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Morphological properties of nanofibrillated cellulose produced using wet grinding as an ultimate fibrillation process

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Abstract Nanofibrillated cellulose (NFC) aqueous suspensions were produced from once-dried bisulfite softwood pulp using enzymatic or 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation pretreatments, followed by wet grinding, as an ultimate fibrillation technique. Two commercial enzyme solutions: cellulase, with the major activity of endoglucanase and exoglucanase, and monocomponent endoglucanase, were compared to facilitate the nanofibrils isolation from cellulose fibers. The influence of their concentrations, as well as the other processing conditions, was analyzed. The morphology of the produced NFC was characterized using optical microscopy, atomic force microscopy (AFM), field emission gun-scanning electron microscopy (FEG-SEM), and morphological fiber analyzer (MorFi). Nanofibrils with a wide size distribution were produced. The average lateral dimensions of 12 ± 7 nm for the most disintegrated enzymatically hydrolyzed NFC and 4 ± 2 nm for TEMPO-oxidized NFC were determined from the AFM height images. The degree of polymerization (DP) decreased and the crystallinity index (CI) increased with an increase of the concentration of both enzyme solutions. TEMPO-oxidation did not have a significant impact on the cellulose CI; however, the DP was strongly affected. The monocomponent endoglucanase solution was found to have a better effect on the nanofibrils isolation rather than their depolymerization.

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Introduction

Production of nanofibrillated cellulose (NFC), otherwise known as microfibrillated cellulose (MFC) or cellulose nanofibers (CNF), has gained an increasing attention during the last decades. This bio-based, biodegradable material has a highly abundant feedstock and possesses unique properties, which make it promising for applications in such fields as: composites, papermaking, coatings, food, medical/pharmaceutical products, etc. [1].

NFC as a new cellulosic material was introduced in the early 1980s by Turbak et al. [2, 3], who produced cellulose with a diameter in nanometer scale by passing a softwood pulp suspension through a high-pressure homogenizer. Depending on the processing conditions, cellulose fibers can be disintegrated to NFC with the lateral dimensions starting from ~3 nm, representing elementary fibrils, to tens of nanometers, which correspond to single microfibrils and their bundles. Typically, NFC has a diameter of few tens of nanometer, length of several micrometers [4, 5] and consists of alternating crystalline and amorphous domains.

Cellulose can be derived from a variety of sources; however, wood is currently the most important industrial source of cellulose [6, 7]. Generally, softwood fibers are 3–4 times longer than hardwood ones [8]. Nevertheless, hardwood fibers have more rigid structure comparing to softwood counterpart, due to their high Runkel ratio (cell wall thickness divided by lumen radius) [9], which is valid for dense hardwoods (e.g., Eucalyptus). Thus, it was reported [7] that softwood pulp requires less mechanical treatment than hardwood homologue to produce equivalent fibrillations.

The common technique to extract nanofibrils from cellulosic fibers is mechanical disintegration using homogenization process. Within this process, cellulose suspension is forced at high pressure through assemblies, where fibrillation occurs. In this context, two types of equipment are employed: homogenizers [4, 5, 10–12] and microfluidizers [11, 13–15]. One of the disadvantages of the last technique is the often clogging of the system when using large fibers [16].

Ultra-fine friction grinding is another mechanical disintegration technique for the production of NFC, which is less common than homogenization. It employs Supermasscolloider device (Masuko Sangyo Co., Ltd., Japan), where cellulose slurry is forced to pass between a static and rotating grinding stones. By the shear forces, generated between the stones, the fiber cell wall is delaminated and the nanofibrils are individualized. Taniguchi and Okamura [17] described the NFC production by passing the natural fiber suspensions of 5–10 wt% through the grinder for 10 passes. As a result, the NFC with a diameter of 20–90 nm was obtained. The other researchers [18–20] reported the NFC production applying grinding technique when using never-dried cellulose as a raw material.

An investigation to compare homogenization, microfluidization, and grinding techniques in terms of the final product properties, relative to energy consumption, was performed by Spence et al. [16]. In their work, the never-dried pulp was disintegrated mechanically in different grade NFC suspensions. However, the NFC production using only mechanical disintegration requires high energy consumption [21, 22]. Therefore, an intensive investigation to facilitate the fibrillation and to reduce energy demand has been undertaken over the last decades. Recent methods involve mainly mechanical, enzymatic, and chemical treatments for the NFC production.

Continuous investigation has been conducted over the last decades ranging from a mild enzymatic hydrolysis of cellulose in order to facilitate the refining process till a strong hydrolysis to convert cellulose in monosaccharides for further production of bioethanol [23]. Only recently, the production of NFC by mild enzymatic hydrolysis of the previously refined bleached softwood pulp, followed by homogenization treatment, was reported [11, 14]. In the both works, a monocomponent endoglucanase solution Novozym 476 (Novozym A/S, Denmark) was used. Some studies [24, 25] reported the use of cellulase solution Celluclast 1.5 L (Novozym A/S, Denmark), which is a mixture of endoglucanase, exoglucanase, and cellobiase [26] with the major activity of endoglucanase and exoglucanase [27]. The comparison of cellulase solutions: Celluclast 1.5 L and Ecopulp Energy (AB Enzymes, Germany), the latest has the major activity of exoglucanase, was also reported for the production of NFC [24].

To clarify the difference between the enzymes, it should be noted that cellulases can be divided into three main classes: endoglucanases (endocellulases), which hydrolyze amorphous regions of cellulose; exoglucanases (cellobiohydrolases), which progressively cleave the ends of cellulose crystalline or amorphous regions, producing disaccharides (cellobiose) and tetrasaccharides; and cellobiases (β-glucosidases), which hydrolyze the di- and tetrasaccharides into glucose [28]. A schematic representation of each enzyme activity on cellulose can be found in the Electronic supplementary material (Fig. S1).

A way for the introduction of carboxyl groups through 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation and the further NFC production from the oxidized fibers was reported by Saito et al. [29]. Due to such treatment, the disintegration of cellulosic fibers is improved because of the electrostatic repulsion formed between negatively charged nanofibrils [30]. In Saito's et al. [29] work, never-dried cellulose samples (bleached sulfite wood pulp, cotton, tunicin, and bacterial cellulose) were disintegrated in individual nanofibrils with a regular width of 3–5 nm using TEMPO-mediated oxidation, followed by homogenization.

The basic principle of TEMPO-treatment is the oxidation of cellulose fibers by nitrosonium ion, generated in situ through the reaction of TEMPO with oxidants, such as hypobromide ions, which in turn are generated from bromide salts, for instance, sodium bromide (NaBr), and sodium hypochlorite (NaClO). The detailed mechanism of the oxidation is presented in the Electronic supplementary material (Fig. S2). TEMPO/NaBr/NaClO system was reported to be used at a pH of 10 [29]. Later, the oxidation using TEMPO/NaClO/$NaClO_2$ system under neutral or weakly acidic conditions was reported [31].

In this work, NFC was produced from once-dried commercial bisulfite softwood pulp. Obviously, the use of never-dried cellulose, comparing to the once-dried, facilitates the fibrillation, since the drying promotes the irreversible hydrogen-bonding between nanofibrils, known as hornification [32]. However, the dry cellulose is much easier to transport. Therefore, the research focuses also on the use of dry cellulose as a raw material for NFC production. Qing et al. [25] compared the use of endoglucanase solution

FiberCare (the grade was not specified) and the mixture of FiberCare and Celluclast 1.5 L for the production of NFC. They did not found a significant difference between these treatments. In our study, the use of cellulase solution Celluclast 1.5 L and endoglucanase solution FiberCare R apart, as well as TEMPO-mediated oxidation, is compared for the production of NFC. The influence of the different enzyme solution concentrations, oxidation, and the other processing conditions on cellulose morphology and the variation of the degree of polymerization (DP) and the crystallinity index (CI), are reported. The optical microscopy, atomic force microscopy (AFM), field emission gun-scanning electron microscopy (FEG-SEM), and morphological fiber analyzer (MorFi) are employed for the morphological analysis. The wet grinding operation using Supermasscolloider, as an ultimate fibrillation technique, is investigated.

Materials and methods

Materials

The high-purity commercial bisulfite softwood pulp (Quality 2100, Domsjö Fabriker AB, Sweden) was used as a source of cellulose. The average fiber dimensions were 28.0 ± 0.1 μm in diameter and 969.7 ± 16.0 μm in length, as determined by the MorFi analysis. The alpha-cellulose content of 93 % and the DP of 780 were specified by the supplier. The commercial enzyme solutions: Celluclast 1.5 L (cellulase, a mixture of endoglucanase, exoglucanase, and cellobiase [26] with the major activity of endoglucanase and exoglucanase [27]), with the declared activity of 700 EGU g^{-1} (endoglucanase units per gram of solution), and FiberCare R (monocomponent endoglucanase)—4700 ECU g^{-1} (endocellulase units per gram of solution) were used for cellulose hydrolysis (both products of Novozymes A/S, Denmark). Since endoglucanase and endocellulase are synonyms, the solutions can be compared in terms of this specific activity. Sodium acetate trihydrate, acetic acid, TEMPO, sodium bromide, sodium hypochlorite solution, and the other chemicals were of laboratory grade and used without further purification.

NFC production methods

Cellulose was soaked in water for 4 h and dispersed in a standard disintegrator according to ISO 5263-1:2004 standard. The obtained cellulose suspension was filtered till 10 wt% on a Büchner funnel using a nylon sieve with the mesh size of 1 μm and beaten in PFI mill according to the procedure described in ISO 5264-2:2011, for a given number of revolutions, typically 40,000.

The enzymatic hydrolysis was performed using cellulase Celluclast 1.5 L and endoglucanase FiberCare R enzyme solutions at different concentrations. The reactions were performed under a mild stirring of 2 wt% cellulose suspensions at a temperature of 50 °C during 2 h in acetate buffer solution of 50 mM and pH of 5, prepared using sodium acetate tryhidrate and acetic acid. The enzymatic activity was stopped by heating the suspension at 80 °C for 15 min. Chloroform at 0.01 wt% was used as a biocide. The used concentrations of enzyme solutions and the other processing conditions of each experiment are specified in Table 1.

TEMPO-mediated oxidation was performed using the non-refined cellulose fibers. The reaction protocol was based on [33]. TEMPO/NaBr/NaClO system was used with 0.1/1/5 millimol of the reactants per gram of cellulose (mmol/g), respectively. TEMPO, ground using a mortar and pestle, and NaBr were mixed with the cellulose suspension before the addition of NaClO water solution with the adjusted pH till 10 using HCl. For the pH adjustments, 3 M HCl and 3 M NaOH were used. The concentration of the obtained cellulose suspension was of 1 wt%. The reaction was carried out at 25 °C at a controlled pH of 10 ± 0.1. The stable pH during the reaction was acquired by the continuous addition of NaOH. When the pH no longer decreased, indicating no longer creation of carboxyl groups, the reaction was quenched by lowering the pH till 7. The TEMPO-oxidized cellulose was filtered on a Büchner funnel using a nylon sieve with the mesh size of 1 μm and washed until the filtrate conductivity reached a value below 5 μS cm^{-1}. Chloroform at 0.01 wt% was used as a biocide.

The cellulose suspensions were fibrillated using ultrafine friction grinder Supermasscolloider (model MKZA6-2, disk model MKG-C 80, Masuko Sangyo Co., Ltd., Japan) and for one sample (enz-F21-H) additionally with homogenizer Panther (model NS3006L, GEA Niro Soavi S.p.A., Italy). Supermasscolloider was used at 2500 rpm; typically, 60 passes of cellulose suspension were performed. The 2 passes through homogenizer were carried out at 1000 and 1500 bar, sequentially.

NFC characterization methods

The size distribution of cellulose fibers and the fines content was determined using MorFi LB-01 fiber analyzer (Techpap, France), hereinafter MorFi analysis. The fiber threshold length and diameter were set in the software to 80 and 2 μm, respectively. The fines content was measured as: (i) percentage in length, indicating the percentage of the fines length versus the total length of all the objects, both fibers and fines; (ii) fines/g, representing a number of fines per gram of all the cellulosic elements in the suspension.

Table 1 Processing parameters for the production of NFC from once-dried bisulfite softwood pulp

Sample name	Processing conditions			
	Refining[a]	Pretreatment[b]	Grinding[c]	Homogenization[d]
enz-C2.1	Yes	Celluclast 1.5 L; 2.1 EGU g^{-1}	Yes	No
enz-C10.5	Yes	Celluclast 1.5 L; 10.5 EGU g^{-1}	Yes	No
enz-C21.0	Yes	Celluclast 1.5 L; 21.0 EGU g^{-1}	Yes	No
enz-F21	Yes	FiberCare R; 21.0 ECU g^{-1}	Yes	No
enz-F21-H	Yes	FiberCare R; 21.0 ECU g^{-1}	Yes	Yes
enz-F210	Yes	FiberCare R; 210.0 ECU g^{-1}	Yes	No
enz-F315	Yes	FiberCare R; 315.0 ECU g^{-1}	Yes	No
ox-TEMPO	No	TEMPO/NaBr/NaClO	Yes	No

[a] Beating in PFI mill for 40,000 revolutions

[b] EGU g^{-1} stands for endoglucanase units per gram of cellulose; ECU g^{-1} stands for endocellulase units per gram of cellulose; TEMPO/NaBr/NaClO — 0.1/1/5 millimol per gram of cellulose, respectively

[c] Disintegration in the grinder (Supermasscolloider MKZA6-2) for 60 passes except for enz-F21-H, where the cellulose suspension of 6 L was passed continuously through the grinder, using recirculation pump, for 1 h

[d] Disintegration in the homogenizer (Panther NS3006L) for 2 passes, at the pressure of 1000 and 1500 bar, sequentially

The pulp freeness (drainability) was measured using Schopper-Riegler tester according to ISO 5267-1:1999 standard.

The optical microscopy images were taken using Carl Zeiss Axio Imager M1 m optical microscope in a transmission mode. The cellulose suspensions were diluted to a concentration of 0.2 wt%, and a drop was placed between the glass slide and a coverslip. The images were captured by AxioCam MRc 5 digital camera.

The AFM was performed in a tapping mode using Dimension Icon Atomic Force Microscope with an OT-ESPA cantilever. The cellulose samples were diluted to the concentration of 10^{-3} wt% and a droplet of suspension was placed on a mica disk and dried at room temperature. Nanoscope III software was used for the evaluation of the nanofibrils diameter from the height profiles of AFM height images. At least four different areas of the sample were scanned and 200 heights were measured for each sample.

The FEG-SEM was performed using ZEISS Ultra 55 microscope, equipped with In-Lens secondary electron detector. An accelerating voltage of 3 kV was used. The samples were deposited on a double-sided adhesive carbon tape, dried under vacuum and sputtered with Au/Pd layer of 2–3 nm before the analysis.

The DP was measured from the intrinsic viscosities (η_{int}) of cellulose, dissolved in copper(II) ethylenediamine (CED), measured according to ISO 5351:2010. The viscometric average degree of polymerization (DPv) was then calculated from the intrinsic viscosity using the Mark–Houwink–Sakurada equation:

$$DPv^{0.905} = 0.75\eta_{int}. \quad (1)$$

For the preparation of cellulose solution in CED, the produced NFC suspensions in water were used directly without drying.

The crystallinity index (CI) of cellulose samples was determined from wide-angle X-ray diffraction (XRD) spectra. The samples were placed into zero-background Si holder and the measurements were performed using PANanalytical X'Pert PRO MPD diffractometer, equipped with an X'celerator detector. The operating conditions were: Copper Ka radiation with a wavelength of 1.5418 Å, double Bragg angle (2θ) of 5–56°, step of 0.067° and the counting time of 360 s. The cellulose CI (%) was determined according to the peak height method [34]:

$$CI = (I_{002} - I_{am})/I_{002} \times 100, \quad (2)$$

where I_{002} is the diffraction intensity of the main crystalline peak at $2\theta \sim 22.5°$ and I_{am} represents the intensity at the minimum taken at $2\theta \sim 18°$ both after subtraction of the background signal.

The carboxyl content of cellulose was determined using the conductometric titration method according to SCAN-CM 65:02. After the protonization, cellulose suspension was titrated with sodium hydroxide solution using the addition step of 400 μL. Carboxyl content was calculated from the equation:

$$X_{COOH} = [(V_2 - V_1)c]/w, \quad (3)$$

where X_{COOH} is in mmol/g, V_1 and V_2 are the volumes (L) of the sodium hydroxide solution consumed at 1st and 2nd intersection points at the titration curve, respectively, c is the concentration (mmol/L) of the sodium hydroxide solution, and w is the oven-dry weight (g) of cellulose.

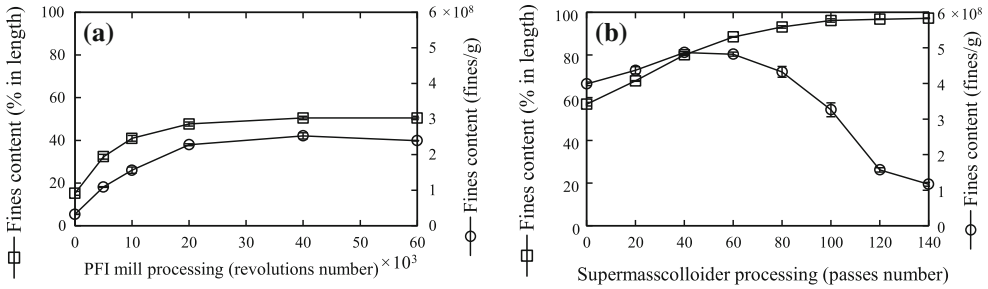

Fig. 1 Dependence of the fines content on **a** the number of revolutions in PFI mill for the raw pulp and **b** the number of passes through the grinder for the sample, previously beaten in PFI mill for 40,000 revolutions and enzymatically treated with endoglucanase solution FiberCare R, 210.0 ECU g^{-1}

Results and discussion

Some preliminary experiments were carried out to determine the sufficient levels of refining (beating) in PFI mill and the final grinding in Supermasscolloider. The refining stage disintegrates the fibers, increases their specific surface area, and makes them more accessible for enzymatic activity. Figure 1a represents the influence of the refining in PFI mill on the fines content of the beaten raw cellulose pulp. The most significant increase of the fines content occurs for the first 20,000 revolutions, reaching a plateau at 40,000. The further fibrillation did not influence much the measured parameters. Thereby, refining for 40,000 revolutions in PFI mill was performed for all the next samples. However, in terms of efficient energy consumption, 20,000 revolutions can be more favorable.

The raw cellulose pulp was beaten using PFI mill for 40,000 revolutions and enzymatically treated with endoglucanase solution FiberCare R at 210.0 ECU g^{-1}, and was used further to study the influence of the grinding operation. Figure 1b shows the dependence of the fines content on the number of passes through Supermasscolloider for this sample. The curves show that during all the grinding process, the cellulose fibrillation occurs. The percentage of the fines increases progressively reaching a plateau at $\sim$97 %. The growth of the fines content indicates the conversion of the fibers to fines and nanofibrils. The number of the fines per gram of cellulose reaches a peak at 40–60 passes and declines with the further grinding. The decline occurs since the nanofibrils production dominates the conversion of the fibers into fines. Since the nanofibrils are not detected by MorFi analyzer due to their small dimensions, the decrease of the fines content is observed.

The fiber dimensions reduce progressively within the multiple processing conditions applied, such as beating in PFI mill, enzymatic hydrolysis, and grinding, as seen from the optical microscopy images in Fig. 2. However, some non-fibrillated flocculated structures remain with increasing the number of passes through the grinder. Therefore, the grinding operation for 60 passes was chosen for all the next samples in order to study the effect of the enzymatic treatment.

The enzymatic hydrolysis using two enzyme solutions, namely Celluclast 1.5 L and FiberCare R, was compared to facilitate the NFC isolation. The difference between the enzyme solutions resides in their composition: Celluclast 1.5 L is a cellulase mixture with the major activity of endoglucanase and exoglucanase, whereas FiberCare R contains a monocomponent endoglucanase.

Figure 3 shows the optical microscopy and atomic force microscopy images of the produced NFC suspensions, treated with different concentrations of cellulase solution Celluclast 1.5 L. Obviously, the increase of the enzymatic concentration results in the stronger cellulose fibrillation in the grinder. The minimal concentration of the applied enzyme produced the fibers, which are partly disintegrated in nanofibrils. The enz-C21.0 sample, despite some non-fibrillated flocculated residuals, comprises the nanofibrils with the lateral dimensions of 29 $\pm$ 25 nm, measured from the height profile of AFM height images.

The optical microscopy and AFM height images of NFC samples, produced when applying endoglucanase solution FiberCare R, are shown in Fig. 4. The better dissociation of cellulose in enz-F21 (Fig. 4a), comparing to enz-C21.0 (Fig. 3g), is observed from the optical microscopy images, despite the applied equivalent concentrations of endoglucanase (endocellulase). The further increase of FiberCare R concentration enhances the hydrolysis, which follows from the smaller cellulose objects, detected in the microscopy images. The enz-F210 and enz-F315 samples possess the lateral dimensions of 18 $\pm$ 10 nm and 18 $\pm$ 13 nm, respectively, as deduced from the AFM height images. However, some residual big particles still remain, as detected by optical microscopy.

The DP and CI of enzymatically hydrolyzed and TEMPO-oxidized NFC before the grinding operation, as well as of the raw cellulose pulp, are compared in Fig. 5a and b. The DP decreases drastically as the enzymatic

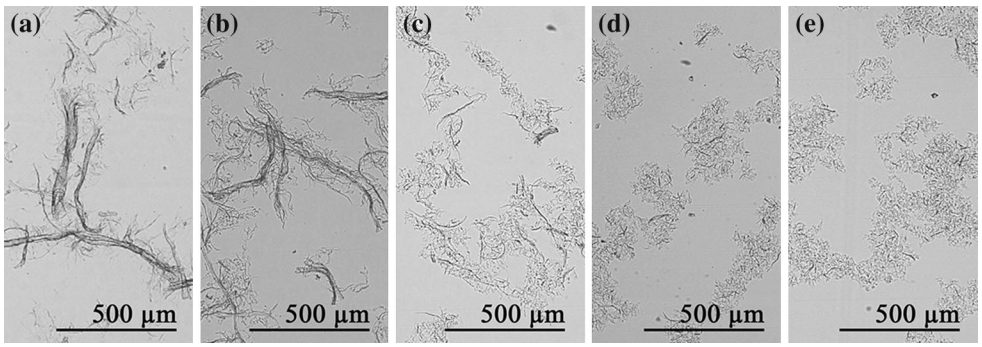

Fig. 2 Optical microscopy images of bisulfite wood pulp: **a** beaten in PFI mill for 40,000 revolutions; **b** refined and enzymatically treated with endoglucanase solution FiberCare R at 210.0 ECU g^{-1}; refined, enzymatically treated, and grinded using Supermasscolloider for: **c** 40 passes; **d** 80 passes; and **e** 120 passes

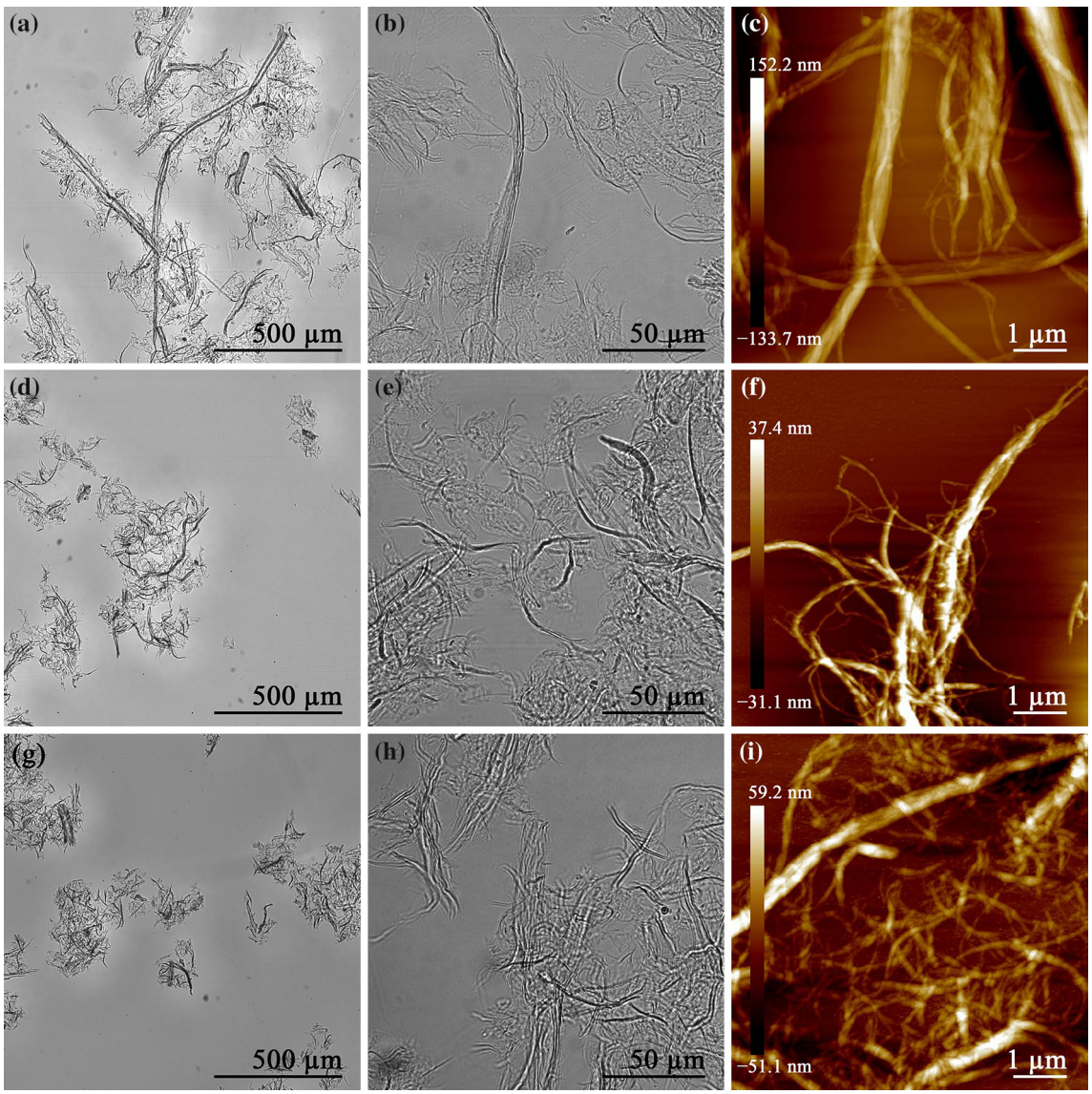

Fig. 3 Optical microscopy (*left* and *middle*) and AFM height (*right*) images of: **a–c** enz-C2.1; **d–f** enz-C10.5; and **g–i** enz-C21.0 samples at different magnifications

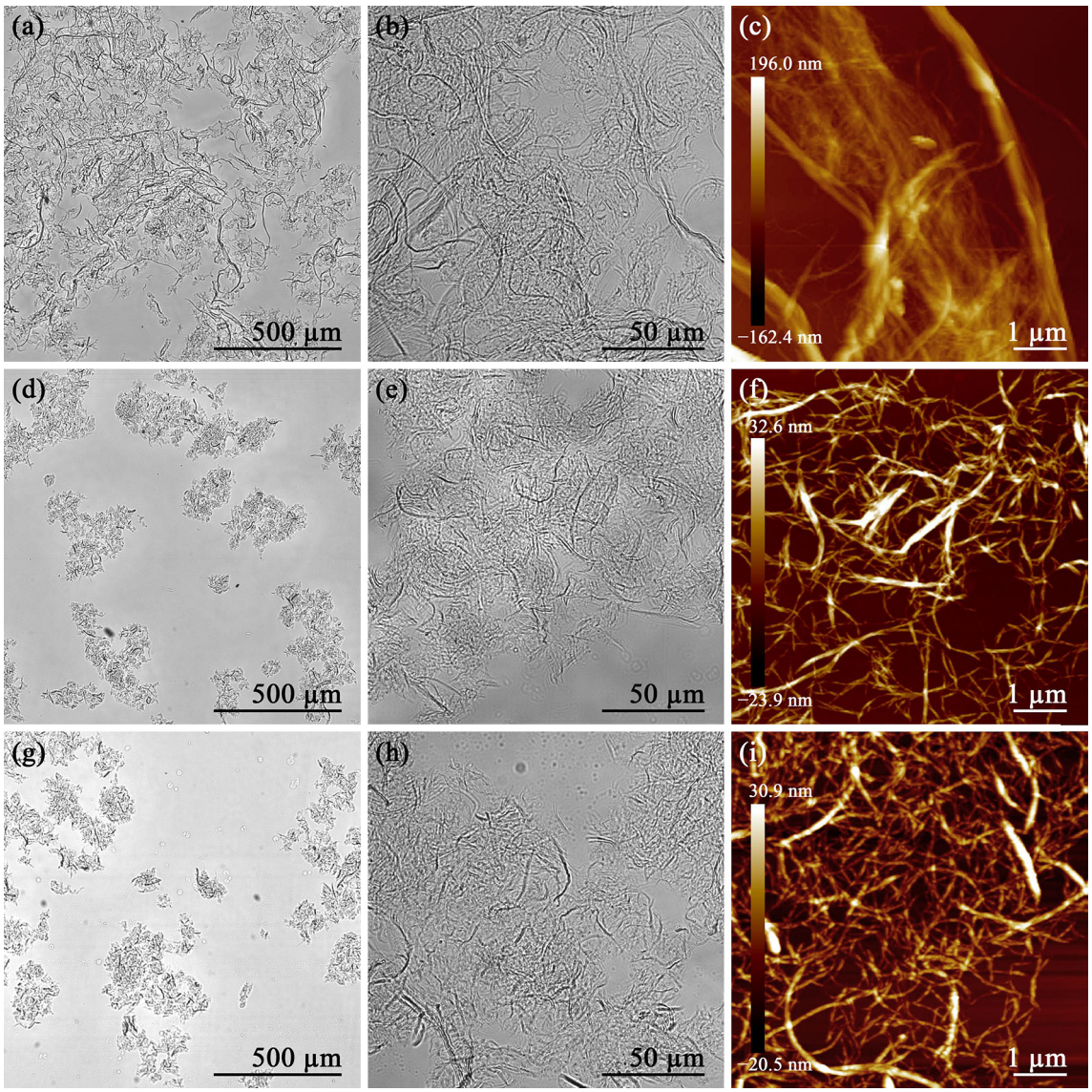

Fig. 4 Optical microscopy (*left* and *middle*) and AFM height (*right*) images of: **a–c** enz-F21; **d–f** enz-F210; and **g–i** enz-F315 samples at different magnifications

concentration increases, which is in agreement with the other studies [24, 25, 35]. As seen from Fig. 5a, enz-C21.0 yields cellulose with lower DP comparing to enz-F21 (290 ± 1 vs. 361 ± 5) when applying the equivalent endoglucanase activity. Apparently, the stronger depolymerization of enz-C21.0 occurs due to the presence of the other active enzymes, for e.g., exoglucanase.

The cellulose CI was reported to vary significantly depending on the measurement method [36]. The peak height method based on XRD spectra, used in this work, is known to produce higher values of crystallinity comparing to the other methods. However, it is used here basically to analyze the crystallinity variation, rather than to determine the exact CI values of cellulose samples.

Figure 5b shows that the cellulose CI rises with the increase of enzymatic charge, as also reported in the other works [25]. The raw cellulose pulp has a CI of 82.4 % and this value increases till 86.5 % for the highest concentration of Celluclast 1.5 L applied (enz-C21.0 sample). It is likely to occur due to the hydrolysis of cellulose amorphous regions by endoglucanase. Moreover, the enzymatic hydrolysis rate of cellulose amorphous sites is around 30 times higher than that of crystalline ones [35]. The CI of enz-F21 was found to be higher comparing to that of enz-C21.0 (88.0 vs. 86.5), which means that FiberCare R attacks the amorphous domains of cellulose more strongly comparing to Celluclast 1.5 L. The difference between enz-F210 and enz-F315 is very poor to compare these

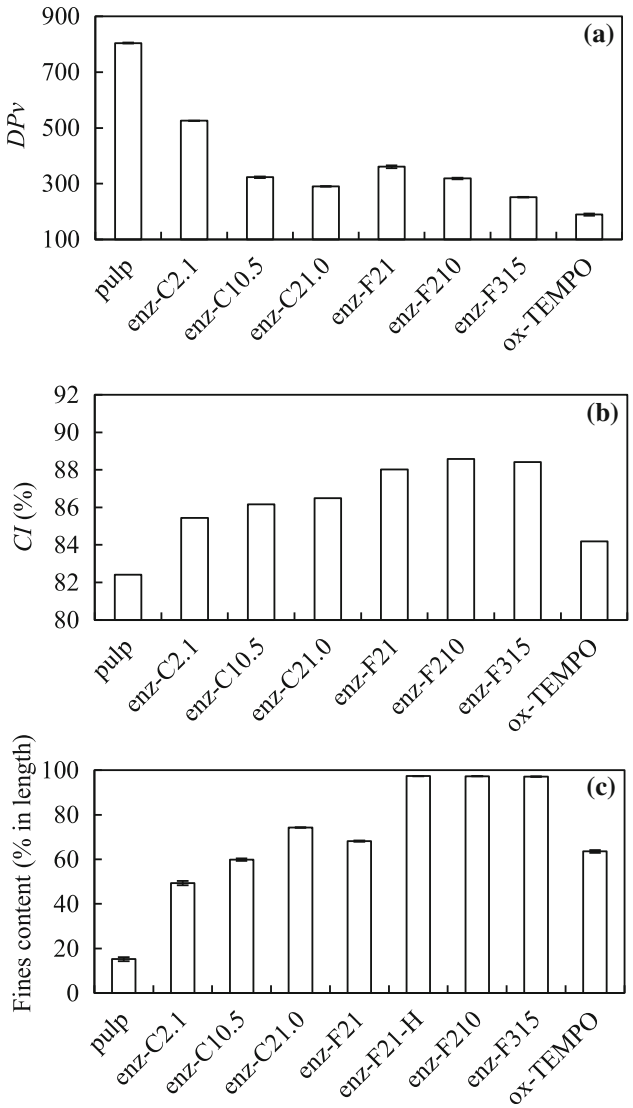

Fig. 5 The comparison of the raw cellulose pulp and the NFC suspensions: **a** degrees of polymerization and **b** crystallinity indexes before grinding in the grinder and **c** fines content of the final NFC suspensions

samples. However, it seems that the saturation of the enzyme takes place, as reported elsewhere [37]. It can follow as well from the similar microscopy images for these samples. However, the decrease of DP for enz-F315, comparing to enz-F210, is still observed.

The fines content of the finally produced NFC suspensions is presented in Fig. 5c. The increase of the enzymatic load enhances the fines content. For enz-F210 and enz-F315 samples, the fines content reaches the value of ~97 % indicating the strong fibrillation level of cellulose.

Comparing to enz-C21.0, the enz-F210 was treated with the higher concentration of endoglucanase, and the lower depolymerization of this sample is observed (290 ± 1 vs. 319 ± 3, respectively, see Fig. 5a). At the same time, the much higher fines content for enz-F210 was observed (Fig. 5c), as well as the smaller residual aggregates (as seen from Fig. 4). We have also reported [38] much stronger viscoelastic properties of the NFC samples, hydrolyzed using FiberCare R, comparing to that of Celluclast 1.5 L while performing the rheological measurements. This indicates the stronger networks of nanofibrils produced when using FiberCare R enzyme. The foregoing suggests that the use of endoglucanase solution FiberCare R allows increasing the enzymatic load, comparing to cellulase solution Celluclast 1.5 L, preserving cellulose from the depolymerization and producing better fibrillated cellulose suspensions.

One sample (enz-F21-H) was mildly hydrolyzed by FiberCare R applying 21 ECU g^{-1}, grinded in Supermass-colloider equipped with a recirculation pump for 1 h (by processing the suspension of 6 L) and finally passed through Panther homogenizer for two times (at 1000 and 1500 bar, respectively). The homogenizer was applied to examine whether it would be effective to disintegrate the residual fibers in the NFC suspension. Figure 6 shows that in enz-F21-H sample, much lower amount of the residual aggregates is present, comparing to all the other

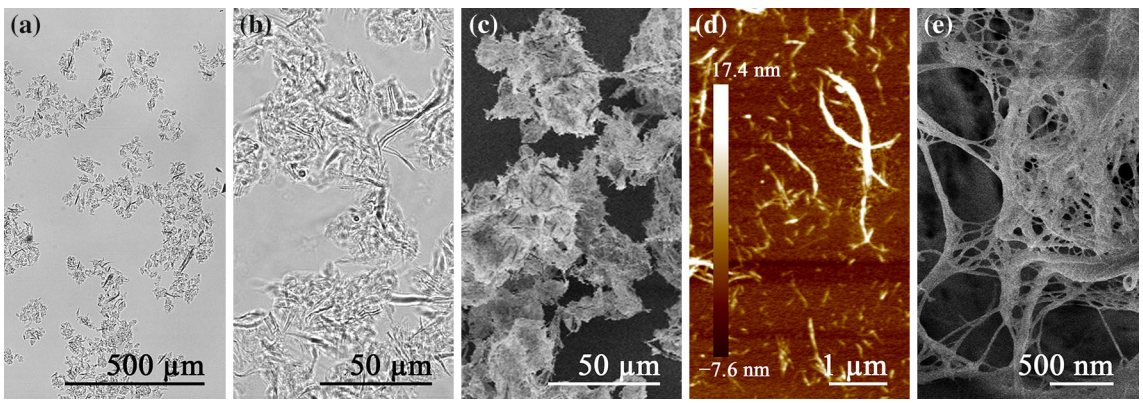

Fig. 6 Optical microscopy (**a, b**), FEG-SEM (**c, e**) and AFM height (**d**) images of enz-F21-H sample at different magnifications

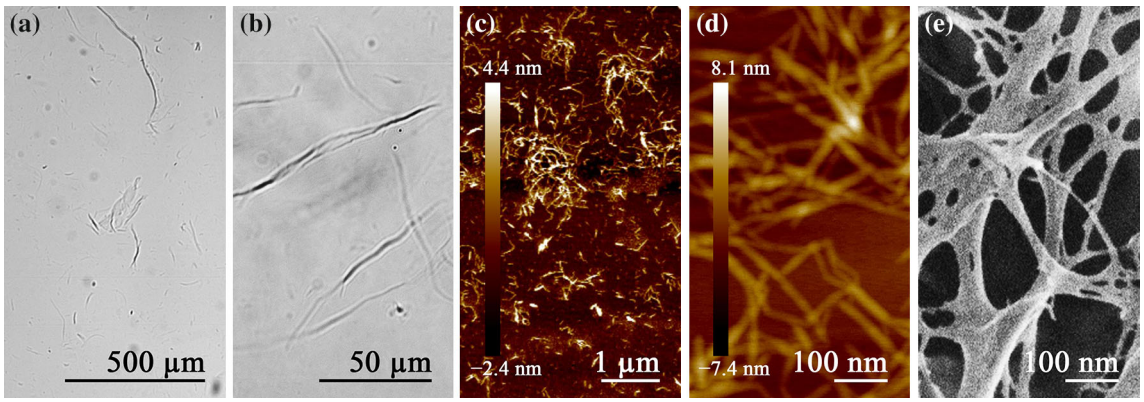

Fig. 7 Optical microscopy (**a, b**), AFM height (**c, e**) and FEG-SEM (**d**) images of ox-TEMPO sample at different magnifications

enzymatically treated samples. However, the optical microscopy (Fig. 6b) and FEG-SEM (Fig. 6c) both demonstrate the presence of flocks of ~50 µm in diameter. These flocks consist of the highly entangled NFC. The individual nanofibrils with the diameter of 12 ± 7 nm are present as well. These dimensions were calculated based on the AFM height images.

As a result of TEMPO-mediated oxidation, the NFC (ox-TEMPO sample) with the carboxyl content of 1.75 ± 0.05 mmol g^{-1} was produced. Just a few passes of the suspension in the grinder were sufficient to obtain a translucent gel; however, with the increase of the passes number till 60, the gel became more viscous. A strong decrease of the DP was observed as a result of TEMPO-mediated oxidation (see Fig. 5), which is in agreement with the previous studies [31, 39]. It occurs due to the presence of sodium hypochlorite in the system, which causes 2,3-scissions of glucose units, forming dialdehyde and dicarboxyl groups [40], which leads to further cellulose chain depolymerization. The CI of ox-TEMPO was not much influenced, which is also in agreement with the other works [33, 41].

The microscopy observations of ox-TEMPO (see Fig. 7) show the produced nanofibrils at different magnifications. The lateral dimensions of 4 ± 2 nm were determined from the AFM height images (see Fig. 7d). The FEG-SEM image (see Fig. 7e) shows some nanofibrils with a similar width and some larger elements, which may be agglomerations formed while drying. Some residual non-fibrillated fibers were detected by optical microscopy (see Fig. 7a and b), which justify the relatively low amount of the fines content for this sample (63.5 %, as seen from Fig. 5c).

Conclusions

The NFC suspensions were successfully produced from once-dried bisulfite softwood pulp using enzymatic and TEMPO-oxidation pretreatments followed by wet grinding. The increase of the enzymatic concentration resulted in the stronger hydrolysis and hence fibrillation, which was accompanied by the decrease of the DP and the increase of the CI.

The enzymatic hydrolysis using endoglucanase solution FiberCare R was found to be more favorable for the NFC production since it led to lower depolymerization with better dissociation of the nanofibrils comparing to cellulase solution Celluclast 1.5 L, which has the major activity of endoglucanase and exoglucanase. Grinding of TEMPO-oxidized suspensions produced nanofibrils with lower lateral dimensions, as well as reduced length, comparing to that of enzymatically prehydrolyzed. All the enzymatically treated NFC samples possessed highly entangled flocculated structures.

In this work, there was also shown that the morphology of the produced NFC suspensions depends on the applied processing conditions, which influence the material properties. More intensive fibrillation results in smaller nanofibrils. Consequently, different NFC grades can be produced. The exact processing conditions should be adapted according to the requirements for the specific application. Efficient energy consumption during the NFC production is still an important issue.

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