bioresources.com

Structural Aspects on the Manufacturing of Cellulose Nanofibers from Wood Pulp Fibers

Carl Moser, Gunnar Henriksson,* and Mikael E. Lindström

The exact mechanism behind the disintegration of chemical pulp fiber into cellulose nanofibers is poorly understood. In this study, samples were subjected to various homogenization cycles, indicating that the mechanism is a stepwise process. In the earlier stages of the mechanical process, a large amount of macrofibrils were created as the larger structures disappeared. Upon mechanical treatment these macrofibrils disappeared despite the increasing yield of cellulose nanofibers. The proposed model expands the understanding of the disintegration pathway and may provide additional insight as to how wood cells are converted into microfibrils.

INTRODUCTION

Cellulose, the most common biopolymer on earth (Klemm *et al.* 2005; Wilson *et al.* 2012; Brinchi *et al.* 2013), consists of β-glucopyranoside units connected with 1,4 β-glucosidic bonds (Meshitsuka and Isogai 1995). Cellulose is synthesized in eukaryotic organisms from the Plantae and Animalia kingdoms (Zhao and Li 2014) and in certain prokaryotic bacteria (Trovatti 2013). In vascular plants, cellulose is synthesized through hexameric rosette-shaped cellulose synthase complexes (CSC) located in the plasma membrane (Hill *et al.* 2014; Li *et al.* 2014). These rosettes contain three unique cellulose synthase (CesA) isomers, which are required in 1:1:1 stoichiometry for CSC assembly (Hill *et al.* 2014). Each elementary fibril is comprised of 18, 24, or 36 cellulose chains with a lateral dimension of 3 to 5 nm in wood (Meier 1962; Ohad and Danon 1964; Heyn 1969; Blackwell and Kolpak 1975; Doblin *et al.* 2002; Somerville 2006; Fernandes *et al.* 2011; Hill *et al.* 2014). The exact mechanism behind the process of cellulose crystallization into elementary fibrils is unknown and widely debated (Mølhøj *et al.* 2002; Somerville 2006). However, the crystallization route is likely flawed due to the twisted nature of the cellulose chains (Conley *et al.* 2016), which generate less ordered and more reactive regions along the elementary fibril (Blackwell and Kolpak 1975; Pääkkö *et al.* 2007). Elementary fibrils agglomerate into microfibrils to reduce the free energy of the surfaces (Peterlin and Ingram 1970), the lateral dimensions of which can range from 10 to 35 nm (Mühlenthaler 1949; Meier 1962; Frey-Wyssling and Mühlethaler 1963; Heyn 1969). An illustration of the pathway at the molecular level and through the ultrastructure to the macroscopic scale was shown in Moser *et al.* (2015).
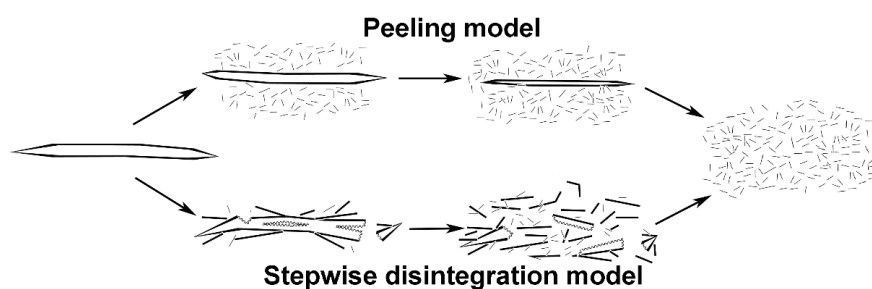
Fig. 1. Two hypothetical models for the disintegration process of chemical pulp fibers into cellulose nanofibers. In the peeling model, cellulose nanofibers are peeling from the fiber surfaces creating cellulose nanofibers and a fiber residue until the material is completely converted into nanofibers, *i.e.*, released microfibrils. In the stepwise disintegration model, the fiber is delaminated and broken into fiber fragments, exposing the fiber surface. Further treatment creates macrofibrils and smaller fiber fragments. These components are eventually disintegrated into nanofibrils.

The plant cell wall is comprised of a primary cell wall and a secondary cell wall. The secondary cell wall consists of three sublayers: S1, S2, and S3. The S2 layer accounts for 80% of the total thickness at 1.6 µm (Bergander and Salmén 2002). The hierarchical levels between the cell walls and the microfibrils are not well understood. There are signs of the presence of fibrillar components in the cell wall with diameters of approximately 1 µm that are referred to as macrofibrils (Meier 1962; Chinga-Carrasco 2011). Fibrillar secondary fines of similar dimensions are present in mechanical pulp and refined chemical pulps, either in free form or on fiber surfaces (Mayr *et al.* 2017).

The manufacturing of cellulose nanofibers (CNF) is a mechanical process that relies on the acceleration of fibers through narrow chambers, slits in homogenizers, or by refining in specialty "ultra-fine" friction grinders. The goal is to come as close to achieving fully liberated elementary fibrils as possible. Depending on pretreatment methods and energy input, the quality of CNF varies significantly (Moser *et al.* 2015). Most CNF contains a variety of structures, such as elementary fibrils, microfibrils, and fiber fragments.

The hierarchic levels in chemical pulp fibers may play an important role in the CNF disintegration process. Principally, there are two "extreme" models for how cellulose nanofibers are manufactured from chemical pulp. In the peeling model, microfibril bundles are peeled from the fiber surfaces until the fiber is completely disintegrated. In the stepwise disintegration model, the fibers are delaminated, cut, and disintegrated into small fiber fragments and macrofibrils before forming microfibrils. A diagram of both models can be seen in Fig. 1. The purpose of this work is to investigate which of these two models best describes the homogenization process and whether macrofibrils have a role as intermediates during the disintegration of fibers to cellulose nanofibers by means of a high-pressure homogenizer.

EXPERIMENTAL

Materials

Bleached softwood kraft pulp (SCA Östrand, Timrå, Sweden) with 14% hemicellulose and less than 0.1% lignin was PFI milled for 10000 revolutions. After refining, the pulp was subjected to enzymatic pretreatment using monocomponent endoglucanase Fibercare® (Novozyme, Kalundborg, Denmark). The fibers were treated with endoglucanase, 25 ECU (Endocellulase Units) per gram fibers, at a

temperature of 55 ℃ for 1 h after which the enzymes were denatured by submersing the pulp in 100 ℃ water for 15 min.

Delamination was facilitated by a high-pressure homogenizer (M110-EH; Microfluidics Corp., Westwood, MA, USA) connected with two different series of chambers, 400/200 µm and 200/100 µm. The pretreated pulp was subjected to one pass at 900 bar in the 400/200-µm chambers followed by passes at 1550 to 1650 bar in the 200/100-µm chambers up to 10 times. The energy consumption varied between 2.3 to 34 MWh/t.

Methods

Characterization

Image analysis on light optical microscopy images (Fig. 2) was manually carried out by two persons that worked independently, and had no knowledge of the sample history, and the photos were given random notations. The frequency of fibers with a specific size was measured for five images taken on a 5 µL, 1 g/L CNF sample. The measurement ranges were 15 to 30 µm in width for fiber fragments and 0.5 to 2 µm in width for macrofibrils.

The CNF yield was measured *via* centrifugation according to Moser *et al.* (2015). Filtration of the various CNF qualities was carried out using a Britt Dynamic Drainage Jar (BDDJ) setup (Paper Research Materials, Seattle, WA, USA). Additional mesh wires with sizes of 30, 60, and 100 µm were used. The CNF samples were filtered on the 100-µm mesh, followed by the 60-µm mesh, and lastly using the 30-µm mesh. Each wire was dried and weighed to determine the gravimetric yield of each fraction.
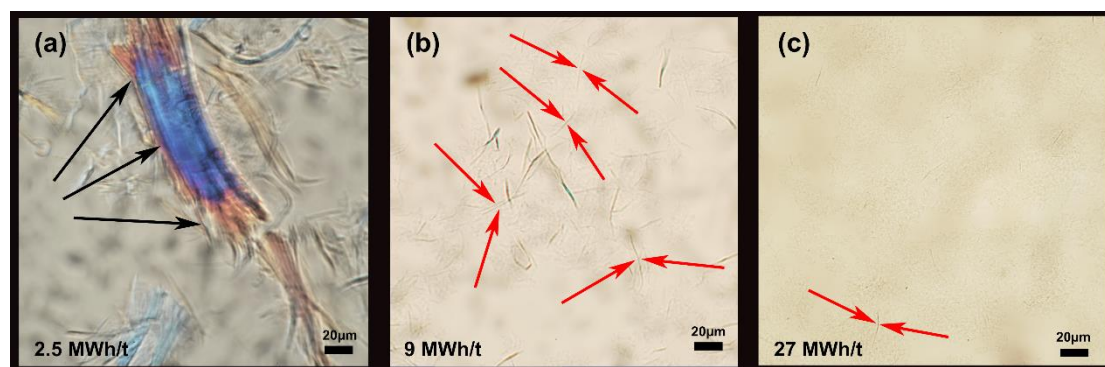

Fig. 2. Light optical microscopy (LOM) images used for image analysis. (a) CNF sample after the initial pass through the homogenizer (2.5 MWh/t); large fiber fragments are visible and highlighted with black arrows, (b) macrofibrils occurring at 9 MWh/t highlighted by red arrows, and (c) few macrofibrils visible after an energy input of 27 MWh/t.

RESULTS AND DISCUSSION

Chemical pulps where homogenized to different degrees by using varying numbers of cycles. The pulps were examined by two methods. The first examination method was sequential filtration, which used a BDDJ system in combination with centrifugation and the second method was manual image analysis of light microscopy images. The other examination was independently conducted by two individuals without knowledge of the individual samples. The individual samples were given neutral names to avoid bias.
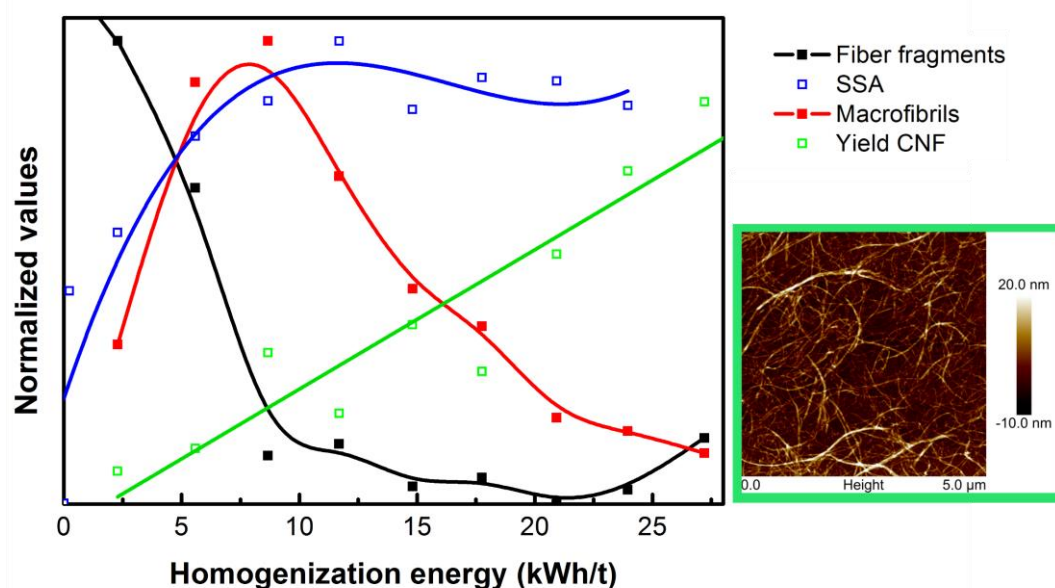
Fig. 3. Image analysis of light optical microscopy pictures showing the disappearance of larger particles and the disintegration of macrofibrils. Image analysis was performed as a blind test for 10 CNF samples homogenized to different degrees. The CNF yield and specific surface area (SSA) values were added as reference values (Moser *et al.* 2016). All values have been normalized with the highest value in its own series. An AFM image is shown with green border to highlight what types of nanofibers are referred to as yield CNF.

Microscopy analysis of the homogenization series (Fig.3) showed that as the energy input increased, the larger fibers and fragments rapidly disappeared. When the larger fibers disappeared, smaller macrofibrils appeared. These macrofibrils had widths of approximately 0.5 to 2 µm and lengths of up to several hundred micrometers, making them some of the smallest fibrous particles visible using optical microscopy. Pure CNF yield linearly increased throughout the homogenization process. However, there was a hierarchical gap between the measured macrofibrils and pure CNF that corresponds to a portion of the pellet after centrifugation and cannot be detected. Larger particles disappeared around 9 MWh/t, corresponding with the results in Fig. 3. The microscopy analysis was verified by filtration of the same samples using a BDDJ system and varying membrane sizes (Fig. 4). The amount of intermediate sized fibers increased, as is illustrated by the higher retention values on the 30-µm membrane for the samples homogenized with 5.4 MWh/t compared to at 2.5 MWh/t (Fig. 4).

During centrifugation (Fig. 4), a pellet accounting for 47 to 89% of the total mass was formed that contained various fibrous structures in between that of the microfibrils and the macrofibrils. These structures could not be efficiently measured within the scope of this work.

The results from Fig. 3 indicated that fibrous compounds, with diameters of 1 to 2 µm and aspect ratios above 100, were common during the homogenization process as intermediates. These had dimensions similar to fibrillar fines in mechanical pulp, and corresponded to a hierarchic level in the plant cell wall between that of the cell wall layer and the microfibrils – a macrofibril (Meier 1962). Both experiments indicated that intermediate-sized particles were rapidly created and disintegrated throughout the homogenization process. Furthermore, SSA rapidly increased during the earlier stages of the homogenization process before a large amount of cellulose nanofibers had been created (Moser *et al.* 2016). Thus, the stepwise disintegration model seemed to describe

the cellulose nanofibers manufacturing process by homogenization better than the peeling model. However, the stepwise disintegration model also created microfibrils early in the process.
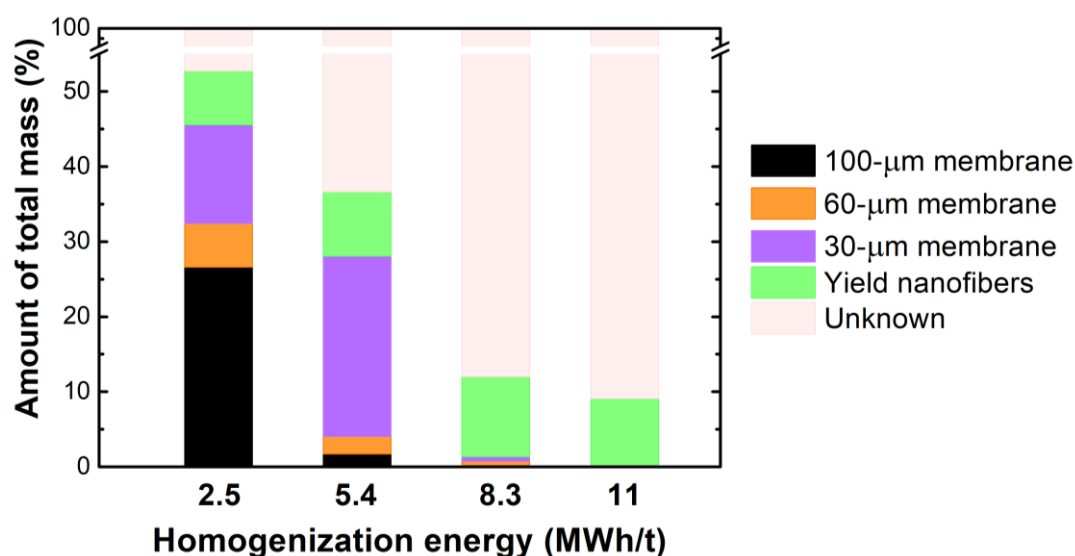

Fig. 4. Britt Jar filtration using various membrane sizes 30-100 μm. The fraction regarded as yield is the centrifugal supernatant and the remaining material "unknown" gathered as a pellet is the unaccounted-for fraction.
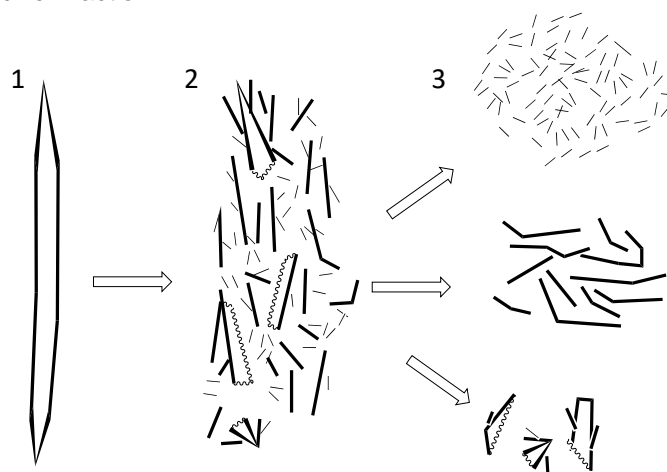

Fig. 5. Possible process for obtaining an assortment of products from chemical pulps. The pulp (1) is initially subjected to a relatively mild disintegration process (2), producing nanofibers, macrofibrils, and fiber fragments. This material is then separated (3) into cellulose nanofibers, macrofibrils, and fiber fragments that can be used in various applications.

In refining there is a distinction between internal and external fibrillation. Internal fibrillation is a result of compressive refining whereas external fibrillation is due to abrasion (Wang *et al.* 2007). The early expansion of surface area during the delamination process (Fig. 3) without full fiber separation resembles the phenomena of internal fibrillation (Moser *et al.* 2016). The proposed mechanism for delamination within the homogenizer is an interfacial acceleration that tears the fibers apart. This is consistent with an extreme form of abrasion that acts from the end of the cell and cuts in the longitudinal direction. The combination of these effects is what provides fiber delamination during homogenization. The fact that macrofibrils were seen during refining and in the mechanical pulp as the smallest documented fines (Meyer and Misch

1937) strengthens the argument that these are naturally occurring hierarchical structures appearing before the microfibrils and subsequently elementary fibrils.

These results indicated that it is possible to separate mildly homogenized chemical pulp into different fractions such as nanofibers and macrofibrils (Fig. 5). Pure macrofibrils exhibited interesting properties for use in various technical applications. For example, fibrous fines in mechanical pulp are regarded as valuable to the strength properties of the papers (Fischer *et al.* 2017).

CONCLUSIONS

The delamination pathway from pulp fibers to cellulose nanofibers is a stepwise process in which the fibers separate into various hierarchical levels before becoming microfibrils at the nanometer scale. The occurrence of macrofibrils, with widths in the range of 0.5 to 2 µm, was evident in the earlier stages of the delamination process.

ACKNOWLEDGEMENTS

REFERENCES CITED

Bergander, A., and Salmén, L. (2002). "Cell wall properties and their effects on the mechanical properties of fibers," *Journal of Materials Science* 37(1), 151-156. DOI: 10.1023/a:1013115925679

Blackwell, J., and Kolpak, F. J. (1975). "The cellulose microfibril as an imperfect array of elementary fibrils," *Macromolecules* 8(3), 322-326. DOI: 10.1021/ma60045a015

Brinchi, L., Cotana, F., Fortunati, E., and Kenny, J. M. (2013). "Production of nanocrystalline cellulose from lignocellulosic biomass: Technology and applications," *Carbohydrate Polymers* 94(1), 154-169. DOI: 10.1016/j.carbpol.2013.01.033

Chinga-Carrasco, G. (2011). "Cellulose fibres, nanofibrils and microfibrils: The morphological sequence of MFC components from a plant physiology and fibre technology point of view," *Nanoscale Research Letters* 6(1), 417-423. DOI: 10.1186/1556-276X-6-417

Conley, K., Godbout, L., Whitehead, M. A., and Van de Ven, T. G. (2016). "Origin of the twist of cellulosic materials," *Carbohydrate Polymers* 135, 285-299. DOI: 10.1016/j.carbpol.2015.08.029

Doblin, M. S., Kurek, I., Jacob-Wilk, D., and Delmer, D. P. (2002). "Cellulose biosynthesis in plants: From genes to rosettes," *Plant & Cell Physiology* 43(12), 1407-1420. DOI: 10.1093/pcp/pcf164

Fernandes, A. N., Thomas, L. H., Altaner, C. M., Callow, P., Forsyth, V. T., Apperley, D. C., Kennedy, C. J., and Jarvis, M. C. (2011). "Nanostructure of cellulose microfibrils in spruce wood," *Proceedings of the National Academy of Sciences of the United States of America* 108(47), 195-203. DOI: 10.1073/pnas.1108942108   This entry is not cited within the text!

Fischer, W. J., Mayr, M., Spirk, S., Reishofer, D., Jagiello, L. A., Schmiedt, R., Colson, J., Zankel, A., and Bauer, W. (2017). "Pulp fines - Characterization, sheet formation, and comparison to microfibrillated cellulose," *Polymers* 9(8), 366. DOI: 10.3390/polym9080366

Frey-Wyssling, A., and Mühlethaler, K. (1963). "Die Elementarfibrillen der Cellulose [English translation]," *Die Makromolekulare Chemie* 62(1), 25-31. DOI: 10.1002/macp.1963.020620103

Heyn, A. N. (1969). "The elementary fibril and supermolecular structure of cellulose in soft wood fiber," *Journal of Ultrastructure Research* 26(1), 52-68. DOI: 10.1016/S0022-5320(69)90035-5

Hill, J. L., Hammudi, M. B., and Tien, M. (2014). "The *Arabidopsis* cellulose synthase complex: A proposed hexamer of CESA trimers in an equimolar stoichiometry," *The Plant Cell* 26(12), 4834-4842. DOI: 10.1105/tpc.114.131193

Klemm, D., Heublein, B., Fink, H. P., and Bohn, A. (2005). "Cellulose: Fascinating biopolymer and sustainable raw material," *ChemInform* 44(22), 3358-3393. DOI: 10.1002/chin.200536238

Li, S., Bashline, L., Lei, L., and Gu, Y. (2014). "Cellulose synthesis and its regulation," *The Arabidopsis Book* 12, e0169. DOI: 10.1199/tab.0169

Mayr, M., Eckhart, R., Winter, H., and Bauer, W. (2017). "A novel approach to determining the contribution of the fiber and fines fraction to the water retention value (WRV) of chemical and mechanical pulps," *Cellulose* 24(7), 3029-3036. DOI: 10.1007/s10570-017-1298-6

Meier, H. (1962). "Chemical and morphological aspects of the fine structure of wood," *Pure and Applied Chemistry* 5(1), 37-52. DOI: 10.1351/pac196205010037

Meshitsuka, G., and Isogai, A. (1995). "Chemical structures of cellulose, hemicelluloses, and lignin," in: *Chemical Modification of Lignocellulosic Materials*, D. Hohn (ed.), Marcel Dekker, New York, NY, pp. 11-34.

Meyer, K. H., and Misch, L. (1937). "Positions des atomes dans le nouveau modèle spatial de la cellulose [English translation]," *Helvetica Chimica Acta* 20(1), 232-244. DOI: 10.1002/hlca.19370200134

Moser, C., Henriksson, G., and Lindström, M. E. (2016). "Specific surface area increase during cellulose nanofiber manufacturing related to energy input," *BioResources* 11(3), 7124-7132. DOI: 10.15376/biores.11.3.7124-7132

Moser, C., Lindström, M., and Henriksson, G. (2015). "Toward industrially feasible methods for following the process of manufacturing cellulose nanofibers," *BioResources* 10(2), 2360-2375. DOI: 10.15376/biores.10.2.2360-2375

Mühlenthaler, K. (1949). "Electron micrographs of plant fibers," *Biochimica et Biophysica Acta* 3, 15-25. DOI: 10.1016/0006-3002(49)90075-X

Mølhøj, M., Pagant, S., and Höfte, H. (2002). "Towards understanding the role of membrane-bound endo-1,4-glucanases in cellulose biosynthesis," *Plant & Cell Physiology* 43(12), 1399-1406. DOI: 10.1093/pcp/pcf163

Ohad, I., and Danon, D. (1964). "On the dimensions of cellulose microfibrils," *Journal of Cell Biology* 22(1), 302-305. DOI: 10.1083/jcb.22.1.302

Pääkkö, M., Ankerfors, M., Kosonen, H., Nykänen, A., Ahola, S., Österberg, M., Ruokolainen, J., Laine, J., Larsson, P. T., Ikkala, O., *et al.* (2007). "Enzymatic hydrolysis combined with mechanical shearing and high-pressure homogenization for nanoscale cellulose fibrils and strong gels," *Biomacromolecules* 8(6), 1934-1941. DOI: 10.1021/bm061215p

Peterlin, A., and Ingram, P. (1970). "Morphology of secondary wall fibrils in cotton," *Textile Research Journal* 40(4), 345-354. DOI: 10.1177/004051757004000407

Somerville, C. (2006). "Cellulose synthesis in higher plants," *Annual Review of Cell and Developmental Biology* 22, 53-78. DOI: 10.1146/annurev.cellbio.22.022206.160206

Trovatti, E. (2013). "Bacterial cellulose," in: *Biopolymer Nanocomposites: Processing, Properties, and Application*s, A. Dufresne, S. Thomas, and L. A. Pothen (eds.), John Wiley & Sons, Hoboken, NJ, USA, pp. 339-367.

Wang, X., Maloney, T., and Paulapuro, H. (2007). "Fibre fibrillation and its impact on sheet properties," *Paperi ja Puu-Paper and Timber* 89(3), 148-151.

Wilson, S. M., Burton, R. A., Collins, H. M., Doblin, M. S., Pettolino, F. A., Shirley, N., Fincher, G. B., and Bacic, A. (2012). "Pattern of deposition of cell wall polysaccharides and transcript abundance of related cell wall synthesis genes during differentiation in barley endosperm," *Plant Physiology* 159(2), 655-670. DOI: 10.1104/pp.111.192682

Zhao, Y., and Li, J. (2014). "Excellent chemical and material cellulose from tunicates: Diversity in cellulose production yield and chemical and morphological structures from different tunicate species," *Cellulose* 21(5), 3427-3441. DOI: 10.1007/s10570-014-0348-6