^+-form could form additional hydrogen bonds with for instance other oxygen atoms or they could form esters with hydroxyl groups (Lindström and Carlsson 1982). The reason can also be the electrostatic repulsion between the charged groups. Lactones have been measured in the case of dried cotton samples (Samuelson and Törnell 1961). The pH level is also an important factor influencing hornification during wet pressing (Carlsson and Lindström 1984). Namely, the lower the pH, the more extensive the loss of fiber swelling.

By contrast, Matsuda *et al.* claim that the primary mechanism of hornification is hydrogen bond formation and not the ester formation (Matsuda *et al.* 1994). This is based on the TAPPI test method T 237 om-88 measurements of carboxyl group contents of the pulp before and after drying. According to the measurements with this method, the carboxyl group content seems to remain unchanged. If esters were formed, a decrease in carboxyl group content should be detectable.

The growth of microfibrillar aggregates during chemical pulping is said to be dependent on alkalinity: the higher the alkalinity, the larger the aggregates (Virtanen *et al.* 2008). However, hemicelluloses seem to influence this (Virtanen *et al.* 2008). When hemicelluloses are retained during the cook the aggregate size does not increase with higher alkalinity levels. Thus, hemicelluloses prevent the aggregate growth during

alkaline cooking. This is also known from the dissolution of cellulose chains of wood fibers to NaOH-water, which is dependent on the localization of cellulose in the cell wall and especially on the hemicellulose matrix (Le Moigne and Navard 2010). The molecular weight of cellulose is not the determining factor of dissolution, as the macrostructure and chemical environment are its key elements. Amorphous cellulose in a featureless ultrathin cellulose film is, thus, readily soluble in very low concentrations of alkali (Kontturi *et al.* 2011). The dissolution of cellulose in alkali is said to be due to the dissociation of the three hydroxyl groups of cellulose (Isogai 1997).

Cell Wall Composition

Cellulose microfibril coalescence during various treatments depends on the origin of the pulp. There is a clear difference between low and high yield pulps due to the loss of the hemicellulose-lignin matrix in low yield pulps (Higgins and McKenzie 1963; Scallan and Tigerström 1992; Laivins and Scallan 1993; Billosta *et al.* 2006; Law *et al.* 2006; Luukko and Maloney 1999). Mechanical and semi-mechanical pulps, such as stone-groundwood pulp (SGW) and chemical thermo-mechanical pulp (CTMP) undergo only slight alterations during drying or recycling (Billosta *et al.* 2006). However, the hornification effects of drying and recycling can be clearly seen with chemical and semi-chemical pulps, such as kraft pulps (Jayme 1944; Maloney and Paulapuro 2000; Billosta *et al.* 2006). A clear difference between sulphite and kraft pulps is also detected, as the sulphite process does not remove the hemicellulose-lignin matrix as extensively as the kraft process (Jayme and Hunger 1957). Cotton, which is essentially pure cellulose, has a strong hornification effect during drying (Fahmy and Mobarak 1971).

Hemicelluloses have been proposed to have a hindering effect on hornification during drying, where hornification was measured as the change in fiber properties, such as WRV, total pore volume, and tensile strength (Oksanen *et al.* 1997). Therefore, hemicelluloses greatly impact the cellulose microfibril structure, pore structure, and cellulose supermolecular structure during drying and chemical pulping (Wan *et al.* 2010). During kraft pulping and drying, the lateral fibril aggregate dimension, average pore diameter, and cellulose crystallinity increase (Wan *et al.* 2010). This phenomenon is more pronounced for pulps with lower hemicellulose content (Hult *et al.* 2001; Duchesne *et al.* 2001 and 2003). This is thought to be due to the increased coalescence of cellulose microfibrils in the absence of hemicelluloses (Oksanen *et al.* 1997; Rebuzzi and Evtuguin 2006). Thus, the removal of hemicelluloses in the fiber matrix seems to give the opportunity for the cellulose fibril surfaces to move close enough to each other to form hydrogen bonds and thus increase the average lateral fibril aggregate dimensions (Oksanen *et al.* 1997; Hult *et al.* 2001; Duchesne *et al.* 2003). Furthermore, a higher xylan content was systematically found to improve the quality of thermomechanical pulp after drying (Cao *et al.* 1998). However, the hemicellulose removal cannot by itself explain the coalescence, since there seems to be no fibril coalescence under low-temperature alkaline conditions that also remove hemicelluloses (Fahlén and Salmén 2003). The additional removal of lignin combined with the hemicellulose removal seems to give the microfibrils even more possibility to coalesce (Ishizawa *et al.* 2009). The coalescence during chemical pulping may also be due to the softening of the lignin network (Fahlén and Salmén 2003). Cellulose microfibril coalescence has also been detected in other cellulosic plants, such as

celery, in the course of removal of their cell wall components, *e.g.*, pectins (Thimm *et al.* 2009).

The effect of hemicelluloses could be partly due to the carboxyl groups of certain hemicelluloses, as the carboxylic groups in their protonated form are said to cause additional bonding within microfibrils (Lindström 1992). Xylan, in contrast to glucomannan, contains carboxyl groups. However, the effect of hemicellulose removal on WRV is equal for both xylan and glucomannan (Oksanen *et al.* 1997). Therefore, this phenomenon cannot be fully explained by the carboxyl groups of xylan.

Temperature

Heating has been acknowledged to change the swelling properties of fibers (Jayme 1944; Renaud 1947). Temperature seems to have an effect on this phenomenon, although the significance of it has been debated. The effect of temperature has been studied during drying (Maloney and Paulapuro 2000), chemical pulping (Fahlén and Salmén 2003), ageing (Kato and Cameron 2002), and heat-treatment on dry handsheets (Matsuda *et al.* 1994).

Lyne and Gallay (1950) assessed the influence of temperature alone by heating sulphite pulp fibers in a saturated water atmosphere at 95 °C prior to drying. The heat-treated samples underwent complementary hornification compared to the samples that were dried without pre-heating. Drying temperature also affects hornification. The change in WRV is more pronounced with fast drying at high temperature, namely over 100 °C, compared to gentle drying at room temperature over a longer period of time (Laine *et al.* 2003a; Kontturi and Vuorinen 2009; Chunilall *et al.* 2010). This is also seen in the pore closure. The median pore size is retained while heating at 25 °C, but it is considerably smaller during drying at 105 °C (Stone and Scallan 1965b). This is said to be due to the more permanent pore closure of the larger pores. The effect of temperature on hornification can be seen already at lower temperatures (Maloney and Paulapuro 2000). However, the fiber saturation point (FSP) changes drastically at temperatures above 70 °C. This is probably due to the increased removal of water between the microfibrils or the increase in molecular rearrangements at temperatures above 70 °C (Weise *et al.* 1996). The capillary pressure in a porous system is also said to be enhanced by increasing temperature (Hanspal and Das 2012). This is logical, because more rapid evaporation results in faster water removal from the pores. It can be concluded that temperature has an influence on the hornification phenomenon.

Temperature must also play a role in the cellulose microfibril coalescence during chemical pulping, as there is said to be no enlargement of fibril aggregates at temperatures below 150 °C even at high alkalinity (Fahlén and Salmén 2003). High temperature increases microfibril mobility and enables the rearrangement of the aggregates.

In thermal treatments of dry handsheets, the higher the temperature is, the lower the WRV will be after the treatment (Matsuda *et al.* 1994). The amount of accessible hydroxyl group measured by deuterium exchange followed by 1H NMR spectroscopy decreases by 10% during heat treatment in 120 °C for one hour. Thus, the thermal treatment decreases cellulose accessibility. The change in WRV also shows remarkable differences as a result of treatment at different temperatures (Kato and Cameron 2002). It must be concluded that in the course of ageing at such high temperatures there is also significant degradation through hydrolysis and oxidation, and this, obviously, has an

effect on fiber properties. Nevertheless, lower chain length resulting from such degradation favors hornification, as the chain mobility increases with decreasing chain length, enabling more extensive coalescence of microfibrils.

Moisture

Dehydration as such is a significant factor in cellulose microfibril coalescence, as it has been proposed to occur to some extent even while drying without heating as well as during wet pressing in the absence of heating (Carlsson and Lindström 1984; Laine *et al.* 2003a). The effect of the moisture content during heat treatment at 100°C is illustrated in Fig. 4 (Maloney and Paulapuro 2000). The heating is conducted in a sealed environment at various moisture contents. The maximum hornification is reached at a moisture content of 0.25g/g. This is the moisture content where capillary water forms in the cell wall, a moisture level also referred to as the "second critical point". Below this moisture content, hornification is promoted by adding more water. Above this moisture content hornification is hindered by adding water, until the moisture content reaches the fiber saturation point at 0.65 g/g. Above this, the moisture content has no effect on the phenomenon. The FSP, as the critical moisture content below which hornification begins, has also been detected at room temperature (Laivins and Scallan 1993). Thus, the coalescence does not require any heat, but water needs to be removed from the cell wall, not only from the spaces between the fibers.

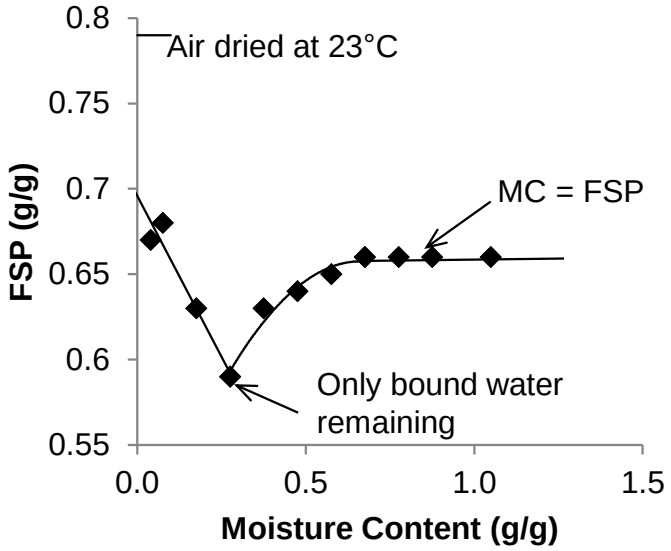

Fig. 4. Effect of moisture content on hornification during heat treatment in a sealed environment (reproduced with data obtained from Maloney; published in Maloney and Paulapuro 2000)

It is also said that water acts not only as an obstructing molecule but also facilitates chain movement (Caulfield and Steffes 1969). As the relative humidity increases, the crystallinity of cellulose increases due to the plasticizing effect of water on cellulose chains, promoting microfibril alignment.

PREVENTING AND REVERSING CELLULOSE MICROFIBRIL COALENCENCE

Beating

In the course of beating, the cell wall is able to take up more water; in other words, swelling is increased (Stone *et al.* 1968). In addition, the elastic modulus is decreased (Scallan and Tigerström 1992). This implies that the fibers are more deformable after beating. Thus, beating can recover some fiber properties that had been altered by hornification (Higgins and McKenzie 1963; Page 1985; Laivins and Scallan 1996; Wang *et al.* 2003). However, many properties caused by hornification cannot be totally reversed by beating (Wang *et al.* 2003). Even though beating reverses the changes in swelling, the pore size distribution between never-dried and dried-then-beaten fibers remains different. The fewer number of small pores in the dried-then-beaten fibers implies that during hornification strong bonds are formed between the microfibrils, causing the pores to close. These bonds are partly resistant to the shear and compression forces of beating. The amount of larger pores increases during beating (Stone *et al.* 1968). According to AFM measurements with image processing, cellulose fibril aggregate size does not change during beating (Fahlén and Salmén 2005). Thus, beating is not able to reverse hornification in this respect either. From an economical point of view, drying-induced changes in the pulp increase the beating time.

Beating affects the fibers in several different ways (Page and De Grâce 1967; Page 1985), *e.g.*, by producing internal fibrillation and fibrillar fines. Internal fibrillation is more pronounced with sulphite pulps than with kraft pulps (Page and De Grâce 1967). Thus, beaten sulphite pulps should be more flexible and chemically reactive compared to beaten kraft pulps. One of the most important factors for the increase of strength properties in beaten pulps is the straightening of the fibers (Page 1985). This is due to the release of kinks, crimps, and curl that had been set in the fiber during drying and then released by swelling and mechanical stress during beating. The paper made of straighter fibers has better stress distribution and therefore better strength properties. The fines produced during beating, so-called secondary fines, have different swelling characteristics compared to the primary fines (Laivins and Scallan 1996). Thus, they change the dewatering properties of the pulp even though they are produced quite moderately in the course of beating (Laivins and Scallan 1996).

The conditions during beating have also an effect on the reversion of the microfibril coalescence. Beating under alkaline conditions proceeds faster compared to beating under acidic conditions (Laivins and Scallan 2000). Swelling of unbleached kraft pulp is increased most by beating the pulp in its alkaline form. Thus, beating in the alkaline form reduces the need for the energy-consuming beating.

Additives

Adding substances that hinder hydrogen bond formation can decrease the microfibril coalescence (Higgins and McKenzie 1963; Laivins and Scallan 1993). However, most of the available additives are not economically feasible due to high concentrations or inadequate effects (Higgins and McKenzie 1963; Laivins and Scallan 1993). In addition, some of the chemicals have to be removed prior to papermaking (Higgins and McKenzie 1963). One of the disadvantages is also that the additives are only

functional during the first drying cycle, as they are lost during the rewetting (Laivins and Scallan 1993).

Introducing a limited amount of hydrophobic groups reduces the formation of hydrogen bonds between the adjacent cellulose chains (Higgins and McKenzie 1963; Zhang *et al.* 2002). This, furthermore, makes the residual hydroxyl groups more accessible. Surface-active agents provide also means to weaken the hydrogen bond formation between microfibrils (Higgins and McKenzie 1963). This is due to the decrease in surface tension. Another approach in preventing the formation of hydrogen bonds is to introduce compounds that form bonds with cellulose that are reversible after rewetting, *e.g.*, glucose or sucrose (Higgins and McKenzie 1963; Laivins and Scallan 1993; Zhang *et al.* 2004). Recently, Aarne *et al.* managed to slightly suppress hornification by adding high molecular weight cationic polyelectrolyte in excess before drying, which overcompensated the charge inside the larger pores and helped reopen them upon rewetting (Aarne *et al.* 2012).

Charge and pH

Charge and pH influence both the reversibility of cellulose microfibril coalescence as well as the hindrance of the coalescence. First, we discuss the reversibility. Some properties lost during microfibril coalescence can be partly restored by the increase of fiber charge. The ionic groups in fibers increase the swelling properties of fibers as well as the specific bond strength (Fors 2000). Therefore, introducing charge in the fibers will increase their swelling and bonding, and thus, provide better strength properties. Carboxymethylation and carboxymethyl cellulose (CMC) adsorption are ways to introduce additional charge to fibers (Rácz and Borsa 1997; Laine *et al.* 2003b). Both of these methods can improve the properties lost due to coalescence even to a larger extent than beating (Laine *et al.* 2003b). However, CMC adsorption, which is fiber surface specific, gives superior strength properties compared to bulk carboxymethylation. CMC adsorption leads to an increase in the relative bond strength. In addition, the counter ion has an influence on the changed properties.

Another way to regain properties lost during cellulose microfibril coalescence is an alkaline treatment. Unbleached chemical pulps dried under acidic conditions can be partly reswollen by an alkaline treatment (Lindström and Carlsson 1982; Lindström 1992). The alkaline treatment has commonly been proposed to improve the bonding properties of the pulp when applied to secondary fibers (Klungness 1974). Measured as a change in WRV, a one hour alkaline cooking in 3% NaOH is said to reverse the hornification of bleached kraft pulp fibers by 55% (Weise *et al.* 1998).

Cellulose microfibril coalescence can be hindered by both high fiber charge and high pH. An increase in fiber charge of never-dried pulps seems to reduce the effect of hornification during drying as measured by a change in strength properties and WRV (Lindström and Carlsson 1982; Dang *et al.* 2007). Fiber charge can be increased by, *e.g.*, carboxymethylation or peroxide treatment (Lindström and Carlsson 1982; Dang *et al.* 2007). To achieve a reduced hornification, the pulps containing carboxylic groups need to be dried with the acidic groups in their ionized form instead of drying with the acidic groups in their protonated form (Lindström and Carlsson 1982; Laivins and Scallan 1993). No consensus has been reached on the reasons behind this effect. It is assumed that carboxylic groups in their protonated form could form additional hydrogen bonds with for

instance other oxygen atoms or they could form esters with hydroxyl groups (Lindström and Carlsson 1982). Another reason can also be the electrostatic repulsion between the microfibrils due to the charged groups. Hornification is said to be prevented by a degree of carboxymethylation that corresponds to the ionic content of approximately 30 meq/100g pulp, when bleached kraft pulp is dried in its ionized form (Lindström 1992; Laivins and Scallan 1993). The pH level during drying also influences the WRV of pulps containing carboxylic groups, *e.g.*, unbleached pulps (Lindström and Carlsson 1982). For unbleached pulps, hornification is more profound at low pH levels (Lindström and Carlsson 1982).

METHODS TO EVALUATE CELLULOSE MICROFIBRIL COALESCENCE

Cellulose microfibril coalescence is said to change the structure of the fiber by creating paracrystalline cellulose that is inaccessible to water (Wickholm *et al.* 1998). In addition, cellulose microfibril coalescence causes the pores to close due to the formation of bonds between adjacent lamellae (Maloney and Paulapuro 1999). These changes are seen as reduced swelling. Swelling is a volumetric enlargement caused by, *e.g.*, the imbibition of liquid by a polymer (Gallay 1950). Amorphous parts of cellulose are able to imbibe water, even though water cannot penetrate inside the crystalline cellulose structures (Müller *et al.* 2000; Aulin *et al.* 2009). Water can, nevertheless, be incorporated between the fibers, adjacent microfibril lamellae, and microfibrils, thus, increasing swelling. In fiber suspensions, the water uptake of the network comprises several different components: water inside the cell wall, water inside the lumen, water held by microfibrils on the fiber surface, and water held between the fibers (Stone and Scallan 1967). This creates a challenge when measuring fiber swelling, as the measure should only contain the water held within the cell wall. This measure is often referred to as the fiber saturation point (FSP) (Stone and Scallan 1967). In the cell wall, water is located inside the pores and associated mainly with the hemicelluloses (Alince 2002). Thus, the total volume of water in the cell wall is not necessarily equal to the pore volume. There are said to be different kinds of pores in the cell wall: small pores between microfibrils and large pores between macrofibrils (Alince 2002). Dry fibers contain almost no pores, the estimated amount being less than 0.02 cm^3/g (Stone and Scallan 1965b; Stone *et al.* 1966). The methods to evaluate cellulose microfibril coalescence are commonly based on the cell wall water measurement. However, this is not a direct measure of the coalescence.

Jayme (1944) introduced the first acknowledged analytical method to evaluate the amount of water inside the cell wall, namely the water retention value (WRV), lately standardized as the ISO 23714:2007 method. This method describes the affinity of pulp to water as measured after centrifugation, which is supposed to remove the excess water and leave behind only the water inside the cell wall (Jayme 1944, 1958). Hornification is evaluated as the percentage change in WRV during a treatment, *e.g.*, drying. This method can also be considered as a measure of cellulose accessibility, even though it is not a direct measurement. Although having its limitations, WRV can be a good and simple measurement for relative changes in the fiber morphology. Problems related to the WRV test are water retention between the fibers and, on the other hand, water removed from the cell wall due to pressing (Maloney *et al.* 1999). For example, in previously frozen kraft

pulps or mechanical pulps, water is retained between the fibers, since these pulps are equipped with ridged cell walls that do not collapse during centrifuging and, therefore, retain water in small interfiber pores. Whereas, highly swollen pulps, such as never-frozen hardwood kraft pulps, allow water to be pressed out of the cell wall during centrifugation.

A more recently applied method is the so called hard-to-remove (HR) water content measured by high resolution thermogravimetric analysis (TGA) (Park *et al.* 2006a). This water is considered to be located close to the fiber surface as well as trapped inside the fiber geometry. TGA is applied for drying the sample until it reaches a weight loss curve of 0.001 %/min. HR is defined as the ratio of water mass to fiber mass at the transition between the constant rate zone and the falling rate zone. The advantage of this method compared to WRV is the small sample size, approximately 10 mg of dry mass, compared to the 1.54 g needed for the standard ISO 23714:2007 WRV measurement. HR values are in alignment with WRV values (Park *et al.* 2006a).

The pore size distribution within the fibers is also applied to evaluate microfibril aggregation (Alince 2002). The study of the porous structure of fibers in their water-swollen state by solute exclusion technique began in the 1960s (Stone and Scallan 1967 and 1968). Solute exclusion can be applied for pulp fibers to evaluate the pore size or to measure FSP (see Fig. 5).

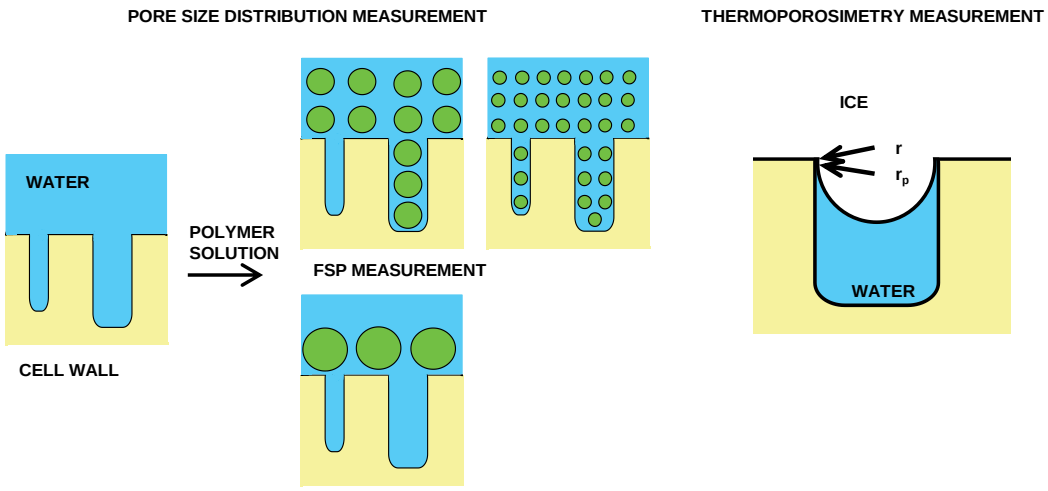

Fig. 5. On the right: The principle of the solute exclusion technique. On the left: Thermoporo-simetry experiment with an excess of water. Pore radius depicted as r_p and surface curvature as r. In a sufficiently small pore, only liquid water exists within the pore.

The measurement is conducted with a probe polymer or a range of probe polymers. If the pores are accessible to the probe polymers, then they will contribute to the dilution of the probe solution. By conducting the measurement with a range of polymers, the pore size distribution can be determined. The FSP measurement applies a single polymer that will not penetrate the cell wall, commonly a 2×10^6 Da dextran polymer with a spherical diameter in solution of 54 nm (Stone and Scallan 1967; Maloney *et al.* 1999). The polymer solution is, thus, diluted by the water associated with the fibers above the FSP. Water within the cell wall will not dilute the solution. Thus, the FSP can be calculated according to Equation (1) by measuring the change in the polymer

concentration while a known amount of polymer solution is added to a known amount of fibers with a known moisture content,

$$\delta_s = \frac{w+q}{p}\left[1-\left(\frac{w}{w+q}\right)\left(\frac{c_i}{c_f}\right)\right] \quad (1)$$

where p is grams of dry fibers, q is grams of water associated with the fibers, w is grams of polymer solution added, c_i is the initial concentration of the solution in grams of solute per gram of solution, c_f is the final concentration, and δ_s is the FSP in grams of water per gram of dry fiber.

The solute exclusion test, which measures the actual amount of water in the cell wall, is considered to be a more accurate method to evaluate hornification compared to the WRV test (Maloney *et al.* 1999). However, there are some limitations to this method, as some pores have limited accessibility within the fiber and water within the depletion layer, the thickness of which is presumably equal to the radius of the probe polymer, is not available for diluting the polymer (Maloney and Paulapuro 1999).

In addition to the solute exclusion technique, there are several different methods to evaluate the pore size distribution. We cover here some of these methods commonly applied for cellulosic materials, *e.g.*, thermoporosimetry conducted with differential scanning calorimetry (DSC) by the isothermal melting technique (Maloney and Paulapuro 1998; Wang *et al.* 2003), DSC combined with a TGA (Park *et al.* 2006b), NMR cryoporosimetry (Gane *et al.* 2004; Östlund *et al.* 2010), and inverse size-exclusion chromatography (ISEC) (Berthold and Salmén 1997). The different methods give approximately the same accuracy with respect to the pore sizes (Gane *et al.* 2004). However, caution must be taken when comparing the values obtained by different methods, as the actual values differ between the methods depending on, *e.g.*, the material analyzed (Gane *et al.* 2004). All of the methods assume that the pores are cylindrical or spherical, which is a limitation to these measurements. This can be, in some cases, overcome to an extent by correction factors (Berthold and Salmén 1997).

DSC enables the controlled temperature adjustment of the sample and simultaneous monitoring of the melting and freezing transitions in a porous sample confined in a liquid (Maloney and Paulapuro 2001). In DSC with isothermal melting, the solvent exchanged sample is first frozen and then the solvent is melted in steps. The stepwise melting is applied for fibrous samples due to the large size of the pores, because it improves the resolution (Maloney and Paulapuro 2001). The pore size is inversely related to the melting depression according to the Gibbs-Thomson equation (2),

$$D = \frac{4VT_0\sigma_{1s}}{H_m\Delta T} = \frac{k}{\Delta T} \quad (2)$$

where D is the pore diameter, V is the molar volume, T_0 is the normal melting point, σ_{1s} is the interfacial tension between the solid and liquid, H_m is the latent heat of melting, and ΔT is the melting temperature depression.

Figure 5 depicts the conditions where the sample is frozen. The shift in the transition from liquid to solid or solid to liquid, *i.e.* the freezing or melting point

depression, is dependent on the radius of curvature of the interface between the solid and liquid phases (Landry 2005). The radius of curvature is related to the pore size. Challenges related to thermoporosimetry include freezing damage to the cell wall, distortion of the pores by crystal growth, a limited range of measurable pores in aqueous systems, and partial solubility of the cell wall components (Maloney and Paulapuro 2001). These issues can be partly overcome by choosing another solvent. However, certain solvents, *e.g.*, cyclohexane, contract the cell wall and may change the pore structure. In DSC with TGA, the samples are dried to different moisture ratios by a thermogravimetric analyzer prior to DSC (Park *et al.* 2006b).

NMR cryoporosimetry follows the same principal as the DSC thermoporosimetry (Gane *et al.* 2004; Petrov and Furó 2009; Östlund *et al.* 2010). In NMR cryoporosimetry, the water-swollen samples are frozen and then melted stepwise. The phase transition temperature shift will provide information on the pore sizes, their distribution, and in some cases even their shape according to the Gibbs-Thomson equation (Eq. 2). This method differs from DSC as the actual melting is not detected but the amount of water that melts at a certain temperature. This is accomplished by the spin-echo pulse sequence that enables the separation of nuclei of mobile and immobile molecules. The magnetization relaxes to zero for the nuclei of immobile molecules. With this technique, only the pores with a radius less than 100 nm are detected.

Inverse size-exclusion chromatography (ISEC) is based on the preparation of chromatographic columns of the analyzed pulp fibers (Berthold and Salmén 1997). The chromatogram is then eluted with probe molecules with standard molecular weights. The elution time of a probe molecule too large to penetrate any pores in the fiber matrix is compared to the elution time of a probe molecule small enough to penetrate all the pores in the matrix. ISEC is a relatively fast measurement.

Solvent-exchange-drying combined with Brunauer-Emmet-Teller (BET) sorption by nitrogen is also applicable for the analysis of surface area and pore volumes (Stone and Scallan 1965c). Solvent exchange preserves the water-swollen structure of fibers during drying, and thus the pore volume can be measured by nitrogen adsorption. However, slight shrinkage can occur and, thus, the pore volume is somewhat under-estimated.

In the future, more specific measurements need to be used for the evaluation of cellulose microfibril coalescence to assess the actual extent of aggregation and the change in accessibility. Deuterium exchange combined with infrared spectroscopy has been applied already in the 1950s for the analysis of cellulose crystal structures (Mann and Marrinan 1956; Jones 1958). The accessibility of cellulose microfibrils has also been calculated from the data obtained by deuterium exchange combined with the infrared spectroscopy for wet samples (Sumi *et al.* 1963). This method has been adapted to a novel method to analyze the actual bond formation during drying by deuterium exchange followed by the fourier transform infrared spectroscopy (FTIR) (Suchy *et al.* 2010b). In this method, the hydrogen in the accessible hydroxyl groups are replaced by deuterium (Fig. 6). Then the sample is subjected to the treatment studied, *e.g.*, drying in deuterium oxide atmosphere. After the treatment, the sample is washed to remove the non-bound deuterium. Deuterium is then detected by FTIR. This is a comparatively easy and rapid method, and it provides direct evidence on the bond formation during cellulose coalescence.

To analyze the morphological and ultrastructural changes that occur during cellulose microfibril coalescence, one can apply electron microscopy. For example, transmission electron microscopy (TEM) with staining of the cell wall polysaccharides can be a powerful tool to look at the changes induced by cellulose microfibril coalescence (Billosta *et al.* 2006). In addition, the NMR spectroscopy has been widely used to measure the dimensions of cellulose microfibril aggregates (Hult *et al.* 2001; Fahlén and Salmén 2003). Atomic force microscopy (AFM) with quantitative imaging can also be used to measure the dimensions of cellulose microfibrils (Fahlén and Salmén 2005; Lee *et al.* 2007). This enables the evaluation of cellulose aggregate formation during various treatments. Recently, the use of AFM was extended to investigate the aggregation of isolated nanofibrillar cellulose on solid supports after drying from a variety of solvents. The results were complemented with the X-ray photoelectron spectroscopy, which suggested a specific tendency of the cellulose chains on the fibril surface to orientate during drying (Johansson *et al.* 2011).

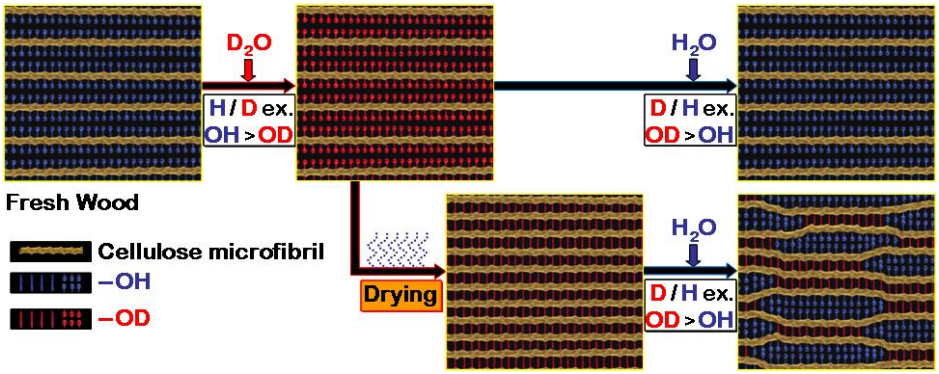

Fig. 6. Schematic of the proposed mechanism during drying in D_2O. Reprinted with permission from Suchy *et al.* 2010b. Copyright 2010 American Chemical Society.

We have now discussed the methods for the analysis of wet samples. The accessibility of dry fibers can also be determined; even though they contain almost no pores (Stone *et al.* 1966). The measurement is commonly based on gas sorption by N_2, Ar, Kr, or water (Klemm *et al.* 1998). Accessibility as a measure, then, depends on the interaction of the gas with the cellulosic sample. Thus, the actual values for accessibility are not comparable between different methods and the choice for the method has to be evaluated carefully.

CONCLUSIONS

Cellulose microfibrils have a high tendency to bond with each other, especially in the absence of obstructing molecules. Wood processing often involves drying, high temperature treatments, and the removal of the hemicellulose-lignin matrix. All of these processes induce microfibril coalescence, often leading to localized aggregation of cellulose strands into microfibrillar bundles. In addition to the commonly acknowledged changes in the papermaking related properties, such as swelling and strength properties,

coalescence reduces cellulose accessibility. This reduction presumably causes difficulties in the subsequent chemical and enzymatic treatments.

In the future there are three issues concerning cellulose microfibril coalescence that need to be acknowledged for novel products: 1) accessibility of the cellulose raw material to various enzymes or chemical species, which may be a decisive parameter in the production of novel products, such an nanocellulose, 2) process parameters, such as dewatering, affecting the novel processes, and 3) product quality requirements of novel products. In addition, better understanding of the phenomenon in various treatments is also important for the papermaking industry.

Furthermore, a need for an accurate and fast determination of the actual accessibility of cellulosic material is evident. Deuteration combined with FTIR appears to be a promising alternative to evaluate the changes during various processing steps.

ACKNOWLEDGEMENTS

R.P. acknowledges financial support from the Academy of Finland project number 138411 Microscopic tools for biorefinery development. E.K. acknowledges Aalto University (Starting Grant) for financial support. Professor Maloney is acknowledged for providing data.

REFERENCES CITED

Aarne, N., Kontturi, E., and Laine, J. (2012). "Influence of adsorbed polyelectrolytes on pore size distribution of a water-swollen biomaterial," *Soft Matter* 8(17), 4740-4749.

Abe, K., Iwamoto, S., and Yano, H. (2007). "Obtaining cellulose nanofibers with a uniform width of 15 nm from wood," *Biomacromolecules* 8(10), 3276-3278.

Åkerholm, M., and Salmén, L. (2001). "Interactions between wood polymers studied by dynamic FT-IR spectroscopy," *Polymer* 42(3), 963-969.

Alince, B. (2002). "Porosity of swollen pulp fibers revisited," *Nord. Pulp Pap. Res. J.* 17(1), 71-73.

Andersson, S., Wikberg, H., Pesonen, E., Maunu, S. L., and Serimaa, R. (2004). "Studies of crystallinity of Scots pine and Norway spruce cellulose," *Trees* 18(3), 346-353.

Atalla, R. H., and VanderHart, D. L. (1984). "Native cellulose: A composite of two distinct crystalline forms," *Science* 223(4633), 283-285.

Aulin, C., Ahola, S., Josefsson, P., Nishino, T., Hirose, Y., Österberg, M., and Wågberg, L. (2009). "Nanoscale cellulose films with different crystallinities and mesostructures – Their surface properties and interaction with water," *Langmuir* 25(13), 7675-7685.

Awano, T., Takabe, K., Fujita, M., and Daniel, G. (2000). "Deposition of glucuronoxylans on the secondary cell wall of Japanese beech as observed by immuno-scanning electron microscopy," *Protoplasma* 212(1-2), 72-79.

Back, E. L. (1967). "Thermal auto-crosslinking in cellulose material," *Pulp Pap. Mag. Can.* 68(4), T165-T171.

Battista, O. A., Coppick, S., Howsmon, J. A., Morehead, F. F., and Sisson, W. A. (1956). "Level-off degree of polymerization," *J. Ind. Eng. Chem*. 48(2), 333-335.

Berthold, J., and Salmén, L. (1997). "Inverse size exclusion chromatography (ISEC) for determining the relative pore size distribution of wood pulps," *Holzforschung* 51(4), 361-368.

Billosta, V., Brändström, J., Cochaux, A., Joseleau, J.-P., and Ruel, K. (2006). "Ultrastructural organization of the wood cell wall can explain modifications caused in fibers during the pulping process," *Cell. Chem. Technol.* 40(3-4), 223-229.

Borrega, M., and Kärenlampi, P. P. (2008). "Mechanical behavior of heat-treated spruce (*Picea abies*) wood at constant moisture content and ambient humidity," *Holz Roh. Werkst.* 66(1), 63-69.

Borrega, M., and Kärenlampi, P. P. (2010). "Hygroscopicity of heat-treated Norway spruce (*Picea abies*) wood," *Eur. J. Wood Prod*. 68(2), 233-235.

Borrega, M., and Kärenlampi, P. P. (2011). "Cell wall porosity in Norway spruce wood as affected by high-temperature drying," *Wood. Fiber Sci*. 43(2), 206-214.

California Department of Conservation (1997). *History of Recycling*, California.

Cao, B., Tschirner, U., and Ramaswamy, S. (1998) "Impact of pulp chemical composition on recycling," *Tappi J.* 81(12), 119-127.

Carlsson, G., and Lindström, T. (1984). "Hornification of cellulosic fibers during wet pressing," *Sven. Papperstidn.* 87(15), R119-R125.

Caulfield, D. F., and Steffes, R. A. (1969). "Water-induced recrystallization of cellulose," *Tappi* 52(7), 1361-1366.

Christiansen, A. W. (1990). "How overdrying wood reduces its bonding to phenol-formaldehyde adhesives: A critical review of the literature. Part I. Physical responses," *Wood Fiber Sci.* 22(4), 441-459.

Chunilall, V., Bush, T., Larsson, P. T., Iversen, T., and Kindness, A. (2010). "A CP/MAS ^{13}C-NMR study of cellulose fibril aggregation in eucalyptus dissolving pulps during drying and the correlation between aggregate dimensions and chemical reactivity," *Holzforschung* 64(6), 693-698.

Dang, Z., Elder, T., Hsieh, J. S., and Ragauskas, A. J. (2007). "Alkaline peroxide treatment of ECF bleached softwood kraft pulps: Part 2. Effect of increased fiber charge on refining, wet-end application, and hornification," *Holzforschung* 61(4), 451-458.

Ding, S.-Y., and Himmel, M. E. (2006). "The maize primary cell wall microfibril: A new model derived from direct visualization," *J. Agric. Food Chem*. 54(3), 597-606.

Donaldson, L. (2007). "Cellulose microfibril aggregates and their size variation with cell wall type," *Wood Sci. Technol.* 41(5), 443-460.

Duchesne, I., Hult, E.-L., Molin, U., Daniel, G., Iversen, T., and Lennholm, H. (2001). "The influence of hemicellulose on fibril aggregation of kraft pulp fibres as revealed by FE-SEM and CP/MAS ^{13}C-NMR," *Cellulose* 8(2), 103-111.

Duchesne, I., Takabe, K., and Daniel, G. (2003). "Ultrastructural localisation of glucomannan in kraft pulp fibres," *Holzforschung* 57(1), 62-68.

Elazzouzi-Haffraoui, S., Nishiyama, Y., Putaux, J.-L., Heux, L., Dubreuil, F., and Rochas, C. (2008). "The shape and size distribution of crystalline nanoparticles prepared by acid hydrolysis of native cellulose," *Biomacromolecules* 9(1), 57-65.

Ellis, J. W., and Bath, J. (1940). "Hydrogen bridging in cellulose as shown by infrared absorption spectra," *J. Am. Chem. Soc.* 62, 2859-2861.

Fahlén, J., and Salmén, L. (2002). "On the lamellar structure of the tracheid cell wall," *Plant Biol.* 4(3), 339-345.

Fahlén, J., and Salmén, L. (2003). "Cross-sectional structure of the secondary wall of wood fibers as affected by processing," *J. Mater. Sci.* 38(1), 119-126.

Fahlén, J., and Salmén, L. (2005). "Pore and matrix distribution in the fiber wall revealed by atomic force microscopy and image analysis," *Biomacromolecules* 6(1), 433-438.

Fahmy, Y., and Mobarak, F. (1971). "On fine structure of cellulose fibers," *Sven. Papperstidn.* 74(1), 2-9.

Fengel, D. (1970). "Ultrastructural behavior of cell wall polysaccharides," *Tappi* 53(3), 497-503.

Fernandes Diniz, J. M. B., Gil, M. H., and Castro, J. A. A. M. (2004). "Hornification – Its origin and interpretation in wood pulps," *Wood Sci. Technol*. 37(6), 489-494.

Fink, H.-P., Hofmann, D., and Purz, H. J. (1990). "Zur Fibrillarstruktur nativer Cellulose," *Acta Polym.* 41(2), 131-137.

Fink, H.-P., and Walenta, E. (1994). "Röntgenbeugungsuntersuchungen zur übermolekularen Struktur von Cellulose im Verarbeitungsprozeß," *Papier* 48(12), 739-748.

Fors, C. (2000). "The effect of fibre charge on web consolidation in papermaking," Licentiate Thesis, Royal Inst. Technol., Stockholm.

French, A. D., and Johnson, G. P. (2009). "Cellulose and the twofold screw axis: Modeling and experimental arguments," *Cellulose* 16(6), 959-973.

Frey-Wyssling, A. (1954). "The fine structure of cellulose microfibrils," *Science* 119(3081), 80-82.

Gallay, W. (1950). "The measurement and significance of swelling of cellulose fibers," *Tappi* 33(9), 425-429.

Gane, P. A. C., Ridgway, C. J., Lehtinen, E., Valiullin, R., Furó, I., Schoelkopf, J., Paulapuro, H., and Daicic, J. (2004). "Comparison of NMR cryoporometry, mercury intrusion porosimetry, and DSC thermoporosimetry in characterizing pore size distributions of compressed finely ground calcium carbonate structures," *Ind. Eng. Chem. Res.* 43(24), 7920-7927.

Gerhards, C. C. (1982). "Effect of moisture content and temperature on the mechanical properties of wood: An analysis of immediate effects," *Wood Fiber Sci.* 14(1), 4-36.

Goring, D. A. I, and Timell, T. E. (1962). "Molecular weight of native celluloses," *Tappi* 45(6), 454-460.

Gross, A. S., and Chu, J.-W. (2010). "On the molecular origin of biomass recalcitrance: The interaction network and solvation structures of cellulose microfibrils," *J. Phys. Chem. B* 114(42), 13333-13341.

Hanspal, N. S., and Das, D. B. (2012). "Dynamic effects on capillary pressure-saturation relationships for two-phase porous flow: implications of temperature," *AIChE J.* 58(6), 1951-1965.

Hearle, J. W. S. (1958). "A fringed fibril theory of structure in crystalline polymers," *J. Polym. Sci.* XXVIII(117), 432-435.

Heyn, A. N. J. (1969). "The elementary fibril and supermolecular structure of cellulose in soft wood fiber," *J. Ultrastruct. Res*. 26(1), 52-68.

Higgins, H. G., and McKenzie, A. W. (1963). "The structure and properties of paper XIV. Effects of drying on cellulose fibres and the problem of maintaining pulp strength," *Appita* 16(6), 145-164.

Hill, S. J., Kirby, N. M., Mudie, S. T., Hawley, A. M., Ingham, B., Franich, R. A., and Newman, R. H. (2010). "Effect of drying and rewetting of wood on cellulose molecular packing," *Holzforschung* 64(4), 421-427.

Hillis, W. E. (1984). "High temperature and chemical effects on wood stability," *Wood Sci. Technol.* 18(4), 281-293.

Hillis, W. E., and Rozsa, A. N. (1985). "High temperature and chemical effects on wood stability," *Wood Sci. Technol.* 19(2), 93-102.

Hofmann, D., Fink, H.-P., and Philipp, B. (1989). "Lateral crystallite size and lattice distortions in cellulose II samples of different origin," *Polymer* 30(2), 237-241.

Hon, D. N.-S., and Shiraishi, N. (1991). *Wood and Cellulosic Chemistry*, Mercel Dekker, Inc., New York.

Howard, R. C. (1990). "The effects of recycling on paper quality," *J. Pulp Pap. Sci*. 16(5), J143-J149.

Howard, R. C. (1991). "The effects of recycling on paper quality," *Paper Technol*. 32(4), 20-25.

Howard, R. C. (1995). "The effects of recycling on pulp quality," *Technol. Pap. Recycling* 180-203.

Hubbe, M. A., Venditti, R. A., and Rojas, O. J. (2007). "What happens to cellulosic fibers during papermaking and recycling? A review," *BioResources* 2(4), 739-788.

Hult, E.-L., Iversen, T., and Sugiyama, J. (2003). "Characterization of the supermolecular structure of cellulose in wood pulp fibres," *Cellulose* 10(2), 103-110.

Hult, E.-L., Larsson, P. T., and Iversen, T. (2001). "Cellulose fibril aggregation – An inherent property of kraft pulps," *Polymer* 42(8), 3309-3314.

Hult, E.-L., Larsson, P. T., and Iversen, T. (2002). "A comparative CP/MAS ^{13}C-NMR study of the supermolecular structure of polysaccharides in sulphite and kraft pulps," *Holzforschung* 56(2), 179-184.

Ishizawa, C. I., Jeoh, T., Adney, W. S., Himmel, M. E., Johnson, D. K., and Davis, M. F. (2009). "Can delignification decrease cellulose digestibility in acid pretreated corn stover?," *Cellulose* 16(4), 677-686.

Isogai, A. (1997). "NMR analysis of cellulose dissolved in aqueous NaOH solutions," *Cellulose* 4(2), 99-107.

Jayme, G. (1944). "Mikro-Quellungsmessungen an Zellstoffen," *Wochenbl. Papierfabr.* 6, 187-194.

Jayme, G. (1958). "Properties of wood celluloses II. Determination and significance of water retention value," *Tappi* 41(11), 180A-183A.

Jayme, G., and Hunger, G. (1957). "The rearrangement of microfibrils in dried cellulose and the implication of this structure alteration on pulp properties," *Fundamentals of Papermaking Fibres*, Cambridge, 263-270.

Johansson, L.-S., Tammelin, T., Campbell, J. M., Setälä, H., and Österberg, M. (2011). "Experimental evidence on medium driven cellulose surface adaption demonstrated using nanofibrillated cellulose," *Soft Matter* 7(22), 10917-10924.

Jones, D. W. (1958). "Crystalline modifications of cellulose. Part III. The derivation and preliminary study of possible crystal structures," *J. Polym. Sci.* XXXII, 371-394.

Kadla, J. F., and Gilbert, R. D. (2000). "Cellulose structure: A review," *Cell. Chem. Technol.* 34(3-4), 197-216.

Kato, K. L., and Cameron, R. E. (1999). "A review of the relationship between thermally-accelerated ageing of paper and hornification," *Cellulose* 6(1), 23-40.

Kato, K. L., and Cameron, R. E. (2002). "Structural aspects of the thermally accelerated ageing of cellulose: Effect of cellulose source and ageing conditions," *Polym. Int.* 51(8), 707-714.

Kerr, A. J., and Goring, D. A. I. (1975). "The ultrastructural arrangement of the wood cell wall," *Cell. Chem. Technol.* 9, 563-573.

Kifetew, G., Thuvander, F., Berglund, L., and Lindberg, H. (1998). "The effect of drying on wood fracture surfaces from specimens loaded in wet condition," *Wood Sci. Technol.* 32(2), 83-94.

Klemm, D., Philipp, B., Heinze, U., and Heinze, W. (1998). *Comprehensive Cellulose Chemistry. Vol. 1. Fundamentals and Analytical Methods*, Wiley-VCH, Weinheim.

Klungness, J. H. (1974). "Recycled fiber properties as affected by contaminants and removal processes," *Tappi* 57(11), 71-75.

Klye, R. C. (1961). "The effect of drying on pulp strengths," *Appita* 14(6), xxi-xxxv.

Kontturi, E., and Vuorinen, T. (2009). "Indirect evidence of supramolecular changes within cellulose microfibrils of chemical pulp fibers upon drying," *Cellulose* 16(1), 65-74.

Kontturi, E., Suchy, M., Penttilä, P., Jean, B., Pirkkalainen, K., Torkkeli, M., and Serimaa, R. (2011). "Amorphous characteristics of an ultrathin cellulose film," *Biomacromolecules* 12(3), 770-777.

Laine, J., Lindström, T., Bremberg, C., and Glad-Nordmark, G. (2003a). "Studies on topochemical modification of cellulosic fibres. Part 4: Toposelectivity of carbomethylation and its effects on the swelling of fibres," *Nord. Pulp Pap. Res. J.* 18(3), 316-324.

Laine, J., Lindström, T., Bremberg, C., and Glad-Nordmark, G. (2003b). "Studies on topochemical modification of cellulosic fibres. Part 5: Comparison of the effects of surface and bulk chemical modification and beating of pulp on paper properties," *Nord. Pulp Pap. Res. J.* 18(3), 325-332.

Laivins, G.V., and Scallan, A.M. (1993). *The mechanism of hornification of wood pulps*, Products of papermaking, Transactions of the Xth fundamental research symposium, Oxford, 1235-1260.

Laivins, G.V., and Scallan, A.M. (1996). "The influence of drying and beating on the swelling of fines," *J. Pulp Pap. Sci.* 22(5), J178-J184.

Laivins, G., and Scallan, T. (2000). "Acidic versus alkaline beating of pulp," *J. Pulp Pap. Sci.* 26(6), 228-233.

Landry, M. R. (2005). "Thermoporometry by differential scanning calorimetry: Experimental considerations and applications," *Thermochim. Acta* 433(1-2), 27-50.

Law, K. N, Song, X. L., and Daneault, C. (2006). "Influence of pulping conditions on the properties of recycled fibers," C*ell. Chem. Technol.* 40(5), 335-343.

Lee, J. M., Heitmann, J. A., and Pawlak, J. J. (2007). "Technique for the measurement of dimensional changes of natural microfibril materials under variable humidity environments," *Mater. Sci. Eng. A* 445-446, 632-640.

Lehtiö, J., Sugiyama, J., Gustavsson, M., Fransson, L., Linder, M., and Teeri, T. T. (2003). "The binding specificity and affinity determinants of family 1 and family 3 cellulose binding modules," *P. Natl. Acad. Sci. USA* 100(2), 484-489.

Le Moigne, N., and Navard, P. (2010). "Dissolution mechanisms of wood cellulose fibres in NaOH-water," *Cellulose* 17(1), 31-45.

Leppänen, K., Andersson, S., Torkkeli, M., Knaapila, M., Kotelnikova, N., and Serimaa, R. (2009). "Structure of cellulose and microcrystalline cellulose from various wood species, cotton and flax studied by X-ray scattering," *Cellulose* 16(6), 999-1015.

Leppänen, K., Bjurhager, I., Peura, M., Kallonen, A., Suuronen, J.-P., Penttilä, P., Love, J., Fagerstedt, K., and Serimaa, R. (2011). "X-ray scattering and microtomography study on the structural changes of never-dried silver birch, European aspen and hybrid aspen during drying," *Holzforschung* 65(6), 865-873.

Li, Q., and Renneckar, S. (2011). "Supramolecular structure characterization of molecularly thin cellulose I nanoparticles," *Biomacromolecules* 12(3), 650-659.

Liitiä, T., Maunu, S. L., Hortling, B., Tamminen, T., Pekkala, O., and Varhimo, A. (2003). "Cellulose crystallinity and ordering of hemicelluloses in pine and birch pulps as revealed by solid-state NMR spectroscopic methods," *Cellulose* 10(4), 307-316.

Lindman, B., Karlström, G., and Stigsson, L. (2010). "On the mechanism of dissolution of cellulose," *J. Mol. Liq*. 156(1), 76-81.

Lindström, T. (1992). "Chemical factors affecting the behaviour of fibres during papermaking," *Nord. Pulp Pap. Res. J.* 4(7), 181-192.

Lindström, T., and Carlsson, G. (1982). "The effect of carboxyl groups and their ionic form during drying on the hornification of cellulose fibers," *Sven. Papperstidn.* 85(3), R146-R151.

Liu, Y.-S., Baker, J.O., Zeng, Y., Himmel, M. E., Haas, T., and Ding, S.-Y. (2011). "Cellobiohydrolase hydrolyzes crystalline cellulose on hydrophobic faces," *J. Biol. Chem.* 286(13), 11195-11201.

Luo, X. L., Zhu, J. Y., Gleisner, R., and Zhan, H. Y. (2011). "Effects of wet-pressing-induced fiber hornification on enzymatic saccharification of lignocelluloses," *Cellulose* 18(4), 1055-1062.

Luukko, K., and Maloney, T. C. (1999). "Swelling of mechanical pulp fines," *Cellulose* 6(2), 123-135.

Lyne, L. M., and Gallay, W. (1950). "The effect of drying and heating on the swelling of cellulose fibers and paper strength," *Tappi* 33(9), 429-435.

Maloney, T. C., Laine, J. E., and Paulapuro, H. (1999). "Comments on the measurement of cell wall water," *Tappi J.* 82(9), 125-127.

Maloney, T. C., and Paulapuro, H. (1998). "Hydration and swelling of pulp fibers measured with differential scanning calorimetry," *Nord. Pulp Pap. Res. J.* 13(1), 31-36.

Maloney, T. C., and Paulapuro, H. (1999). "The formation of pores in the cell wall," *J. Pulp Pap. Sci.* 25(12), 430-436.

Maloney, T. C., and Paulapuro, H. (2000). "The effect of drying conditions on the swelling and bonding properties of bleached kraft hardwood pulp," *Appita Annual Conference Proceedings* 1, 41-46.

Maloney, T. C., and Paulapuro, H. (2001). "Thermoporosimetry of pulp fibers," Trans. 12th Fundamental Research Symposium, Oxford, 897-926.

Mann, J., and Marrinan, H. J. (1956). "The reaction between cellulose and heavy water, Part 3. – A quantitative study by infra-red spectroscopy," *Trans. Faraday Soc.* 52, 492-497.

Mark, H. (1940). "Intermicellar hole and tube system in fiber structure," *J. Phys. Chem.* 44, 764-788.

Matsuda, Y., Isogai, A., and Onabe, F. (1994). "Effects of thermal and hydrothermal treatments on the reswelling capabilities of pulps and papersheets," *J. Pulp Pap. Sci.* 20(11), J323-J327.

McKee, R. C. (1971). "Effect of repulping on sheet properties & fiber characteristics," *Pap. Trade J.* 155(21), 34-40.

Michell, A. J., and Higgins, H. G. (1999). "The absence of free hydroxyl groups in cellulose," *Cellulose* 6(1), 89-91.

Minor, J. L. (1994). "Hornification – its origin and meaning," *Progr. Pap. Recycling* 3(2), 93-95.

Müller, M., Czihak, C., Schober, H., Nishiyama, Y., and Vogl, G. (2000). "All disordered regions of native cellulose show common low-frequency dynamics," *Macromolecules* 33(5), 1834-1840.

Nazhad, M. (2005). "Recycled fiber quality – A review," *J. Ind. Eng. Chem.* 11(3), 314-329.

Nazhad, M., and Paszner, L. (1994). "Fundamentals of strength loss in recycled paper," *Tappi J.* 77(9), 171-179.

Newman, R. H. (2004). "Carbon-13 NMR evidence for cocrystallization of cellulose as a mechanism for hornification of bleached kraft pulp," *Cellulose* 11(1), 45-52.

Nishiyama, Y., Kim, U.-J., Kim, D.-Y., Katsumata, K. S., May, R. P., and Langan, P. (2003b). "Periodic disorder along ramie cellulose microfibrils," *Biomacromolecules* 4(4), 1013-1017.

Nishiyama, Y., Langan, P., and Chanzy, H. (2002). "Crystal structure and hydrogen-bonding system in cellulose Iβ from Synchrotron X-ray and neutron fiber diffraction," *J. Am. Chem. Soc.* 124(31), 9074-9082.

Nishiyama, Y., Sugiyama, J., Chanzy, H., and Langan, P. (2003a). "Crystal structure and hydrogen bonding system in cellulose I_α from Synchrotron X-ray and neutron fiber diffraction," *J. Am. Chem. Soc.* 125(47), 14300-14306.

Nishiyama, Y., Johnson, G. P., French, A. D., Forsyth, V. T., and Langan, P. (2008). "Neutron crystallography, molecular dynamics, and quantum mechanical studies of the nature of hydrogen bonding in cellulose I_β," *Biomacromolecules* 9(11), 3133-3140.

Okita, Y., Saito, T., and Isogai, A. (2010). "Entire surface oxidation of various cellulose microfibrils by TEMPO-mediated oxidation," *Biomacromolecules* 11(6), 1696-1700.

Oksanen, T., Buchert, J., and Viikari, L. (1997). "The role of hemicelluloses in the hornification of bleached kraft pulps," *Holzforschung* 51(4), 355-360.

Olsson, A.-M., and Salmén, L. (2004). "The softening behavior of hemicelluloses related to moisture," *ACS Sym. Ser.* 864(13), 184-197.

Östlund, Å., Köhnke, T., Nordstierna, L., and Nydén, M. (2010). "NMR cryoporometry to study the fiber wall structure and the effect of drying," *Cellulose* 17(2), 321-328.

Page, D. H. (1985). "The mechanism of strength development of dried pulps by beating," *Sven. Papperstidn*. 88(3), R30-R35.

Page, D. H., and De Grâce, J. H. (1967). "The delamination of fiber walls by beating and refining," *Tappi* 50(10), 489-495.

Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A., and Johnson, D. K. (2010). "Cellulose crystallinity index: Measurement techniques and their impact on interpreting cellulase performance," *Biotechnol. Biofuels* 3(1), 10.

Park, S., Venditti, R. A., Jameel, H., and Pawlak, J. J. (2006a). "A novel method to evaluate fibre hornification by high resolution thermogravimetric analysis," *Appita J.* 59(6), 481-485.

Park, S., Venditti, R. A., Jameel, H., and Pawlak, J. J. (2006b). "Changes in pore size distribution during the drying of cellulose fibers as measured by differential scanning calorimetry," *Carbohyd. Polym.* 66(1), 97-103.

Petrov, O. V., and Furó, I. (2009). "NMR cryoporometry: Principles, applications and potential," *Prog. Nucl. Mag. Res. Sp.* 54(2), 97-122.

Pääkkö, M., Ankerfors, M., Kosonen, H., Nykänen, A., Ahola, S., Österberg, M., Ruokolainen, J., Laine, J., Larsson, P. T., Ikkala, O., and Lindström, T. (2007). "Enzymatic hydrolysis combined with mechanical shearing and high-pressure homogenization for nanoscale cellulose fibrils and strong gels," *Biomacromolecules* 8(6), 1934-1941.

Rácz, I., and Borsa, J. (1997). "Swelling of carboxymetylated cellulose fibres," *Cellulose* 4(4), 293-303.

Rebuzzi, F., and Evtuguin, D. V. (2006). "Effect of glucuronoxylan on the hornification of *Eucalyptus globulus* bleached pulps," *Macromol. Symp.* 232(1), 121-128.

Renaud, R. (1947). "Le raffinage. Emploi de la centrifugation dans le contrôle du gonflement," *Association Technique de l'Industrie Papetiere* 1, 14-17.

Saito, T., Hirota, M., Tamura, N., Kimura, S., Fukuzumi, H., Heux, L., and Isogai, A. (2009). "Individualization of nano-sized plant cellulose fibrils by direct surface carboxylation using TEMPO catalyst under neutral conditions," *Biomacromolecules* 10(7), 1992-1996.

Saito, T., Nishiyama, Y., Putaux, J.-L., Vignon, M., and Isogai, A. (2006). "Homogenous suspensions of individualized microfibrils from TEMPO-catalyzed oxidation of native cellulose," *Biomacromolecules* 7(6), 1687-1691.

Salmén, L., and Olsson, A.-M. (1998). "Interaction between hemicelluloses, lignin and cellulose: Structure-property relationships," *J. Pulp Pap. Sci*. 24(3), 99-103.

Samejima, M., Sugiyama, J., Igarashi, K., and Eriksson, K.-E. L. (1998). "Enzymatic hydrolysis of bacterial cellulose," *Carbohyd. Res.* 305(2), 281-288.

Samuelson, O., and Törnell, B. (1961). "Influence of lactones upon the determination of carboxyl groups in cellulose," *Sven. Papperstidn.* 64(6), 198-203.

Scallan, A. M. (1971). "A quantitative picture of the fringed micellar model of cellulose," *Text. Res. J.* 41(8), 647-653.

Scallan, A. M. (1974). "The structure of the cell wall of wood – A consequence of anisotropic inter-microfibrillar bonding?," *Wood Sci*. 6(3), 266-271.

Scallan, A. M., and Tigerström, A. C. (1992). "Swelling and elasticity of the cell walls of pulp fibres," *J. Pulp Pap. Sci.* 18(5), J188-J193.

Sepall, O., and Mason, S. G. (1961). "Hydrogen exchange between cellulose and water II. Interconversion of accessible and inaccessible regions," *Can. J. Chem.* 39, 1944-1955.

Sheikhi, P., Talaeipour, M., Hemasi, A. H., Eslam, H. K., and Gumuskaya, E. (2010). "Effect of drying and chemical treatment on bagasse soda pulp properties during recycling," *BioResources* 5(3), 1702-1716.

Steege, H.-H., and Philipp, B. (1974). "Herstellung, Charakterisierung und Anvendung mikrokristalliner Zellulose," *Zellst. Pap.* 23(3), 68-73.

Stone, J. E., and Scallan, A. M. (1965a). "Effect of component removal upon the porous structure of the cell wall of wood*," J. Polym. Sci.: Part C* 11, 13-25.

Stone, J. E., and Scallan, A. M. (1965b). "Influence of drying on the pore structures of the cell wall," *Consolidation of the Paper Web*, London, Vol. 1., 145-174.

Stone, J. E., and Scallan, A. M. (1965c). "A study of cell wall structure by nitrogen adsorption," *Pulp. Pap. Mag. Can*. 66(8), T407-T414.

Stone, J. E., and Scallan, A. M. (1967). "The effect of component removal upon the porous structure of the cell wall of wood. II. Swelling in water and the fiber saturation point," *Tappi* 50(10), 496-501.

Stone, J. E., and Scallan, A. M. (1968). "A structural model for the cell wall of water-swollen wood pulp fibres based on their accessibility to macromolecules," *Cell. Chem. Technol.* 2(3), 343-358.

Stone, J. E., Scallan, A. M., and Aberson, G. M. A. (1966). "The wall density of native cellulose fibres," *Pulp Pap. Mag. Can*. 67(5), T263-T268.

Stone, J. E., Scallan, A. M., and Abrahamson, B. (1968). "Influence of beating on cell wall swelling and internal fibrillation," *Sven. papperstidn.* 71(19), 687-694.

Suchy, M., Kontturi, E., and Vuorinen, T. (2010a). "Impact of drying on wood ultrastructure: Similarities in cell wall alteration between native wood and isolated wood-based fibers," *Biomacromolecules* 11(8), 2161-2168.

Suchy, M., Virtanen, J., Kontturi, E., and Vuorinen, T. (2010b). "Impact of drying on wood ultrastructure observed by deuterium exchange and photoacoustic FT-IR spectroscopy," *Biomacromolecules* 11(2), 515-520.

Sumi, Y., Hale, R. D., and Rånby, B. G. (1963). "The accessibility of native cellulose microfibrils," *Tappi* 47(10), 126-130.

Thimm, J. C., Burritt, D. J., Ducker, W. A., and Melton, L. D. (2009). "Pectins influence microfibril aggregation in celery cell walls: An atomic force microscopy study," *J. Struct. Biol.* 168(2), 337-344.

Virtanen, T., Maunu, S. L., Tamminen, T., Hortling, B., and Liitiä, T. (2008). "Changes in fiber ultrastructure during various kraft pulping conditions evaluated by ^{13}C CPMAS NMR spectroscopy," *Carbohyd. Polym.* 73(1), 156-163.

Wågberg, L., Decher, G., Norgren, M., Lindström, T., Ankerfors, M., and Axnäs, K. (2008). "The build-up of polyelectrolyte multilayers of microfibrillated cellulose and cationic polyelectrolytes," *Langmuir* 24(3), 784-795.

Wan, J., Wang, Y., and Xiao, Q. (2010). "Effects of hemicellulose removal on cellulose fiber structure and recycling characteristics of eucalyptus pulp," *Bioresource Technol.* 101(12), 4577-4583.

Wang, X., Maloney, T. C., and Paulapuro, H. (2003). "Internal fibrillation in never-dried and once-dried chemical pulps," *Appita J.* 56(6), 455-459.

Weise, U. (1998). "Hornification – mechanisms and terminology," *Pap. Puu* 80(2), 110-115.

Weise, U., Hiltunen, E., and Paulapuro, H. (1998). "Verhornung von Zellstoff und Maßnahmen zu ihrer Reversion," *Das Papier* 52(10A), V14-V19.

Weise, U., Maloney, T., and Paulapuro, H. (1996). "Quantification of water in different states of interaction with wood pulp fibres," *Cellulose* 3(1), 189-202.

Wickholm, K., Larsson, P. T., and Iversen, T. (1998). "Assignment of non-crystalline forms in cellulose I by CP/MAS ^{13}C NMR spectroscopy," *Carbohyd. Res.* 312(3), 123-129.

Wistara, N., and Young, R. A. (1999). "Properties and treatments of pulps from recycled paper. Part I. Physical and chemical properties of pulps," *Cellulose* 6(4), 291-324.

Wistara, N., Zhang, X., and Young, R. A. (1999). "Properties and treatments of pulps from recycled paper. Part II. Surface properties and crystallinity of fibers and fines," *Cellulose* 6(4), 325-348.

Zhang, M., Hubbe, M. A., Venditti, R. A., and Heitmann, J. A. (2002). "Can recycled kraft fibres benefit from chemical addition before they are first dried?," *Appita J.* 55(2), 135-144.

Zhang, M., Hubbe, M. A., Venditti, R. A., and Heitmann, J. A. (2004). "Effects of sugar addition before drying on the wet flexibility of redispersed kraft fibres," *J. Pulp Pap. Sci.* 30(1), 29-34.

Article submitted: June 29, 2012; Peer review completed: August 2, 2012; Revised version received: August 27, 2012; Accepted: August 30, 2012; Published: September 11, 2012.