A Novel Nano Cellulose Preparation Method and Size Fraction by Cross Flow Ultra-Filtration

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Abstract: A novel energy-efficient method called nanopulping (patent pending) to produce nanocellulose from chemical pulp, and a novel cross-flow ultra-filtration method to separate microfibrils fractions of different size were applied in this study. Pretreatment with endoglucanase or 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) oxidation seems to enhance the nanopulping process. Results were evaluated with atomic force microscope and ultrafiltration. The nanopulping produced a relatively inhomogeneous material with larger particles/fibers in addition to nanofibers. However, by ultrafiltration of the material it was possible to obtain more homogeneous material in different dimensions with methods industrially acceptable.

Keywords: Cross flow ultra-filtration, Endoglucanase pretreatment, Microfibrillated cellulose, Nanocellulose, Nanopulping, TEMPO pretreatment.

1. INTRODUCTION

Cellulose is interesting as a sustainable material due to its biodegradability and that it originates from renewable resources. There are different sources for cellulose such as trees, cotton, tunicates and bacterial cellulose, but the property of cellulose varies depending on sources and production methods. The traditional ways to utilize cellulose technically have been as “fibers”, i.e., more or less intact cell walls with lengths from approx. 0.5 mm to several centimeters for instance in paper board and textiles. Another usage has been totally released polymers of cellulose fibres for cellulose derivatives, such as cellulose acetate. During the last decades, however, there has been an interest in producing and using cellulose as nanosized fibers. This nanocellulose is interesting due to the high surface to volume ratio and high specific strength and might be useable in composites, foams, strong films and many other applications. Nanocellulose is divided into three types: microfibrillated cellulose (MFC), nanocrystalline cellulose (NCC), and bacterial nanocellulose (BNC) [1]. MFC has an average diameter of 5-60 nm and a length of several micrometers [1] and has interesting properties which can be utilized in different end products. During the last years the numbers of articles and patents concerning microfibrillated cellulose have increased [2]. NCC was discovered as rodlike particles in 1949 [3] and is relatively smaller than MFC but still technically interesting and is sometimes referred to as cellulose whiskers or cellulose nanoparticles [1].

Around the beginning of 1980s the first work about nanocellulose from wood was published, in 1983 the first articles was published [4, 5] and patents were issued in 1982 and 1983 [6-8]. It was a method where pulp was treated in several passes through a homogenizer with a high pressure drop [4, 5]. Since then other mechanical treatments have been tested. Super-grinding is a method where natural fibres pass a special designed grinder in cycles to produce MFC [9]. Grinding was also used in a study by Abe et al. [10], where they produced MFC from chemically treated wood powder. Grinding has also been used after homogenization to produce MFC from kraft pulp fibres [11]. By refining kraft pulp to different degrees before homogenization, Nakagaito and Yano [12] showed that MFC with different properties are created. In 2004 Zimmerman et al. [13] reported the use of microfluidizer for microfibrillation of sulphite pulp. Cryocrushing was a method developed in 2005 by Chakraborty et al. [14], where wood pulp was refined and placed in liquid nitrogen and then subjected to crush with mortar. Beads have been used to microfibrillate cellulose particles [15], likewise another apparatus with spherical elements was addressed to work as for production of MFC [16]. Furthermore ultrasonic treatment on natural fibres in water produces MFC according to Zhao et al. [17]. In 2011, Chen et al. [18] chemically treated sieved wood powder before ultrasonification and recived cellulose nanofibres of 5-20 nm in with.

The problem with producing nanocellulose commercially has been the high energy-input needed in order to receive a homogeneous product. The energy input to produce MFC can be between 20 000-30 000 kWh/t [2]. Different attempts have been made trying to solve the energy problem. Chemical pretreatment, refining and enzymatic pretreatment have been used in combination or as single stage treatments to break up the structure of the raw material. This aims at making the technique for producing microfibrillated cellulose less energy demanding.

It has been shown that by including a hydrophilic additive in the liquid before adding the cellulose pulp e.g. carboxymethyl cellulose, the fibrillation was improved. Moreover, it was stated that it
was necessary to use a liquid that swells the cellulose, for getting significant microfibrillation [6]. Some different pre-treatments have been tried such as oxidation and enzymatic treatment [19-21]. In 2006, Saito et al. [19] reported a new way to produce MFC with the use of 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) oxidation on never-dried cellulose (i.e. produced cellulose pulp that never has been dried after production). By introducing negative charge on the cellulose the swelling of cellulose increased and it was possible to produce MFC with a last step of mechanical treatment. In 2007 Henriksson et al. [20] showed that the use of enzymatic hydrolysis with endoglucanase enhanced the separation of microfibrills done by mechanical treatment. Compared to acid hydrolysis as a pre-treatment the former resulted in higher aspect ratio of the MFC. Enzymatic pre-treatment to produce MFC was also done by Pääkkö et al. [21]. By using dissolving pulp in a chemical treatment step before homogenization in a microfluidizer, Ankerfors and Lindström have produced MFC with an energy consumption of 1000-1500 kWh/t [22, 23].

Although repeated homogenizations produce a relatively homogeneous nanocellulose, other more energy efficient preparation methods for nanocellulose might produce more inhomogeneous material. This can be a drawback for certain applications, but on the other side might fractions with somewhat larger particles/fibers have interesting properties for some applications. Therefore are efficient techniques for size fractionation of nanocellulose required. Recently, Madani et al. [24] presented a study where they separated microfibrilated cellulose into fractions with different length by different methods involving hydrocyclone, pressurized screening or centrifugation in a viscoplastic carried fluid. It was shown that the size of the nanocellulose had importance for its effect as wet end additive to paper. Different attempts have tried by other researchers trying to produce low cost homogenous nanocellulose, but further technical progresses can be done to widen the way of producing nanocellulose. In this work, we present two technical inventions for commercially feasible ways to produce nanocellulose, firstly nanopulping, a novel and energy efficient way to disintegrate pulp into nanocellulose based on an intensive acceleration of the fibers in a flow in combination with a sudden pressure release, and fractionation of the nanocellulose into several fractions using a one-step cross flow ultra-filtration.

2. MATERIAL AND METHODS

2.1. Materials

Three softwood kraft pulps were supplied from Metso Paper (Sundsvall, Sweden). Two samples were ozone treated and one sample was a reference pulp i.e. not treated with ozone. The two ozone treated pulps was made from oxygen delignified softwood pulp with original kappa number 10.0. Two different ozone dosage was used, 1 and 5 kg/odt pulp, which gave a kappa number of 8.7 and 4.5 respectively. The reference pulp was washed before use. The ozone treated samples were used without washing.

Monocomponent C-type endoglucanase labeled as Novozyme 476 was kindly provided from Novozyme ( Bagsvaerd, Denmark), and used without further purification. The cellulolytic activity was determined by the manufacturer and was expressed in Endo Cellulase Units (ECU) per unit mass of material. The enzymatic activity of the endoglucanase was 5000 ECU/g. TEMPO ((2,2,6,6-Tetramethylpiperidin-1-yl)oxyl) was purchased from Aldrich-Sigma (Stockholm, Sweden). All other chemicals were of analytical grade.

2.2. Pretreatment of the Reference Pulp

**Tempo pretreatment** 0.075 g TEMPO was subjected to ultrasonication (150 W and 50 Hz at room temperature) in 50 ml deionized water for 30 minutes to achieve a uniform solution, and then the TEMPO solution was combined with 0.75 g sodium bromide (NaBr) dissolved in 100 ml deionized water. The TEMPO/NaBr mixture was added to 30 g dry weight reference pulp suspended in 2 L deionized water. The TEMPO-mediated oxidation of the pulp slurry was started by adding 120 mL 10 % NaClO at room temperature. The pH was controlled at 10.5 by adjusting with sodium hydroxide solution. The pH was monitored every 30 minutes for two hours. Once the TEMPO treatment was finished, the fibrous TEMPO-oxidized product was washed thoroughly with water in Büchner filtration.

**Enzyme pretreatment** 0.165 g monocomponent endoglucanase was added to 200 mL phosphate buffer (pH 7, 11 mM NaH 2PO 4 and 9 mM Na 2HPO 4), and 30 g dry weight reference pulp. The pulp and enzyme were mixed thoroughly and put in a heat-resistant plastic bag, and then submerged in a 50 °C water bath for one hour. The sample was kneaded every 15 minutes to obtain a homogeneous distribution of the enzyme. After incubation, the sample was washed on a Büchner funnel with hot deionized water at 100 °C, and submerged in a 100 °C water-bath for 20 minutes to deactivate the enzyme. The treated pulp was then filtered in a Büchner funnel and washed with deionized water.

2.3. Nanopulping Procedure

All samples were nanopulped, i.e., the reference pulp, two O3 treated pulps, the TEMPO treated pulp and the endoglucanase treated pulp. For the nanopulping procedure, 1000 mL suspension with 1 g dry content of each sample was contained in individual autoclaves. The special-made autoclave lid was carefully closed and tightened to avoid any leakage before it was put in the glycol bath. The autoclave heater was set to 170 °C, and the temperature during use was 168-171 °C. The autoclave was revolving inside the oil bath for 12 minutes. Then the autoclave was mounted in the nozzle of the Nanopulper, i.e. a cyclone for collecting the material, and the valve was opened to ~25 % for 10 seconds, after which the valve was opened to ~60 % for another 10 seconds and finally the valve was completely opened, until the high pressure noise was gone, 5-20 seconds. The sample was finally collected and stored in a cold room with a temperature of 4 °C before ultrafiltration. See Fig. (1).

![Fig. (1). Picture and scheme of nanopulping equipment.](image-url)
2.4. Ultrafiltration Process

To separate the different sizes of nanopulped material, an ultrafiltration set with a feed tank, a gear pump, a cross-flow filtration unit and a ceramic membrane was used. The ceramic membranes had a total filter area of 816 cm² and a total volume of 0.76 L. The cross-flow separation unit was operated in the batch mode, and the retentate was recirculated to the feed tank while permeate was continuously drained (Fig. 2). 5 L nanopulped sample was first homogenized through the system by running the pump with the pressure valve completely open. The pressure valve was closed until the pressure reached 0.5 bar. The permeate drain-valve was opened to collect the filtrated sample. The valve was left open until 1 L of filtration permeate was gathered, due to the dead volume ~4 L in the tank. Towards the end of the filtration when the rate of permeate was slowing down the pressure was increased to 1 bar. At the end of each run the membrane was washed with water again to ensure that the starting flow rate was the same. The membrane was kept submerged in deionized water cleaned to avoid fibers from drying inside the membrane resulting in a worse permeate rate. At the beginning of each run, the membrane was washed with water again to ensure that the starting flow rate was the same.

Membrane Permeate rate, the rate of the concentration of the product in the permeate to the concentration of the product in the original sample, was calculated as following:

\[
\text{Membrane Permeate rate} = \frac{\text{Conc. of Permeat}}{\text{Conc. of NP}} \times 100\%
\]

2.5. Atomic Force Microscope Characterization

MultiMode atomic force microscope (Bruker Instruments, USA) with silicon etched cantilevers (Ultralevers, Park Scientific Instruments) and a high aspect ratio tip (typical radius of curvature 10 nm) were used to image the surface of nanofibrillated cellulose in tapping mode at room temperature of 25 °C in an ambient atmosphere. Several images were obtained in one sample and each image consisted of 512 lines with 512 points per line collected at a scan rate of 1 Hz with 5 μm scan size. The set point amplitude ratio (operational amplitude to free amplitude) was fixed around 0.9. Nanoscope IIIa was employed as scanning probe microscope controller. The data was processed with Nanoscope 5.3 software.

3. RESULTS AND DISCUSSION

3.1. Energy Consumption for Nanopulping

In the Table 1 below the pulp at a consistency of 1 % by weight is heated from the pulp mill’s system temperature of 90 °C to 160 °C. This gives an energy consumption of 2,7356 GJ/t or 760 kWh/t. It is possible to recovery some of the energy by flashing from 160 °C to 130 °C resulting in a total energy consumption for the process of 1,607 GJ/t or 446 kWh/t. This can be compared with the values reported by Ankerfors and Lindström et al. of 1000-1500 kWh/t to produced MFC [22, 23].

<table>
<thead>
<tr>
<th>Pulp mill system temp</th>
<th>Temp of nanopulp</th>
<th>Cp water</th>
<th>Cp fiber</th>
<th>Pulp consistency</th>
<th>m (water)</th>
<th>m (fiber)</th>
<th>q (water)</th>
<th>q (fiber)</th>
<th>q (total) without heat recovery</th>
<th>hₜ (steam, 12bar)</th>
<th>hₜ (liq, 160 °C)</th>
<th>dH</th>
<th>Steamconc.</th>
<th>Energy recovery 160°C to 130°C</th>
<th>Total energy consumption = q(total)-energy recovery 160°C to 130°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 °C</td>
<td>160 °C</td>
<td>418 kJ/kg/°C</td>
<td>1.46 kJ/kg/°C</td>
<td>10 %</td>
<td>9 m³/BTD</td>
<td>1 BDT</td>
<td>2633400 kJ</td>
<td>102200 kJ</td>
<td>2735600 kJ = 2.7356 GJ corresponding to 760 kWh/t</td>
<td>2784 kJ/kg</td>
<td>675.5 kJ/kg</td>
<td>2108.5 kJ/kg</td>
<td>1297 kg/BTD</td>
<td>1128600 kJ = 1.1286 GJ</td>
<td>1.607 GJ corresponding to 446.4 kWh/t</td>
</tr>
</tbody>
</table>
3.2. Effect of Different Pretreatment Methods

From the concentration of samples after nanopulping, we can see that the concentration was reduced, which means some fibers were lost during the nanopulping procedure. Comparing the permeate rate of ozone treated pulp with reference pulp, the permeate rate was reduced instead of increasing. According to a study on ozone modification of lignin in alkali-fiberized wood [25] ozone is a selective oxidant, with lignin being preferentially degraded and solubilized. According to that study there was no evidence for carbohydrate chain degradation in ozonated pulp. From the results in Table 2 it is assumed that the ozone pretreatment played a role of bleaching by delignification, but this pretreatment was not enough since the fiber was not converted into smaller fibrillar fragment when nanopulped. From the data, we can see the ozone treatment is not a suitable pretreatment for preparation of nanofibrillated cellulose.

The monocomponent endoglucanase treatment showed an increase in permeate rate with 27 % compared to the 22 % from the reference, thus making this the only pretreatment that showed an actual increase in fibrillation after being nanopulped. Monocomponent endoglucanase can be used to increase the reactivity and accessibility of dissolving pulp in the viscose process [26]. After the endoglucanase pretreatment, the surface structure of the cellulose was more accessible, through which the nanopulping efficiency was increased. More nanofibrillated cellulose was formed and the permeate rate was increased.

The low permeate rate of the TEMPO-treated sample is still somewhat troubling when comparing the results that is obtained from experiments using a TEMPO oxidized pulp in the homogenizer shows huge increase in fibrillation [27], and it seems as if TEMPO treatment needs to be combined with other pretreatments in order to increase fibrillation for nanopulping. Future studies on the reason behind the extremely low permeate rate would be of interest.

3.3. Separation of Different Fractions by Ultra-Filtration

The nanofibrillated cellulose suspension was run through a ceramic membrane tube with certain pore size under high pressure. The suspension circulates trough the tube and the high pressure force the smaller fibers and water to pass through the membrane, resulting in a permeate of fibers with the desired size. The size of the resulting permeate fibers from the filtration depends on the size of the pores in the ceramic membrane. Following are some sample images characterized with AFM, scan size 5 μm x 5 μm. From Fig. 3a we can see that were both long and short fiber in the sample before ultrafiltration. It was obvious that the nanopulping method had produced a wide range of sizes of microfibrillated cellulose from dimensions of 25-30 nm wide and 0.5 μm long up to almost intact, but often fibrillated. Fig. 3b and Fig. 3c were the permeate from membranes with different pore size. Apparently, the nanofibrillated cellulose can be separated to different fractions with more homogenous distribution by ultrafiltration. In this way a palette of fibrils of different sizes can be obtained and the method for fractionation is easy to scale up. These fractions can be important in many applications including the manufacture of novel materials. As can be seen in Fig. 3b) there are smaller fractions that might be separated further by the use of the smaller cut off, hence creating an even more homogenous fraction.

<table>
<thead>
<tr>
<th>Table 2. Results for Ozone Treated Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reference</strong></td>
</tr>
<tr>
<td>Conc. after NP (g/l)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Average</td>
</tr>
<tr>
<td>Standard deviation</td>
</tr>
</tbody>
</table>

| Membrane Permeate Rate (%) | 18.14 | 15.14 | 15.81 |

**Fig. (3). a) AFM image of fiber after nanopulping; b) Permeate with higher cut off; e) Permeate with smaller cut off.**
**4. CONCLUSION**

Nanopulping, a novel method for the preparation of nanocellulose fiber and particles, has been developed. The method is based on acceleration of chemical pulps, where the acceleration is caused by a pressure drop. The efficiency of the method is increased if the pulp is pretreated by various methods of which enzymatic treatment with monocomponent endoglucanase appeared to be the most efficient. Cross flow ultra-filtration on ceramic membranes can separate nano-pulped pulp into fractions with more homogenous size distribution. Both nanopulping and separation technologies seem to be industrial feasible.

**CONFLICT OF INTEREST**

The author(s) confirm that this article content has no conflicts of interest.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


**Table 3. Results for Endoglucanase and Tempo Treated Samples**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Endoglucanase Pretreatment</th>
<th>Tempo Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. after NP (g/l)</td>
<td>Conc. of Perm.(g/l)</td>
<td>Conc. after NP (g/l)</td>
</tr>
<tr>
<td>Conc. after NP (g/l)</td>
<td>Conc. of Perm.(g/l)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.0147</td>
<td>0.2187</td>
</tr>
<tr>
<td>2</td>
<td>1.0207</td>
<td>0.2183</td>
</tr>
<tr>
<td>3</td>
<td>1.0313</td>
<td>0.2447</td>
</tr>
<tr>
<td>Average</td>
<td>1.0222</td>
<td>0.2272</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.0084</td>
<td>0.0151</td>
</tr>
</tbody>
</table>

Membrane Permeate Rate (%): 22.23 27.09 15.29