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**Development of Aspen Bleached
Chemithermomechanical Pulp
Towards Nanostructures**

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Declaration:

Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Kärt Kärner

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Haava pleegitatud keemilis-termilise puitmassi töötlemine nanostruktuuride saamiseks

KÄRT KÄRNER

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List of Publications

The list of author's publications, based on which the thesis has been prepared:

- I Kärner, K.; Talviste, R., Viipsi, K., Elomaa, M.; Kallavus, U. (2014) Study of the effect of mechanical treatment and supercritical CO₂ extraction on aspen BCTMP by surface charge measurements and SEM. *Cellulose Chemistry and Technology*, 48:535–544.
- II Kärner, K.; Elomaa, M.; Kallavus, U. (2014) Lignin and outer cell wall removal from aspen pulp by using supercritical CO₂ extraction. *In: Proceedings of the 9th International Conference of DAAAM Baltic Industrial Engineering: 24–26st April 2014*, Tallinn, Estonia. TUT Press: 372–377.
- III Kärner, K.; Elomaa, M.; Kallavus, U. (2016) Fibrillation of BCTMP of aspen by alkaline cold pre-treatment and vibration milling. *Materials Science (Medžiagotyra)*, 22 (3): 358–363.
- IV Kärner, K., Elomaa, M, Kallavus, U, Tõnsuaadu, K. (2017) Controlled nanocrystalline precipitation of hydroxyapatite on the surface of microfibrillated cellulosic fibres. *International Journal of Recent Scientific Research*, 8 (10): 20803–20809.

Copies of these publications are included in APPENDIX.

Author's Contribution to the Publications

Contribution to the papers in this thesis are:

- I The author was responsible for planning and conducting the experiments, the interpretation of the results, the calculation work and composing the paper.
- II The author carried out the experiments and composed the paper.
- III The author carried out the experiments and composed the paper.
- IV The author carried out the experiments and composed the paper.

Introduction

As today's science and technology aims to be green, researchers all over the world have focused an enormous amount of scientific research towards bio-based materials because of their cost effectiveness, eco-friendliness, and renewability. This rapidly expanding field is generating many exciting new materials with novel properties and promises to yield advanced applications in diverse fields.

Cellulose is especially important, as it is the most abundant biopolymer on earth. It has attractive the characteristics of being both recyclable and renewable and non-toxic (Dufrense, 2013). It consists of microfibrils which are bundles of elementary crystallites bridged by amorphous phases. Because a complete purification of cellulose demands a lot of energy and chemicals, there are many products in which the cellulose used is only partly purified. For example, products such as bleached chemithermomechanical pulp (BCTMP) have been developed to be used as a component in paper making. A characteristic property of mechanical pulp is that it retains most of the wood lignin (Lönnerberg, 2009).

Microfibrillated cellulose (MFC), generally produced from pulp, is a novel innovation, where cellulose fibres are fibrillated (meaning that the mother fibre is split into a higher number of thinner fibres/fibrils). MFC has unique and potentially useful features. It combines important cellulose properties, such as hydrophilicity, broad chemical modification capacity and the formation of versatile semi-crystalline fibre morphologies with the specific features of nano-sized materials, such as a high surface area with a large number of available surface OH groups, good water holding capacity, and a higher aspect ratio compared with cellulose. MFC has several applications, *e.g.*, (nano) paper, a barrier in greaseproof paper, a reinforcing component in paper coatings, composite reinforcement. Due to its nontoxic, hydrophilic and rheological properties, nanocellulose is also used in food, pharmaceuticals, and cosmetics (Hjørnevik, 2016; Kangas, 2014).

Research on micro/nanocellulose has gradually increased since the year 2000, and there are research groups all over the world aiming to produce this novel material in many ways and by using different new sources. Currently, there are approximately 10 nanocellulose-producing companies, some pre-commercial institutions, and some pilot and lab-scale production facilities, all over the world, and this field is rapidly expanding. A few institutions are even able to produce more than 1000 kg per day (Miller, 2015).

Why is nanocellulose generally important? First, it is a biomaterial and biomaterials are generally recognized to form a better basis for a sustainable industry than petrochemicals. Second, the traditional method of utilizing cellulose has not fully utilized the elemental fine structure of this abundant polysaccharide.

Nanocellulose applications range from bulk applications, such as a rheological modifier, composite reinforcement or paper additive, to high-end applications, such as tissue engineering, drug delivery, and functional materials. Nanocellulose many potential commercial applications, including composites and foams for automotive, aerospace, and building construction, viscosity modifiers for cosmetics and oil drilling fluids, and high-performance fillers for paper, packaging, paints, plastics, and cement (bioplus.com) Additionally, due to its excellent biological properties (biocompatible, biodegradable, and low toxicity) MFC has many usages in biomedicine, *e.g.*, it can be used in scaffolds and in drug delivery, as well as for skin and bone tissue repair and

wound healing (Lind and Dufrense, 2014). However, the main drawback today is still the relatively high price, being between 1,500 and 20,000 USD/kg (Cellulose Lab, 2017).

The main goal of this research was to study the possibility of producing microfibrillated cellulose (MFC) from aspen pulp in an environmentally friendly and simple way, while preserving the natural crystalline form of cellulose. Much work has been done globally to produce new types of products from cellulose, especially in recent years. Typically, highly purified cellulose from wood or even bacterial cellulose has been used as the starting material. In an effort to not repeat already reported procedures and because of an existing relationship with a local producer, AS Estonian Cell, aspen pulp was selected as the starting material. Estonian Cell has an aspen pulp mill in Kunda, Estonia and an annual production capacity of 173 000 tons of aspen pulp (Estonian Cell, 2018). The characteristics of the pulp are described in more detail in chapter 1.3.1. If mechanical pulp could be refined to nanostructures in an economical manner, many novel applications would be possible.

The production of cellulose to nanocellulose is expensive due to the high energy consumption needed for the mechanical processing, expensive apparatus and expensive chemicals (Khalil *et al.*, 2013). Therefore, the main goal of this study is the development of a simple, energy efficient and environmentally friendly method to produce microfibrillated cellulose (MFC) from bleached chemithermomechanical pulp (BCTMP) of aspen wood manufactured by Estonian Cell Company. This new product could be less expensive and potentially has some properties common with nanocellulose, especially the large surface area and reactivity. By using different chemical and mechanical treatments, it is possible to peel off the outer lignin-rich cell wall layers of wood fibres and expose the inner layer, as it consists mostly of cellulose. Additionally, the inner layer consists of bundles of microfibrillar cellulose. It is a good starting material for further peeling of nanocellulose bundles. In addition, to utilize the negative surface charge of cellulose, one goal is to bind an additive, *e.g.*, hydroxyapatite (HAp), onto the produced MFC.

In addition, results of this thesis have been presented at different scientific conferences

Kärner, K.; Talviste, R.; Viipsi, K.; Kallavus, U. (2013) Enhancing the Surface Charge of BCTMP of Aspen with supercritical CO₂ treatment. In: Baltic Polymer Symposium 2013: Baltic Polymer Symposium 2013, Trakai, Lithuania, September 18–21, 2013. Ed. Vilnius University. 87.

Kärner, K.; Elomaa, M.; Kallavus, U. (2014) Fibrillation of BCTMP of aspen by alkaline cold pretreatment and vibration milling. Kaunas, Lithuania, September 22–24, 2014.

Abbreviations

AGA	Aktiebolaget Gasaccumulator
BCTMP	Bleached Chemithermomechanical pulp
BET	Brunauer-Emett-Teller particle surface area determination method
BNC	Bacterial Nanocellulose
CML	Compound Middle Lamella
CNC	Cellulose Nanocrystals
CNF	Cellulose Nanofibrils
FM	Fine Material
HAp	Hydroxyapatite
HMW	High Molar Weight
IKA	registered trademark of IKA-Werke GmbH & Co. KG
KVPS	Potassium Polyvinyl Sulphate
LP	Larger Particles
MCC	Microcrystalline Cellulose
MF	Microfibril
MFC	Microfibrillated Cellulose
ML	Middle Lamella
Mw	Molar Weight
NCC	Nanocrystalline Cellulose
OM	Optical Microscope
OTB	Ortho-Toluidine Blue
P	Primary Wall
p-DADMAC	Poly-Diallyldimethylammonium Chloride
scCO ₂	Supercritical Carbon Dioxide
SEM	Scanning Electron Microscope
TAPPI	Technical Association of Pulp and Paper Industry
VM	Vibration Mill
wt%	Weight Percentage
XRD	X-ray diffraction
TEMPO	(2, 2, 6, 6-tetramethyl-piperidine-1-oxil)

Terms

Cellulose molecule	Linear polysaccharide consisting of approximately 1000 anhydroglucose units (Cellulose from wood pulp has typical chain lengths between 300 and 1700 units; cotton and other plant fibres as well as bacterial cellulose have chain lengths ranging from 800 to 10,000 units (Pääkkö, 2013).
Compound middle lamella	Middle lamella with primary wall and first layer of secondary cell wall (Hafrén <i>et al.</i> , 2005).
Elementary fibril	Consists of 36 cellulose molecules, width of 3.5 nm (Chinga-Carrasco, 2011).
Fibre	Consists of macrofibrils, with widths of 30–40 µm, lengths of 1–3 mm.
Fibrillation	Fibre split into higher number of thinner fibres/fibrils.
Hemicelluloses	Second component of wood; group of different sugar molecules in wood, gives flexibility to wood.
Lignin	third component of wood, consists of aromatic units, acts as adhesive in wood.
Macrofibril	Consists of bundles of microfibrils, widths of 5–30 nm, length over 1 µm.
Microfibrillated cellulose (MFC)	Gel-like material, consists of cellulose microfibrils, composed of expanded high-volume cellulose, moderately degraded, greatly expanded in surface area, Diameters of 20–60 nm, length in µm-s (Klemm <i>et al.</i> , 2011).
Microfibril	Consists of elementary fibrils, with widths usually 28–35 nm (Chinga-Carrasco, 2011).
Microlamella	Consists of 1–6 microfibrils. Wide but thin structural unit where cellulose microfibrils are attached side by side.
Nanocellulose	At least one dimension less than 100 nm.
Nanocrystalline cellulose (NCC)	Similar to MFC, has lower aspect ratio than MFC, consists of rod-like cellulose crystals, diameters of 5–70 nm, lengths of 100–250nm (Klemm <i>et al.</i> , 2011).
Polyelectrolyte titration	Titration technique for surface charge measurement based on the fact that polyelectrolytes can form complexes with oppositely charged polyelectrolytes (direct titration) or surfaces (indirect titration) (Terayama <i>et al.</i> , 1952).
Simons' stain	Mixture of direct blue and direct orange dye, used to examine changes in the physical structure of pulp fibres under the microscope. When treated with a mixed solution of orange and blue dyes, internal fibrillation will be orange (Yu <i>et al.</i> , 1994).
S1	Outermost layer of the secondary cell wall of a wood fibre.
S2	Middle layer of the secondary cell wall of a wood fibre.
S3	Inner layer of the secondary cell wall of a wood fibre.

1 LITERATURE REVIEW

1.1 Wood and its main components

Wood is the natural tissue material of trees. It can be considered a natural composite material consisting mainly of cellulose, hemicelluloses, and lignin. They are all physically and chemically attached to each other. The proportion of the three polymers varies between species. A simplified picture is that cellulose forms a skeleton of oriented macromolecules, which is surrounded by other substances functioning as the matrix (hemicelluloses) and binding (lignin) materials. Approximately 40 to 45 % of the dry substance in most wood species consists of cellulose, located predominantly in the secondary cell wall (S2, Figure 2) in the form of cellulose microfibrils (Sjöström, 1993; Hon and Shirashi, 2001).

Wood is a renewable, biodegradable, environmentally friendly, multifunctional material; it has played a major role throughout human history. People have used wood for fuel, construction material, for making weapons, cooking food, making shelters, furniture, packaging, artworks, paper and for countless other uses.

Currently, new alternative usages are emerging. For example, nanocellulose is used in food, cosmetics, medical appliances, films, foams, composite materials, hygiene products, paper and electronics.

1.1.1 Cellulose

Cellulose is the most important single component in wood what provides the wood's strength. Cellulose is also the most abundant organic material on earth. It is an essential polymer in pulping and papermaking as the web of cellulose fibres forms the body of the paper (Salmi *et al.*, 2006). The behaviour of the cellulose surface and its interactions with different chemicals is important in current and future applications such as papermaking, composites, and nanocomposites (Gardner *et al.*, 2008).

Cellulose is a homopolysaccharide composed of β -D-glucopyranose units that are linked together by (1 \rightarrow 4)-glycosidic linkages. The polymer chains are bound together in structures by intermolecular hydrogen bonding (Budd and Herrington, 1989).

The length of the native cellulose molecule is at least 5000 nm and its chain consists of approximately 10000 glucose units. The smallest building element of the cellulose skeleton is an elementary fibril. This is a bundle of 36 parallel cellulose molecules, which are held together by hydrogen bonds (Sjöström, 1993).

The cellulose morphology represents a well-organized architecture of fibrillar elements. The elementary fibril of native cellulose is the smallest morphological unit, with a diameter of approximately 3.5 nm. During the growth of the tree, the cellulose molecules are arranged together in the form of microfibrils, which are 10–20 nm wide and lengths can reach to micrometres. Microfibrils are combined to greater fibrils and lamellae (bundles of microfibrils, sometimes called macrofibrils) and they finally build up the cellulose fibres (Klemm *et al.*, 2005).

Within the fibrils, cellulose chains are tightly packed together forming highly crystalline regions (crystallites), which are accompanied by disordered, amorphous regions, making cellulose semi-crystalline (Sjöström, 1993). The cellulose crystallites in the ordered regions exist in several polymorphs depending on the source, method of extraction or treatment. Cellulose I, so-called native cellulose, has two crystalline forms, I α and I β -, that can coexist in various proportions. Cellulose I β is dominant in plant cell wall cellulose and in tunicates. Cellulose II, *i.e.*, the regenerated cellulose

crystal structure, is formed by two processes, regeneration (solubilization and recrystallization) and mercerization. Cellulose II is the most stable structure with technical relevance (viscose products). Treatment with liquid ammonia or with certain amines such as 1,2-diaminoethane (ethylenediamine, EDA) allows the preparation of cellulose III from either cellulose I or cellulose II. Cellulose III, treated at high temperature in glycerol, is transformed into cellulose IV (Pääkkö, 2013; Pérez and Samain, 2010).

Cellulose has a long history in the pulp and paper industry. However, as it has been stated that traditional wood and pulp products do not meet the needs of modern society, but materials based on cellulose nanoparticles and structures have the potential to do so (Moon *et al.*, 2010). Cellulose nanomaterials have been developed to provide new functionalities, to improve existing products, to lower the need for resources and to enable sustainability (Qvintus *et al.*, 2015).

1.1.2 Hemicelluloses

Hemicelluloses are a group of amorphous polysaccharides. They are produced from glucose as well as from the other sugars. Hemicelluloses are thus a mixture of various polymerized sugar molecules. In some cases, the polymers are straight-chained, such as cellulose, but polymers with short side chains are also common (Sjöström, 1993).

Hemicelluloses bind up cellulose and lignin and thus give flexibility and strength to the fibres. In contrast to cellulose, which is a homopolysaccharide, hemicelluloses are heteropolysaccharides. They function together with cellulose as the supporting material in the cell walls. Compared with cellulose, hemicelluloses have a low degree of polymerization (ca. 200). The proportion of hemicelluloses of the wood dry weight is usually between 20 and 30 %. The main monomers in hemicelluloses are D-glucose, D-mannose, D-xylose, and L-arabinose (Rowell, 2013).

1.1.3 Lignin

The third main wood component is lignin. Lignins are polymers of phenylpropane units linked together in different ways. The lignin concentration is highest in the middle lamella (ML), but most of the lignin is in the inner fibre wall (S2) due to its dominant size. Normal softwood contains 26–32 % of lignin and hardwood contains 20–25 % lignin (Sjöström, 1993).

Lignin is a highly branched, substituted, mononuclear aromatic polymer. In the lignin structure, depending on the source, there are, in various ratios different monomers, coniferyl alcohol, sinapyl alcohol and paracoumaryl alcohol. Lignins are a class of complex, high molar weight polymers whose exact structure varies. It is an amorphous polymer that acts as a binding agent to hold cells together (Eklund and Lindström, 1991).

1.2 The structure of wood fibre

“Fibre” refers to libriform cells in hardwoods and the tracheid cells in softwoods. There are also other cells that are present in minor proportions and are not considered fibres, for example, the vessel elements in hardwood and parenchyma cells in softwoods and hardwoods (Fardim, 2002; Sjöström, 1993).

Natural fibres are composite materials, resulting from the assembly of microfibrils and hemicelluloses embedded in a matrix mainly composed of lignin. For instance, in wood cellulose the polymer chains are packed into elementary fibrils with lateral diameters of about 3–5 nm and lengths of 10–20 nm. They are further aggregated

together with hemicelluloses by hydrogen bonding to form what are classically known as microfibrils and their aggregates with diameters of about 5 to 30 nm. The microfibrils are further packed into larger aggregates, finally forming macroscopic wood cellulose fibres, see Figure 1 (Pääkkö *et al.*, 2013; Krässig, 1993).

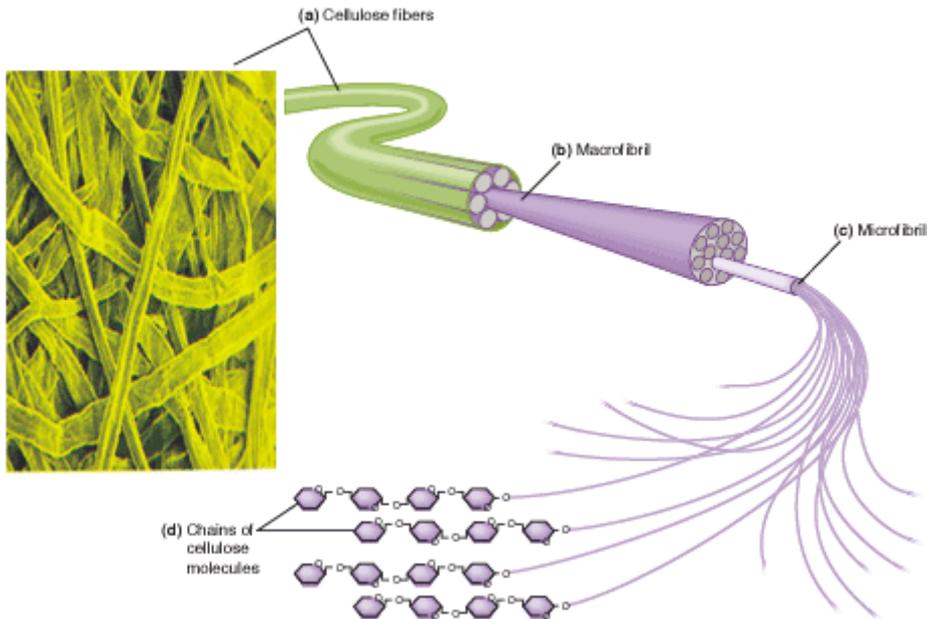


Figure 1. Cellulose fibres (a), macrofibrils (b), microfibrils (c), and molecules (d) (Nutrition Resources, 2018).

The structure of the fibre consists of several different layers. The three main layers are the ML, the primary wall (P), and the secondary wall. The P and ML regions in lignified wood fibres are not easily separated and are therefore often jointly referred to as compound middle lamella (CML). The secondary wall is divided into sub layers, *i.e.*, S1, S2, and S3. The thickness of the fibre cell wall can vary between 3–15 μm depending on the wood species (Hafrén *et al.*, 2005; Klemm *et al.*, 2005).

Wood is built up hierarchically, starting from the smallest building blocks of D-glucose units, which are linked together by glycosidic linkages to form the cellulose polymer. Cellulose chains are then connected to elementary fibrils and microfibrils, which are the main component of the cell wall, indicated by fine lines in Figure 2. Additionally, the orientation of cellulose microfibrils is specific to each layer. For example, the orientation of microfibrils in the P is quite random, in the S1 layer microfibrils are oriented horizontally in relation to the fibre axis, and in the S2 layer, they are almost vertically oriented. The microfibril angle of the cellulose in the S2 layer is 14–19°. Consequently, the different cell wall layers are easily identified by using scanning electron microscopy (SEM) (Rowell, 2013; Krässig, 1993).

The chemical constituency is also different in different layers; the lignin content is highest in the ML and the P, whereas the cellulose content is highest in the S2 layer. Therefore, to obtain pure cellulose from wood, it is necessary to remove the outer cell wall layers. The proof of successful treatment is a clearly visible S2 layer with a parallel orientation of cellulose microfibrils (Rowell, 2013; Sjöström, 1993).

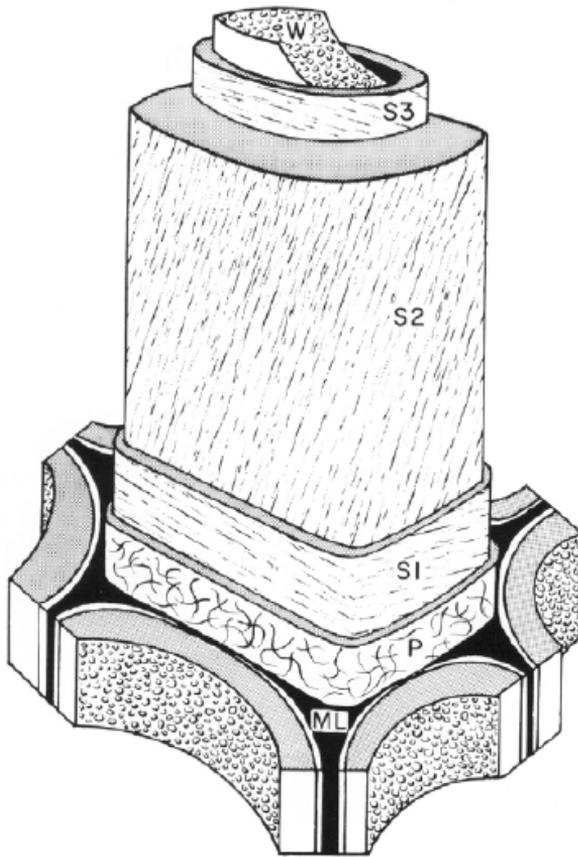


Figure 2. Simplified structure of a wood cell, showing the middle lamella (ML), the primary wall (P), the outer (S₁), middle (S₂) and inner (S₃) layers of the secondary wall (Coté, 1967).

The CML is located between the fibres and its thickness is 0.1–1.0 μm , depending on the tree species. It consists of 60–80 % of lignin (Sjöström, 1993).

The P consists of a rough network of microfibrils but is highly encrusted with lignin. The thickness is 0.1–0.3 μm . The P contains cellulose and hemicelluloses in a matrix of lignin. The P layer is formed during the surface growth of the cell wall. The orientation of microfibrils in the P is random. A characteristic property is their high resistance to pulping and bleaching chemicals.

The secondary wall is built up from three different layers, *i.e.*, the transition S1 lamella, the thick central S2 region and S3 lamella towards the lumen. S1, S2 and S3, all have different orientations of microfibrils. The thickest part of secondary wall is the S2 layer, which constitutes ca. 80 % of the wood fibre and hence provides most of the fibre strength. The thickness of the S2 layer varies from 0.5 μm to 4 μm . The microfibrils of the S2 layer run more or less parallel to the long axis of the cell, whereas the microfibrils of the S1 and S3 layers run more or less horizontally. The S layer is formed during the thickening of the cell wall. In the central part of each cell, there is a usually empty space called the lumen.

Generally, the S2 layer increases with increasing wall thickness, whereas S1 and S3 remain fairly constant. Because of its greater thickness, the S2 layer is largely responsible for the physical and mechanical properties of the cell walls. S2 is also the most important layer of the fibre (Hon and Shirashi, 2001; Eklund and Lindström, 1991).

The structure of the cell wall largely determines the properties of the individual fibres as well as the wood as a whole.

1.3 Wood pulp

Pulp is the basic wood product, used mainly for papermaking, but is also processed to various cellulose derivatives, such as rayon and cellophane (Sjöström, 1993). Pulp is a mixture of fibres, fibre fragments and small cells (parenchyma or ray cells), usually called pulp fines.

The extraction of pulp fibres from wood can be done using chemical, mechanical, thermal and chemimechanical processes. The finished product may be either bleached or non-bleached, depending on the customer requirements.

In mechanical pulping, the fibres are separated from each other by physically grinding or rupturing the wood, not by dissolving lignin like in chemical treatment. The process degrades the lignin and hemicellulose into small, water-soluble molecules that can be washed away from the cellulose fibres without depolymerizing the cellulose fibres (chemically depolymerizing the cellulose weakens the fibres).

The two main industrial processes used for mechanical pulping are grinding and refining. Grinding is done by pressurising the wood logs against a rotating pulp stone with water acting as the cooling and lubricating agent. The refining process, on the other hand, utilizes a narrow gap between a rotor and a stationary disc to separate the fibres from the wood chips. In addition, during the refining process, the fibres are defibrillated. The most common process is thermomechanical pulping where the refining chamber is pressurised with steam (Lönnerberg, 2009).

There are a number of related hybrid pulping methods. These hybrid methods include thermomechanical pulping, also known as TMP, and chemithermomechanical pulping, also known as CTMP. The chemical and thermal treatments reduce the amount of energy required subsequently by the mechanical treatment, and also reduce the amount of strength loss suffered by the fibres (Lönnerberg, 2009).

1.3.1 Bleached chemithermomechanical pulp

The main method of producing bleached high-yield pulps is chemithermomechanical (CTMP) processing and subsequent bleaching with peroxide under alkaline conditions. It is known that fibre fractionation of the CTMP takes place in the lignin-rich ML or P. This makes its properties different from other pulps such as chemical pulps and other mechanical pulps. CTMP differs from chemical pulps in their size, shape, surface chemistry, flexibility, bonding and especially lignin content. Additionally, a considerable amount of original lignin (84.1 %) and hemicelluloses (72.0 %) are retained during BCTMP process (Fu *et al.*, 2015).

Bleached hardwood CTMP (BCTMP) can now be used in a high-quality wood-free paper.

The use of BCTMP in a wood-free paper not only reduces the cost of the raw material but also improves paper quality, *i.e.* higher bulk, higher opacity and better paper formation (Li *et al.*, 2006).

During pulping and bleaching, a great amount of lignin is removed. According to pulping theory, delignification starts from the lumen side of the fibres and then proceeds outwards to the ML. If the ML is removed by further treatments, it is reasonable to believe, that most of the lignin in the P is also removed (Li and Reeve, 2002).

The BCTMP flow chart from the Estonian Cell Company is shown in Figure 3. Estonian Cell is also a pulp factory that promises to produce the highest quality pulp with minimal negative environmental impacts. Its annual production is 173 000 tons of aspen pulp (Estonian Cell, 2018).

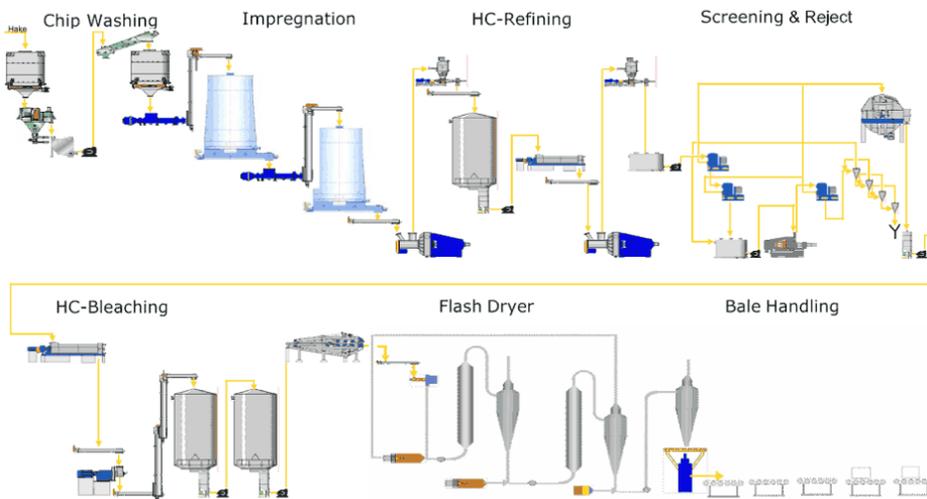


Figure 3. Main Flow sheet of BCTMP (Estonian cell, 2018).

1.4 Changes occurring to fibres during refining

During refining, the changes to fibres are listed as follows: fibrillation (external fibrillation, internal fibrillation (swelling)), fines formation, fibre shortening and fibre straightening.

Refining is a mechanical treatment of fibres with metallic bars in the presence of water. It leads to the mechanical degradation of fibres and changes in their structure. New surfaces are obtained because of external and internal fibrillation (Eklund and Lindström, 1991). However, the severe refining causes fibre shortening (Banavath *et al.*, 2011). In papermaking, the fibre surfaces are peeled off during beating/refining and increasing amounts of charged fines are formed.

Unrefined pulps have stiff and smooth fibres and poor bonding ability, but refined pulps have flexible, fibrillated fibres and good bonding ability.

1.4.1 Internal fibrillation

Internal fibrillation is revealed by loosening of the bonding of the cell layers, caused by the cyclic compression action of forces inside the refiner. Internal fibrillation has been extensively studied because it is believed by several investigators to be the most important effect of refining. The breakage of the inner bonds, which are between the

cellulosic fibrils, between the fibrils and hemicellulose, between the cellulose and lignin, and between the hemicellulose and lignin, causes the pore structure inside the cell wall to expand and swell. The swelling is an indication of internal fibrillation during refining. Internal fibrillation loosens the fibre wall, further enhancing the susceptibility of the fibre for further treatment or homogenization (Hubbe *et al.*, 2007).

1.4.2 External fibrillation

External fibrillation refers to the peeling off of the external layers, thus opening the S2 layer for further chemical, physical or mechanical treatment. It is important because the bond strength between fibres increases remarkably due to external fibrillation. The most significant effect of external fibrillation is the increasing of the specific surface area of the fibrils and the removal of the CML material (Shao *et al.*, 2007). External fibrillation has received less attention because, during refining, it is associated with internal fibrillation and fine formation, and the simultaneous changes made it difficult to judge the role of external fibrillation.

1.4.3 Simons' stain test

It is possible to determine the rate of internal fibrillation by using Simons' stain. It is a two-colour differential stain, a mixture of Direct Blue 1 and Direct Orange 15 at a ratio of 10:1. The Direct Blue 1 has a lower molar weight, 992.82 g/mol. Direct Orange 15 forms an extended polymer; it has high molar weight. When fibres are treated with a mixed solution of orange and blue dyes, unbeaten fibres will stain *blue* and beaten fibres orange. Beaten fibres will stain orange because of the internal fibrillation. If both stains are present, orange dominates. The blue stain has a smaller molecule size, so that it could penetrate very small capillaries where the orange could not penetrate (Yu *et al.*, 1994).

1.4.4 Fines formation

Two types of fines are considered present in the pulp, which are primary fines and secondary fines. Primary fines are present in unbeaten pulp and come from ray cells and parenchyma cells (Ferreira *et al.*, 1999; Wistara and Young, 1999) while secondary fines are produced during the refining, as a result of external fibrillation or fibre shortening (Hartman, 1984). These fines consist of fragments of primary and secondary walls with sizes approximately 20 µm (Heijennesson *et al.*, 1995). Fines have high surface charges and can improve the bonding of fibres.

1.5 Characteristic properties of aspen wood and pulp

1.5.1 Aspen wood

Aspen, especially *Populus Tremula*, is one of the most abundant pulpwoods in Europe due to its rapid growth and good propagation. Aspen is used for furniture, toothpicks, matchsticks, boxes and crates, panelling and, chipboard. Additionally, this plentiful tree has been a popular source of paper pulp since the late 1940's. In Estonia, aspen is one of the most common tree species (holding fourth place after pine, birch and spruce) (www.estonia.ee).

Aspen has an average fibre length of 0.9 mm (ranges from 0.2 to 1.6 mm) and an average width of 19µm (ranges from 13 to 30 µm). Aspen is a diffuse-porous hardwood. The pores are small and evenly distributed throughout the annual growth increments. The heartwood of aspen is white to light brown or creamy, the sapwood is typically whiter and blends into the heartwood with no clear line or demarcation. The

rays are extremely fine and are barely visible, even with a magnifying glass. The vessel elements of aspen are medium long to long (up to 0.9 mm), perforations are simple and tyloses are rare. Intervessel pits alternate and are crowded, with small oval apertures and oval hexagonal outlines. Pits leading to ray parenchyma are relatively large, oval to oval-angular, simple and appear in 2–3 (4) rows in horizontal groups. The pitting areas are usually widely spaced. Aspen does not contain any resin (Ilvessalo-Pfäffli, 1995, Mackes and Lynch, 2001).

Aspen wood mainly contains the following hemicelluloses: xylose, smaller amounts of arabinose, rhamnose, uronic acids and at least one of the hexoses, *i.e.*, glucose, mannose, and galactose (Jones *et al.*, 1957).

1.5.2 Aspen pulp

Aspen wood is easily pulped by all commercial processes. It has an excellent length to diameter ratio and the fibre thickness is characterized as thin to medium. Aspen pulp is used to produce book-, newsprint-, and fine printing papers. The highest quality groundwood pulps are produced from aspen. Chemimechanical pulps produced from aspen are primarily used for hardboards and fibreboards. Because aspen has a relatively low density, it is desirable for producing of low- to medium-density fibreboards (Mackes and Lynch, 2001).

1.5.3 Bleached chemithermomechanical pulp of aspen

Today, aspen BCTMP is mainly used as a substitute for chemical pulps. Many paper standards have limited the amount of lignin in certain permanent grades of paper to 1 % or less. Because aspen BCTMP contains 17 % lignin, its usage has tended to be limited to approximately 3 % of the fibre furnish. Depending on the final product or paper grade, aspen BCTMP content can be up to 30 % (Nielsen, 2001).

In addition, aspen wood cellulose is a promising starting material for the preparation of cellulose hydrogels. The main benefit of Aspen BCTMP is that it allows continuing the “green” functionalization process of cellulose fibre as this production is sulphur free and utilizes a chlorine-free bleaching. Although the pulping process aims to separate cellulose fibres from solid wood, some hemicelluloses are also partly or completely removed together with the lignin.

1.6 Nanocellulose

Nanocellulose can be divided into three main types, as follows, based on their preparation method, size and other properties: microfibrillated cellulose (MFC), nanocrystalline cellulose (NCC) and bacterial nanocellulose (BNC). MFC is oriented to get long fibrils, with both amorphous and crystalline parts, NCC has a rather short and rod-like structure with only crystalline parts (Klemm *et al.*, 2011; Lavoine, 2012; Kangas, 2014). One indication of the expected industrial potential of nanocellulose is the explosive growth of scientific literature, see Figure 4.

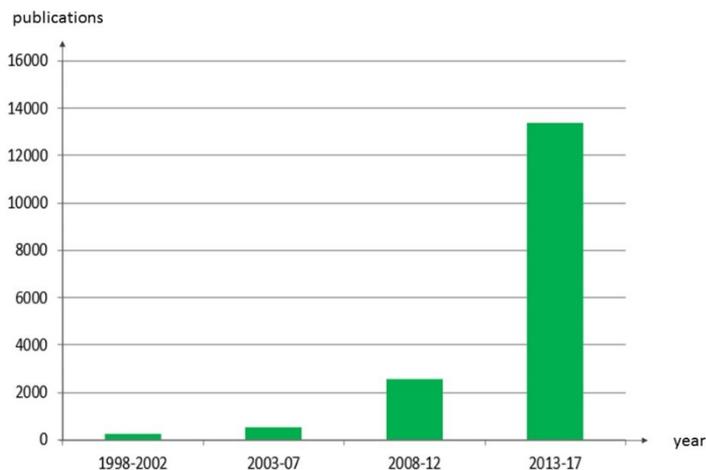


Figure 4. Scientific papers with word nanocellulose found since 1998. Search performed by Google Scholar 28.1.2018.

According to TAPPI, there are numerous things that are referred to as nanocellulose, including NCC, CNC, CNF, MFC, MCC (see list of abbreviations), but only two materials fit this definition, *i.e.*, cellulose nanocrystals (CNC) and cellulose nanofibrils (CNF). Other materials are also referred as nanocellulose, even though they are not nano-scale (Miller, 2015).

MFC (also called nanofibrillated cellulose, nanofibers, nanofibrils, microfibrils) is a material usually made from wood cellulose fibres by detaching individual cellulose microfibrils from each other. MFC can be viewed as a cellulosic material, composed of expanded high-volume cellulose, moderately degraded and greatly expanded in surface area. It was invented in 1983 by Turback and Herrick at ITT Rayonier (Turbak *et al.*, 1983). Depending on the processing conditions, cellulose fibres can be disintegrated to flexible MFC with lateral dimensions starting from ca. 5 nm, representing elementary fibrils, to tens of nanometres, which correspond to single microfibrils and their bundles. Typically, MFC has a diameter of 5–60 nm and a length of a few micrometres. The size distribution of the fibres is wide, and even if some fibres have diameters in nanoscale, there are many bigger fibres as well. Moreover, the fibres are in a network structure and are interconnected to each other; see Figure 5 (Karppinen, 2016). The MFC material may be composed of nanofibrils, fibrillar fines, fibre fragments and fibres; it is not necessarily a nano-material, but it contains nano-structures (Chinga-Carrasco, 2011). MFC is an interesting industrial material; however, the production via pure cellulose is complex and energy demanding.

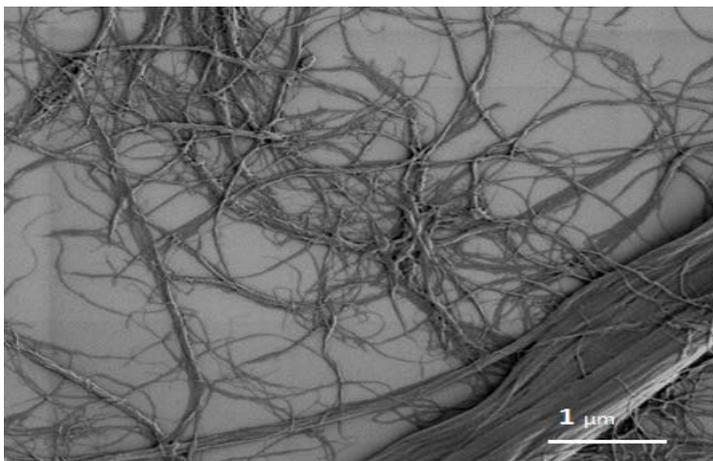


Figure 5. Microfibrillated cellulose consists of long and thin fibres which are tied to a three-dimensional network (Karppinen, 2016).

NCC is generally manufactured from wood pulps but also from alternative sources. Concentrated sulphuric acid is commonly used, which degrades the amorphous regions of cellulose and leaves the crystalline ones intact. By such treatment, rod-like rigid NCC with sulphate groups at their surface are produced. The morphology of NCC generally depends on the source of the cellulose. The crystal width is 2–20 nm and the lengths vary from 100–600 nm to over 1 μm (Lavoine, 2012).

BNC is high-purity cellulose generated by bacteria in an aqueous culture media containing a sugar source. The time of such a process ranges from a few days up to two weeks. BNC has the same chemical composition as plant cellulose. However, it is free of functional groups other than alcohol, *e.g.*, carboxyl or carbonyl, which are usually introduced to wood or plant-derived cellulose during the purification process. BCC also has biomedical applications. The widths of the bacterial cellulose fibrils are 20–100 nm and they consist of even finer cellulose nanofibrils with widths of approximately 2–4 nm (Klemm *et al.*, 2011).

In Europe, the tendency is to produce MFC-type nanocellulose, but in the USA, the focus is more on the NCC-type. Depending on the packaging size, water content and production method, nanocellulose prices are between 1,500 and 20,000 USD/kg (Cellulose Lab, 2017).

1.6.1 Preparation of nanocellulose

Cellulose micro/nanofibres can be obtained by using various methods. These procedures include the following: grinding, refining or high-pressure homogenization processes, microfluidization, the ultrasonic technique, chemical and biological treatments, synthetic and electrospinning methods, ball-milling, PFI milling, combinations of beating, rubbing, *etc.* (Tonoli *et al.*, 2012; Lavione *et al.*, 2012; Klemm *et al.*, 2011). These methods can be divided into two groups, *i.e.*, conventional and non-conventional. The first includes a homogenizer, microfluidizer and grinding, and the other includes extrusion, refining, blending, steam explosion, ultrasonication, ball milling, cryocrushing and aqueous counter collision (Nechyporchuk *et al.*, 2016).

Moreover, a combination of processes, such as refining, cryocrushing, enzymatic treatment, ultrasonication, and acid hydrolysis, has been studied to produce MFC at a high yield (Uetani *et al.*, 2011). To produce a gel-like material, the concentration of the suspension is especially important; the effective concentration is 0.125–5.9 wt% (Lavoine *et al.*, 2012).

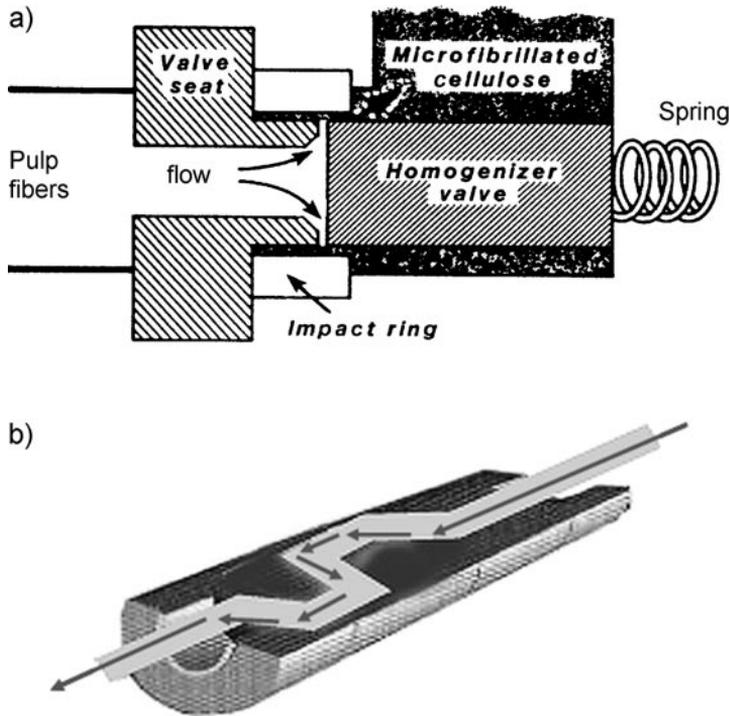


Figure 6. Devices for MFC production: a) high-pressure homogenizer (Klemm *et al.*, 2011).

The oldest device for MFC production is the high-pressure homogenizer (Figure 6, a), in which fibres are pressed through a slit between the valve seat and the pressurized homogenizer valve (Klemm *et al.*, 2011). During this treatment, strongly entangled networks of nanofibrils, having both crystalline and amorphous domains, are produced due to high shearing forces. They possess high aspect ratios and form gels in water with shear-thinning and thixotropic behaviour. For this treatment, kraft pulping is usually used, where almost all lignin is already removed (almost pure cellulose). However, the process needs 5–10 passes to provide MFC with gel-like characteristics and energy consumption is therefore relatively high.

One of the most popular device for MFC production today is the microfluidizer (Figure 6, b). In the microfluidization process, the suspension is subject to high pressure to pass through a narrow Y- or Z-type geometry interaction chamber.

One widely used device is a grinder. The principle consists of the breakdown of the cell wall structure owing to the shearing forces generated by the grinding stones. The pulp is passed between a static grind stone and a rotating grind stone revolving at approximately 1500 rpm (Lavoine *et al.*, 2012). However, this process can degrade the

pulp fibres and decrease their length, which might affect the reinforcement and physical properties of the MFC.

Another option is to use different mills, including the PFI mill, Valley Beater, Jorco mill, Conical refiner, Cylindrical refiner, which are mainly used for refining (Gharehkhani *et al.*, 2014). The PFI, for example, can beat from 5 to 40 g of pulp in concentrations from 5 to 50 % (max. 450 ml of suspension). Pulps are refined between a stainless-steel roll with bars and a rotating disk with a smooth bed where pulps are distributed over the disk wall uniformly. However, fibre shredding might occur, rather than delamination.

Ball milling is another technique that was reported very recently to produce CNF. In this method, a cellulose suspension is placed in a hollow cylindrical container, partially filled with balls (*e.g.*, ceramic, zirconia or metal). While the container rotates, cellulose is disintegrated by the high energy collision between the balls. Zhang *et al.* studied the process of MFC production from a once-dried, bleached softwood kraft pulp suspension at a solid concentration of 1 wt% using ball milling (Zhang *et al.*, 2015). They showed the influence of the process conditions, such as the ball size and ball-to-cellulose weight ratio, on the morphology of the produced MFC. An average diameter of 100 nm was reported for the disintegrated fibres.

1.6.2 The most important properties of nanocellulose

The mechanical treatment during MFC preparation leads to external and internal fibrillation and increased surface area of fibrils. The two most important properties of MFC are that it is normally a viscous and shear thinning aqueous gel at a very low concentration (2–7 wt%) and it can form a transparent film once it is dried. Therefore, MFC has a very large surface area (typically 100–200 m²/g) and extensive hydrogen bonding ability (Lavoine *et al.*, 2012).

Cellulose nanomaterials are considered more than 10 times stronger compared with wood or pulp. The strength of cellulose nanomaterials has been estimated by measuring nanomaterial film. However, the results depend very much on the film preparation method, testing methods and film properties.

Because MFC is more flexible, has longer fibres and has a larger surface area than any other fibres, it is being developed for use in many scales, ranging from addition in food, biodegradable food packaging, pigment, selective delivery/separation, tissue-engineering, nanocomposites upon impregnation by polymers, and other medical and pharmaceutical applications.

1.7 Pretreatment of cellulosic fibres

Pretreatment is often used to decrease the energy consumption. If the energy consumption without any chemical pretreatment is 20 000–30 000 kWh/tonne by using a microfluidizer then with chemical treatment it can be as low as 1000 kWh/tonne (Siró and Plackett, 2010; Ankerfors, 2012).

One possible aim of pretreatment is the introduction of charged groups into the pulp fibres as it enhances delamination of the fibre walls. For example, through the introduction of carboxymethyl groups, a fully delaminated carboxymethylated MFC may be produced. Such groups should be in the form of their sodium salts to cause as much swelling of the pulp as possible. Swollen pulps have lower cell-wall cohesion than less swollen pulps and should, therefore, be easier to delaminate. Hence, holocellulose

pulps, which contain anionic polysaccharides, are very easy to delaminate (Klemm *et al.*, 2011).

Chemical pretreatments include TEMPO-oxidation, enzymatic treatment and carboxymethylation. In all those treatments, the charge density on the fibre surface increases and charge repulsion leads to a decrease in the fibre-fibre friction, and therefore, there is less flocculation and clogging in subsequent mechanical treatment (Henriksson *et al.*, 2007; Lavoine *et al.*, 2012).

Acidic or basic treatment of pulp is traditionally used to extract hemicelluloses, lignin and other impurities from cellulose pulp. Such treatment can give a beneficial effect to pulp disintegration, even though it also partially dissolves cellulose (Nechporchuk *et al.*, 2016).

Another option is alkaline pretreatment, as it is well known for its efficiency in the delignification and is relatively more environmentally friendly than most of the other bleaching methods. Alkaline treatment can be done in different ways; one option is to use, *e.g.*, sodium hydroxide with elevated temperatures, another option is to use lowered temperatures. Cai *et al.* used lowered temperatures and dissolved cellulose completely in 7 % NaOH / 12 % urea aqueous solutions at $-10\text{ }^{\circ}\text{C}$ in 2 min, but those chemicals in milder conditions (less concentrated alkaline solution, higher temperature and shorter treatment time) might be used just for pretreatment for nanocellulose production (Cai *et al.*, 2015). However, this treatment might change the crystalline structure of the cellulose (Gupta *et al.*, 2013). Paper III includes a description of alkaline cold treatment with a urea cellulose treatment and its suitability as a pretreatment.

Complete lignin removal is impossible to obtain with solely alkaline pretreatment due to the recalcitrant structure of lignin attached to the holocellulose matrix.

1.8 The importance of drying

To preserve the nanocellulose web-like structure, the drying technique is especially important. Numerous different methods for water removing are possible, including vacuum drying, freeze-drying and supercritical drying. In freeze drying, the aqueous gel is frozen, *e.g.*, by liquid nitrogen or liquid propane and is then kept in the vacuum oven for drying. In vacuum drying, the sample is dried by sublimation directly by the vacuum. In supercritical drying, water in aqueous gel is exchanged with organic solvent first, and then the sample is transferred in a supercritical dryer with CO_2 as the co-solvent (Jin *et al.*, 2008).

1.9 Supercritical carbon dioxide treatment of cellulosic fibres

Supercritical carbon dioxide (scCO_2) treatment can be viewed as one of the most environmentally friendly wood treatments. The idea is to carry out all types of chemical synthesis, processing, usage and disposal without using any hazardous substances. The low values of its critical temperature ($31.1\text{ }^{\circ}\text{C}$) and pressure (73.8 bar) allows for easy control of processes involving scCO_2 , and the processes are energy efficient, as the establishing of supercritical conditions requires little energy; this is in good agreement with green chemistry principle of energy conservation. In addition, it is in good agreement with green chemistry principles because of the non-toxic nature of scCO_2 . Additionally, scCO_2 is non-flammable, chemically inert and inexpensive.

Carbon dioxide under supercritical conditions is commonly used for delignification of wood (Li *et al.*, 1988). Treatments with fluids are necessary at high pressure to permit

the reaction of the solvent mixture with lignin present in the wood. Organic solvents (acetic acid, ethanol, methanol) are often used as co-solvents (Pasquini *et al.*, 2005).

ScCO₂ treatment as a helpful process for fibre cellulose fibrillation is studied in Paper I and Paper II.

1.10 The importance of surface charge

Wood fibres have a negative charge at all pH values due to the presence of acidic groups (carboxyl, sulphonic acid, phenolic or hydroxyl), which can be either surface or bulk charges depending on their location. Charged groups are either a natural part of cell wall constituents or come during the pulping or bleaching of fibres. The negative surface charge of BCTMP is most likely due to carboxylic groups, as sulphonic groups are not expected to be present. The number of charged groups depends on the origin of the and on the chemical and mechanical treatment during the pulping and refining (Horvath *et al.*, 2006).

The surface chemistry of MFC is especially important as it also involves pre- and/or post-treatments. Different methods have been used to measure the charge of cellulosic fibres, including conductometric titration, colloidal titration, potentiometric titration and polyelectrolyte titration (Lavoine *et al.*, 2012; Mocchiutti *et al.*, 2007).

The surface charge importance was discussed in Paper I and it was successfully measured by using the titration technique in Paper I and Paper IV.

1.11 Hydroxyapatite and its precipitation on cellulose

Hydroxyapatite (HAp), Ca₁₀(PO₄)₆(OH)₂, is an important inorganic material in biology and chemistry as it is the major component of tooth enamel and bone mineral. That is why it has found applications in bone tissue engineering as well as in drug and gene delivery.

Among the numerous methods for apatite synthesis (Nayak *et al.*, 2010; Gomez-Morales *et al.*, 2013; Eslami *et al.*, 2008), the most popular and widely researched technique is wet precipitation because of its simplicity and cost. However, the composition, physiochemical properties, crystal size and morphology of synthetic apatites are extremely sensitive to preparative conditions; The impurities such as calcium phosphate compounds such as amorphous calcium phosphates (ACP), octacalcium phosphate, and calciumhydrogenphosphate dehydrate, might also occur (Nayak *et al.*, 2010; Santos *et al.*, 2004; Markovic *et al.*, 2004).

Cellulose-HAp composites have great potential for application in tissue engineering or bone regeneration. Cellulose and apatite mixtures have also been used for making scaffolds (Müller *et al.*, 2006). Biomedical applications of HAp covered cellulose often use bacterial cellulose (Nge *et al.*, 2006; Zimmermann *et al.*, 2011) or modified celluloses (Petrauskaite *et al.*, 2013) and bio-mimetic conditions for precipitations (Nge *et al.*, 2006; Qu *et al.*, 2012).

The concept of apatite formation on these bioactive materials is generally twofold, as follows: the existence of the surface functional groups (hydroxyl and carboxyl groups in this study) that induce the heterogeneous nucleation of apatite, and the increased supersaturation of the surrounding fluid that accelerates the nucleation process and growth (Nge *et al.*, 2007).

Paper IV gives an example showing how BCTMP can be refined towards nanostructures and used further as a support to hydroxyapatite.

1.12 Summary of the literature review and the objectives of the thesis

Bleached chemithermomechanical pulp (BCTMP) is a useful material for higher grade paper manufacturing. However, by applying further treatments, it is possible to obtain new potential applications for this industrial pulp. Regarding BCTMP, almost all lignin is still on the surface of wood fibres, which hinders the inter-fibre bonding and bonding to additives. During mechanical pulping, the outer cell wall layers have not been removed and lignin is still on the surface of wood fibres, mostly in the most lignin-rich areas in wood fibre of the ML region and P. These fibres are hydrophobic because lignin is hydrophobic. If the outer cell wall layers are removed (ML, P and S1 layer), the S2 layer becomes visible with nice cellulose microfibrils oriented more or less parallel to the fibre axis. This S2 layer has the highest content of cellulose and is therefore hydrophilic.

As a rule, kraft pulp (almost pure cellulose) is used for nanocellulose preparation. The morphology of kraft pulp fibres differs completely from mechanical pulp fibres. On the surface of the kraft pulp fibre, the S2 layer is already visible. Conversely, for mechanical pulp, the S2 layer is hidden under outer cell wall layers. For nanocellulose preparation, it is, therefore, more convenient to use kraft pulp. The major disadvantage of the usage of kraft pulp is that a heavy chemical treatment has been used and the yield of pulp is much lower compared to the mechanical pulp. Therefore, to make a far more environmentally friendly product, BCTMP has been examined in this doctoral work.

For cellulose fibrillation, two sequent processes are usually needed: chemical pretreatment to make fibres softer and then mechanical treatment to provoke fibrillation. Cellulosic fibres are often pre-treated with different chemicals, such as NaClO_2 , NaBr, NaOH and TEMPO. Pretreatments also include treatments with acidic, enzymatic or ionic liquids and organosolvents. However, these treatments are quite expensive and are not very environmentally friendly. One possible chemical pretreatment is cold alkaline treatment by using an NaOH, KOH and urea aqueous solution, which has been recently introduced as one possible dissolution method for cellulose (Cai *et al.*, 2005). It is an effective, economic and environmentally friendly method. Cai *et al.* dissolved cellulose completely in a 7 % NaOH / 12 % urea aqueous solutions at $-10\text{ }^\circ\text{C}$ in 2 min, but the chemicals in milder conditions might be used just for pretreatment for nanocellulose preparation (Cai *et al.*, 2015). The mechanical treatment is also done usually by using sophisticated and expensive apparatus, *e.g.*, microfluidizer. However, it could be replaced by a simpler and less energy consuming apparatus, *e.g.*, a vibration mill. Supercritical CO_2 (sc CO_2) has also been used for delignification of wood (Li *et al.*, 1988), but it might be helpful for peeling off the outer cell wall layers and fibrillation in an environmentally friendly way.

Therefore, the overall objective of this study is to provoke structural changes in BCTMP to release the most cellulose-abundant layer, S2 from aspen wood fibre by using different environmentally friendly and simple treatments. Therefore, various treatments are tested to find the most suitable alternative.

Thus, the aims of this doctoral work are:

- (i) - to determine how sc CO_2 extraction together with mild chemical and mechanical treatment influences the surface charge and structure of aspen BCTMP fibres, especially peeling of the outer cell wall layers and fibrillation;
- (ii)- to produce MFC by using alkaline cold treatment and vibration milling;

(iii) - to test the suitability of the BCTMP for a support of chemical modifications;

(iv) - to synthesize apatite on the surface of our own produced MFC from the BCTMP of aspen.

The activities related to abovementioned objectives are as follows:

- performing fibrillation of aspen BCTMP by using mild chemomechanical treatment with scCO₂ treatment,
- performing fibrillation of the pulp by using (scCO₂) treatment with and without co-solvents,
- using alkaline cold pretreatment and vibration milling to obtain MFC,
- precipitation of hydroxyapatite on the surface of MFC fibres and find the optimal dose for covering the MFC,
- investigation the morphology of fibres by using optical (OM) and scanning electron microscopes (SEM),
- studying the structure of the obtained MFC by X-ray diffraction (XRD) measurements,
- establishing the relationship between the surface morphology and surface charge (area) of the produced MFC.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Starting material

The starting material for all the experiments was the BCTMP of aspen from Estonian Cell Company. Oven-dried aspen BCTMP was used for the scCO₂ treatments (Paper I and Paper II) and never-dried aspen BCTMP was used for the alkaline cold treatments (Paper III and Paper IV). Treatment scheme is presented in Figure 7.

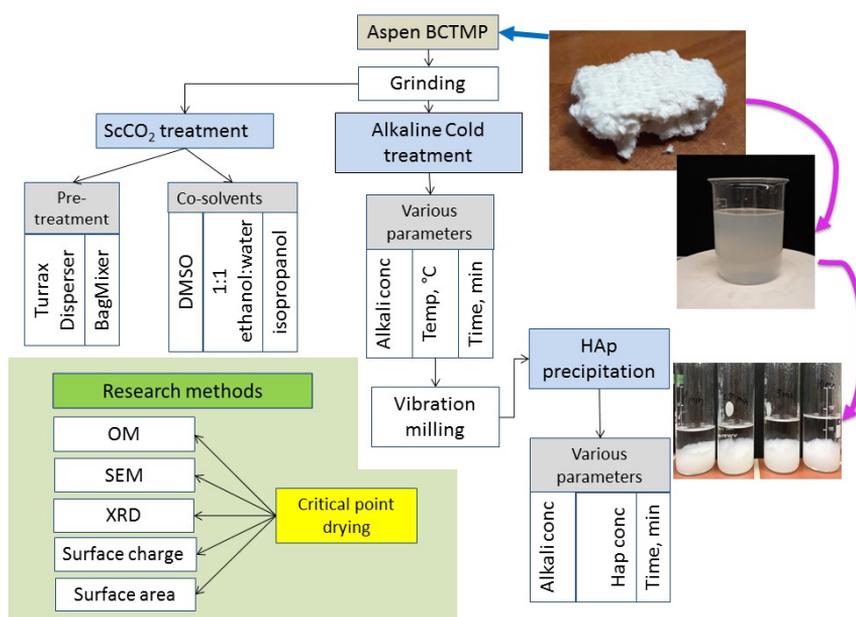


Figure 7. Treatment scheme.

2.1.2 Chemicals

NaOH, KOH, analytical grade urea and HCl were supplied from Sigma Aldrich and were used for chemical treatment of BCTMP. For the surface charge measurements, polydiallyldimethylammoniumchloride (p-DADMAC) was used as the cationic polyelectrolyte and potassium hydroxide (KOH) as titrant. P-DADMAC was supplied by Sigma Aldrich as a 20 wt% concentrate. Molar weight (Mw) of p-DADMAC was 100000–200000 g/mol. Commercially available technical grade acetone with 99.5 % purity from AP Chemicals was used in the solvent exchange process. For staining reactions, Direct Blue 1 and Direct orange 15 were supplied by Sigma Aldrich. For HAp precipitation, Ca(NO₃)₂, H₃PO₄, N₂ and He were used, which were of laboratory grade. Ethanol (96.6 % vol) and acetone (95.5 % vol) were of laboratory grade and were used without further purification. Commercially available technical grade CO₂ with a purity of 99.7 % from AGA was used in the supercritical CO₂ drying process. Distilled water was readily used from the laboratory's own distilled water system.

2.2 Methods

An outline of the various treatments and investigation methods is presented in Table 1 and the main investigation methods (SEM, OM) are explained in more detail below.

Table 1. Investigation methods

Pulp treatment	Analysing method	Paper
scCO ₂ treatment with co-solvents, mechanical treatment, chemical treatment	OM, SEM, surface charge measurement	Paper I
scCO ₂ treatment with co-solvents	OM, SEM	Paper II
Alkaline cold treatment, vibration milling	OM, SEM	Paper III
Alkaline cold treatment, vibration milling, Hap precipitation	OM, SEM, surface charge and surface area measurements, XRD	Paper IV

2.2.1 Experimental apparatus and procedures

Fibrillation of bleached chemithermomechanical pulp by using supercritical CO₂ treatment

To open the S2 layer of the wood cell, mild chemo-mechanical treatment with the supercritical carbon dioxide (scCO₂) treatment was used in Paper I due to the fact that the scCO₂ treatment can be viewed as one of the most environmentally friendly wood treatment methods. This was used in order to fibrillate aspen pulp fibres without using any hazardous substances.

A commercially available disperser, T 25-digit Ultra Turrax (Figure 10) from IKA laboratory equipment was used as part of the mechanical treatment process of the cellulosic fibres. Figure 11 shows the working principle of the Ultra Turrax exchangeable nozzle. The device produces strong shear- and thrust forces with high acceleration acting on the medium. The operating speed (3400–25000) of the device automatically draws the dispersed medium axially through the slits in the rotor/stator arrangement. The spacing between the rotor and the stator of the exact nozzle used was 1 mm. For dispersing, 0.5 wt% of pulp dispersions were used.

Commercially available BagMixer 400W from Interscience was used for the homogenization and mechanical treatment process of the cellulose fibres. For the BagMixer, a 0.5 wt% pulp dispersion was used. The working mechanisms of the Ultra Turrax and the BagMixer are described in more detail in Paper I.

A supercritical CO₂ experimental extraction system SES-UK 1, (Figure 8) was built up to carry out the scCO₂ extraction experiments. The reactor system and procedures are thoroughly described in Paper I and Paper II.



Figure 8. Experimental device for $scCO_2$ extraction (Detailed description in Paper I and II).

Fibrillation of bleached chemithermomechanical pulp by using alkaline cold treatment and vibration milling

In Paper III, the aim was to determine the optimal conditions for BCTMP fibrillation by using lowered temperatures with alkaline and urea mixtures. A full factorial model of the experiments was used to find the optimal conditions for fibrillation. For mechanical cold pretreatment, Ultra Turrax T25 (Figure 10 and Figure 11) was again used. The experiments are characterized in Paper III.

In paper IV, the best reaction conditions, which were determined in Paper III, were repeated. The conditions for cold treatment in Paper IV were therefore as follows: 7 wt% of alkali at $-3\text{ }^\circ\text{C}$ for 3 min. The BCTMP sample was cooled and stirred with Ultra Turrax T25 at 5000 rpm. Then, the sample was neutralized with hot ($70\text{ }^\circ\text{C}$ 3 wt%) HCl solution and washed with distilled water on the batiste material (Figure 9) to avoid the loss of very fine material.

Further mechanical treatment was done by using a vibration mill (VM, Figure 12) with different milling mediums such as water, acetone and ethanol. At this stage, it was expected to fibrillate the loosened cell wall layers and release the nanofibrillar network.

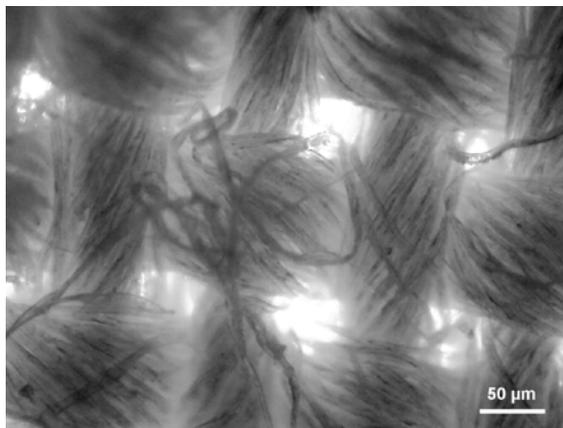


Figure 9. OM micrograph of the batiste material used as a sieve for material washing.



Figure 10. Cold pretreatment with Ultra Turrax T25 disperser.

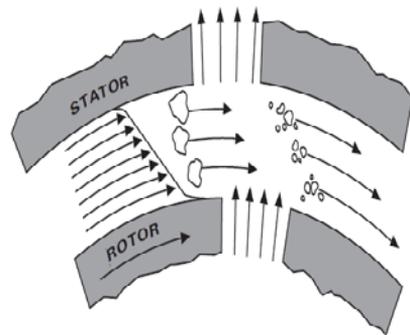


Figure 11. Working principle of the disperser unit (from the equipment manual).



Figure 12. Vibration mill closed (left) and opened (right) with 2 glass balls with diameters of 8 mm.

Hydroxyapatite precipitation

For the hydroxyapatite (HAp) precipitation, a Mettler Toledo T90 titrator was used as a pH controller and two Watson Marlow Sci 323 peristaltic pumps were used for the calcium nitrate and phosphoric acid additions. The HAp synthesis system can be seen in Figure 13.



Figure 13. HAp synthesis devices consisting of a pH controller, magnetic stirrer with temperature controller, two peristaltic pumps and two additional solvents.

Surface charge measurement

A Mettler Toledo T90 titrator was used for the surface charge measurements. It consists of a titrator with a terminal, pH board, integrated burette drive and magnetic stirrer. It is expandable with two sensor boards (pH and/or conductivity) and up to seven additional burette drives (for dosing and titration purposes), (see Figure 15). Surface charge measurement is more thoroughly described in Paper I. The method is also used in Paper IV.

Critical point drying

To preserve the fibres' original structure, critical point drying was used. Before critical point drying, samples of the water solution were transformed into acetone via the solvent exchange method by using acetone-water solutions (30, 50, 70, 90, 100, 100 wt% of acetone).

The critical point drying was performed in a commercially available Balzers CPD 030 device (Figure 14), where liquid CO₂ is used as the drying medium because of its low critical point values for temperature and pressure. For drying, a sample in acetone was placed in the chamber which was cooled to 5–7 °C to ensure liquid CO₂, sealed, pressured from the CO₂ and flushed with liquid CO₂ 6–7 times. Finally, the chamber was heated to 40 °C, kept for 20 min and gassed out.



Figure 14. Balzers CPD 030 critical point dryer.



Figure 15. Mettler Toledo T90 titrator for surface charge measurements.

SEM, OM, XRD, surface area measurements.

To investigate fibre's morphology, the main study was performed by using scanning electron microscope (SEM). The SEM studies were carried out with Zeiss EVO MA 15 at an accelerating voltage 12 to 15 keV. The samples were attached with the double adhesive tape to the stub and coated with the Ag/Pd conductive layer with the Fine Coat Ion Sutter JFC-1100.

For viewing the fibres after each treatment step and the examination of external and internal fibrillation (Simons' stain procedure) the FX version of the Nikon optical microscope was used in transmitted light.

To see, if the alkaline pretreatment or HAp precipitation in alkaline environment changed the original crystalline form of the cellulose, an x-ray diffractometer was used. For the X-ray diffraction (XRD) patterns of cellulose and HAp Rigaku, an Ultima IV

diffractometer was used with monochromatic Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$, 40 kV at 40 mA). Data were gathered in the 2θ range of 10° – 50° with scan speed of $2^\circ/\text{min}$ and scan step 0.02° using the silicon strip detector D/teX Ultra. The X-ray diffractograms were analysed using the software on the Rigaku system (PDXL 1.4.0.3).

For the surface area measurements, a sorptometer Kelvin 1042 built by Costech International was used.

Staining procedure

To investigate the internal fibrillation by using an optical microscope, Simons' stain was used. Two separate solutions, one consisting of 1 % direct blue 1 dye and the other of 0.2 % HMW (>25,000) direct orange 15 dye, were prepared. The solutions were mixed at a ratio of 1:1. Eight drops of the mixed dye solution were applied to the fibres on a slide. The slide was dried at 75°C , washed with distilled water, and examined in transmitted light.

3 DEVELOPMENT OF ASPEN BLEACHED CHEMITHERMO-MECHANICAL PULP TOWARDS NANOSTRUCTURES

As morphology is the crucial parameter to control during MFC preparation from wood pulp, the extent of fibrillation of BCTMP, as well as peeling effect of individual fibres, were mostly characterized by using scanning electron microscope (SEM).

The surface charge and surface area measurements were also very helpful, especially at the final stage, when apatite nanoparticles were precipitated on the surface of MFC fibres.

The structural changes caused by the mild chemical treatment and scCO₂ treatment are described in Paper I and Paper II. The structural changes towards microfibrillated cellulose by using alkaline cold treatment and vibration milling are described in Paper III and Paper IV. In Paper IV, the precipitation of hydroxyapatite on the surface of our final product was carried out to test the applicability of this fibrillated product for further modifications.

3.1 Study of bleached chemithermomechanical pulp fibres, the starting material

A SEM micrograph of the starting material of our experiments can be seen in Figure 16 and Figure 17. On the overview image, it can be seen that there are single fibres as well as fibre bundles. Among the other objects, the most predominant are fibres with CML and pieces of CML remnants, which appear smooth and patch-like (Lie *et al.*, 2006). The surfaces of the mechanical pulp fibres are very heterogeneous. There is also only a small amount of the cellulose micro-fibrillar structure visible. The main components as fibres, fibre fragments, fines and non-fibrillar material can be seen in Figure 16. Figure 17 shows the single BCTMP fibre of the starting material. It is a typical mechanical pulp fibre, where all wood fibre layers are present. As the CML is the outermost layer, only this layer can be seen. It is recognizable by its rectangular shape. On the surface of BCTMP fibres, a large piece of non-fibrillar lignin-rich patches can be seen. It has been shown that those pieces belong to the CML (Hafrén *et al.*, 2005; Li *et al.*, 2010; Li *et al.*, 2006). During pulping, the fibre separation occurs mainly in the CML region. This is evident because after mechanical pulping, the outer cell wall layers (ML and P) are still on the surface of the wood fibres. It can be seen that outer cell wall layers are unbroken, no cracks can be seen in the CML, and the layers are strongly attached to the fibre main body. There is almost no surface fibrillation visible in the starting material, and very little amount of microfibrils (MFs) can be seen as a result of the refining process during the BCTMP preparation.

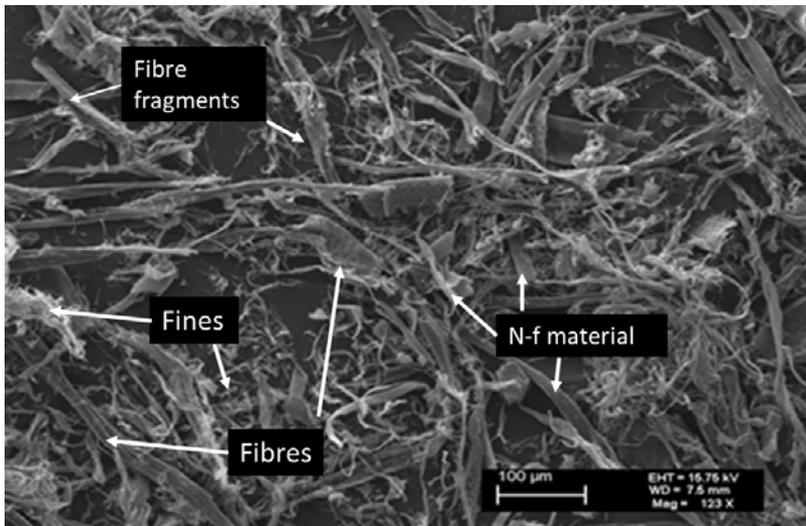


Figure 16. SEM micrograph of the overview image of the dried and ground BCTMP, showing fibres, fibre fragments, non-fibrillar (N-f) material and fines.

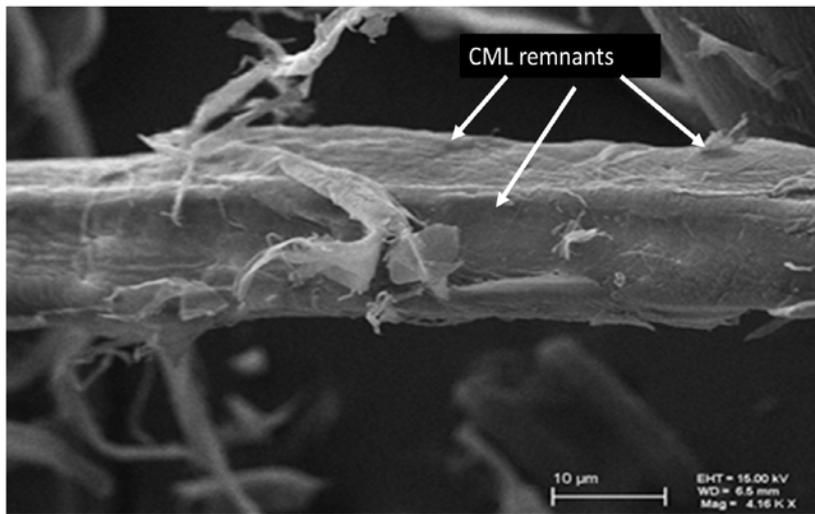


Figure 17. SEM micrograph of the initial sample, BCTMP fibre, covered with remnants of compound middle lamella (CML).

3.2 Fibrillation of bleached chemithermomechanical pulp by using supercritical CO₂ treatment

The results of the scCO₂ treatment and the influence of different co-solvents is described in detail in Paper I and Paper II.

The effective result of the scCO₂ treated fibre is shown in Figure 18. As seen, the outer cell wall layers CML are broken and start to peel off from the fibre body. Additionally, the cellulose fibres are hairier and fibrillated, especially scCO₂ treatments

with different co-solvents, which shows that scCO₂ has influenced the fibres. During the scCO₂ treatment, extensive break up of fibres, as well as fibrillation, occurred.

In Paper I, it was also shown, that the surface charge is strongly influenced by the fibre's appearance; more fibrillated fibres cause a higher surface charge.

In Paper II, different co-solvents were tested to improve the fibrillation during the scCO₂ treatment. It was shown that ethanol: water 1:1 and isopropanol are rather effective for fibre fibrillation (see Figure 19 to Figure 21). It was also shown that the addition of a co-solvent enhances the removal of the P and S1 layers, as well as the fibrillation of S2 layer, which might be due to the removal of some lignin as the co-solvents react with the lignin (Pasquini *et al.*, 2005). As it can be seen, there are numerous cellulose microfibrils (MF) visible after scCO₂ treatment with isopropanol (see Figure 19). These MFs are also the proof of S2 layer and removal of CML as no MFs can be seen in other layers (Hafrén *et al.*, 2005). Pure S2 layer with parallel oriented cellulose MFs are also easily recognizable in Figure 20. After a very effective scCO₂ treatment with 1:1 ethanol: water co-solvent even nanofibers (NF) appeared, see Figure 21.

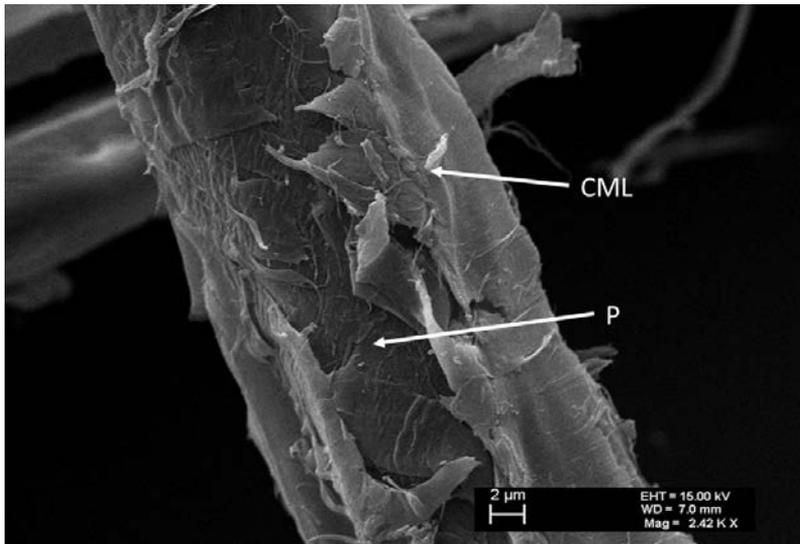


Figure 18. SEM micrograph of the peeling effect showing compound middle lamella (CML) and primary wall (P) after the scCO₂ treatment at 150 °C, 2900 psi, 1 h.

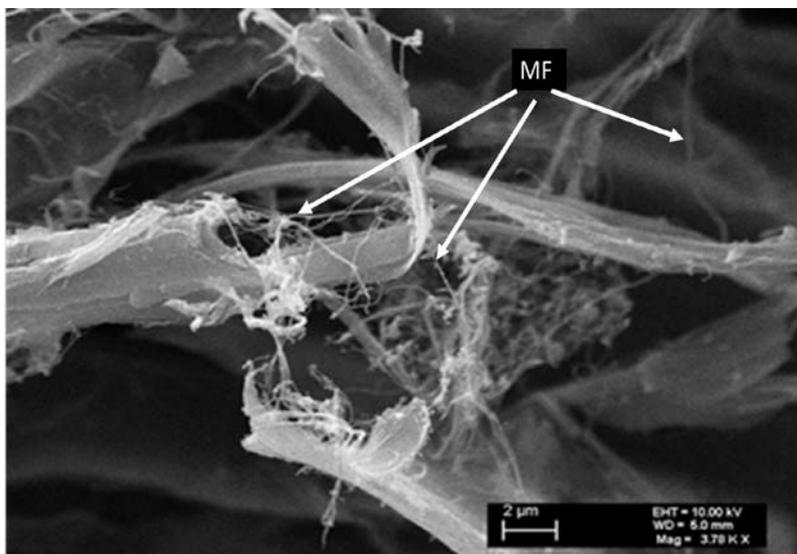


Figure 19. SEM micrograph of the cellulose microfibrils (MF) after the $scCO_2$ treatment with isopropanol as the co-solvent at 150 °C, 2900 psi, 1 h.

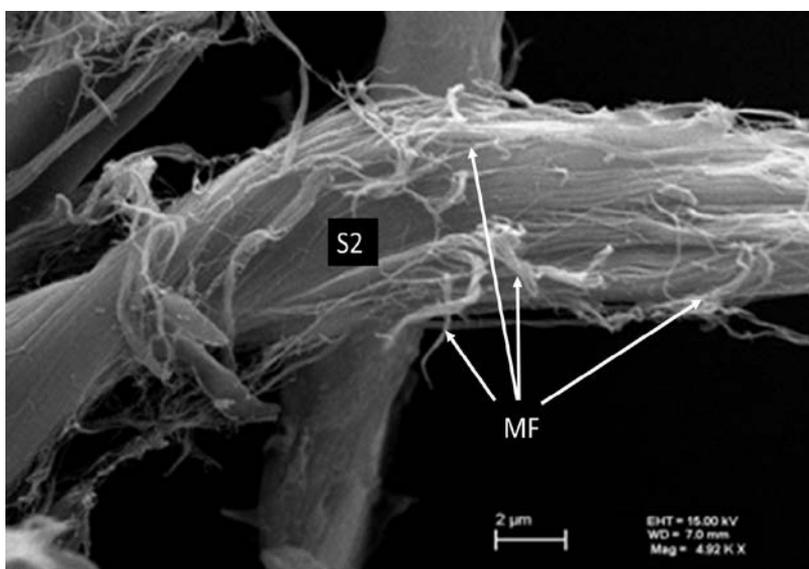


Figure 20. SEM micrograph of cellulose microfibrils (MF) and S2 layer after the $scCO_2$ treatment with 1:1 ethanol: water co-solvent at 150 °C, 2900 psi, 1 h.

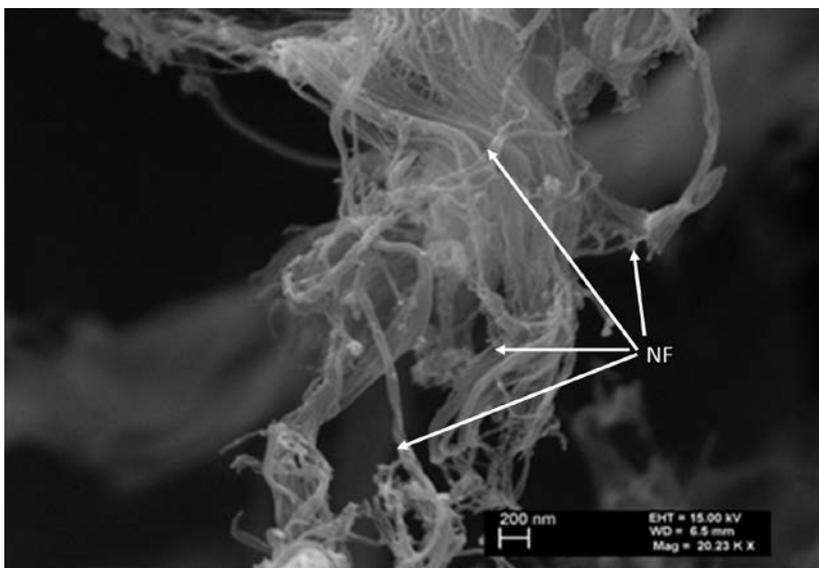


Figure 21. SEM micrograph showing nanofibers (NF) after the $scCO_2$ treatment with 1: 1 ethanol: water co-solvent, at 150 °C, 2900 psi, 1 h.

3.3 Fibrillation of bleached chemithermomechanical pulp by using cold pretreatment with vibration milling

3.3.1 Step 1: chemical pretreatment

Different chemical pretreatments were performed (mild, heavy, optimal) in Paper III to determine the best potential for further mechanical treatment. The most effective pretreatment was to treat the material for a short time with a strong alkali at a low temperature (7 wt% of alkali at -3 °C for 3 min). The effective result of the chemical pretreatment is shown in Figure 24 and Figure 25 where the S2 layer is clearly visible with parallel oriented cellulose MFs.

3.3.2 Step 2: vibration milling after chemical pretreatment

The effectiveness and suitability of the chemical pretreatment were further tested with vibration milling (VM) with different environments in Paper III.

Figure 22 shows the 3 basic stages of microfibrillated cellulose (MFC) preparation. On the left is the untreated BCTMP of aspen, on the right is the sample after optimal chemical pretreatment and at the bottom, is the sample after VM treatment.

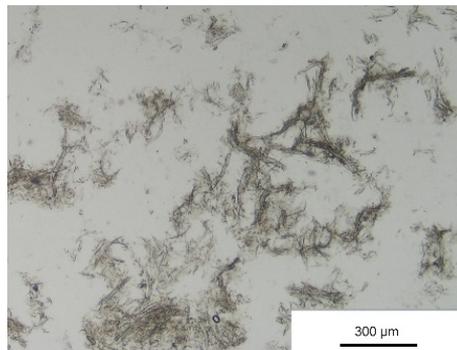
As it is seen in Figure 22, after cold pretreatment, the fibres turned softer and were swollen, but the fibres basically maintained their full volume. The vibrator milling caused large fibres to disappear. As it was shown in Paper III via SEM images, VM resulted in the appearance of MFs and microfibrillar bundles, the bulk came more uniform and the S2 layer of the cell wall was opened and fibrillated. The obtained MFC after chemical and mechanical treatment is seen in Figure 23.



BCTMP.



BCTMP after chemical treatment.



BCTMP after chemical and mechanical treatment.

Figure 22. Optical microscope images of the steps from pulp to MFC.

3.4 Microfibrillated cellulose, fibrils, lamellae

A typical obtained MFC is shown in Figure 23. There are cellulose MFs, as well as fine material (FM) and larger particles (LP). As can be seen, the whole material is greatly expanded in surface area. Although MFC mostly consists of microfibrils, it still has material that might be inhomogeneous, containing, *e.g.*, fibres, fibre fragments, fines and fibrils. The MFC is not necessarily fully a nano-material, but it contains nano-structures, *i.e.*, nanofibrils. It agrees with previous studies (Chinga-Carrasco, 2011) that also find the same result even though MFC is obtained by other methods. Of course, the final structure greatly depends on the treatment time, amount of sample in the VM container and the amount of liquid. At the same time, it should be mentioned that if the treatment time in the VM is too long, it might cause shortening of the fibres and fibrils and a gluing effect sometimes occurs rather than a separation effect.

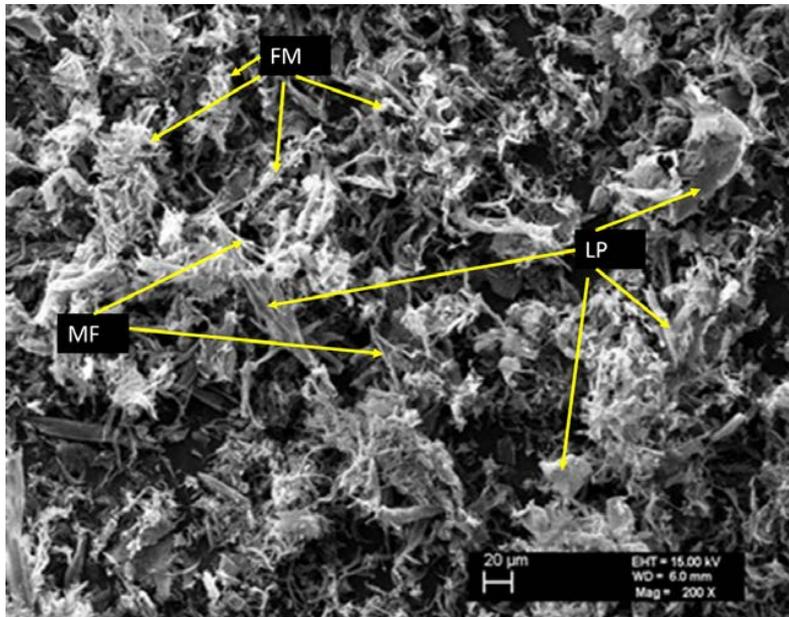


Figure 23. SEM micrograph of MFC after chemical and mechanical treatment showing fine material (FM) and microfibrils (MF) as well as larger particles (LP), e.g., fibres and fibre fragments.

The pure S2 cell wall layer with untangled microfibrils is visible in Figure 24 –Figure 25 indicated with the white arrows. As can be seen, MFs have very high aspect ratios. This amazing result is gained only by using the alkaline cold treatment ($-3\text{ }^{\circ}\text{C}$, 7 wt% of alkali 3 min) (Paper III).

The interesting result of chemical treatment as lamellae is seen in Figure 26 indicated with the white arrow. They are also typical structure elements of the S2 layer, composing of several microfibrils attached side by side, forming thin but wide communities. Lamellae were very typical after mild alkaline cold treatment ($-3\text{ }^{\circ}\text{C}$, 3 wt% of alkali and 7 min of treatment) (Paper III).

A combination where MFs and lamellae are both present in the same micrograph can be seen in Figure 27. MFs are indicated with the white arrows and lamellae with the yellow arrows. This result appeared after treatment in $-3\text{ }^{\circ}\text{C}$, 7 wt% of alkali 3 min and vibration milling (Paper III).

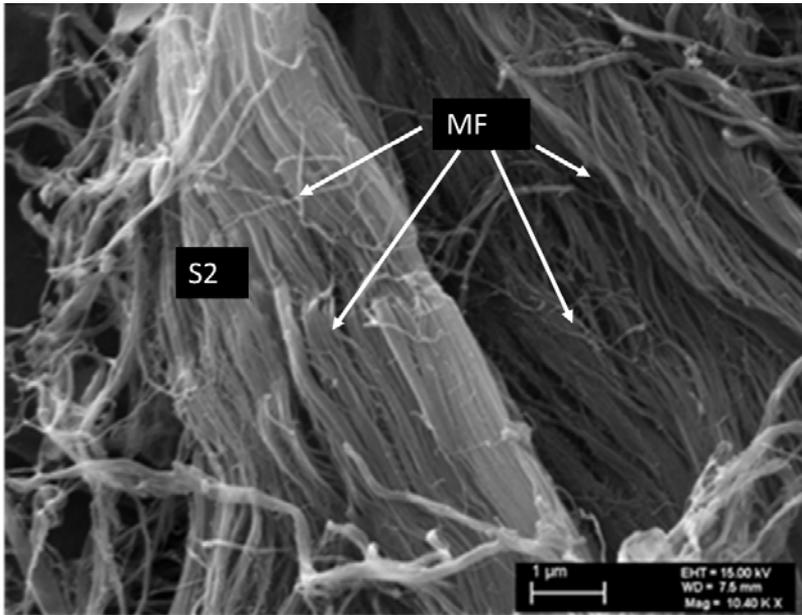


Figure 24. SEM micrograph of the S2 layer with parallel oriented cellulose microfibrils (MF) after the alkaline cold pretreatment.

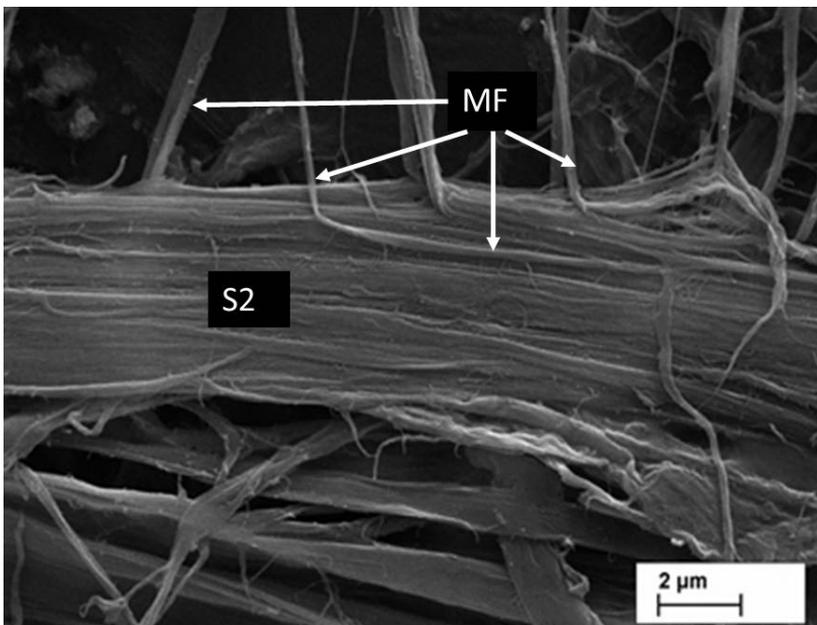


Figure 25. SEM micrograph of microfibrils (MF) after the alkaline cold pretreatment.

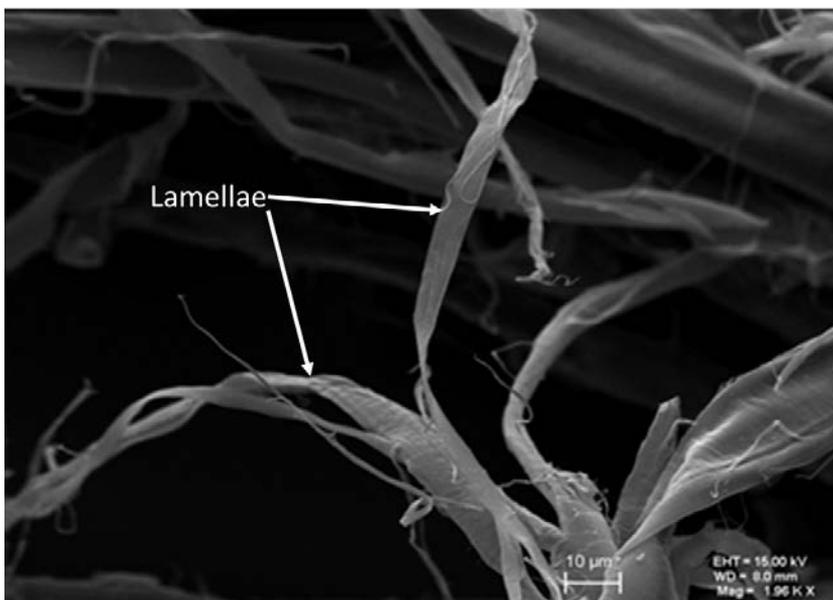


Figure 26. SEM micrograph of lamellae after the mild alkaline cold treatment (Paper III).

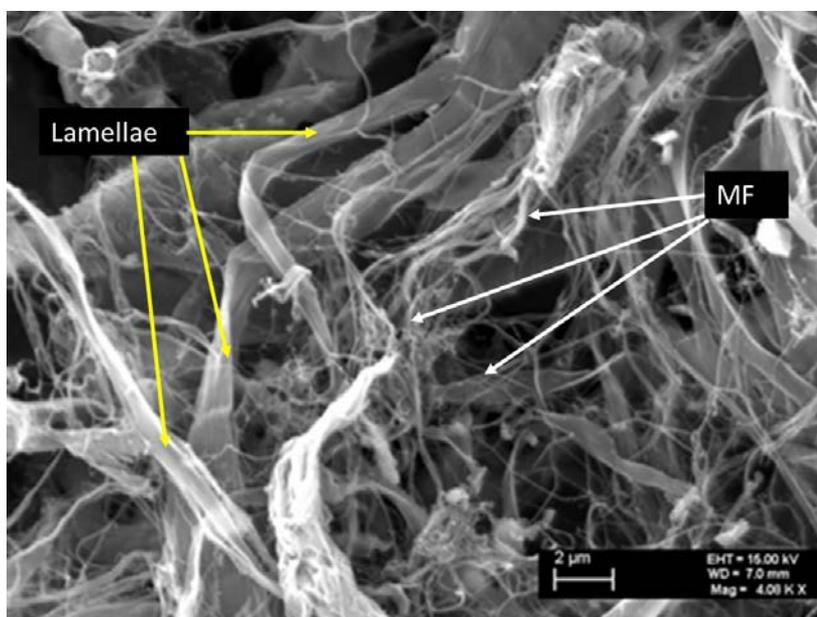


Figure 27. SEM micrograph of microfibrils (white arrow) and lamellae (yellow arrow) after the alkaline cold pretreatment (7 wt% of alkali, -3°C , 3 min) and vibration milling.

3.5 Internal and external fibrillation

The internal fibrillation is shown in Figure 28 where the right-hand picture is obtained by using Simons' stain. Simons' stain shows the fibrillated or damaged parts as orange and the non-fibrillated parts as blue. The external fibrillation is shown in Figure 29. As orange dye has HMW, it only penetrates the fibres, with a large pore sizes (Yu *et al.*, 1994). As seen from Figure 28 and Figure 29, the treatment has been quite effective, as the orange-coloured fraction is large.

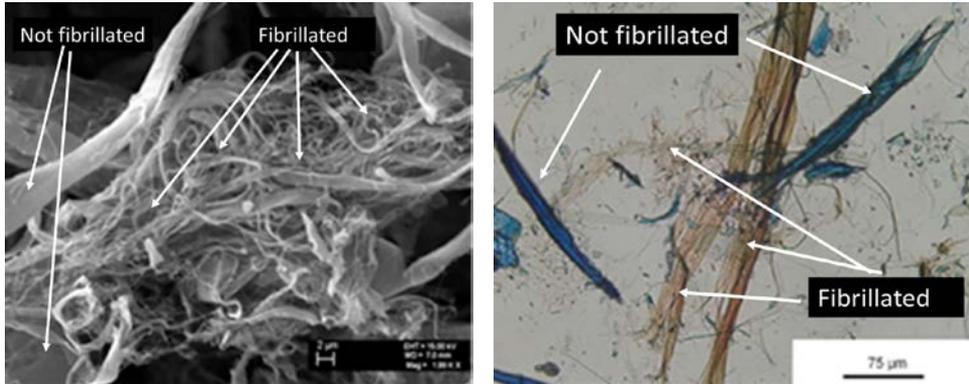


Figure 28. Internal fibrillation showed by SEM (left) and OM (right), where the fibrillated part is stained orange and the non- fibrillated part is stained blue.

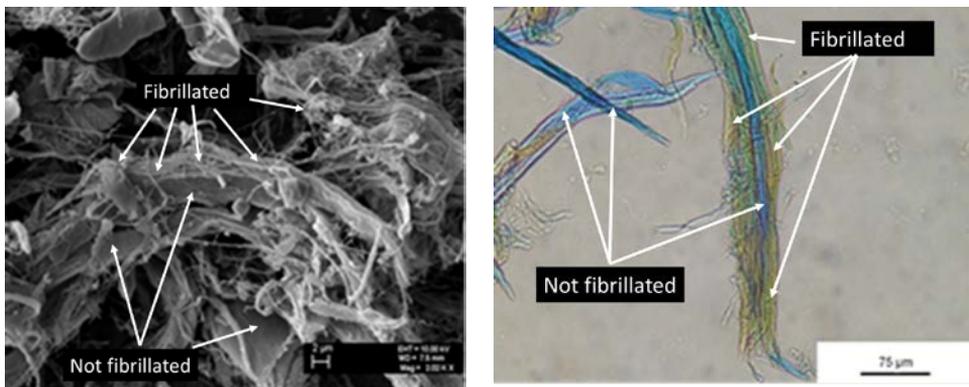


Figure 29. External fibrillation showed by SEM (left) and OM (right), where the fibrillated outer part of fibre is stained orange and the non- fibrillated internal part is stained blue.

3.6 Hydroxyapatite precipitation on the surface of microfibrillated cellulose

HAp was precipitated on the MFC samples during 1 min, 2.5 min, 5 min and 10 min in Paper IV, leading to concentrations of 0.64 wt%, 1.32 wt%, 3.4 wt% and 11.1 wt% of apatite, respectively.

The correlation between the surface charge and surface area with the HAp precipitation was also investigated in Paper IV.

The short-time HAp precipitation (2.5 min) is demonstrated in Figure 30. It can be seen that this precipitation time leads to formation of HAp crystallites and agglomerates which are quite regularly attached on the surface of MFC.

As was shown in Paper IV, after 10 min of HAp precipitation, the surface of MFC was totally covered with HAp, see Figure 31. It also correlated with the surface area, as surface area increased continuously after each step (chemical pretreatment and mechanical VM treatment) and even during the initial HAp precipitation as the sample became more “airy”. The surface area increased until the cellulose surface was covered with HAp, and then dropped sharply. As it was pointed out in Paper IV, this concentration can be considered also as optimal concentration for HAp precipitation as further precipitation would start to form several HAp layers on top of HAp instead of the MFC as there is not vacant area on the MFC. If HAp layers start so overlap, it is also likely that some HAp falls off from the surface (Paper IV).

The surface charge was also in connection with the sample’s appearance and constituencies. The surface charge increased incrementally when material became finer. The surface charge dropped sharply and even became negative after the HAp addition. It is in good agreement with previous studies that declare the connection between fines content and surface charge (Bhardwaj *et al.*, 2006; Banavath *et al.*, 2011; Horvath *et al.*, 2006).

In paper IV, it was shown that 10 min (11 %) of HAp precipitation is the optimal time for our MFC material to totally cover the sample. After this point, the HAp starts to attach itself to other HAp forming more layers.

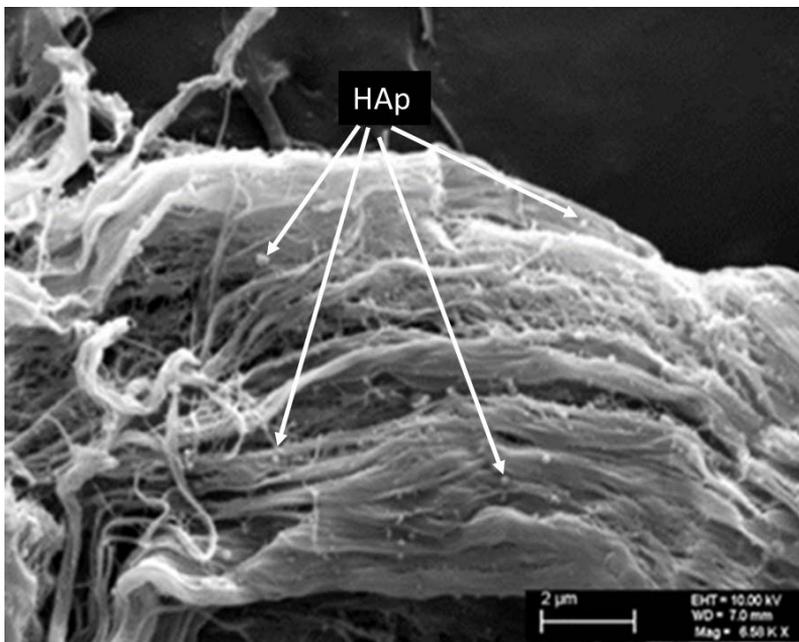


Figure 30. SEM micrograph of hydroxyapatite (HAp) crystallites precipitated on the surface of MFC for 2.5 min (1.32 %) (Paper IV).

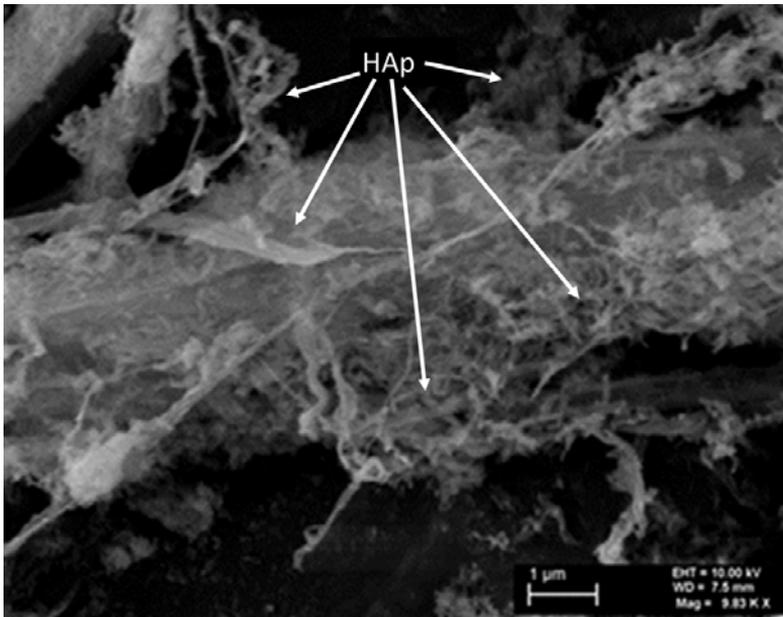


Figure 31. SEM micrograph of hydroxyapatite (HAp) precipitated on the surface of MFC for 10 min (11 %) (Paper IV).

3.7 Surface charge measurements

The surface charge density measurements were conducted by using the indirect polyelectrolytic titration technique to evaluate the effectiveness of different chemical, mechanical and supercritical treatments. As the method that was used for surface charge measurements is somewhat different from conventional polyelectrolyte titration methods, the numeric results are not suitable for comparisons with other earlier published works but are acceptable for comparing our samples with each other. Paper I and IV, it can be seen that the surface charge density is strongly connected with the fibres appearance. The surface charge density of the initial sample (BCTMP of aspen) is relatively low. The surface charge density increased after effective chemical, mechanical and scCO_2 treatments, as a more fine material was introduced to the fibre surfaces. It is in agreement with the previous studies that find the same connection (Bhardwaj *et al.*, 2006; Banavath *et al.*, 2011). Additionally, if the chemical treatment is not very effective and fines are removed via washing the sample after the treatment, the surface charge may even decrease compared with the initial sample. It is also in correlation with the SEM images, as those fibres show relatively smooth surfaces and the content of fines is even less than in the untreated BCTMP.

It can be seen in Paper IV that the surface charge density decreased after adding the apatite. Moreover, the charge turned negative and continued to decrease as the apatite concentration increased. This occurs because of the bonding of all available cellulose charged particles to the apatite structure and performing the synthesis in a basic environment ($\text{pH}=9$). Apatite itself has a negative charge when pH is above 7.

3.8 Surface area

The surface area determined by BET was measured for the cold treatment and vibration milling samples and during apatite synthesis in Paper IV.

As it was shown in Paper IV, the surface area was quite small for the initial sample ((8.62) m²/g), *i.e.*, the untreated BCTMP of aspen. However, it is somewhat larger than shown by Fu *et al.*, (2.63 m²/g, respectively) (Fu *et al.*, 2015). Surface area increased step by step during treatments and HAp precipitation until 5 min of HAp precipitation (3.4 % of HAp) to its maximum value 200.63 m²/g due to the additional surface between fibres and then dropped after 10 min of HAp precipitation as the cellulose surface was then totally covered with HAp (HAp content > 11 %).

3.9 Cellulose crystallinity

To see the changes in crystallinity during the alkaline pretreatment or HAp precipitation, in Paper IV diffraction analyses were performed.

The diffraction patterns of the unmodified MFC, with precipitated and pure HAp, are shown in Figure 32. As can be seen, the crystal form of cellulose did not change during the chemical treatments, mechanical treatments nor the HAp precipitation step, which it is probably because of the rather a mild chemical treatment of the fibres. Three peaks on curves a–f at 2 θ = 15.1, 16.7 and 22.5 deg confirmed that only cellulose I is present in the sample (Lu and Hsieh, 2010; Gupta *et al.*, 2013), and the basic environment of HAp synthesis did not change the crystalline structure of the sample. The fact that cellulose crystallinity did not change during treatments is a good sign and therefore the number of possible applications is larger.

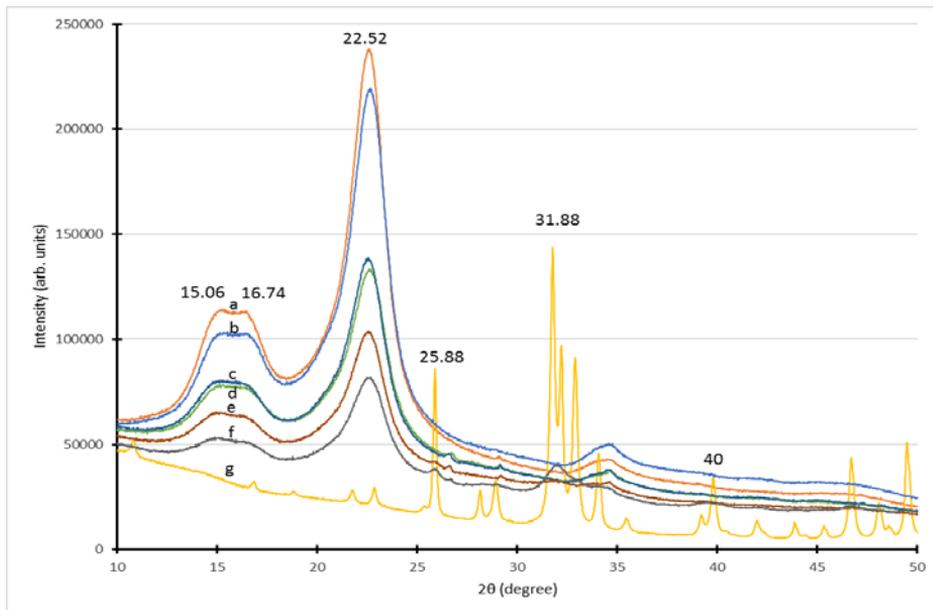


Figure 32. XRD of (a)-starting material BCTMP; (b)-BCTMP with chemical pretreatment; (c)-MFC; (d) 1min HAp precipitation; (e) 5min HAp precipitation; (f) 10 min HAp precipitation; (g) pure HAp.

3.10 Production yield, energy consumption and environmentally friendliness

According to the calculations, the yield by using alkaline cold treatment and vibration milling for getting this partly fibrillated cellulosic product from BCTMP as the starting material, is relatively high, *i.e.*, 65 %. Chen *et al.* obtained yields between 61 and 91 by producing highly crystalline nanocellulose from alpha cellulose (Chen *et al.*, 2017). However, these starting materials are very different.

If we consider that thermomechanical pulping itself gives very high yields (up to 95 %), then the whole process starting from wood to nanocellulose is rather effective. What is more, during supercritical CO₂ treatment there is almost no loss of material at all.

The approximate energy consumption during mechanical treatment was calculated per tonne and it was the same as for the treatment with the microfluidizer (Siró and Plackett, 2010). On the other hand, this comparison is not appropriate, as the mass of the sample in this study was very small, *e.g.*, the capacity of the vibration mill was only a 2 g sample. In scaling up for pilot or mass production level, the price per unit changes considerably.

The pretreatment step was certainly cheaper, as the chemicals used in this study, were much cheaper than conventionally used chemicals, *e.g.*, TEMPO or enzymes. Additionally, the pretreatment was very fast and therefore time- and energy saving.

Because of the fibrillar structure, the gained material might be more environmentally friendly and easier to handle than, *e.g.*, NCC with its needle-like structure.

CONCLUSIONS

The main goal of this study was a development of a simple, energy efficient and environmentally friendly method to produce microfibrillated cellulose (MFC) from bleached chemithermomechanical pulp (BCTMP) of aspen manufactured by Estonian Cell Company. MFC is conventionally prepared by using rather expensive and not environmentally friendly chemicals for pretreatment and sophisticated apparatus which require a lot of electrical energy for subsequent mechanical treatment.

Typically, nanocellulose is made from pure cellulose. In this work, preparation of MFC from aspen BCTMP in simple and environmentally friendly ways is reported such as supercritical CO₂ treatment and vibration milling. Exploited equipment is conventional and easy to use what makes this technology available for small businesses. MFC was obtained by using different chemical and mechanical methods, including alkaline treatment combined with mechanical treatment, and supercritical CO₂ treatment. This work demonstrated that the outer layer of wood fibre can be removed in several different ways. Although both methods were successful, alkaline cold treatment following by vibration milling was found to be the best method for MFC preparation as it is easier to scale up for pilot plant and industrial level.

Based on this work, the conclusions can be driven in more detail:

1. Supercritical CO₂ treatment was done with and without different co-solvents and some aid of mechanical treatment (Paper I and Paper II). All the methods led to peeling off the outer cell wall layers. This treatment was chosen because the fibrillation was created without using any hazardous chemicals.
 - a) By using this method, the best results in fibrillation were gained with a 1:1 ethanol: water co-solvent.
 - b) DMSO and urea did not produce fine fibrillation of fibres.
 - c) Best results for further fibrillation were obtained when mechanical and chemical treatments were applied after scCO₂ treatment.
2. The influence of various aqueous solutions of NaOH, KOH, urea and ethanol at lowered temperature were tested for pretreatment (Paper IV). This method was chosen due to its simplicity because of the short treatment time and because high pressures are not needed.
 - a) Very mild chemical treatment effectively released lamellae, but fine fibrillation was not present.
 - b) Heavy chemical treatment melted the fibre surface and no fibrils were present.
 - c) The best result was gained with a strong alkali (7 %) for a short time (3 min) at -3 °C.
3. The pretreatment was followed by vibration milling, which aimed to peel off the outer cell wall layers and to fibrillate the aspen wood fibre internally.
4. Finally, different amounts of HAp were successfully precipitated on the cellulose surface (Paper IV).

The scientific novelty of the work

1. In this work aspen BCTMP was used as a starting material for MFC preparation thereby adding the value to this mechanical pulp which is conventionally predominantly used in the paper industry.

2. Supercritical CO₂ treatment is considered as one of the most environmentally friendly methods for MFC preparation.
3. The experimental device for supercritical CO₂ was constructed at laboratory (Paper I and Paper II).
4. A completely new approach is the use of alkaline cold treatment as a pretreatment stage of MFC preparation.
5. In this work partially fibrillated MFC was prepared contrary to well-known completely fibrillated nanocellulose. This approach extends the utilization of produced MFC for various less sophisticated applications where partial fibrillation of the fibres is sufficient.

Future perspectives

1. Newly prepared fibrillated material has a great potential to be used in the applications where high surface charge/area is demanded such as a degradable template for growth of nanostructures of ZnO, TiO₂ and SiO₂ by using *e.g.*, sol-gel method. That possibility was demonstrated in this work where HAp crystallites were successfully precipitated on the surface of MFC.
2. Commercially interesting new products and applications (based on literature) could include food supplements (with HAp), biodegradable food packaging materials, antibacterial products with nanosilver, thin films and, water absorbing, retaining and releasing materials (hygiene products), composite materials for various industries, light materials with increased strength (building) *etc.*

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LÜHIKOKKUVÕTE

Haava pleegitatud keemilis-termilise puitmassi töötlemine nanostruktuuride saamiseks

Käesolevas doktoritöös uuriti võimalusi kuidas valmistada Eestis AS Estonian Cell' is toodetud haava pleegitatud keemilis-termilisest puidumassist (BCTMP) mikrofibrileeritud tselluloosi (MFC) võimalikult lihtsalt, soodsalt ja keskkonnasõbralikult; kirjeldati saadud materjali valmistamist, omadusi ja hinnati selle sobivust potentsiaalses rakenduses. Tavapäraselt toodetakse nanotselluloosi keemilisest puitmassist, mis on tselluloosi sisalduse mõttes küll puhtam, kuid mille saagis tootmisel on oluliselt väiksem kui näiteks mehaanilisel teel toodetud puitmass. Lisaks on kasutatud kemikaalide hulk tunduvalt suurem kui näiteks keemilis-termilise puitmassi tootmisel.

MFC saamiseks eemaldati haava BCTMP kiududelt ligniin, mida on rohkesti puidukiudude välimistes kihtides (P ja S1 kiht), ja kiudude vaheplaadis (CML). Erinevate tööstlustega avati puidukiu S2 kiht, kus tselluloosi sisaldus on suurem ja mikrofibrillide orientatsioon korrapärasem. Välimiste kihtide eemaldamiseks kasutati superkriitilise süsinikdioksiidiga (scCO₂) tööstlust koos kerge keemilis-mehaanilise mõjutamisega, keemilist tööstlust leeliselises keskkonnas madalatel temperatuuridel ja mehaanilist tööstlust vibratsioonveskis. Selleks, et ära kasutada puidukiudude peenestamisel tekkivaid pinnalaenguid ja neid siduda vastasmärgilist laengut omava ainega, sadestati MFC kiududele hüdroksüapatiidi (HAP) kristalliite.

Kiudude morfoloogiat uuriti skaneeriva elektronmikroskoobiga (SEM). Erinevates etappides mõõdeti pinnalaengut polüelektrolüütilise tiitrimise meetodil, eripinda BET meetodil ja tselluloosi kristallilisust röntgendifraktomeetria abil. Hinnati kiudude välimuse seost eripinna ja pinnalaenguga.

Antud töös leiti, et kerge mehaaniline tööstlus ja sellele järgnev scCO₂ tööstlus koos erinevate kaaslahustitega (etanol, isopropüülalkohol) aitas tugevalt kaasa CML, P ja S1 kihtide eemaldamisele. Originaalse tulemusena õnnestus täielikult eemaldada CML ja P kiht ja avada rakuseina S2 kiht oma iseloomulike tselluloosi mikrofibrillidega.

Külmatööstlus leeliselises keskkonnas koos edasise vibratsioonveski kasutamisega osutus efektiivseks vahendiks MFC saamiseks. NaOH, KOH ja karbamiidi kasutamine eeltööstlusena muutis haava puidukiud pehmemaks ja kergemini töödeldavaks, edasine mehaaniline tööstlus vibratsioonveskis aga eraldas peened MFC kiud. Töös selgitati välja optimaalsed parameetrid külmatööstluseks (aeg, temperatuur ja leelise kontsentratsioon), samamoodi sobiv proovi mass nii eeltööstluseks kui vibratsioonveskis tööstluseks, kuulide materjal ja läbimõõt, konteineri maht ja muud olulised parameetrid. Lisaks katsetati erinevaid keskkondi (vesi, etanol, piiritus) vibratsioonveskis tööstluse jaoks, mille tulemusel selgitati välja optimaalne töörežiim. Katseliselt leiti minimaalne HAP kihi paksus, mis kompenseeriks täielikult MFC kiudude pinnalaengut.

Uurimise tulemusel leiti, et nii pinnalaeng kui eripind on tihedalt seotud kiudude välimusega. Algproovil, kus peaaegu puudusid peened osakesed, oli pinnalaeng väike. Kiudude peenestusastme suurenemisel pinnalaeng suurenes. HAP-i lisamisel muutus pinnalaeng aga järsult negatiivseks, mis näitab HAP-i liiga lahuses. Töötlemata proovil oli eripind väike (12 m²/g), töödeldud peenestatud proovil aga tunduvalt suurem (201 m²/g). Kui puidukiudude negatiivsed laengud olid 11 % ulatuses ära kasutatud, siis edaspidine HAP

lisamine enam eripinda ei muutnud. Positiivseks võib lugeda fakti, et antud töötluste tulemusel tselluloosi kristallilisus ei muutunud.

Selliste omadustega materjal võib leida kasutust keskkonnasõbralikult, lihtsalt ja soodsalt valmistatava MFC-na näiteks isolatsioonmaterjalis või toidulisandites. Nende materjalide valmistamisel on oluline suur eripind ja kiudude naturaalne olemus.

ABSTRACT

Development of Aspen Bleached Chemithermomechanical Pulp Towards Nanostructures

Environmentally friendly materials and their (energy) efficient production as well as biodegradability are becoming more and more important. Therefore, cellulose as the most abundant natural polymer on Earth, is becoming more popular. To create new more valuable products of cellulose a lot of research has been focused to the development of nanocellulose. Nanocellulose means a family of various new products in which cellulose is in form of nano-scaled particles.

This study is devoted to the development of simple, energy efficient and environmentally friendly method to produce microfibrillated cellulose (MFC) from bleached chemithermomechanical pulp (BCTMP) of aspen manufactured by Estonian Cell Company. Applied processes and properties of obtained MFC were described in detail and suitability of certain application was investigated.

MFC is conventionally produced from kraft pulp which is chemically quite pure cellulose. However, it has lower yield and greater amount of chemicals used during pulping compared to mechanical pulp, *e.g.*, BCTMP.

On the surface of BCTMP fibres some of the wood lignin is retained in the remnants of (middle lamella) ML and in the primary wall (P). This blocks the release of fine fibrils from the inner layer (S2) of the fibre, even by aggressive mechanical treatment. Therefore, the first step towards preparation of finely fibrillated MFC was the removal of remnant lignin from fibres and thereby opening rich in cellulose S2 layer where orientation of microfibrils is more regular. For the removal of outer cell wall layers wide-ranging methods were used including mild chemomechanical treatment together with supercritical CO₂ (with or without different co-solvents), alkaline cold treatment with vibration milling in different environments.

Investigation of morphology of fibres was performed by using scanning electron microscope. Surface charge was measured in different stages of fibrillation by using polyelectrolytic titration method, surface area by using BET method and crystallinity by using X-ray diffraction analysis. The correlation between fibres' morphology and surface charge and surface area was detected.

In this research it was found that mild mechanical treatment and subsequent supercritical CO₂ treatment with different co-solvents (ethanol, isopropanol) promotes the detachment of outer layers of BCTMP fibres. The original result was obtained that remnants of ML, P and additionally S1 layer were removed completely from the BCTMP fibres bringing out S2 layer with its parallel oriented cellulose microfibrils.

The cold alkaline treatment followed by vibration mill treatment was very effective for producing MFC. NaOH, KOH and urea used as pretreatment turned fibres softer and swollen. Following vibration milling released successfully fine MFC fibrils. Optimal parameters (time, temperature and the concentration of alkali) as well as sample mass for pretreatment and vibration milling, material of balls and their diameter, volume of milling container and other important parameters were worked out. Additionally, different milling environments were tested and most suitable working regime was established.

To demonstrate possible application of the worked-out material, composite with hydroxyapatite (HAp) crystallites was produced utilizing abundant negative surface charges of fibres created by refining and fibrillation of fibres. The optimal thickness of

the HAp layer to compensate completely the negative surface charge of MFC was established experimentally.

Research results of our study showed that surface charge and surface area were both strongly connected to the appearance of microfibrils. For the initial sample of BCTMP where fine particles are rarely present, the surface charge was small. As fibrillation turned finer, surface charge increased. After the addition of HAp, surface charge became negative due to the excess of HAp in solution. For untreated sample, the surface area was small ($12 \text{ m}^2/\text{g}$), but it was considerably higher for milled sample ($201 \text{ m}^2/\text{g}$). After fibres' charges were compensated up to range of 11 %, the addition of more HAp did not change further the value of surface charge. During the treatment, the crystallinity of cellulose remained unchanged which was one of the important targets of this research.

Material with such properties could be used in applications what elaborate specific properties of MFC like the nanocellulose but substantially simpler, cheaper and friendlier to environment in production, *e.g.*, specific insulation materials or pharmaceuticals. In these materials the main considerations are utilization of high surface area and natural origin of produced MFC.

APPENDIX

Paper I

Kärner, K.; Elomaa, M.; Kallavus, U. (2014). Study of the effect of mechanical treatment and supercritical CO₂ extraction on aspen BCTMP by surface charge measurements and SEM. *Cellulose Chemistry and Technology*, 48:535–544.

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STUDY OF THE EFFECT OF MECHANICAL TREATMENT AND SUPERCRITICAL CO₂ EXTRACTION ON ASPEN BCTMP BY SURFACE CHARGE MEASUREMENTS AND SEM

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Cellulose is the most abundant organic polymer and is utilized in many forms in industry. Sustainable use of materials includes the utilization of renewable resources like cellulose and selecting methods that allow saving energy and avoiding pollution. Because a complete purification of cellulose demands a lot of energy and chemicals, there are many products in which cellulose is used only partly purified. For example, products like bleached chemi-thermo mechanical pulp (BCTMP) have been developed to be used as a component in paper making. In this article an attempt to further refine aspen BCTMP is reported. The aim is to find an energy effective and environmentally friendly way to affect the surfaces of BCTMP fibres, in other words, to peel and fibrillate the pulped aspen wood cells. Agitation in a bag mixer and supercritical carbon dioxide (scCO₂) extraction were selected as methods to treat the fibres. The effects of the treatment were analysed by the surface charge measurement and scanning electron microscopy (SEM). The surface charge of pulp fibres is important as it influences interfibre and fibre-additive interactions in the pulping process, as well as in other applications. In this work, the effect of mechanical treatment scCO₂ extraction on the surface charge of aspen BCTMP was evaluated. Dried and ground BCTMP was dispersed in water, treated with a BagMixer (BM) with a nylon separator, extracted with scCO₂ with and without ethanol-water co-solvent. The influence of 1% sodium hydroxide was also studied. Polyelectrolyte titration was used to measure the surface charge. The processes were carried out at various times and in different order of succession. A relatively high surface charge was gained by a gentle mechanical and chemical treatment. SEM analysis showed the relation of surface fibrillation to the surface charge of the fibres. Washing with sodium hydroxide removed both the fibrils from the surfaces and the surface charge. The results can be used as a guide to further fibrillation of aspen BCTMP for various applications. Fibrillated intermediates are aimed for processing to, for instance, aerogels and other nanostructured materials.

Keywords: SEM, surface charge, cellulose, titration, supercritical CO₂, electrolyte, polyelectrolyte, p-DADMAC, aspen, aspen pulp fibres, BCTMP, thermo-mechanical treatment, fibre surface

INTRODUCTION

Sustainable use of materials has been agreed to be imperative in the 21st century. This is the reason why so much attention has been paid to novel bio-based materials. Especially cellulose, the most abundant biopolymer on earth, has been the subject of very active research. Nanomaterials have been developed to provide new functionalities, to improve existing products, to lower the need for resources and to enable sustainability. Nanocellulose, a material composed of nano-sized cellulose fibrils, has unique and potentially useful features. It combines important cellulose properties, such as

hydrophilicity, broad chemical modification capacity and the formation of versatile semi-crystalline fibre morphologies with the specific features of nano-sized materials.¹ Nanocellulose has a number of applications, e.g. (nano)paper, barrier in greaseproof paper, reinforcing component in paper coatings, composite reinforcement. Due to its nontoxic, hydrophilic and rheological properties, nanocellulose is also used in food, pharmaceuticals and cosmetics.¹

Wood fibres (wood cells) are composed of three main chemical components: cellulose, hemicelluloses and lignin, which are all

differently influenced during the pulping process. When talking about fibres, only libriform cells are considered as fibres in hardwood. Typical lengths of wood fibres are 1 to 3 mm and typical widths are 10 to 50 μm ;² for aspen (*Populus tremula*) dimensions are approximately 0.9 mm and 19 μm , respectively.³ The fibre wall thickness is roughly between 1 and 5 μm . The fibre wall consists of defined layers, including the primary wall (P) and several secondary wall layers (S1, S2 and S3), between the fibres there is lignin-rich middle lamella. Each cell wall is characterised by a specific arrangement of fibrils. Wood fibre consists of cellulose microfibrils, which have a maximum diameter of 35 nm.⁴ Microfibrils are composed of bundles of elementary fibrils, with a diameter of approximately 3.5 nm.⁵ Microfibrils are arranged differently in various layers of the fibre wall structure, i.e. in primary and secondary walls.

Nanocellulose can be divided to three main types: microfibrillated cellulose (MFC), nanocrystalline cellulose (NCC) and bacterial nanocellulose (BNC). A further classification of these materials is given in a comprehensive review by Klemm *et al.*¹ MFC (also called nanofibrillated cellulose, nanofibres, nanofibrils, microfibrils)² is a material usually made of wood cellulose fibres by detaching individual cellulose microfibrils from each other. The length of fibrils is relatively high (up to 1 μm), fibre diameter is in the range of 5-60 nm.¹ MFC is industrially an interesting material; however, the production via pure cellulose is complex and energy demanding. As the industrial production of nanocelluloses is growing, the price of NCC is predicted to lower to the level of a few Euros per kilo, as for the grades of MFC and MFC, they have prices of up to hundreds of Euros per kilo.⁶

Bleached chemi-thermo mechanical pulp (BCTMP) is a useful material for higher grade paper manufacturing.⁷ However, by applying further treatments, it is possible to obtain new potential applications for this industrial pulp. In BCTMP, almost all lignin is still on the surface of wood fibres, hindering the inter-fibre bonding and bonding to additives. During mechanical pulping, outer cell wall layers have not been removed and lignin is still on the surface of wood fibres, mostly in the middle lamella region and primary wall, as they are the most lignin-rich areas in wood fibre.^{7,8} These fibres are hydrophobic, because lignin is hydrophobic. If outer cell wall layers are removed (middle-lamella, primary wall and S1

layer), the S2 layer becomes visible with nice cellulose microfibrils oriented more or less parallel to fibre axis. This S2 layer has the highest content of cellulose and is therefore hydrophilic.

As a rule, kraft pulp is used for nanocellulose preparation. The morphology of kraft pulp fibres differs completely from mechanical pulp fibres. On the surface of kraft pulp fibre, the S2 layer is already visible, for mechanical pulp on the contrary S2 layer is hidden under outer cell wall layers.⁷ For nanocellulose preparation, it is therefore more convenient to use kraft pulp. The major disadvantage of the usage of kraft pulp is that a heavy chemical treatment has been used and the yield of pulp is much lower compared to the mechanical pulp. Therefore, in order to make a far more environmentally friendly product, BCTMP has been examined.

The purpose of this work is to examine possibilities for further refining aspen BCTMP for various potential applications. ScCO₂ extraction and mechanical treatment are tested for peeling off outer cell wall layers.¹² Co-solvents, e.g. ethanol-water mixtures, are tested for delignification. These kinds of treatments are well-known from organosolv pulping.¹³ Ethanol treatment facilitates fibre separation in the middle lamella region.¹ The selectivity of delignification might also be improved by the addition of NaOH, by increasing alkali charge from 5% to 35% and by using ethanol up to 40% by volume. The residual lignin content has been reported to decrease from 20% to 3%.^{10,11}

The aim of the scCO₂ extraction is to treat wood without using any hazardous chemicals. ScCO₂ is becoming an important commercial and industrial solvent, due to its role in chemical extraction, in addition to its low toxicity and environmental impact. ScCO₂ is also non-flammable, chemically inert and inexpensive.¹² ScCO₂ extraction has been used to remove lignin and small molecular weight extractives from wood. Organic solvents (acetic acid, ethanol and methanol) are used as co-solvents. The use of scCO₂ extraction before pulping enhances the penetration of pulping chemicals.^{13,14,15}

Wood pulp fibres are negatively charged at all pH values due to the presence of acidic groups (carboxyl, sulphonic acid, phenolic or hydroxyl), which either derive from cell wall constituents or are introduced during pulping or bleaching of fibres. The negative surface charge of BCTMP is most probably due to carboxylic groups and sulphonic groups are not expected to be present.

The charged groups are located either on the fibre surface or inside the cell wall; therefore they are referred to as surface and bulk charges. The number of charged groups depends on the origin of the fibre and on the chemical and mechanical treatment during pulping and refining. The surface charge of pulp has been reported to be 0.5-50 $\mu\text{eq}/100\text{ g fibre}$.^{16,17}

Surface charges influence fibre-fibre bonding and interactions with high molecular mass additives. Surface charge of cellulosic fibres increases linearly with increasing refining (beating).^{18,19,20} It is because the refining process opens up the fibre surface and, as a consequence, there is more area for fibre-fibre contacts and subsequently more bonds between fibres.¹⁸ The fines produced early in the refining have more charged groups than the fines produced later in the refining process.²¹ As mechanical pulp contains more fine material than chemical pulp, the surface charge of mechanical pulp tends to be higher.²²

A well-known method for determining the surface charge of cellulosic fibres is polyelectrolyte titration. The procedure is based on the fact that polyelectrolytes can form complexes with oppositely charged polyelectrolytes or surfaces. It is possible to determine the charges on cellulosic fibres by measuring the amount of the cationic polymers adsorbed on cellulosic fibres. The method can be used to determine the anionic surface charge of fibres if a cationic polyelectrolyte has a sufficiently high molecular weight that it does not penetrate the cell wall.²²

Direct polyelectrolyte titration was pioneered by Terayama,²³ who determined the concentration of a cationic polyelectrolyte solution by titration with an anionic polyelectrolyte in the presence of a cationic indicator. Polyelectrolyte titration was later applied to cellulosic fibres (indirect titration).²⁴

Linear polyelectrolytes of high charge density are used to apply this method.^{23,24} In a typical titration, the pulp mixture is first treated with a known excess of highly cationic polymer, such as p-DADMAC. Then it is filtered or centrifuged to remove solids (the content of fines plays a major role in polyelectrolyte titrations, as it increases the amount of polymer required to neutralize the charge).²² A small amount of indicator dye (usually toluidine blue-O-OTB) is added and then the blue solution is back-titrated with an anionic polyelectrolyte, such as potassium poly-

vinylsulfate (KPVS) to a purple-pink endpoint. Complexation takes place between the dye and the negatively charged polymer, causing the colour-change to pink. We have modified the back titration and used, instead of anionic polymer, potassium hydroxide and, instead of indicator dye, potentiometric indication to determine the amount of the cationic polymer.

The most important factors that affect surface charge values are surface and polyelectrolyte charge density, ionic strength and molecular weight of the polyelectrolyte.¹³ It was concluded that at least three criteria must be met for a 1:1 stoichiometry to prevail when measuring the surface charge content: 1) a high charge density of the cationic polyelectrolyte, 2) a high molecular mass of the cationic polyelectrolyte, and 3) sufficiently low electrolyte concentration. There must also be no non-electrostatic interactions between the polyelectrolyte and the fibre.²²

To fulfil these criteria, high molecular mass polydiallyldimethylammonium chloride (p-DADMAC) was used as cationic polymer and the titrations were carried out at low electrolyte concentrations.

This study is focused on wood fibres; therefore solid particles were removed for further investigation and fine particles containing liquid were not used during titrations.

The aim of this study was to find out how scCO_2 extraction, together with mild chemical and mechanical treatment, influences the surface charge and structure of aspen BTCMP fibres. Surface charge results were combined with SEM analysis for assessing the effects on the fibres, considering further fibrillation.

EXPERIMENTAL

Materials

Aspen BCTMP was obtained from Estonian Cell. All pulps were oven dried at 105 °C and ground in a coffee-grinder. Commercially available technical grade CO_2 with a purity of 99.7% from AGA was used in the scCO_2 extraction process. Technical Acetone with 99.5% purity from APChemicals was used in the solvent exchange process. Polydiallyldimethylammoniumchloride (p-DADMAC) was used as cationic polyelectrolyte and potassium hydroxide (KOH) as titrant. P-DADMAC was supplied by Sigma Aldrich as a 20 wt% concentrate. Mw of p-DADMAC was 100000-200000 g/mol. NaOH was supplied by Sigma Aldrich, with M = 40000 g/mol. KOH and ethanol were of laboratory grade

and used without further purification. Distilled water was readily used from the laboratory distilled water system.

Equipment

Commercially available disperser T 25 digit Ultra Turrax from IKA laboratory equipment was used as part of the mechanical treatment process of the cellulosic fibres. The device is composed of the digital disperser, an extension arm, a support screw and an exchangeable nozzle. The T-25 digital model is designed to work at high circumferential speeds, ranging between 6–24 m/s, which accounts for 3400–24000 rpm.

Commercially available BagMixer 400W from Interscience was used for the homogenisation and mechanical treatment process of the cellulose fibres. The blending occurs during a back and forth movement of two patented paddles. As one of the paddles is in the forward position, the other is in the backward position. The blending speed is set to a constant value of 8 strokes/min by the manufacturer. One cycle of the machine can maximally last for 360 seconds and the machine automatically comes to a stop as the timer has counted the set time. The device allows for processing volumes ranging between 50–400 ml. The processed medium is held in a blending bag, which is mounted inside the stainless steel body.

A supercritical CO₂ extraction system SES-UK 1 was started up to carry out the SCCO₂ extraction experiments.

Figure 1 depicts the reactor system composed of the liquid CO₂ source (1), liquid CO₂ pump (2), a valve for injection of a co-solvent that can be used as a valve for closing off the reactor from the pump (3), the reactor (4), which is placed inside a thermostat (9), a triplet (5) that separates the outlet into two valves (6 and 7), of which 6 is used for releasing the solvent from the system and 7 is the back-pressure regulator.

The Supercritical 24 constant flow-constant pressure dual piston pump is used for providing the supercritical processing system with sufficient pressure to operate in the supercritical region of CO₂. The pump is built to operate in two distinct modes: constant pressure and constant flow regimes. Pressure is adjustable from 0 to 10000 Psi with an error of 0.01. In the constant pressure mode, a maximum value for the flow rate is set for the pump, which then is one of the

determining factors of the pace at which the set constant pressure threshold is achieved.

Commercially available Carbolite PF30 convection oven is used as thermostat in the supercritical processing system. The maximum temperature of the system is 200 °C. The reactor is placed inside the thermostat.

The two valves at the end of the system serve as the back-pressure regulator and a way to release the solvent from the reactor. The system allows for precise control of pressure and temperature, which are the most important parameters defining the supercritical state. The processing time is also adjustable and variable at need. The system can operate under pressures up to 190 bars and temperatures up to 200 °C.

Commercially available titrator, Mettler Toledo T90 was used; it consists of a titrator with terminal, pH board, integrated burette drive and magnetic stirrer. It is expandable with two sensor boards (pH and/or conductivity) and up to seven additional burette drives (for dosing and titration purposes).

Methods

The experimental part of this study consisted of two main parts: pre-treatment processes of wood pulp and the surface charge measurement. SEM images of the samples were also taken in order to evaluate the correlation between surface charge and visual appearance.

The pre-treatment process consisted of the following steps: dispersing in water, treatment with BagMixer (BM) with a nylon separator, supercritical CO₂ extraction with and without ethanol:water co-solvent, mild chemical treatment with 1 wt% NaOH solution. For pre-treatment procedures, an experimental plan was composed in order to vary the position of different treatments to each other and to find out the optimum conditions for the highest surface charge (best fibrillation).

After each treatment, surface charge was measured and SEM images were taken. For surface charge measurements, pH was measured with Mettler Toledo electrode DG-112 Pro calibrated with Mettler Toledo pH buffer solutions (4.01; 7.00; 9.21) and connected to a Mettler Toledo automatic titrator T90 under N₂ flow.

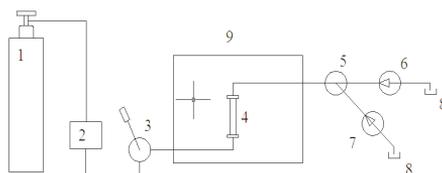


Figure 1: A set-up of the SC extraction system

The dry-grinding of the aspen pulp was carried out in an ordinary grinder under atmospheric conditions. The initial sample was then dispersed in distilled water at concentrations of 0.5 wt% and 1 wt% of pulp in water. The dispersing was carried out at 12000 rpm for 5 minutes.

The pulp was separated by filtration in the Büchner funnel by using a metallic sieve with a mesh size of 50 μm . Then the pulp was weighed and used for titrations and further treatments. The filtrate of the pulp dispersion was not used during the titrations as the interest was to investigate the pulp fibres rather than dispersion.

After dispersing, the sample with water was mechanically treated for six minutes by the BM; the sample was placed inside the bag with the nylon separator in the middle, with a mesh size of 300 μm . For the BM treatment, the pulp was put inside the bag and BM pushed the pulp through the sieve, the blending occurred during a back and forth movement of two patented paddles.

For supercritical CO_2 treatment, dry samples were prepared in stainless steel netting and placed inside the reactor. The samples from the dispersing or bag mixer treatment, i.e. in water solution, were dried at the critical point after the solvent exchange from water to acetone. The samples for the SC treatment process were then again placed in stainless steel netting and into the reactor.

In the case of using water-ethanol 1:1 solution as a co-solvent, it was injected into the system at the point where temperature reached its plateau value. In current experiments, 40 μl were injected.

ScCO_2 experimental equipment is imaged in Figure 2. Working parameters were chosen as follows: pressure 160 bars, temperature 120 $^\circ\text{C}$ and treatment time of 50 minutes starting from the point where temperature reached 60 $^\circ\text{C}$.

For SEM studies, dry samples were placed with a fine pipette to a metallic stub, using a double-sided adhesive tape. Then the samples were placed into an ion sputtering chamber JFC-

1100 for one minute and 40 seconds and coated with gold and platinum under vacuum to enhance electrical conductivity. Coating was restricted to 1.9 kV and 10 mA. The SEM examination was carried out with EVO MA 15 at accelerating voltage from 12 to 15 keV.

To carry out critical point drying of wet pulp samples, solvent exchange to acetone was performed, followed by SEM analyses. Solvent exchange is a method for shifting material from one solvent to another. The samples in water solutions were transformed into acetone via solvent exchange using acetone-water solutions (30, 50, 70, 90, 100, 100 wt% of acetone). During each step, segregation was assured by mixing in an ultrasonic bath for 2-3 minutes. The wood fibre mass was then filtered (50 μm pore size stainless steel filter) and placed in the next solution until no water was left.

A typical critical point drying was conducted in the Balzers apparatus. The wood fibre mass in acetone was placed in the chamber, which was cooled to 5-7 $^\circ\text{C}$, sealed, pressured and flushed with liquid CO_2 3-4 times. Finally, the chamber was heated to 40 $^\circ\text{C}$ and gassed out.

Surface charge measurements

The titrations were performed using a high-precision, microprocessor-controlled auto-titrator (Mettler Toledo T90) connected with a pH glass electrode, DGi 112 Pro. The electrode was calibrated using buffer solutions of pH 4.01, 7.00, and 9.21. During the titrations, a propeller stirrer was used to mix the pulp suspension. The rate of titrant addition was controlled at 0.05 ml/s. The data were recorded. Titrations were performed under N_2 flow to prevent the absorption of CO_2 into the test samples during the titrations.

Preliminary experiments were made with and without pulp in fibre dispersions and no remarkable changes in the results were noticed by two different techniques.

Therefore, it was not necessary to remove the pulp from the dispersion.

For titrations, 0.25 g of dry sample was weighed, then 47.5 ml of 1% p-DADMAC solution was added. The mixture was stirred with a propeller stirrer to completely neutralize the anionic charge in the pulp by poly-DADMAC.



Figure 2: Sc experimental devices

When a wet sample was taken, 1.75 g of the sample was weighed and 48.25 g of 1% p-DADMAC was added. Further procedures were the same as described above. The wet sample mass was calculated under the same conditions and it was 7 times heavier than the dry sample.

The surface charge of the samples was calculated using the following equation:

$$q = (V_2 - V_1) \times C \times 1000/W \quad (1)$$

where: q is the specific surface charge (mmol/kg); $(V_2 - V_1)$ is the difference between volumes of KOH used for p-DADMAC solution with and without pulp; C is the titration agent concentration; 1000 is the conversion factor; w is the weight of a sample (oven dry basis).

To insure the integrity of the results, all the experiments were duplicated.

RESULTS AND DISCUSSION

Table 1 summarises the surface charge values after different mechanical and thermal treatments of aspen BCTMP.

A sample of titration curve is exemplified in Figure 3. The equivalence point of titration was calculated by a computer program. It is the point where inflection is most abrupt and this indicates the situation where protons in the solution were replaced with KOH ions. Based on the data of the equivalence point, surface charge was calculated.

Experiment 1

In the first experiment, the initial sample (1.1), dried and ground BCTMP (3 g) was dispersed in

The stirring lasted until the pH of the dispersion was stable, approximately for 10-15 minutes.

Then the pulp mixture was titrated with 0.06 M KOH solution until the equivalence (end) point. Fibre charge was calculated according to Bhardwaj *et al.*¹⁹

water. Then, BM processing was used (1.2) and finally the sample was treated under scCO₂ conditions (1.3).

The surface charge of the initial sample (1.1) is quite low, 5.78 mmol/kg. It increased immensely after the BM treatment ($q = 65.1$ mmol/kg). The increase in surface charge continued after scCO₂ treatment ($q = 75.12$ mmol/kg).

The mechanical treatment in BM in combination with scCO₂ treatment had a good effect on the surface charge of pulp fibres; this kind of pulp processing could be a very effective way to generate surface charges. As can be seen from the SEM image, there are many fine fibrils that cause charges (see Figure 4).

Experiment 2

In the second experiment, the initial sample (3 g) was treated under scCO₂ conditions (2.1). Next, it was dispersed in water (2.2) and finally treated with BM (2.4).

An upsurge in the surface charge after scCO₂ treatment ($q = 78.08$ mmol/kg) occurred, with a slight decrease after dispersing in water due to loss of fine material ($q = 54.95$ mmol/kg), but there was again an increase in the surface charge after BM treatment ($q = 64.43$ mmol/kg).

Experiment 3

In the third experiment, the initial sample (3 g) was processed under scCO₂ conditions with 1:1 ethanol water co-solvent (3.1). Then, it was treated with 1% NaOH, and the pulp mixture was

put into the Büchner funnel with a metallic sieve with a mesh size of 50 μm and washed with distilled water. Once the pH neutrality was attained (3.3), the sample was submitted to BM treatment (3.5). An abrupt surface charge increase after scCO_2 treatment with 1:1 ethanol water co-solvent ($q = 83.77 \text{ mmol/kg}$) occurred. Surface

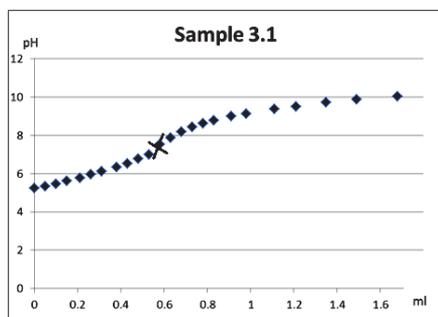


Figure 3: Illustration of a titration curve (the capital X indicates the equivalence point)

charge plummeted after NaOH treatment and washing with distilled water. There was no detectable equivalence point in the titration curve. However, surface charge showed an upward trend after BM treatment ($q = 3.39 \text{ mmol/kg}$).

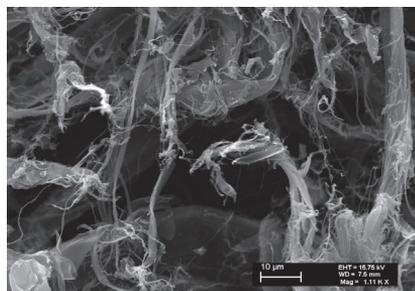


Figure 4: SEM image of sample 1.4 treated in BM and with scCO_2

Table 1
Surface charge for differently treated pulp fibres

Treatment	Surface charge, mmol/kg
Dried and ground pulp (1.1)	5.78 \pm 0.1
Dried and ground, BagMixer (BM) (1.2)	65.1 \pm 0.1
Dried and ground, BM, ScCO_2 (1.4)	75.12 \pm 0.1
ScCO_2 (2.1)	78.08 \pm 0.1
ScCO_2 , dispersed in water (2.2)	54.95 \pm 0.1
ScCO_2 , dispersed in water, BM (2.4)	64.43 \pm 0.1
ScCO_2 , with 1:1 ethanol water co-solvent (3.1)	83.77 \pm 0.1
ScCO_2 , with 1:1 ethanol water co-solvent; 1% NaOH, washed with distilled water (3.3)	-
ScCO_2 , with 1:1 ethanol water co-solvent; 1% NaOH, washed with distilled water; BM (3.5)	3.39 \pm 0.1
ScCO_2 ; dispersed in water; BM; scCO_2 (4.2)	63.99 \pm 0.1
ScCO_2 , with 1:1 ethanol water co-solvent; 1% NaOH, washed with distilled water; ScCO_2 (5.2)	-
ScCO_2 , with 1:1 ethanol water co-solvent; 1% NaOH, washed with distilled water; ScCO_2 , BM (5.4)	18.34 \pm 0.1

Experiment 4

In the fourth experiment, the initial sample (3 g) was treated under scCO_2 conditions. Then, it was dispersed in water and treated with BM. Finally, the sample was treated with scCO_2 (4.2) again.

Experiments 2 and 4 are similar; the only difference was that in Experiment 4 there was a second supercritical CO_2 treatment at the end. However, the second scCO_2 treatment did not play a role in the surface charge value. The surface charge for sample 2.4 was 64.43 mmol/kg

and for sample 4.2 (treated with scCO_2 twice) it was 63.99 mmol/kg.

Experiment 5

In the fifth experiment, the initial sample (3 g) was treated under scCO_2 conditions with 1:1 ethanol water co-solvent. Then, it was treated with 1% NaOH and washed with distilled water in the Büchner funnel until neutral pH. Following this, the sample was submitted to BM treatment (5.4). The fifth experiment resembled the third experiment; the only difference was the scCO_2

treatment process between 1% NaOH treatment and BM treatment.

However, the surface charge values of samples 3 and 5 are quite different (3.39 mmol/kg and

18.34 mmol/kg, respectively). This means that the scCO₂ treatment has an influence even between mechanical treatments.

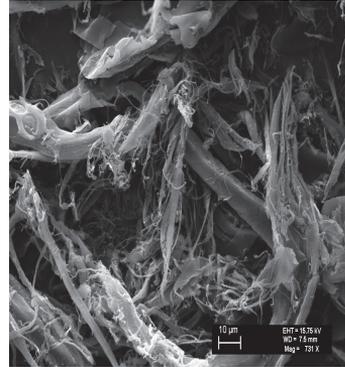
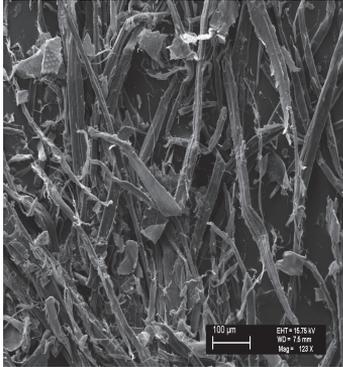


Figure 5: Samples 1.1 (left) and 1.2 (right) showing the influence of BM treatment

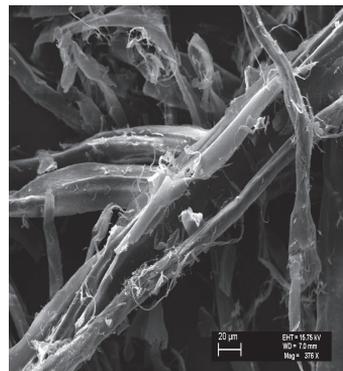
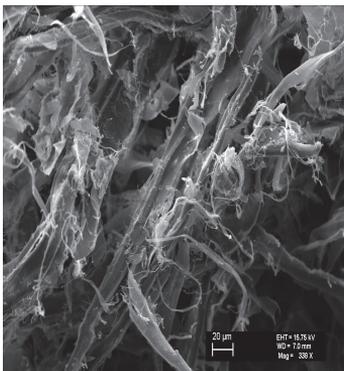


Figure 6: Samples 2.1 (left) and 3.1 (right) showing similar results after scCO₂ treatment regardless of ethanol-water solvent used for sample 3.1

Effect of BagMixer treatment on the surface charge

The result was quite significant before and after BM treatment, in samples 1.1 and 1.2, respectively. Surface charge increased considerably, comparing the surface charge before and after BM treatment. This is in agreement with SEM images, which show that there is an abundance of lamellas, unbroken fibres, but no fibrillous parts, in sample 1.1, while in sample 1.2, on the contrary, the fibres are broken and there are more fibrillous parts. It can be concluded that fibrils were separated from the fibres during mechanical treatment. The influence of BM treatment is depicted in Figure 5.

Effect of scCO₂ extraction on surface charge

The surface charge showed the largest values for samples after scCO₂ treatment. However, there was no significant difference between samples 2.1 and 3.1, treated under scCO₂ conditions and by scCO₂ treatment with 1:1 ethanol-water co-solvent, respectively. This means that either ethanol-water did not influence the surface charge, or the amounts of co-solvent were too small.

Figure 6 shows similar results for samples 2.1 and 3.1 despite the 1:1 ethanol-water treatment of sample 3.1. SEM images for the samples also yielded similar results. The results of scCO₂ treatment are illustrated in Fig. 6.

Effect of supercritical CO₂ and BagMixer treatment on surface charge

A strong influence of scCO₂ in combination with BM treatment was found. The surface charge for sample 1.4 was 75.13 mmol/kg and for sample 4.2 it was 63.99 mmol/kg. Nevertheless, the surface charge value did not rise after the second scCO₂ treatment (sample 4.2). The influence of scCO₂ in combination with BM treatment is illustrated in Figure 7.

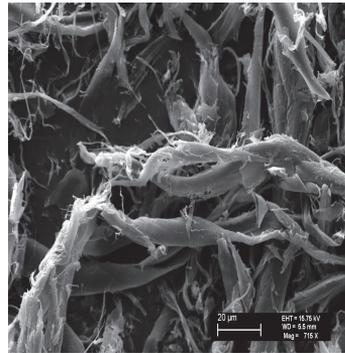
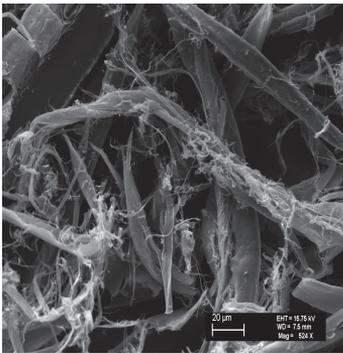


Figure 7: Samples 1.4 (left) and 4.2 (right), displaying the influence of scCO₂ in combination with BM

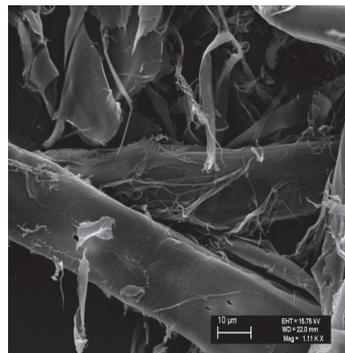
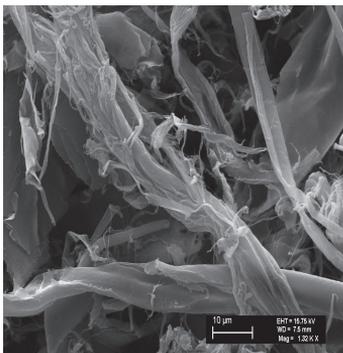


Figure 8: Samples 3.3 (left) and 3.5 (right), showing large lamellas with no fibrillous part after 1% NaOH treatment

That might be also the consequence of washing, as all the fine material that induces higher charge values is swept away from the fibre surface.

CONCLUSION

The main objective of this study was to investigate how different thermo-mechanical treatments and their order influence the surface charge of cellulose fibres in aspen BCTMP. Various treatments of pulps were conducted and surface charges were measured. SEM images

Effect of 1% NaOH on surface charge

Surface charge was the lowest for the samples that were treated with 1% NaOH and then washed until neutral reaction. Samples 3.3 and 5.2 did not show any result and sample 3.5 had the lowest determined values.

These results were in good agreement with the SEM images of samples 3.3 and 3.5, which have no fibrillous structure or fines that could cause significant fibre charge (Figure 8).

were also taken to investigate the correlation between surface charge value and visual appearance.

A new experimental method was practiced in order to measure the surface charge of cellulose fibres. It was a combination between the polyelectrolyte titration technique and potentiometric titration. In this method, cationic polyelectrolyte, p-DADMAC was used (polyelectrolytic titration) for pulp dispersions and KOH solution was used as titrant, then pH was measured for end point determination

(potentiometric titration). According to these data, the surface charge of the samples was calculated. This method helped to analyse how different treatments influenced the surface charge of cellulosic fibres.

Dried and ground BCTMP pulp of aspen was dispersed in water, treated with BM with a nylon separator, treated with scCO₂ with and without 1:1 ethanol water co-solvent and treated with 1% NaOH. The processes occurred at various times and order of succession. After each treatment the surface charge was measured and SEM images were taken.

There was a good agreement between the titration results and SEM pictures, as much of the fibrillous structure was seen where the surface charge was higher and smooth surfaces of fibres were found where the surface charge of cellulose fibres had a lower value. This suggests that the surface charge of cellulosic fibres could be measured by using this new experimental method.

It is not possible to compare the surface charge results with those of other authors, as the method is completely different from those used by other authors who measured the charge of filtrate and removed the pulp before titrations. It was of interest to measure the surface charge of the pulp and therefore the filtrate was never used for titrations. However, there is a good correlation between the changes in fibre morphology and the electrokinetic character of the fibres.

In future studies, the surface charge of the pulp could be measured by using all suspension material, including the filtrate. By measuring the surface charge of the filtrate, the result should be somehow different; the surface charge should be higher, as the filtrate contains much fine material that gives surface charges.

In the future, it is possible to bind some other material with pulp fibres that has high surface charge. One possibility is to make cellulose hydrogel and bind it with hydroxyapatite.

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Paper II

Kärner, K.; Elomaa, M.; Kallavus, U. (2014) Lignin and outer cell wall removal from aspen pulp by using supercritical CO₂ extraction. In: *Proceedings of the 9th International Conference of DAAAM Baltic Industrial Engineering: 24–26st April 2014, Tallinn, Estonia*. TUT Press: 372–377.

LIGNIN AND OUTER CELL WALL REMOVAL FROM ASPEN PULP FIBRES BY USING SUPERCRITICAL CO₂ EXTRACTION

Kärner, K.; Elomaa, M. & Kallavus, U.

Abstract: Supercritical carbon dioxide extraction (scCO₂) is chosen to a treatment to remove lignin and to peel off outer cell wall layers to expose S2 layer of aspen wood fibres. The aim is to find an effective and environmentally friendly method to advance the fibrillation of the BCTMP pulp. The effects of the treatment are analysed by using scanning electron microscopy (SEM). The chemicals used together with scCO₂ extraction include 1:1 ethanol: water co-solvent, isopropyl alcohol, and dimethyl sulfoxide (DMSO) with urea. The results show that supercritical CO₂ extraction helps to peel the fibres of mechanical pulp. Best results are gained with 1:1 ethanol: water co-solvent.

Key words: Cellulose; Lignin; Super-critical CO₂; Aspen; BCTMP, Microfibrils,

1. INTRODUCTION

Cellulose is one of the most abundant biopolymers on earth; it has attractive characteristics of being both recyclable and renewable. It consists of microfibrils that are bundles of elementary crystallites bridged by amorphous phases. Because a complete purification of cellulose demands a lot of energy and chemicals, there are many products in which cellulose is used only partly purified. For example, products like bleached chemi-thermo mechanical pulp (BCTMP) have been developed to be used as a component in paper making. A characteristic property of mechanical pulp is that it retains most of the wood lignin.

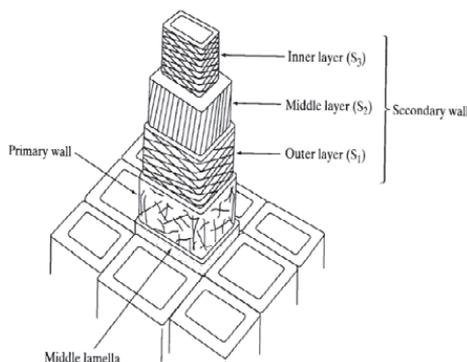


Fig.1. Wood cell wall layers

The structure of fibre consists of several different layers; three main layers are the middle lamella, the primary wall and the secondary wall. Secondary wall is divided into sub layers: S₁, S₂ and S₃. Different cell wall layers can be identified by the orientation of cellulose microfibrils; it is specific to each layer, see Figure 1. Lignin and cellulose contents are also different in different layers; the lignin content is highest in the middle lamella and the primary wall, whereas cellulose content is highest in the S₂ layer. Therefore, in order to get pure cellulose from wood fibre, it is necessary to remove outer cell wall layers. The proof of successful treatment is a clearly visible S₂ layers with parallel orientation of cellulose microfibrils.

Lignin that is present on the surface of BCTMP forms a physical barrier against inter fibre bonding, moreover, it hinders the inter-fibre hydrogen bonding due to the hydrophobic nature of lignin [1]. Therefore, it is essential to remove the lignin-rich material in order to fibrillate fibres and to produce e.g. nanocellulose. The

hydrophobic lignin-rich material is also one of the main reasons for low inter-fibre bonding strength of mechanical pulp fibres [1]. On the contrary, when more cellulose fibrils are exposed, and more fibril separation takes place in the secondary wall, then inter-fibre bonding is enhanced. At the same time, by removing specifically the lignin-rich material on the fibre surface it will not reduce much the yield [1].

scCO₂ is becoming an important commercial and industrial solvent due to its role in chemical extraction in addition to its low toxicity and environmental impact [2]. scCO₂ is also non-flammable, chemically inert and inexpensive. scCO₂ extraction under sub and supercritical conditions has been used to remove lignin and small molecular weight extractives from wood. Organic solvents (acetic acid, methanol, ethanol and butanol) have been used as co-solvents, they enhance the influence of sc CO₂ [3]. Organic solvents together with water are also used in organosolv pulping process. The use of scCO₂ extraction before pulping enhances the penetration of pulping chemicals [4]. One of the most effective modifiers in supercritical conditions might be isopropyl alcohol, it has been successfully used and it resulted delignification of 4 % [4]. Because 100% ethanol is not very nucleophilic it has been used as co-solvent with water (ethanol-water ratio 30-70%). The addition of water promotes the delignification reactions but reduces the ability of the solvent to dissolve the lignin separated by the process [7]. It has also been suggested that adding of co-solvent (ethanol-water 1:1 solution) to the extraction should dissolve lignin [7].

It is essential to use high pressures to permit the reaction of the solvent mixture (or fluid) with the lignin present in the wood [7]. Delignification effect of wood takes place under the pressure of 350 atmospheres. However, temperature does not affect the delignification rate so much, it can be between 80 and 140 °C [4]. The reaction temperature cannot be over 200°C,

as the degradation of polysaccharides present in the wood starts around 200°C and is very significant over 250°C. Therefore, in order to preserve cellulosic fraction as much as possible, those limits must be considered [7]. 60-120 minutes treatment times for extraction have been reported [5]. However, most of the delignification takes place during the first 30 minutes [7]. In order to produce microcrystalline cellulose, dimethyl sulfoxide (DMSO) has been used together with urea. In this case urea helps the reaction agents to penetrate deeper into the fibre. Urea also dissolves well in DMSO [6].

The aim of this work is to examine if it is possible to use scCO₂ extraction, possibly intensified with co-solvents, to remove residual lignin and to peel off outer cell wall layers.

2. EXPERIMENTAL

2.1 Materials

Aspen BCTMP was obtained from Estonian Cell. All pulps were oven dried at 105°C and grinded in a coffee-grinder. Commercially available technical grade CO₂ with a purity of 99.7% from AGA was used in the scCO₂ extraction process. Technical Acetone with 99.5% purity from APChemicals was used in the solvent exchange process. Isopropyl alcohol was from Sigma Aldrich with purity ≥99.7 % and with Mw=60.10. Ethanol and dimethyl sulfoxide (DMSO) were of laboratory grade and used without further purification. To enhance the influence of DMSO during the scCO₂ treatment, urea was used; it was 99.0-100.5 % pure from Sigma Aldrich. Distilled water was readily used from laboratory's own distilled water system.

2.2 Equipment

A supercritical CO₂ experimental device SES-UK 1 from Norlab OÜ was used to carry out the SCCO₂ extraction experiments. Figure 2 depicts the reactor system.

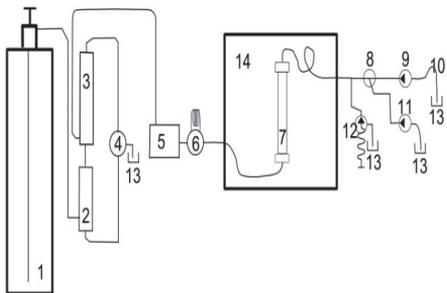


Fig.2. A set-up of the supercritical extraction system. CO₂ cylinder (1), liquid/gas outlet line (2,3,4) high pressure dual piston pump (5) six-way injector (6), reactor (7), three way joint (8), output vents (9) safety valve (12) flow outs (13), thermostat oven (14).

The Supercritical 24 constant flow-constant pressure dual piston pump was used the sufficient pressure to operate in the supercritical conditions of CO₂. The pump is built to operate in two distinct modes: constant pressure and constant flow regimes. Pressure is adjustable from 0 to 10000 Psi with an error of 1%. In the constant pressure mode a maximum value for the flow rate is set for the pump which is then one of the determining factors of pace at which the set constant pressure threshold is achieved.

Commercially available Carbolite PF30 convection oven is used as thermostat of the supercritical processing system. The maximum temperature of the system is 200°C. The reactor is placed inside the thermostat.

The two valves at the end of the system serve as the back-pressure regulator and a way to release the solvent from the reactor. The system allows for precise control of pressure and temperature that are the most important parameters defining the supercritical state. The processing time is also adjustable and variable at need. The system can operate under pressures up to 190 bars and temperatures up to 200°C.

The critical point drying was carried out in a commercially available Balzers CPD 030 device. The specimen chamber is

incorporated into the body of the device. Heating and cooling of the specimen chamber are monitored with a digital temperature meter. Liquid CO₂ is used as the drying medium because of its low critical point values for temperature and pressure.

Blazer's critical point dryer was applied for drying wet samples before microscopy.

Fine Coat Ion Sputter JFC-1100 was applied for SEM samples.

All SEM analyses were carried out with a Zeiss EVO MA 15 scanning electron microscope; as it was used for imaging and morphological characterization, the secondary electron regime was used. The specific SEM allows for accelerating voltages ranging from 0.2 – 30 keV, magnifications up to 1 million and corresponding resolutions down to some nanometres. The field of view is defined to be 6 nm at the analytical working distance. The microscope allows for investigation of specimen with heights up to 145 mm.

2.3 Methods

For supercritical CO₂ extraction an accurately weighed dry sample (3 g) was put into stainless steel netting and placed inside the reactor. Stainless steel reactor was placed inside the thermostat (see Figure 1). Then thermostat was closed and pressure and temperature risen.

When solutions were used (ethanol: water co-solvent, isopropyl alcohol, DMSO with urea), samples were first mixed with 10 ml of solution and then placed to the reactor.

The extraction conditions were chosen close to the maximum parameters to ensure treatment throughout the wood fibre - 2900 psi and 120 °C. Treatment was set to 60 minutes starting from the point where pressure and temperature had reached their desired values.

Before SEM analysis, solvent exchange to acetone and critical point drying were performed. Solvent exchange is a method for shifting material from one solvent to another. Samples in water solutions were transformed into acetone via solvent

exchange using acetone-water solutions (30, 50, 70, 90, 100, 100w% of acetone).

A typical critical point drying was conducted in the Blazer's apparatus. The wood fibre mass in acetone was placed in the chamber which was cooled to 5 - 7 °C to ensure liquid CO₂, sealed, pressured from the CO₂ vessel and flushed with liquid CO₂ 3 - 4 times. Finally the chamber was heated to 40 °C and gassed out.

For SEM studies, dry samples were placed with fine pipette to a metallic stub using a double-sided adhesive tape. Then the samples were placed into ion sputtering chamber for two minutes and coated with gold and platinum under vacuum to enhance electrical conductivity. Coating was carried out at 1.9 kV and 10 mA.

The SEM examination was carried out with EVO MA 15 at accelerating voltage of 12 to 15 keV. Two sets of images were taken for each sample: an overview image with 200 x magnification and close images of separate fibres with about 1000 x magnification. Specific points of interest were imaged with magnifications up to 4500 x.

3. RESULTS AND DISCUSSION

Our initial sample is bleached chemi-thermo mechanical aspen pulp (BCTMP). Emphasis is set to the fibres and changes on their surfaces caused by the scCO₂ extraction. The S2 layer of the cell wall is the thickest layer and contains the most of the cellulose. It is thus desirable to remove the middle lamella and two layers (P and S1) to open up the S2 layer for further treatment or processing. On the surface of BCTMP fibres, large pieces of non-fibrillar lignin-rich material can be seen, see Fig. 3. This is mainly because after mechanical pulping, outer cell wall layers (middle lamella and primary wall) are still on the surface of wood fibres. On Figure 3, the middle lamella is easily recognizable by its rectangular shape. It can be seen that outer cell wall layers are unbroken, no cracks can be seen in the middle lamella, layers are strongly attached to the fibre main

body. Figure 3 also shows that there are non-fibrillar patches present and they derive from two adjacent fibres. This is the most lignin-rich area, which glues fibres together in wood (middle lamella contains about 70 % of lignin).

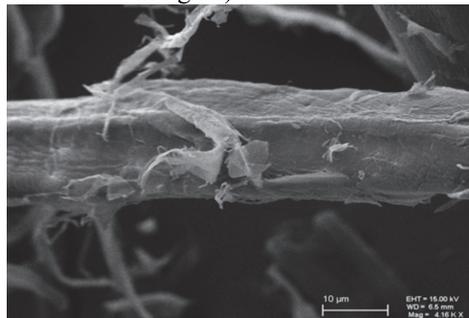


Fig. 3. Starting material-dried and grinded BCTMP.

After scCO₂ extraction, fibres look somewhat different than before the treatment. It can be seen from Figure 4 that outer cell wall layers, especially middle lamella, start to peel off from the wood fibre. It seems that outer cell wall is loosened. There are a lot of cracks in outer cell wall layers, splitting is likely to happen. It can be presumed, that after some mechanical treatment those almost loose patches come off completely.

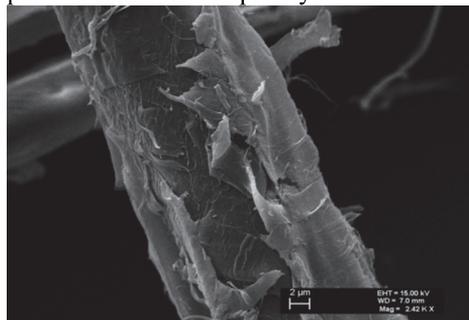


Fig. 4. Peeling effect after scCO₂ treatment.

The supercritical treatment is even more effective when ethanol is added to the reaction system. In our treatment 1:1 ethanol: water co-solvent was used and the pulp was moistened with this solution before the sc CO₂ extraction. As can be seen from Figure 5, middle lamella and

primary wall are both successfully removed from aspen pulp fibre. S2 layer is clearly visible and it can be easily identified by parallel orientation of cellulose microfibrils. It suggests that the addition of a co-solvent (1:1 ethanol-water solution) reacts with lignin and loosens the whole structure so that most lignin-rich layers split out from the main fibre.

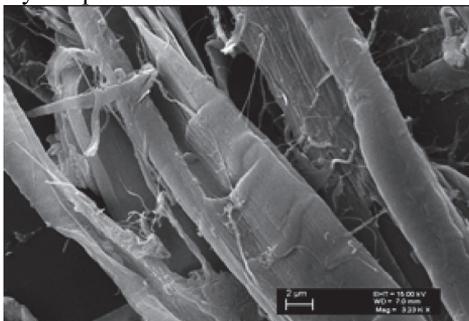


Fig. 5. Peeling effect after sc CO₂ treatment with ethanol: water cosolvent

The supercritical CO₂ extraction was not always very effective. For example treatments with isopropyl alcohol and dimethyl sulfoxide did not have very good effect on fibrillation and outer cell wall removal. The result is moderate after isopropyl alcohol (see Figure 6) as there is no sign of fibrillation. On the other hand, there is a little visible peeling. The whole fibres look quite unbroken. Thus isopropyl alcohol was not the best co-solvent to scCO₂ in the procedure used.

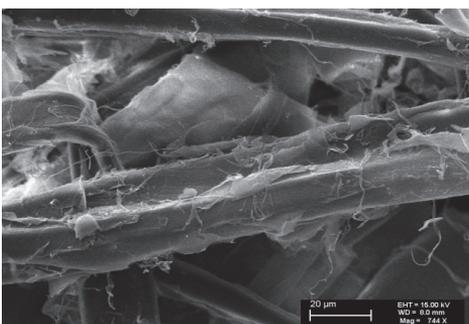


Fig. 6. Fibres looking almost the same as without treatment.

Supercritical CO₂ with DMSO and urea did not give any remarkable effect. On the contrary, it seemed that all residual lignin that was present in outer cell wall layers, has been “melted” on the fibre surface during the scCO₂ treatment, see Figure 7. Fibre surface looks quite smooth and there is no sign of peeling or fibrillation. It might be that DMSO and urea are not suitable for fibre fibrillation and lignin removal at higher temperatures. It is reasonable to assume that at high temperatures, lignin in the middle lamella melts and spreads over the fibre surface.

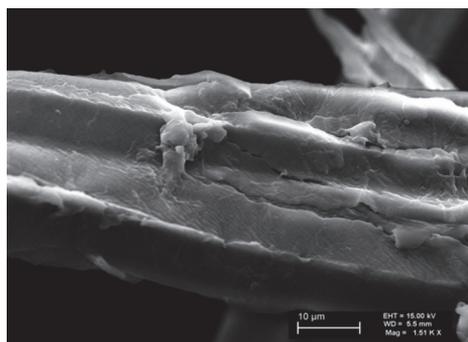


Fig. 7. DMSO with urea.

4. CONCLUSION

Results showed that supercritical CO₂ treatment has an influence on fibre peeling on mechanical pulp. It is possible to remove outer cell wall layers by using scCO₂ extraction. As the treatment loosens and in some cases even removes outer cell wall layers, it can be presumed that lignin content is also decreased as the lignin content is especially high in those removed layers. Best results were gained with 1:1 ethanol: water co-solvent. It was shown that scCO₂ extraction changes the appearance of wood fibres. Nevertheless, DMSO did not cause any residual lignin removal or peeling of the fibre outer cell walls. On the contrary, lignin was like a smooth film covering the fibre surface. Sc CO₂ treatment is a good starting point for fibre fibrillation; the process might be enhanced by further mechanical treatment.

5. ACKNOWLEDGEMENTS

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Paper III

Kärner, K.; Elomaa, M.; Kallavus, U. (2016) Fibrillation of BCTMP of aspen by alkaline cold pre-treatment and vibration milling. *Materials Science (Medžiagotyra)*, 22 (3): 358–363.

Fibrillation of Aspen by Alkaline Cold Pre-treatment and Vibration Milling

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In this article an attempt to fibrillate aspen bleached chemi-thermo mechanical pulp (BCTMP) fibre in an environmentally friendly way is reported. The effects of various NaOH, KOH, urea and ethanol aqueous solutions at lowered temperature were tested for pre-treatment. The pre-treatment was followed by vibration milling aiming to peel off outer cell wall layers and to fibrillate S2 layer of the aspen wood fibre. The effects of the treatments were evaluated by scanning electron microscopy (SEM). The results show that it is possible to fibrillate BCTMP aspen fibres by using alkaline aqueous solutions at low temperatures followed by a mechanical treatment. A strong dependence on fibrillation of cellulose on temperature, time and alkali concentration was established.

Keywords: SEM, cellulose, fibrillation, alkaline cold treatment, nanocellulose, MFC, vibration milling.

1. INTRODUCTION

Photosynthetic organisms such as plants, algae, and some bacteria produce more than 180 billion tonnes of organic matter each year due to the photosynthesis; cellulose makes up half of this biomass [1]. It consists of microfibrils that are bundles of elementary crystallites bridged by amorphous phases. Cellulose is the most important component of wood; it gives strength to wood. Cellulose is a homopolysaccharide composed of β -D-glucopyranose units that are linked together by (1 \rightarrow 4)-glycosidic bonds. Two D-glucopyranose units form a cellulose unit that is the repeating unit in the cellulose chain. The polymer chains are bond together in twisted rope-like structures by intermolecular hydrogen bonding. The most abundant cellulose form is native cellulose also known as cellulose I which has two distinct crystalline forms, cellulose I α and cellulose I β . Cellulose I can be transferred irreversibly to stable crystalline form of cellulose II by regeneration and mercerization process by using NaOH [1].

The smallest building element of the cellulose skeleton is an elementary fibril which is a bundle of 36 parallel cellulose molecules, which are held together by hydrogen bonds. During growth of the tree, the cellulose molecules are arranged together in the form of microfibrils, which are 10–20 nm wide. Microfibrils are combined to greater fibrils and lamella (bundle of microfibrils, sometimes called macrofibrils) and they finally build up cellulose fibres. Mostly cellulose makes up the cell walls of the fibres.

Pulping is a process to separate wood fibres; it can be roughly divided into two: chemical pulping and mechanical pulping. Because a complete purification of cellulose demands a lot of energy and chemicals, there are many products in which cellulose is used only partly purified. For example, products like bleached chemi-thermo mechanical pulp (BCTMP) have been developed to

be used as a component in paper making. A characteristic property of mechanical pulp is that it retains most of the wood lignin present in the middle lamella and in the primary wall. This material that is present on the surface of BCTMP forms a physical barrier to inter fibre bonding due to the hydrophobic nature of lignin [2].

Therefore, it is essential to remove the lignin-rich material in order to fibrillate fibres and to produce e.g. nanocellulose. The hydrophobic lignin-rich material is also one of the main reasons for low inter-fibre bonding strength of mechanical pulp fibres [2]. On the contrary, when more cellulose fibrils are exposed, and more fibre separation takes place in the secondary wall, then inter-fibre bonding is likely to happen. At the same time, by removing specifically the lignin-rich material on the fibre surface it will not reduce much the yield [2].

Microfibrillated cellulose (MFC) or nanocellulose was invented by Turbak already in 1983 by using high pressure homogenizer [3]. MFC is a type of cellulose where wood pulp fibres have been rapidly expanded in surface area and opened into their sub-structural microfibrils by using chemical pre-treatment and mechanical treatment. Fully delaminated nanocellulose consists of long (1–2 μ m) microfibrils (5–20 nm in diameter) and has the appearance of a highly viscous, shear-thinning transparent gel. MFC is a material produced from a fibrillation process, which may contain fibres, fibre fragments, fibrils and nanofibrils. Various chemical pre-treatments have been reported including TEMPO-mediated oxidation, enzymatic-assisted hydrolysis, acid hydrolyses, carboxymethylation and acetylation [1, 8, 9–12]. Several mechanical devices are also used to cause cell wall delamination including high pressure homogenizer, microfluidizer, grinder, blender, PFI mill, mechanical stirrer, ultrasonication, cryocrushing, electrospinning [3–7, 11] However, when pulp fibre suspension is homogenized, it needs often several passes in order to increase fibrillation but that often leads to high energy consumption. Therefore, a combination of processes of chemical and mechanical treatments have been developed to facilitate mechanical treatment; by making fibres less stiff and cohesive it is possible to reduce

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the number of passes of mechanical treatment and therefore energy consumption [9, 12]. In MFC production values around 20,000–30,000 kWh/t are not uncommon (without pre-treatment) but while combining the mechanical treatment with certain pre-treatments, it is possible to decrease the energy consumption to 1000 kWh/t [13]. All those abovementioned methods or combination of those can lead to different types of nanocellulose materials.

MFC is a spectacular material with broad spectrum of potential applications such as paper, coatings, barriers, food, cosmetics, pharmaceuticals, and electronics. Micro- and nanofibrillated fibre bundles obtain high reaction ability. The price rises remarkably during the wood and pulp treatment processes, e.g. if wood costs \$ 0.1–0.2 /kg, then pulp \$ 0.6–0.8 /kg and nanocellulose \$ 20–100 /kg [4].

The cellulose pulp obtained from refining is generally inhomogeneous on the nano scale still mostly consisting of crystalline cellulose microfibrils but with random amorphous domains within the structure. Furthermore, the size of fibres in mechanical pulp varies on the micron scale while the size of microfibrils ranges only from 10–40 nm. The aim of fibrillating is to homogenize the size of cellulose microfibrils and the removal of the primary (P) and first secondary layer (S1) in order to expose the S2 layer [30].

During the fibrillation process the cellulosic pulp fibres undergo both internal and external fibrillation. External fibrillation refers to the peeling off of the external layers thus opening the S2 layer for further chemical, physical or mechanical treatment. The internal fibrillation loosens the fibre wall further enhancing the susceptibility of the fibre for further treatment or homogenization. However, MFC is usually obtained from different kraft pulps where lignin is already removed and S2 layer is visible which facilitates the further treatment of MFC preparation.

Cellulose is known to be insoluble in water and most of organic solvents. However, in some applications it is important to dissolve cellulose, e.g. to produce regenerated cellulose but this task is normally complicated. Cellulose cannot be dissolved easily in common cheap solvents because of its inter- and intramolecular hydrogen bonding [18]. Cellulose can be dissolved with solvent that can release effectively the interchain hydrogen bonding in cellulose [18]. Traditional dissolution methods have some important limitations, mainly in costs and environmental issues. Therefore, it is essential to use alternative methods [17].

Alkaline pretreatment is well known for its efficiency in the delignification and is relatively more environmentally friendly than most of the other bleaching methods.

Complete lignin removal is impossible to obtain with solely alkaline pretreatment due to the recalcitrant structure of lignin attached to the holocellulose matrix.

In alkaline conditions both cellulose swelling and dissolution are possible. However, they are totally different processes as the cellulose swelling maintains the gross structure of cellulose at the same time changing its physical properties and sample volume. On the other hand,

cellulose dissolution is a transition process from a two-phase system to a one-phase system (clear solution) where the original supramolecular cellulose structure is destroyed [15]. Hence, same solvents can act as swelling or dissolution agents depending on conditions and cellulose properties [15].

It has been shown that cellulose can be dissolved completely in 7 % NaOH/12 % urea aqueous solutions at -10 °C in 2 minutes [14] or in a two-step process by using first 14 wt.% aqueous and 24 wt.% urea solution at 0 °C followed by 7 wt.% NaOH and 12 wt.% urea solution [19]. However, according to Cui and Zhang [14] KOH / urea aqueous solutions do not dissolve in any concentration at low temperatures, it causes only swelling. At the same time, the optimum swelling conditions appear in 8–9 wt.% NaOH and followed by freezing into solid mass by holding it at -20 °C [16]. Moreover, by treating samples further in 5 wt.% NaOH solution at room temperature can also cause complete dissolution of cellulose [16]. It is important to know, that there is a critical temperature (T_c) for cellulose dissolution -12.6 °C (freezing point) which means that by lowering the temperature further, does not dissolve more cellulose [20].

Schwanninger et al. 2004 [22] studied the effect of vibratory ball milling in short time (up to 60 min) and analysed results with FT-IR spectroscopy, it was found that this kind of mechanical treatment has a deep influence on wood and cellulose. The most obvious changes were caused by the decrease in the degree of crystallinity and degree of polymerization of cellulose; it was less affected by temperature increase and oxidation or radical reactions.

Microfibrillated cellulose (MFC) material is commonly inhomogeneous; it might contain nanofibrils, fibrillar fines, fibres and fibre fragments. Typical lengths of pulp fibres there are 1 to 3 mm and with widths 10–50 µm, diameter of microfibrils is approximately 28 nm [23].

In this study neither a complete dissolution of cellulose nor degradation of the cellulose molecules was aimed. Despite of chosen solvents capable to dissolve cellulose, the conditions were selected so, that part of the cellulose remained in the form of native fibres. Therefore, the reaction parameters were changed compared to the complete dissolution values – the alkali concentrations were chosen less concentrated, temperature not that low and treatments were carried out in shorter period of time. The goal of the first step was simply to peel off outer cell wall layers of aspen BCTMP fibre and partially fibrillate exposed S2 layer. It was also important to save the natural crystalline form of cellulose (cellulose I). Further mechanical treatment is expected to fibrillate loosen cell wall layers and release nanofibrillar network. The ultimate aim was to get microfibrillated cellulose (MFC) by using above described alternative to well-known treatments method.

2. EXPERIMENTAL

2.1. Materials

Aspen BCTMP was obtained from Estonian Cell Company. Commercially available technical grade acetone with 99.5% purity from APChemicals was used in the

solvent exchange process. NaOH [40.0 g/mol], KOH [56.11 g/mol], analytical grade urea [99.0–101.0 %] and HCl [Mw = 36.46 g/mol, ≥ 37 %] were supplied from Sigma Aldrich. Ethanol [96.6 % vol] and acetone [95.5 % vol] were of laboratory grade and used without further purification. Distilled water was readily used from the laboratory's own distilled water system.

For mechanical treatment a vibration mill Narva Erbisdard DDR- 6M9458 with glass balls with diameter of 10.2 mm were used.

2.2. Methods

For experimental part full factorial model of experiments, where 3 variables are changed at the same time to minimize the amount of needed experiments, was used in order to find optimal conditions for fibrillation.

For chemical pre-treatment, a mixture of aqueous solutions as solvent of cellulose was prepared by directly mixing alkali hydroxide, urea, ethanol and distilled water. Alkali concentration varied from 3–7 wt.%; and urea concentration was 12 wt.% in all experiments. The amount of ethanol was kept also 20 g in each experiment, KOH and NaOH ratio was maintained 2 : 1. The reaction times were chosen 3 min and 7 min, reaction temperatures – 3 °C and – 7 °C. The mixture of desired alkali hydroxide, urea, and ethanol was pre-cooled to desired temperature and then 5 g of never-dried grinded BCTMP of aspen was added to the pre-cooled solution and stirred for desired time. After the cooling stage, sample was quickly neutralized with pre-heated 3 % HCl solution and then heated up to 70 °C, after that the sample was washed with distilled water.

In order to fibrillate aspen pulp fibres, vibration mill was used. For Vibration mill treatment, 2.4 g chemically pre-treated sample with 2 g of distilled water was put into vibration mill with 2 glass balls and milled for 30 minutes.

The most fibrillated sample after first step (cold treatment) was milled in ethanol and acetone in vibration mill by using the same conditions as mentioned above; milling without any liquid was also performed.

To carry out critical point drying of wet pulp samples, solvent exchange to acetone was carried out. Samples in water solutions were transformed into acetone using a range of acetone-water solutions (30, 50, 70, 90, 100, 100 wt.% of acetone). During each step segregation was assured by mixing in an ultrasonic bath for 2–3 minutes.

Critical point drying was conducted in the Balzers apparatus. The wood fibre mass in 100 % acetone solution was placed into the chamber which was cooled to 5–7 °C, sealed, pressured and flushed with liquid CO₂ 3–4 times. Finally, the chamber was heated up to 40 °C and gassed out. For SEM studies, dry samples were placed with fine tweezers to a metallic stub using a double-sided adhesive tape. Then the samples were placed into ion sputtering chamber, JFC-1100 for 1 min and 40 s and coated with gold/platinum layer under vacuum to enhance electrical conductivity. Coating was restricted to 1.9 kV and 10 mA. The SEM examination was carried out with EVO MA 15 at accelerating voltage 12 to 15 keV.

3. RESULTS AND DISCUSSION

To understand the influence of chemical and mechanical treatment, the samples were examined as they were; no additional sieving or fine particle separation was performed.

By changing the NaOH and KOH concentrations, treatment temperatures and treatment times, a series of NaOH/KOH/urea/ethanol solutions were tested. Different reaction conditions that were used during experiments are presented in Table 1. Although the complete dissolution of cellulose was not the aim of this study, it was also tested that BCTMP of aspen dissolves completely in conditions that have been used before [14].

Preliminary tests in our laboratory showed that NaOH caused softening and swelling of aspen BCTM fibres, KOH softening not swelling, NaOH + KOH softening + fibrillation. Therefore, in order to get well fibrillated fibres, the mixture of KOH and NaOH were used together with urea and ethanol. Ethanol prevented also the cooling of the reaction solvents.

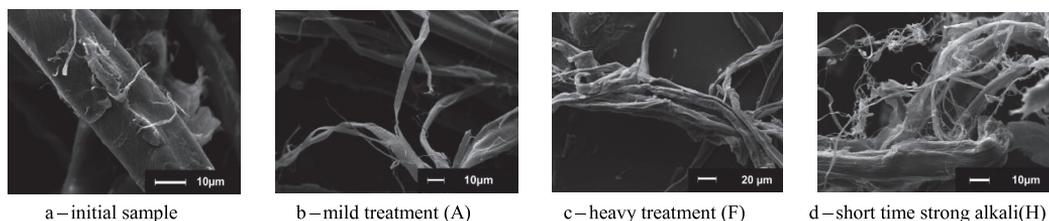


Fig. 1. The influence of cold treatment

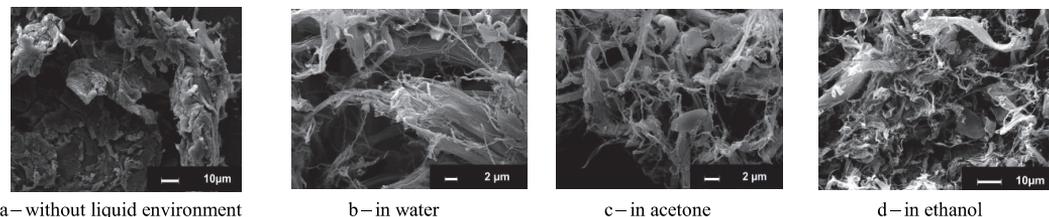


Fig. 2 The influence of mechanical treatment after the cold treatment

Table 1. Parameters of KOH/NaOH + urea solutions (edge-points of factorial model)

Exp.	Concentration of alkali, wt.%	Time, min	Temperature, °C
A	3	3	-3
B	3	7	-3
C	3	7	-7
D	3	3	-7
E	7	3	-7
F	7	7	-7
G	7	7	-3
H	7	3	-3

In initial sample, aspen BCTMP (Fig. 1. The influence of cold treatment), rectangularly shaped wood fibres were mostly unbroken, covered with P and S1 layer and remnants of middle lamella. As it can be seen from the Fig. 1. The influence of cold treatment, outer layers are strongly attached to the fibre main body. There is no sign of fine fibrillation. This is very typical morphology to mechanical pulp (including thermomechanical pulp, chemi-thermomechanical pulp, alkaline peroxide mechanical pulp and BCTMP) fibres, having outer cell wall layers and large pieces of non-fibrillar material on its surface [25, 26].

As outer layers of aspen BCTMP contain substantial amount of residual lignin and hemicelluloses that keep fibre together, then it is necessary to remove outer layers in order to further fibrillate fibres. It is essential that the cellulose-rich inner layer S2 will become visible after several further treatments and cellulose microfibrils will be separated from each other.

3.1. Influence after cold treatment

After mild treatment A (Fig. 1. The influence of cold treatment b) numerous thin but wide cellulose lamellar structures were detached from the fibre surface. Treatment of fibres with 3 wt.% of alkali at -3 °C and for 3 min (Fig. 1. The influence of cold treatment) effectively released lamellae but fine fibrillation was not present.

In conditions of heavy chemical treatment F (Fig. 1. The influence of cold treatment c), when sample was treated in hardest conditions: 7 wt.% of alkali at -7 °C and for 7 min (F, Table 1) there were no lamellas or fibrils visible. Fibres were distorted, wrinkled and the surface of fibres was partially dissolved. No fine fibrillation was present. It should consider as overtreatment but differently than it was referred in [25].

According to another edge of the factorial model sample was treated in short time (3 min) but with strong alkali (7 %) according to data in Table 1 H (Fig. 1. The influence of cold treatment d). Here, numerous bundles of microfibrils were separated from the fibre surface with the diameter of 10–50 nm and the sample showed good potential for the further treatment.

3.2. Influence after mechanical treatment

Performing vibration milling of aspen BCTMP fibres after chemical influence revealed the effect of weakening of strong intra- and intermolecular hydrogen bonding in

cellulose and lead to the formation of microfibrillated cellulose bundles. Fibre morphology revealed in SEM directly after chemical treatment allowed only following most obvious changes in fibre surface morphology but did not give information about internal changes inside the fibre cell wall. The time of mechanical treatment with vibrator mill was highly dependent on following parameters: volume of sample container, size and amount of vibrator mill balls, amount of sample and solution that was used during milling. The treatment time 30 min – was chosen experimentally as well as the amount of the sample and solution used during the experiments according to the best results achieved so far in our laboratory.

Vibration milling without any liquid additive had no influence (Fig. 2 The influence of mechanical treatment after the cold treatment a) on the formation of any fine structures. Conversely, mechanical treatment “glued” fibres together and large mass of undefined lumps appeared.

Therefore, it is reasonable to carry out milling in liquid environment, preferably in polar solvent like water, ethanol, or acetone. For this experiment the most favourable sample F (Fig. 1. The influence of cold treatment d) was chosen as it showed the best potential for further delamination by mechanical treatment. Milling in water led to the effective release of microfibrils from the surface layer of the fibre (Fig. 2 The influence of mechanical treatment after the cold treatment b). The diameter of finest fibrils was measured less than 20 nm. Also opening of the S2 layer with strongly orientated cellulose microfibrillar structure occurred. At the same time fines from the remnants of P and middle lamella stayed intact. Those fibre parameters are in good accordance with suggested MFC dimensions elsewhere [23, 24, 29, 30].

Vibration milling in the non-diluted acetone environment (Fig. 2 The influence of mechanical treatment after the cold treatment c) showed that fibrillation was not as fine as obtained with water. It is in good accordance to the results of [32]. The sample was well fibrillated but bundles of fibrils were couple of times wider compared of those obtained with the milling in water. Also some opening of S2 layer occurred. Non-orientated structures from compound middle lamella stayed intact.

An interesting effect occurred when samples were treated with vibration mill in the environment of ethanol (Fig. 2 The influence of mechanical treatment after the cold treatment d). Sample was almost all disintegrated into fibrillar bundles and fines. There were no large pieces of fibres visible. Fibrils obtained were as fine as with mechanical treatment in water. Fines from compound middle lamella were not affected by this treatment. According to the [32] the most significant changes in pine wood cell wall structures occurred with the treatment of wood with aqueous solution of ethanol, but current experiment showed the suitability of this solvent to the hardwood pulp fibres as well.

Cold treatment and vibration milling effects on cellulose have been investigated before by using instrumental methods of analysis (UV, FT-IR); those analyses have provided information about changes in cellulose crystallinity and constituents. [22] Morphology of mechanical pulp fibres have been studied by *Li et al.* (a) and (b) [25, 26] and outer cell wall removal by *Kärner et*

al. [27] but the morphological changes after cold and mechanical treatment by using SEM evaluation have not been investigated yet by other authors.

Obtained material has great opportunity to be used in different potential applications such as aerogels [28], food and emulsion/dispersion applications, medical, cosmetic, pharmaceutical, and hygiene/absorbent products, nanopaper, nanocomposites [29, 30]. Due to the very fine fibrillar structure, obtained material has relatively high surface charge and therefore high reaction activity [31].

Therefore, it could be treated with suitable additive to obtain new functional material, e. g. antibacterial aerogel, by adding silver nanoparticles.

The results are unique because other authors have used different mechanical methods for pulp treatment. The use of full factorial model is also rather new in this field.

4. CONCLUSIONS

The effectiveness of chemical pre-treatment followed by vibration milling was evaluated by the changes in fibre's morphology and fibrillation using scanning electron microscopy. Even changes after certain treatments are caused by chemical interaction on molecular structure of aspen BCTMP fibres' chemical constituents, it is possible to visualise the result of these changes by looking closely at the fibres' morphology, delamination, opening of internal layers, external and internal fibrillation.

After cold treatment fibres turned softer, middle lamella, P and S1 layers detached from the top of the fibre, but fibres basically remained their full volume.

If chemical pre-treatment is successful and S2 layer is opened (visible in SEM), there is a great potential that mechanical treatment will cause cellulosic fibres to delaminate and form microfibrillated cellulose network.

Vibrator milling caused large fibres to disappear. This resulted in appearance of microfibrils and microfibrillar bundles, bulk came more uniform and S2 layer of the cell wall was opened and fibrillated. The effectiveness of whole treatment can be readily decided after vibration milling solely by fibre morphology visualized by SEM.

Some cold treatments seemed to be rather effective (cold treatment H). Applying mechanical treatment in suitable environment after that resulted in deep delamination of the fibre cell wall. Additionally, some potential of successful mechanical treatment can be predicted based on the chemical treatment step, e. g if there are signs of overtreatment, no further mechanical treatment will help to release microfibrils.

Vibrational milling in water and ethanol lead to similar results in respect to the formation of microfibrils and microfibrillar bundles. However, milling in the ethanol environment favoured disappearance of big fibre pieces.

It was noticed during the experiments, that even though some effective treatments caused the appearance of nice micro- and nanofibres, there is always some fraction of primary wall and middle lamellae remainders that cannot be subjected to any chemical or mechanical treatment. As those parts contain more lignin than cellulose, and the cellulose macromolecular network there is not strongly

orientated, the fibrillation of these structures by current technology is not successful.

In this study, it was shown that it is possible to get MFC from BCTMP of aspen by using two-step process: chemical pre-treatment in alkaline environment followed by mechanical treatment with vibration mill. Resulting material contained very fine microfibrillar bundles; it was relatively uniform and promising alternative to MFC made from kraft pulp.

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Paper IV

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Research Article

CONTROLLED NANOCRYSTALLINE PRECIPITATION OF HYDROXYAPATITE ON THE SURFACE OF MICROFIBRILLATED CELLULOSE FIBERS

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ABSTRACT

In this study hydroxyapatite nanocrystals were synthesized on the surface of microfibrillated cellulose (MFC) fibers by using a wet chemical precipitation technique. MFC was prepared from bleached chemi-thermomechanical pulp (BCTMP) of aspen. KOH was used for pH regulation during apatite synthesis at 60 °C. Samples were dried under supercritical CO₂ and analyzed by scanning electron microscope (SEM). Measurements of surface area and surface charge, x-ray diffractometry and chemical analyses were performed. It was found that about 11 wt % of apatite is optimal dose for complete covering of the surface of MFC fibers. Synthesized apatite had Ca/P ratio of 1.6, which is (XRD) close to that of the stoichiometric ratio of hydroxyapatite.

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INTRODUCTION

Hydroxyapatite (HAp), also known as hydroxylapatite, Ca₁₀(PO₄)₆(OH)₂, is the major component of bone mineral and tooth enamel; it is an important inorganic material in biology and chemistry. HAp has outstanding properties concerning its medical uses. It is biocompatible, bioactive, non-toxic and non-inflammatory (Demirchan *et al.* 2012). That is why it has found applications in bone tissue engineering as well as in drug and gene delivery. Many other potential applications were reported basing to the absorptive properties of HAp. Those include absorption of CO (Nasr-Esfahani *et al.* 2012) and absorption of fluorine (Pandi *et al.* 2014).

Cellulose is the most abundant organic material and also an applicable material for making organic-inorganic hybrid materials. It is also known to be renewable, biodegradable and non-toxic (Klemm 2011). In recent years, cellulose-HAp composites have gained much attention. Cellulose-HAp composites have great potential for application in tissue engineering or bone regeneration. Cellulose and apatite mixtures have also been used for making scaffolds (Müller *et al.* 2006). Biomedical applications of HAp covered cellulose

use often bacterial cellulose (Nge *et al.* 2006, Zimmermann *et al.* 2011) or modified celluloses (Petrauskaitė *et al.* 2013) and bio-mimetic conditions for precipitations (Nge *et al.* 2006, Qu *et al.* 2012).

The most popular and widely explored technique for synthesis of HAp is the precipitation technique. This technique is also called as wet precipitation or chemical precipitation or aqueous precipitation and has chosen widely to synthesize HAp in contrast to other techniques (Nayak *et al.* 2010, Santos, *et al.* 2004, Markovic, *et al.* 2004). The important parameters for wet methods are pH (ranging from 6 to 11), temperature (ranging from room temperature to 200°C) maturation time and temperature. Composition and concentration of the reactants and their mixing rate are used to control the precipitation process (Santos *et al.* 2004, Ibrahim *et al.* 2000, Ferraz *et al.* 2004).

In our recent work was reported a partially nano-fibrillated cellulose product made of bleached chemo-thermo-mechanical pulp (BCTMP) (Kärner *et al.* 2016). Now our interest was to examine if that product is a suitable support for HAp. We know that our cellulose has negative surface charge (Kärner *et al.*

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2014) and high specific surface area. These two properties should be beneficial for growing HA on the material.

The electrokinetic properties of cellulose fibers are determined by the chemical composition of the fiber surface, the presence or absence of surface functional groups, the fiber porosity and the swelling behavior. Wood pulp fibers are negatively charged at all pH values due to the presence of acidic groups (carboxyl, sulphonic acid, phenolic or hydroxyl), which either derive from cell wall constituents or are introduced during pulping and bleaching of fibers.

HAp surface has both positive and negative ions such as Ca^{2+} and PO_4^{3-} , which can electrostatically bind with basic and acidic macromolecules (He *et al.* 2012) depending on the pH value of environment.

In cellulose the charged groups are located either on the fiber surface or inside the cell wall, therefore, they are referred to as surface and bulk charges. The number of charged groups depends on the origin of the fiber and on the chemical and mechanical treatment during pulping and refining. Surface charges influence the fiber-fiber bonding and interactions with high molecular mass additives. Surface charge of cellulosic fibers increases with increasing refining (beating) (Banavath, *et al.* 2011, Bhardwaj, *et al.* 2014, Bhardwaj, *et al.* 2006).

Hydroxyl groups are anionic functional groups with high affinity for Ca^{2+} . It has been stated that cellulose hydroxyl groups possess a strong negative dipole that could chelate free Ca^{2+} in the CaCl_2 solution and form a coordinated bond, after which PO_4^{3-} could bond with the cellulose-associated calcium (Farbod, *et al.* 2014).

The concept of apatite formation on the bioactive materials generally includes twofold: the existence of the surface functional groups (hydroxyl and carboxyl groups in this study) that induce the heterogeneous nucleation of apatite, and the increased super saturation of the surrounding fluid that accelerates the nucleation process and growth (Nge *et al.* 2007).

It has been reported that calcium ions initiate a heterogeneous nucleation of HAp formation on the negatively charged surface (Morimune-Moriya, *et al.* 2015).

A well-known method for surface charge determination is polyelectrolytic titration; this method was pioneered by Terayama (Terayama *et al.* 1952) who determined the concentration of a cationic polyelectrolyte solution by titration with an anionic polyelectrolyte in the presence of a cationic indicator. Surface area of nanocellulose, as well as of nanoapatite, is strongly dependent on the drying methods and the solvent exchange. The latter gives surface areas about twice compared with regular freeze drying (Jin *et al.* 2004).

Many sophisticated works have been published about the covering of cellulose scaffolds with Hap (Petrauskaitė *et al.* 2013, Zimmermann, *et al.* 2011, Wei, *et al.* 2004, Beladi *et al.* 2017). However, direct wet precipitation conditions are seldom used. In this study, MFC from BCTMP of aspen was prepared and different amounts of apatite were synthesized on the surface of cellulosic fibers. Our intention was to test how our aspen pulp based nano-fibrillated cellulose can be coated by

HAp. The whole process, including the production of MFC from BCTMP of aspen, was carried out in an environmentally friendly and cost-effective way. It was demonstrated that the deposition of HAp on aspen pulp derived nano-fibrillar cellulose is possible by a rather straightforward controlled nanocrystalline precipitation.

MATERIALS AND METHODS

Materials

Bleached chemo-thermo-mechanical pulp (BCTMP) of aspen was obtained from Estonian Cell. Aspen BCTMP was used as never-dried. NaOH, KOH, analytical grade urea and HCl were supplied from Sigma Aldrich and were used for chemical treatment of BCTMP. Polydiallyldimethylammoniumchloride (p-DADMAC) was used as cationic polyelectrolyte and potassium hydroxide (KOH) as titrant. P-DADMAC was supplied from Sigma Aldrich as a 20 wt% concentrate. Mw of p-DADMAC was 100000-200000 g/mol. Commercially available technical grade acetone with 99.5% purity from APChemicals was used in the solvent exchange process. $\text{Ca}(\text{NO}_3)_2$, H_3PO_4 , N_2 , He and ethanol were of laboratory grade. Ethanol [96.6 % vol] and acetone [95.5 % vol] were of laboratory grade and used without further purification. Commercially available technical grade CO_2 with a purity of 99.7% from AGA was used in the supercritical CO_2 drying process. Distilled water was readily used from the laboratory's own distilled water system.

Equipment

For chemical pretreatment of BCTMP fibers commercially available disperser T 25 digit Ultra Turrax from IKA laboratory equipment was used. For mechanical treatment of MFC a vibration mill Narva Erbisdard DDR- 6M9458 with glass balls with diameter of 10.2 mm were used. For apatite synthesis, Mettler Toledo T90 titrator was used as a pH controller and two Watson Marlow Sci 323 peristaltic pumps were used for calcium nitrate and phosphoric acid additions. Critical point drying was conducted in the Balzers apparatus. Mettler Toledo T90 titrator was used for surface charge measurements; it consists of a titrator with terminal, pH board, integrated burette drive and magnetic stirrer. It is expandable with two sensor boards (pH and/or conductivity) and up to seven additional burette drives (for dosing and titration purposes). For surface area measurements a sorptometer Kelvin 1042 built by Costech International was used. SEM studies were carried out with EVO MA 15 at accelerating voltage 12 to 15 keV. X-ray diffraction (XRD) patterns of cellulose and HAp were recorded by using a Rigaku Ultima IV diffractometer with monochromatic $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$, 40 kV at 40 mA). Data were gathered in the 2θ range of 10° - 50° with scan speed of $2^\circ/\text{min}$ and scan step 0.02° using the silicon strip detector D/teX Ultra. The X-ray diffractograms were analyzed using the software on the Rigaku system (PDXL 1.4.0.3).

Methods

Microfibrillated cellulose from aspen wood pulp was obtained by using the same methodology as described in our previous article (Kärner, *et al.* 2015).

For apatite precipitation the $\text{Ca}(\text{NO}_3)_2$ and H_3PO_4 solutions (0.01 M) were added simultaneously into MFC-water slurry (0.1 L, pH=9.7) at rate of 2.0 and 1.2 ml/min, respectively, by peristaltic pumps, keeping Ca/P mole ratio equal to 1.67. The precipitation reaction can be written as:



pH of the synthesis environment was controlled by the titrator with electrode DG-112 Pro (pH stating using KOH 0.01M), calibrated with Mettler Toledo pH buffer solutions (4.01;7.00; 9.21) under N_2 flow. The temperature was maintained at 60 ± 2 °C. The obtained suspension was stirred for 1 hour at 60 °C and matured for 24 hours at room temperature. Apatite was precipitated in various concentrations; the concentration of apatite was controlled by the addition time of the solutions: 1 min, 2.5 min, 5 min and 10 min.

Ca and P content of the apatite samples were determined by standard analytical methods. Content of Ca was determined after thermal oxidation of MFC and dissolution of the residue in diluted HCl by AAS (Varian SpectraAA 55B Flame AAS) and phosphorus by photo colorimetry method as phosphomolybdate yellow complex (Biochrom Libras 70PC UV/Visible Spectrophotometer).

Apatite precipitation process on MFC is described in Figure 1.

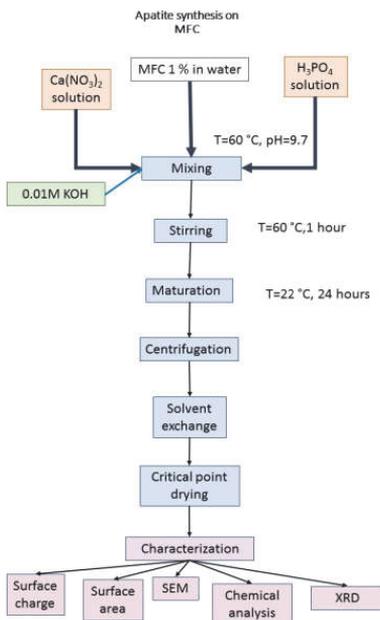


Figure 1 Apatite precipitation process and characterization methods

Surface charge was measured by using the same method as in our earlier work (Kärner *et al* 2013) by using 1 wt % p-DADMAC solution and 0.06 M KOH to find equivalence (end) point. After that surface charge was calculated according to Bhardwaj *et al*. 2006.

Specific surface area (SSA) measurement was performed using a Sorptometer KELVIN 1042. Helium was used as the carrier gas and nitrogen as the adsorptive gas. The specific surface area (SBET) was calculated according to the Brunauer-

Emmett-Teller (BET) theory. 0.02-0.06 g of MFC sample was degassed at 105 °C for 22 min to 169 min prior to the analysis followed by N_2 adsorption at -196 °C. BET analysis was carried out for a relative vapor pressure of 0.01-0.3 at -196 °C.

RESULTS AND DISCUSSION

Specifications of the samples are listed in Table 1. BCTMP of aspen was our initial sample without any treatment. MFC is chemically and mechanically treated BCTMP.

The results of specific surface area measurements can be seen from Figure 2. Apatite was precipitated on MFC samples 1 min, 2.5 min, 5 min and 10 min, leading to concentrations of 0.64 wt %, 1.32wt %, 3.4 wt% and 11.1 wt% of apatite respectively.

Table1 Samples and their apatite contents

Sample	Sample name	Apatite concentration, weight%
1	BCTMP	0
2	MFC	0
3	MFC HAp 1 min	0.64
4	MFC HAp 2.5 min	1.32
5	MFC HAp 5 min	3.4
6	MFC HAp 10 min	11.1

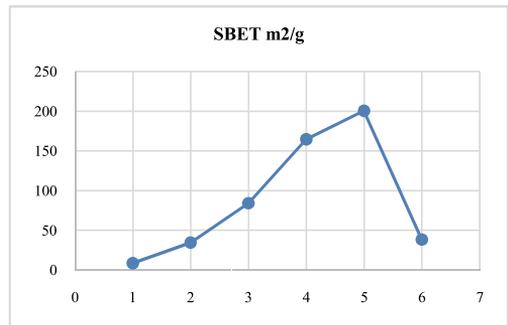


Figure 2 Surface area of MFC and apatite on MFC samples, listed in Table 1

The specific surface area determined by BET was 11.9-200.6 m²/g. Surface area value increases in the beginning of apatite precipitation due to additional surface between cellulose fibrils. As it can be seen from

Figure 2, surface area increased continuously until 5 min (3.4 wt %) of apatite precipitation. The maximum BET surface area value for MFC-HAp mixture (3.4 wt% HAp) was 201 m²/g.

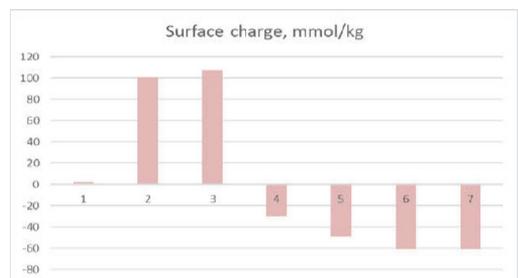


Figure 3 Surface charge of cellulose and apatite samples: 1) BCTMP, 2) chemically treated BCTMP, 3) MFC (chemically and mechanically treated BCTMP), 4) MFC with 1 min HAp precipitation; 5) MFC with 2.5 min HAp precipitation; 6) MFC with 5 min HAp precipitation; 7) MFC with 10 min HAp precipitation.

When the cellulose surface was totally covered by apatite (11%) the surface area value dropped.

Surface charge of MFC-apatite samples (Figure 4) is quite small for untreated BCTMP of aspen. However, it is in good correlation with our previous studies, where surface charge of initial sample (BCTMP) was also very low, 5.78 mmol/kg (Kärner, et al 2014).

An upsurge in the surface charge occurred after chemical treatment of BCTMP. The increase in surface charge continued after mechanical treatment. These were quite expected results, as there are more fine particles after chemical and mechanical treatment and they are responsible for higher surface charge values (Bhardwaj et al. 2006, Banavath, et al. 2011).

Surface charge decreased after adding of apatite, moreover, the charge turned negative and continued to decrease as apatite concentration increased. It can happen because of bonding of all available cellulose charged particles to apatite structure and carrying out the synthesis in basic environment (pH=9). Apatite itself has a negative charge, when pH is above 7. As our surface charge measurement method is not completely same as the conventional polyelectrolytic titration method (Horvath, et al. 2006, Bhardwaj et al. 2006, Banavath, et al. 2011), the results of surface charges measurements are specific only for the comparison of our samples.

The diffraction patterns of unmodified MFC, with precipitated and pure HAP are shown in Figure 3. Three peaks on curves a, b, and c at $2\theta = 15.1, 16.7, 22.5$ deg confirmed that only cellulose I is present in the sample (Lu and Hsieh 2010), and the basic environment of HAP synthesis has not changed the crystalline structure of the sample. Weak characteristic to HAP (curve d) diffraction peaks at $2\theta = 25.88, 31.88$ and 40 deg in curves b and c evidence the precipitation of HAP particles onto the surface of cellulose fibrillated structure (there was no any loose precipitated HAP particles in the reaction volume).

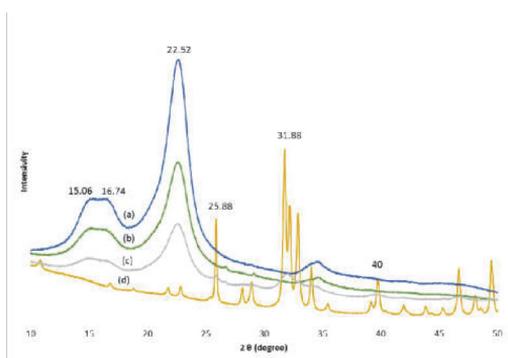


Figure 4 XRD of (a)starting MFC; (b)HAP1min HAP precipitation; (c)HAP10min HAP precipitation; (d)pure HAP

The chemical composition of precipitated HAP was calculated from the mean values of Ca and P contents. The calculated Ca/P molar ratio was 1.60, and is close to the stoichiometric value of 1.67.

Figure 5 shows the microstructure of initial sample, industrial BCTMP of aspen. It is a typical mechanical pulp showing diverse constituents of this kind of wood pulp. There are large

fibers as well as small particles: fines, fiber fragments, fibrils, lamellas. The most characteristic to any mechanical pulp is the presence of remnants of compound middle lamella easily recognizable by its rectangular shape. The surface of fibers is not showing particular fibrillation. For this sample the surface area and surface charge were both relatively small: $8.6 \text{ m}^2/\text{g}$ and $2.5 \text{ mmol}/\text{kg}$, respectively.

In Figure 6 the microstructure of chemically and mechanically treated BCTMP microfibrillated cellulose is shown. There is much more of fine structure visible compared to BCTMP. Effective chemical and mechanical treatment liberated a part of remnants of compound middle lamella, primary wall and S1 layer exposing S2 layer of wood fiber with its characteristic parallel fibril orientation under a low angle toward fiber axis. Additionally, numerous fine fibrils have been released from the fiber's surfaces. The material is enough fibrillated to be a good starting point for precipitation of additives onto the MFC surface. Measurements of surface area and surface charge ($34.5 \text{ m}^2/\text{g}$ and $108 \text{ mmol}/\text{kg}$, respectively) showed potential for a good bonding between fibers and other additives.

On the next figures (Fig. 7, 8, 9, 10) gradual attachment of Hap precipitated crystals is demonstrated. After 1 minute HAP precipitation (Fig.7) with the concentration of 0.64 wt %, the occurrence of HAP crystals is hardly visible and the crystals are very minute. Even though the concentration of apatite was relatively slow, surface area has increased to $84.2 \text{ m}^2/\text{g}$, but surface charge went slightly negative reaching the value of $-30.4 \text{ mmol}/\text{kg}$.

In the next Figure 8 when precipitation lasted for 2.5 min, numerous HAP crystals and agglomerates are visible attaching the surface quite regularly. HAP tends to form agglomerates rather than single crystals. The distance between apatite structures is about 200-300 nm, with crystal size approximately of 70 nm. Surface area has even more increased up to $164.8 \text{ m}^2/\text{g}$, but surface charge continued to drop to the value of $-49.3 \text{ mmol}/\text{kg}$ (Fig.4).

The precipitation time of 5 min did not make major difference in the visible settlement of HAP crystals onto the surface of cellulose but the surface area continued to grow to the maximum value $201 \text{ m}^2/\text{g}$, and surface charge decreased continuously to the value of $-60.9 \text{ mmol}/\text{kg}$.

A drastic change occurred after 10 minutes of HAP precipitation onto the MFC (Figure 9).

The surface of MFC is totally covered with very thin but airy apatite layer. Apatite crystals and agglomerates were sparsely distributed covering the surface excellently. Airy layer formed due to the partially released but still attached to the surface nanofibrils of cellulose where HAP crystals and agglomerates were attached. After last precipitation experiment, surface area dropped sharply to the value of $38.4 \text{ m}^2/\text{g}$ and surface charge remained almost the same compared with 5 minutes of apatite precipitation, to the value of $-60.9 \text{ mmol}/\text{kg}$.

This confirms that for our MFC product, 10 minutes of apatite precipitation is the optimum time to get primarily bound HAP crystals to make use of the surface charge of cellulose. It is not reasonable to add more apatite, because the active surface is already covered and further process will be as a sole

precipitation of apatite from the solution. It has been proved before, that there are accessible primary hydroxyl groups in cellulose that only take part in ion-ion reactions with HAp, the others (inaccessible hydroxyl groups) do not take part to it (Nge *et al.* 2006). It was shown in this experiment, that after 10 minutes of precipitation (11.1 wt % of HAp) those accessible hydroxyl groups were bonded with apatite, as the surface of MFC was totally covered with thin airy layer of HAp crystals. If the precipitation time is further increased, the apatite layers start to overlap with each other as there is no vacant area on MCF surface. It is also likely that some HAp might fall off from the surface. In all of our experiments, the maturation time after apatite precipitation was only 24 hours, therefore HAp crystals are not very large, longer time of maturation (as used earlier by Nge *et al.* 2006, Morimune-Moriya *et al.* 2015) would have probably caused bigger crystals.

CONCLUSIONS

In this study, HAp was successfully incorporated into the MFC matrix by a wet chemical precipitation technique. Synthesized samples were characterized by using SEM, surface area and surface charge measurements, XRD and chemical analysis. Good bonding between apatite and cellulose surface was established. SEM micrographs showed that the precipitated HAp may consist of crystallites with the size of 1-1.5 μ m. To the end of experiment cellulose fibers were completely covered with fine airy layer of apatite crystals in 10 minutes (11.1 wt % of apatite). This can also be considered as optimal time or an amount as after that concentration apatite will not precipitate on the surface of cellulose but starts to form several apatite layers on top of each other. Surface charge was also small for initial sample, but started to increase after chemical and mechanical treatments.

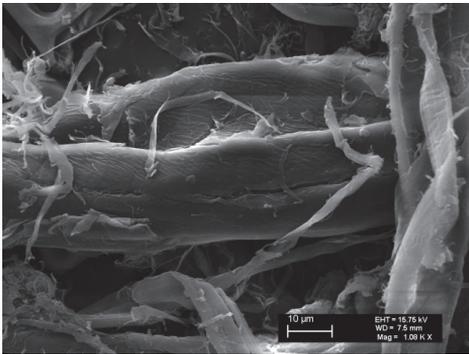


Figure 5 BCTMP

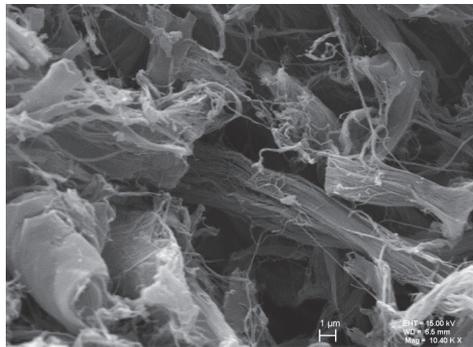


Figure 6MFC

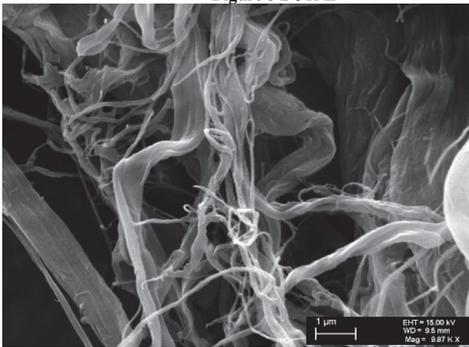


Figure 7 HAp 1 min

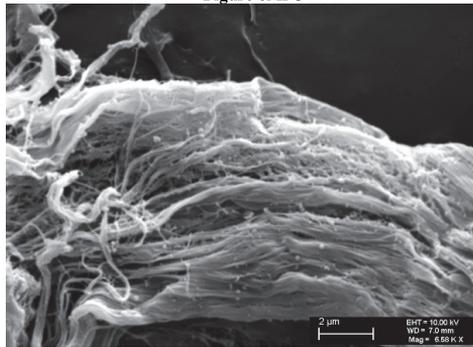


Figure 8 HAp 2.5 min

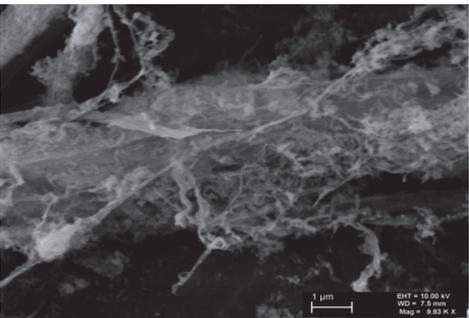


Figure 9 HAp 10 min

Surface charge of the samples with the precipitated HAp depend on the amount of apatite bound and dropped as the concentration of apatite increased.

XRD analyses showed that the crystallinity of cellulose did not change during the treatments. On grounds of the chemical analysis the formed apatite had a Ca/P ratio 1.6, which is close to stoichiometric hydroxyapatite (with Ca/P ratio 1.67).

The resulting material can be assumed to be potentially biocompatible, non-toxic, have interesting adsorption properties and economical price. However, further research is needed for assessment of possible applications.

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